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APWSS



WEEDS AND ENVIRONMENTAL IMPACT

NOVEMBER 22-27, 1999
BANGKOK, THAILAND

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A.N.RAC

Proceedings I (A)

Keynote Address

1. The ethics of agriculture and weed science
   R.L. Zimdahl ... 3
2. Weed science in year 2000s in Asian-Pacific region
   J. Harada ... 9

Invited Papers

1. Behavior and phytotoxic activity of herbicides and allelochemicals in soil
   K. Kobayashi ... 15
2. Fulfilling needs for transgenic herbicide-resistant rice while preventing and mitigating introgression into weedy rices
   J. Gressel ... 21
3. Herbicide modes of action, recent progress and new challenges
   H. Matsumoto ... 39
4. On ecological issues in the development of sustainable weed management
   M. Mortimer ... 45
5. Some aspects of tropical weed seed banks : determination, dynamics and depletion
   S.W. Adkins ... 51

Oral Presentation

1. Weed Biology

1. A survey of weeds in rainfed rice-onion cropping systems in central Luzon, Philippines
   A.M. Baltazar, E.C. Martin, M.C. Casimero, F.V. Bariuan, S.R. Obien, and S.K. De Datta .. 65
2. Competitive ability of spring wheat as influenced by relative time of weed emergence
   S.M.R. Karim, R.E.L. Naylor, and G.P. Whytock ... 71
3. Development of an competitive enzyme-linked immunosorbent assay for butamifos
   Y. Kagawa, K. Watanabe, K. Morimune, M. Kawata, and S. Kanai ... 77
4. Effects of delaying starter fertilizer application on rice (*Oryza sativa* L.) -barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.) competition
   L.L. Ranasinghe and G.D. Crabtree ... 83
5. Infestation and identification of perennial Gramineous weeds in paddy fields of Japan
   H. Morita ... 89
6. Phytotoxic activity of Mexican sunflower (*Tithonia diversifolia*) in soil and its allelopathic potential
   S. Tongma, K. Kobayashi, and K. Usui ... 93
7. Plant characteristics associated with rice (*Oryza sativa* L.) -barnyardgrass (*Echinocloa crus-galli* L. Beauv.) competition
   L.L. Ranasinghe and G.D. Crabtree ... 99
8. The spatio-temporal pattern of distribution of weedy rice accessions in Sungei Burong Rice Granary of Tanjong Karang, Selangor
   M.A. Bakar and B.B. Bakar ... 105

9. Weed morphology effects on competitiveness for light in direct-seeded rice
B.P. Caton, A.M. Mortimer, T.C. Foin, J.E. Hill, K.D. Gibson, and A.J. Fischer..... *116*

2. Weed Ecology

1. A preliminary study and survey on Gramineae of Thailand
W. Nanakorn and M. Norsangsri..... *123*
2. Effects of *Salvinia molesta* (D.S.Mitchell) on growth and yield of rice (*Oryza sativa* L.)
A.S.K. Abeysekera, B. Marambe, and E.M.M.S. Ekanayake..... *130*
3. Morphologic and genetic variations in upland and lowland ecotypes of purple nutsedge (*Cyperus rotundus* L.) in rainfed rice-onion systems
M.C. Casimero, A.M. Baltazar, J.S. Manuel, S.R. Obien, and S.K. De Datta..... *134*
4. Research on damage and population change of weeds at spring wheat fields in Qinghai
Q. Xuelin, X. Cunyue, and G. Qingyun..... *140*
5. The change of weed community in wheat-maize cropping system and its control in Beijing
I. Che and S. Li *143*
6. The impact of cropping practices on rice-weed communities
J.D. Janiya, M.A. Dizon, M. Mortimer, C. Piggin, and J. Hill..... *146*
7. The use of weed density to predict loss in wheat yield due to weed competition
E.E. Hassanein, H.T. Al-Marsafy, M.M. Ibrahim, and S.M. Shebl..... *152*

3. Weed Control and Management

1. Based upon chemical weed control and attaching importance to integrated weed management
Z.P. Zhang..... *158*
2. Control and demography of wild radish (*Raphanus raphanistrum*) resistant to ALS inhibitors herbicides
A. Cheam, S. Lee, D. Bowran, and A. Hashem..... *164*
3. Current advances and future needs in integrated weed management in early 21 century in China
C.X. Zhang, Y.X. Qian, Z.W. Fu, and X.E. Hu..... *168*
4. Current status of weed control in rice in Sri Lanka
A.S.K. Abeysekera..... *174*
5. Effect of dinitroaniline herbicides on the control of weeds in groundnut (*Arachis hypogaea* L.)
S.S. Punia and R.K. Malik..... *180*
6. Effect of mulching practices on weed management, growth and yield of cotton
R. Raman, G. Kuppuswamy, and R. Krishnamoorthy..... *186*
7. Effect of sowing methods and weed control on controlling wild oats and productivity of wheat in Egypt
A.A. Salim and H.M. Ibrahim..... *189*
8. Effect of tillage and time of sowing on the efficacy of herbicides against *Phalaris minor* in wheat
G. Mahajan, L.S. Brar, and V. Sardana..... *193*
9. Effect of tillage practices, seeding rates and herbicides on weed infestations in direct-seeded rice
M. Azmi and A.M. Mortimer..... *199*

10. Effects of mixtures of cyclosulfamuron and pendimethalin on weed control in rice in Southeast Asia
L. Ros, J.K. Lim, S. Chantarasa-ard, C.Y. Liew, and F. Antameng 205
11. Effects of seeding rate and cultivar of field pea on the weed incidence and productivity of intercrops of field pea and canola
P. Soetedjo, L.D. Martin, and A.J.V. Janes 213
12. Effects of continuous no-tillage applied glyphosate herbicide on weed community of irrigated lowland rice
Z. Lamid, Azwir and G. Adlis 219
13. Establishment of irrigated lowland rice under zero and conventional tillage systems in the Philippines
C.O. Garcia, C.M. Piggin, J.D. Janiya, M.A. Bell, E. Castro, E. Razote, and J.E. Hill 226
14. Impact of weed control on growth and yield of urdbean (*Vigna mungo*)
R. Raman and G. Kuppuswamy 232
15. Integrated weed management in tea plantations in North East India
A. Bose 235
16. Integrated weed management practices on nodulation and yield of mungbean (*Vigna radiata* (L.))
R. Raman 239
17. Long term effect of soil solarization on weed competition and seed yield of soybean
N.T. Yaduraju, T.K. Das, and Seema 242
18. Performance of three rice varieties planted by Chinese seed drill under different weed management
J.D. Ranjit 246
19. Resistance biotype of *Monochoria korsakowii* against sulfonylurea herbicides in the reclaimed paddy fields in Korea
T.S. Park, C.S. Kim, J.P. Park, Y.K. Oh, and K.U. Kim 251
20. Selective control of purple nutsedge (*Cyperus rotundus*) in maize and asparagus
A. Rahman and T.K. James 255
21. Soil solarization and glyphosate for purple nutsedge (*Cyperus rotundus*) control
R.K. Nishimoto and O. Kawabata 261
22. Status of weed management research in intercropping in India-An overview
A.N. Tewari 266
23. Studies on some biological characteristics of purple nutsedge and its control in cotton fields
Z.P. Zhang, X.E. Hu, Y.X. Qian, and Z.Y. Wang 276
24. Sulphosate, paraquat and combination of paraquat/diuron herbicides for land preparation in no-tillage upland rice (*Oryza sativa*)
N. Sriyani 282
25. The techniques of weed chemical control in fields seeded on unfallowing wheat fields
Y. Mansheng, D. Wei, W. Hongfu, and G. Pingyi 288
26. Use of some cultivation methods on the milpa excluding some weed species
T.T.P. Lan 292
27. Utilisation and management of orchard ground cover in the Mekong Delta, Vietnam
P.V. Mele and T.V. Phen 296

28. Weed control efficacy and soil persistence of new pendimethalin formulations
B.R. Jeong, Y.D. Lee, and S.D. Song 303
29. Weed management strategies in onion grown after rice
A.M. Baltazar, E.C. Martin, M.C. Casimero, F.V. Bariuan, S.R. Obien, and S.K. De Datta 308

4. Herbicides

4.1 Application Techniques

1. Drizzle herbicide applications for vegetation management on forest trails in Hawaii
P. Motooka, C. Koga, A. Kiyono, G. Kawakami, G. Nagai, L. Ching, G. Shishido, and J. Powley 317
2. Field evaluation of a formulation of imazapyr/glyphosate for weed control in rubber plantations in Thailand
C. Boonsrirat, W. Noopradit, and S. Chantarasa-ard 322
3. Organosilicone adjuvants for agrochemical applications
J.S. Sun and G. Policello 327
4. Optimizing herbicide application rate in summer maize (*Zea mays*)
H. Ni, L. Zhang, and Y. Chen 333
5. Safety evaluation for herbicides used in soybeans
X.F. Wong and C.H. Guan 338
6. Study on influence of three herbicides to corn seedling
H. Chunyan, C. Tiebao, W. Yu, and S. Baohong 344
7. The application of 2,4-D sustain released in controlling *Hydrilla verticillata*
H. Abdullah and M. Mansor 347
8. Weed control efficacy of glyphosate herbicides with the use of controlled droplet application (CDA) sprayer
K.G. Prematilaka, A. Gamage, and H.S.N. Pieris 353

4.2 Physiology and Biochemistry

1. Effect of carfentrazone-ethyl on absorption and translocation of (^{14}C) glyphosate
J.C. Chun, S.E. Kim, Y.S. Park, K.Y. Kim, and M.H. Kim 361
2. Effectiveness of herbicides and slashing on weed control in young rubber plantations
A.F.M. Alif 368
3. Efficacy of clomazone 36ME, sulfentrazon 48SC and sulfentrazone 75DF in dry and rainy season soybean (*Glycine max*)
P. Lueang-a-papong and P. Khawtepawan 374
4. Field evaluation of flufenacet for weed control in Philippine rice production condition
F. Palis, F. Vasquez, H. Dupo, E. Llabores, H. Cabanilla, and J. Aspuria 379
5. Interaction between a propanil-containing herbicide and an organophosphate insecticide on rice (*Oryza sativa L.*)
B. Marambe 383
6. Interactions of metsulfuron-methyl and glyphosate applied to foliage of woody borreria (*Diodia ocimifolia*)
O.K. Eng, R. Amartalingam, D. Omar, and M.R.A. Halim 389

7. Quizalofop-P-tefuryl plus certain broadleaf herbicides in rainy season soybean *(Glycine max)*
P. Lueang-a-papong, A. Tadakitisarn, and N. Hongsbhanich 395
8. Structure-activity relationships of new benzenesulfonylureas in rice (*Oryza sativa*) and barnyardgrass *(Echinochloa crus-galli* var. *oryzicola)*
I.T. Hwang, Y.K. Ko, T.J. Kim, D-W. Kim, and K.Y. Cho 403
9. Studies on effective action of sethoxydim-bentazon mixture to mono-and dicotyledonous weeds
G.B. Ye and Z.P. Zhang 408
10. Studies on joint-action of herbicide combination of atrazine with acetochlor
Z. Zongjian, Y. Yuting, and L. Changju 414
11. The effects of herbicide glyphosate and 2,4-D mixtures on weed depression in soybean field
J. Moenandir and E. Murniningtias 419
12. Use of haloxyfop-R-methyl ester for annual grass weed control in groundnut (*Arachis hypogea*)
P. Lueang-a-papong, and S. Singhara Na Ayudhaya 424

4.3 Newly Developing Herbicides

1. BAS 625 H - A new post-emergence herbicide for grass weed control in rice
C. Finley, U. Schofl, B. Sievernich, A. Landes, M. Landes, W. Unglaub, and W. Nuyken ... 431
2. BAY FOE 5043 (flufenacet) - A new selective herbicide for the use in corn, cereals, potatoes and other crops
H. Fursch 437
3. Bispyribac-sodium, a new selective rice herbicide in direct-seeded rice in Vietnam
D.V. Chin 443
4. Bispyribac sodium for weed control in Philippine rice production system-dry seeded, dry sown rice
F. Palis, F. Vasquez, H. Dupo, E. Llabores, and J. Aspuria 447
5. CGA 279'233, a new grass herbicide in different rice production systems
J.L. Allard, K.F. Kon, J. Allen, and C. Liithy 449
6. CGA 279'233, a new timing-flexible grass component of one-shot herbicides in transplanted rice in Japan and Korea
K.F. Kon, S. Murakami, Y. Morishimo, A. Hosoda, Y.W. Kim, S.T. Kim, and J-L. Allard 455
7. Clomazone+propanil for weed control in rice (*Oryza sativa*)
R.A. Estores and M.J.B. Medina 460
8. Effects of primisulfuron on quackgrass (*Elytrigia repens*) populations in corn (*Zea mays*)
P.C. Bhowmik 466
9. Flazasulfuron (SL-160) for weed control in bermudagrass in golf turf
X. Guang and M. Jian-xia 472
10. Imazamox : A new herbicide for soybean
C. Tiebao, H. Chunyan, W. Yu, and S. Baohong 476
11. Imazapic : A new broad spectrum herbicide for weed control on sugarcane in Thailand
S. Chantarasa-ard, L. Sasipreyajun, N. Armes, and L.J. Kim 480
12. Imazapyr/glyphosate : An effective new herbicide for general weed control (GWC) in oil palm and rubber plantations
C.Y. Liew, M. Sharma, R. Balasubramaniam, and L.J. Kim 487

13. Post-emergence grass weed control with BAS 625 H in Asian rice cultures (results from Thailand, Korea, Malaysia, Vietnam and Taiwan 1994-98)
U. Schofl, A. Landes, S.L. Kwon, T. Wanchai, B. Pimol, and L.S. Ho..... 493
14. Sulfentrazone for pre-plant weed control in pineapple (*Ananas comosus* L.)
R.A. Estores, M.J.B. Medina, and S.G. McDaniel..... 499
15. The performance of some new selective herbicides for weed control in wheat under different seed rates in Egypt
A.A. Salim, M. EL-Haroun, and G.M. Mekhail..... 505

Proceeding I (B)

5. Environment

5.1 Fate of Herbicides

1. Soil persistence and movement studies with imazethapyr and imazapyr
T.K. James, A. Rahman, and J. Mellsop..... 513
2. Residual effects of quinclorac on field crops grown in paddy fields of Sri Lanka
B. Marambe and L. Amarasinghe..... 519

6. Resistance

1. Characterization of glyphosate resistant *Eleusine indica* biotypes from Malaysia
M. Tran, S. Baerson, R. Brinker, L. Casagrande, M. Faletti, Y. Feng, M. Nemeth, T. Reynolds, D. Rodriguez, D. Schafer, D. Stalker, N. Taylor, Y. Teng, and G. Dill..... 527
2. Habitat management and inheritance of sulfonylurea resistance in *Lindernia micrantha*, an annual paddy weed in Japan
K. Itoh, G.X. Wang, H. Shibaike, and K. Matsuo..... 537

7. Biocontrol

1. Effects of *Exserohirum monoceras* on growths of barnyardgrass and rice seedlings by inoculation on roots
A. Ogawa, H. Shibayama, and M. Gohbara..... 547
2. *Fusarium moniliforme* Sheldon : A potential mycoherbicide for wild oat (*Avena ludoviciana* Dur.) in Iran
H. Kazemi and P. Shimi..... 552
3. Pathogenicity of *Alternaria alternata* to crofton weed (*Eupatorium adenophorum*)
S. Qiang and B. Summerell..... 556
4. Research on some fungi to control barnyard grass (*Echinochloa crus-galli* (L.) Beau) and red sprangletop (*Leptochloa chinensis* (L.) Nees) in rice
H.L. Thi, L.H. Man, and D.V. Chin..... 562
5. Screening of the selective biocontrol agents for *Leptochloa chinensis* (L.) Nees in Thailand
P. Smitamana, S. Panyafoo, and A.K. Watson..... 567

Poster Presentation

1. Weed Biology and Ecology

1. Biological activities of plant growth inhibitors from *Duranta repens*
   S. Hiradate, N. Nakajima, S. Zungsontiporn, and Y. Fujii 577
2. Development, germination, and dormancy of small-white flower morningglory (*Ipomoea obscura* (L.) Ker-Gawl) seed
   A. Julakasewee, R. Suwanketnikom, and S. Chandakul 583
3. Germination and growth of horse purslane (*Trianthema portulacastrum*)
   C. Prakongvongs 588
4. Insect antifeedants in Cyperaceae : Utilization of constituents from weed as agrochemical lead
   M. Morimoto, Y. Fujii, M. Urakawa, S. Tamaki, T. Morita, and K. Komai 594
5. Investigation of weed and weed fungal pathogen in soybean production area
   R. Pongpisutta, K. Sirivejabandhu, and T. Pornprom 600
6. Involvement of abscisic acid in temperature-sensory mechanisms for controlling the seasonal timing of seed germination in winter annual weeds
   T. Yoshioka, T. Gonai, S. Satoh, and T. Hashiba 604
7. Is *Poa crassinervis* Honda endemic to Japan?
   S. Umemoto 610
8. Mode of action of a new plant growth inhibitor, durantanin II
   N. Nakajima, S. Hiradate, and Y. Fujii 614
9. On the succession of weed communities in five kinds of crop fields
   T. Heling 619
10. Plant interaction in vegetation of a model levee of paddy field
    K. Nakatani and Y. Fujii 627
11. Progress of study on weed in wheat field in Jiangsu province
    D. Yao 632
12. Seedling identification of morningglory weeds in pineapple
    A. Julakasewee, R. Suwanketnikom, and Y. Nimruksa 635
13. Some agronomic characteristics and seed domancy study of 12 wild rice (*Oryza rufipogon*) accessions from different origins
    P. Mongkolbunjong, S. Juntakool, and H. Hyakutake 639
14. Weed seedling survival under flooded conditions
    O.S. Namuco and C. Piggin 645
15. Weed shifts in response to herbicide applications in cotton
    W. Jianrong, J. Ronglong, C. Bieboand, and L. Chunhong 650

2. Weed Control and Management

1. Allelochemicals in rice straw
   C.W. Lee, K. Yoneyama, M. Ogasawara, Y. Takeuchi, and M. Konnai 659
2. Application of the nicosulfuron for itchgrass control in maize
   N. Waranit and R. Suwanketnikom 663

3. Effect of crop rotations on the control of wild oat and other weeds in wheat in Middle Egypt
E.E. Hassanein, G.M. Mekhail, H.M. Ibrahim, and M.K. Zahran..... 668
4. Effect of irrigation on the performance and leaching behaviour of isoproturon and metoxuron for the control of *gulli danda* (*Phalaris minor* in) wheat
N. Kumar, U.S. Walia, H.S. Brar, and L.S. Brar..... 674
5. Effect of some herbicides on small-white flower morninggolory (*Ipomoea obscura* (L.) Ker-Gawl) control in pineapple
A. Julakasewee and R. Suwanketnikom..... 680
6. Evaluation of dinitroaniline herbicides against isoproturon resistant *Phalaris minor* in wheat (*Triticum aestivum*) under zero and conventional tillage
R.M. Sirohi, A. Yadav, and R.K. Malik..... 685
7. Herbicidal efficacy of bispyribac-sodium against rice weeds
Y. Ono, K. Yanagisawa, S. Kitamura, and A. Kawamoto..... 691
8. Identification of phytotoxins from manilagrass (*Zoysia matrella* (L.) Merr.) plants
C. Laosinwattana, K. Yoneyama, Y. Takeuchi, M. Ogasawara, and M. Konnai..... 695
9. Integrated weed management in paddy fields
W. Jinglun..... 701
10. Itchgrass control in corn with primisulfuron
S. Changsaluk and R. Suwanketnikom..... 706
11. Management of *Cynodon dactylon* in sugar cane
A.A. Makkawi and C.E. Price..... 712
12. No-till irrigated corn production in Thailand
S. Boonpradub and T. Welsh..... 719
13. Rice-weed competitiveness
S. Moolsri, P. Kow-in, W. Chaitep and M. Mortimer..... 721
14. Studies on resistance of *Solanum phoreinocarpum* and *Bidens pilosa* to herbicide metsulfuron-methyl
W. Lin, M. Hong, and N. Zhu..... 727
15. Study on relationships between *Beckmannia syzigachne* (Stued.) Fernald and other weeds in rape field and fallow following rice
C. Jianjun, W. Gingya, and L. Yanghan..... 732
16. Study on the occurrence of *Sclerochloa kengiana* and it's chemical control in wheat
Z. Cheng-mei and X. Guang..... 739
17. The integrated weed management system (IWMS) in soybean field
L. Gui..... 742
18. Vegetation analysis to study rangeland quality and sources of potential noxious weeds
P.V. Mele and H. Beeckman..... 745
19. Weed control and management of resistant goosegrass (*Eleusine indica*) in Malaysia
T.Y. Thai and T. K. Chiong..... 753
20. Weed population dynamics in coffee plantation managed by different soil conservation techniques
N. Sriyani, H. Suprapto, H. Susanto A.T. Lubis, T. Adachi, and M. Senge..... 759

21. Weed seedbank response to herbicides use in paddy rice fields
R. Sago 764
22. Yield and cost of production in reduced-tillage sugarcane
S. Chinawong and C. Cheosamutr 770

3. Herbicides

3.1 Application Techniques

1. Effects of spray volumes and dosages on propanil/2,4-D toxicity and weed control in pre-germinated direct-seeded rice
K. Nakornsri and P. Vongsaroj 779
2. Postemergence application of broadleaf herbicides on growth of onion (*Allium cepa* L.) and garlic (*A. sativum* L.)
S. Kongsaengdao and K. Suwanrak 785

3.2 Physiology and Biochemistry

1. Isolation and characterization of glufosinate tolerance in soybean cell lines
T. Pornprom, S. Surawattananon, and P. Srinives 793
2. Tolerance mechanism of pepper cultivars to bentazon
B.S. Ahn, J.Y. Pyon, and E.K. Ryu 797

3.3 Newly developing herbicides

1. Dimethenamid-a new herbicide for the use in various seeded and transplanted crops in Asia
E. Lu, A. Landes, C. Finley, and U. Schofl 805

4. Environment

1. Influence of chlorsulfuron residue on growth and development of transplanted rice
L. Yuanlai and Y. Jianqiang 813
2. The mobility and degradation of glufosinate-ammonium in tropical soils
I.B. Sahid and M. Nur-Razana 818

5. Others

1. A biotype of *Limnocharis flava* multiple-resistant to 2,4-D and bensulfuron-methyl in Malaysia and its control
S. Nakayama, A. Man, and R.A. Ghani 827
2. Allelopathic effects of acetone extraction of *Wedelia chinese*
T. Xing-shan, F. Li, and L. Gang-yue 833
3. Development of immunoassay for detection of the herbicide thiobencarb residues in water
S. Miyake, Y. Yamaguchi, K. Ohde, S. Ito, Y. Yuasa, and Y. Ishii 836
4. Effect of cyanobacteria extract on germination and growth of some plants
S. Surawattananon, N. Sanevas, and K. Namwongprom. 841
5. Effects of soil environmental conditions on decomposition rate of herbicide pretilachlor in soils
Y.H. Moon and Y.S. Kim 844

6. Fauna and activity of insects visiting *Lindernia* flowers in paddy fields in Northern Japan
T. Matsumura, G. Wang, K. Goukon, and K. Itoh..... 850
7. *In vitro* selection and propagation of salt tolerant *Sesbania rostrata* Brem. & Oberm.
J. Jungklang, S. Surawattananon, M. Nanakorn, and K. Namwongprom..... 856
8. Traditional use of Cogongrass *(Imperata cylindrica)* in Japan and future perspective on its utilization
T. Tōminaga..... 860

Author Index..... 865

5. Environment

SOIL PERSISTENCE AND MOVEMENT STUDIES WITH IMAZETHAPYR AND IMAZAPYR

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Abstract: The persistence of two imidazolinone herbicides, imazethapyr (Spinnaker 240 AS) and imazapyr (Arsenal 250 AS) were determined under field conditions in a Horotiu sandy loam soil of volcanic origin (61% sand, 14% clay, 7.3% organic carbon, bulk density 0.71 kg/L, pH 5.7). Soil samples were collected from the 0 - 10 cm depth at monthly intervals and bioassayed in the glasshouse. Standard calibration curves for radish (*Raphanus sativus* L.) and oats (*Avena sativa* L.) showed that radish was more sensitive than oats to both herbicides. The detection limits for imazethapyr were 5 µg/kg for radish and 20 µg/kg for oats while the corresponding data for imazapyr were 10 µg/kg and 20 µg/kg. Radish was generally able to detect herbicide residues in the soil for about a month longer than the oats could. At the highest rates evaluated (imazethapyr 384 g ai/ha, imazapyr 1000 g ai/ha) and using the radish bioassay, it was found that detectable residues of imazethapyr disappeared within 4 months while those of imazapyr disappeared within 5 months after application. In packed soil columns and using the same bioassay plants as indicators, both herbicides were shown to be highly mobile, with 76 mm of rain moving them up to 20 cm down the columns.

Keywords: imazethapyr, imazapyr, persistence, movement, degradation.

Introduction

The imidazolinone herbicides were developed in the late 1970's and 1980's for use in a range of crops including oil palms, rubber trees, sugarcane, forestry, soya beans, cereals, sunflower and lentils (Shaner, 1991). Recently, these herbicides are again being re-evaluated due the development of imidazolinone-tolerant (IT) crops. Unlike some other herbicide resistant crops many of the IT crops were developed by conventional cross breeding. IT crops to date include, maize, rice, sugar beet, wheat, canola and soya beans (Shaner, 1996; Owen, 1997). One of the major concerns associated with herbicide tolerant crops is the continuous use of single (or closely related) herbicides and the possible accumulation in the environment. This is of particular concern when the herbicides involved are either persistent or mobile or both. In this situation the possibility of groundwater contamination or residue carry over to subsequent crops is greatly increased. The imidazolinone herbicides imazethapyr and imazapyr are the active ingredients of a combination product Lightning, which is currently being evaluated for use with IT maize in New Zealand. Both these herbicides are claimed to be persistent but relatively immobile in the soil environment (Shaner 1991).

Bioassay is a commonly used method for studying the location and amount of herbicide in the soil. Sensitive bio-indicators are able to detect low levels of herbicide without the need for the elaborate extraction and determination equipment associated with chemical assays. Many different species have been used as indicators of imidazolinone herbicides including red beetroot (Jourdan *et al.*, 1998), wheat (Vizantinopoulos and Lolos, 1995) and lentils (El Azzouzi *et al.*, 1998). The choice of indicator species used is determined by many factors including sensitivity to the herbicide, ease of growth, environment and availability of seed.

The aim of the work reported here was to study the persistence and movement of imazethapyr and imazapyr in a common cropping soil through field and glasshouse experiments using bioassay techniques.

Materials and Methods

Bioassay calibration curves

Calibration curves for the activity of imazethapyr (Spinnaker 240 AS) and imazapyr (Arsenal 250 AS) in the soil were determined on three occasions using two bioassay species, viz. radish (*Raphanus sativus*) and

oats (*Avena sativa*). The test soil was a Horotiu sandy loam, freshly taken from a site at the Blands Horticultural Research Station near Hamilton. This soil is of volcanic origin and has 61% sand, 23% silt, 14% clay, 7.3% organic carbon, a bulk density of 0.71 kg/L and a pH of 5.7. A range of concentrations (0.001 to 0.2 mg/kg) for both imazapyr and imazethapyr, was used to establish a standard calibration curve for each test species. The herbicide standard solutions were prepared by mixing the formulated product with water in volumetric flasks. These solutions were added to field moist soil at the rate of 20 mL/kg of oven dry soil equivalent. This soil was thoroughly mixed by shaking in a polythene bag and then stored overnight to equilibrate before being used for potting out four replicates of the test species. Soon after emergence the plants are reduced to eight per pot. Visual observations of plant damage were made throughout the duration of the experiments and the plant foliage was harvested for dry matter yields 4 to 5 weeks after planting. Calibration curves were produced on three separate occasions over the duration of the study.

Field study on herbicide persistence

This experiment was conducted during spring and summer of 1995/96 on the same Horotiu sandy loam site as described for the bioassay calibration experiments. Individual plots were 10 m x 2 m and were replicated four times in a randomised block design. The herbicides were each applied at three rates (Tables 2 and 3) to freshly cultivated ground on 6.12.95 with a CO_2 powered precision sprayer delivering 300 L/ha at 200 kPa. Soil samples from the top 10 cm were collected from each plot at monthly intervals for the duration of the study. Each sample was thoroughly mixed and then used to plant out one pot of each of the two bioassay species and placed in a glasshouse. The bioassay plants were grown and assessed as for the bioassay calibration experiments described above. Average rainfall for the intervals between samplings for the field study are presented in Table 2. On completion of the soil sampling for the glasshouse bioassay (i.e. when no residues could be detected in the soil samples) an *in situ* bioassay was conducted on the treated plots using the same test species. The bioassay plants were sown on 8.8.96, harvested on 15.10.96 when their size was similar to that of the glasshouse grown plants. A set number of plants from each plot was harvested, dried and weighed as for the glasshouse bioassays.

Glasshouse studies on herbicide movement

The movement experiments were performed in 30 cm long columns made from polyvinyl chloride (PVC) pipe of 10 cm inside diameter (Rahman and James, 1989). Each column was cut longitudinally to make a 4 cm wide gap which was covered with clear acetate sheet for the movement part of the experiment. Bulk soil was collected and treated as for the bioassay calibrations described above. This field moist soil was gently packed into the columns to a height of 28 cm. After allowing the column to settle for 24 h, 100 g of soil pre-mixed with the required rate of herbicide (Figs. 2 and 3) was placed on top of the column, leaving a small gap for adding water. The amount of herbicide applied to the treated soil layer was calculated from the soil surface area at the top of the column and was mixed using the same method as the bioassay calibrations described above.. Two nylon string markers were placed between the treated layer and the untreated soil in the column to act as a reference point for measuring herbicide movement at the end of the experiment. The soil in the column was topped with a filter paper and 5 mm of washed sand to allow an evenly distributed water load to the treated soil. Water was added to each soil column with a pipette in 10 or 20 mL aliquots over a 3 day period until it had uniformly reached the bottom of soil column (760 mL). Untreated controls were treated in a similar way. Upon completion of the watering regime, the columns were left to equilibrate for a further 24 h, after which the filter paper and sand were removed. The columns were topped with vermiculite and capped. They were then laid on their side and opened longitudinally by removing the acetate sheet. The soil was scraped to level it with the side walls, starting from the bottom to avoid contamination, in preparation for bioassays in the glasshouse. Seeds of the bioassay species, radish and oats were sown in triplicate at 2 cm intervals along the length of the column. The columns were then placed in a heated glasshouse with day temperatures of 20-24°C and night temperature down to 16°C. No supplementary light was provided. This experiment was conducted in autumn (April-May) 1999 and the soils were kept moist by mist watering daily. Three replicates were allocated to each treatment and the columns were arranged in a completely randomised design. After 3 weeks of growth in the glasshouse, plants were assessed for herbicide damage and then harvested in 2 cm increments for dry weight determination. The distance that the herbicide leached was determined as the distance from the string markers to the first plants that had a fresh weight of within 85% of equivalent plants in the untreated columns. The data were subjected to analysis of variance to separate the treatment means.

Results and Discussion

Bioassay calibration curves

The data from the three bioassay calibrations were similar and the dry weights were therefore averaged. Radish was more susceptible to both herbicides than that of oats (Fig. 1). The relative phytotoxicity of the two herbicides can be determined from the ED_{50} values (effective dose or amount of herbicide required to reduce dry matter production of the bioassay species to 50% of untreated) for the individual herbicides. The ED_{50} values are presented in Table 1. Both imazethapyr and imazapyr had similar toxicity to radish but oats was more susceptible to imazapyr.

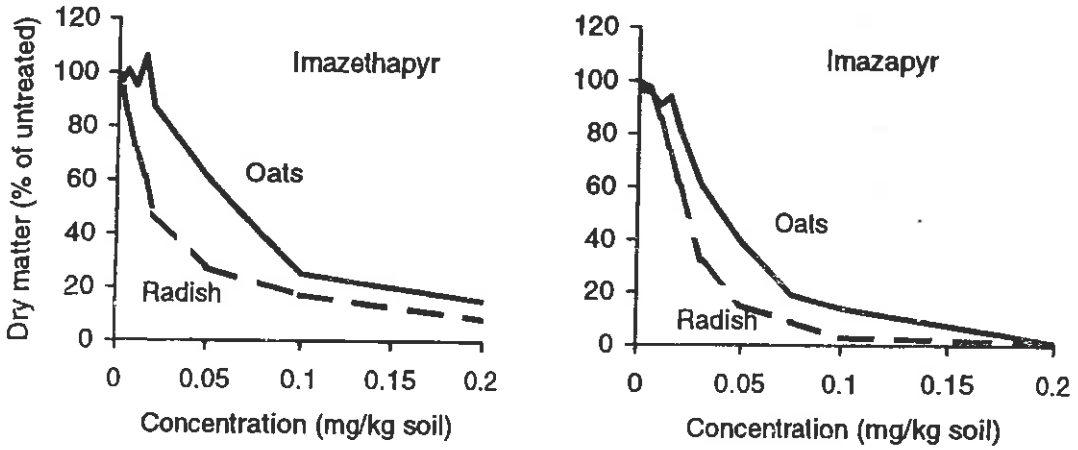

Fig. 1 Bioassay calibration for imazethapyr and imazapyr - data average of three experiments.

Table 1 Relative phytotoxicities (ED_{50} values) of the two herbicides to the two bioassay species.

	ED_{50} values (mg/kg soil)	
Herbicide	Radish	Oats
imazethapyr	0.02	0.07
imazapyr	0.025	0.04

Field study on herbicide persistence

At the rates evaluated in the field study, imazethapyr was less persistent in the soil than imazapyr, which persisted for about a month longer (Tables 2 and 3). Radish was a more sensitive bioassay species and was generally able to detect herbicide residues in the soil for about a month longer than the oats could. At the highest rates evaluated and using the more sensitive radish bioassay, it was found that detectable residues of imazethapyr had dissipated from the top 10 cm of soil within 4 months while those of imazapyr dissipated within 5 months. The *in situ* bioassay on the treated plots confirmed these results as the dry matter weights of the bioassay plants were not significantly different from untreated control, (Table 4). Close inspection of the plants throughout the trial period showed no visual damage that would indicate the presence of herbicide residues in the soil

The *in situ* bioassay is one method used for validating the glasshouse bioassay results. One of the problems associated with glasshouse bioassays is that the soil samples are usually taken to a greater depth than the herbicide may have leached. Prior to being used for the bioassay these soil samples are thoroughly mixed and this can result in a significant dilution of the herbicide, particularly if it remains concentrated near the soil surface. An *in situ* bioassay will overcome this problem. The result of the *in situ* bioassay conducted in this study showed that 9 months after the herbicides were applied, no residues could be found in the field.

Table 2 Effect of herbicide treatments on the bioassay species radish; dry matter production as a percentage of untreated. –

Treatment	Rate (g ai/ha)	Dry matter - % of untreated					
		Days after treatment					
		28	56	84	112	140	168
Untreated	-	100	100	100	100	100	100
imazethapyr	96	52*	88	97	115	97	103
imazethapyr	192	26*	62*	79*	99	94	89
imazethapyr	384	17*	47*	72*	94	94	101
imazapyr	250	15*	77*	94	101	103	95
imazapyr	500	1*	39*	75*	103	93	94
imazapyr	1000	1*	3*	19*	66*	87	93
Rainfall (mm)[a]		120	66	176	115	138	146

* Figures are significantly different from untreated control at the 95% confidence level.

[a] Rainfall figure for the month prior to the sampling dates

Table 3 Effect of herbicide treatments on the bioassay species oats; dry matter production as a percentage of untreated.

Treatment	Rate (g ai/ha)	Dry matter - % of untreated					
		Days after treatment					
		28	56	84	112	140	168
Untreated	-	100	100	100	100	100	100
imazethapyr	96	87	103	99	101	92	99
imazethapyr	192	56*	86*	94	86	99	98
imazethapyr	384	40*	81*	95	95	95	95
imazapyr	250	43*	98	90	93	96	99
imazapyr	500	9*	73*	98	94	103	104
imazapyr	1000	2*	8*	36*	70*	91	99

* Figures are significantly different from untreated control at the 95% confidence level.

Table 4 Plant dry weights from the *in situ* bioassay planted on 8.8.96 and harvested on 15.10.96.

Treatment	Rate (g ai/ha)	Dry matter - % of untreated	
		Radish	Oats
Untreated	-	100	100
imazethapyr	96	111	89
imazethapyr	192	91	79
imazethapyr	384	103	106
imazapyr	250	117	79
imazapyr	500	77	94
imazapyr	1000	99	76
LSD 5%		34	28

The persistence of imazapyr reported here is considerably shorter than reported previously by Rahman *et al.* (1993) where rates of 500 and 1000 g ai/ha persisted for more than 6 and 9 months respectively using the same bioassay methods. Other workers have also found longer persistence. El Azzouzi *et al.* (1998) reported half lives of 25 – 58 days for imazapyr in different soils while Vizantinopoulos and Lolos (1994) found half lives of 7.8 to 50 months for the same herbicide. In our study attention must be drawn to the rainfall data

presented in Table 2 as the amount of rain over the duration of the study was very high, with several months recording higher than average precipitation. The effect of this high rainfall would be to increase the movement of the herbicides. Not only is this likely to leach some the herbicides completely out of the soil layer being sampled but it would also distribute the herbicides over a greater depth and thus also reduce their phytotoxicity in the soil.

Glasshouse studies on herbicide movement

The results of the movement study are presented in Figs 2 and 3. Here, the dry weights of the bioassay plants (radish) have been plotted against the distance from the string markers placed at the bottom of the treated soil layer. Thus, the graphs are a representation of the effect of the leached herbicide on the bioassay plants. When compared to the growth of the plants in an untreated column (top line) it can be seen that the herbicides evaluated in this study severely reduced plant growth for up to 20 cm down the column. Both herbicides were moved similar distances by the same quantity of water and there were also only small differences due to herbicide rate. This demonstrates that both imazethapyr and imazapyr can be easily leached. Application rate appears to have little effect on the distance that the herbicide leached but is certain to have a significant effect on the amount of herbicide leached. In this study a large proportion of the active herbicide must have been leached out of the top layer of the soil as the radish plants were frequently growing better at the top of the column, especially at the lowest herbicide rate (Figs 2 and 3). The amount of water applied in this study was equivalent to 97 mm of rain and this was sufficient to thoroughly wet the soil in the column and allow for a small amount of excess to leach out the bottom. Thus it could be concluded that moderate to heavy rain soon after application would leach these herbicides well into the root zones of plants and possibly further.

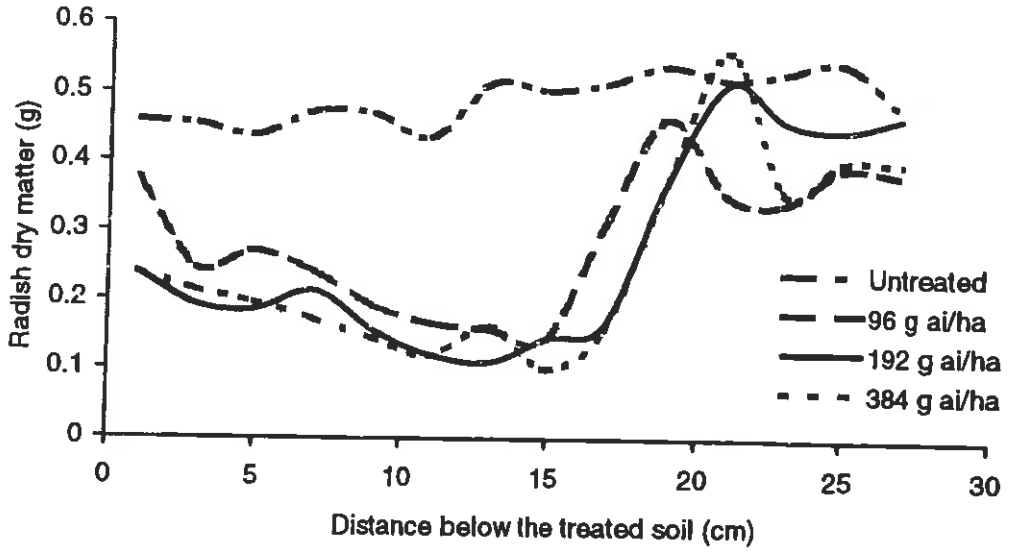

Fig. 2 Dry weights of radish plants grown at 2 cm intervals along the soil column after treatment with imazethapyr and subsequent watering.

These results are similar to those reported earlier by Rahman *et al.* (1993) where it was shown that imazapyr was highly mobile in a soil column under similar conditions. In another New Zealand soil, McDowell *et al.* (1997) found that imazapyr leached below 25 cm after 4 months while Vizantinopoulos and Lolos (1994) found that imazapyr leached to 45 cm in clay loam when total rainfall was 873 mm.

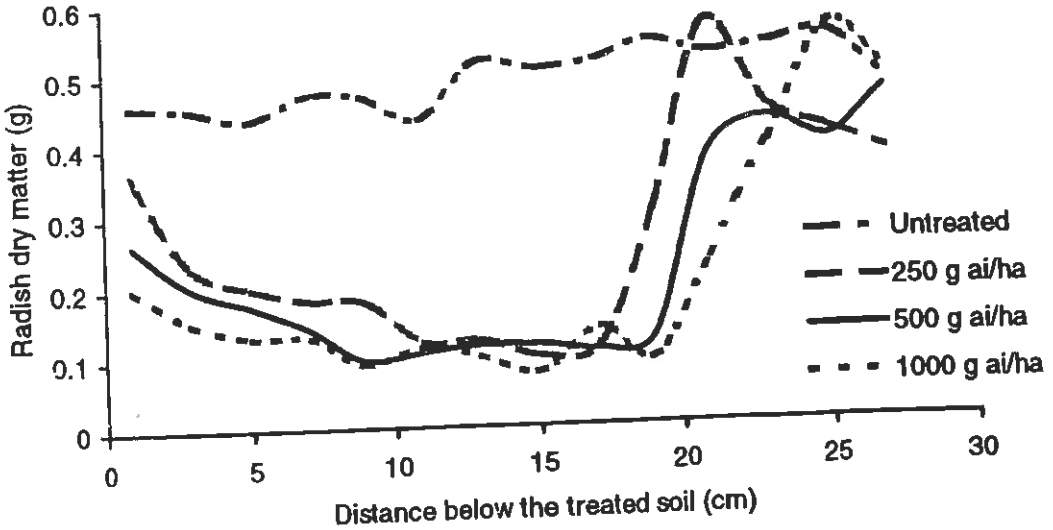

Fig. 3 Dry weights of radish plants grown at 2 cm intervals along the soil column after treatment with imazapyr and subsequent watering.

Conclusions

The findings presented here show that in a volcanic, sandy soil the imidazolinone herbicides imazapyr and imazethapyr were both persistent and highly mobile. The mobility of the herbicides is supported by several other studies reported recently but is contrary to some early descriptions of the herbicides behaviour in the environment. As these herbicides have been shown to be very persistent under certain conditions, their field use rates need to be accurately determined for a range of conditions to avoid carryover into subsequent seasons. Their demonstrated high potential to move through the soil profile with moderate to heavy rainfall also means that they should be used with caution to avoid off target movement.

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RESIDUAL EFFECTS OF QUINCLORAC ON FIELD CROPS GROWN IN PADDY FIELDS OF SRI LANKA

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Abstract: Cultivation of other field crops in paddy (rice) fields, especially crops of family Solanaceae, is not recommended following application of quinclorac, which is a major grass-killer herbicide. This is due to the fear of detrimental residual effects of the herbicides on crops. However, scientifically valid information on the residual effects of quinclorac in rice fields. Thus, experiments were conducted to investigate the possible residual effects of the herbicide quinclorac (Facet SC, 25% a.i ; BASF, Germany) on the field crops grown in rice fields of Sri Lanka. The commercial formulation of the herbicide was applied at rates of 0, 200, 300, 400, and 500 g a.i./ha on to plots cultivated to a rice crop (105-110 days old) grown at the onset of the long rainy season (November-December). Three field crops were established at 2, 6, 10, and 14 weeks after harvesting rice, in the following season (at the onset of short rainy season: May), namely chili and tomato (transplanted), and mung bean (direct seeded) on the same plots cultivated to rice previously. Pot experiments were also conducted to elucidate the impact of soil applied Quinclorac (at same rates) on seed germination and seedling growth of crops.

The results indicated that quinclorac could have residual effects when chili and tomato are planted within 4 months of application of the herbicide. However, when these crops were planted at 6 weeks after harvesting of rice (*i.e.* 6 months after application of the herbicide) there were no phytotoxic effects on the selected crops and no significant reduction in crop yields were observed. Mung bean did not show any significant reduction in growth and yield even when planted soon after application of quinclorac.

Thus, the results of the present study suggest that fields crop such as chili and tomato could be cultivated successfully in rice fields previously treated with quinclorac when the seedlings are transplanted at least 6 months after application of the herbicide. If the situation demands early planting of crops, mung bean would be a better selection for rice fields treated previously with quinclorac.

Key words: quinclorac, residual effects, chili, tomato, mung bean, rice, soils

Introduction

Use of herbicides is the most common weed control technique adopted by rice farmers in Sri Lanka. However, despite the heavy use of herbicides, weeds such as *Echinochloa spp.* has become a serious threat to rice cultivation in Sri Lanka (Marambe *et al.* 1997). In early 1990's, a quinclorac herbicide was introduced to Sri Lanka for the successful control of *Echinochloa spp* in lowland rice culture (Amarasinghe *et al.* 1999). A wide application window, compatibility with insecticides in the carbamate group or organo-phosphates, and rain fastness has made this herbicide a popular selection among rice farmers in the dry zone region of the country. However, despite the effectiveness of the herbicide in controlling *Echinochloa spp.* in rice-production systems, due to the suspected residual effects of quinclorac, certain crops such as tomato and chili are not recommended as rotation crops after rice in quinclorac-treated rice fields (Keerthi Liyanage, BASF-Finlay Pvt. Ltd., Sri Lanka: personal communication; WSSA, 1994; Grossmann, 1998). It is important to note that in Sri Lanka, a considerable number of farmers, especially under irrigation schemes, cultivate rice during the long-rainy season (Maha season; October to February) and other field crops such as chili in the short-rainy season (Yala season; April to August). This is mainly due to the scarcity of water for rice cultivation in short rainy seasons (Karunasena *et al.*, 1997).

It is well known that large number of factors, including climatic and edaphic factor influences residual effects of herbicides. In tropical conditions where soil moisture is adequate, these residual effects are often not long lasting. Therefore, it could be speculated that Facet has no prolonged residual effects. However, there is no such information available under Sri Lankan condition. Therefore, the major objective of the present study was to investigate the residual phytotoxicity of quinclorac (Facet SC 25% a.i.; BASF, Germany) on growth and development of several non-rice crops grown in Sri Lanka.

Materials and Methods

The experiments were conducted in the Experimental Station of the Faculty of Agriculture, University of Peradeniya, Sri Lanka (480m amsl, annual rainfall 900 mm, average day temperature 31±1.8°C). Both field and pot experiments were carried out to determine the residual effects of the herbicide. Field experiments were carried out in a randomized complete block design with three replicates. The pot experiments were conducted in a complete randomized design with three replicates.

The rice fields were cultivated at the beginning of the long rainy season (November-December). The quinclorac herbicide (Facet SC, 25% a.i., BASF, Germany) was applied at four rates, namely (a) 200 g a.i./ha, (b) 300 g a.i./ha, (c) 400 g a.i./ha and (d) 500 g a.i./ha, at 10 days after planting of rice (when the *Echinochloa spp* were at 2-3 leaf stage). The plot size was 5 x 4 m^2 and soil was a low humic gley soil (pH 5.9). Plots without herbicide treatment was used as the control. The rice variety (BG 352, 105-110 days old) was harvested in mid April, and at 2, 6, 10 and 14 weeks after harvesting rice, the plots were divided into 3 equal parts and soil beds were prepared to establish 3 different field crops. The selected three crops were chili (*Capsicum annum* var. MI 2, family: Solanaceae), tomato (*Lycopersicon esculentum* var. KWR, family: Solanaceae), and mung bean (*Vigna radiata* var. MI 5, family: Fabaceae). These crops were selected as they are commonly cultivated other-field-crops (OFCs) in paddy fields during the short rainy season in the dry and intermediate zones of Sri Lanka. Mung bean seeds were directly sown and the other crops were transplanted. The seeds of the selected crops were also planted in plastic trays filled with topsoil treated with or without the herbicide to investigate the impact of the herbicide-residues on seed germination and emergence.

The effects of the herbicide residues were evaluated using germination percentage, survival % of healthy seedlings (without wilting of leaves and stems, stunting), dry weight and the final yield of crops. The results were statistically analyzed using the SAS computer software package and the treatment means were compared by the Least Significant Difference (LSD) at p=0.05.

Results and Discussion

Pot experiments

The seed germination of all three crops (estimated by the radicle protrusion) was not affected by the treatments, and all the seedlings emerged within 3-5 days after planting.

The results of the pot experiments indicated that chili and tomato seedlings could not survive in a soil environment 1 week after treatment with quinclorac (Table 1). The survival percentage of these two Solanaceous crops was less than 10% when compared to the non-treated control. However, results on the survival percentage of healthy seedlings of mung bean indicate that quinclorac did have any significant impact on the stand establishment of this crop. Similar results were reported by Grossmann (1998) who indicated that, although many crops are tolerant to quinclorac, tomato is one of the highly susceptible species.

The seedling survival of the test plants established in soils sampled from rice fields previously treated with quinclorac is presented in Table 2. The results showed that the survival % of seedlings of chili and tomato was poor in soils up to 4 months after treatment of quinclorac. However, thereafter Similar to the previous findings (Table 1), mung bean did not show any significant reduction of the survival rate at all tested concentrations irrespective of the time taken for establishment of the crop after quinclorac treatment.

Table 1. Healthy seedling % of the field crops grown in top-soil at 1 week after treatment with quinclorac.

Quinclorac Treatment	Chili	Tomato	Mung bean
Control	100	100	100
200 g a.i./ha	5	10	95
300 g a.i./ha	10	10	100
400 g a.i./ha	0	10	90
500 g a.i./ha	0	0	90

* Healthy seedling % was estimated as the percentage of seedlings in treated plots at 3 weeks after establishment without any wilting symptoms compared to control plots

Table 2. Healthy Seedling % of Other Field Crops Grown in Rice-soil different months after treatment with Quinclorac.

Quinclorac Treatment	Chili			Tomato			Mung bean		
	4*	6	8	4	6	8	4	6	8
Control	100	100	100	100	100	100	100	100	100
200 g a.i./ha	70	100	100	100	90	90	100	100	100
300 g a.i./ha	60	100	95	100	100	100	100	100	100
400 g a.i./ha	55	90	90	100	100	100	100	100	100
500 g a.i./ha	55	90	90	100	90	90	100	100	100
LSD (p=0.05)	12.5	NS	NS	NS	NS	NS	NS	NS	NS

* 4, 6 and 8 indicate the months after application of quinclorac; plants without wilting, stunting, or malformation were considered as healthy seedling LSD - least significant difference; NS - non significant

Field experiments

In chili and tomato, the survival rate of the plants was poor when the seeds were planted at 2 weeks after the rice crop was harvested (approximately 4 ½ months after application of quinclorac in the rice crop). The results indicated that the dry weight of chili and tomato plants at time of flowering (Table 3) was significantly reduced when the seedlings are established at 2 weeks after harvesting of rice in paddy fields previously treated with quinclorac.

Table 3. Dry weight at time of flowering of Other Field Crops grown in rice fields at different weeks after harvesting rice (% of control). Rice fields were treated with quinclorac at 10 days after planting.

Quinclorac Treatment	Chili				Tomato				Mung bean			
	2*	6	10	14	2	6	10	14	2	6	10	14
Control	100	100	100	100	100	100	100	100	100	100	100	100
200 g a.i./ha	65	90	90	95	75	95	95	95	100	100	100	100
300 g a.i./ha	60	100	100	100	75	100	100	100	100	100	100	100
400 g a.i./ha	60	100	85	100	80	100	100	100	100	100	100	100
500 g a.i./ha	65	100	100	100	75	100	100	100	100	100	100	100
LSD (p=0.05)	8.4	NS	NS	NS	14.0	NS	NS	NS	NS	NS	NS	NS

* 2, 6, 10 and 14 are weeks after harvesting rice; LSD - least significant difference NS – non significant

However, these crops did not show any phytotoxic symptoms or reduction in dry weight when they were established in the rice fields at 6 weeks after harvesting of rice (approximately 6 months after application of the herbicide to the rice field). The results also indicated that chili is more sensitive to the residual effects of quinclorac when compared to tomato. Mung bean did not show any growth retardation or phytotoxic symptoms when grown in rice fields treated with different rates of quinclorac, irrespective of the time of planting after the harvest of rice (Table 3).

The final yield of the selected crops (Tables 4, 5 and 6) also showed a similar pattern to that of the dry weight. The final yield of chili and tomato were significantly reduced when they were established 2 weeks after harvesting rice, in paddy fields previously treated with quinclorac. However, there was no significant reduction in yield of the selected field crops when they were established in the quinclorac-treated rice fields at 6 weeks after harvesting of rice (approximately 6 months after application of the herbicide). Mung bean did not show any response to the residual effects of quinclorac.

Table 4. Final yield of chili (Green chili recorded in eight harvests and estimated in mt/ha) grown in rice fields at different weeks after harvesting rice. Rice fields were treated with quinclorac at 10 days after planting.

Quinclorac Treatment	Weeks after harvesting rice			
	2	6	10	14
Control	5.81	6.14	5.77	5.88
200 g a.i./ha	3.41	6.01	5.61	5.82
300 g a.i./ha	3.42	6.16	5.70	5.74
400 g a.i./ha	2.96	6.21	5.57	5.91
500 g a.i./ha	3.23	6.09	5.72	5.80
LSD* (p=0.05)	0.56	NS	NS	NS

* LSD - least significant difference; NS - non significant

Table 5. Final yield of tomato (estimated in mt/ha) grown in rice fields at different weeks after harvesting rice. Rice fields were treated with quinclorac at 10 days after planting.

Quinclorac Treatment	Weeks after harvesting rice			
	2	6	10	14
Control	7.62	7.44	7.01	7.38
200 g a.i./ha	5.33	7.51	6.98	7.31
300 g a.i./ha	5.28	7.31	6.84	7.19
400 g a.i./ha	5.32	7.29	7.08	7.28
500 g a.i./ha	4.95	7.36	7.10	7.26
LSD* (p=0.05)	0.79	NS	NS	NS

* LSD - least significant difference; NS - non significant

The results indicated that the two Solanaceous crops selected for the study is susceptible to the residual effects of quinclorac up to 4-6 months after application of the herbicide to rice fields. The results are in agreement with Grossmann (1998) who also reported on the susceptibility of several crops to quinclorac. WSSA (1994) reported that crops susceptible to quinclorac may not be established in fields even after 1 year of treatment with the herbicide. However, the results of the present study indicated that, under Sri Lankan conditions, the Solanaceous crops tested could be established in fields at 6 months after

application of quinclorac to a rice crop. The results also indicated that mung bean could be successfully grown in paddy fields irrespective of the time of application of quinclorac.

Table 6. Final yield of mung bean (estimated in mt/ha) grown in rice fields at different weeks after harvesting rice. Rice fields were treated with quinclorac at 10 days after planting.

Quinclorac Treatment	Weeks after harvesting rice			
	2	6	10	14
Control	0.87	0.92	0.90	0.85
200 g a.i./ha	0.89	0.88	0.92	0.86
300 g a.i./ha	0.83	0.90	0.93	0.88
400 g a.i./ha	0.91	0.93	0.89	0.86
500 g a.i./ha	0.85	0.91	0.91	0.84
LSD* (p=0.05)	NS	NS	NS	NS

* LSD - least significant difference; NS - non significant

Conclusions

Chili and Tomato should not be grown in rice fields or in soils during the first 4 months after treatment with quinclorac (Facet SC, 25% a.i) at all tested concentrations. However, these crops could be grown successfully in rice fields/soils 6 months after application of quinclorac (in situations where the herbicide has been applied to a 3 ½ month rice crop). Mung bean would be an ideal crop to be cultivated in rice fields previously treated with the herbicide regardless of the time of application.

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6. Resistance

Characterization of Glyphosate Resistant *Eleusine indica* Biotypes from Malaysia

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ABSTRACT

Glyphosate resistant biotypes of *Eleusine indica* (goosegrass) were collected from two locations in Malaysia. The fields from which these biotypes were collected have been treated an average of 8 times per year with glyphosate for the past ten years. The glyphosate resistance observed in field trials was confirmed in pot dose experiments, in which the LD_{50} value for resistant (R) biotypes was approximately 2 to 4-fold greater as compared to the sensitive (S) biotype. Based on the these results, an experimental program was designed to investigate the mechanism of glyphosate resistance in *Eleusine indica*. Parameters evaluated included uptake, translocation and metabolism of glyphosate *in planta,* as well as the biochemical and molecular analysis of crown tissue extracts. There was no significant difference in the uptake and translocation of ^{14}C glyphosate in the R and S biotypes. The biochemical data generated from these experiments showed a 5-fold difference in the $I_{50[glyphosate]}$s of the R and S 5-enolpyruvylshikimate-3-phosphate synthase (EPSPSs). RFLP analysis of the sensitive and resistant biotype EPSPS-related sequences revealed distinct polymorphisms associated with the R biotype.

INTRODUCTION

Glyphosate (N-phosphonomethyl-glycine) is the active ingredient of one of the world's most popular foliar-applied, broad-spectrum, herbicides (Malik *et al.*, 1989), Glyphosate is highly effective against the majority of annual and perennial grasses and broad-leaved weeds. Glyphosate inhibits the enzyme EPSPS, which is the sixth committed step in the shikimic acid pathway for aromatic amino acid biosynthesis in plants and microorganisms (Steinrücken and Amrhein, 1980). The specificity of glyphosate to EPSPS contributes to the selective action of the herbicide toward plants and its safety to animals.

Today there exist over 109 herbicide-resistant weed biotypes, and approximately half of these are resistant to the triazine family of herbicides (Holt and Le Baron, 1990; Le Baron, 1991; Shaner, 1995). Spontaneous resistance is generally thought to occur as a

consequence of selection pressure exerted by repeated use of herbicides with a single target site and a specific mode of action. This, coupled with long residual activity, frequent applications without rotation to other herbicides and cultural control practices may lead to conditions which preferentially discriminate between individual resistant and susceptible plants. The result would be to shift the proportions of plant types within a population such that field failure of specific weed control practices is observed.

Evaluation of these resistance criteria, along with current and historical use data, have resulted in the conclusion that glyphosate is a herbicide with a low risk for the development of weed resistance (Benbrook, 1991). Glyphosate has been used for over 20 years in various pre-plant, directed, spot and post-harvest weed management systems. In recent reports, data were presented which showed that annual ryegrass (*Lolium rigidum*) samples collected from two field sites in Australia demonstrated resistance to glyphosate (Pratley *et al.* 1996, Powles et. al., 1998 and Feng, et. al., 1999). These were the first reports of confirmed resistance to glyphosate.

The intent of this document is to report the results of studies on the weed *Elusine indica* collected from an area near Jahor, Malaysia which is suspected of being resistant to glyphosate.

MATERIALS AND METHODS

Seed and Plant Sources. Seed samples of putative glyphosate resistant and susceptible *Elusine indica* were obtained from orchards located in the Jahor region of Malaysia. Plants of both biotypes were propagated in the greenhouse. Plants were grown in either 6.25 or 10 cm square pots filled with fertilized potting soil. These were maintained in a greenhouse with approximate day/night temperatures of $28^0/20^0$ C, relative humidity from 30 to 70% and supplemental lighting on a 14 hour photoperiod. Light intensity ranged from 500 to 1300 uE. Pots were both top watered and subirrigated. Subirrigation only was employed after herbicide applications.

Herbicide Applications and Injury Assessments. In titration experiments, herbicides were applied to plants in early tillering stage. Herbicide applications were conducted with a calibrated track sprayer equipped with a 8001E TeeJet tip at 40 cm height delivering a diluent volume of 234 L/ha at 207 kPa. Immediately after treatment the plants were returned to the greenhouse. Control was evaluated after approximately three weeks by comparisons to untreated plants. Control ratings were based on the number

of dead plants and by visual assessments of growth reduction, chlorosis, necrosis and floral malformations using a scale of 0 to 100 percent with 0 indicating no damage and 100 percent indicating plant death. Glyphosate was applied in a step-wise titration from 4000 to 61 g ae/ha. Four replicate pots were used for each glyphosate rate.

Experimental Design and Statistical Analyses. Titration studies were arranged in randomized complete block designs. The model fit to the data was a sigmoid curve with the functional form:

$$y = a + b/[1+\exp(-(x-c)/d))]$$

with y = % control and x = rate. The model was fit to the data and numerically solved for x using Mathcad Professional software, for y = 50 and y = 90. The 95% confidence bounds were estimated from the plotted curve of y versus x. In many cases, the estimated upper 95% confidence bound for the CD90 was infinity since the curves had a tendency to asymptote around 90. These were termed not available.

Plant Propagation for Biochemical Studies. Seed from the sensitive and resistant Jahor biotype were germinated and transplanted into 6.25 cm square pots. After seven weeks, 80 sensitive and 71 resistant plants were divided into clone pairs and re-potted into 10 cm square pots. At sixty days post germination, one clone of each set was challenged with glyphosate. Sensitive plants were dosed at 500 g ae/ha and resistant plants were treated at 2,000 g ae/ha. The second clone of each set was left untreated. Phytotoxicity was assessed 2 and 4 weeks after treatment. Five plants each of the sensitive and resistant biotypes were selected for second generation selfing. Thirty plants from each biotype were selected for biochemical studies and divided into clone pairs. Final selection of seven plants were made and glyphosate and surfactant control applications were conducted.

Uptake, Translocation Studies: *Elusine indica* plants were grown under greenhouse conditions until they were at the 5-6 tiller stage. At this point, the plants were moved to a controlled environment growth chamber where the radioactive experiments were performed. For Radiolabeled studies, glyphosate formulations were prepared at 0.25 and 0.5 kg/ha and spiked with [^{14}C] glyphosate (methylene labeled, free acid, 38.9 mCi/mmol, Monsanto). These formulations were sprayed on a 5-7 cm area of the leaf lamina from the second oldest tiller using an IVEK microsprayer (spray volume =3ul). At various times after treatment (6H, 2D and 4D), the treated leaf lamina was excised and washed with ddH_2O followed by methanol. The wash solutions were retained and counted by liquid scintillation spectroscopy. The treated leaf area, aerial portions, crown, and root were collected and oxidized to determine the radioactivity contained within each

plant part. Total loaded glyphosate includes all glyphosate found inside the plant (i.e. treated leaf, aerial parts, crown and roots). Total mobilized glyphosate represents all of the glyphosate which moved away from the treated leaf lamina to other plant parts (i.e. aerial parts, crown and root).

Metabolism Studies. Five plants each of sensitive and resistant goosegrass biotypes were treated with ^{14}C-glyphosate by spotting 260,101 dpm/uL, equivalent to 0.5 kg/ha a.e. on a single leaf. Plants were harvested after 48 hr, and separated into treated leaf, leaves, crown and roots. Glyphosate and its metabolites were analyzed by HPLC (Waters system, Milford, MA, USA) using a cation exchange column PRP-X400 (Hamilton, Reno, Nevada, USA) with a mobile phase of 5 mM phosphate buffer, pH 1.9, and a flow rate of 1 ml/min to separate glyphosate from potential metabolites. Radio activity was detected by a Flow Scintillation Analyzer (Packard, Downers Grove, Il., USA).

Shikimic Acid Analysis: Shikimic acid was extracted from the frozen tissues with 0.25 N sulfuric acid. The extracts were analyzed by HPLC (Waters system, Milford, MA, USA) with a C-18 reversed phase column, 250X4.6 mm (Alltech Associates, Inc., Deerfield, IL., USA). A gradient was used with1 % methanol in 20 mM phosphate buffer, pH 2.2 for 5 min, then increased to 10% methanol in 5 min. Flow rate was 1 ml/min and shikimic acid was detected at 210 nm by a Photodiode array detector (Waters, Milford, MA, USA). Shikimic acid was eluted as a single peak at about 5.2 min.

EPSPS Biochemistry. Crown regions were dissected from whole plants and pulverized under liquid nitrogen. Tissues were stored at -80°C until extraction. Extracts were prepared from 0.5 g tissue per sample by Polytron™ homogenization in 25 ml of 4°C buffer (100 mM TrisCl, 10 % glycerol, 1 mM EDTA, 1 mM benzamidine, 1 mM dithiothreitol, 1 mM 4-(2-Aminoethyl)-benzenesulfonyl floride HCl, 0.1 mM leupeptin, ph 7.4). Debris was separated by 0.2 µ filtration and the resulting supernatant concentrated and desalted using a Millipore ™ UF-15 unit with Biomax 10K membrane. Final volumes were approximately 0.5 ml. Protein concentrations were determined spectrophotometrically using Biorad™ protein assay reagent. EPSPS specific activities were determined by assaying 10 µl extract at 25°C for 5- 15 min. (50 µl reactions included 50 mM HEPES, ph 7.0, 5 mM KF, 1 mM shikimate-3-phosphate, 0.5 mM phosphoenolpyruvate (PEP), and 0.1 mM ammonium molybdate.) Reactions were quenched with 50 µl of 9:1 ethanol: 0.1 M acetic acid. 30 µl of the quenched reaction were injected onto a Keystone™ AX-100 anion exchange column equilibrated with 0.235 M potassium phosphate buffer, ph 6.5, and eluted isocraticly with the same. A Packard™ C525 radioactive flow detector was used to determine turnover of [^{14}C]-PEP in the reaction.

For determination of $I_{50(glyphosate)}$ values, the assays were performed in the presence of increasing concentrations of glyphosate and the resulting activities analyzed and ploted using GraFit (Erithacus Software ©).

RFLP Analysis. Total cellular DNA was isolated from crown tissues of individual plants using the Plant DNAzol Reagent (Life Technologies, Rockville, MD.). Approximately 1 gm of flash frozen, powdered sample was mixed with 3.0 mls. Plant DNAzol reagent and 30 uls. of a 100 mg./ml.solution of RNase A, then incubated at room temperature for 10 minutes with gentle shaking. The remainder of the extraction procedure was carried out per manufacturer's instructions, with an additional chloroform : isoamyl alchohol (24:1 v/v) extraction step performed prior to ethanol precipitation. DNA integrity was confirmed by agarose gel electrophoresis of undigested samples, and concentrations and purity of the preparations were determined spectrophotometrically.

For each sample, 10 ugs. of cellular DNA was digested with either SspI, SphI, ScaI, or EcorV, as described by Watson and Thompson, 1986. Following overnight incubation, digests were ethanol precipitated, resuspended in TE buffer, and subjected to electrophoresis on a 0.8% agarose gel. DNAs were then transferred to a nylon membrane (Hybond-N, Amersham) in 20X SSC, and immobilized by UV crosslinking. The membranes were then pre-hybridized and hybridized in 6X SSC, 0.2% SDS, 5X Denhardt's solution, 100 ug/ml herring sperm DNA, and 2-5 ng/ml radio-labeled probes for 16-24 hours. Membranes were washed two times for 10 minutes at room temperature in 2X SSC, 0.2% SDS, followed by two 30 min washes at 65°C in 0.1X SSC, 0.2% SDS, then subjected to autoradiography.

RESULTS AND DISCUSSION

The response of the resistant and susceptible biotypes of *Elusine indica* treated with a range of glyphosate doses is summarized in Table 1. The 95% confidence intervals around both the 50% and 90% control levels observed indicated that the differences between the biotypes was significant. These results indicate that under the conditions of this experiment, the resistant biotype was able to tolerate approximately 2 to 3 times more glyphosate than the susceptible biotype.

Table 1. Results of dose response experiments with glyphosate susceptible and resistant *Elusine indica* biotypes.

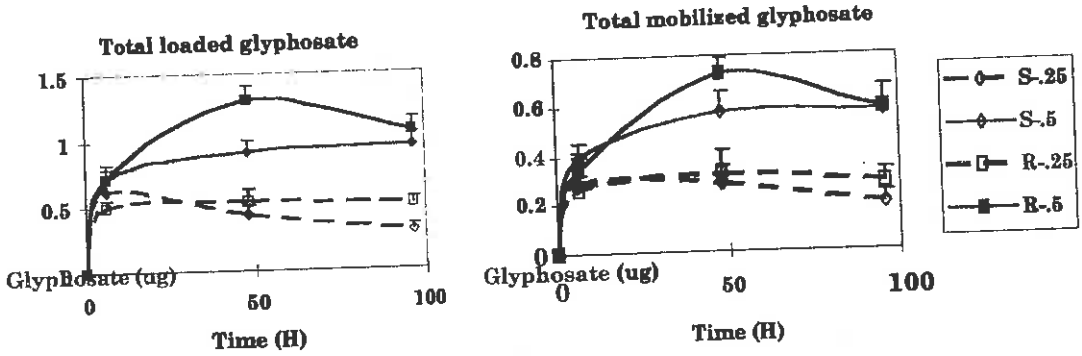
	CD50[a]	CD90[c]

Description	g ae/ha	R/S[b]	g ae/ha	R/S[b]
Jahor S	200 (190, 210)		290 (270, 380)	
Jahor R	390 (330, 460)	2	810 (620, na)	2.8

a. Dosage of herbicide required for 50% control with 95% confidence bounds.
b. Indicates ratio of resistant and sensitive biotypes.
c. Dosage of herbicide required for 90% control with 95% confidence bounds.

Some minor differences in uptake and translocation of ^{14}C glyphosate were observed between the two biotypes. The results in Figure 1. show that the resistant biotype appeared to have more glyphosate in the treated leaf following glyphosate application at 0.5 kg/ha but not at the 0.25 kg/ha rate. Similarly, the resistant biotype appeared to translocate more glyphosate away from the treated leaf at the higher glyphosate application rate. These results are similar to previous observations with glyphosate resistant crops which contain a gene that encodes a glyphosate resistant EPSPS. While the resistant biotype did uptake and translocate slightly more glyphosate than the susceptible biotype, this was speculated not to be the reason for the observed resistance.

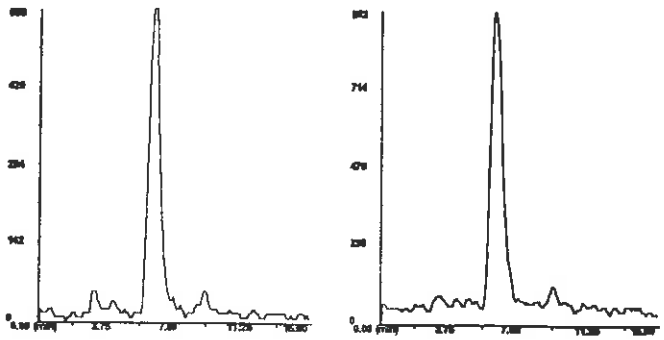
Figure 1. Plot of total ^{14}Cglyphosate loading and mobilization in glyphosate susceptible and resistant *Elusine indica* biotypes.



In Figure 2. the radioactive HPLC traces of ^{14}C glyphosate applied to both resistant and susceptible plants are shown. There was no significant breakdown of the ^{14}C glyphosate in either biotype and the recovery of total ^{14}C glyphosate was the same in both biotypes. These data indicate that the

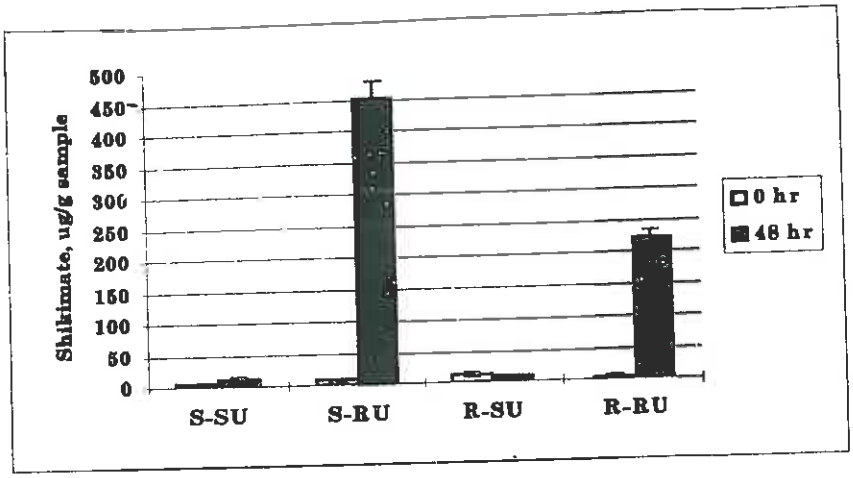
metabolism of glyphosate is not a factor involved in the resistance mechanism.

Figure 2. Radioactive HPLC traces of ^{14}Cglyphosate extracted from glyphosate susceptible and resistant *Elusine indica* biotypes.



The analysis of shikimate levels is reported in Figure 3. These data show that in both the resistant and susceptible biotypes that shikimate levels are similar before the application of glyphosate. The data also indicate that shikimate levels increase significantly 48 hours after glyphosate application. However, the resistant biotype had shikimate levels approximately half that of the susceptible biotype. The increase in shikimate levels after the application of glyphosate is an indication that glyphosate is reaching the target enzyme (EPSPS) in both biotypes. Even though the resistant biotype accumulates approximately 2-fold less shikimate, this does not confirm that the target enzyme, EPSPS, is resistant. This is based on previously published data whereby the shikimate levels of plants containing a resistant EPSPS were measured after the application of glyphosate and there was no accumulation of shikimate (Singh and Shaner, 1998).

Figure 3. Plot of shikimate levels extracted from glyphosate susceptible and resistant *Elusine indica* biotypes at 0 and 48 hours following glyphosate application.



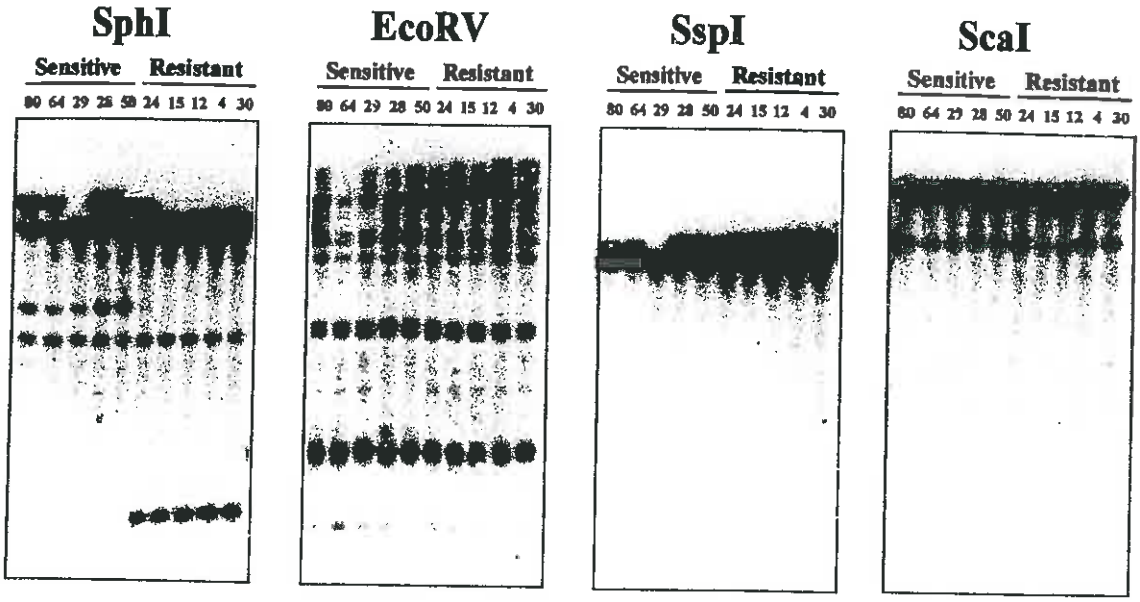
The biochemical characterization of the EPSPS from the resistant and sensitive biotypes found that there was a five fold difference in sensitivity to glyphosate between the two biotypes (Table 2). This data indicates that there could be differences in the EPSPS enzyme of the two biotypes. The EPSPS's from both biotypes are currently being purified and more detailed kinetics are in progress.

Table 2. $I_{50[glyphosate]}$ values for resistant and susceptible *Elusine indica* biotypes.

Biotype	$I_{50\ [glyphosate]}$	Difference
Jahor S	**3 uM**	
Jahor R	**16 uM**	**5.33**

The RFLP results in Figure 4 indicate that EPSPS comprises a small gene family in all individuals tested, possibly two-to-three members. Interestingly, SphI digests revealed both R-specific and S-specific polymorphism's inherited in all individuals of each class. These RFLPs will be used as a tool to identify hybrid individuals in crossing experiments, as well as population studies of F_2 descendants from these hybrids designed to link the EPSPS-related marker with glyphosate resistance.

Figure 4. RFLP analysis of glyphosate susceptible and resistant *Elusine indica* biotypes.



CONCLUSIONS

The data described in this report indicate that differences occur in the EPSPS enzyme, either at the structural and/or functional level, between the glyphosate resistant and sensitive biotypes of *Elusine indica*. Several additional experiments will be conducted to test this hypothesis, including an analysis of the expressed EPSPS sequences in both the resistant and sensitive biotype, in conjunction with kinetic analyses of the cloned enzymes expressed *in vitro*. The inheritance of the resistance trait will be also be evaluated in segregating hybrid populations to determine if correlations exist between resistance and the presence of a specific EPSPS allele. These same studies could address the question of whether the inheritance of resistance to glyphosate is monogenic in nature, or if additional factors may be involved. The EPSPS-related DNA polymorphisms identified in the RFLP analysis represent a useful tool for identifying the hybrid individuals required for these experiments. At the same time, field experiments have been designed to establish practical control measures for the management of resistant Elusine indica.

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HABITAT MANAGEMENT AND INHERITANCE OF SULFONYLUREA RESISTANCE IN *Lindernia micrantha*, AN ANNUAL PADDY WEED IN JAPAN

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Abstract: Biotypes resistant to sulfonylurea herbicides, including bensulfuron-methyl, pyrazosulfuron -ethyl and imazosulfuron, were discovered in naturally occurring populations of *Lindernia micrantha* D. Don Scrophulariaceae (Japanese name: Aze-tohgarashi) grown in paddy fields. These populations were distributed and growing separately from central Japan to Northern Honshu Islamd. However all of those paddy fields had been treated with SU-based herbicides for several consecutive years. In three year pot experiments, a few seedlings of *L. micrantha* emerged under poorly (ill)-drained paddy fields. In the F_1 from crosses of S×S, S×R, R×S and R×R, and the back crosses,the results indicated that a single dominant gene controls the sulfonylurea resistance in *L. micrantha*.

Key words: herbicide resistance, sulfonylurea, ALS, paddy field, *Lindernia*

Introduction

Since their introduction in the early 1980s, sulfonylurea (SU) herbicides have been widely used in major cereal-growing areas to control or suppress broad leaves and sedges. With the repeated use of the same SU-based herbicides, several weed species have developed resistance to such SU herbicides. SU reisitant biotypes to chlorsulfuron were first reported in *Kochia scoparia* L. (Priminani *et al.*, 19 90) and *Lactuca serriola* L. (Mallory-Smith *et al.*, 1990). Since then, resistance to acetolactate synthase (ALS) inhibitors has been reported in 50 additional weed species. At least one or more resistant biotypes of a species has been confirmed in the USA, Canada, Australia, Denmark, UK and Israel (Heap, 1998). Most of the ALS inhibitor resistant weed species grew in winter wheat fields

where both chlorsulfuron and a metsulfuron-methyl had been applied continuously for three to five years.

Both in California, USA, and Australia, SU-resistant paddy weeds have been detected in *Cyperus difformis* L. and *Sagittaria montevidensis* L. subsp. *calycina* since 1992 (Saari *et al.*, 1994).

During the past decade in Japan, BSM and pyrazosulfuron-ethyl (PSE), which are called on "one-shot treatment herbicides," have been widely used for herbicides in paddy fields. Examples include BSM/mefenaset (Zark), BSM/esprocarb (Fujigrass), BSM/dimepiperate (Push) and PSE/mefenaset (Act) in Japan. In 1995, an SU-resistant biotype of paddy weed was first detected in *Monochoria korsakowii* Regel et Maack, Pontederiaceae (Japanese neme: Mizuaoi) in Hokkaido Prefecture. In 1996, biotypes resistant to SU herbicides were also detected in some annual paddy weeds of Scrophulariaceae in the Tohoku district. Examples include *Lindernia micrantha* D. Don, and *L. dubia* Pennell subsp. *dubia* (Japanese name: Taketo-azena) in Yamagata Prefecture and *L. dubia* subsp. *major* Pennell (Japanese name: Amerika-azena) in Akita and Miyagi Prefectures. *L. procumbens* (Krock.) Philcox. (Japanese name: Azena) and *Limnophila sessiliflora* Blume (Japanese name: Kikumo) were not controlled by SU herbicides or barnyard grass-killers in the Tohoku and Hokuriku district. In recent years, *Scripus juncoides* Roxb. var. *ohwianus* T. Koyama (Japanese name: Inu-hotarui), a serious perennial weed in Hokkaido and Tohoku district, and *Rotala indica* Koehne (Japanese name: Kikashigusa) in Akita Prefecture. *Elatine triandura* Schk. (Japanese name: Mizo-hakobe) in Saitama Prefecture, and *Monochoria vaginalis* (Burm. f.) Kunth. (Japanese name: Konagi) in Akita and Ibaraki Prefectures. All common annual paddy weeds, were found resistant to SU herbicides (see Table 1).

In recent years, the number of paddy weed species resistant to SU herbicides has gradually increased. In this paper, we would like to report on the habitat management and inheritance of SU -resistant *L. micrantha* for basic reseach.

Materials and Methods

An experiment was conducted using 50× 50× 30cm concrete pots in the rice growing season from early May to late August in 1998 and 1999. About 50,000 seeds of SU-resistant *L. micrantha* from Kawanishi Town were sown in autumn 1997, the pots simulating well-drained paddy conditions (only rice growing season from May 17 to July 2, and from July 10 to Sept. 11) were submerged. Pots simulating poorly (ill)-drained paddy conditions (all years submerged) were treated at the NIAES field, in Tsukuba City, Ibaraki Prefecture. After puddles formed in the soil, six hills of rice were trans-

planted in mid of May. BSM/mefenacet granule (30kg/ha) was applied one week after transplanting. All seedlings were picked up and counted once a week in the 25× 25cm quadrat in the center of the pot with six replications.

A crossing test was carried out between a SU-resistant biotype of *L. micrantha* from Kawanishi Town and a susceptible biotype from Omagari City. One day before flower opening, all anthers were emasculated, and the flower was bagged. On the morning of the flowering day, the top of the stigma was touched with pollen grain of different flowers. The F_1 seeds from crosses of S×S, S×R, R×S and R×R, were collected in July 1997. These seeds were stocked in a refrigerator, planted in 200 cm^2 pots filled with sterile paddy soil in early Feb. 1998, kept in a green house. After seedlings were counted on Feb. 20, 21 g a.i./ha of PSE was applied. The number of surviving plants was counted on April 7. The remaining plants of S×R progenies were grown and crossed with susceptible pollens (back cross). The seeds were collected in late June and planted on Oct. 30. Back crossed seedlings were counted on Nov. 14, and 75 g a.i./ha of BSM was applied. Surviving plants were counted on Dec. 25. The x^2 test was conducted to test the deviation of observed segregation ratio from the expected ratio on a one locus - two alleles model.

Results and Discussion

Seasonal changes in the germination of buried seeds of SU-resistant *L. micrantha* are shown in Figs. 1 and 2. A total 832 seedlings per square meter emerged under well-drained paddy conditions (rice growing season was irrigated), however, only 28 seedlings emerged under poorly-drained paddy conditions in 1998. A total 1982 seedlings per square meter emerged under well-drained paddy conditions and no seedlings emerged in 1999. The seeds of *L. micrantha* germinate only on the surface of paddy soil. The data showed that *L. micrantha* seeds rarely germinate and emerge under 2 or 3 cm of submerged and reducted soil condition. After the seedlings emerged, submerged conditions did not disturb the plant growth. Japan reconstructed large-scale paddy fields for long-term land conservation after World War 2. At that time, field water was drained using tile drainage. There has thus been a transition from poorly-drained paddy fields to well-drained paddy fields. *L. micrantha* can now grow easily in paddy fields.

Flowers of SU-resistant plants (R) were sequentially crossed with susceptible plants (S). Some baggs had sterile seeds. Over 50 seeds per capsule were used in the experiment. The results are shown in Table 2. All seedlings from the F_1 progenies resulting from S×S died after being treated with PSE,

although some progenies from crosses S×R, R×S and R×R survived. This suggests that dominant nucleic genes control the inheritance manner of the SU resistant *L. micrantha*. The numbers of survivors in each line varied significantly because *1.* seedlings were very small, *2.* surface soil in pots was exfoiated, *3.* algaes developed, and *4. Daphnia* spp. and small aquatic insects were present.

After PSE treatment, remaining S×R progenies were cultivated, and the pollen grains were crossed with susceptible ovules. The results provided good evidence that a single dominant gene controls the SU resistance in *L. micrantha* (Table 3). The results of line (7) of $3\times F_1(R)$, and line (9) and line (11) of $F_1(R)\times F_1(R)$ in Table 3 did not satisfy the x^2 test. The reason as given before.

A field survey was conducted in the rice-growing season, early July 1996 in Kawanishi Town of Yamagata Prefecture, and in 1997 in Nangai Village, Akita Prefecture, Kazo City, Saitama Prefecture, and Omiya Town, Kyoto Prefecture in separate districts of central and northern Japan, which were vigorously infested SU-resistant biotypes of *L. micrantha*. The weeds were observed in 88 (8.3%) of a total of 1055 patches (Itoh *et al.* 1999). The season is considered to be the period three to four weeks after herbicide treatment. The differences in distribution patterns of infested paddy fields depended on the weed control method adopted by individual farmers suggesting consecutive use of one-shot treatment herbicides including SUs, such as, BSM and PSE. The herbicides intensified the infestation of the resistant biotypes of SUs (Itoh *et al.*, 1997). From the agricultural background in the rice cultivation of the surveyed areas, the infestation of *L. micranta* in rice fields was commonly caused by consecutive use of one-shot herbicides, presence of vacant spots in paddy fields for the limitation of rice cultivated areas of individual farmers, because of rice over production in Japan, and weed seeds attaching to and being transported by agricultural machines.

It was concluded that the resistant biotypes of *L. micrantha* are about 100 times more resistant to four kinds of SU herbicides than the susceptible ones. All resistant weeds were adequately control -led by a combination of soil treatment herbicides, and foliar and soil treatment herbicides (Itoh *et al.*, 1999).

To institute appropriate controls, a rapid, clear method of diagnosing resistant weeds is needed. In that regard, a report titled "Light requirements for rapidly diagnosing SU-resistant weeds of *Lindernia* spp. (Scrophulariaceae)" is now being published (Uchino *et al.*, 1999). The evolution of resistance to ALS-inhibitor was investigated in *L. micrantha* using molecular markers. The results indicated that the populations of resistant biotypes contained less variation than those of susceptible ones. In some cases, the populations of resistant biotypes were nested in the populations of susceptible biotypes. We concluded that the multiple founding events of resistant biotypes occurred in *L. micrantha* pulation (Shibaike *et al.*, 1999). The population genetics of SU-reistant species must be

examined in more detail.

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Table 1. The list of paddy weed species reported on the resistance to sulfonylurea herbicides in Japan

Species	Family	Japanese name	Location	References
Elatine triandra	Elatinaceae	Mizohakobe	Saitama Pref.	Hata *et al*., 1998
Limnophila sessiliflora	Scrophulariaceae	Kikumo	Akita Pref.	Wang *et al*., 1998
Lindernia dubia ssp. *dubia*	Scrophulariaceae	Taketo-azena	Tohoku district, Hokuriku district	Itoh & Wang, 1997; Uchino *et al*. 1999
L. dubia ssp. *major*		Amerika-azca	Tohoku district, Hokuriku district, Kanto district	Hata *et al*. 1998; Itoh & Wang, 1997; Seitoh *et al*., 1997; Uchino *et al*., 1999
L. micrantha		Aze-tohgarashi	Tohoku distirct, (Kyoto Pref., Saitama Pref.)	Itoh & Wang, 1997; Itoh *et al*., 1999; Shibaike *et al*. 1999
L. procumbens		Azena	Tohoku district, Hokuriku district	Itoh & Wang, 1997; Uchino *et al*.. 1999
Monochoria korsakowii	Pontederiaceae	Mizuaoi	Hokkaido	Kohara *et al*., 1996; Wang *et al*., 1997
M. vaginalis		Konagi	Akita Pref., Ibaraki Pref.	Koarai & Morita, 1999
Rotala indica	Lythraceae	Kikashigusa	Akita Pref.	Itoh *et al*., 1998
Scripus juncoides var. *ohwianus*	Cyperaceae	Inu-hotarui	Hokkaido, Tohoku district	Kohara *et al*., 1999; Yoshida *et al*., 1999

Table 2. Segregation ratio in F_1 progenied of 4 kind of crosses between sulfonylurea resistant (R) and susceptible (S) biotypes of *Lindernia micrantha*

Parental biotype ♀ × ♂	Total No. of Seedlings*	No. of Survivals**
S × S	*303*	*0*
S × S	*184*	*0*
S × S	*107*	*0*
S × R	*23*	*21*
S × R	*67*	*27*
S × R	*66*	*14*
S × R	*107*	*27*
R × S	*51*	*49*
R × S	*36*	*35*
R × S	*127*	*3*
R × R	*58*	*33*
R × R	*53*	*11*
R × R	*56*	*4*

*: Feb.6, 1998 Seeded, Feb. 19, Observed
**:Feb. 20, Treated PSE, April 7, Observed

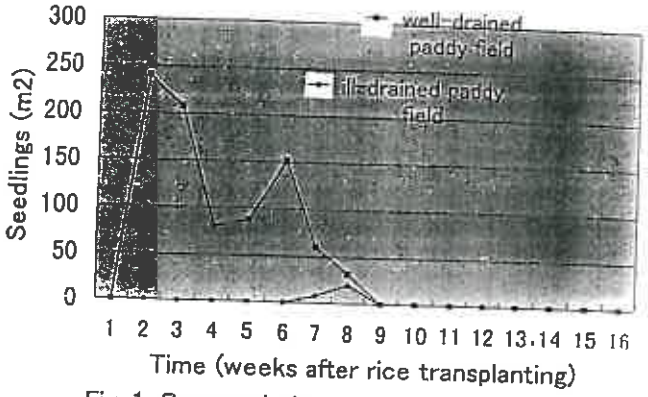

Fig. 1. Seasonal change of *Lindernia micrantha* resistant to SU herbicides in 1998

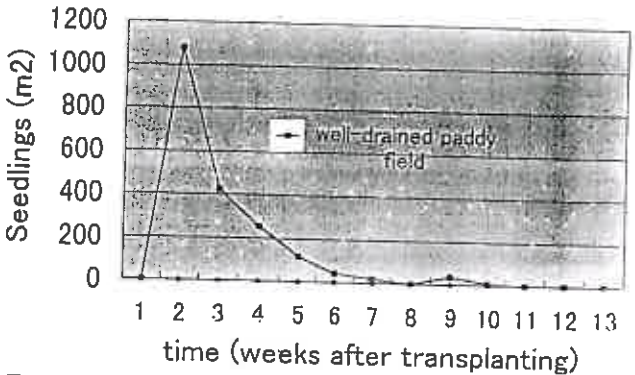

Fig.2. Seasonal change of *Lindernia micrantha* resistant to SU herbicides in 1999

Table 3. Segregation ratios in back cross and in F_2 progenies of cross between each survived plants of S × R

Parental biotype ♀×♂		Total No. of Seedlings*	No. of Survival**	No. of Dead Seedlings**	Expected ratio (R:S) ***	x^2	P
(1)	S × F_1(R)	*246*	*107*	*139*	*1:1*	*4.163*	*> 0.01*
(2)	S × F_1(R)	*165*	*85*	*80*	*1:1*	*0.152*	*> 0.05*
(3)	S × F_1(R)	*97*	*43*	*54*	*1:1*	*1.247*	*> 0.05*
(4)	S × F_1(R)	*187*	*104*	*83*	*1:1*	*2.356*	*> 0.05*
(5)	S × F_1(R)	*168*	*87*	*81*	*1:1*	*0.214*	*> 0.05*
(6)	S × F_1(R)	*317*	*138*	*179*	*1:1*	*5.303*	*> 0.01*
(7)	S × F_1(R)	351	116	235	1:1	40.345	< 0.001
(8)	F_1(R) × F_1(R)	*86*	*70*	*16*	*3:1*	*2.302*	*> 0.05*
(9)	F_1(R) × F_1(R)	187	104	83	3:1	37.480	< 0.001
(10)	F_1(R) × F_1(R)	*198*	*150*	*48*	*3:1*	*0.061*	*> 0.05*
(11)	F_1(R) × F_1(R)	262	159	103	3:1	28.625	< 0.001

*: Oct. 30, 1998 Seeded, Nov. 14, Observed, **: Nov. 15, BSM Treated, Dec. 25, Observed,
***: Single dominant gene

7. Biocontrol

EFFECTS OF *EXSEROHILUM MONOCERAS* ON GROWTHS OF BARNYARDGRASS AND RICE SEEDLINGS BY INOCULATION ON ROOTS

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Summary : *Exserohilum monoceras* is the most potential fungus as a bioherbicide for barnyardgrass. The difference of growths in shoots and roots of barnyardgrass and rice seedlings were studied after inoculating *E. monoceras* on their roots. Growth of barnyardgrass was severely inhibited by the inoculation, especially in its branch root formation, but the degrees of injurious effects were different by its strain or species. On the other hand, the effect by the inoculation wasn't remarkable in rice. Anatomical observation on seminal roots of barnyardgrass indicated that hyphae of *E. monoceras* were invading inter- and intra- cortex cells of roots in 5days after the inoculation, but, in rice, the almost all of hyphae were stopped invading into the internal tissues by papilla formation at periderm cells of roots.

INTRODUCTION

Now the almost agricultural technologies had aimed to increase in productive efficiency and enhance quality of crops, and to decrease labor works under cost-saving technologies, and, many diseases, insects and weeds which had the chemical tolerance appeared as a result of farmers using a large amount of chemicals[1)], so we need to develop alternative technologies, as biological ones, for the sustainable agriculture which is environmentally friendly. We had evaluated the most potential fungus, *Exserohilum monoceras*, as a bioherbicide for controlling barnyardgrass. Barnyardgrass is the most troublesome weed in paddy fields in many Asian countries, and it has been difficult to control without chemical herbicides.

Almost reported papers for *E. monoceras* had experimented by inoculating it on shoots of barnyardgrass plants[5,6,7,8,9)]. But there is a report that *E. monoceras* was isolated from the soil of paddy field around growing barnyardgrass. The objective of this research was to investigate the difference of effects of *E. monoceras* on growths of barnyardgrass and rice seedlings inoculated on their roots.

MATERIALS AND METHODS

Barnyardgrass used in this test were *Echnochloa oryzicola* (3 strains:CHIKUGO in Fukuoka,

KURI in Saga, KONOSU in Saitama), *E. crus-galli* var. *crus-galli* (4 strains:CHIKUGO in Fukuoka, USA in Oita, TAKEKOBA and KAGAMI in Saga), and *E. crus-galli* var. *formosensis* (CHIKUGO in Fukuoka). Rice, cv. NIPPONBARE, was used in it for comparing with barnyardgrass.

The spore suspension of *E. monoceras* with sterilized distilled water and surfactant were injected with pipette into the surface of the corner on 0.5% sugar and 0.5% agar media in plant boxes (6 x 6 x 10 cm H). In untreated control, these plant boxes were injected only with sterilized distilled water and surfactant. After these boxes were incubated into growth chamber (Nippon Medical and Chem. Inst. Co., LH-200-RDSCT) at 28C in light condition for 8 days, 9 seeds of each of above-mentioned barnyardgrass strain or species, of which glumes were removed, or rice were germinated after sterilization, and were seeded on the agar media at even space (Figure 1). These boxes were covered with black plastic bags surrounding their sides and bottoms to keep roots of seedlings dark, and they were put into the growth chamber at 28C in light condition. On 5th day after seedling, lengths or numbers of shoots, roots and branches on seminal roots of seedlings were measured. And, in *E. oryzicola* and rice, seedlings growing at 2.1 and 3.0 cm from the injected point were measured for lengths and numbers of branches on seminal roots by every 1 cm from their bases.

After measuring, seminal roots were used as materials for anatomical observation by paraffin section to find the aspect of hyphae's invasion into root tissues. For this observation, the growing period of rice in plant boxes were extended the longer to 10 and 20 days after seeding. These materials were stained with safranine and fast green solutions for microscopic observation.

Experiments with plant boxes were conducted three times by three replications. The effects of *E. monoceras* on shoots and roots were statistically evaluated using the coefficient of correlation, and those on number and length of branches on seminal roots by every 1 cm from their bases were done using the *t*-test, to compare treated seedlings with untreated control ones.

RESULTS

The radius of fungal colony expanded to about 5.5 cm on 5th day after seeding, where barnyardgrass and rice seedlings had grown until second leaf stage. By inoculating with *E. monoceras* on barnyardgrass roots, its seminal and crown ones in the colony in agar media appeared to have some brown lesions, while their shoots and other seedlings growing outside of the colony didn't have any external symptoms. Around these lesions on the surface of barnyardgrass roots, hyphae of *E. monoceras* were observed to be invading into their inner tissues. As seedlings grew the nearer to the spore-injected point, their shoots and roots were the shorter, so those of seedlings in treated boxes

were the shorter at the average than those in untreated ones. The branch formation on seminal roots was especially affected by fungal inoculation in all strains and species of barnyardgrass.

The degrees of effects on shoots and roots by the inoculation were different among species of barnyardgrass, as CHIKUGO strains of *E. oryzicola*, *E. crus-galli* var. *crus-galli* and *E. crus-galli* var. *formosensis*, and the effect was the largest as a whole in *E. crus-galli* var. *formosensis* even in seedlings growing at the outside of colony in treated boxes. The degrees of effects by the inoculation were somewhat different even among strains, as CHIKUGO, KURI and KONOSU strains of *E. oryzicola*, and the correlation between distances from injected point and growths of shoots and roots of seedlings in CHIKUGO strain was significant at the highest among them. The degrees of effects by the inoculation were also different among CHIKUGO, USA, KAGAMI and KOHDA strains of *E. crus-galli* var. *crus-galli*, and the correlation between distances from injected point and growths of shoots and roots of seedlings in CHIKUGO strain was significant at the highest among them. On the other hand, the effect of the inoculation wasn't remarkable in rice.

The mean number of branches per cm on all parts from base to tip of seminal roots was fewer in inoculated barnyardgrass seedlings growing at 2.1 and 3.0 cm from injected point in plant boxes than that in untreated ones. The mean length of branches per cm on all parts of these seminal roots was also shorter than that in untreated ones. On the other hand, there was no difference in rice between treated and untreated seedlings on the mean number of branches per cm of seminal roots, except at the part of 1 to 2 cm from the base of them. The mean lengths of these branches at each position of seminal roots were different between treated and untreated seedlings.

By anatomical observation of infected seminal roots of barnyardgrass, hyphae were found to form appressoria on the surface of roots, and to invade into epidermis, periderm and cortex, successively. Some hyphae were found to succeed in invading into central cylinder, but many of them were disturbed to penetrate into it by papilla formation at endodermis, which was a reaction of dynamic resistance of the plant against pathogen penetration[3]. Hyphae formed appressoria on the surface of seminal roots of rice and they had penetrated into epidermis, but almost all of them had been disturbed to penetrate into inner tissues by papilla formation at periderm. Even on 10th and 20th days after seeding, almost all of them weren't successful invading into peridermal cells through papilla there.

DISCUSSION

Results from this study indicated that the growth of shoots and roots of barnyardgrass seedlings

was inhibited by the fungal inoculation on roots, and degrees of inhibition were different by its strain and species. The diffusion of phytotoxin from *E. monoceras* into agar was clearly suggested as seedlings of CHIKUGO strain of *E. crus-galli* var. *formosensis* seeded at the outside of colony in treated boxes were also inhibited in their growth[7]. There wasn't remarkable effects on the growth of shoots and roots of treated rice seedlings, although there was a slight effect on the mean length of branches per cm on seminal roots of treated ones. Branch root formation used to be easily affected by internal and external factors[4], and branch roots of barnyardgrass were sensitively injured by fungal inoculation in this experiment, too. However, there seemed to be little effects on the growth of rice during tested periods in this experiment. Hyphae had invaded around and into cells of central cylinder of barnyardgrass roots, while they were disturbed to invade into rice roots at periderm. Therefore, it was supposed that this reaction of dynamic resistance was a factor to cause differences in injurious effects between barnyardgrass and rice. These differential effects on barnyardgrass and rice will make *E. monoceras* a useful fungus as a potential bioherbicide to control barnyardgrass in paddy fields.

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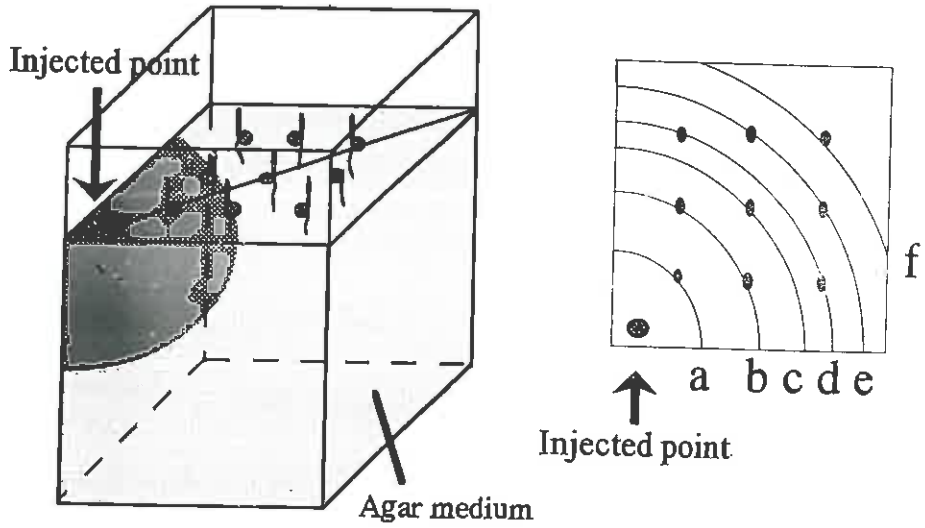

Figure 1. Seeding places of barnyardgrass and rice and injected point of *E. monoceras* in plant box. Distances of places from the injected point are, a:2.1 cm, b:3.0 cm, c:4.2 cm, d:4.7 cm, e:5.4 cm. f:6.3 cm.

FUSARIUM MONILIFORME SHELDON :A POTENTIAL MYCOHERBICIDE FOR WILD OAT (*AVENA LUDOVICIANA* DUR.) IN IRAN

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Abstract : Tehran and Markazi provinces were surveyed for potential biocontrol agents on wild oat (*Avena ludoviciana* Dur.) during 1993 and 1994. *Fusarium moniliforme* Sheldon was found to be pathogenic on this grass. A host range test was run on various crops to determine their susceptibility to the disease . Results showed that wheat (Var. Qods and Falat) , barley (Var. Valfjr and Kavir), maize (SC704) , rye (*Secale cereale*), millet (*Setaria italica*), faba beans (Var.Barekat), red beans (Var.Naz), green beans (Var.Top crop), sunflower (Var. Record), soybeans (Var. Williams), rape (Var.Ceres), cotton (Var.Sahel), safflower (Var.2811), cucumber (Var.Dora F1) ,water melon (Var.Krimson sweet), berseem clover , and sainfoin (Karaj echotype) were resistant with 0% kill, wild oat was very susceptible with 78% kill , and Johnsongrass (*Sorghum halepense*) 19% kill , crested wheatgrass (*Agropyrum desertorum*) 24% kill and tomato (Var. Red cloud) 17% kill were susceptible to the pathogen .

Key words : Wild oat , *Avena ludoviciana* , *Fusarium moniliforme* , Mycoherbicide .

Introduction

Many microbes (bacteria and fungi) are pathogenic to weeds and / or produce phytotoxic compounds that have potential in biological weed control programs (Hoagland , 1990) . Many species of fungi , especially *Fusarium* spp. are common and widespread throughout the world in plants , plant products , and soils . Grain crops (Abbas and Mirocha , 1985 ; Abbas *et al.*, 1989 ; Abbas *et al.*, 1984 ; Vesonder , 1986) and weeds (Jones and Hancock , 1990 ; McCain , 1978) are frequently infected pathogenically and saprophytically by *Fusarium* spp.

Fusaria such as *F.lateritium* , *F.oxysporum* and *F.solani* isolated from infected plant parts and soil have a role in biological control of weeds such as velvetleaf (*Abutilon theophrasti* Medicus) , prickly sida (*Sida spinosa* L.) and Texas gourd [*Cucurbita texana* (Scheele) Gray] (Boyette *et al.*, 1984 ; Boyette and Walker , 1985 ; Jones and Hancock , 1990) :

Members of the genus *Fusarium* produce a range of phytotoxic compounds that are chemically diverse and possess a broad range of activities and metabolic effects . For example , moniliformin causes growth inhibition , necrosis , and chlorosis on many plants (Duke , 1986 ; Hoagland , 1990) . Fusaric acid is a well - known phytotoxin isolated from *Fusarium* - infected plants and causes wilt of several plants (Chakrabari and Basuchaudharg , 1980 ; Davis , 1969 ; Kuo and Scheffer , 1964) .

Wild oat (*Avena ludoviciana* Dur .) is an economically important weed especially in wheat fields of Iran and can reduce yields up to almost 100% . Much money is spent in controling this noxious weed , chemically . Biocontrol of wild oat will be an environmentally friendly means of controlling this very common weed .

Materials and Methods

For two years (1994 and 1995) from spring to fall , farms in Tehran and Markazi provinces were surveyed to collect wild oat (*Avena ludoviciana* Dur.) plants were transferred to laboratory where two methods of investigation were followed :

1- Samples were separated from infected parts of plants and washed under running water for 15 minutes . Next , surfaces 5 mm pieces of each sample were sterilizes in 1-2 % sodium hypochlorite solution for 3

minutes , washed three times with sterile distilled water , and dried on sterile filter paper . The prepared samples were put in Potato - Dextrose Agar (PDA) , Corn Meal Agar (CMA) , and Nash & Snyder culture media and kept for 24-48 hours in an incubator under 24 C°.

2-Pieces of the plant at the border of infection area (including parts of infected and healthy area) were cut , put in water Agar (WA) and Nash & Snyder culture media , and kept under 24 C for 24-48 hours . The grown fungi were transferred to PDA subculture under sterile conditions and then purified by single spore method . The purified fungi were then identified .The identified fungus was tested for pathogenesity on wild oat as by following method :

Four 20 cm diameter black plastic pots were filled with sterile soil . In each pot 6 seeds of wild oat were planted .Two pots were inoculated with the pathogen and the other two pots were kept as control . Inoculation was carried out through three methods :

1- Inoculation of sterile soil before seeding

2- Direct inoculation of the plant at 2-4 leaf stage

3- Dipping the root system of the plant at 2-4 leaf stage in the inoculum suspension .

The control pots were treated with sterile distilled water (Dhingra and Sinclair , 1994) . The greenhouse temperature varied between 25 - 32 C°.To create high humidity , the pots were kept under plastic housing after inoculation . The pathogen thus developing was once again identified . After pathogenesity of the fungus was proved , a host - range test was run in the greenhouse using crops and plants as shown in table 1 . The following method was followed :

20 cm diameter black plastic pots were filled with sterile soil . Four or 9 seeds of the crop were planted in each pot (4 for large - seeds and 9 for small - seeds) . The greenhouse temperature ranged between 25 - 32 C°. Inoculation of plants was done as explained above .

The response of plants to the pathogen was determined according to Abbas & Boyette , 1992 and Abbas *et al.*, 1991 . Percent kill of plants was also determined according to Abbas & Boyette , 1992 . All host - range tests were run twice .

Results and Discussion

Wild oat infected with *F.moniliforme* was collected from wheat and barley fields in the second half of April . Pathogenesity tests were positive . The results of host - range tests are shown in table 1 . Amongst plants tested , wild oat was very sensitive with 78 % kill , Johnsongrass , crested wheatgrass and tomato were sensitive with 19% , 24% and 17% kill to the fungus respectively . Other test plants were resistant with zero percentage of kill . Above results indicate that *F.moniliforme* maybe considered as a potential biocontrol agent for wild oat in Iran .

F.moniliforme exudes mycotoxins . The phytotoxicity of this fungus on crops and weeds has been related to fumonisin B_1 which is one of the most important toxins produced by this fungus (Abbas and Boyette , 1992) .

The phytotoxicity of *F.moniliforme* on wild oat has been quite outstanding in our findings , and this could very well be related to metabolites such as fumonosin B_1 or other toxins which should be taken note of . The toxic specifications of these metabolites will be subject of our future research .

Table 1 Sensitivity and percent kill of test plants to *Fusarium moniliforme* Sheldon .

Test plant	* Sensitivity to *F.moniliforme*	% Kill
Wheat (*Triticum aestivum* L.) var.Falat	R	0
Wheat (*Triticum aestivum* L.) var.Qods	R	0
Barley (*Hordeum vulgare* L.) var. Valfajr	R	0
Barley (*Hordeum vulgare* L.) var. Kavir	R	0
Maize (*Zea mays* L.)var. SC704	R	0
Wild oat (*Avena ludoviciana* Dur.)	VS	78
Rye (*Secale cereale* L.)	R	0

continued

Table 1 Sensitivity and percent kill of test plants to *Fusarium moniliforme* Sheldon .

Johnsongrass[*Sorghum halepense* (L.) Pers.]	S	19
Millet [*Setaria italica* (L.) P.Beauv.]	R	0
Faba beans (*Vicia faba* L.)var.Barekat	R	0
Green beans (*Phaseolus vulgaris* L.)var.Top crop	R	0
Kidney beans(*Phaseolus vulgaris* L.)var.Naz	R	0
Sunflower(*Helianthus annuus* L.) var.Record	R	0
Soybeans [*Glycine max*(L.) Merrill]var.Williams	R	0
Rape (*Brassica napus* L.) var.Ceres	R	0
Cotton (*Gossypium herbaceum* L.)var.Sahel	R	0
Safflower (*Carthamus tinctorius* L.) var.2811	R	0
Tomato (*Lycopersicon esculentum* Mill.) var.Red cloud	S	17
Cucumber (*Cucumis sativus* L.) var.Dora F_1	R	0
Water melon (*Citrulus vulgaris* Schrad.) var. Krimson sweet	R	0
Berseem clover (*Trifolium alexandrium* L.)	R	0
Sainfoin (*Onobrychis Verae* Sirj.)Karaj echotype	R	0
Crested wheatgrass[*Agropyrum desertorum* (fisch.) Schults]	S	24

* R= resistant(no visible symptoms) ; S= sensitive(one or more symptoms such as necrosis or stunting); and VS = very sensitive(all symptoms) .

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PATHOGENICITY OF *ALTERNARIA ALTERNATA* TO CROFTON WEED (*EUPATORIUM ADENOPHORUM*)

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Abstract: Three isolates of *Alternaria alternata* (Fr.) Keissler were obtained from crofton weed(*Eupatorium adenophorum* Spreng.) in Sydney, Australia. Their pathogenicity to the weed was confirmed by the survey and check of the leaf lesion spots of plants in the wild and of the inoculated plants in a glasshouse. The process of the leaf infected by conidium and mycelium fragment was observed. The time the infection process of mycelium fragment took was seven times shorter than that of conidium. Mycelium fragment did not only infect the leaf through stomata same as conidium, but also directly through the epidermis at cell junction. Those three isolates were compared in the morphology, growth rate and virulence of mycelium. Isolate 501 was the most virulent since its growth rate was much quicker than the others. Mycelium fragment of isolate 501 may have potential to be developed as biological agent for crofton weed.

Key words: ***Alternaria alternata, Eupatorium adenophorum*, pathogenicity, mycoherbicide, mycelium fragment,**

Introduction

Crofton weed(*Eupatorium adenophorum* Spreng. syn. *Ageratina adenophora* (Spreng.) K.& R.) is a significant troublesome weed in coastal areas of Queensland and New South Wales, Australia and through parts of the tropics and subtropics of the world(Auld 1969 and 1981, Fuller 19881, Mari'n, Negrillo et al. 1983, Morris 1989). Control of this weed by conventional methods is difficult(Auld 1981; Morris 1989) and biological control by fungi is being investigated(Morris 1989). One species of *Phaeoramularia* has been investigated for possible development as a mycoherbicide for the weed(Dodd 1961, Morris 1989 and 1991, McKenzie 1990, Gou et al. 1991, Guo et al. 1992, and Wang & Summerell 1995 unpub. data).

One isolate of *Alternaria* sp. was obtained from the leaf lesion spots with the irregular dark brown part caused by *Phaeoramularia*. On the basis of symptomatological, morphological and cultural characteristics, the fungus was identified as *Alternaria alternata* and deposited at the New South Wales Agriculture Plant Disease Herbarium as DAR71534. Later on, a large number of pure dark brown leaf lesion spots were observed in the natural crofton weed populations in the different places of Sydney and Wollongong. Other two isolates with same morphological characteristics as *A. alternata* were collected from the leaf lesion spots of the diseased plants in natural occurrence.

Alternaria alternata is one of the most important pathogens in the genus *Alternaria*. It has a wide range of hosts. Up to date, it has been found that 115 species in 43 families can be infected by this fungus(Rotem, 1994). However, crofton weed has not been included in the list.

The study reported here was designed to determine whether the isolates of *A. alternata* have Pathogenicity to crofton weed and whether they have potential to be developed as a biological control agent for the weed.

Materials and Methods

Pathogenicity of *A. alternata* to crofton weed

1)Isolation Three isolates 402, 501 and 503 were separately obtained from the following sources: leaf lesion spots caused by *Phaeoramularia* sp. at the Royal Botanic Gardens, Sydney in 1994, dark brown leaf lesion spots on green leaf at Dulwich Hill, Sydney in 1995, and dark brown leaf lesion spots on brown dead leaf at Dulwich Hill, Sydney in 1995.

Leaf segments(1cm^2) from diseased crofton weed plants were surface disinfested for 5 min in 1.0% (v/v) NaOCl. The segments were rinsed twice in sterile distilled water, placed on water agar(WA) and incubated at 25^{0}C in the dark for 7 days. The resulting colonies were subcultured on carnation leaf-piece agar (CLA) and incubated for 8days. Isolates were purified by the single spore technique and grown on CLA and potato dextrose agar(PDA).

2) Pathogenicity tests Single spore isolates were cultured on CLA for 8days, and subcultured onto PDA at 25^{0}C with 12hrs photoperiod for 6 days. Conidia were collected by flooding cultures with sterile water and rubbing the surface with a soft brush. The conidial suspension was filtered through a layer of nylon cloth (0.3mm aperture) and the concentration was adjusted to 1x10^6/ml. The conidial suspension was sprayed onto 10 two month-old plants to run-off with a hand atomizer. The inoculated plants were immediately placed in a humidity chamber for 2days. After 4 weeks, the new leaf lesions on those plants were compared with the original leaf lesions and the fungus was re-isolated and identified to see whether it still was *A. alternata*.

Two dozen 2 month-old plants were inoculated with the conidial suspension containing a mixture of conidia of *A. alternata*(10^6 /ml) and *Phaeoramularia* sp. (10^3/ml), and placed in a humidity chamber for 3 days. These plants were maintained for 8 months. Three different color leaf lesion spots were sampled at monthly intervals: green(young lesion), dark brown and light plus dark brown. Seven lesion spots of each lesion types were sampled respectively at each sampling date. Leaf lesions were cultured on quarter strength PDA. After incubation for 24hrs, the cultures were observed for the presence of conidia of *Phaeoramularia* sp as well as for the presence of *A. alternata*. The similar experimental procedure was applied to checking leaf lesion spots in the field too.

3)Infection of plant After plants were inoculated with conidia and received dew for 4days, leaf pieces(1cm^2) were immersed in alcohol and acetic acid solution (1:1v/v) for 3hrs or more to remove chlorophyll within leaf cell and subsequently stained by alcoholic lactophenol cotton blue for 1hrs or more and examined using a light microscope.

SEM(scanning electron microscopy) was also done to observe the infection process on the leaves. Segments 4mm long were fixed in FAA and rinsed in o.1mol/ml PBS buffer. The dehydration schedule consisted of 20min in each of 50, 70, 95 and 100% ethanol. The segments were taken through three changes of 100% ethanol. Critical-point drying was carried out for 45min with CO_2 in HITACHI HCP-2 model instrument. Samples were placed on copper sample stubs and sputter-coated with gold-palladium in an argon atmosphere with a EIKO IB.3 instrument. Amary-1840 scanning electron microscope was used at 30 kv to observe and photograph specimens.

After mycelial suspension were sprayed onto plants for 14 hrs, leaves were treated and examined in the above same method.

Culture of fungus

The single-conidium isolate of *A. alternata* was grown on PDA for seven days. The isolate was subcultured into a 250ml flask contained liquid growth medium (167ml) and incubated on an orbital shaker (110 rotations per minute) in the dark at 25^{0}C. Two kinds of liquid media were used. One (SCSC) was consisted of soybean(15g), corn flour(15g), sucrose(30g) and $CaCO_3$(3g) in one litre of media. The other (PSK) was made from 300ml home made potato broth, 20g sucrose and 1g KH_2PO_4 per litre.

In order to stimulate production of conidia, twelve flasks of mycelia cultured in SCSC medium were filtered and rinsed with sterile water separately. Six flasks of mycelia were homogenized in a blender for 10min. The others were not treated. They were respectively poured onto thirty six 9cm petri-dishes covered by sterile filter paper. Each medium treatment was divided into two groups. One group was incubated under a combination of white and NUV light/dark circle with a 12hrs photoperiod while the other was incubated in the dark. All cultures were kept at 25C for 2days. The petri-dishes were recovered for 2days to air dry. The cultures were twice washed with 10ml 0.1% Tween 80 solution. Numbers of conidia were counted with a haemocymetre to compare the number of conidia produced by the different treatment.

Comparison of three isolates

1) Rate of growth One single spore of each of three isolates 402, 501 and 503 was inoculated on PDA in a 9cm plastic petri-dish at 25C in the dark. Seven replicates were conducted respectively. After 1, 4, and 5days incubation, the colonies were examined and details of germination of conidium was observed and diameter of mycelia colony was measured.

The rate of growth of three isolates in submerged-culture in SCSC liquid medium at 25C under dark for 4days was estimated by blending the mycelium for 10 min, and counting the mycelium fragments with a haemocytometre. The color and shape of the mycelia were also observed. There were seven replicates established.

2)Virulence of the fungus Mycelia of three isolates were prepared as above and were homogenized in a blender for 10min. Two dilution of mycelium fragments were prepared. One treatment was to dilute 100ml mycelial fragment suspension with 20ml distilled water. The other was done to adjust the same concentration of mycelial fragment suspension diluted with distilled water. For each treatment for seven potted, 2.5-month old plants with five pairs of leaves were inoculated respectively with the above mycelial suspension of three isolates through the above two different dilution systems. Another seven plants were sprayed with their

conidial suspension ($2x10^6$ conidia/ml) respectively. Three controls were sprayed with water. All plants were placed in a humidity chamber for 20hrs and then kept in a glasshouse at 15/28c(night/day). The plants were examined after 4days and then at weekly intervals for diseased symptoms. The height of plant and number of diseased leaves were measured and recorded. The experiments were repeated.

The Duncan's test was employed to compare the difference between the treatments.

Results

Pathogenicity

The leaf lesion spots caused by *A. alternata* were dark brown, circular to irregular in shape, and 1-5mm in diameter sometimes with narrow black dark brown circular margin(Fig.1). The inocule of mycelium was also able to infect petiole and stem, and cause those tissue to fester.

The conidium of the fungus was obclavate, dark brown, 35-40 μ m long by 15 μ m wide, minutely verrucose and with five transverse and two to four longitudinal septa. On PDA and CLA, conidium was catenate, beakless(but pseudorastrate in light dark color). The chains of conidium consisted of 10 conidia or more ,usually branched. However, the chains had much fewer conidia on the surface of the diseased leaf than on the media(Fig.2)

After being cultured for 24hrs, the clusters of olivaceous conidia of *Phaeoramularia* sp. were found to form on the surface of the young green and the mixture of light and dark brown lesion spots. However, on the leaf segment with the dark brown lesion spot, only white hyphae were obsérved. After 5days, mycelia and conidia of *A. alternata* grew from leaf segments with the dark brown spots and with the mixture of light and dark spots. Only *Phaeoramularia* sp. was isolated from the segments with the young green lesion spots.

Culture of fungus

A. alternata grew well in both media(SCSC and PSK) tested. Mycelium darkened in PSK medium 2day earlier than in SCSC medium. However, the volume and weight of fresh mycelia growing in SCSC medium were about 30% greater than in PSK medium.

There was significant difference in the number of conidia when incubated in either light or dark(Tab.1) with light promoting sporulation markedly. Homogenized mycelial fragments from the same number of flasks produced much more conidia than other treatments did whether in light or not(Tab.1).

Table 1. Sporulation of homogenized and non-homogenized mycelium of *A. alternata* in constant light and continuous dark condition.

Treatment	Number of conidia(ml^{-1})	
	light	dark
Homogenized mycelium	$13x10^8$*a	$1x10^8$c
mycelium	$5.6x10^8$b	$0.2x10^8$d

*The number of conidia were produced from 500ml SCSC liquid medium.
The number of conidia followed by different letter in each column and row are significantly different $P<0.05$.

Infection of plant

Germ tube usually grew out of the end of pseudorastrate with only one germ tube per conidium. On rare occasions two ones or more were observed(Fig.3). The hypha thickened to form a penetration structure at the stomata. This slightly thickened hyphae also appeared at cell junctions over which the hyphae was growing(Fig.3). The hypha penetrated stomata(Fig.4) and then branched in the substomatal cavity(Fig.3). Apart from stomatal penetration, no other penetration ways were observed. The total infection process by conidia took four days. One week after inoculation with conidia only a few small leaf lesions, brown in color, were present on the leaf surface.

Following inoculation with mycelial fragments extensive amounts of new hyphae developed on the leaf surface after 14hrs(Fig.4). Penetration structure similar to those produced by conidia developed at the top of new hypha and at the site of the hypha across cell junction usually(Fig.6). In addition to stomatal penetration, penetration structures formed at cell junction, directly penetrated leaf epidermis there and entered the leaf mesophyll tissue(Fig.7,8). It demonstrated a hypha entered through the epidermis at cell junction. Fourteen hrs after inoculation, hyphae were observed in mesophyllous tissue(Fig.7) and necrotic spots were observed on the surface of the infected leaf. Some parts of the infected leaf began to wither. The process of infection took 20hrs.

Comparison of three isolates

Mycelia of isolate 501 pigmented earliest while isolate 402 was last. Mycelium of all isolate produced clustered lumps when grown in an orbital shaker at 110 rotary per min. However, the morphology of mycelia was somewhat different from each other in SCSC submerged-culture at 130 rotary per min. Isolate 402 was cotton-like, but some of isolate 501 and most of isolate 503 were clustered(Tab.2).

The experiments testing the diameter of single-pore colony on PDA and the number of mycelial fragments in SCSC liquid medium demonstrated that the growth rate of isolate 402 was significantly slower than isolate 501 and 503 (Tab.2).

Table 2. The main characteristics of isolate 402, 501 and 503 of *A. alternata* were compared.

Isolate	D of single-spore colony on PDA after 6d(cm)	Cultural characteristics in SCSC liquid medium after 4d in 130 rotary pm			Pathogenicity(percentage of diseased leaves after 6w %)		
					Conidium	Mycelium fragment	
		Colour	Shape of mycelia	No. of m.f.* ml^{-1}		Under dilution with same volume of water	at same concentration
402	7.31a	white	cotton-like	7.2×10^6a	17.12a	91.35a	100a
501	7.99b	light black	part of cluster	9.46×10^6b	21.68b	100b	100a
503	7.70b	little light black	most of cluster	8.4×10^6b	15.31a	absent	87.76b

*m.f.: mycelial fragments

The number of conidia followed by different letter in each column are significantly different $P<0.05$.

The percentage of diseased leaves caused by isolate 501 was markedly greater than that caused by isolate 402 and 503. There was not significant difference between isolate 402 and 503 (Tab.2).

Under same concentration of the mycelial fragments, mycelial suspension of isolate 402 and 501 was more virulent than isolate 503(Tab.2). There was marked difference in virulence between isolate 402 and 501(Tab.2). However, There was a significant difference in virulence between isolate 402 and 501 mycelia when the suspension was diluted by the same volume of water with isolate 501 growing more quickly(Tab.2). Among these two experiments, isolate 501 mycelia caused serious disease of plants and killed all inoculated plants after 5wks.

Discussion

A. alternata was shown to naturally infect crofton weed in a glasshouse and capable of causing a leaf spot disease. It had been isolated and found from the leaf lesion spots of crofton weed growing in two different place(Dulwich Hill and Wollongong) in Sydney. This suggests that *A. alternata* may be a natural pathogen to crofton weed.

Examination of the processes of infection in the glasshouse showed that the infection of crofton weed by conidium of *A. alternaria* might need initial infection by *Phaeoramularia* sp. *A. alternaria* had never been isolated from young leaf lesion spots and had more commonly been isolated from older leaf lesion spots that had been initiated. It was possible that the fungus infected plants through wounds or some other form of damage(Rotem, 1965 and Zitter & Hsu 1990). Inoculation of plants with conidium demonstrated that conidium was only mildly pathogenic to crofton weed. It seems that there was the commonness of *A. alternata* in lesions produced by *Phaeoramularia* sp. on crofton weed(Rotem, 1994).

The predominant site of penetration by mycelium differed considerably from conidium occurring predominantly through the epidermis. Conidium entered leaf only through stomata, which occurred at penetration of bean leaf observed by Saad and Hagedorn(1969). This suggests that conidium is weakly pathoenic to crofton weed in the view of penetration way(Rotem, 1994). However, mycelium was capable of direct penetration through epidermis predominantly at cell junction, suggesting that mycelium may have the property more commonly associated with more virulent pathogens. The experiments of inoculation of plants have shown that mycelium can cause much more disease than conidium. Moreover, infection process by mycelium took much shorter time than conidium. Mycelial infection required a much shorter dew duration than conidium. A short wetting duration and quick speed of infection are requisite for the development of pathogens into mycoherbicides(Charudattan, 1991). Mycelium of *A. alternata* appears to have a great potential to be developed as biological herbicide for crofton weed. The fungus has been studies as the potential biocontrol agent for other weeds(Aneja & Singh 1989; Yang at al. 1993).

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Caption Fig . l-8.

Fig.1. Leaf lesions on crofton weed. Left: light brown spots caused by *Phaeoramularia* sp.(P). Centre: mixture of right and dark brown spots caused by both *Phaeoramularia* sp. and *Alternana alternata*(PA). Right: Dark brown spot caused by *A. aliernata* (A). Bar=20 mm. Fig.2. Catenate conidia(arrow) formed on the leaf surface of the diseased plants. Bar=80 μ m. Fig.3. Hyphae(h) formed from conidium(c) 4 days after inoculation. Infection predominantly through stomata(st) although penetration structures(ps) occurred at the junction of cells(j). gm=germ tube. Bar=20 μ m. Fig.4. SEM photo: Infection predominantly through stomata(st). Fig.5. Now hyphae(h) formed from mycelial fragments(mf) after l4 hrs with penetration structures(ps) developed at the junction of cells(j). Bar=20 μ m. Fig.6. Mycelial penetration of the leaf through the junction of cells(j) rather than through stomata(st), 14 hrs after inoculation. Note the penetration of hyphae(h) through epidermis into mesophyll tissue. hm=hypha in mesophyll tissue. Bar=20 μ m. Fig.7. Hyphae(hm) in mesophyll tissue of infected leaf. mc=mesophyll cell. Bar=20 μ m. Fig. 8. SEM photo: mycelial penetration of the leaf through the junction of cells.

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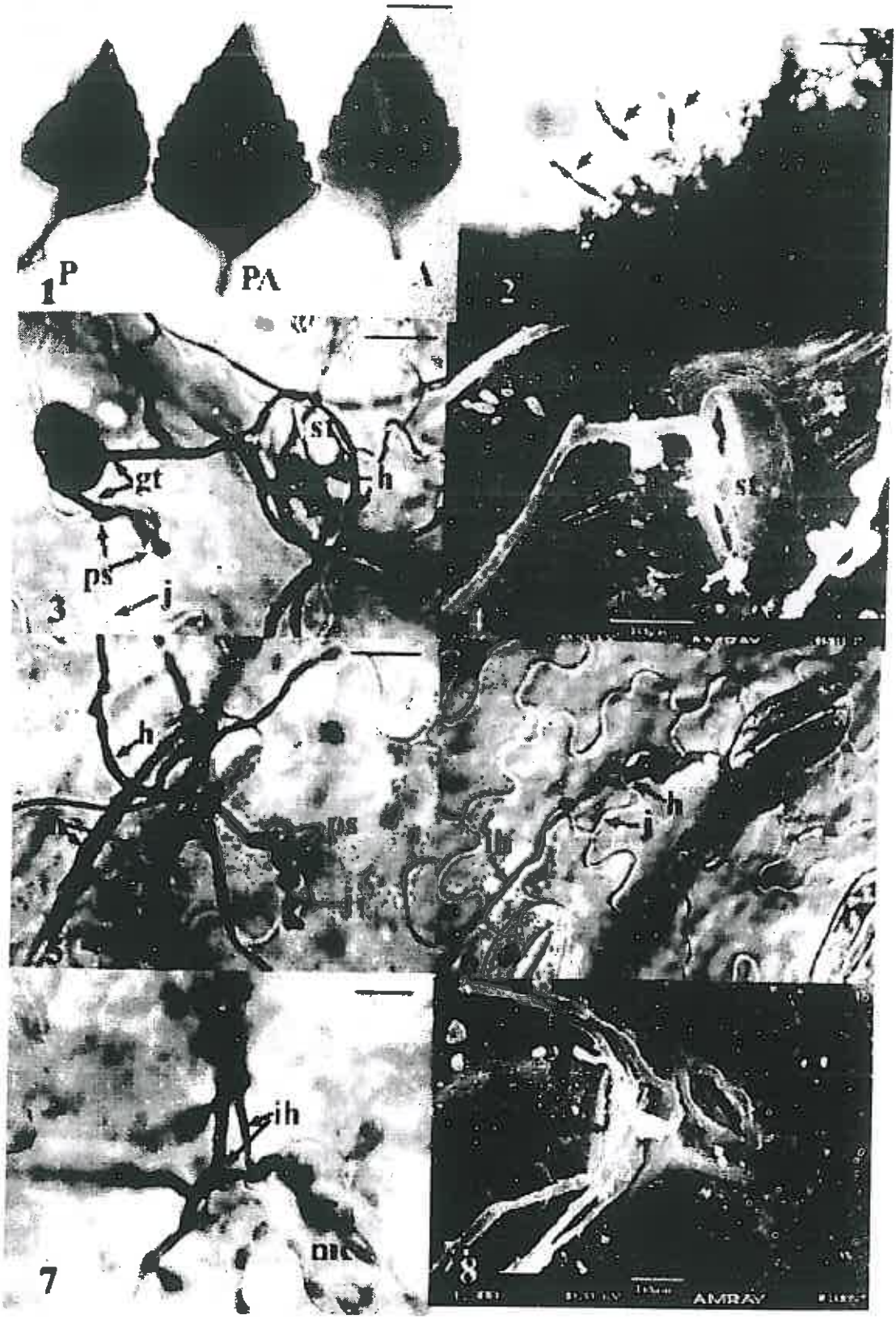

RESEARCH ON SOME FUNGI TO CONTROL BARNYARD GRASS (*ECHINOCHLOA CRUS - GALLI* (L.) BEAU) AND RED SPRANGLETOP (*LEPTOCHLOA CHINENSIS* (L.) NEES) IN RICE

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Abstract: Mekong Delta and Red River delta are important rice growing areas in Vietnam. They contribute about 70 % of rice production for whole country. Weeds are a major pest causing substantial yield loss, mainly due to grassy weeds. Two major grassy weeds in rice production are *Echinochloa crus-galli*, and *Leptochloa chinensis*. Traditionally, farmers do hand weeding to control weeds but recently herbicide use has increased sharply. This trend causes concern with regard to herbicide resistant weeds and environmental pollution. In order to minimize the trend of high dependency on herbicides to control weeds, the Australian Center for International Agricultural Research (ACIAR) and three partners in Vietnam namely National Institute of Plant Protection (NIPP), Cuulong Delta Rice Research Institute (CLRRI) and Cantho University (CU) have collaborated in a project entitled "Biological control of grassy weeds with fungi as bioherbicides". Diseased plants of grasses in Red River delta were collected and isolated by NIPP. Similar collections were done in the Mekong delta by CLRRI and CU. Promising fungi were sent to the International Mycological Institute in U.K. for identification. Research results at CLRRI revealed that the most promising fungi for controlling *E. crus - galli* are *Exserohilum monoceras* and *Cocholiobolus lunatus*. The promising fungus on *L. chinensis* is *Setosphaeria sp.cf.rostrata*. Spore concentrations of 3×10^4, 3×10^5 and 1×10^6 were inoculated onto the three-leaf stage of *E. crus - galli*. At the rate of 7×10^7 spores per square meter of *E. monoceras* and *C. lunatus*, 100% death after 14 days was recorded with a dew period of 48 hours. Rice varieties IR 50404 and CR 203 are not affected by the fungi. The fungus *Setosphaeria sp.cf rostrata* was found to be effective in controlling *L. chinensis* and safe to rice variety IR64.

Key words: Bioherbicides, Biocontrol, , *Echinochloa crus-galli, Leptochloa chinensis*.

Introduction

Weeds are an important pest in rice production, particularly in direct seeding. Herbicides have been used popularly in rice production by farmers because there are many selective rice herbicides available in the market, their price is acceptable to middle and even poor farmers. Most farmers do not like to work in the field for a long time with boring handweeding and labour is in supply due to the migration of farmers from rural areas to cities for seeking the better jobs. The trend of dependency on herbicides has been observed resulting in accumulation of herbicidal residues in the environment. Farmers tend to use the same compound for many years, which can create the new problems of herbicide resistant weeds. One way to reverse those trends is to explore the possibility of using biological control method as one component of integrated weed management. Fungus is the most feasible one because the mass production of spores can be done in small scale industry to produce mycoherbicides to meet the demand of particular areas (Watson, 1991). The Cuulong Delta Rice Research Institute (CLRRI) has collaborated with NSW Agriculture, Orange Agricultural Institute, NSW, Australia to study this subject since 1995. The objectives of this study are: (1) To survey the disease symptom on *Echinochloa species* and *Leptochloa chinensis* and to isolate the causal fungi. (2) To conduct pathogenesis to weed but no to rice. The primary results are presented briefly in this paper.

Materials and methods

Collection of diseased weeds, isolation and identification of fungi.

In Summer - Autumn of 1997, the major target weeds with disease symptoms were collected from rice fields in Omon and Thotnot district of Cantho province. Diseased weeds collected at an early and late stages of rice growth. Particularly, disease symptoms as stem rot, top leaf and leaf spot (Morin *et al.*, 1994). The infected part of weed were surface sterilized with 0.1 % $HgCl_2$ and incubated on fresh Potato - Dextrose - Agar (PDA). The pathogens were isolated using standard isolation techniques. Pure cultures were maintained in storage. Identification of the isolated fungi was based on the monograph of Barnett and Hunter (1972) utilizing the light microscopic.

Pathogenicity testing of isolates.

Healthy young seedlings (4- to 6- leaf stage) of *Echinochloa* species, *Leptochloa chinensis* and of rice (IR64-Indica , IR50404 and CR 203; OM67-Japonica) were planted in pots and maintained in the greenhouse. Spores were prepared from 10 to 15 day-old cultures, filtered in nylon cloth and the concentration adjusted to 3×10^4 and 10^6 spores per ml (Tween 80 was added). Grasses and rices were inoculated by spraying using a hand sprayer and were placed in a dark dew chamber for 2 day (T^0=28^{0}C, A^0=98%) and transferred to the greenhouse for observation of disease symptoms. The pathogenicity of each microorganism against weeds and rices was evaluated in accordance with the 4- grade system from - (no effect) to +++ (death) (Te *et al.*, 1977).

Results and Discussion

Identification of fungi:

The code names of isolates during the process of research at CLRRI are E-10-97 and L-8-97. The specimens of those isolates were sent to IMI for identification. The specialist there identified E-10-97 as *Cochliobolus lunatus* R.R. and L-8-97 as *Setosphaeria sp.cf. rostrata.*

Effect of fungal spore concentration on infected leaves of *Leptochloa chinensis* and *Echinochloa* spp

The *Echinochloa* spp got low infected leaves percentage by *Cochiliobolus lunatus* and they did not died at the spore population of 3×10^4 ml^{-1}. Under the spore population of 3×10^5 ml^{-1} the weed species viz., *Echinochloa* spp. *and Leptochloa chinensis* all were affected from 95 - 96 % died at 14 days after incubation and these fungi showed only a slight effect on rices (Table 1 and table 2).

Table 1. Effect of *Cochiliobolus lunatus* on infected leaves (%) of *Echinochloa* spp. and rices.

Spore population (ml^{-1})	*Echinochloa* species									Rices					
	E. crus -galli			*E.glabrescens*			*E. colona*			IR 64			OM 67		
	*3 DAI**	*7 DAI*	*14 DAI*	*3 DAI*	*7 DAI*	*14 DAI*	*3 DAI*	*7 DAI*	*14 DAI*	*3 DAI*	*7 DAI*	*14 DAI*	*3 DAI*	*7 DAI*	*14 DAI*
$3x\ 10^4$	35	42	49	32	37	45	25	32	40	0	5	5	0	7	10
$3x\ 10^5$	39	65	96	37	63	90	36	75	85	0	10	15	0	12	15

*DAI: days after inoculation

Table 2. Effect of *Septosphaeria rostrata* on infected leaves (%) of *Leptochloa chinensis* and rices.

Spore population (ml^{-1})	*Leptochloa chinensis*			Rice var. IR 64			Rice var. OM 67		
	3DAI	7DAI	14DAI	3DAI	7DAI	14DAI	3DAI	7DAI	14DAI
3×10^5	36	75	95	0	25	25	0	20	25
10^6	98	100	100	40.5	30.1	11.4	41.5	28.2	17.7

Effect of causal agent fungus *Cocholiobolus lunatus* on infected leaves of barnyard grass

Based on the above results, we have also tested on the application of the fungi at a concentration of $1x10^6$ ml at 7, 14, 21 and 28 days after sowing . The plants were remained damp as with dew for 48 hours after inoculation in order for disease to develop (due to the result of initial incubation study). The results from table 3 and 4 showed that after 3 DAI, the percentage of infected leaves obtained as 87.3 % and 99.4 % for *Cocholiobolus lunatus* and *S. rostrata* at 7DAS, respectively. This revealed that the inoculation of fungal pathogen should be done at early stage to get more effectiveness (2 -4 leaf stage).

Table 3. Percentage of infected leaves (%) of *Echinochloa crus - galli* by *Cocholiobolus lunatus* at different times of inoculation.

Treatments	Days after inoculation (DAI)			
	3	7	14	21
T1. Spray water at 7DAS*	0.0	0.0	0.0	0.0
T2. Spray 10^6 spores/ml at 7DAS	87.3	90.0	90.0	90.0
T3. Spray water at 14DAS	12.7	7.3	22.7	22.3
T4. Spray 10^6 spores/ml at 14DAS	83.0	90.0	90.0	90.0
T5. Spray water at 21DAS	15.7	24.7	23.3	22.0
T6. Spray 10^6 spores/ml at 21DAS	78.0	90.0	90.0	90.0
T7. Spray water at 28DAS	27.0	26.7	22.7	28.3
T8. Spray 10^6 spores/ml at 28DAS	71.3	90.0	90.0	90.0

*DAS: Days after sowing

Effect of causal agent fungus *Setosphaeria rostrata* on infected leaves of *Leptochloa chinensis.*

Table 4. Percentage of infected leaves (%) of *Leptochloa. chinensis* by *Setosphaeria rostrata* at different times of inoculation.

Treatments	*Leptochloa chinensis*			
	3	7	14	21
T1. Spray water at 7DAS	0.0	0.0	0.0	0.0
T2. Spray 10^6 spores/ml at 7DAS	99.4	100.0	100.0	100.0
T3. Spray water at 14DAS	3.7	1.7	15.1	14.5
T4. Spray 10^6 spores/ml at 14DAS	97.7	100.0	100.0	100.0
T5. Spray water at 21DAS	7.5	17.5	15.9	14.0
T6. Spray 10^6 spores/ml at 21DAS	95.0	100.0	100.0	100.0
T7. Spray water at 28DAS	20.4	21.1	15.2	23.3
T8. Spray 10^6 spores/ml at 28DAS	89.2	100.0	100.0	100.0

*DAS=days after sowing

Effect of *Cocholiobolus lunatus, Exserohilum monoceras* (H6) and *Exserohilum monoceras* (85.1) on Barnyard grass and Red sprangletop

The plant height of barnyard grass decreased gradually from 5 days after inoculation onwards as compared to control and completely died at 15 DAI. Both varieties were unaffected by the fungi (Table 5)

The percentage of dead barnyard grass plants recorded was 75 %, 35 % and 75% at 10 days after inoculation for *Cocholiobolus lunatus , Exserohilum monoceras (H6)* and *Exserohilum monoceras (85.1),* respectively. At 15 DAI, all *E. crus- galli* were dead from all treatments of the fungi. Among treatments on rice, there was only 5 % of rice var. IR 50404 was affected by *C. lunatus*.

Table 5. Treatmental influence on plant height and death plant percentage (%) of barnyard grass and rices at different times of inoculation .

Treatments	Plant height (cm)			Dead plants (%).		
	5 DAI*	10 DAI	15 DAI	5 DAI	10 DAI	15 DAI
A. Effect on *E. crus - galli*						
Treated with *C. lunatus*	7.7	1.5	0.0	10.0	75.0	100.0
Treated with *E. monoceras* (H6)	5.4	4.7	0.0	0.0	35.0	100.0
Treated with *E. monoceras* (85.1)	6.2	2.2	0.0	0.0	75.0	100.0
Untreated (check)	24.0	45.4	58.3	0.0	0.0	0.0
B. Effect on rice var. IR50404						
Treated with *C. lunatus*	13.7	21.4	31.1	5.0	5.0	5.0
Treated with *E. monoceras* (H6)	12.9	21.1	28.1	0.0	0.0	0.0
Treated with *E. monoceras* (85.1)	13.9	22.7	34.0	0.0	0.0	0.0
Untreated (check)	14.8	23.0	31.0	0.0	0.0	0.0
B. Effect on rice var. CR 203						
Treated with *C. lunatus*	14.8	23.0	31.0	0.0	0.0	0.0
Treated with *E. monoceras* (H6)	13.3	19.7	29.6	0.0	0.0	0.0
Treated with *E. monoceras* (85.1)	15.4	25.4	32.5	0.0	0.0	0.0
Untreated (check)	23.0	29.4	36.4	0.0	0.0	0.0

Effect of *S. rostrata* on rice var. IR 64:

Rice variety IR 64 is not affected by the fungi. Panicle length, number of panicle / hill, unfilled grain percentage, 1000-grain weight and dry matter accumulation of rice are similar in treated and untreated treatments (table 6).

Table 6. Yield components and dry matter accumulation of rice var. IR 64

Treatments	Panicle length (cm)	No. panicle / hill	Unfilled grain percentage (%)	1000-grain weight (g.)	Dry matter accumulation (g. /pot)
T1. Spray water at 7DAS*	23.7	8.7	14.4	26.9	30.0
T2. Spray 10^6 spores/ml at 7DAS	20.2	7.3	12.8	24.1	35.6
T3. Spray water at 14DAS	23.2	6.7	12.4	27.2	31.8
T4. Spray 10^6 spores/ml at 14DAS	20.5	5.7	17.5	26.1	30.1
T5. Spray water at 21DAS	23.0	6.3	10.7	26.2	32.4
T6. Spray 10^6 spores/ml at 21DAS	21.3	6.0	13.0	24.8	27.5
T7. Spray water at 28DAS	22.9	7.0	12.9	26.7	33.7
T8. Spray 10^6 spores/ml at 28DAS	20.6	7.7	12.0	27.6	30.9

Discussion

Some species of fungi living in rice ecosystems can have the pathogenic activity on weeds in rice fields. Those fungi can be collected, propagated and produced mycoherbicides and used as one component of the integrated weed management (IWM). Results in this research revealed that the inoculation for 14-15 days is sufficient to cause the epidemic effect on weeds because the sufficient time of incubation is required for fungi to cause substantial epidemic effect to kill weeds effectively. *C. lunatus* controls *E. crus-galli* and - *S. rostrata* can kill *L. chinensis* effectively and safe to rice both Indica rice var. (IR 50404 , IR 64 and CR 203) and japonica rice var.(OM67). They are environmental friendly and sufficiently good marketing prospects to serve farming community. More research on application and formulation for use in the field is required.

Acknowledgements

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SCREENING OF THE SELECTIVE BIOCONTROL AGENTS FOR *LEPTOCHLOA CHINENSIS* (L.) NEES IN THAILAND

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ABSTRACT : Weeds are an integral part of all arable crops and serve as a major constraint preventing the crops from achieving their yield potential. Furthermore, weeds also serve as the wild hosts for many insects and pathogens, which may cause serious loss to economic crops. Weed problems are serious in tropical regions since environmental factors favor their growth and propagation. Various management strategies are available for suppression of weeds in the cultivated lands, including cultural techniques, hand weeding, mechanical control and chemical control using herbicides. However, application of chemical control has resulted in the development of herbicide resistance in many weed species as well as increased environmental and societal concerns related to pesticides use. Recently, biological control using indigenous plant pathogens against weeds has been evaluated and proved to be an effective approach in various parts of the world. However, the selection of the suitable agents is very important and a major factor in its success. The two main keys for the selection of biological weed control agents are effectiveness and selectivity, aspects of this study. Pathogens were collected from the weeds found in and near the rice fields of Chiang Mai Valley, isolated, and characterized. Pure cultures of the pathogens were sprayed on the target weeds, rice varieties, wheat, barley, maize, and sorghum in order to evaluate the host/pathogen interactions. Isolated pathogens were grouped in the genera *Bipolaris*, *Curvularia*, *Drechslera*, *Exserohilum*, *Fusarium* and *Pyricularia*. Most of the screened pathogens could also infect the economic Poaceae hosts, although with different symptoms and severity. However, one *Exserohilum* sp. isolate was highly virulent on *Leptochloa chinensis*, and produced only mild symptom on the economic Poaceae crops.

Introduction

Red sprangletop, *Leptochloa chinensis* (L.) Nees., is an important weed species found in the paddy field and ranked as the same outbreak level as *Echinochloa crus-galli* (L.) Beauv. (Holm *et al.*, 1977). In Thailand, it is a serious weed in lowland rice (Holm *et al.*, 1977 and Noda *et al.*, 1984), and listed as a principal and a common weed in several countries (Holm *et al.*, 1977). After germination its grows profusely in nearly waterlogged or even flooded soil condition and moreover, it is very difficult to control (Soerijani, 1987). At present, chemical control using herbicides associated with cultural techniques, hand weeding, and mechanical control are applied for the control of *L. chinensis*. However application of chemical control has lead to the development of herbicide resistance in many weed species as well as increased environmental and societal concerns related to pesticides use (IRRI, 1994). Recently, biological control is becoming an important method of weed control that is capable of contributing to the protection of the environment and promoting sustainable agriculture (Wongsaroaj, 1995). Biological weed control also has a great potential in reducing chemical inputs and providing viable, economic, effective weed control component in rice (Watson, 1994). In Japan, Gohbara and Yamaguchi (1994) reported effective biocontrol of barnyard grass with *Drechslera monoceras* and biocontrol of water chestnut using *Epicoccosorus nematosporus*. Zhang et al. (1996) similarly indicated the effective biological control agent of *Echinochloa* species in rice in the Philippines with *Exserohilum monoceras*. In this study, pathogens of grass weeds were collected and their effectiveness and selectivity as biological weed control agents determined.

Materials and Methods

Isolation and characterization of fungi.

Diseased leaves of *L. chinensis* and *E. crass-galli* were collected in and near the rice fields of Chiang Mai Valley, Thailand from late 1997 to early 1999. Infected tissues were cut to appropriate sizes, surface sterilized with 2% calcium hypochlorite solution and placed on moist filter paper in petri dishes. After 24 h. of incubation, lesions were examined under a microscope and conidial masses were picked up using capillary tubes, streaked on 4% water agar (WA), and incubated for 24 h. Single spore isolates were transferred to potato dextrose agar (PDA; 200 g of potatoes, 20 g of dextrose, 17 g of agar and 1 liter of water) and the recovered fungi identified and characterized (Ellis, 1971; Hanlin, 1989). For long term storage, pure cultures of original isolates were maintained on filter paper followed by IRRI methods at –20° C as stock cultures.

Inoculum production.

To revive stored cultures, small pieces of mycelium from stock culture of each fungus were placed on PDA and cultured for 7 days. Agar plugs from the margins of young colonies were used as seed inoculum (Zhang *et al.*, 1996) and placed in the center of each petri dish containing PDA, the conidial production medium. Inoculated plates were sealed with parafilm strips and incubated at room temperature for 7 days. Before conidial harvesting, plates were placed under alternating 12 h near ultraviolet (NUV) light / dark for 2 days. Inoculum was prepared by pouring distilled water into the petri dish and gently scraping the surface of the colonies with a glass slide. The conidial suspensions were filtered though a layer of cheesecloth and conidial concentration was determined using a haemocytometer. The final conidial concentration was adjusted to 10^5 to 10^6 conidia per milliliter in 1% gelatin before application.

Pathogenicity test.

The target weeds were *L. chinensis, E. crus-galli* and *Ischaemum rugosum*. A single lot of seeds of each species collected from the rice field in Chiang Mai Valley, except *I. rugosum* collected from Pitsanulok province, Thailand, were used in all experiments. Seeds of each species were planted in plastic trays filled with water-saturated soil and kept at room temperature for a week. After germination, eight seedlings were transferred to 10 cm-diameter pots and kept in a greenhouse. Seedling at the three-to four-leaf stage, ca. 3 weeks old, were treated with urea fertilizer before placing in a dew chamber, and then sprayed with the conidial suspension until run off. Inoculated plants were incubated at 25±2° C under 100% relative humidity conditions for 24 h, and then returned to the greenhouse. After 7 days, symptoms on the weeds were observed and disease severity was assessed as percentage of plants infected and percentage of plants killed.

Virulence and specificity.

After the initial screening, *Exserohilum* sp. (LE) and unknown isolate 1 (LU 1) were selected and tested on the economic Poaceae hosts in order to evaluated the host-parasite interaction in the greenhouse. The target weeds and the economic Poaceae hosts e.g. rice (*Oryza sativa*) var. RD6, KDML and upland rice, wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), maize (*Zea mays*) and sorghum (*Sorghum bicolor*) were used in these experiments. Seeds of each species were incubated in moist chambers at room temperature for 48 h. Ten newly germinated seeds of each species were planted in plastic trays filled with water saturated soil and fertilized. After two weeks, the test plants were treated with urea and inoculated with 10^5 to 10^6 conidia per ml of *Exserohilum* sp. (LE) and unknown 1 (LU 1) isolates to run-off. Inoculated test plants were placed in a dew chamber with relative humidity of 95-100% and 25 ± 2° C for 24 h. Seven days after inoculation the disease reaction of weed hosts and the economic Poaceae crops to each the test pathogens were evaluated. Disease severity was assessed as percentage of infected plants, percentage of killed plant and percentage leaf area damage.

Results

Isolation and identification of fungi.

Six different species of pathogens were isolated and characterized as shown in Table 1. Seven isolates of *Drechslera* sp., five *Curvularia* sp. isolates, one *Exserohilum* sp. isolates and one unknown were isolated from *L. chinensis*. Four *Drechslera* sp. isolates, two *Curvularia* sp. isolates, one *Fusarium* sp. isolate, one *Bipolaris* sp. isolate, one *Pyricularia* sp. isolate and one unknown were isolated from *E. crus-galli*.

Pathogenicity test of selected fungi.

The fungal isolates from *L. chinensis* and *E. crus-galli* could infect all of the target weed plants at very high percentages except *Curvularia* sp. isolate 2 (LC2) and (EC4) (Table 2). After 24-h of dew period, necrotic spot symptoms had appeared and a blight-like reaction was observed 3 days after inoculation. Subsequently, lesions expanded, merged, and turned to blight. Some mortality was observed after 7 days, especially *L. chinensis* inoculated with *Exserohilum* sp. isolate 1 (LE 1) at 93%, Unknown 1(LU 1) at 90%, *Curvularia* sp. isolate 1 (LC 1) at 38%, and *Drechslera* sp. isolate 6 (LD 6) at 27%. *Exserohilum* sp. was more virulent on *L. chinensis* leaves than on *E. crus-galli* or *I. rugosum*. *E. crus-galli* seedlings were all killed by unknown 1 (LU1) and some isolates of *Drechslera* also caused mortality, but at lower percentages.

Inoculation on the test plants.

The selected *Exserohilum* sp. (LE 1) isolate could infect and kill *L. chinensis* seedlings at 80-100% (Table 3). This isolate also infected maize, barley, sorghum, wheat, and rice seedlings, although at different levels of severity. Sorghum, maize and barley were affected by the tested pathogens. Only minute symptom were detected on wheat and all tested rice cultivars (Table 3). Unknown isolate (LU 1) was highly virulent on seedlings and one-month-old *L. chinensis* plants, killing seedling 80 and 90%, respectively. Among the tested Poaceae crop plants tested, sorghum was the most susceptible to this pathogen, while wheat and barley were not infected by this isolate (Table 3).

Discussion

Six different pathogenic fungi in the genera *Bipolaris, Curvularia, Drechslera, Exserohilum, Fusarium* and *Pyricularia* were found to have naturally infected *L. chinensis* and *E. crus-galli* leaves. Whereas Zhang et al. (1996) in the Philippines had previously isolated six different species of pathogenic fungi; two *Exserohilum* sp., two *Curvularia*, one *Bipolaris* sp. and *Dactylaria* sp. from naturally infected *Echinochloa* plants. In Japan, various species of fungi in the genera *Fusarium, Rhizoctonia, Phoma,* and *Drechslera* were found to have naturally infected barnyard grass leaves (Gohbara, 1994). TeBeest (1991) reviewed the weed biocontrol projects with pathogenic fungi in the genera *Colletotrichum, Fusarium, Bipolaris, Phomopsis, Ascochyta, Alternaria,* and *Cercospora* from many weed hosts.

The pathogenic fungal species isolated from naturally infected *L. chinensis* and *E. crus-galli* could highly infect all of the target weeds plant, but not all pathogens could kill the target weeds. Furthermore, due to the wide host range of *Fusarium, Bipolaris* and *Pyricularia* isolates, they were not intensively tested and applied in these experiments. The selected *Exserohilum, Drechslera, Curvularia* and the unknown isolates have exhibited suitable characteristics for biocontrol agents. There is a close relationship between dew period and the effectiveness of the tested fungi in terms of percent mortality and percent of leaf area damage as was previously described with *Exserohilum monoceras* that related efficacy with duration, frequency, and timing of dew period (Zhang and Watson, 1997a). Similar conclusions were obtained in other bioherbicide projects including *Colletotrichum gloeosporioides f. sp. aeschynomene* (TeBeest et al., 1978). Zhang and Watson (1997b) reported the potency of applying the biocontrol agent on different growth stages of *Echinochloa* sp. seedlings and stated that the 1.5 leaf stage plantlets were completely controlled, but younger or older seedling were not totally killed. This experiment showed the same results for the *Exserohilum* isolate but the unknown isolate could damage the *Leptochloa* seedling better than the older stage. Although *Exserohilum* and the unknown isolate, LU 1 proved to be highly virulent, killing or severely damaging seedling of *L. chinensis*,

they were also caused some damage on the economic Poaceae crops, especially on sorghum and maize. This isolate did not infect the upland rice and only mild symptom could be observed on the tested lowland rice seedlings. Further experiments on the older stages of rice could be therefore tested before it could apply as the biocontrol agent in the rice growing areas.

Table 1 Morphological characters of weed pathogens found in Chiang Mai Valley.

Host	Pathogen	Spore size (μ)[1]	Mycelium character[2]	
			Color	Colony
L. chinensis	*Drechslera* sp. (LD 1)	11.75±1.03×67.08 ±7.38	Greenish black	Smooth
	Drechslera sp. (LD 2)	12.55±10.98×94.08 ± 12.58	Black	Puffy in some area
	Drechslera sp. (LD 3)	13.12±1.35 ×71.32 ± 12.37	Gray	Tiny puffy
	Drechslera sp. (LD 4)	9.18±1.10×20.0 ±1.68	Gray	Smooth
	Drechslera sp. (LD 5)	15.13±1.68×59.0 ±6.68	Gray	Puffy
	Drechslera sp. (LD 6)	13.08±0.98 × 63.38 ± 11.03	Dark brown	Smooth
	Drechslera sp. (LD 7)	9.25±1.16 × 22.08±1.75	Brown	Smooth
	Exserohilum sp. (LE 1)	13.92±1.82 × 62.17±14.33	Greenish black	Smooth
	Curvularia sp. (LC 1)	12.5±1.25 × 98.75±2.04	Greenish black	Puffy
	Curvularia sp. (LC 2)	8.08±1.23 × 16.8±1.05	Greenish black	Smooth
	Curvularia sp. (LC 3)	9.83±1.38 × 18.72±2.16	Black	Smooth
	Curvularia sp. (LC 4)	9.83±0.93 × 20.75±1.55	Black	Smooth
	Curvularia sp. (LC 5)	9.71±0.85 × 19.54±2.49	Greenish black	Tiny puffy
	Unknown 1 (LU)	14.29±1.63 × 77.25±18.86	Black	Smooth
E. crus-galli	*Drechslera* sp. (ED 1)	9.71±1.38 × 19.75±2.49	Greenish black	Tiny puffy
	Drechslera sp. (ED 2)	16.25±1.83 × 97.22 ±9.03	Greenish black	Tiny puffy
	Drechslera sp. (ED 3)	18.00±2.03 × 100.4±8.48	Dark brown	Puffy
	Drechslera sp. (ED 4)	10.00±0.87 × 24.54±3.57	Brownish	Puffy, smooth edge
	Curvularia sp. (EC 1)	9.95±1.55 × 18.43±1.73	Dark brown	Not puffy
	Curvularia sp. (EC 2)	9.21±1.21 × 21.21±2.04	Brown	Puffy, smooth edge
	Unknown 1 (EU)	14.42±1.56 × 103.50±6.34	Grayish black	Puffy, smooth edge
	Fusarium sp. (EF 1)	3.51±0.88 × 13.76±4.25	Whitish pink	Puffy
	Bipolaris sp. (EB 1)	12.72±1.44 × 29.88±6.6	Grayish black	Tiny puffy
	Pyricularia sp. (EP 1)	5.95±1.27 × 23.96±4.07	Whitish gray	Smooth

[1]Average from 30 conidia. [2]Observed after 7 days of culture on PDA

Table 2 Pathogenicity tests of the fungi isolated from weed hosts on the three weeds.

Pathogen	% infected plant			% killed plant			Symptom		
	Lep.	Ech.	Isc.	Lep.	Ech.	Isc.	*L chinensis*	*E. crus-galli*	*I. rugosum*
Drechslera sp.(LD 1,2,3,4,5)	100	100	100	0	0	0	Brown stripe or spot, merge lesion, severe.	Brown spot, merge lesion	Small brown spot, merge
Drechslera sp. (LD 6)	100	100	100	27.27	0	0	Brown stripe, merge lesion, blight.	Small brown spot.	Small brown spot
Exserohilum sp. (LE 1)	100	100	100	92.86	0	0	Brown stripes, yellowish brown leaf and blight.	Small brown spot, merge lesion.	Small black spot
Curvularia sp. (LC 1)	100	100	100	38.46	0	0	Brown stripe, merge lesion, blight.	Small brown spot.	Brown spot
Curvularia sp. (LC 2)	26.67	0	25	0	0	0	Small brown spot.	No symptom.	Small brown spot
Curvularia sp. (LC 4)	100	62.5	88.89	0	0	0	Brown spot, merge lesion.	Brown spot.	Tiny brown spot
Unknown 1 (LU 1)	100	100	100	90	0	0	Brown stripe, merge lesion, blight.	Small brown spot, merge lesion.	Small brown spot
Drechslera sp. (ED 1)	93.33	100	20	0	18.18	0	Black-brown stripe.	No symptom	No symptom
Drechslera sp. (ED 2)	100	100	50	0	0	0	Brown stripe.	Brown eye shape spot.	Brown spot with yel. edge
Curvularia sp. (EC 4)	60	100	42.86	0	0	0	Brown spot, merge lesion.	Irregular brown spot, merge lesion.	Brown spot, merge lesion
Unknown 1 (EU 1)	100	100	42.86	13.33	100	0	Brown stripe	Light brown eye shape spot	Brown spot

Table 3 Percent infected plant, percent killed plant and percent leaf area damage of weed host and the economic Poaceae hosts seedling after treated with *Exserohilum* sp. isolated 1 (LE 1) and unknown 1 (LU 1).

Pathogen	Criterion	Weed hosts		Economic Poaceae hosts						
		Lep. seedling[1]	*Lep.* 1month[2]	Maize	Barley	Sorghum	Wheat	UL Rice	RD6	KDML
Exserohilum sp.	%infected plant	100	100	100	100	100	30	0	29	8
	%killed plant	100	80	0	0	13.63	0	0	0	0
	%leaf area damage	100	80-100	5-25	5	10	0.5-1	0	0.5-1	0.5-1
Unknown (LU 1)	%infected plant	100	100	100	0	100	0	4.67	23.81	4.76
	%killed plant	80	90	0	0	4.76	0	0	0	0
	%leaf area damage	50-100	50-100	10-50	0	5.2	0	0.5	5-10	5

[1]seedling at the one- and two- leaf stage

[2]seedling at the three- and four- leaf stage

Furthermore, other isolates of *Exserohilum* should be tested and screened. Zhang and Watson (1996a) selected one of six species of pathogens- *Exserohilum monoceras* as a potential biological control agent for *Echinochloa* species. Other species were pathogenic to rice. As reported by Evans (1987) as cited in Watson (1994), various pathogens were isolated from diseased *R. cochinchinensis*, but most were also pathogenic on maize. In our study, barley and wheat were not infected by the unknown isolate, LU 1, and were only slightly damaged by the *Exserohilum* isolate. These responses could be due to barley and wheat not being widely grown in Thailand. Although the tested *Exserohilum* and unknown isolate, LU 1, are virulent and can effectively control *L. chinensis*, more information and data must be collected before their potential as safe and effective biological control agents can be fully assessed.

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Poster Presentation

1. Weed Biology and Ecology

BIOLOGICAL ACTIVITIES OF PLANT GROWTH INHIBITORS FROM *Duranta repens*

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Abstract: *Duranta repens* Linn. (Syn.: *Duranta plumieri* Jacq) is a candidate of allelopathic plants. To isolate the plant growth inhibitor(s) in *D. repens*, air-dried leaves of this plant were collected and extracted with 80% MeOH. Successive bioassay-directed purification of the extracts gave three pure plant growth inhibitors. Chemical structures of these compounds were identified as durantanins I, II and III, which are saponins with molecular weight of 1222, 1354 and 1222, respectively. Inhibitory effects of durantanins I - III on elongation growth of some test-plants were measured. Differences of the inhibitory effects among durantanins I - III were negligible for all plant species examined. The durantanins I - III inhibited elongation growth of plant roots stronger than that of shoots. Amaranthaceae plants, such as common amaranth (*Amaranthus retroflexus*) and green amaranth (*Amaranthus viridis*) were found to be more tolerant against durantanins I – III than the others. Around 50 % of the root growth of seedlings of leaf mustard (*Brassica juncea var. cernua*) was inhibited by 50 μM of durantanins I – III. The total content of durantanins I - III in the leaves was 1.0 to 1.5 % (air-dry basis). The inhibitory activity of crude extract of *D. repens* was well explained by durantanins I – III. Inhibitory activity of durantanin II was higher than that of the other candidate allelochemicals, such as vanillic acid, syringic acid, ferulic acid and coumarin. These results indicate possible role of durantanins I – III as allelochemicals.

Key words: *Duranta repens*, allelopathy, plant growth inhibitors, triterpenoid saponins, durantanins I - III.

Introduction

Duranta repens is a tree-type ornamental plant grown in tropical countries. It is generally 2 to 4 meters in height and its large number of leaves create shade year round. Weeds and shrubs growing around it are not healthy, suggesting the presence of water-soluble plant growth inhibitor(s) in the leaves. This suggests a possible allelopathic phenomenon (Zungsontiporn, 1995). This report describes the isolation and determination of candidates of allelochemicals from the leaves of *D. repens*, and shows the biological activity of these chemicals as plant growth inhibitor for some plants.

Materials and Methods

Isolation and chemical determination of plant growth inhibitor(s) from *D. repens*

Air-dried leaves of *D. repens* (from Bangkok, Thailand) were extracted with 80% MeOH. After evaporation at 40℃ in vacuum condition, the extract was suspended in distilled water. Inhibitory effect of the extract on elongation growth of leaf mustard was examined as described below. The water suspension of the extract was purified to follow the inhibitory activity by using liquid-liquid extraction, open-column activated charcoal (Charcoal, Activated, 031-02135, Wako Pure Chem. Ind., Ltd.) and silica-gel (Wakogel C-200, 74-149 μm, Wako Pure Chem. Ind., Ltd.) chromatography, solid phase extraction (reversed-phase, Analytichem Bondesil C18, 40 μm, preparative grade, Varian) and preparative HPLC (Shim-pack PREP-ODS(H), 20×250 mm, Shimadzu). Purified fractions were applied to thin layer chromatography (TLC, Merck, Silica Gel 60 F-254, No.5715, 0.25 mm thickness) and detected with vapor of iodine. Chemical structures of purified compounds were determined by NMR (JEOL JNM α-600, CD_3OD) and FAB-MS (JEOL SX102A).

Measurement of plant growth inhibiting activity

Test solutions were placed into test tubes and dried completely under vacuum condition at 40℃. One milliliter of hot agar aqueous solution (40-50℃, 0.5%) was added to the test tube, and the sample was dissolved or suspended. After solidification of the agar solution, six seeds of white clover (*Trifolium repens*), lettuce (*Lactuca sativa*), leaf mustard, common amaranth, green amaranth, barnyard grass (*Echinochloa crus-galli*) or rice (*Oriza sativa*) were placed on it. After incubation at 25℃ in the dark (for crude and impure samples) or light condition (for purified samples, 2000 Lux) for 5 days, the inhibitory effect on elongation growth was observed by measuring the length of hypocotyls and roots.

Results and Discussion

The crude extract of *D. repens* showed strong inhibitory effects on elongation growth of root of

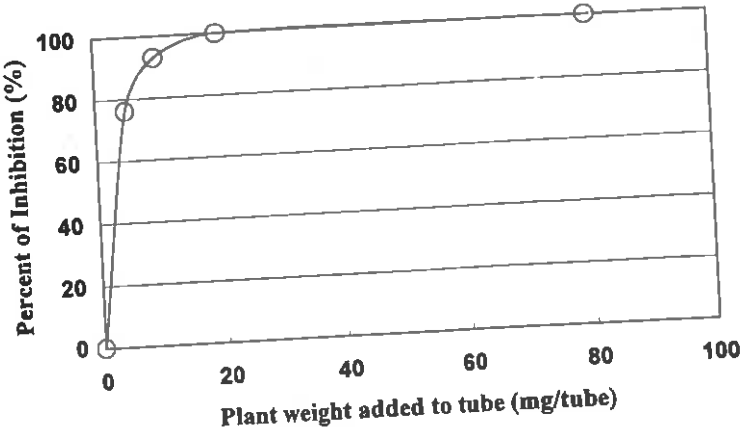


Fig. 1. Inhibitory effect of clude extract of *D. repens* on elongation growth of root of leaf mustard.

leaf mustard (Fig. 1). It was clear that the shoot and root growth of leaf mustard was completely inhibited by the plant growth inhibitor(s) involved in 20 mg of the leaf. The inhibitory activity was moved to H_2O fraction when the water suspension of the crude extract was shaken with hexane (Fig. 2). Likewise, active fractions were purified successively by using liquid-liquid extraction, open-column chromatography, solid-phase extraction and preparative HPLC. Three active compounds (I, II and III) were obtained from the HPLC at retention time of 52, 58, and 68 min. Thin layer chromatography (developed with n-BuOH:AcOH:H_2O = 3:1:1 or pyridine:EtOAc:AcOH:H_2O = 5:5:1:3) and ^1H- and ^{13}C-NMR analyses indicated that these compounds were pure.

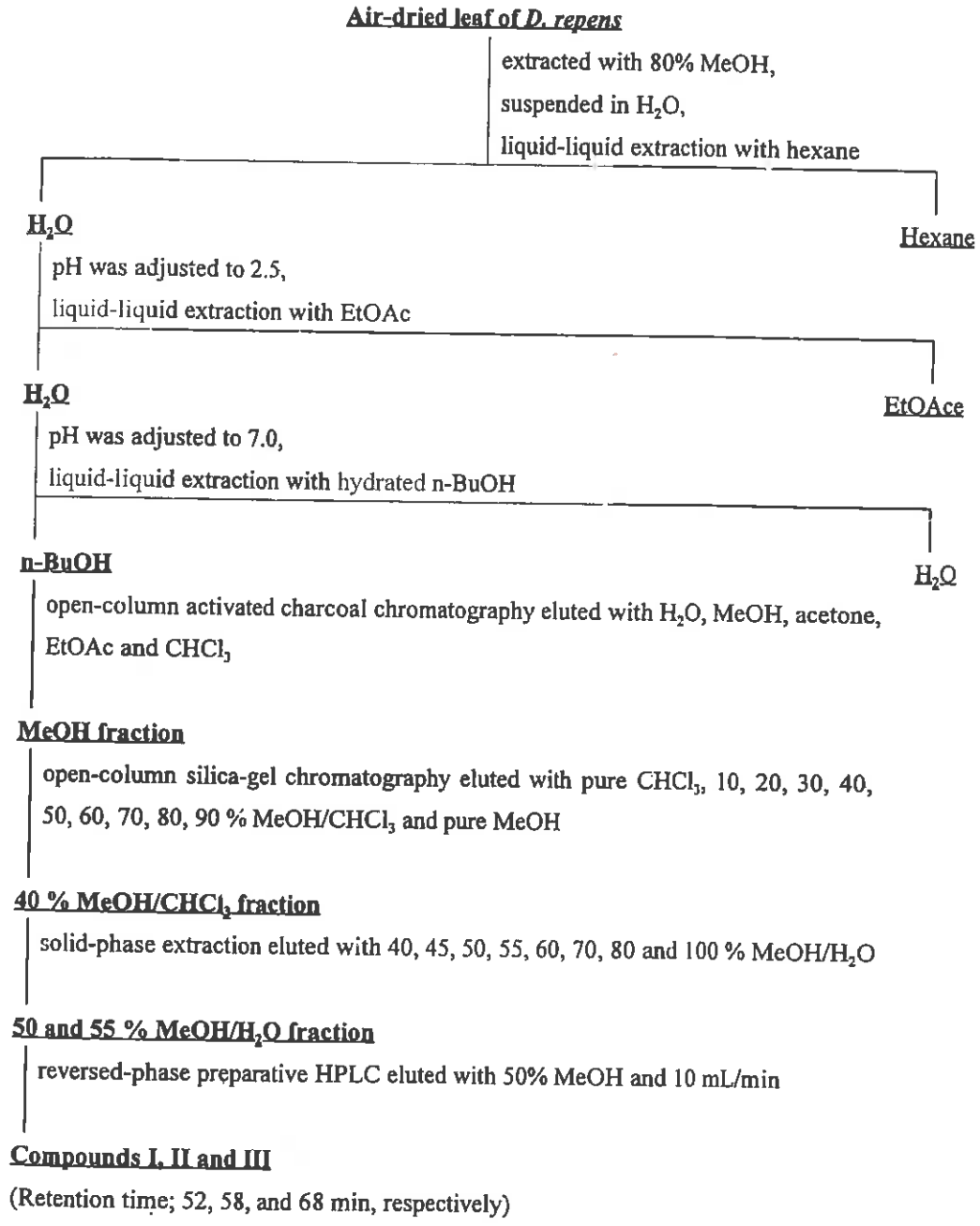
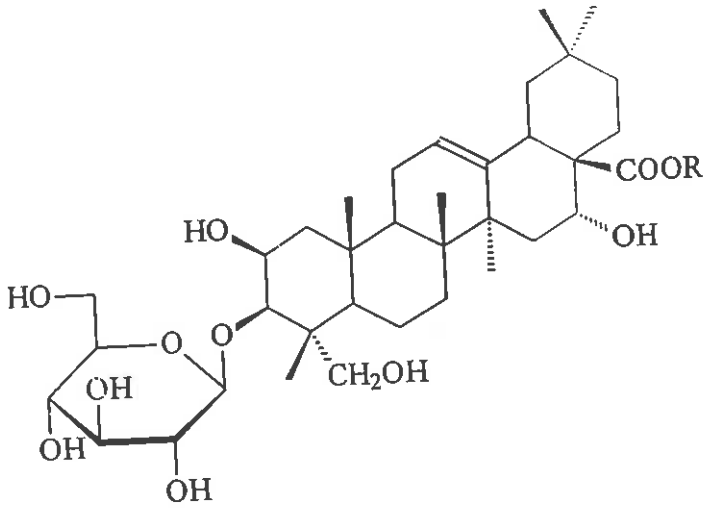


Fig. 2. Isolation of plant growth inhibiting compounds from *D. repens*.

To elucidate the chemical structure, these compounds were analyzed by 1D and 2D NMR and FAB-MS. It was cleared that compounds I, II and III were durantanins I, II and III, respectively (Fig. 3). Durantanins I, II and III are saponins with molecular weight of 1222, 1354 and 1222, respectively (Hiradate et al., in press).

Contents of durantanins I, II and III in the leaves of *D. repens* were measured by reversed-phase HPLC (Table 1). It was found that a large amount of durantanins was contained in the leaves. The total content of durantanins I, II and III reached to 13 g kg^{-1} or 10 mmol kg^{-1}.

Differences of the inhibitory activity among durantanins I - III were small for all test-plant species examined (Fig, 4). However, durantanins I – III inhibited elongation growth of leaf mustard root stronger than that of shoots. Similar trends were observed for other test-plants (data not shown). Sensitivity of plants to durantanins I – III was different depending on the species. Amaranthaceae plants, such as common amaranth and green amaranth, were found to be more tolerant to durantanins I – III than white clover, lettuce, leaf mustard, barnyard grass and rice. Around 50 % of the root growth of leaf mustard was inhibited by 50 μM of durantanins I – III.



Durantanin I: R = α-L-Rha-(1→3')-β-D-Api-(1→4)-α-L-Rha-(1→2)-β-L-Ara

Durantanin II: R = α-L-Rha-(1→3)-β-D-Xyl-(1→4)-[β-D-Api-(1→3)]-α-L-Rha-(1→2)-β-L-Ara

Durantanin III: R = α-L-Rha-(1→3)-β-D-Xyl-(1→4)-α-L-Rha-(1→2)-β-L-Ara

Fig. 3. Chemical structure of durantanins I, II and III.

Table 1. Content of durantanins I, II and III in the leaves of *D. repens* (air-dry basis).

	Content in the leaves	
	(g kg^{-1})	(mmol kg^{-1})
Durantanin I	2.83±0.14	2.31±0.12
Durantanin II	8.67±0.36	6.41±0.27
Durantanin III	1.53±0.20	1.25±0.17
Total (average)	13.0	9.97

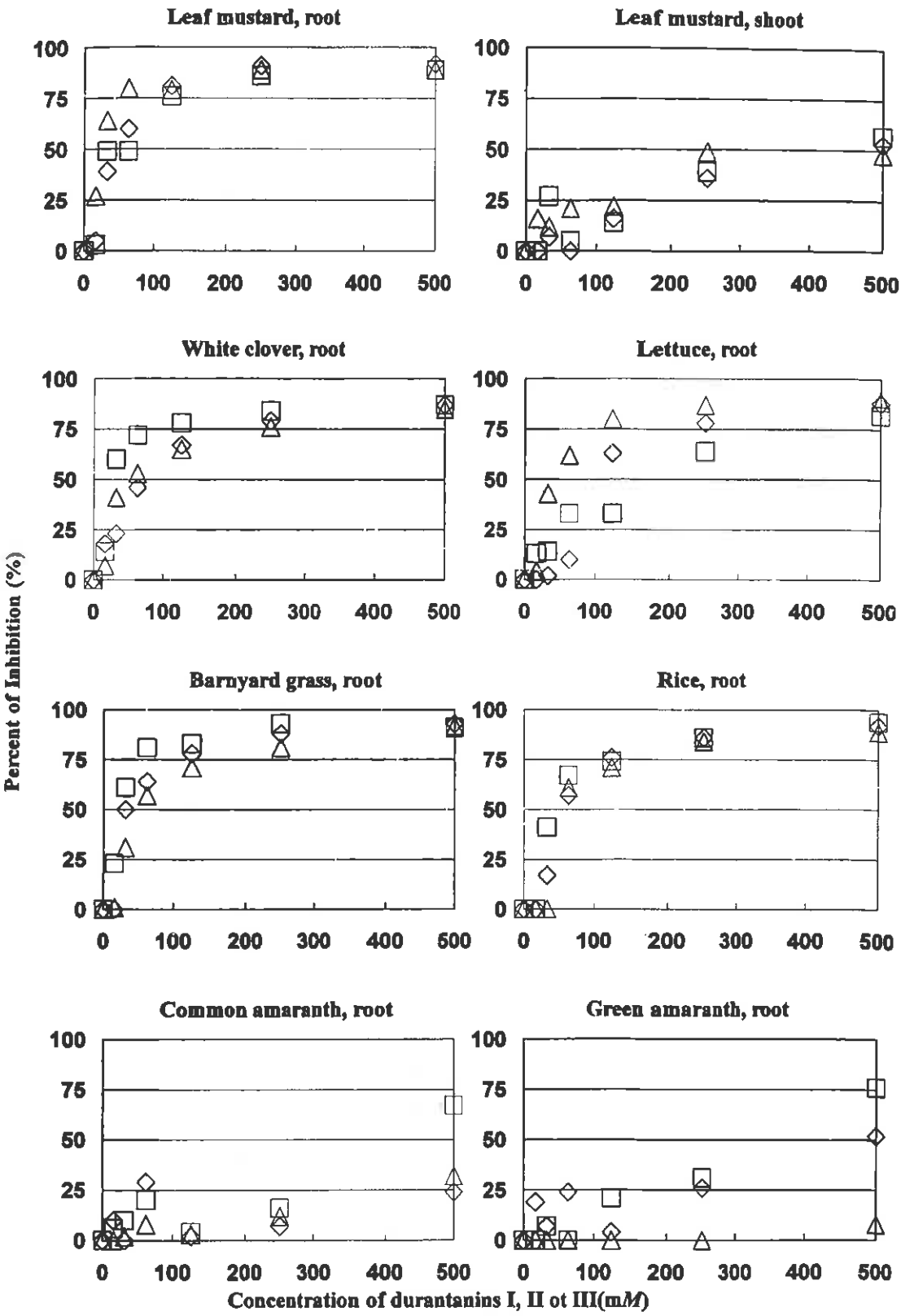


Fig. 4. Inhibitory effects of durantanin I (○), II (□) and III (△) on elongation growth of leaf mustard, white clover, lettuce, barnyard grass, rice, common amaranth and green amaranth.

The inhibitory activity of 63 μM of durantanins I – III on the growth of leaf mustard in Fig. 4 was in the same level as that of 5 mg of air-dried leaf of *D. repens* in Fig. 1. From the result of the quantitative analysis of durantanins I – III (Table 1), 5 mg of air-dried leaf should contain about 50 nmol of durantanins I – III. This means concentration of durantanins I – III in the test tube of Fig. 1 was about 50 μM. Therefore, it is concluded that the main plant growth inhibitors in the crude extract of *D. repens* are durantanins I, II and III.

Inhibitory activity of durantanin II was higher than that of the other candidate of allelochemicals, such as vanillic acid, syringic acid, ferulic acid and coumarin (Fig. 5).

Durantanins I, II and III are all freely soluble in water. This feature is advantageous for durantanins I – III to dissolve in rainwater and reach to weeds under the tree of *D. repens*. The amounts of durantanins I – III are larger than the other common candidate of allelochemicals. Furthermore, durantanins I – III have a higher plant growth inhibiting activity. All these results indicate possible role of durantanins I – III as allelochemicals in natural environments.

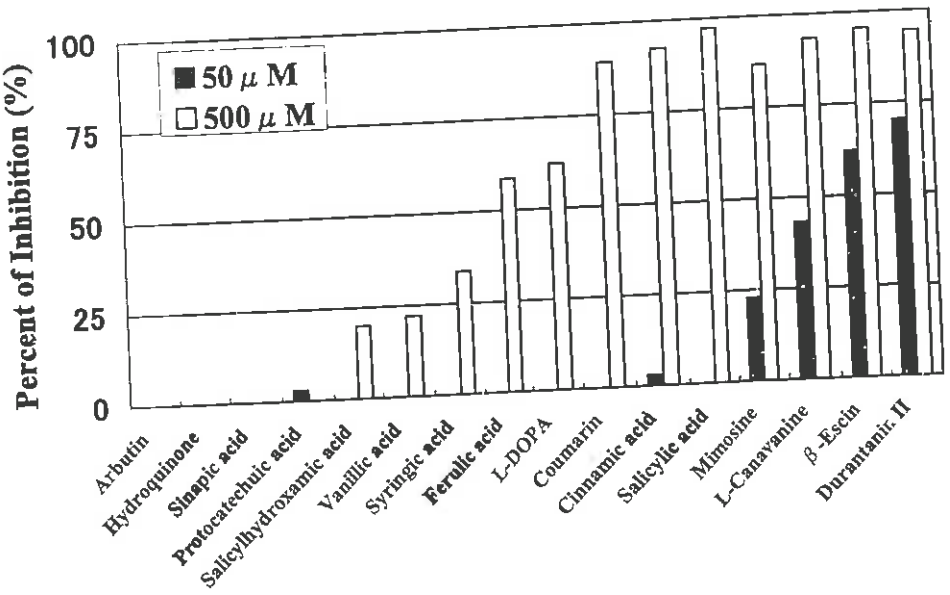


Fig. 5. Inhibitory effects of plant growth inhibitors on elongation growth of roots of barnyard grass.

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DEVELOPMENT, GERMINATION, AND DORMANCY OF SMALL-WHITE FLOWER MORNINGGLORY (*Ipomoea obscura* (L.) Ker-Gawl) SEED

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Abstract : The studies were carried out in two experiments, the first involving seed development and the second involving the cause and method of dispelling dormancy. Results revealed that seed development of small-white flower morningglory followed two obvious phases via the growth and food reserve accumulation phase and ripening phase. Physiological maturity was attained at 21 days after flowering and study on the cause and method of dormancy breaking showed that seed dormancy of this plant was the impermeability of seed coat to water. Acid scarification by using H_2SO_4 (conc.) for 80 minutes gave the highest number of germination and coefficient of velocity.

Key words : small-white flower morningglory, *Ipomoea obscura* (L.) Ker-Gawl, seed dormancy, acid scarification, hard seed

Introduction

Members of the genus Ipomoea are cosmopolitan weeds of the tropics and temperate zones. Cole and Coats (1973) found that *I. purpurea* (L.) Roth (tall morningglory) seed germinated and became established over a wide temperature range. Houston (1970) has characterized *Ipomoea* spp. as the ten worst weeds of field crops. They are one of the economic importance in crops such as upland cotton (*Gossypium hirsutum* L.) (Buchanan and Burns, 1968) and soya beans (*Glycine max* (L.) Merr.) (Wilson and Cole, 1968) where they can cause harvest losses of over 65 % (Oliver, 1974; Oliver *et al.*, 1976). Cole (1976) reported 46 % yield loss of cowpea (*Vigna sinensis* (L.) Endl.) due to *I. Purpurea* competition. In corn (*Zea mays* L.), the twining habit of *Ipomoea* spp. interferes with mechanical harvest and results in addition to those caused by earlier competition. Loasinwattana (1994) reported that small-white flower morningglory was troublesome perennial vines that infest pineapple production for industry in the eastern region of Thailand, and this plant may become greater problems than other weeds due to its tolerance to herbicides.

Ipomoea spp. often produce hard seed (Johnson *et al.*, 1977; Elmore *et al.*, 1990). This makes an experiment with these species difficulty, especially when uniform germination of a high percentage of seed is needed. Anderson (1968) reported that mechanical scarification or scarification with strong acid increased hard seed germination of several plant species. Therefore, to facilitate control studies and to partially define the most effective time of herbicide application, the experiments designed to investigate in seed development, seed germination and dormancy, and the cause and method of dispelling dormancy of *I. obscura* seed.

Materials and Methods

The studies were conducted in two experiments, the first involving seed development and the second involving the cause and method of dispelling dormancy. The experiments were carried out at the Department of Agronomy, Kasetsart University from November 1996 to June 1997. In the first experiment, seedlings of small-white flower morningglory were planted in the pots (15 cm. diameter and 15 cm. height) to observe the reproductive phase of the plants. Their fruits (and seeds) were harvested according to the tag marking on blooming flowers in order to examine the seed age and fruit development after flowering date. Fresh weight, dry weight, and moisture contents of 10 to 30 days (after flowering) seeds were recorded to evaluate a physiological maturity and a field maturity of the seeds (100 seeds). As well as some germination data were also recorded to provide more information about seed dormancy of this plant.

In the second experiment, method of dispelling dormancy was developed from the method, which was introduced by Horak and Wax (1991). According to this method, seeds were harvested after the field maturity period and then scarified in a concentrate of H_2SO_4 for 10, 20, 30, 40, 50, 60, 70, 80 and 90

minutes, rinsed in flowing tap water for 5 minutes, 25 scarified seeds were put down in a 100-by-15 mm petri dish placing with two layers of 9 cm in diameter of towel paper, and 8 ml of distilled water was added. During the course of the experiment, the water was added as needed. Germination tests were conducted in the dark at a constant temperature of 27°C. Germinated seeds (seeds were considered to be germinated when the radicle had emerged at 1 mm long) were counted and removed every day for 14 days at which time of the experiment was concluded. A completely randomized design with four replications was used in the experiment. Germination percentage and coefficient of velocity (CV) were calculated from the experimental data of each treatment. Coefficient of velocity was calculated as:

$$CV = 100[\Sigma N_i / \Sigma N_i T_i]$$

Where N is the number of seed germination on date i, and T is the number of days after seeding.

Results and Discussion

Fig. 1 shows certain physiological changes in seed development of small-white flower morningglory from 10 to 30 days after flowering. Results from seed development study indicated that the physiological maturity of seed was attained at 21 days after flowering (Fig. 2) which were depending on the maximum seed dry weight and the percent of germination. Development of hard seed began to increase rapidly after 21 to 22 days of flowering and caused more than 90 percent of non-imbibed seed at 27 days after flowering. Pricking seed coats with a needle increased germination of small-white flower morningglory seed in moist towel paper from less than 5 to more than 95 percent (data did not show). Therefore, the results revealed that seed dormancy of this plant was caused by the impermeability of seed coat to water.

Results from the second experiment were shown in Fig. 3. Germination percentage and coefficient of velocity of non-scarified seed were low at 7 % and 11.4, respectively (Fig. 3), whereas the scarified seed had significantly increasing germination percentage and coefficient of velocity. Both germination and CV increasing were affected by soaking seed in H_2SO_4 from 10 to 20 minutes. Soaking periods, which were longer than 40 minutes did not increase the percent of germination but increased the CV.

Results from this study indicated that seed maturity of small-white flower morningglory was attained at 21 days after flowering and hard seed was developed after maturity. The study on the cause and method of dormancy breaking showed that the seed coat was the major barrier of seed germination or the impermeability of seed coat to water. It was suggested that this dormancy was a form of innate dormancy. Low CV values of the controlled seed indicated that germination events were less frequent and the speed of germination was slower than that of acid scarified seed. The hard seed coat might allow the seed to survive and germinate over a number of seasons, enhancing species survival.

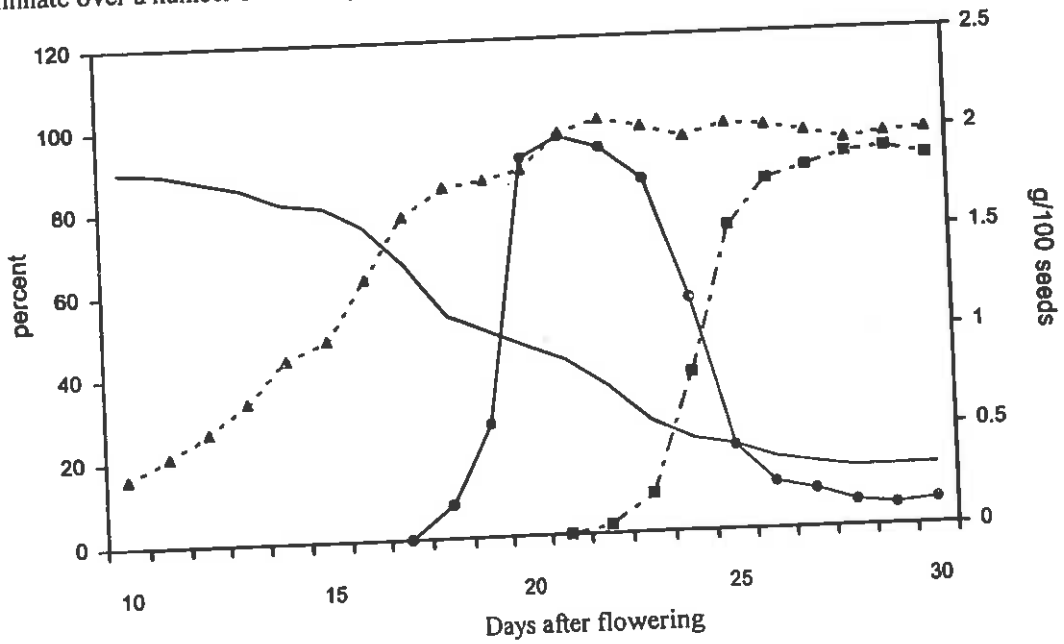

Fig. 1 Physiological of small-white flower morningglory seeds changed after flowering, dry weight (▲--▲), moisture content (—), germination (●--●) and hard seed development (■--■)

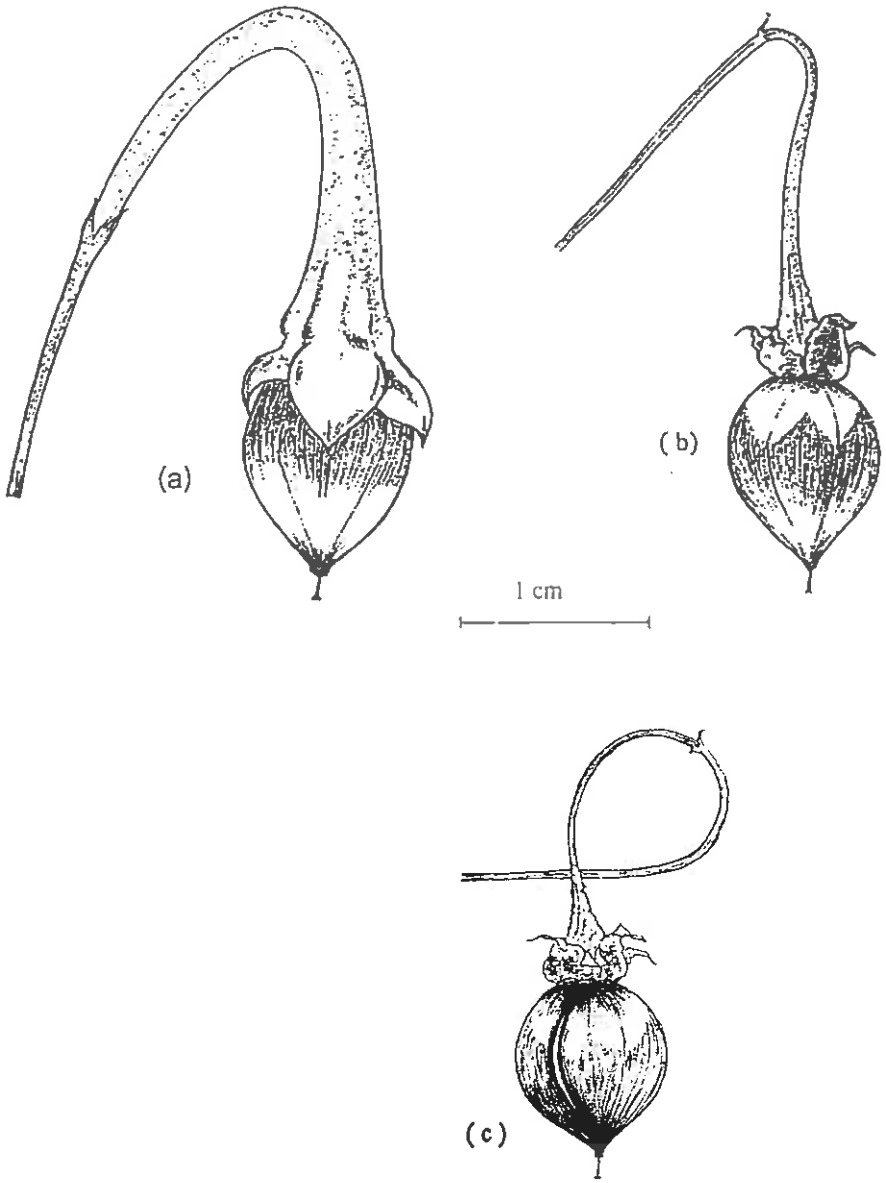

Fig. 2 Fruit development of small-white flower morningglory at 18 days(a), 21 days(b) and 27 days(c) after flowering.

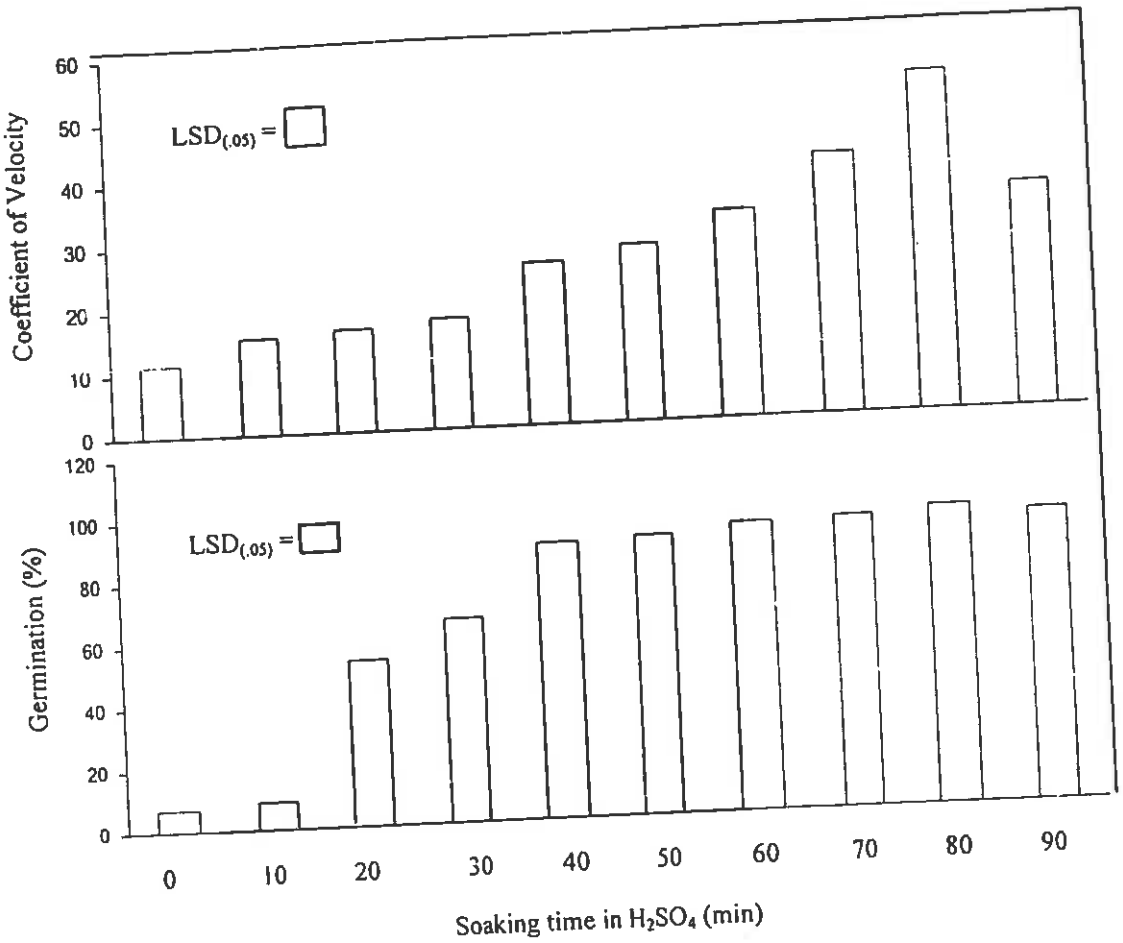

Fig. 3 Effects of chemical scarification on germination percentage and germination velocity of small-white flower morninggiory seed.

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The authors would like to acknowledge Assoc. Prof. Dr. J. Duangpatra for her permission to use the seed laboratory throughout this work. Gratitude is also expressed to KURDI for their financial support of this research.

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GERMINATION AND GROWTH OF HORSE PURSLANE (*TRIANTHEMA PORTULACASTRUM*)

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Abstract: Horse purslane seed germination were conducted in the laboratory of Weed Science Building, Bangkok from December 1997 to November 1998. The seeds germination were tested in the petri dish under three difference conditions of light: light, dark and light/dark conditions at room temperature. The trials were both done immediately and once a month, totally 12 months after seeds had harvested from the field. The other germination tests were evaluated by burying seeds at 0, 2, 4, 6, 8, and 10 cm. depth in the soil. The results showed that there were significant differences of germination between seeds under light and dark conditions. The highly seed germination were 76.6% under light condition. The germination of seeds on soil surface were higher than seeds buried in the soil for the new harvested seeds from the third to fifth month. Growth of horse purslane was investigated in dry season during November 1998 to March 1999 in Bangkok. Seedlings were established after 3 to 10 days of seed sowing on the soil. The height was increased from 2.4 cm. in the first week of seedling to 102 cm. in the fourteenth week, with the branches of 368 per plant. Flower bud was developed within 3 weeks after seed germination and a mature fruit had been obtained within 6 weeks. A mature horse purslane could produce up to 3,330 fruits per plant and 6-10 seeds per fruit.

Key word: germination, horse purslane, *Trianthema portulacastrum,petri dish.*

Introduction

Horse purslane *(Trianthema portulacastrum* Linn.) is widely distributed in the tropics, mainly in southeast Asia. (Harada et al.,1997). It is one of the major weed in both agricultural and urban areas which are distributed throughout Thailand from near sea level to above 800 metres (Prakongvongs,1998). According to its abundance in vegetable plantation, dry plant of horse purslane is used to incorporate into the soil of Chinese radish plot for increasing its yield.(Kongsaengdao et al.,1997). It is also reported as one of the main weed in sugarcane field. The decayed horse purselane at the composition of 2.5, 5.0, 7.5 and 10% w/w enhanced growth of sugarcane whereas these compositions inhibited the germination of peanut, soybean, mungbean and chinese cabbage (Suwanarak et al.,1995).

Although the infestation/troublesome and allelopathic property are recognized, there is little known about germination and growth aspects. .Horse purslane is an annual weed, seed germination is an important role of reproduction and dissemination. Knowledge about germination behavior of seeds, propagules and other aspects of weed biology help to gain insight into the mechanism of specialization and distribution of the species. Such studies will offer an opportunity to locate vulnerable points in the life histories of weeds when they can be successfully attacked for their control (Misra, 1969).

The objectives of this study are to evaluate the germination behavior under various conditions of light and depths of seeds buried in the soil. In addition, the growth behavior and development were studied.

Materials and Methods

Germination studies

Seeds used in this experiment were collected from sugarcane field in Kanchanaburi province Thailand. Mature seeds were divided into 13 sets, and kept in a paper bag at room temperature. The trials were conducted in Bangkok from December 1997 to November 1998.

The germination experiment was conducted both in the petri dishes and in the soil. In the petri dishes: seeds were placed on a filter paper with 10 ml. of distilled water in each petri dish. The tests were conducted under three different conditions of light which were light condition, dark

condition and light/dark condition at room temperature. The experiment was conducted immediately after seeds were harvested by using the first set of seeds. And the rest of 12 sets were periodical monthly tested, totally 12 months The trails were consisted of 10 replications of 100 seeds each. The germination record was made 2 weeks after the initiation of the experiment. Protrusion of the radicles through the seed coat was used as criterion for germination. The data were recorded as percentage of germination and a completely randomized experiment desire was used.

On the other hand, the germination in various depth level of soil was done by covering seeds with nylon bag and buried in soil within cylindrical plastic pots of 7 by 11 cm.,.at the specific depth of 0, 2, 4, 6, 8, and 10 cm. in a green house. The pots were daily watered as needed. After 2 weeks soil in each pot was emptied to determine the number of germinated seeds. The trails were consisted of 10 replications of 100 seeds each. The monthly tests of germination, germination record, and statistical analysis were done as the previous described.

Growth studies

Seeds were sown on the soil in the pot of 40 cm. by diameter and 45 cm. at height, totally 70 pots. After emergence, one vigor seedling was allowed to establish in each pot. The experiment had been investigated from November 1998 to March 1999. The growth was recorded every week for 15 weeks in term of shoot height, number of branches, data of flowering, fruiting and seed ripening. In addition, number of seed per fruit and fruit per plant were also recorded.

Result and discussion

Germination experiment

Germination of horse purslane seeds under different conditions of light in the petri dishes were shown in Table 1. Continuous light throughout the experiment increased the germination percentage of seeds tested in every month. The germination under light condition was higher than the other two light conditions, and significant difference from the seeds germinated under dark and light/dark conditions were noticed. There was low correlation between light intensity and germination, but long time exposure of light and wave length were important to stimulate germination.(Jone,1961).In this experiment, the best germination of 76.6 % was obtained from the 12 months seeds storage which were tested under light condition. Germination of 1 to 3 months seeds storage under light/dark condition were higher than seeds under dark condition, whereas germination of 7 to 12 months seeds storage under dark condition were higher than in the condition of light/dark . Even though seeds could germinate in both dark and light conditions, the maximum germination was gained under light condition which was the same as maximum of 66.75% was obtained from giant mimosa seeds germination at the continuous with intensity of 1,200 luxes while in the complete darkness the rate of germination was only 40.40%(Bhunthumnavin,1977).

Germination of seeds buried in soil at depths of 0, 2, 4, 6, 8 and 10 cm: the storage seeds of 1 to 5 months showed the highest germination on soil surface, with the germination of 5.8%, 9.5%, 32.3%, 12.3% and 11.6% at 1, 2, 3, 4 and 5 months respectively. However for the 6 to 11months seeds storage, there were almost no differences on germination between seeds left on soil surface and seeds buried at various depths as previous described. For the long time storage seeds of 12 month, germination of buried seeds were higher than seeds left on the surface (Table 2). According to this experiment, in field condition horse purslane seeds could germinate both on the soil surface and buried in the soil, especially seeds storage up to 12 months could germinate even in the depth of 10 cm.

Seeds storage germination: Under continuous light condition, seed germination was gradually increased upon the period of storage, and the storage seeds of 12 month gave the highest germination of 76.6 %, whereas the least germination were obtained from freshly harvested seeds. In both darkness and light /dark conditions, the highest germination were occurred from 6 months storage with 41.1% and 39.6% respectively, while the fresh seeds also gave the lowest germination (Table 3). The germination of storage seeds buried in the soil, the 6 month storage were also showed the highest peak of germination in both on soil surface and in every designed depth (Figure 1). It is to be expected that the seeds could have a short period of dormancy after harvesting.

Table 1. Percentage germination of horse purslane seeds under light dark and light/dark conditions.

Conditions	Months after harvesting												
	0	1	2	3	4	5	6	7	8	9	10	11	12
Light	9.6 a	33.2 a	54.7 a	71.2 a	68.0 a	66.5 a	64.5 a	56.3 a	67.6 a	56.7 a	67.1 a	68 3 a	76.6 a
Dark	0.0 c	9.2 b	15.7 c	22.2 c	21.9 b	14.8 b	41.1 b	17.1 b	21.6 b	24.2 b	21.7 b	31.6 b	39.0 b
Light/Dark	1.6 b	8.7 b	26.1 b	39.1 b	26.3 b	12.7 b	39.6 b	12.0 c	17.1 c	19.3 c	13.5 c	15.2 c	24.0 c

In a column, means followed by a common letter are not significantly different at 95 by DMRT

Table 2. Percentage germination of horse purslane buried seeds in various soil depths.

Soil depths (cm)	Months after harvesting												
	0	1	2	3	4	5	6	7	8	9	10	11	12
0	0.0 a	5.8 a	9.5 a	32.3 a	12.3 a	11.6 a	20.6 a	12.2 a	13.3 a	18.3 a	16.7 a	12.9 a	4.8 c
2	0.0a	3.3 bc	3.5 bc	17.3 b	2.9 b	4.4 bc	22.3 a	7.6 a	7.8 b	12.1 b	10.7 b	10.8 ab	14.4 b
4	0.2 a	4.9 ab	5.3 b	13.2 bc	4.7 b	5.0 b	22.7 a	10.4 a	9.4 ab	14.3 ab	14.0 ab	11.0 ab	15.2 ab
6	0.0 a	2.3 c	3.6 bc	10.4 cd	2.8 b	2.8 c	19.8 a	7.8 a	11.6 ab	17.6 a	15.0 ab	12.0 ab	19.9 ab
8	0.2 a	1.6 c	2.8 c	7.4 d	4.5 b	2.7 bc	16.1 a	9.9 a	7.8 b	13.3 ab	16.8 a	11.0 ab	18.2 ab
1 0	0.4 a	2.1 c	3.8 bc	11.6 c	4.8 b	2.9 bc	20.0 ab	8.0 a	7.7 b	13.5 ab	14.9 ab	8.0 b	19.8 a

In a column, means followed by a common letter are not significantly different at the 5 % level by DMRT.

Table 3. Germination of horse purslane seeds after different periods of storage at room temperature.

Months of storage	Conditions		
	Light	Dark	Light/Dark
0	9.6 f	0.0 f	1.6 g
1	33.2 e	9.2 e	8.7 f
2	54.7 d	15.7 d	26.1 b
3	71.2 b	22.2 c	39.1 a
4	68.0 bc	21.9 c	26.3 b
5	66.5 bc	14.8 d	12.7 e
6	64.5 c	41.1 a	39.6 a
7	56.3 d	17.1 d	12.0 e
8	67.6 bc	21.6 c	17.1 cd
9	56.7 d	24.2 c	19.3 c
10	67.1 bc	21.7 c	13.5 de
11	68.3 bc	31.6 b	15.2 cde
12	76.6 a	39.0 a	24.0 b

In a column,means followed by a common letter are not significantly different at 5% by DMRT

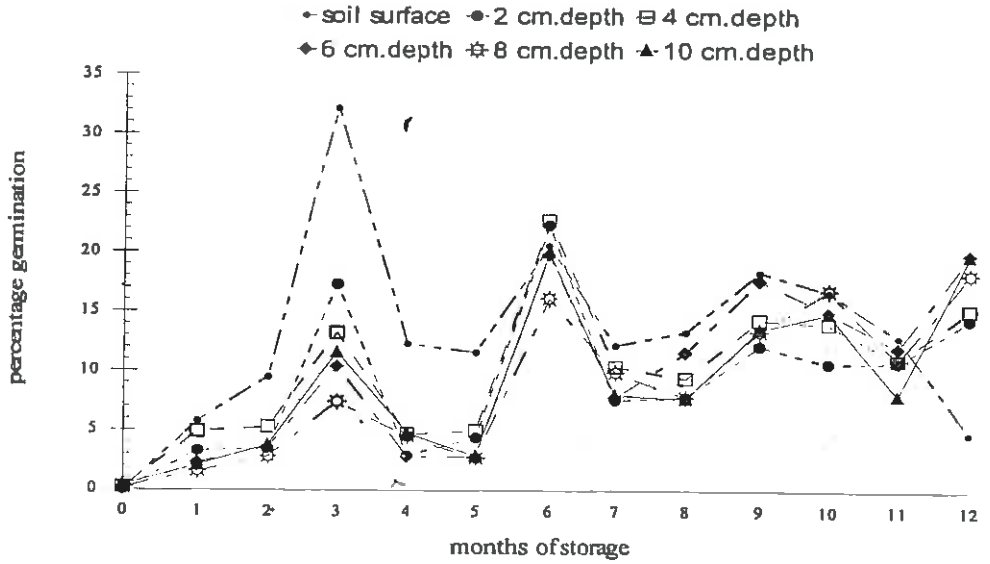

Figure 1. Germination of horse purslane storage seeds buried at different depths in the soil.

Growth and development

Seedling emerged after 3 - 10 days of seeds sowing on the soil. The first true leaf appeared about 5 days after seed emergence. The early stage of growth consisted principally of leaves/branches production and shoots with elongation. The first terminal flowery bud was developed at the age of about 3 weeks, also with the first pair of branches. The typical period of flower blooming is only one day which is the same as flower blooming period of water hyacinth (Prakongvohgs and Suwanasingha, 1989). Fruit maturity required about 3 weeks after the development of flower bud.

The plant height, number of branches, dry weight and number of fruits per plant were showed in table 4. The typical plant at 1 week had an average height of 2.4 cm., and increased to 102.0 cm. at the age of 14 weeks. The average number of branches increase from 3.1 at 3 weeks to 308.0 at the 14 weeks. The dry weight was 0.004 g./plant at 1week, and increased up to 35.3 g./plant at 14 weeks. The yield was increased from 1 fruit/plant at 3 weeks to 2,921 fruit/plant at the age of 14 weeks. The average seeds / fruit was 8.6, with the weight of 1.54g/1,000 seeds.

Table 4.Average plant height, number of branches, dry weight and seed production of horse purslane at different dates after emergence in the green house.

Weeks after emergence	Height (cm.)	Branches/plant	D.W./plant (g)	Fruits/plant
1	2.4 ± 0.3	-	0.004 ± 0.00008	-
2	6.9 ± 1.0	-	0.06 ± 0.02	-
3	13.1 ± 1.7	3.1 ± 0.7	0.3 ± 0.08	1 ± 0.0
4	25.1 ± 2.4	4.0 ± 0.0	1.3 ± 0.3	14.2 ± 5.8
5	40.5 ± 4.9	39.0 ± 4.2	4.9 ± 0.8	80.0 ± 4.2
6	56.4 ± 6.2	105. 7 ± 15.6	11.2 ± 3.5	229.2 ± 77.2
7	49.7 ± 14.3	113.0 ± 23.2	9.8 ± 2.6	323.5 ± 61.3
8	70.0 ± 8.7	155.7 ± 50.6	16.7 ± 5.6	543.7 ± 79.3
9	70.3 ± 11.9	227.0 ± 63.9	14.23 ± 4.4	737.7 ± 94.6
10	80.0 ± 10.4	229.0 ± 64.2	17.9 ± 5.1	1225.0 ± 302.8
11	93.5 ± 9.2	435.0 ± 98.5	35.7 ± 5.2	2609.5 ± 29.0
12	84.7 ± 12.4	419.0 ± 78.0	33.7 ± 9.7	2764.3 ± 623.1
13	86.5 ± 13.5	421.0 ± 92.5	34.5 ± 3.6	2746.5 ± 352.8
14	102.0 ± 1.4	308.0 ± 72.1	35.3 ± 3.4	2921.0 ± 257.4

D.W. – dry weight,
Values are mean ± S.D. of 5 plants.

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INSECT ANTIFEEDANTS IN CYPERACEAE: UTILIZATION OF CONSTITUENTS FROM WEED AS AGROCHEMICAL LEAD

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Abstract: Cyperaceae are widely distributed weeds from the upland to paddy fields and from the temperate zone to torrid zone. Some of them are only slightly damaged by phytophagous insects in their habitat. It was reported that a kind of plants have a specific defense system against phytophagous insects. We investigated the plant defense factor on a chemical basis, and isolated and identified some novel quinones and a coumaran (2,3-dihydrobenzofuran) as insect antifeedants. These constituents mainly exist in the basal stem (bulb) and tuber. These organs are important for the monocotyledon, because they include the dormant bud and provide storage of carbohydrates and protein. The natural products from Cyperaceae (*Cyperus* spp.) are effective as an antifeedant against the common cutworm (*Spodoptera litura* F.). A quinone from *C. distans* showed the strongest antifeedant activity (ED_{50} 2.6 x 10^{-9} mol/cm^2). Moreover, we investigated the coumarans based on a structure-activity relationship (SAR), though this chemical structure was easy to synthesize. The chemical factors showed that the activity was increased by introduction of an acetyl group and methoxyl group. These results showed that the weeds can be used in the place of agrochemicals, not only as pure compounds but also as rough extracts like a natural oil additive.

Key words: Cyperaceae, insect antifeedants, cyperaquinone, benzofuran

Introduction

Though the phytophagous insect comprise major of insect in the world, plant are not completely consumed by them. We now know that many plants have a defense system against phytophagous organisms. There are seasonal escape, the life stage finished by emergence of the insect and a physiological defense system as the hard body and increasing trichome density on the leaf surface. The other plant defense system is utilization of plant secondary metabolites acting as insecticides, repellents and antifeedants. It is very difficult for pest control that introduce previous discribed plant defense system to a crop. However, it is very easy to utilize chemicals produced by plants for the same reason.

Material and Methods

Collection and extraction of plant material

Plants used in the antifeedant bioassay and separations of active compounds, collected during the July to December, 1995-98 at Nara, Kyoto and Osaka Pref. in Japan and in March of 1999 at Phuket, Chiangmai in Thailand.

For each plant, the leaves, stems and basal bulbs and roots were separated, minced, and soaked in hexane at 4°C for 3 days. After filtration, the extracts were evaporated under reduced pressure. These extracts were dissolved in acetone then used for the antifeedant bioassay against the common cutworm. For each test, 3 replicates were performed, and the results are given as the average index and treated disk feeding rate. Isolation of active compounds were done using ordinary manner. The extract was chromatographed on silica gel using hexane-ethyl acetate (10:1) as the eluent to give the natural product and identified by its MS and NMR spectrum data.

Insect rearing

Common cutworms (*Spodoptera litura* F. Lepidoptera Noctuidae) were reared on an artificial diet (Insecta LF, Nihon Nosan Kogyo Co.) in a controlled environmental room at 26.5°C and 60 % humidity.

Dual choice leaf disk bioassay

The experimental setup used leaf disks of 2 cm diameter prepared with a cork borer from fresh sweet potato (*Ipomoea batatas*) leaves that have been cultivated at the Kinki University farm (Nara Pref.) without using agrochemicals as the pesticide and chemical fertilizer. Two disks treated with a specific amount of test sample in acetone solution, and two other disks were the control that were treated with only acetone of the same volume. These 4 treated disks sat in a cross-position in the same petri dish. After complete removal of the carrier solvent, 15 larvae of common cutworm (3rd. instars) were released into the dish. The dishes were then placed in an insect rearing room at 26.5°C under dark for 5 h.

Evaluation of antifeedant activity (measurement of consumed areas)

The damaged leaf disks were taped onto copy paper due to the monotone data conversion. The photocopied monotone data was used to avoid errors and this was converted to digital data files with a digital scanner. The digital data analysis was performed on a Macintosh computer using the public domain NIH Image program in pixel units (developed at the U.S. National Institute of Health and available on the internet by anonymous FTP from codon.nih.gov, or download from http://rsb.info.nih.gov/nih-image/download.html). For each experiment, the data file of an intact disk was measured and compared to that of the treated disk. Today, there are many indices of evaluation for antifeedant activity. We adopted the antifeedant index described by Alkafahi *et al.* (1989). This index illustrated as AFI = % of treated disks

consumed / (% of treated disks consumed + % of control disks consumed) x 100. When the same amount was consumed in the treated and control disks, an AFI value of 50 was calculated (Escoubas *et al.*, 1993). However, this index can not show the direct toxicity of test samples against insects, because toxic samples cause less insect activity, therefore, an insect can not consume both the treated and control disks. In spite of potential antifeedant activity, the results do not show antifeedant activity in this case. We regarded the results including this property of the bioassay. The ED_{50} values of the test samples were calculated by computer using the probit method.

TLC plate bioassay

TLC silica gel plates with a fluorescent indicator (Merck Silica Gel 60 F_{254} 0.25 mm thin) cut into 4 x 10 cm pieces. The Cyperaceae extract containing the hexane solution were applied as a band on the TLC plate. This plate was developed with the appropriate solvent system for adequate separation. Distilled water added to the artificial diet made a paste that was thinly applied onto a TLC plate. This prepared TLC plate was placed in a plastic cup with 50 larvae (4th instar) for 1 day at 26.5° C. The location of the areas uneaten by the larvae were compared with the Rf values of the compounds detected on the reference TLC plate. The reference TLC plate was developed under the same conditions, and the analysis was detected with UV light (254 nm) and sprayed with 50% H_2SO_4 when heated at 150° C. The compounds having antifeedant properties remained in silica gel residue (Escoubas *et al.*, 1992).

Evaluation of fish toxicity

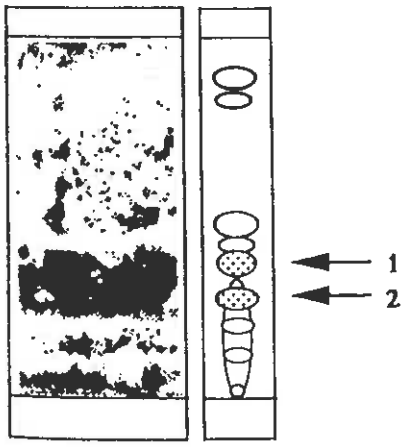
The test samples involving 1% $(Me)_2SO$ incubated water ($NaHCO_3$ 65mg, $CaCl_2$ 223mg, $MgSO_4$ 60mg, KCl 10mg / litter) were prepared for the fish toxicity bioassay. Twenty water fleas (*Daphnia magna*), less than 2 days after hatching were released into 100 ml of the incubated water at 20° C. We visually observed the death or immobilization end point as measured in this test. Similar to the evaluation of the antifeedant activity, the TLm values of, the test samples were calculated by computer using the probit method.

Synthesis of coumaran (2,3-dihydrobenzofuran) derivatives

A study of the construction of the 2,3-dihydrobenzofuran moiety by the condensation of phenol and alkylbromide has been conducted by Yamaguchi and co-workers (Yamaguchi *et al.*, 1987). However, it is also important to find analogues with higher activity than that of the natural product and to elucidate the structural requirements for agents which inhibit feeding.

Results and Discussion

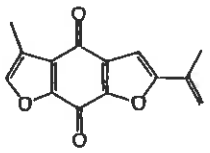
Some of Cyperaceae extracts showed antifeedant activity against the common cutworm. Though many species showed potential antifeedant activity, we initially evaluated two species, *Cyperus nipponicus* from Japan and *C. distance* from Thailand. These hexane extracts consisted of a few major compounds, and we regarded to identify the active compounds easily. The results of the TLC plate bioassay for the extract of *C. nipponicus* clearly gave information about the antifeedant (Fig. 1). And we separated two antifeedants, cyperaquinone and remirol from the crude extract base using the TLC bioassay result. Similarly, we identified scabequinone in the extract of *C. distans* (Morimoto *et al.*, 1999a). In 1999, we obtained the cyperaquinone derivative, dihydrocyperaquinone, from *C. tuberosus* in Thailand. This species had two quinones, cyperaquinone and dihydrocyperaquinone, almost the same amount. Dihydrocyperaquinone is a stronger inhibitor of oxidative phospholylation than rotenone (MacLeod *et al.*, 1978). This compound has shown antifeedant activity against the common cutworm as expected. For the structure-activity relationship (SAR), scabequinone hydrogenated the unsaturated bond on the furan ring with palladium carbon. The results of bioassay for these various cyperaquione derivatives, indicated two factors related to the potential antifeedant activity against the common cutworm (Fig. 2). They were the existence of the ring ether and only one furan ring. The antifeedant activity of scabequinone drastically decreased with reduction of the furan ring. Moreover, dihydrocyperaquinone, having only one furan ring was stronger than cyperaquinone, that has two furan rings. In fish toxicity, these test compounds tended to show the same rate of thier antifeedant activities (Table 1).



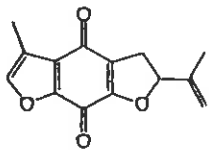
Cyperus nipponicus
(hexane : ethylacetate 10:1)

1: remirol 2: cyperaquinone

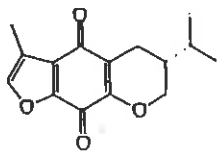
Fig. 1 TLC plate assay of hexane extract from *C. nipponicus*: black on left TLC plate uneaten area and right TLC is reference under same conditions.



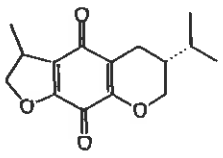
cyperaquinone



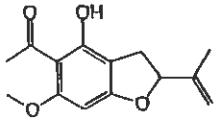
dihydrocyperaquinone



scabequinone



dihydroscabequinone



remirol

Fig. 2 Natural products in Cyperaceae and their derivatives

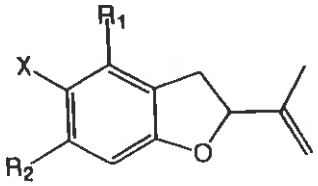
Table 1 Insect antifeedant activity and fish toxicity of natural products in Cyperaceae and its derivative

test compounds	fish toxicity (*D. magna*)	antifeedant activity (*S. litura*)	
	*TLm (ppm)	ED_{50} (mol / cm^2)	pED_{50}
cyperaquinone	>10.0 **	1.7×10^{-7}	6.77
dihydrocyperaquinone	3.7	1.0×10^{-7}	6.98
scabequinone	4.5	2.6×10^{-9}	8.59
dihydroscabequinone	>10.0	$>1.3 \times 10^{-6}$	<5.89
remirol	3.5	1.3×10^{-7}	6.89

* TLm value at 3 hr after treatment ** no survival 1 day after treatment

The various derivatives of cyperaquiones are difficult to prepare by synthesis for testing of the SAR, because these neutral products have a complex chemical structure. However, one of anitfeedants in Cyperaceae, remirol has a simply chemical structure (2,3-dihydrobenzofuran) and has been reported to have an synthetic route. We elucidated the various derivatives of 2,3-dihydrobenzofuran for antifeedant bioassay (Morimoto *et al*., 1999b). Consequently, the introduction of substituents and the acetyl group to the benzene ring increased property of antifeedant activity for entry compounds. For introduction on the benzene ring, the most effective substituent was the methylether. This result is similar to many insect antifeedants having a methylether moiety in the chemical structure.

Table 2 SAR of 2,3-dihydrobenzofuran derivatives (remirol analogue) with synthesis



feature of chemical structure			antifeedant activity	
X	R_1	R_2	ED_{50}	pED_{50}
H	H	H	1.1×10^{-6}	5.96
H	OH	OH	4.8×10^{-7}	6.32
H	CH_3	CH_3	6.3×10^{-7}	6.20
H	OCH_3	OCH_3	5.3×10^{-8}	7.28
H	OC_2H_5	OC_2H_5	4.0×10^{-7}	6.40
Ac	H	H	7.4×10^{-8}	7.13
Ac	OCH_3	OCH_3	2.8×10^{-8}	7.55
Ac	H	OCH_3	4.2×10^{-8}	7.38

It was not enough to just control the crop pest by application of the synthetic acetylcoumaran (4,6-dimethoxy-5-acetyl-2,3-dihydrobenzofuran, 4,6-dimethoxy-7-acetyl-2,3-dihydrobenzofuran mixed) agent at a test farm. One other factor is its poor environmental stability. We are now investigating to pest

control agents made from plants or crude extracts. Cyperaceae has some activities against insects, for instance, α-cyperone, sesquiterpen ketone produced by *C. rotundus* has insecticidal activity against the diamond back moth (Dadang *et al.*, 1996), and *C. iria* produced the same JH III using insect (Toong *et al.*, 1988). These species were also able to utilize pest control materials. Moreover, we need to investigate the mode of action of these natural products as insect antifeedants.

Acknowledgments:

The authors are grateful to Phongsak Pholitri (Thailand Weed Science Division) and Yuka Kobayashi (Kyoto University) for identification of Cyperacea in Thailand.

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INVESTIGATION OF WEED AND WEED FUNGAL PATHOGEN IN SOYBEAN PRODUCTION AREA

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Abstract: Several broadleaf and grass weeds are the serious problem in soybean (*Glycine max*) production area at the Asian Vegetable Research and Development Center (AVRDC), Kamphaengsaen, Nakhon Pathom, Thailand. Fourteen and nineteen varieties of weeds were observed in the rainy and the winter seasons, respectively. During of the investigation in both seasons, leaf spot, anthracnose and powdery mildew showed on nine varieties such as *Ipomoea pes-tigridis*, *Ludwigia hyssopifolia*, *Euphorbia heterophylla*, *Melochia corchorifolia*, *Aeschynomene americana*, *Borreria laevis*, *Rottboellia cochinchinensis*, *Physalis minima* and *Echinochloa crus-galli*. The disease tissues were isolated by tissue transplanting method and found the interesting fungi as follows : *Colletotrichum*, *Glomerella*, *Phomopsis*, *Nigrospora*, *Drechslera*, *Helminthosporium*, *Oidium* and *Ovulariopsis*. All of them are plant pathogens. Especially, the genera of *Phomopsis*, *Colletotrichum* and *Glomerella* have been reported inducing soybean diseases. It is suggested that these weeds may be alternate hosts in soybean crops.

Key words: leaf spot, anthracnose, powdery mildew, alternate hosts

Introduction

Weeds cause crop losses by competition and reducing crop quality. They also increase cost of operation as harvesting, drying and cleaning. Moreover, they increase pest infestation, especially plant pathogen which can survive on weed as host. It has been reported *Pseudocercospora nigricans* be a foliar pathogen causing mildew, lesions and mottling on mature sicklepod leaves (Hofmeister and Charudattan, 1987; Pitelli *et al*., 1998). *Anchusa italica* was identified as an important source of inoculum of *Puccinia recondita* on wheat in Morocco (Ezzahiri *et al*.,1992). Hartman and Lee (1995) reported that velvetleaf was a host of *Septoria glycines* causing brown spot of soybean. *Cynodon dactylon* showed covered smut symptom by *Sporisorium sorghi* in sorghum crop in Nigeria (Marley, 1995). *Exerohilum monoceras* was found causing necrotic streaks on *Echinochloa colona* leaves in rice fields at the International Rice Research Institute (IRRI) in the Philippines (Zhang *et al*., 1996).

Soybean is an important oil crop in Thailand which covered 271,360 ha and yielded 359,000 tons in 1996-1997 (OAE, 1998). So far, the production is not enough for consuming in the country. There are many reasons for that. The most important one is soybean diseases such as rust, powdery mildew, leaf spot, pod and stem blight, wilt and anthracnose. Many kinds of fungicides are introduced to control those diseases increasing residual in environment. As far as pollution has been concerned. We should find any strategies to control those causal agents by avoiding it. Thus, the following of this study might help to find some pathogens controlling the weeds or some weeds be alternate hosts of those pathogens.

The purposes of this research were (1) survey the kind of weeds and (2) identification the pathogens causing diseases on the weeds in soybean crops.

Material and Methods

Field survey

Survey was taken place in soybean crops at the Asian Vegetable Research and Development Center (AVRDC), Kasetsart University, Kamphaengsaen Campus, Nakhon Pathom, Thailand. Broadleaf and grass weeds spread during the rainy season (August-September 1998) and the winter season (November 1998-January 1999). The surveys were recorded at 15-d intervals for identification of weeds and diseases found on weeds and soybean.

Isolation and identification of fungi

To isolate the disease tissues from the weeds and soybean leaves, tissue transplanting method was used via cutting infected leaves into 5 x 5 mm and soaking in sodium hypochlorite solution (1%) for surface disinfestation . Then, they were placed on potato dextrose agar (PDA) and incubated at 25°C under nearUV-light for 12 hrs. The cultures were observed for 5-7 days after plating.

Coverslip culture technique was selected in order to identify the fungal pathogens. Appropriate PDA medium was poured approximately 3 mm deep in a 100 mm petridish. A minute portion of pure culture was put on the solidified medium as a spot inoculum in the center of each of four quardrants. A sterile 18 mm square coverslip was cut into the medium, through each inoculum at a 45° angle to the agar surface. The culture was incubated at 25°C under nearUV-light for 12 hrs. Each coverslip was removed by using sterile forceps and gently heating for fixing the fungus. It was left on a drop of lactophenol. A second drop of lactophenol was placed on it and a clean 18 mm square coverslip was put on the drop of lactophenol. Four or five drops of Permount (trade name) were left on that coverslip. It was completed for a permanent slide by covering with a 22 square coverslip.

Freehand section method was used for identification in some pathogens which can produce conidium on plant tissue.

Results and Discussion

Field survey

Fourteen varieties of both broadleaf and grass weeds were obtained in the rainy season. Ten varieties of broadleaf weeds were sweet broom (*Scoparia dulcis* L.), *Lagascea mollis* Cav., tiger's foot morning glory (*Ipomoea pes-tigridis* L.), botton weed (*Borreria laevis* Lamk. Griseb.), goose berry (*Physalis minima* L.), waterprimrose (*Ludwigia hyssopifolia* G.Don), wild poinsettia (*Euphorbia heterophylla* L.), sensitive weed (*Mimosa pudica* L.), american jointvetch (*Aeschynomene americana* L.), and red weed (*Melochia corchorifolia* L.). Four varieties of grass weeds were *Eriochloca procera* Steud., itchgrass (*Rottboellia cochinchinensis* Lour. W.D. Clayton), wiregrass (*Eleusine indica* L. Gaertn.) and southern crabgrass (*Digiteria ciliaris* Retz. Koel.).

In the winter season, the weeds observed in the soybean production area were nineteen varieties. Twelve varieties were broadleaf weeds including tiger's foot morning glory, botton weed, country mallow (*Abutilon hirtum* Sweet.), goose berry, spiny pigweed (*Amaranthus spinosus* L.), wind spider (*Cleome viscosa* L.), wild poinsettia, sensitive weed, morning glory (*Ipomoea glacillis* R. Br.), american jointvetch, red weed and cowitch (*Mucana pruriens* DC.). Seven varieties were grass weeds which were identified as barnyardgrass (*Echinochloa crus-galli* L. Beauv.), itchgrass, chinese sprangletop (*Leptochoa chinensis* L. Nees), jungle rice (*Echinochloa colona* L. Link), crowfoot grass (*Dactyloctenium aegyptium* L.), finger grass (*Chloris barbata* Sw.) and feather lovegrass (*Eragrostis tenella* L. Beauv.).

From the results, there were some weeds in both seasons such as tiger's foot morning glory, botton weed, goose berry, wild poinsettia, sensitive weed, american jointvetch, red weed, and itchgrass. Interestingly, leaf spot, anthracnose, and powdery mildew were important plant diseases which showed on leaves of these weeds except sensitive weed (Table 1 and 2). Hence, not only they compete with soybean, but they also take a role as pathogen hosts in soybean field.

Isolation and identification of fungi

The fungal pathogens which were isolated from various symptoms of the weeds and soybean were identified into 3 classes as Ascomycetes, Hyphomycetes and Deuteromycetes (Table 1 and 2).

Glomerella sp., a genus of class Ascomycetes, is perfect stage of *Colletotrichum* causing anthracnose of soybean, botton weed, itchgrass, waterprimrose and wild poinsettia. It has been reported *Glomerella glycines* specificity to infect soybean and some weed varieties (Hartman *et al.*, 1986; David, 1991).

Nigrospora sp. is a genus of class Hyphomycetes. Commonly found as a parasite of cereals, it was infrequently recorded from soils (Gilman, 1957). It was isolated from leaf spot symptoms of soybean, american jointvetch, gooseberry and itchgrass.

Fungi in class Deuteromycetes, *Oidium* sp. and *Ovulariopsis* sp. were the powdery mildew-causing agent of gooseberry and wild poinsettia, respectively. Grigaliunaite *et al.* (1995) reported *Sphaerotheca*, *Erysiphe* and *Oidium* spp. affecting annual, biennial and perennial weeds in Lithuania. Leaf spot diseases were caused by many fungi from this class. For instance, *Drechslera* sp. showed on itchgrass and soybean, *Curvularia* sp. was discovered on botton weed, and *Helminthosporium* sp. was

found on barnyardgrass. Nevertheless, *Phomopsis* sp. covered on soybean, red weed, tiger's foot morning glory and waterprimrose. The causing agents of anthracnose, *Colletotrichum gloeosporioides* and *C. truncatum* were observed on waterprimrose and tiger's foot morning glory, respectively. It has been reported about the both species of *Colletotrichum* causing anthracnose of soybean and several weeds (Hepperly *et al.*,1980; Hartman *et al.*, 1986; David, 1991). Roy *et al.* (1994) reported fungi, *Phomopsis*, *Colletotrichum*, *Alternaria*, and *Curvularia*, were isolated from surface-disinfested foliar tissue samples of 35 weed species. They were found on 31, 23, 20 and 6 weed species, respectively. Weed species yielding the greatest diversity of fungi were *Xanthium strumarium*, *Ipomoea purpurea*, *Sida spinosa*, *Cassia tora*, *Jacquemontia tamnifolia*, *Euphorbia maculata*, *Amaranthus hybridus*, *Sesbania exaltata*, *Acalypha virginica*, *Eclipta alba* and *Ambrosia artemisiifolia*. Soybean and itchgrass were infected by *Curvularia*, *Drechslera* and *Helminthosporium* (Zhang and Watson, 1997). From this study, we isolated *Drechslera* sp. from itchgrass in the rainy season and showed leaf spot symptom on soybean leaves in the winter. The characteristic comparison of two *Drechslera* isolates were similar in colonial color and conidial characteristics. So, itchgrass may be serve as alternate host for the disease causing agent of soybean.

The advantage of this research was fungal collection which challenge to study some strains as weed biocontrol. Many fungal pathogens of weeds lost virulence in long term artificial cultures and will not infect their specific host while they were inoculated. It has been reported about some species of the common fungal pathogens; *Alternaria*, *Cercospora* and *Helminthosporium*, generally are too weakly virulent to kill their weed hosts even when they were applied as inundatory inoculum to seedling plants. This problem may be relevant to fungal genetic. Thus, it should be seek for the strategies to collect these fungal cultures which the pathogenicity still be stable. Prior to the initiation of a classical biocontrol plan, a careful pathogenicity to soybean should be investigated.

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Table 1 Disease symptoms and fungal pathogens found on soybean and several weeds in the rainy season.

Plant/weeds	Scientific name	Disease symptoms	Fungal pathogen
Soybean	*Glycine max* L. Merr.	Leaf spot	*Phomopsis* sp.
		Anthracnose	*Glomerella* sp.
Broadleaf weeds:			
American jointvetch	*Aeschynomene americana* L.	Leaf spot	*Nigrospora* sp
Botton weed	*Borreria laevis* Lamk. Griseb.	Anthracnose	*Glomerrella* sp.
		Leaf spot	*Curvularia* sp.
Redweed	*Melochia corchorifolia* L.	Leaf spot	*Phomopsis* sp
Tiger's foot morning glory	*Ipomoea pes-tigridis* L.	Leaf spot	*Phomopsis* sp.
		Anthracnose	*Colletotrichum truncatum*
Waterprimrose	*Ludwigia hyssopifolia* G.Don	Leaf spot	*Phomopsis* sp.
		Anthracnose	*Glomerella* sp./*Colletotrichum gloeosporioides*
Wild poinsettia	*Euphorbia heterophylla* L.	Anthracnose	*Glomerella sp.*
Grass weeds:			
Itchgrass	*Rottboellia cochinchinensis*	Anthracnose	*Glomerella sp.*
	Lour. W.D. Clayton	Leaf spot	*Drechslera sp.*

Table 2 Disease symptoms and fungal pathogens found on soybean and several weeds in the winter season.

Plant/weeds	Scientific name	Disease symptoms	Fungal pathogen
Soybean	*Glycine max*	Leaf spot	*Nigrospora* sp.
			Drechslera sp.
Broadleaf weeds:			
Gooseberry	*Physalis minima* L.	Leaf spot	*Nigrospora* sp.
		Powdery mildew	*Oidium* sp.
Wild poinsettia	*Euphorbia heterophilla* L.	Powdery mildew	*Ovulariopsis* sp.
Grass weeds:			
Barnyardgrass	*Echinochloa crus-galli* L. Beauv.	Leaf spot	*Helminthosporium* sp.
Itchgrass	*Rottboellia cochinchinensis*	Leaf spot	*Nigrospora* sp.
	Lour. W.D. Clayton		*Drechslera* sp.

INVOLVEMENT OF ABSCISIC ACID IN TEMPERATURE-SENSORY MECHANISMS FOR CONTROLLING SEASONAL TIMING OF SEED GERMINATION IN WINTER ANNUAL WEEDS

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Abstract: High soil temperatures in summer inhibited seed germination of *Stellaria neglecta*, an obligate winter annual. Seeds of *Lactuca sativa* at 33 °C, with a high abscisic acid (ABA) content, did not germinate, but those treated with fluridone, an inhibitior of ABA biosynthesis, germinated. Seed germination in many winter annual weeds was also promoted by fluridone. The present study suggests that the temperature-dependent changes in ABA contents in soil-buried seeds play a decisive role for the detection of appropriate germination timing in winter annual weeds.

Key words: abscisic acid (ABA), conditional dormancy, fluridone, thermoinhibition of seed germination, winter annual weed.

Introduction

Weeds have adapted their life cycles to seasonal changes in environment, especially temperature. Winter annual weeds possess a pattern of life cycle; seeds germinate in autumn, the emerged plants grow vegetatively in winter, flower in the following spring and set seeds. That is, they pass the summer as soil-buried seeds.

Many ecological studies using buried seeds have revealed that germination of winter annuals in summer is prevented by the high soil temperatures (Baskin and Baskin, 1998). However, how the seeds sense the temperatures remains unclear.

In *Lactuca sativa*, which is often used for researches on germination physiology, exogenous ABA lowers the maximum temperature at which seed germination is possible (Reynolds and Thompson, 1971). We reported previously (Yoshioka *et al.*, 1998) that germination of *L. sativa* seeds at supraoptimal temperatures is promoted by the application of fluridone.

Stellaria neglecta is a typical obligate winter annual (Baskin and Baskin, 1976; Miura *et al.*, 1995), and distributes over temperate regions as an important weed in crop fields and fruit cultivation. In the present study, we examined the ecophysiological role of ABA in the detection of appropriate timing of seed germination in winter annual weeds using *S. neglecta* and *L. sativa*.

Materials and Methods

Plant materials

Seeds of *L. sativa* cv. Grand Rapids, purchased from Takii & Company Ltd., were used for physiological experiments. For ecological experiments, seeds of some winter annual weeds were collected in the suburbs of Sendai, Japan, at ripening seasons in 1995-1997.

Field-tests

Seeds of *S. neglecta*, harvested on 7-14 May 1997 and placed in nylon bags, were buried 1 cm deep in soil at an experimental field of Tohoku University on 14 May 1997. They were periodically exhumed and used for germination tests described below.

Germination tests

Fifty seeds were sown in a 5-cm petri dish on two layers of filter paper wetted with 1.8 mL of water or 30 μM fluridone. The petri dishes were wrapped with aluminium foil, and incubated at designated temperatures in darkness. At various times after the start of incubation, germinated seeds were scored.

To check the viability of seeds, we cut seeds in half, and incubated them in 14.9 M 2, 3, 5-triphenyltetrazolium chloride for 12 h; the embryos of viable seeds gave a red colour. The percentage of germination was calculated based on the number of viable seeds.

ABA measurements

Lactuca. sativa seeds (1 g dry weight) were incubated at 23 or 33 °C with or without 30 μM fluridone in darkness. The seeds were collected at designated times and stored at -30 °C until use.

S-(+)-ABA content was assayed according to Yoshioka *et al.* (1998). Each frozen sample was pulverized in liquid nitorogen, and extracted with 80% methanol at 4 °C over night. As an internal standard, trans, trans-ABA was added to the extract. The ABA extract was passed through solid phase extraction columns (Supelclean LC-18 and LC-NH_2) and eluted. The eluate was concentrated under vacuum and the concentrate was taken up in 100 μL of 20% methanol.

A part of the solution (10 μL) was analyzed by HPLC (Shimadzu LC-10, column, Wakosil-II 5C18 HG; step-wise linear gradient elution with methanol; detection, UV 254 nm). Using the remaining solution, the S-(+)-ABA fraction was separated by HPLC, methylated with diazomethane, and identified by GC-MS (Shimadzu QP-2000, column: DB-1, full-mass spectral monitoring).

Chemicals

Fluridone is a herbicide that inhibits phytoene desaturase, which converts phytoene to phytofluene in the pathway of carotenoid biosynthesis (Bartels and Watson, 1978). Carotenoids are the main precursors of ABA in plants. Thus, the inhibition of carotenogenesis also prevents the biosynthesis of ABA. Fluridone was a gift from Mr. Kazuma Yamauchi of Dow Chemical Japan Co., and S-(+)-ABA from Professor Yasuo Kamuro of Gifu University.

Results

Seasonal changes in soil temperature and temperature range allowing germination of *S. neglecta* seeds.

The seeds of *S. neglecta* harvested in May were in a state of primary dormancy. Seeds buried in the soil were then conditionally dormant in June-August; the maximum temperatures for germination gradually rose from 17 to 22 °C while daily minimum temperatures in the soil were just above the germinable temperatures (Fig. 1-A). In September, the soil temperature lowered to the temperature range allowing germination, and a flush of seedling emergence occurred.

When exhumed seeds were tested for germination in 30 μM fluridone, the maximum temperatures allowing germination in summer was 5-8 °C higher than in water, and overlapped temperature range in the soil. Figure 1-B shows the stimulation by fluridone of seed germination in the habitat in July. If the exhumed seeds were immersed in fluridone solution for 30 min and sown again in the field, 60% of them germinated within 14 days, whereas none of the water-treated seeds germinated.

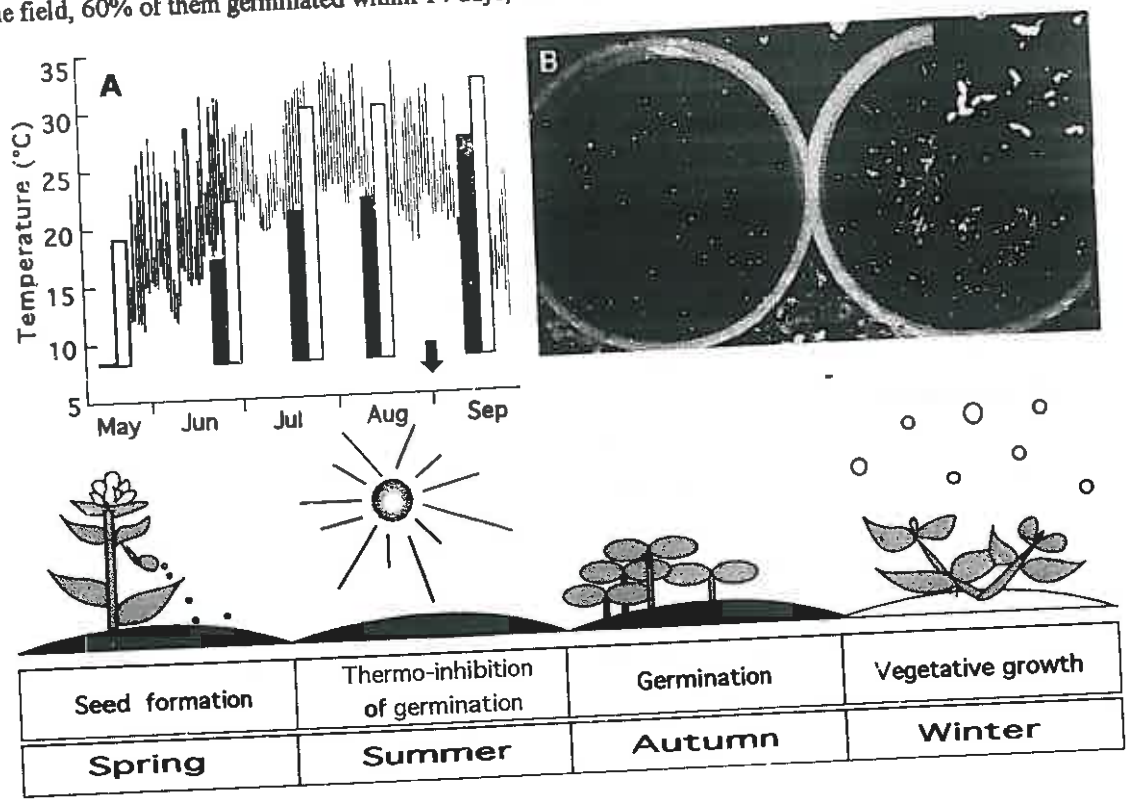

Fig. 1. Seasonal changes in soil temperature, and temperature range allowing germination in *Stellaria neglecta*. A, Freshly harvested seeds were buried in the soil and exhumed monthly from May to September 1997. The exhumed seeds were incubated in the presence (□) or absence (■) of 30 μM fluridone at 8, 13, 18, 23, 28, and 33 °C for 14 days in darkness. The columns show the temperature ranges at which more than 5% seeds germinated. The vertical lines indicate the daily soil temperatures at a depth of 1 cm. A flush of seedling emergence was observed in late August to early September (⬇). B, In July, the exhumed seeds were immersed in water (left) or 30 μM fluridone (right) for 30 min and buried again in the field, and then their germination was checked on 14th day after the burial.

ABA contents in the seeds incubated at a supraoptimal temperature for germination.

Dry seeds of *L. sativa* contained 133 ng g^{-1} of ABA. In the seeds incubated with water at 33 °C, ABA contents remained constant at the initial level (Fig. 2-A). In the seeds incubated with fluridone at the

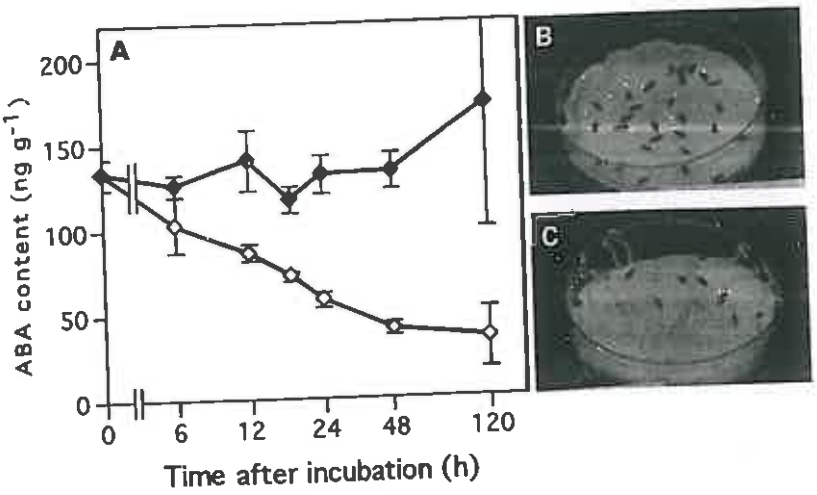

Fig. 2. Effects of fluridone on ABA contents and germination of *Lactuca sativa* seeds 33 °C. **A**, Seeds (1 g dry weight) were incubated in the presence (◇) or absence (◆) of 30 μM fluridone at 33 °C, and their ABA contents were determined at intervals. Note that the abscissa is shown on a log scale. **B**, Thermoinhibition of lettuce seed germination. Seeds were incubated without fluridone at 33 °C for 10 days. **C**, Relief of thermoinhibition with 30 μM fluridone application. Seeds were incubated with fluridone at 33 °C for 10 days.

same temperature, the ABA contents gradually decreased and reached 40 ng g^{-1} after 120 h of incubation. The seeds incubated in water did not germinate (Fig. 2-B), but those incubated in fluridone did (Fig. 2-C).

We also examined the seasonal changes of ABA contents in buried seeds of *S. neglecta* (data not shown). The ABA contents were 335 and 939 ng g^{-1} in June and July, respectively, when the seed germination was inhibited, but 64 ng g^{-1} in September.

Table 1. Promotion by fluridone of seed germination at supraoptimal temperatures in various winter annual weeds. In each weed, 50 seeds were incubated with or without 30 μM fluridone at optimal (13 or 18 °C) or supraoptimal (28 or 33 °C) temperatures for 14 days in darkness.

	Germination (%)			
	Optimal temperature		Supraoptimal temperature	
Species	−fluridone	+fluridone	−fluridone	+fluridone
Caryopyllaceae				
Cerastium glomeratum	91.6 ± 2.2	NT	0.0 ± 0.0	13.5 ± 1.6
Stellaria neglecta	98.1 ± 1.9	100 ± 0.0	12.7 ± 7.5	100 ± 0.0
Compositae				
Conyza canadensis	70.1 ± 7.1	NT	17.9 ± 3.7	84.0 ± 1.0
Gnaphalium pensylvanicum	61.3 ± 4.7	60.0 ± 3.5	8.7 ± 1.8	66.7 ± 8.2
Youngia japonica	48.1 ± 4.0	50.9 ± 4.5	15.1 ± 1.9	56.7 ± 1.1
Senecio vulgaris	10.7 ± 2.4	98.7 ± 1.3	1.3 ± 0.7	88.7 ± 3.5
Cruciferae				
Lepidium virginicum	34.7 ± 1.3	77.3 ± 5.8	1.8 ± 1.8	63.2 ± 4.4
Gramineae				
Poa annua	51.8 ± 3.0	53.8 ± 8.0	24.2 ± 6.0	55.4 ± 2.3
Leguminosae				
Vicia angustifolia	97.1 ± 1.0	NT	18.7 ± 1.8	93.7 ± 1.2
Lactuca sativa cv. Grand Rapids	100 ± 0.0	100 ± 0.0	4.0 ± 2.0	94.7 ± 2.7

†NT; not tested. ‡Data are the means of triplicate ± SE.

Responses of the seeds of various winter annual weeds to temperature and fluridone.

Table 1 shows the effects of fluridone on seed germination in 9 winter annual weeds and *L. sativa* at optimal and supraoptimal temperatures. All *L. sativa* seeds germinated both with and without fluridone at 23 °C (optimal temperature), but only 4.0% did without fluridone at 33 °C (supraoptimal temperature). After the application of fluridone, 94.7 % germinated at 33 °C. In all winter annual weeds examined, fluridone stimulated seed germination at 33 °C, although the effect varied with the species.

Discussion

Germination of *S. neglecta* seeds was inhibited during summer because the maximum temperatures allowing germination were lower than the minimum soil temperatures. The seeds germinated in autumn when the temperature range for germination and those in the soil overlapped. These results indicates that the buried seeds of *S. neglecta* determine the appropriate germination timing by sensing the soil temperature, and such response to soil temperature is consistent with that observed in many winter annuals (Baskin and Baskin, 1998). By the application of fluridone, an inhibitor of ABA biosynthesis, the maximum temperature allowing germination rose, and germination occurred even in the summer. Abscisic acid may be used to sense the germinable temperature. Actually, flush of seedling emergence synchronized with a steep decrease in ABA content in the buried seeds.

At a supraoptimal temperature for germination, the ABA content in the *L. sativa* seeds was consistently high, but decreased in the presence of fluridone. This means that the metabolism and synthesis of ABA are balanced in the thermo-inhibited seeds and that continuous synthesis of ABA is necessary for the inhibition of germination at the high temperature. In the present study, we could not give direct evidence for the induction of ABA biosynthesis at a high temperature. Recently, genes of zeaxanthin epoxidase and 9-cis-epoxi-carotenoid dioxigenase, which catalyze the key steps in ABA biosynthesis, have been cloned (Marin *et al.*, 1996; Schwartz, 1997). Thus, further research on the expression of these genes in response to temperature is awaited.

In addition, the promotion of germination by the application of fluridone at supraoptimal temperatures was observed in all winter annual weeds tested. We suppose that seeds of winter annual weeds regulate their ABA contents by sensing soil temperatures, and inhibit their germination until the appropriate season for germination. This temperature-sensory mechanism mediated by ABA may play a decisive role in the control of the life cycle in winter annual weeds.

Existence of seed banks attributes to the success of regeneration of weed populations. Stimulation of buried seed germination would be an effective method to reduce the size of weed seed banks, if the resultant seedlings are eliminated. The seeds induced to germinate by fluridone developed albino seedlings and soon died. Therefore, soil application of herbicides that inhibit carotenoid biosynthesis could be a new tool for weed management.

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IS *Poa crassinervis* Honda ENDEMIC TO JAPAN?

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Abstract: In order to examine the endemic occurrence of *P. crassinervis* as a weed in ruderal habitats of Japan, the herbarium specimens deposited in three herbaria of China, two institutes of Vietnam, and two herbaria of Japan were carefully examined, and several field surveys were conducted in Kyushu island where the type specimen was collected. *P. crassinervis* was clearly identified as separating from *P. annua*, because *P. crassinervis* has three unique morphological characters: (1) the panicle branches are rather thick and obliquely straight to ascending, (2) the second glume and lemmas of the spikelet are narrowly elliptic and not pigmented, (3) the lateral nerve of each lemma is densely appressed to pubescent on more than half the length from the floret base. Herbarium specimens reveal that *P. crassinervis* was commonly distributed in southern, western and central Japan and four sites in coastal provinces of southeastern China. In addition to this, field surveys around the type locality of *P. crassinervis* suggested that this species might be introduced from other regions into Japan. Although the indigenous domain of this species is unclear, the endemism of *P. crassinervis* to Japan should be reconsidered.

Key words: *Poa crassinervis*, *Poa annua*, endemism, China Continent, herbarium specimen

Introduction

The taxonomy of a cosmopolitan species, *Poa annua* L. of the family Gramineae, in a wide sense, is somewhat confusing. In East Asia, *P. crassinervis* Honda which is a annual species and very close to *P. annua* is also distributed. Both species have brought about serious weed problems in agricultural, industrial, amusement and urban habitats of Japan.

The origin and natural distribution of *Poa crassinervis* Honda is uncertain and this species has been considered to be endemic to Japan. Since Dr. Honda (1926) described this species in Kumamoto of Kyushu Island of western Japan during the mid 1920s, *P. crassinervis* has become a widespread occupation as a common weed in western Japan, frequently together with *P. annua*.

The purpose of this paper is to reinvestigate the geographic distribution of *P. crassinervis* by using of herbarium specimens deposited in two herbaria of Japan, two herbaria of the Peoples' Republic of China and three herbaria of the Socialist Republic of Vietnam and to discuss the endemism of *P. crassinervis*.

Materials and Methods

In order to understand the original geographic distribution of *P. crassinervis* around the Japan Archipelago, eighty-eight sheets of herbarium specimens of *Poa* deposited in the Herbarium of the Institute of Botany and the Herbarium of the Kunming Institute of Botany of Academica Sinica of China, six sheets of herbarium specimen deposited in the National Center for Natural Science and Technology, Vietnam National University of Hanoi and the Institute of Tropical Biology of Ho Chi Minh City and 74 and 20 sheets of herbarium specimens in Kyoto and Okayama Universities of Japan were carefully examined by microscope and identified using the combined criteria given by Tateoka (1987).

The list of herbarium specimens examined is not provided here. These investigations were carried out in 1998 for China and Vietnam and in 1999 for Japan.

Results and Discussion

P. crassinervis Honda was described as a new species, taxonomically very close to a cosmopolitan weed, *P. annua* L.(Tateoka, 1985; 1987, Ohwi, 1975). This taxon is frequently difficult to distinguish from *P. annua* because some Japanese taxonomists have given ambiguous and improper keys for identification. For example, T. Koyama of a grass taxonomist in 1964 stressed on the length

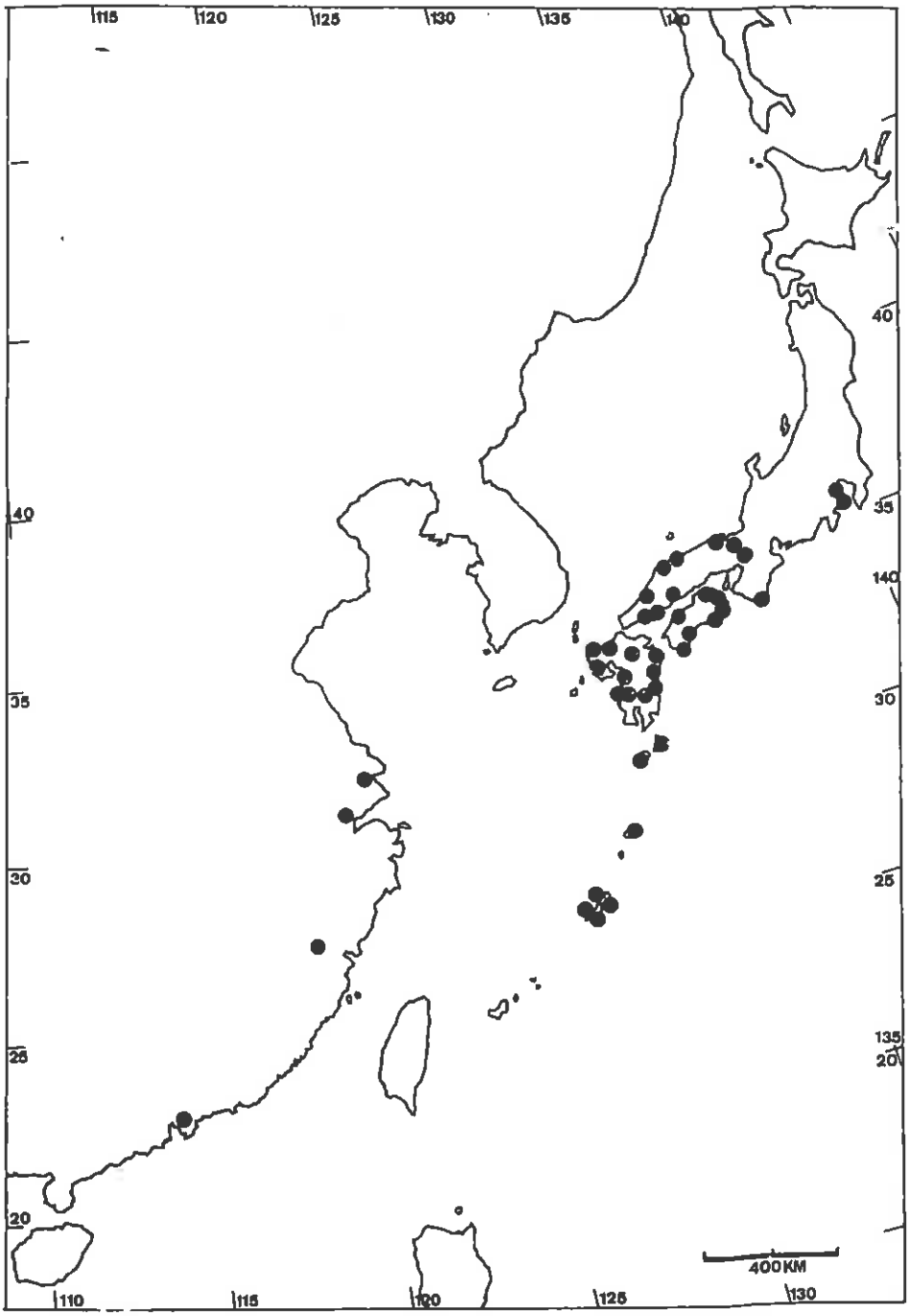

Fig. 1. Geographical distribution of *Poa crassinervis* Honda in East Asia. Each collection site is shown with (●).

of the leaf ligule between two species for morphological identification, but this character is so variable and overlapped among many populations that two species could not be easily distinguished. Ohwi (1975) regarded *P. crassinervis* as a good species separating from *P. annua* in such a few characters set as the shape of the lemma and the nerve on the lemma. Unfortunately, their successors came to consider the nerve on the lemma as the one and only key character to identify. As a result, a confusion of identification, not of taxonomy, had and sometimes has been invited among plant researchers.

Under these undesirable situations, Tateoka (1987) examined the identity and distribution of *P. crassinervis* using the herbarium specimens and the natural populations and clearly presented the morphological characters set for identification. According to him, in distinguishing of *P. crassinervis* from *P. annua*, three unique morphological characters of *P. crassinervis* are emphasized as follows: (1)

the panicle branches are rather thick and obliquely straight to ascending, (2) the second glume and lemmas of the spikelet are narrowly elliptic and not pigmented, (3) the lateral nerve of each lemma is densely appressed to pubescent on more than half the length from the floret base. Moreover, the plant has rather thick and obliquely straight-ascending panicles. These should be considered as the combined criteria and are hence easily distinguished from those of *P. annua*. Identifications are also easily carried out even if using dried plants on herbarium specimens, as well as living materials.

The natural distribution and origin of *Poa crassinervis* is not so clear. Since Honda (1926) first described *P. crassinervis* as a new species based on a herbarium specimen collected in Kyushu island of southwestern Japan, the collection localities of this species by many plant collectors were limited within the ruderal habitats of western and central Japan, without any exception. Consequently, many botanists and weed biologists believe that *P. crassinervis* is a weed endemic to Japan.

The geographical distribution of *P. crassinervis* is shown in Fig. 1, based on herbarium specimens deposited in two herbaria of China, two herbaria of Japan and Tateoka (1987). The collection localities of *P. crassinervis* in the China Continent, outside of Japan, seem to be rather restricted to the coastal provinces of the China Continent: *i.e.* Shanghai in 1949, Lonjin of Zhejiang in 1957, Shunchian of Fujian in 1970s and Hongkong of Guangdong in 1970. In China, *P. crassinervis* was distributed in upland field and lawn. In Okinawa prefecture of southernmost Japan, *P. crassinervis* is distributed in ruderal habitats such as agricultural fields, lawns and roadsides.

In addition to this, field surveys around the type locality of *P. crassinervis* in Kyushu Island of western Japan suggested that this species might be introduced from other regions. In Ashikita County of southern Kumamoto prefecture where *P. crassinervis* was first collected, this species is still a common weed in winter, of wheat fields, ornamental greenhouses, parking places, paddy fields, farm roads and flower beds. However, the distribution of *P. crassinervis* was rather limited and the habitats were less disturbed when compared with those of *P. annua*, and seen as fugitive. Therefore, it is reasonable that the endemism of *P. crassinervis* to Japan should be reconsidered.

Tateoka (1987) noted that *P. crassinervis* was not recognized in China and Korea. In South Korea including Chejudo Island, no plants as identified *P. crassinervis* were observed (Dr. T. Enomoto of Okayama University, *pers. comm.*). The endemism of this species in Japan is supported by Osada (1993). However, Tateoka (1987) pointed out that *P. crassinervis* might be a weed endemic to Japan but was very mysterious because of its sympatric occurring and virtual niche equality with *P. annua*. He supposed the mutation origin of *P. crassinervis* from *P. annua*.

An new and extended distribution of *P. crassinervis* westward from Japan to the coastal provinces of China, needs some re-examinations on the endemism of this species to Japan. Circumstances may provide three hypotheses: (1) *P. crassinervis* in the southeastern coastal provinces of China is a result of accidental or historical introductions from Japan and this species is all the same endemic to Japan, (2) *P. crassinervis* was originally distributed around the southeastern coastal regions of China and should not endemic to only Japan and (3) *P. crassinervis* was formed by *P. annua* and *P. infirma* complex.

The first hypothesis that *P. crassinervis* is endemic to the Japan Archipelago has been supported by many plant taxonomists and weed biologists of Japan. In fact, *P. crassinervis* was collected only in western Japan until the author's investigation since Honda (1926) described as a new species endemic to Kyushu. No record of this species was listed in other regions of the world. It is assumed that *P. crassinervis* has evolved by a few gene mutations from *P. annua*, which is supposed to taxonomically close to *P. crassinervis*. Tateoka (1987) observed a few sterile hybrids between two species in natural populations, and it suggested that two species was partly reproductively isolated. If *P. crassinervis* is only distributed in Japan, then this species is neo-endemic. In the genus of *Poa* of Japan, many endemic species are also enumerated.

Thus, the endemic-to-Japan hypothesis is one of the most persuasive stories. One or two hundred years ago, *P. crassinervis* had a geographically narrow distribution restricted to Kyushu Island of western Japan. Traffic revolution and agricultural reconstruction, in particularly after World War II, has promoted the dispersal of this species into other regions of western Japan. Expansion of this species into such regions may be partly due to the misidentification or ignorance of plant professionals. Naturalization into the China Continent and Okinawa prefecture of Japan of *P. crassinervis* was achieved by the increase in international trading among Japan and continental countries and the military affairs.

The second hypothesis that *P. crassinervis* is endemic to the China Continent and its related regions is here proposed by the author, based on herbarium investigations. The geographical distribution shown in Fig. 1 suggests *P. crassinervis* is supposed to have the original distribution around the warm temperate broad-leaved forest and its southern regions in southeastern coastal China. This means *P. crassinervis* was evolved as an adaptation to warmer climate from a mother species, *P.*

annua. It is speculated that this species requires more temperature for vegetative growth and reproduction than *P. annua*. It is probable that *P. crassinervis* constitutes an element of the Shouyoujurin Zone, a kind of tamed versions or formations of the warm temperate broad-leaved forest zone

P. crassinervis then reached into western Japan as a result of historical human activities and/or meteorological and non-anthropological events. After a few populations succeeded in naturalizing onto Kyushu Island and neighboring areas of Japan, the distribution of this species had been suppressed within the regions by vegetation management and edaphic factors. However, recent changes in agriculture and socio-economic structure have promoted the widespread occurrence of *P. crassinervis* in warm temperate regions of western Japan. Because this species needs higher temperature conditions for reproduction as above mentioned, then *P. crassinervis* is impossible to invade beyond Tohoku district in northern Japan. Genetic variation of *P. crassinervis* in Japan is speculated to be medium to low, as in the case of populations of naturalized plants.

The third hypothesis is that *P. crassinervis* was formed by hybridization of *P. annua* with another unknown species. *Poa* is a large genus counting more than five hundred species in the world. Most of species are perennial and the remnants are annual. One of annual species is *P. infirma* H. B. K. whose number of somatic chromosome is 14. This diploid species was originally distributed in the British Isles and become widespread by human activities into the Mediterranean Sea (Hubbard, 1968). Afterwards, this species was forced to disperse via the Canary Islands to Africa, South America. In Asia, it is also distributed Australia (e.g. http://biodiversity.uno.edu/delta/pooid).

Diploid *P. infirma* can cross with tetraploid *P. annua* to form hybrids in natural conditions (Hubbard, 1968). According to the morphological description of *P. infirma*, the lateral nerve of each lemma is densely pubescent on more than half the length from the floret base, as in the case of *P. crassinervis*. Somatic chromosome numbers of *P. crassinervis* and *P. annua* in Japan are tetraploid and 28 (Tateoka, 1985). If this nerve character was repeatedly introduced to *P. annua*, for example, by introgressive hybridization, then a plant having *P. crassinervis* features may be evolved. If so, the origin of *P. crassinervis* is in pan-temperate to pan-subtropical zones, and the distribution of this species in Japan and China as obtained in Fig. 1. is a easternmost part of the whole domain. However, no plants resembling *P. crassinervis* have been found in any monographs of the world, except western Japan and southeastern coastal China of this case. A possibility of species overlooking or species misidentification is not ignored.

Under the existing circumstances, the second hypothesis is the most persuasive because 99 % of ruderal weeds in Japan were introduced from outside the Japan Archipelago and the present vegetation has been built up by historical migrations from the China Continent and other regions, just like social cultures.

To elucidate the endemism of *P. crassinervis* and to determine the real origin of this weed, further studies including both the geographical distribution and archaeological survey and molecular biological analysis using living materials from the China Continent and other regions are necessary.

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MODE OF ACTION OF A NEW PLANT GROWTH INHIBITOR, DURANTANIN II

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Abstract: A new plant growth inhibitor, durantanin II, is a triterpenoide saponin isolated from *Duranta repens*. In this study, mode of action of durantanin II was investigated. Cucumber seeds (*Cucumis sativus*. L. cv. Aonagajibai) were sown on 0.5% agar containing 0, 63, 125, 250, 500 and 1000 μM durantanin II and incubated at 25℃ in the light. The 125 and 250 μM durantanin II inhibited the root elongation and promoted horizontal hypertrophy of the root. Cucumbers treated with 500 and 1000 μM durantanin II hardly grew after germination. Horizontal enlargement of root cells in cucumber seedlings treated with 125 and 250 μM was observed by a optical microscope. Because of these morphogenesis induced by durantanin II, it was likely that durantanin II affected the action of plant hormones. The effect of durantanin II on plant growth was similar to that of ethylene, suggesting that durantanin II might enhance ethylene synthesis. An ethylene synthesis inhibitor, aminooxyacetic acid, however, did not affect the inhibitory activity of durantanin II. Therefore, the mode of action of durantanin II would have no relation to promotion of ethylene synthesis. On the other hand, enhanced elongation growth of cucumber segments induced by 1 μM 3-indoleacetic acid, 50 μM gibberellic acid or 0.01 μM brassinolide was inhibited more than 70% by 250 μM durantanin II. Therefore, durantanin II would inhibit growth promotion stimulated by plant hormones, such as auxins, gibberellins and brassinosteroids.

Key words: triterpenoide saponin, *Duranta repens*, plant growth inhibitor, durantanin II, mode of action

Introduction

Saponins are widely distributed in a plant kingdom, and a large number of saponins is isolated from various spices of plants. The significance of them for plants is little known, but several saponins have been reported to have phytotoxic or allelopathic properties (Hoagland et al., 1996). Durantanin II is a triterpenoid saponin isolated from *D. repens* (Hiradate et al., in press). This substance inhibited growth of various spices of plants, and few weeds grow under a tree of *D. repens*. Therefore, it would be possible that *D. repens* has allelopathic effect, and durantanin II is an allelochemical of *D. repens* (Hiradate et al., in press). In this report, property and mode of action of durantanin II was studied.

Materials and Methods

Cucumber growth-test

Cucumber seeds were sown on 0.5 % agar containing 0, 63, 125, 250, 500 and 1000 μ M durantanin II and incubated at 25℃ in the light (approximately 50 μ mol/m^{2}s) for 14 days. The growth of cucumbers was observed by measuring the length of root. The shoots and roots were cut and sliced in about 100 μ m thickness by using a microslicer (Dosaka EM Co.,Ltd) for observation with a optical microscope (Nikon, Diaphot-TMD). The sections were stained with toluidine blue or iodine/potassium iodide solution.

Growth-test of lettuce root

The test was performed by the method of Tsurumi and Ishizawa (1997) with some modification. Lettuce seeds (*Lactuca sativa* L. cv. Great Lakes) were sown on a filter paper wetted with water and incubated at 25℃ in the light (approximately 50 μ mol/m^{2}s). After 28 hours, Germinated seedlings with roots length of 1.5 to 2.5 mm were selected Ten seedlings were placed on a filter paper in each petri dish containing 1 ml of test solution. Test solutions were prepared by dissolving various concentrations (0, 32, 63, 125, 250, and 500 μ M) of durantanin II and aminooxyacetic acid (AOA) in distilled water. After 24 hours of incubation at 25℃ in the dark, the length of the roots was measured.

Elongation-test of cucumber section

The test was carried out by the method of Kazama and Katsumi (1973). Cucumber seeds were sown in vermiculite and incubated at 25℃ in the light (approximately 50 μ mol/m^{2}s) for 4 days. Sections were excised at 5 mm distance from the cotyledonary node of the cucumber seedlings. Ten sections were floated in each petri dish containing 2 ml of test solution. The test solutions were composed of designated concentrations of plant hormone (indol-3-acetic acid, gibberellic acid or brassinolide) and various concentrations (0, 32, 63, 125, 250, and 500 μ M) of durantanin II. After 20 hours of incubation at the same condition as described above, length of the sections were measured with a micrometer under a low power binocular microscope (Nikon, SMZ-10).

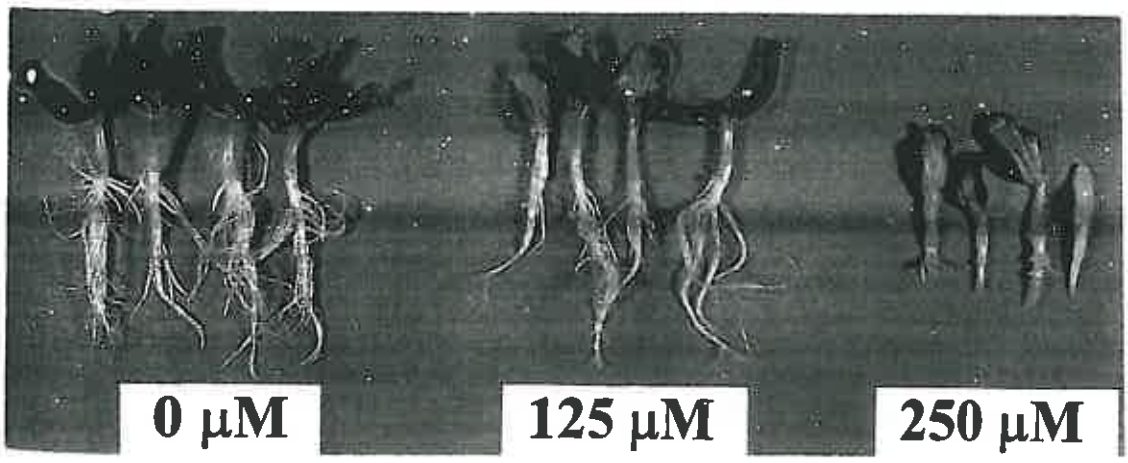

Fig. 1. Cucumber seedlings treated with durantanin II after 14 days.

Results and Discussion

In cucumber growth-test, the 125 and 250 μ M durantanin II inhibited the root growth, and promoted horizontal hypertrophy of the root (Fig. 1). Cucumber treated with 500 and 1000 μ M durantanin II did not grow after germination. Optical microscopic observation revealed that the horizontal enlargement of root cells had occurred in cucumber seedlings treated with 125 and 250 μ M durantanin II. In addition, starch grains were accumulated in the root cells of cucumber treated with 125 and 250 μ M durantanin II. It was evident that durantanin II inhibits growth of cucumber root and promotes horizontal enlargement and starch accumulation in the root cells. Because of these effects of durantanin II on plant morphogenesis, it was likely that durantanin II affected the action of plant hormones. Especially, the inhibitory effect of durantanin II on plant growth is similar to that of ethylene. Therefore, durantanin II is seemed to promote ethylene synthesis in plants to inhibit the elongation growth. To clarify the relationship between ethylene synthesis and durantanin II, the growth-test of lettuce root was conducted. Durantanin II inhibited root elongation of lettuce and AOA did not affected the inhibitory effect of durantanin II (Fig. 2). The AOA is an ethylene synthesis inhibitor. This result suggests that inhibitory effect of durantanin II was not caused by promotion of ethylene synthesis.

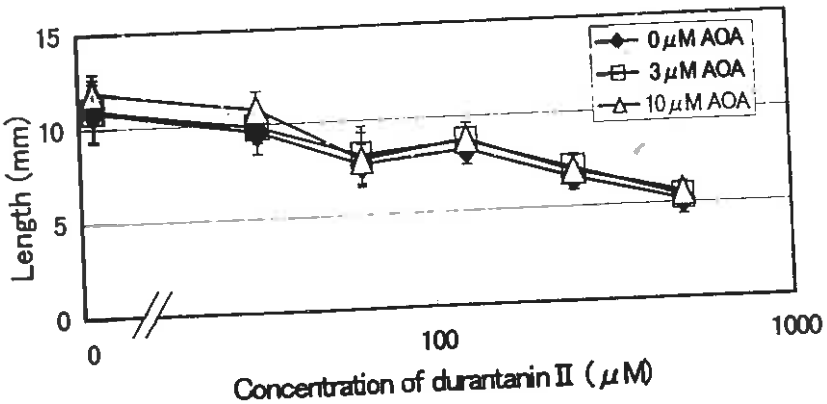

Fig. 2. Effect of AOA on durantanin II-induced inhibition of root growth of lettuce. Values are the means with SD (n=10).

There is other .possibility that durantanin II inhibits growth promotion action induced by other plant hormones (auxins, gibberellins and brassinosteroids). Then, interaction between plant hormone and durantanin II was investigated with the elongation-test of cucumber sections. One micromolar of indole-acetic acid (IAA), one of auxins, and 0.01 μ M brassinolide (BL), one of brassinosteroids, promoted elongation of the sections (Fig. 3-A, B). The elongation was inhibited around 70% by the 250 μ M durantanin II. In the case of gibberellins, 50 μ M gibberellic acid (GA_3) slightly promoted elongation of cucumber sections, and durantanin II decreased the promotion (Fig. 3-C). Therefore, durantanin II would inhibit elongation growth promoted by IAA, BL and GA_3, but it had no specific inhibitory effect on any of these hormones.

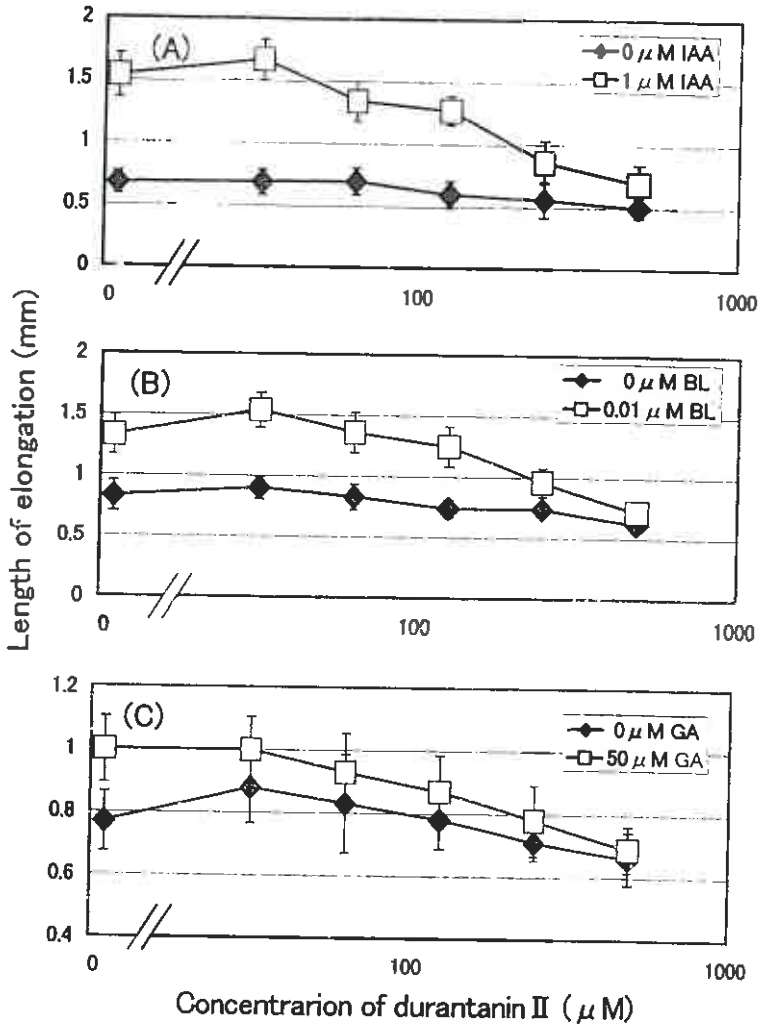

Fig. 3. Effect of durantanin II on elongation of cucumber hypocotyl sections induced by IAA (A), BL (B), or GA_3 (C). Values are the means with SD (n=10).

Tsurumi and Tsujino (1995) reported that choromosaponin I (CS I), one of triterpenoide saponins, promoted growth of lettuce root at concentration of 1~3 mM. The CS I inhibits both synthesis and response of ethylene in lettuce seedlings (Tsurumi and Ishizawa, 1997). Oppositely, durantanin II inhibited the growth of plants, and the inhibitory effects was similar to those of ethylene. However, durantanin II had no effect on ethylene production. On the other hand, α-tomatine, a steroidal saponin, inhibits IAA-induced elongation of wheat coleoptile segments because α-tomatine inhibits the alteration of membrane caused by IAA (Roddick, 1975). It is known that saponins increase the permeability of membranes and cell walls for macromolecules (Meiners et al., 1991). And plant hormones induce alteration of membrane and cell wall to promote plant growth. Durantanin II is likely to affect the alteration of membranes and cell walls induced by plant hormones.

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On the Succession of Weed Communities in Five Kinds of Crop Fields

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Abstract: During the Eighth Five-Year Plan Period of China, the composition, damage and succession of weed communities and the contributing factors in rice, wheat, corn, cotton and soybean fields located in different ecological regions were systematically investigated. The results showed that owing to the changes of tillage and cropping systems, tranfer of labor power in rural area, especially to the longterm and continuous use of a simple herbicide, the succession of weed communities in crop fields has been accelerated. There is a 4~8 years cycle of weed community succession in crop fields. Under such conditions, new weed species, which are difficult to control, will continuously present, the damage of broadleaf weeds in dicotyledonous crop fields and perennial disastrous weeds will be more and more serious.

Key words: Crop field, weed community, succession

1. Preface

Normally, 33,333,000 hectares of rice, 30,000,000 hectares of wheat, 20,000,000 hectares of maize, 6,666,000 hectares of soybean and 6,666,000 hectares of cotton were planted in China, 80% of total plant area of crop in China. In our country, weeds damaged 40,000,000 hectares. Among them, 20,000,000 hectares were serious damaged. Occurrence of weed damage was in 5 main crop field greatly, a lot of yield was loss. 15,333,000 hectares of rice was damaged, 10.3 billion kg of yield was loss, loss rate of 13.4%. 10,000,000 hectares of wheat was damaged, 4 billion kg of yield was loss, loss rate of 15.0%. 6,666,000 hectares of maize was damaged, 2.5 billion kg of yield was loss, loss rate of 10.4%. 2 million hectares of soybean was damaged, 500 million kg of yield was loss, loss rate of 14.8%. It was very important to research on weed China.

For scientific research on week in crop field, since 1950, herbicide control experiment and demonstration was started. In 1981-1985, investigation of weed damage was made. In 1986-1990, occurrence regulation and systematic control of *Avena fatua, Alopecurus aequalis, Echinochloa crusgalli, Cyperus rotundus* was studied. In 1991-1995, main target is weed population damage in 5 main crops field. The regulation of weed population change, weed control technology of one-application herbicide, ecological control of weed and agriochemical remind were studied. This article was one of the main achievement s of research work in 1991-1995.

2. Method

Using fixed spots and sites systematic experiment and survey at a large area in different ecological area of 5 main crops fields, weed population components, damage, changing reason and main weed changing regulation were studied.

2.1 Weed population change at rice field

Weed Research Center, Jiangshu Academy of Agriculture was responsible for researching work at transplant rice field at lower reaches of Yangzhi River. Plant Protection Institute, Jilin Academy of Agriculture was responsible for researching work at directplant rice field at main planting area.

2.2 Weed population change at wheat Field

Shanghai Academy of Agriculture was responsible for researching work at winter wheat area at lower reaches of Yangzhi River. Plant Protection Institute, Qinghai Academy of Agriculture and Forestry was responsible for researching work at spring wheat area at Qingzhang Higland.

2.3 Weed population change at summer maize field

Food and Oil Crop Institute, Hebei Academy of Agriculture was responsible for researching work at summer maize area at Huabei Plain.

2.4 Weed population change at soybean field.

Plant Protection Institute, Helongjiang Academy of Agriculture was responsible for researching work at main planting area of soybean at Dongbei Plain.

2.5 Weed population change at cotton field

Plant Protection Institute, China Academy of Agriculture was responsible for researching work at planting area of cotton at Jianghan Plain at Hubei Province.

3 Results and analysis

3.1 Component and change of weed population at rice field

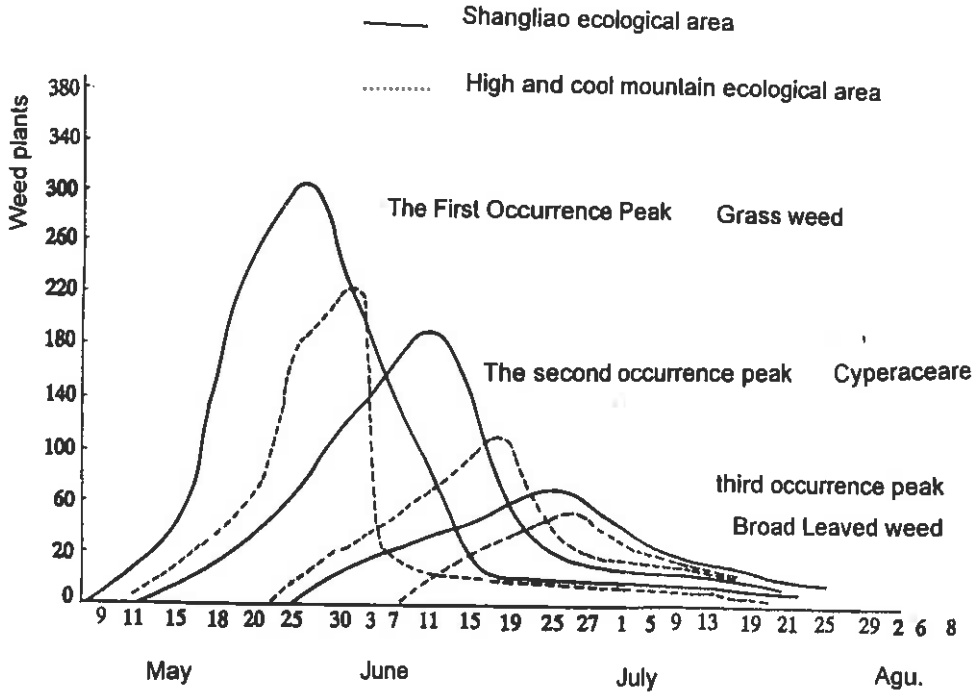
3.1.1 Transplant rice field: There are 69 species of weed at rice field at Jiangshu province, *E. crusgalli, Ludwigia prostrata, Leptochloa chinsis, Potamogeton distinctus,Juncellus serotinus, Eclipta alba, Cyperus difformis, Scirpus juncoides, Scirpus planiculmis, Paspalum distichum, Sagittaia pygamaea*, there are 8 main damage weed populations. The grass family weeds, annual and perennial Cyperus, and dicotyledonous weed component(%) were studied, see table 1. 19.8%, 31.4%, 30.7%, 18.1% at Xiuhuai; 13.0%,32.7%,32.2% 22.1% at Lixiahe; 38.6%, 39.6%, 14.4%.7.3% at Yuanhai; 26.8%, 30.8% 3.0%.39.4% at Jiang Rice Area; 8.8%, 33.0%,1.2%, 57.0% at Taihu Rice area, respectively. At resent year, the tendency of weed population change were: 1) damage of *S. Pygamaea, Lindernia procumbens, Monochoria vaginalis, Rotala indica, J. Serotinus, P. Distinctus, S. Planiculmis*, was increased. 2) Weed population change was accelerated.

Table 1 Components of Weed Population at Rice Field in Jiangshu

Rice Area	Grass Family	annual cyperus	perennial cyperus	dicotyle-donous
Xiuhuai	19.8	31.4	30.7	18.1
Lixiahe	13.0	32.7	32.2	22.1
Yuanhai	38.8	39.5	14.4	7.3
Yuanjiang	26.8	30.8	3.0	39.4
Taihu	8.8	33.0	1.2	57.0

(Jiangshu Academy of Agriculture)

3.1.2 Direct plant rice field : Weed investigation of 31 species at Jilin showed that annual weed of 18 species, perennial weed of 13 species. 5 species were serious damage. Frequency of *E. crusgalli*, population was above 60%. At high mountain area, *E.crusgalli, S. planiculmis* were superior species in these population. The second group were *Sagittaia sagittifolia, P. Distinctus, Monochoria korsakowii*, about 40%. Besides *E. crusgalli and S. Planiculmis*, population of *M. Korsakowii and Eleocharis yokoscensis* was 30-40% in Shongliao plain. The first occurrence peak of weed was from at the late of May to at the early of June at field, *E. crusgalli* mainly. The secend occurrence peak of weed was at the middle of June , *S. planiculmis, M. korsakowii, S. sagittifolia and P. distinctus* mainly . The third occurrence peak of weed was at the early of July, *Najas minor, Spirogyranitide* mainly, see graph 1.

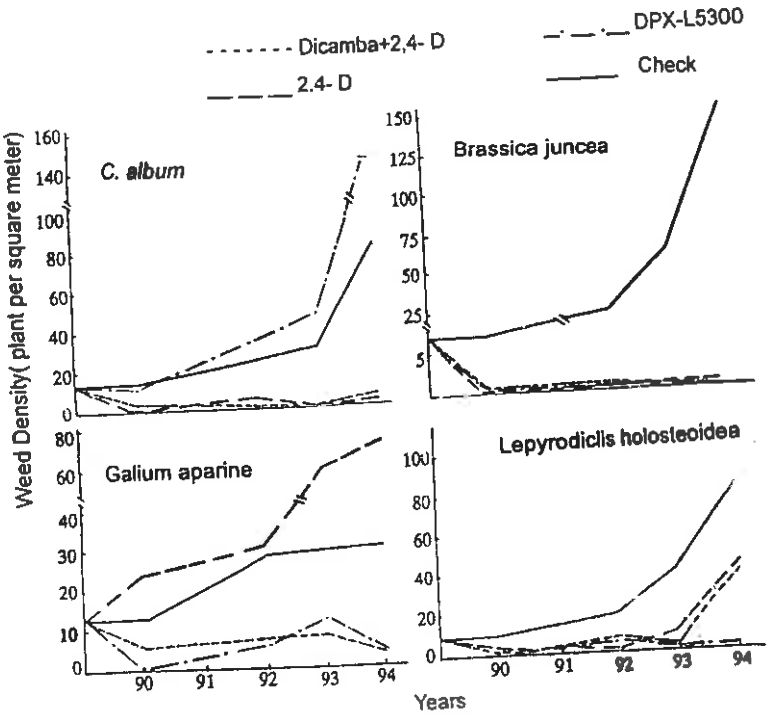


Graph 1: Weed Growth at Direct Planting Rice Field

3.2 Weed population change at wheat field

3.2.1 Winter wheat field: Shanghai Academy of Agriculture studied the change of weed population at winter field from 1981-1991. The original dominate weeds, *Alopecurus aequalis, Malachium aquaticum* decreased by 26.3%, but original secondary weeds, *Beckmannia syzigachne, Alopecurus japonicus, Polypogon fugax* increased by 34.0%, 21.8%, 19.8% at occurrence area respectively. The result was: damage area of weed at winter wheat field increased by 23.4% during 10 years.The main reason, which created this situation, was application of a single herbicide, chlorotoluron for a long time; continuous cropping of rice-wheat for a long time; less green manure; no tillage or less tillage,. All of those, increased the change of weed population.

3.2.2 Spring wheat field: In Qinghai, there are 182 species of weed, *A. fatua, Chenopodium album, Elsholtzia densa, Convolvulua arvensis, Polygonum aviculare, Lepyrodiclis holosteoides, Cephalanoplos setosum, Sonchus brachyotus* mainly. There were 8 main damage weed population. The change of weed population during 10 years were: 1)component of weed population did not change a lot, but the serious damage rate of weed was decreased from 21.4% to11.6% by extensive chemical control of weed in a large area, weed damage was controlled evidently; 2) damage index of weed, *A. fatua, E. densa, P. Aviculare, C. album* decreased 15.0%, 8.0%, 9.5%, 7.4%, respectively, but damage index of perennial weed, *C. Arvensis, S. Brachyotus, C. setosum* increased 1.9%, 1.5%, 1.9%, respectively. For researching population change of 10 species of weed with systematic survey of 7 herbicides at fixed spot and site during 5 years, the result see graph 2



Graph 2 Effect of herbicides on weed population change after applying continuous (Qinghai Academy of Agriculture and Forestry)

A. fatua was controlled applying Triallate continuous in 5 years, but dicotyledonous weed increased 4 times. Sensitivity dicotyledonous weed, *C. Album, Brassica juncea, Thlaspi arvense, L. holosteoides*, were controlled very well after using 2.4-D five years, but *A. fatua* increased 14 times, dicotyledonous weed which was resistance chemical, *Galium aparine, Galeopsis bifida, Polygonum convolvulus* increased 1-3.5 times, 8.6 times for *Hypecoum leptoearpum*. *C. album* increased 9.6 times after using DPX-L5300 for 5 years continuous. These kind of resistance chemical weeds became dominate population speedy, serious damage, so that some of herbicides was loss of control effect.

3.3 Weed population change at summer maize field

Investigation showed that: there are 44 species of weed at summer maize field in Hebei Province, monocotyledonous weed of 12, dicotyledonous weed of 32. Occurrence frequency of *Digitaria sanguinalis* was 92.3%. Next group was *Portulaca oleracea, Eleusine indica, Acalypha australis Alternanthera retroflexus*. There are 5 main weed population. According to improving of irrigation and increasing of multiple crop index during 1970-1980, dominate weed population was changed from *C. album* at spring maize field to *D. Sanguinalis, E. indica* at summer maize field. Because of chemical control extension, density of weed population changed evidently, the density of *D. Sanguinalis, E. indica* changed from 246 plants per square meter to 54 plants per square meter, and from 114 plants per square meter to 25 plants per square meter. Some new weed, wild Chinese sorghum, *Eriochola villosa* increased at fields. Occurrence of weeds at field were: intercropping field> no tillage field>harrowing field>covering field.

3.4 Weed population change at soybean field.

There are three regions for planting soybean in Helongjiang, east part, north part and west part. 810 spots were investigated, results showed that: there were not a big change in weed population component during 10 years. The main weeds were: *E. crusgalli, A. retroflexus, C. album, E. patrini, Commelina commnnis, Equisetum arvense, Xanthium sibiricum, Polygonum bungeanum, S. brachyotus, A. australis,* compared with 10 years ago see table 2. The quantity and damage of annual grass weed decreased. Frequency(%) and damage index of *E. crusgalli* decreased evidently at north, east and west parts of Helongjiang Province, from 96%, 95% and 98% to 80%, 56% and 77% of frequency, and 10,55 and 35 to 7, 23 and 24 .of damage index. The damage of *A.fatua, Setaria viridis and Setaria lute* decreased obviously. On the other hand, occurrence and damage of annual weed, *C. album, A. retrofilexus, C. communis, E. patrini,* and perennial weeds, *S. brachylus and Cirsium segetum* increased evidently. Frequency of *C. album* increased from 36% to 44%, from 11 to 18 of damage index; frequency of *A. retroflexus* increased from 37% to 50%, from 15 to 27 of damage index. The main reason of this results were: herbicides controlled grass weed were applied at a large scale and for a long time; the grass weeds was controlled very well, but broad leaved weed increased greatly.

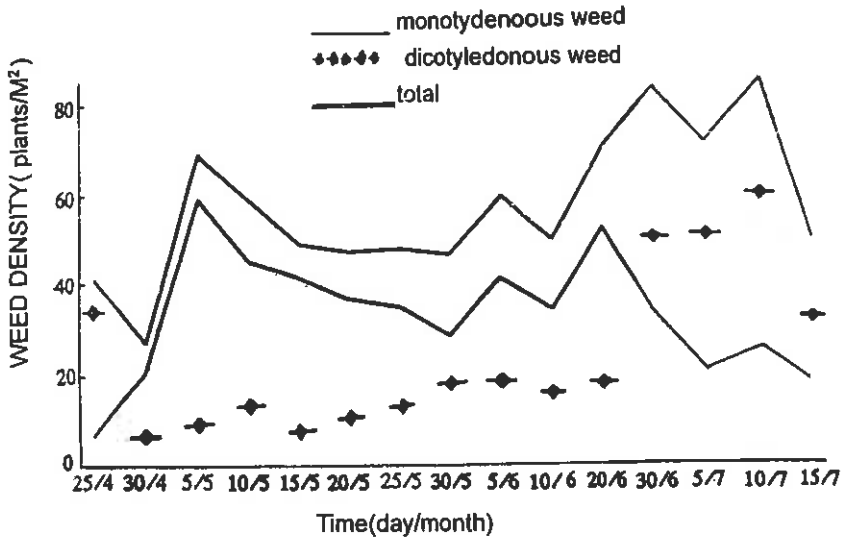
Table 2 Occurrence Frequency of Weed at Soybean field in Helongjiang

Name of Weeds	Frequency(%)					
	West		North		East	
	1982	1992	1982	1992	1982	1992
Echinochloa cnrus-galli	96	80	95	56	98	77
Setaria viridis	22	13	52	3	4	8
Avena falua	53	10	28	11	1	<1
Chenopodium ablum	33	65	42	26	33	12
Amaralhus relroflexus	48	72	35	54	30	24
Polygonum bungeanum	42	15	25	23	23	19
Polygonum lapathifolium	3	19	3	<1	23	18
Commelina communis	25	37	40	39	66	66
Elshollxia patrini	29	40	<1	53	32	34
Xanthium strumarium	36	31	35	14	34	15
Equiselum arvense	53	40	55	69	59	36
Sonchus brachylus	17	25	23	10	42	32
Setaria lute	34	2	47	11	<1	9
Phragmites communis	<1	<1	<1	<1	15	18
Acalypha australis	<1	4	15	30	25	14
Polygonum convolvnlus	22	32	14	23	2	2
Galeopsis bifida	22	9	21	13	3	
Amethystea colerulea	8	42	36	17	10	4
Roripa palustris	1	1	<1	15	37	12
Girisium setosum	9	7	2	14	19	4
Girisium segetum	10	19	<1	<1	12	14
Artemisia sp.	4	15	<1	7	24	6
Teloxys aristata	7	13	<1	1	<1	<1
Stellaria media	<1	<1	5	26	<1	< 1

(Heilongjiang Academy of Agriculture)

3.5 Weed population change at cotton field

72 species of weed was investigated at cotton field in Jianghan Plain of Hubei, 44 species at wheat-cotton field. The main weeds were *D. Sanguinalis, E. indica, L. chinensis, A. retroflexus, Eclipt. alba, A. australis, Cyperus. rotundus, Veronica didyma, Mazus japonicus and Pinellia ternata* etc. Compared the results between "8.5" and "7.5", occurrence of grass weed such as *D. Sanguinalis* decreased by 18.1%, but occurrence of broad leaved weed such as *A. retroflexus and V. didyma* increased by 9%. The dominate weeds changed into *V. didyma and L. chinensis* at seedling and boll period of cotton. The regulation of weed population change at cotton field see graph 3.



Graph 3 Weed Growth At Cotton Field

4 Conclusion and Disscussion

It was the first time for us to systematic study the components, damage and change of weed population during "8.5" in 5 main crops fields, rice, wheat, maize, soybean and cotton, at different ecological regions using fixed spots and sites experiment, and survey in a large scale.

Because of the improvement of cultivate measure, adjustment of industrial construction in country, labor power transformation, especially application of a single herbicide for a long time continuous, the change of weed population was accelerated at farm field. The change cycle of weed population was 4-8 years. Some new damage weed and perennial weed were appeared at farm field such as *S. brachylus, C. segetum, C. arvensis and S. planiculmis*, which were very difficult to control. Occurrence and damage of broad leaved weed at dicotyledonous crops field would be more serious. This is a new challenge to weed control in future.

This researching work was only a achievement at a identified stage. Research on change of weed population was a task for a long-term. Recommendation was: systematic study should be enforced on different ecological regions so as to predict the weed damage, and to make the integrated measure for weed control.

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PLANT INTERACTION IN VEGETATION OF A MODEL LEVEE OF PADDY FIELD

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Abstract: The native bio diversity of plant species on levees of paddy rice fields was believed to be maintained through traditional management practices such as cutting to control weeds. The authors are investigating the ecological characteristics of the species constructing vegetation of levee and analyze the interaction of plants in the levee vegetation to estimate the role of traditional management for maintaining the plant diversity. We constructed a model levee where four native perennial species, *Artemisia princeps* Pampan., *Lotus corniculatus* L. var. *japonicus* Regel, *Imperata cylindrica* (L.) Beauv var. *koenigii* (Retz.) Durand et Schinz and *Equisetum arvense* L. were planted. We analyzed the effects of cutting practices in this model system and also tried to clarify environmental adaptability and allelopathic characteristics of these plant species. The dry weight of underground organs of every species was reduced by a shade treatment. Except for the case of *E.arvense*, the growth of the underground organs recovered somewhat after exposure to light resulting from a cutting treatment. *E.arvense* has tolerance to swampy condition and *A.princeps* has tolerance to desiccation. *L.corniculatus* showed the highest allelopathic activity inhibiting the radicle growth or spore germination of the other three species. The allelopathic activity, estimated by the inhibition of radicle growth of *Digitaria ciliaris,* increased when two species were mixed. These results indicate that allelopathic activity within the plant community on a levee may be increased with coexistence of plural plant species.

Key words: levee of paddy rice field, interaction in vegetation, biomass changes of under ground organs, tolerance for water stresses, allelopathic activity

Introduction

The management of levees of rice paddy fields is one of the most important problems in paddy farming in Japan. Because, there are many subjects to be solved in paddy farm management as follow as rural depopulation, rural aging, becoming huge of levees by basis arrange of paddy fields and difficulty of mechanization or elimination at management of levee (Tomosho *et al,* 1994). Many studies and technological developments have been held about reduction of labor and costs for levee management. On the other hand, it has been noticed recently that native bio diversity of plant species on levees was maintained through traditional management practices such as cutting to control weeds in Japan (Okubo and Maenaka,1995, Yamaguchi and Umemoto, 1996). But the interaction of plants in vegetation of levee has not been studied.

The authors started the analysis of the interaction of plants in the levee vegetation to estimate and preserve the role of traditional management for maintaining the plant diversity in levee. We investigated

the effects of cutting practices in a model system and also tried to clarify the ecological characteristics such as environmental adaptability and allelopathic characteristics of the species constructing vegetation of levee, using four native perennial weed species

Materials and Methods

(1) The effects of cutting treatment for construction of vegetation in the model levee

The model levees were build along the paddy test field with paddy soil. Those levees are 10 meters in length, 60 cm in breadth, and 25 cm in height. Four native perennial species, Artemisia *princeps* Pampan., *Lotus corniculatus* L. var. *japonicus* Regel, *Imperara cylindrica* (L.) Beauv var. *koenigii* (Retz.) Durand et Schinz and *Equisetum arvense* L. were combined and planted in a test plot as follows Table1. Nine seedlings of material species grew up to about 10 cm shoot height were transplanted in a test plot with 60 cm x 60 cm at 16th July in 1997. Weeds except material species occurred in test plots were removed by hand. The shoots of all material plants were cut at 8th Aug. in 1997.

The test to clarify the effect of the number of cutting treatment in a year at the levee was held in 1998. One of the treatment (a) was four times of cutting in a year, 22 th April, 29th June, 11th Aug. and 2nd Oct., and another one (b) was only 2times of cutting in a year, 22 th April and 2nd Oct.. The amount of aerial part biomass and cover degree were measured and compared between the treatment districts.

Table 1. Combination of material species at model levee

Plot NO	Species	Plot NO	Species	Plot NO	Species	Plot NO	Species
1	Ar	5	Ar-Lo	9	Lo-Eq	13	Ar-Im-Eq
2	Lo	6	Ar-Im	10	Im-Eq	14	Lo-Im-Eq
3	Im	7	Ar-Eq	11	Ar-Lo-Im	15	Ar-Lo-Im-Eq
4	Eq	8	Lo-Im	12	Ar-Lo-Eq	16	Control

Ar: *Artemisia princeps*
Lo: *Lotus corniculatus* L.
Im: *Imperata cylindrica* L.
Eq: *Equisetum arvense* L.

(2) The effects of the change of light condition for material plants by shading and cutting treatment

The seedling plants of each species grew up to 10cm shoot height were transplanted to the unglazed pots 10cm diameter. The pots were placed in a greenhouse and set into cover with cheese cloth for shading treatments. Three levels of shading treatments were done as follows: (a) Control: no shading; 72% of quantum at outside, (b) S1: shading with a cheese cloth; 52% of quantum in the control, (c) S3: shading with three cheese cloth; 9% of quantum in the control. Shading treatments were started at 3days after transplanting. Cutting treatment was held at 23days after shading treatment, and all shades were removed and all material plants were exposed just after the cutting treatment. The growth of each organ was measured and compared at cutting date and 44days after the cutting treatment.

(3) Tolerance of material plant species against water stress condition

At the first, the bulk density of the soil used in this experiment was measured. The seedling cases with 700ml were filled with this soil. Next, Several levels of soil moisture content were set up with pouring water. The soil moisture percentage on an oven dry weight were set up to 17.4, 24.8, 29.3and 48.3% for the drought resistant test and 55.2, 72.2, 84.0 and 94.0(flooding condition)% were set up for the wet endurance test. Two under ground propagating organs were put into each seedling case filled the soil. The seedling cases were placed into an incubator in which temperature was controlled to 25°C and daylength was 12 hours. The soil moistures were checked by FDR soil moisture meter and controlled by pouring

water through experiment period. The growth of each organ of material plants was measured at the end of experiment after 40 days.

(4) Allelopathic characteristics of material plant species

(a) The reciprocal allelopathic activity in the material species were examined by Plant box method (Fujii and Shibuya, 1991b, Fujii and Shibuya, 1992) for the secretes from alive roots and by Sandwich method (Fujii and Shibuya, 1991a) for the secretes from dried leaves. Test plants were seedling plants of *A.princeps, L.corniculatus and I.cylindrica* hastening germination by incubation at 22-25 °C with moisture and spores of *E.arvense*. The allelopathic activity was measured by the inhibitory effect of radicle growth or spore germination. (b) The changes of allelopathic activity by mixing of two material plant species were estimated by the inhibition of radicle growth of *Digitaria* as a principal annual weed in levee, with Plant box method and Sandwich method.

Results and Discussion

(1) The effects of cutting treatment for construction of vegetation in the model levee

The cover degrees of almost test plots except the plot No.1 (*A.princeps* only) used to be reduced by increasing of cutting times. Especially, the cover degree and the number of plants of *L.corniculatus* were reduced by four times cutting compared with two times cutting. The cover degree percentage of *A.princeps* or *E.arvense* in a plot was reduced with *I.cylindrica* (plot No.6, 10, 11, 13, 14, 15) (Fig.1). The test plots which total cover degree of a test plot was more than 95% and the biomass produced in a test plot was less than 320g, were plot No1a (*A.princeps* only and 4 times cutting) and two times cutting plots including *E.arvense* (plot No.10b, 13b, 15b). These results indicate that cover degree and produced biomass of levee vegetation is affected more intensely by the characteristics of species as the speed of regrowth after cuttig or competitive ability than the number of species constructing levee.

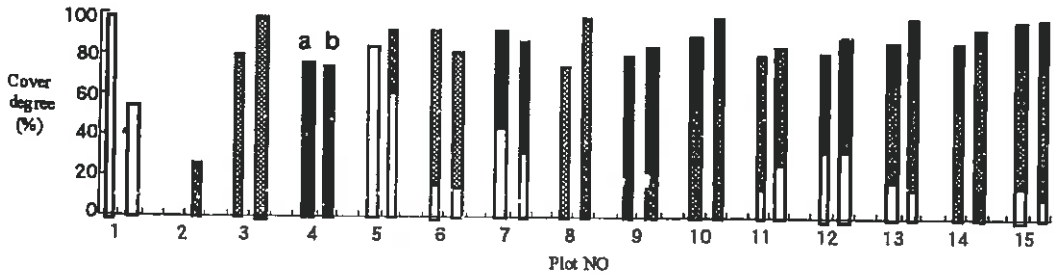


Fig.1 Cover degree of material species in model levee
This data was investigated at '98.9.21. Plot No follows as Table 1.
a: 4 times cutting, b:2 times cutting
□ :*A. princeps* , ■ :*L. corniculatus*, ■ :*I cylindrica* , ■ :*E. arvense*

(2) The effects of the change of light condition for material plants by shading and cutting treatment

The dry weight of underground organs of every species was reduced by shade treatment. Except for the case of *E.arvense*, the growth of the underground organs recovered somewhat after exposure to light

resulting from a cutting treatment(Fig.2). The leaf area per plant of every species except *A.princeps* was decreased by shading treatment. The leaf area per plant of *A.princeps* was increased by shading with a cheese cloth (S1) and obviously recovered after exposing treatment at the shading treatment plot with 3 cheese cloth (S3). These results suggest that the changes of light condition by cutting affects the growth of plants in paddy levee and the effects were different as species.

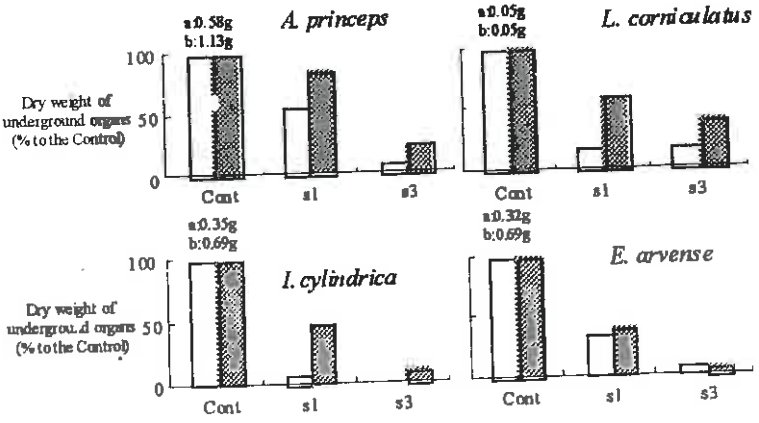


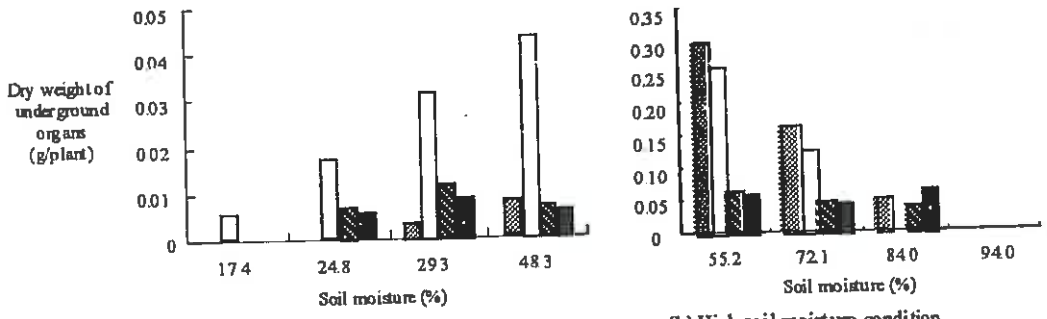
Fig.2 Effects of shading and cutting on the formation of underground organs

Numerals in figures show actual dry weight of underground organs in control plot (g/plant).
Cont: no shading; 72% of quantum at outside, S1: shading with a cheese cloth, 52% of quantum in the control, S3: shading with three cheese cloth, 9% of quantum in the control
□ a. Cutting time, ■ b: End of the Experiment

(3)Tolerance of material plant species against water stress condition

The viability of *A.princeps* was high and the rhizome formation was occurred under low soil moisture condition, although it was inhibited under the some condition. The viability of *E.arvense* was decreased by the drought and the tuber formation was inhibited as same as *I.cylindrica* and *L.corniculatus* (Fig.3a). These results suggest that *A.princeps* has tolerance to desiccation.

On the other hand, the viability of *E.arvense* was high, and the tuber formation was not inhibited under high soil moisture condition, although rhizome formation of *A.princeps* or *I.cylindrica* was inhibited. This result suggests that *E.arvense* has tolerance to swampy condition (Fig.3b).



(a) Low soil moisture condition (b) High soil moisture condition

Fig.3 Effects of soil moisture on the formation of undergound organs

▨ :*I. cylindrica*, □ :*A. princeps*, ▩ :*L. corniculatus*, ■ :*E. arvense*

(4)Allelopathic characteristics of material plant species

L.corniculatus inhibited the radicle growth of *A.princeps*, *L.corniculatus* and *I.cylindrica*, and spore germination of *E.arvense* on Plant box method experiments and Sandwich method experiments (Table2). These results show that *L.corniculatus* has the highest allelopathic activity in material plant species.

The allelopathic activity, estimated by the inhibition of radicle growth of *Digitaria ciliaris*, increased when two material species were mixed on Plant box method experiments and Sandwich method experiments. This result indicates that allelopathic activity within the plant community on a levee may be increased with coexistence of plural plant species.

Table 2. Alellopathic activity between the material species (Sandwich method)

Test species		*A. princeps*		*L. corniculatus*		*I. cylindrica*		*B. arvense*	
		radicle growth ratio to the control(%)		radicle growth ratio to the control(%)		radicle growth ratio to the control(%)		Spore germination*	
Material species	Dry weight of material leaves	10mg	50mg	10mg	50mg	10mg	50mg	10mg	50mg
A. princeps		100.0	100.0	81.3	64.2	64.0	60.1	+	+
L. corniculatus		70.3	49.5	54.1	46.8	64.0	60.1	-	-
I. cylindrica		90.5	80.1	90.7	77.2	72.3	53.5	+	+
B. arvense		86.9	84.7	91.8	65.3	77.8	63.4	+	++

*, -: not germinated, +: germinated, ++: fissioned after spore germination

The actual condition and structure of vegetation in levee was investigated and some characteristics concerned with interaction between plants in levee were clarified. We will have to analyze how the characteristics of plant species affect to the change of construction of vegetation in levee in the near future.

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PROGRESS OF STUDY ON WEED IN WHEAT FIELD IN JIANGSU PROVINCE

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Abstract: The article expounds the main species, distributions and damage of weeds in wheat fields of Jiangsu Province of P.R.China and discusses the biological and ecological characteristics of 12 malignant weeds, and the important effective factors in change of weed population. It also proposes the prospects for future research on weed population and community in wheat fields.

Key words: wheat field, weed, Jiangsu Province

Wheat is main cereal crops in Jiangsu Province and wheat-growing areas is 60 percent of total cultivated area. From 1995 to 1996, areas sown to winter wheat were more 2.8 million-hectare and total yield is more 12.2 billion-kilogram. It is evident that the production of winter wheat in Jiangsu plays an important role in home. But weeds in wheat field disadvantage the production. According to the investigation of Nation Farmland Weed Study Group, Wheat-grass areas are more 30 percent of total wheat-growing areas and yield loss is about 5 million kilograms due to weeds.

Jiangsu Province lies in the Changjiang River and Huaihe River valley with warm climate, rainy and clear seasons. So, wheat-grass is fairly serious. From 1950s, weed chemical control had been taken seriously on the basis of conventional management. Some herbicides were imported in 1960s. New herbicides and weed biology were studied in 1970s. Until 1980s, we researched and developed the techniques that weed biology and ecology combine with herbicide application. Obviously, the extensive research on weed occurrence, harm and its chemical control have taken a great pushing effect on weed management, yield increase and cultivation reform.

Because of varied soil and cultivation, the weed species in wheat are various in Jiangsu Province. More 160 species common weed belongs to 32 families and most of them are annual weed growing in winter. But its community structure and distribution are manifold in accordance with the natural environment and cultivation of different wheat plantation region. Especially, dominant species had changed two times from 1980s because of one-crop farming, till-less and successive application of single herbicide. In the middle of 1980s, *Alopecurus aequalis* Sobol., *Vicia sativa* L. and *Malachium aquaticum* L. were replaced by *Alopecurus japonicus* Steud. and *Galium aparine* L.. Presently, in southern region of Huaihe River, *A. aequalis* Sobol. (*A. japonicus* Steud.) or *Sclerochloa kengiana* Tzvel. or *Beckmannia syzigachne* Fernald. is dominant species in wheat field after rice stubble, *Avena fatua* L., *G. aparine* L., *Cerastium viscosum* L. and *Veronica persica* L. in wheat field after non-irrigated crop. But in northern region of Huaihe River, *Descurainia sophia* L. and *G. aparine* L.are main weeds in wheat field after non-irrigated crop, *V. persica* L., *C. viscosum* L. and *G. aparine* L. in wheat field after cotton stubble. Within the whole province, weed in wheat field after rice stubble is more than in wheat field after non-irrigated crop due to soil moisture. The study on biological and ecological characteristics of weeds including malignant weeds enhanced the weed control in wheat field. Now, main malignant weeds in wheat field as

follows: *A. aequalis* Sobol., *A. japonicus* Steud., *S. kengiana* Tzvel., *B. syzigachne* Fernald., *A. fatua* L., *G. aparine* L., *V. persica* L., *V. sativa* L., *Capsella bursa-pastoris* L., *D. sophia* L., *Polypogon fugax* Nees ex Steud and *Lithospermum arvense* L.. Occurring factors, growing law, breeding characteristics and herbicide-resistance of *A. fatua* L., *S. kengiana* Tzvel., *B. syzigachne* Fernald., *A. aequalis* Sobol., *V. sativa* L., *G. aparine* L. and *L. arvense* L. were researched. The relation of malignant weeds such as *A. aequalis* Sobol., *S. kengiana* Tzvel., *A. fatua* L., *G. aparine* L., *V. sativa* L., *D. sophia* L. and *V. persica* L. with wheat yield loss was studied. These studies provided scientific basis for weed investigation and weed management (including techniques, critical value, index, tactics, means and intensity).

Among the malignant weeds, *A. aequalis* Sobol. and *A. japonicus* Steud. scatter in wheat field after rice stubble in plain, which is characterized by lower, wetter and acid clay. They possess big reproductive capacity, high occurring frequency, high density and strong competition. Moreover, *A. japonicus* Steud. have stronger competition to wheat and resistant to common herbicides than *A. aequalis* Sobol.. This is also main reason that *A. japonicus* Steud. replaced gradually *A. aequalis* Sobol.. Rate of germination of *A. aequalis* Sobol. could increase in accordance with tilling depth, which would lead to later peak and longer of occurrence. This could add difficulties to centralized management of weed. But continuous till-less could gather its most seeds at the surface of soil in order to control. Rotation of wheat with inter-tilled crop or green manure crop is also. In addition, critical value of economical control to *A. aequalis* Sobol. is 12.6 plants per 0.11 square meters at wheat yield 4125Kg/ha.

S. kengiana Tzvel. distributes in sandy or loamy wheat field after rice stubble with pH7.0-8.5. Generally, it has two occurring peaks that they are about four months apart. Now, it has been proved that its dissemination is related closely to fertilizer, irrigation and purity of wheat seed and that its competitive critical period to wheat is about 20-50 days after wheat emerging because effective tiller of wheat is depressed. Critical value of its economical control is 98.7 plants per square meters at wheat yield 4500Kg/ha. For shorter valid term of existing herbicides, its development is increasing.

A. fatua L. is widespread distributed due to its stronger adaptability to soil acidity. Formerly, it harmed the wheat after non-irrigated crop only. The case was different from the past from the end of 1980s. In 1990, its occurring areas are more 70 thousand ha that was 16 percent of total wheat areas in Yangzhou city. The main reasons were considered as follows: 1) New ecotype adapting to rotation of wheat and rice had developed. 2) Sulfouylurea herbicides were applied successively. 3) Warming winter was favor of its over-winter. 4) It was spread in the course of seed exchange. From returning green stage to jointing stage is its competitive critical period to wheat and critical value of its economical control is 5 plants per 0.11 square meters. In the aspect of its integrated management, comprehensive utilization of chemical control, ecological control and tillage could control it effectively.

G. aparine L. that harmed to wheat in the northern region of Huaihe River in 1970s is main broadleaf dominant species in the whole province, especially in region of Lixia Revier and hilly land. Its harmfulness to crop is more serious in wheat field after rice stubble than after non-irrigated crop. Few herbicides could be used effectively because of its weak susceptibility to most herbicides. Its critical value of economical control is 10 plants per square meters.

V. persica L. suits to alkaline soil or sand. Its widespread occurrence, high fruitage, serious harmfulness, vegetative propagation and strong resistance to most herbicides add many difficulties to its control. Its critical value of economical control is 5 plants per 0.11 square meters.

B. syzigachne Fernald. occurred in wheat in 1985 because of its extremely high fertility and rapid spread. But its harmfulness to wheat steep rose in a few years. Now, It distributes in clay wheat field after

rice stubble with pH value 7.0-7.5. In some wheat fields, it has replaced the *A. aequalis* Sobol. and *A. japonicus* Steud. and has become main malignant weed. The main reasons of its increasing development are as follows: 1) Method of planting wheat on rice stubble directly provides suitable ecological environment for *B. syzigachne* Fernald.. Rotation of wheat and rice satisfies the needs for it breeding. 2) Slack sowing and management enhance its occurrence. 3) We applied sulfouylurea herbicides successively. It is the March that *B. syzigachne* Fernald. competes intensely with winter wheat and that is critical period of controlling it. It was tried that early seeding, rotation of wheat and rape, good irrigation and tillage could reduce its harmfulness.

V. sativa L. can suit to soil acidity and texture in large scope. Vigorous growing and high natural seed shedding cause remarkable unfavorable influence on wheat. Generally, *C. bursa-pastoris* L. grows in sandy and loam with pH 7.0-8.5, especially in wheat field after non-irrigated crop. It is important to control it before spring because of its early florescence. *D. sophia* L. often occurs in saline-alkali soil and harms to wheat after non-irrigated crop.

P. fugax Nees ex Steud often scatters in lower and wetter wheat field. Its critical value of economical control is 107.69 plants per square meters. *L. arvense* L. is most common in clay and loam with pH7.0-7.5, especially in wheat after soybean. It is important spreading way by mixing up with wheat seed, wind and water.

Concerned factors influenced weed occurrence in wheat field, we often consider that these factors are important: 1) The earlier we plant wheat, the more the weeds emerge. 2) The more it rains in the period of sowing, the faster the weeds germinate. 3) Farm manure without decomposed thoroughly is favor of weed seeds diffusing and invading again in field. 4) Extensive cultivation would lead more serious harmfulness of weed to crop. 5) Occurring rate would be slow in loam and it is not the case otherwise. 6) The higher the temperature is in the period of sowing, the faster the weeds germinate. 7) Cultivating method influences the weed quantity but not emerging rate notably. 8) Change of cultivation may favor of some weed development.

Summarily, weed species and community distribution are different in accordance to natural environment and rotation in different wheat plantation region. For varied situations, our task of sustained controlling weed in wheat field will be arduous. These studies must be reinforced further, including studies on weed biology and ecology. 1) Investigate and forecast the weeds in different wheat plantation region under consideration of rotation and herbicide application. 2) Explore the feasibility of foresight weed management by studying on critical value and mixture population dynamic. 3) We should pay attention to economical cultivation that put forward the new problems to our practical work.

(110 references are omitted)

SEEDLING IDENTIFICATION OF MORNINGGLORY WEEDS IN PINEAPPLE

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Abstract : The objective of this study was to identify the seedling of morningglory weeds in pineapple production system. Three morningglory species, *Ipomoea obscura* (L.) Ker-Gawl. (small-white flower morningglory), *Ipomoea triloba* L. (three lobe morningglory) and *Ipomoea maxima* (L.f.) Don ex Sweet were identified according to van Ooststroom and Hoogland (1953) in Flora Malesiana Ser. 1 Vol. 4. However, seedling of these three species can be identified easily by the difference of cotyledon shape. In the eastern region of Thailand such as Rayong and Chonburi Provinces, they are the pineapple production area, the small-white flower morningglory was the most serious weed compared with the other two species.

Keywords : morningglory weed, *Ipomoea obscura* (L.) Ker-Gawl, *Ipomoea triloba* L., *Ipomoea maxima* (L.f.) Don ex Sweet, pineapple

Introduction

In Thailand, the convolvulaceae, well known as morningglory family is represented by 22 genera and about 102 species (Kerr, 1951; 1954). Most of them are herbaceous or woody twines, and commonly found all over the country. They are sun-loving plant occupying at the edge of forests, along road sides or in waste areas. These habitats have tendency to be disturbed by human activities, such as buildings, road construction and agriculture expansion, etc. (Na Songkla and Khunwasi, 1993). They were reported by Loasinwattana (1994) that their invasions in pineapple areas in the eastern region of Thailand were very difficult to be controlled by herbicide applications. However, they are called in Thai vernacular in the same word as " Sa-uek" though they are different species, and which species is differed from the others is not to be exactly known. Radanachaless and Maxwell (1997) reported that the morningglory weeds in Thailand composed of 10 *Ipomoea* spp. and 2 *Merrimia* spp., but the name " Sa-uek" was referred to *Ipomoea gracilis* R.Br., *Ipomoea obscura* (L.) Ker-Gawl, *Merrimia aegyptia* (L.) Urb. and *Merrimia hederacea*. Whereas Na Songkla and Khunwasi (1993) reported that this name was referred to *Merrimia emarginata*, *Merrimia gemella* and *Merrimia hederacea*. Samitinand (1980) reported that *Ipomoea maxima* and *Merrimia emarginata* were also called in Thai vernacular as " Sa-uek". Therefore, the objective of this study was to identify the morningglory weeds in the pineapple production area, in Rayong and Chonburi provinces, which is approximately 200 km east of Bangkok. The results of this study will be useful for the other researchers especially in the field of weed biology, weed competition and weed control in the case of species identification.

Materials and Methods

The morningglory weeds in pineapple production area of Siam Agro-Industry Co,Ltd. (public) were surveyed their invasions in June, 1997. Seed of each specie found in the field was collected. Seedlings were planted in the greenhouse at the Department of Agronomy, Kasetsart University in order to examine the taxonomic characters of each specie and to identify according to 5 keys:

1. Backer and Bakhuizen Van Den Brink (1965); Flora of Java Vol. 2
2. Busban and Chumphol (1993); Thai Forest Bulletin (Botany) No. 20
3. Elmore *et. al.* (1990); Reviews of Weed Science Vol. 5
4. van Ooststroom and Hoogland (1953); Flora Malesiana Ser. 1. Vol. 4
5. Small (1972); Manual of The Southern Flora

Each specie had been studied from fresh material as well as the herbarium specimens of the Department of Agriculture in October 1997, and seedlings were planted again in November 1997 to study some characters such as cotyledon shape of each specie. Identification at seedling stage was necessary for a research dealing with pre-emergence herbicide application.

Results and Discussion

Most of morningglory weeds in the pineapple production area were *Ipomoea* spp such as *I. obscura* (L.) Ker-Gawl (small-white flower morningglory), *I. triloba* (L.) (three-lobe morningglory) and *I. maxima* (L.f.) Don ex Sweet. It was found that *I. obscura* (L.) Ker-Gawl was the most serious weed compared with the other two species. The most suitable key for identification of these species was Flora Malesiana Ser. 1 Vol. 4 by Van Oostsroom and Hoogland (1953) which can be summarized as following.

Ipomoea spp.

Flowers axillary, solitary or in 1-α-flowered cymes, sometimes in panicles; bracts small or large; sepals equal or unequal, often enlarged after anthesis; corolla actinomorphic, rarely sub-zygomorphic, small or large, campanulate, funnel-shaped, or hypocrateriform, (often very) shallowly 5-lobed, with a distinctly confined midpetaline area over the middle of each lobe; stamens shorter or longer than corolla; pollen spinulose; ovary 2- or 3-celled with 2 ovules in each cell, or 4-celled with 1 ovule in each cell; style 1; stigmas 2, rarely 3, globose or semiglobose; capsule 3-4- or 6-valved, or irregularly dehiscent; seeds 1-6, glabrous or hairy. Leaves simple, entire or palmatilobed-palmatipartite, rarely pinnatipartite. Sinistrorsely twining, creeping or sometimes erect herbs or shrubs.

1 Sepals obtuse, acute or acuminate, whether or not with a small subulate mucro, but not distinctly corniculate; corolla mostly funnel-shaped or subcampanulate, rarely hypocrateri-form; stamens mostly included, very rarely longer than corolla-tube.....2

2 b Corolla large to small, mostly funnel-shaped or campanulate, 10 cm long at most, but usually much shorter, rarely hypocrateriform, in latter case not longer than 6 cm, not white; sepals obtuse, acute or acuminate; seeds glabrous or hairy. Scandent herbs or rarely shrubs, or the stems prostrate of erect 3

3 b Plants otherwise; wild or cultivated, in latter case not planted for the edible tubers(when tubers are present).....4

4 a Calyx (sometimes slightly) hairy; stamens shorter than corolla..... 5

5 b Otherwise. Peduncle mostly longer; corolla 1.5 cm long at least.....6

6 b Otherwise. Flowers whether or not crowded at the top of the peduncle, not distinctly in volucrate 7

7 b Corolla glabrous; seeds pubescent or glabrous..... 8

8 b Sepals rounded or acute, often with a short subulate mucro, but not long-acumiate.....9

9 b Sepals oblong or narrowly oblong-ovate, acute, minutely mucronate, long-ciliate along one or both margins (hairs inserted on tubercles), pale green, 7-9 mm long, or outer one slightly shorter; corolla pink or pale purple, often with a darker center and pale midpetaline areas, 1¾ – 3 cm long; tube cylindric-obconical, strongly narrowed at base; lobse rounded hairs in lower half; ovary globose, pilose; fruit subglobose, crowned by thickened style-base pilose, 5-6 mm long. Leaves ovate, with cordate base, entire or 2-3-lobed, glabrous or slightly hairy.....10

10 a Cymes with very short branches, therefore dense, glabrous, 1-7-flowered; peduncle 1-10 cm, stout; pedicels 3-10 mm, firm, angular, glabrous, subverrucose, thickened at apex, sepals minutely mucronate; corolla 1¾ – 2 cm long; seeds glabrous or thinly hairy on ventral rib. Stem angular, galbrous or sparsely hairy; leaves 2 ½ – 12 cm by 2-10 cm; petiole 18 cm, glabrous, sometimes finely verrucose. Laticiferous, often much-branched. 1.00-3.00; I – XII; from tropical America; formerly introduced into Java; at present established in W.C.E. Mad.; 1-750; brushwoods, living fences, sugar-cane plantations, roadsides, fields, waste places.....*I. triloba* L. .....

4 b Calyx, outside of corolla, ovary, and fruit glabrous.....11

11 b Calyx otherwise.....12

12 b Stamens and style not longer than corolla-tube.....13

13 a Leaves in outline broadly ovate-orbicular, deeply palmatifid to palmatipartite, glabrous, not fleshy; lobes 5-9, entire; corolla funnel-shaped, shortly lobed, 4 ½ – 6 cm long; seeds pilose. Stem (when conditions are favourable) twining. Root(s) tuberous, All green parts glabrous.....14

14 c Otherwise again.....15

15 a Corolla 2-3 cm long, white, very pale yellow or pale purple, with a dark red or dark purple centre; sepals appressed against the corolla, subverrucose-rugose, ovate-oval-elliptic-oblong, acute or obtuse-emarginate, in latter case mucronulate, 3-6 mm long; fruit ovoid, globose or depressed-globose. 6-8 mm high; seeds hairy; pedicels thicker than the peduncle, finely warty. Leaves ovate or

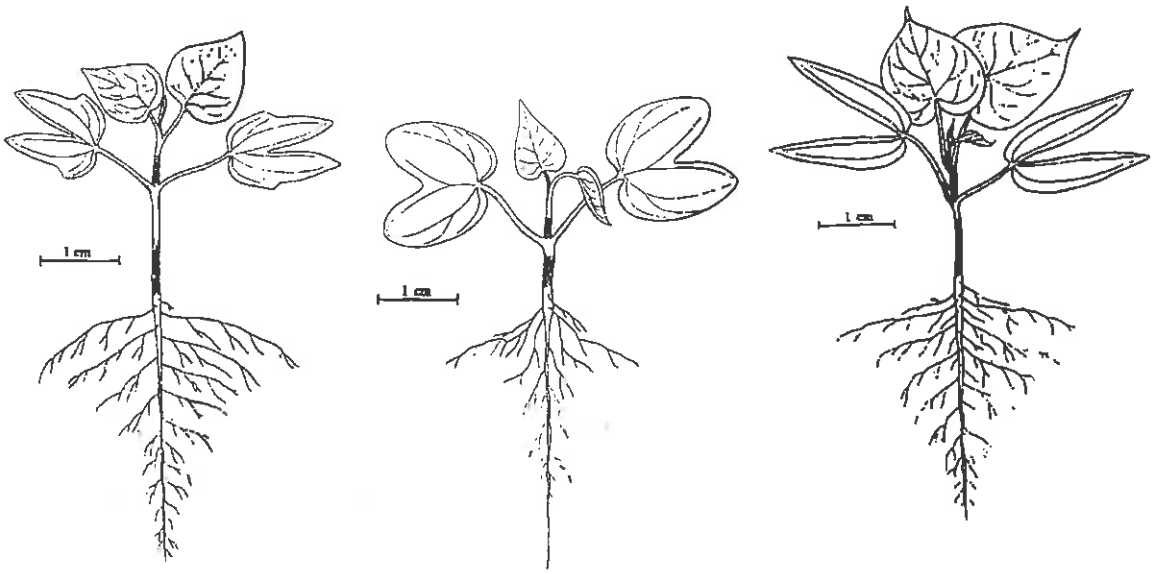
broadly ovate, with a cordate base, acuminate, acute or obtusish, un-lobed, sometimes subangular. 2-9 cm long, glabrous or ciliate. Stem thin, twining.....16

16 a Cymes 5-11-flowered, dense; peduncle 1½ – 14 cm. stout (often thicker than the stem), not rarely compressed; pedicels ¾ - 1 cm; sepals broadly oval, obtuse-emarginate, minutely mucronate, warty without, their margins thinner, corolla 2-2 ½ cm long, light purple or almost white, with a dark purple centre; fruit depressed-globose; seeds very finely white-hairy; marginal hairs longer. Stem glabrous, or occasionally patently pilose; leaves entire or sub-angular, shortly pubescent near margin, somewhat fleshy, 2-8½ cm by 1¼ - 9 cm; petiole 1½ - 4 cm. 1.50 – 2.50; I – XII; W.C.E.,Mad.; ¼ - 50; humid, often somewhat brackish places, but also on non-saline soil; lawns, fields, living fences, sugar-cane plantations, brushwoods, waste places (*I. sepiaria* Koen. ex Roxb.).....*I. maxima* (L.f.) G. Don ex Sweet

b Cymes 1-2-, rarely 3-flowered, peduncle 1½ - 14 cm, thin, glabrous or finely hairy; pedicels 1-2 cm; sepals ovate-elliptic-oblong, subacute, subverrucose-rugose, glabrous or rarely hairy; corolla 2-3 cm long, white or very pale yellow, with a dark red centre; ovary concial; fruit ovoid-globose, conical towards apex; seeds densely covered with white velvety hairs. Stem on the younger parts sparsely to rather densely covered with white patent fine, often rather long, white hairs; leaves entire, glabrous or ciliate, 2-10 cm by 2-9 cm; petiole 3½ - 10 cm. Often several-stemmed. 1.00 – 2.00; I– XII; W.C.E., Mad.; 1- 1300; brushwoods, hedges, open forest, waste places..... ..... *I. obscura* (L.) Ker-Gawl.

However, from the results of this work, seedlings of these three species can be identified easily by the difference of their cotyledon shapes (Fig. 1).

For future works, biology and herbicide susceptibility or resistance of the small-white flower morningglory will be studied in order to determine the cause of infestation and the control of this weed in the pineapple production area which found only in the eastern region of Thailand.



Ipomoea triloba L.
(Threelobe morningglory)

Ipomoea maxima (L.f.) Don ex Sweet.

Ipomoea obscura (L.) Ker-Gawl.
(Small-white flower morningglory)

Fig. 1 Seedlings after germination of three *Ipomoea* spp. with the difference of their in cotyledon shape, at 15 days.

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SOME AGRONOMIC CHARACTERISTICS AND SEED DOMANCY STUDY OF 12 WILD RICE (*Oryza rufipogon*) ACCESSIONS FROM DIFFERENT ORIGINS.

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Abstract: Agronomic characteristics and seed dormancy of 12 wild rice (*O. rufipogon*) accessions were investigated at Agronomy Department Kasetsart University, Bangkhen campus from 1997 to 1998. These accessions, collected from different origins, were grouping into 3 groups. Group I : wild rice from Asia (India, Sri Lanka, Myanmar, and Malaysia, Group II : weedy rice from Thailand and Group III : wild rice form South America (British Guiana, Manaus and Brazil). It was found that the phenotype within each group differs in maturity, flowering period, 100 seed weight and dormancy period. Three hull color groups : straaw, darkbrown and black were recognized.

Key words: Wild rice, agronomic characteristic, seed dormancy.

INTRODUCTION

Wild rices or wild relatives of rice play the two significant roles in rice production. First, as a weed in paddy field. The infestation of weedy wild rice has been reported in many countries and very difficult to control, since the infestation is persistent and spreads rapidly due to easy grain shattering and deep seed dormancy (Baldwin, 1978; Cox, 1978). Second, as an important reservoir of useful genes for rice improvement for example, genes for resistance to pests and diseases, brown plant hopper, bacterial blight and also the cytoplasmic male sterility gene which was a key component in the development of hybrid rice production in China (Vaughan, 1994).

As an important tool for rice production as mentioned, therefore, some agronomic characteristics and seed dormancy of wild rice accessions had been evaluated. The informations from this experiment should be useful in devising strategies for control weedy wild rice or improve rice production.

MATERIALS AND METHODS

Accessions

Twelve accessions of wild relatives of rice were selected for study, five accessions from Asia, another four from Thailand, both belonging to O. *rufipogon*. The last three collected from south America which belonging *to* O. *glumaepatula* (Table 1). The cultivar Nipponbare, LMN 111 and KDML 105 were included as the control.

Field procedure

The experiment was conducted in greenhouse at Agronomy department, Kasetsart University, Bangkok during July 1997-June 1998. The randomized complete block design with 4 replications was employed. Seeds from each accession were pre-drying at 40°C for 10 days in order to overcome seed dormancy, germinating on petri-dish until the seedling reach 2-3 leaf stage. The three seedlings were then transplanted into the 12 inch diameter pot filled with the loamy paddy soil from paddy field nearby the experiment site. Before transplanting, 9.875 gm/pot of 16-16-16 was

applied and top dressing with 3 gm/pot at the panicle initiation stage. Pesticides were applied as necessary.

Data collecting

Agronomic characteristics

- Plant height
- Number of tiller
- Days to 75 % heading
- Number of panicle
- Panicle length

Seed characteristics

- 100 seed weight
- seed size
- seed color
- Awn length

Seed dormancy

Dormancy status of freshly harvested seed was determined by germination test without any special treatment. Four lots of 25 seeds were placed on germination blotters at 10 days intervals in order to monitor the rate of dormancy loss. Seedling counts were made according to the ISTA Rules (1996). At the end of testing period, the ungerminated seeds were bisected and their viability determined by staining in 0.1 % w/v tetrazolium salt. The samples were classified as "non dormant" when the germination percentage exceeded 80 % (Anusompanich *et. al.*, 1990).

RESULTS AND DISCUSSION

According to the different origin, these accessions were then grouping into 3 groups (Table 1). In each of characters investigated, the distribution of measurements was compared with those accessions and control varieties.

Agronomic characteristics

The variation of some agronomic characters were presented in Table 2. Plant height measured at 60 days after transplanting varied from 67 cm (India No.1) to 150 cm (LMN 111, the deep water variety). In contrast to plant height, there were a tendency that an accession with short plant type has higher tillering capacity and produced highest number of panicle per plant as found in accesion from India No. 1 and followed by the one from Saraburi. These relationship has been noticed by Hyakutake *et al* (1984). Moreover, it was clearly showed that wild relatives of rice have higher tillering capacity than cultivated varieties.

The great interesting was the percentage of fruitful culms. It was found that most of accessions had high productivity such as accession from Chean Yai, British Guiana, India No. 1 and Sri Lanka. These advantage characters along with the tolerance of plant to environmental stress make them to be the troblesome weed in the plantation area (Huey and Baldwin, 1978).

Of even greater interest and significance was flowering period which varied from 8 days to 93 days, whereas the cultivated varities need only 7 days and 22 days to complete the flowering. This prolong heading is one of inherent characters of wild type of the Gramineae family found in many tropical grasses (Boonman, 1980).

Seed Characters

Seed characteristics of wild rice accessions were sumeried and presented in Table 3. Most of wild rice accessions were heavy shattering, only two accessions, Nakorn Si Thammarat and Brazil were moderate shattering and owned straw and black hull color respectively, whereas the main color was deepbrown. Long awning, the typical wild character of rice, varied from 5.94 cm to 11.88 cm and compared to awnless in cultivated varieties.

It was noticed that, most of the wild rice accessions produced lower seed weight than cultivated varieties. However, all accessions from South America showed the interesting 100 seed weight when compared to cultivated varieties.

Seed dormancy

The monthly germination percentage of all accessions were presented in Table 4 from data taken at 10 day intervals. There was no emergence for most of wild rice accessions in the first 2 months after harvest. It was noticed that, dormancy of Brazil accession was initially somewhat less intense and was released most rapidly within one month as same as cultivated rice, Niponbare. From the distribution of germination, therefore it can be concluded that most of the wild rice accessions had high degree of dormancy, particularly the accessions in group I, which prolonged their germination longer than 6 months whereas cultivated rice, LMN 111 and KDML 105 had shallow dormancy. Cultivar Niponbare was counted as non-dormant variety.

Conclusion

Wild rice accessions differed in various agronomically traits. The marked differences are found in tiller number, panicle number, percentage of fruitful culm and flowering period. Seed of wild accessions showed the common and distinguishing characteristic of troblesome weed are : red pericarp, early and heavy grain shattering and persistent and deep dormancy. These differences were mainly due to the genetic variability in each type as reported by Morishima et al (1980).

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Table 1 Details of wild rice accessions selected for study.

Accession No.	Species	Strain	Origin
	Group I		
1	*O. rufipogon*	W-0106	India
2	*O. rufipogon*	W-0120	India
3	*O. rufipogon*	W-0593	Malaysia
4	*O. rufipogon*	W-0630	Myanmar
5	*O. rufipogon*	-	Sri Lanka
	Group II		
6	*O. rufipogon*	WR-1	Thailand (Saraburi)
7	*O. rufipogon*	WR-3	Thailand (Singburi)
8	*O. rufipogon*	WR-5	Thailand (Nakhon Si Thammarat)
9	*O. rufipogon*	WR-6	Thailand (Chean Yai)
	Group III		
10	*O. glumaepatula*	W-1183	British Guiona
11	*O. glumaepatula*	W-1193	Manaus
12	*O. glumaepatula*	WR-1714	Brazil
	Control		
13	*O. sativa*	Nipponbare	Japan
14	*O. sativa*	LMN 111	Thailand (Deep water variety)
15	*O. sativa*	KDML 105	Thailand (Low land variety)

Table 2 The comparison of some agronomic characters of wild rice and cultivated varieties.

Accession No.	Origin	Plant* height (cm)	Tiller* No./plt	Panicle No./plt	Percentage of fruitful culm (%)	Panicle length (cm)	Days to 75 % heading (d)	Flowering period (d)
1	India (No. 1)	67 h	80 a	61 a	76	18.8 fg	118	46
2	India (No. 2)	106 ef	52 bc	25 dg	48	19.5 efg	164	45
3	Malaysia	114 cde	54 b	24 dg	44	18.8 fg	196	45
4	Myanmar	70 h	50 bcd	30 cde	60	18.8 fg	97	17
5	Sri Lanka	76 h	46 be	34 cd	74	19.3 efg	160	92
6	Saraburi	85 gh	77 a	49 b	64	23.3 abc	157	66
7	Singburi	96 fg	39 def	21 eh	54	24.3 abc	177	22
8	Nakhon Si	126 bcd	41 cf	21 eh	51	21.9 cde	182	28
9	Chean Yai	96 fg	35 fg	37 c	100	20.2 def	163	77
10	British Guiana	129 bc	21 h	19 fgh	90	25.2 ab	177	93
11	Manaus	111 def	37 efg	19 eh	51	18.0 fg	169	41
12	Brazil	135 ab	34 fg	23 efg	68	22.6 bcd	82	8
13	Nipponbare	77 h	17 h	26 cf	100	16.6 g	60	22
14	LMN 111	150 a	18 h	11 h	61	25.3 ab	164	7
15	KDML 105	118 be	26 gh	15 gh	58	25.9 a	131	7
	C.V. %	9.3	14.6	21.3		7.3		

Means followed by a common letter are not significantly different at the 5 % level by DMRT

*Data measured at 60 days after transplanting.

Table 3 Seed characterics of wild rice accessions and cultivated rice varieties.

Accession No.	Origin	Shattering	Awning (cm)	Seed size (mm)			100 Seed wt. (gm)	Hull color
				Length	Width	Thickness		
1	India No.1	Heavy	10.56 b	5.64	2.03	1.55	1.61 g	Deep brown
2	India No.2	Heavy	10.74 b	6.07	2.04	1.47	1.82 ef	Deep brown
3	Malaysia	Heavy	10.14 b	5.96	2.01	1.42	1.81 ef	Deep brown
4	Myanmar	Heavy	10.24 b	5.88	2.08	1.56	1.94 d	Deep brown
5	Sri Lanka	Heavy	8.15 c	5.74	1.88	1.33	1.46 h	Deep brown
6	Saraburi	Heavy	11.88 a	5.64	2.30	1.69	1.92 d	Brown
7	Singburi	Heavy	6.85 de	5.72	1.72	1.40	1.41 h	Deep brown
8	Nakhon Si	Moderate	5.94 e	5.55	1.92	1.48	1.87 de	Straw
9	Chean Yai	Moderate	10.13 b	5.46	2.22	1.58	1.76 g	Deep brown
10	British Guiana	Heavy	9.85 b	6.81	2.07	1.41	1.91 d	Deep brown
11	Manaus	Heavy	7.92 c	7.18	2.13	1.39	2.24 c	Deep brown
12	Brazil	Moderate	7.63 cd	5.91	2.43	1.74	2.26 c	Black
13	Nipponbare	Absent	Awnless	5.10	2.65	1.95	2.20 c	Straw
15	LMN 111	Absent	Awnless	7.06	2.20	1.82	2.61 a	Straw
15	KDML 105	Absent	Awnless	7.06	2.05	1.73	2.37 b	Straw
	C.V.%		5.9				2.4	

Means followed by a common letter are not significantly different at the 5 % level by DMRT.

Table 4 Changes in seed germination percentage and viability after seed harvesting up to 6 months

Accession No.	Origin	Storage time (month)							Viability
		0	1	2	3	4	5	6	
1	India No. 1	0	0	0	0	0	5	38	90
2	India No. 2	0	0	0	0	0	11	18	88
3	Malaysia	0	0	0	0	0	31	49	100
4	Myanmar	0	0	0	1	0	3	14	89
5	Sri Lanka	0	0	0	0	0	7	27	100
6	Saraburi	0	5	0	2	63	82	95	93
7	Singburi	0	0	0	0	-	-	-	-
8	Nakhon Sri	0	0	18	36	-	-	-	-
9	Chean Yai	0	2	5	8	21	58	73	80
10	British Guiana	0	0	0	2	33	-	-	-
11	Manaus	0	0	1	3	32	49	45	-
12	Brazil	0	96	-	-	-	-	-	95
13	Nipponbare	93	-	-	-	-	-	-	85
14	LMN 111	0	92	-	-	-	-	-	88
15	KDML 105	0	60	89	-	-	-	-	92

WEED SEEDLING SURVIVAL UNDER FLOODED CONDITIONS

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Abstract: A field study at the International Rice Research Institute examined the effect of seedling age and depth of flooding on rice and weed survival under flooded conditions. The experiment used one rice (cv UPLRi-7) and 19 weed species, two flood water depths and two seedling ages. Grass weeds particularly the *Echinochloa spp.* and weedy rice, and the sedges matured under flooding at both 5 or 10 cm compared with most broadleaf weeds. The age and weed height at flooding were critical for establishment and survival. No upland-adapted broadleaf and grass weeds survived flooding up to 28 days.

Key words: **flooding, submergence, weed survival**

Introduction

Flooding is used to control weeds (Moody and De Datta, 1982; Zimdahl, 1999) in irrigated rice fields and in rainfed rice areas where impounding water is economically feasible. Continuous soil flooding even at shallow depths submerges the whole or part of the plant thus suppresses growth of many weeds (Diop and Moody, 1984; Smith and Fox, 1973). Flooding slowed stand establishment, growth, and development of *Cyperus iria, Digitaria setigera* and *Trianthema portulacastrum* (Civico and Moody, 1979). Flooding may not control all weeds. Some species can be persistent even under continuous flooding, and therefore, affect rice yield by effective competition. Since growth and development of most weeds usually coincide with that of rice when directly seeded or when the seedlings are recovering from transplanting shock, water management at the early crop growth is important. Most studies (Civico and Moody, 1979; Diop and Moody, 1984;Ku and Mortimer, 1997; Rao and Moody, 1995; Singh et al., 1991; Smith and Fox, 1973; Vanderzee and Kennedy, 1981) to elucidate flooding response of some weed species were done in controlled environments. The effects of flooding and submergence on many weeds have not been thoroughly studied in the field. Factors such as seedling age and depth and duration of flooding are important in the survival or death of species with limited flooding tolerance (McIntyre et al.,1989).

This field study examined the effect of seedling age and depth of flooding on the establishment and survival of rice and some of its most important weeds.

Materials and Methods

The study was conducted in an irrigated field at the International Rice Research Institute. The experiment was arranged in a randomized complete block design with three replications. The treatments were 20 species, two flood water depths (5 cm, 10 cm), and two seedling ages (7, 14 days after sowing, DAS). Six broadleaf weeds (*Aegeratum conyzoides, Amaranthus spinosus, Eclipta prostrata, Sphenoclea zeylanica, Ludwigia octovalvis, Trianthema portulacastrum),* three sedges *(Cyperus difformis, Cyperus iria, Fimbrystylis milliacea),* and 11 grass weeds *(Ischaemum. rugosum, Leptochloa chinensis, Dactyloctenium aegyptium, Digitaria ciliaris, Rottboellia cochinchinensis, Echinochloa colona, Echinochloa crus-galli, Echinochloa glabrescens, Eleusine indica,* weedy rice) and rice cv UPLRI-7 were studied.

Seeds of all species were taken from seed lots collected within IRRI fields from two to six months prior to the conduct of the study. Seeds were sown at approximately 1/2 cm depth in 0.5 L pots containing sterilized soil. To generate two seedling ages, seeds were sown at 7-day interval. They were germinated under saturated condition in the greenhouse. Results of previous experiments (unpublished) showed that a saturated soil was conducive for seed germination, emergence and establishment of the weed species used in the current study. Emerged seedlings were thinned to 20-30 seedlings per pot. At 7 or 14 DAS, each pot was pushed gently unto the flooded paddy soil in the field so that each was submerged either at five or 10 cm depth. The field was permanently flooded for the duration of the experiment. For monitoring irrigation and flood level, a ruler was placed in each pot. Fresh irrigation water run continuously through all replications through an inlet-outlet PVC pipes. The outlet valve also released excess rainwater.

The initial number of seedlings were recorded prior to transfer of pots to the field *i.e.* at 7 or 14 DAS. Surviving seedlings were counted at 3, 7, 10, 14, 21, 28, 35, 42, 48, and 55 days after flooding (DAF). For each observation date, percent seedling or plant survival was computed as:

% Survival = (number of remaining plant at n DAF/total seedlings at 0 DAF) * 100

A surviving seedling is one which has intact leaves and stem and anchored on the soil. It is considered established on the first contact of its leaf tip with the water surface (McIntyre *et al.*, 1989). The number of days to leaf tip first contact with the water surface was recorded for some species.

Results and Discussion

Although we monitored seedling survival until 55 DAF, we are presenting only the survival data for 7, 14, and 28 DAF. We chose these observation dates because (1) at 7 DAF, the stand of a few species have significantly decreased, (2) at 14 DAF, several species have completely died, and (3) some species that survived 28 days of flooding have reached their reproductive stage.

Table 1 shows the percentage survival of rice and weeds at 7, 14, and 28 DAF. UPLRi-7 and weedy rice had comparable flood tolerance with percent survival of 86% to 99% for UPLRi-7 and 77% to 99% for weedy rice. Smith and Fox (1973) also found a similar response of cultivated rice and red rice when subjected to 1.3 – 5.1 cm flood. The other weed species responded differently to flooding. Among the broadleaf weeds, only *E. prostrata, L. octovalvis, and S. zeylanica* survived 5 cm and 10 cm of flooding for 28 days. Except for *D. aegyptium, D. ciliaris, and R. cochinchinensis*, the rest of the grass weeds survived flooding for 28 days. *C. diffornis, C. iria, and F. milliacea* had higher survival compared with some broadleaves and grasses. Garcia (1931) reported that *C. diffornis* was very tolerant of submergence. In this study, *A. conyzoides, A. spinosus, D. aegyptium, D. ciliaris, and R. cochinchinensis* were most sensitive to flooding since they died between 7 and 28 DAF. For most species, the 7 DAS seedlings survived much better than the 14-day old seedlings regardless of flood depth at 7 DAF. However, as flooding continued , the interaction among species by flood depth by seedling age became highly significant. At 14 and 28 DAF, the 10-cm flood inhibited stand establishment and appeared more detrimental to survival of 7 DAS seedlings compared to the 14 DAS seedlings. As the seedlings became older, it is possible that the surviving species have adjusted to submergence. According to Voezenek and van der Veen (1994), several days of flooding or partial submergence may stimulate growth in some aquatic and semi-aquatic species through shoot elongation and aerenchyma and adventitious root formation. The relatively high survival of grass weeds, particularly weedy rice and the *E. colona, E, crus-galli, and E. glabrescens* may also be attributed to their stage of establishment. Except for *L. chinensis*, all the grass weeds had leaves which extended beyond the water surface at the start of the flooding treatment (Table 2). The survival of aquatic and semi-aquatic plants may also be attributed to their ability to keep their foliage above water.

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Table 1. Effect of seedling age and flooding depth on survival of rice and some important rice weeds

Species and Seedling age	7 Days after flooding		14 Days after flooding		28 Days after flooding	
	5 cm flood depth	10 cm flood depth	5 cm flood depth	10 cm flood depth	5 cm flood depth	10 cm flood depth
Seedling age at start of looding = 7 days after sowing						
Rice cv UPLRi-7	99	96	99	86	97	86
A. conyzoidez	84	93	13	5	0	0
A. spinosus	69	84	0	0	0	0
E. prostrata	93	92	68	20	30	34
L. octovalvis	90	90	34	63	11	12
P. oleracea	72	81	1	19	0	0
S. zeylanica	96	70	61	25	32	16
D. aegyptium	92	96	0	0	0	0
D. ciliaris	76	21	21	0	0	0
E. colona	100	94	75	37	71	33
E. crus-galli	100	80	89	27	89	25
E. glabrescens	96	100	81	77	81	58
E. indica	98	88	80	7	4	0
I. rugosum	100	94	65	73	57	32
L. chinensis	92	89	85	61	26	37
R. cochinchinensis	67	47	22	0	0	0
Weedy rice	94	92	94	77	94	77
C. difformis	100	100	97	100	67	93
C. iria	93	94	54	76	27	29
F. milliacea	95	96	97	79	71	42
Seedling age at start of flooding = 14 days after sowing						
Rice cv UPLRi-7	92	89	88	89	88	89
A. conyzoidez	47	50	0	0	0	0
A. spinosus	33	44	30	0	0	0
E. prostrata	91	93	73	68	51	25
L. octovalvis	77	94	63	54	20	25
P. oleracea	61	65	53	48	1	4
S. zeylanica	87	64	71	42	48	30
D. aegyptium	57	1	27	0	27	0
D. ciliaris	88	66	50	22	50	22
E. colona	100	100	99	99	99	99
E. crus-galli	97	83	86	83	86	83
E. glabrescens	84	92	82	87	82	87
E. indica	71	86	33	0	31	0
I. rugosum	79	86	69	74	45	65
L. chinensis	48	74	38	28	38	22
R. cochinchinensis	49	79	26	17	7	0
Weedy rice	98	100	98	98	98	98
C. difformis	96	91	95	84	86	53
C. iria	82	74	78	30	78	22
F. milliacea	78	93	78	93	58	84
LSD (5%)	31		30		29	
LSD (1%)	41		40		38	

Table 2. Days to leaf first contact with water surface of some species

Seedling age at start of flooding →	7 Days after sowing		14 Days after sowing	
Flooding depth →	5 cm	10 cm	5 cm	10 cm
Broadleaves				
Aegeratum conyzoides	X *	X	X	X
Amaranthus spinosus	X	X	X	X
Eclipta prostrta	17	14	14	18
Portulaca oleracea	X	X	X	X
Sphenochlea zeylanica	17	24	10	22
Sedges				
Cyperus iria	21	21	2	2
Fimbristylis milliacea	25	–	11	10
Grasses				
Echinochloa colona	0	0	0	0
Echinochloa crus-galli	0	0	0	0
Echinochloa glabrescens	0	0	0	0
Ischaemum rugosum	0	0	0	0
Leptochloa chinensis	21	7	0	0
Rottboellia cochinchinensis	0	0	0	0
Weedy rice	0	0	0	0
UPLRi-7	0	0	0	0

X = leaf tip did not reach water surface

WEED SHIFTS IN RESPONSE TO HERBICIDE APPLICATIONS IN COTTON

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Abstract: Common used herbicides such as acetochlor haloxfop, and glyphosate were applied pre emergence, post-emergence and post-emergence directed respectively. They were used once a year at the dosage of 562.5g.ai/ha, 93.75g.ai/ha, 750g.ai/ha and for three years. The shifts of weeds in response to herbicide usage were observed. Weed density in acetochlor treated plots was much less than that of contrast ones, but uncontrolled weeds especially broadleaf ones increased yearly, and to the third year, weed bio-mass in the plots was also exceeded that in control plots. Grasses occurrence annually decreased greatly in haloxyfop treated plots, and almost none of them were observed in the third year. With the competitiveness of grasses declining, the broadleaf weeds got more growth advantage, and both weed density and bio-mass in the plots were much more than that of none herbicide treated plots in the second year and third year. In glyphosate treated plots, both grasses and broadleaf weeds increased yearly, with broadleaf ones superior to grasses.

Key words :weed; shift; herbicide; cotton

Introduction

Weeds in crops are effectively controlled with the application of herbicides. But, with the consequence use of single herbicide, the non-target weeds increase gradually and dominate the others while the target weeds get controlled.

Field test conducted by Feng Wen xu in 1990 showed that grasses in wheat such as *Alopecurus japonicus Steud. Alopecurus aequalis Sobol. Sclerochloa kengiana(Chwi)Tzvel* were controlled with the consequence use of herbicide chlorotoluron, in response to which broadleaf weeds such as *Stellaria media (L.) Cyr. Galium aprarine L. Var.tenerum(Gren.et Godr)Rcbb.* increased greatly. On the other hand, while broadleaf weeds were controlled by herbicide MCPA, grasses increased little. It was concluded that grasses were more competitive in wheat.

What is about in cotton? To answer this question, three types of common used herbicides such as acetochlor, haloxfop and glyphosate were applied successively in cotton from 1996 to 1998, and changes of weed community in response to herbicides were observed systematically.

Material and methods

Plots for test was selected as of same soil parameter and of bounds of common weed species scattering in average. Five treatments were as follows:

(1)Acetochlor, 50%EC, spraying pre planting at the dosage of 562.5g.ai. /ha.

(2)Haloxfop, 12.5%EC, spraying after cotton planting when grasses were of 3-4 foliages at the dosage of 97.35g.ai. /ha.

(3)Glyphosate, 10%WS, spraying post directed when cotton heights were over 35cm at the dosage of 50g.ai. /ha.

(4) Hand weeding control.

(5) No weeding control.

Herbicides were applied in 450 liters of water per hectare to (2) and (3) treatment and it is 900 liters to (1) treatment. Plots of each treatment repeated three times and all plots were randomly designed.

Three spots, 0.25m^2 each, were fixed in each plot. Weed species, plant number and bio-mass in the plots were calculated pre and 45 days after herbicide application with treatment (2) and (3) and 45 days, 75 days after planting with treatment (3) and (4).

Five representative plants were sampled from each spot that five ones are arranged in each plot to measure the seed number, plant height and plant weight of weeds.

Results and analysis

1. The changes of weed species and weed density in no weeding control

The test field, locating in Xinyang experiment station of Jiang su academy of agricultural sciences, had been planted in with upland crops for several years. The main weed species in later spring and summer were grasses such as *Digitaria sanguinalis (L.), Scop Echinochloa spp., Eleusine indica (L.) Gaertn.* Some broadleaf weeds such as *Amaranthus retroflexus L., Eclipta alba* were also prevalent ones sometime. Weed species varied with year. *D. Sanguinalis (L.) Scop* dominated the others in 1996 and 1998, the plant ratios of grasses contrary to broadleaf weeds were 1:0.73 and 1:0.56 45 days after planting respectively, and it increased to 1:0.64 and 1:0.42 75 days after planting, with broadleaf weeds decreasing much more comparatively. In 1997, however, the ratios were 1:1.6 and 1:1.59 45 days and 75 days after planting respectively, with broadleaf weeds especially *A. retroflexus* being some dominant. Weed densities also varied annually, counting 278.8 plants/m^2, 132 plants/m^2 and 185.2 plants/m^2 45 days after planting from 1996 to 1998 respectively. Increasing a little, it calculated as 278.8 plants/m^2, 212.0 plants/m^2 and 276 plants/m^2 respectively 75 days after planting, with it being sparse in 1997.

The main factors affecting weed community were rainfall and soil moisture. *D.sanguinalis(L)Scop., E.indica, A.retroflexus L., E.alba* are all dry tolerant species, with broadleaf A.*retroflexus L* more tolerant to dry condition than grasses species *D.sanguinalis (L.) Scop*. So the occurrence and growth of weeds mentioned above are all interfered from dry and thin rainfall condition, with *A.retroflexus L.* being affected some slighter than *D.sanguinalis (L.) Scop* and growing better comparatively. In contrast, all the weeds mentioned above occur and grow better in wet and heavy rainfall condition, with grasses *D.sanguinalis(L.)Scop* and *E.spp.* being of more growth advantages. The soil in test field was sandy, it had poor moisture conservation capability especially in upland cropping system as it was. So the amount and lasting period of rainfall affected the weed species and density sharply. According to rainfall

data from Dafeng county, the plum rain was heavy and lasted a long period in 1996. From later May to early July, the rainfall reached 599.5 mm much more than the usual plum fall of 400 mm. As a result, weed density and bio-mass increased greatly and crabgrass dominated the others this year. The situation was almost the same in 1998, it drizzled a lot from Apple and the soil sustained moist till August. Things were different in 1997. Rainfall in June was thin, only 38 mm. The plum fall was only 154 mm from July 11 to July 22. As a result, the weed density and bio-mass lessened a lot, and broadleaf *A.retroflexus L.* dominated the others.

2.Weed population structure and its variation under the selection pressures of herbicides

2.1Changes of weed species and their proportions

Successive application of a single herbicide may cause great changes in weed population structure. The degrees of the changes are different among herbicides because of the special selection pressure of each herbicide.

Haloxyfop is a specific for grasses, which reduces grass occurrence gradually year by year. It was investigated that 45 days after cotton planted (before the application of herbicide), the ratio of grass amount to that of broadleaf weeds was 1:0.88 in 1996, which was reduced to 1:14.3 in 1997 and 1:263.2 in 1998. 75 days after planting (30 days after the application of herbicides), the ratio was 1:16.7 in 1996, and no grasses occurred in 1997 and 1998. Among the broadleaf weeds in the field, *Amaranthus retroflexus* L. was the dominant one, it increased year by year and accounted for the proportions of 75.9%, 92.2% and 98.4% in the years of 1996~1998 respectively. Some other species such as *Portulaca oleracea* L. and *Eclipta prostrata* L. shrank their portions gradually. Therefore, with the successive use of haloxyfop for 2 years, the weed community in the cotton field developed to a broadleaf weed community with *Amaranthus retroflexus* L. as its dominator.

Acetochlor is a pre-emergence applied herbicide. It controls annual grasses chiefly and part of broadleaf weeds besides. After acetochlor was used, the portion of the grasses decreased, but the decreasing rate was not so rapid as that when haloxyfop was used. 35 days after cotton planted, the ratios of grass amount to that of broadleaf weeds were 1:1, 1:2.9 and 1:8.3 respectively in the three years.

Glyphosate is a burning up foliage spray, it controls both grasses and broadleaf weeds, but different species or the same species in different leaf stage have different sensitivities to it, therefore, the same dosage of glyphosate may cause different death rates among weeds. In addition, glyphosate affects the amounts of the seeds of the survived weeds. According to the investigation in 1998, the seed amounts of the grasses of *Digitaria sanguinalis* (L.) Scop.,*Eleusine indica*(L.) Gaertn. and *Echinochloa crusgalli*(L.) Beauv.var. *hispidula*(Retz.) Hack. were 6706, 8718 and 3947 per plant respectively in the no treating plots, while in the glyphosate treating plots, the seed amounts of the grasses mentioned above were only 4664, 8542 and 2000 respectively, with the amounts of *Digitaria sanguinalis* (L.) Scop. and *Echinochloa crusgalli*(L.) Beauv.var. *hispidula*(Retz.) Hack. decreasing the most. As to the main broadleaf weeds of *Amaranthus retroflexus* L., the seed amounts were 14052 and 17238 per plant respectively when haloxyfop and glyphosate were applied. This suggested that, due to its lower density and the relatively slighter competition within and among species, the reproduction growth of the *Amaranthus retroflexus* L. survived glyphosate was superior to that survived haloxyfop . Besides, the seed amount of *Amaranthus retroflexus* L. in no treating plots was only 12242, much lower than those in glyphosate

and haloxyfop treating plots, which indicated that the reproduction growth of *Amaranthus retroflexus* L. was inhibited in the competition with the grasses such as *Digitaria sanguinalis* (L.) Scop. and the like.

The difference between the effects of glyphosate on the nutrition and reproduction growth of grasses and broadleaf weeds also led to the gradually increasing portion of broadleaf weeds. 45 days after planting, the ratios of grasses to broadleaf weeds were 1:0.58, 1:1.7 and 1:2.13 in turn in the successive three years.

2.2 Changes of weed amounts in the field

Selective herbicides control weeds with selection. When a single herbicide was successively used for years, some weeds may not be controlled, especially the weed amounts may even exceed that in no treating plots sometime.

After haloxyfop was applied, the grasses were controlled effectively, but the broadleaf weeds such as *Amaranthus retroflexus* L. and so on increased greatly. As a result, the total amount of weeds increased year by year. 45 days after cotton planted (before the application of herbicides), the densities of the weeds occurred in the three years were 239.2, 396 and 1536.8 per square meter respectively, 75 days after planting, the weed densities were 168, 392.8 and 1556.0 per square meter respectively. The weed densities in the third year greatly exceeded those in the previous two years.

Acetochlor controls grasses chiefly and broadleaf weeds including *Amaranthus retroflexus* L. besides. With the effect of acetochlor, the occurrence of grasses and broadleaf weeds are both controlled. At the term end of 1996, the density of weeds in the field was only 21.4 per square meter. But the survived broadleaf weeds such as *Amaranthus retroflexus* L. and so on increased year by year. The first reason for this was that, due to the lower amount of the survived broadleaf weeds and the slighter competition within and among species, the nutrition and reproduction growth of the individuals increased rapidly, and consequently the amount of seeds greatly exceeded that of the grasses such as *Digitaria sanguinalis* (L.) Scop. and the like. The second reason was that the endurance of broadleaf weeds to acetochlor was increasingly. At the term end of 1997and 1998, the total amounts of the weeds reached to 44.0 and 108 per square meter respectively. Whereas the amounts of grasses were almost stable in the three years, which were 10.7, 11.1 and 12.0 per square meter respectively.

Since some grasses and broadleaf weeds can sustain glyphosate and maintain certain reproduction ability, the weed seed bank exports well. 45 days after cotton planted, the weed densities were 96.4, 24.4 and 641.4 per square meter respectively in the years, and the increasing margin of broadleaf weeds was superior to that of grasses.

Additionally, according to sampling investigation on the biomass of species of weeds in 1998, the effects of herbicides on weed biomass had the same trend as that on weed amounts, but the effects were greater and the difference among the treatments more remarkable than the later (Table 1).

Table 1 Changes of weed biomass in the successive three years of different herbicides being used

Sort of Weed	Treatment							
	Haloxyfop		Acetochlor		Glyphosate		No treating	
	Time after cotton planted(day)							
	45	75	45	75	45	75	45	75
	Biomass(g/m^2)							

Grass	6.44	37.32	6.9	193.3	162.0	262.2	394.9	4279.6
Broadleaf weed	67.1	4506.2	110.6	5800.0	727.7	480.0	185.3	713.3
Total	73.5	4543.6	117.5	5939.3	889.7	742.2	580.2	4992.9

Sort of weed	Treatment							
	Haloxyfop		Acetochlor		Glyphosate		No treating	
	Time after cotton planted (day)							
	45	75	45	75	45	75	45	75
	Biomass (g/m^2)							
Grass			6.9	193.3	162.0	262.2	394.9	4279.6
Broadleaf weed	67.1	4506.2	110.6	5800.0	727.7	480.0	185.3	713.3
Total	73.5	4543.6	117.5	5939.3	889.7	742.2	580.2	4992.9

Discussion

1.There must be a certain kind of weed community under a certain kind of herbicide pressure.

Bio-community is the regular combination of all the organisms that impact each other and with the environment. According to the theory of polyclimax, a community, if it can keep stable, finish reproduction and succession separately, can be regarded as a climax. Without herbicide pressure, successive cropping system of cotton is a weed-crop system that the weed community is dominated by grasses such as *sanguinalis(L.)Scop* and *E.spp.* in company with some annual broadleaf and prenial ones. Not only cotton, other summer crops such as soybean, maize are of the same situation, which, in the weeds' eye view, could be recognized as a climax without herbicide pressure and under successive upland cropping condition.

A lot of events that change randomly and affect the stability of climax constantly face bio-community. While sensitive weeds are controlled with herbicide, it results in new niches being formed and later the shift of climax balance. According to the factor compensation principle that a weed community is formed along only those species that can fit the herbicide pressure or environment could occupy the niches. The successive application of a special herbicide gives a repetitively selected chance to those weed species that are most suitable to the pressure. As a result, a special weed community is formed under the special herbicide pressure.

2.The occurrence and spreading of problematic weeds can only be effectively managed through integrated weed management system.

What we control weed for is to reduce the competitiveness of weed to crop and produce a environment that is in favor of crop growth. Herbicide application can do it well. As the experiment results show, while sensitive portion of a weed community is effectively controlled with selective herbicide, the tolerant ones get some growth advantage. The stronger the herbicide selectivity is. the faster the tolerant species spread. It results in great loss of the herbicide efficiency and then crop yield. So as with weed management, two things, both weed control and weed prevention, must be kept in mind. That is. not only we must pay attention to competitiveness of current weeds, also to the

possible future weeds caused by the weed control practice we take now. To get the goal be fulfilled, we must understand the mutual relationship and change rules of the factors among weed-crop system theoretically. And in practice, all possible means should be take to disturb and interfere with the growth and spreading of the temporarily and possible future problematic weeds. For example, when the grasses in cotton are controlled with grasses selective herbicides such as haloxfop, quzalofop, other methods as hand weeding or mechanical weeding must be used later to control the residues especially broadleaf ones. Or, when annual grasses even with some annual broadleaf weeds are controlled with acetochlor used pre planting, the survivals should be controlled with burn down herbicide such as glyphosate being sprayed post directed among rows at later growth stage. In fact, although grasses selected herbicides such as haloxfop, quzalofop have been widely used successively in cotton for years, the weed composition in cotton changed not so greatly as show in the experiment. It is the result of integrated weed management that all possible means including tillage, culture, mechanical, chemical and manual methods have been used.



2. Weed Control and Management

ALLELOCHEMICALS IN RICE STRAW

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Abstract: Four allelochemicals, momilactone A (37.1 mg) and B (12.9 mg), and oryzalexin A (12.1 mg) and C (3.4 mg), were purified from 80% methanol extract of rice straw (*Oryza sativa* L. cv. Haresugata, 20 kg, dry weight) through the conventional solvent partitioning, silica-gel and ODS column chromatographies, and high performance liquid chromatography (HPLC). The structures were confirmed by spectroscopic analyses (NMR and MS). Among them, momilactone B (M-B) exhibited the strongest inhibition on germination and growth of weed species tested. M-B at 5 μM decreased the germination of *Amaranthus lividus* L. to 50%, and at 50 μM more than 50% inhibition was observed for root and shoot growth of *Digitaria sanguinalis*(L.) Scop. and *Amaranthus lividus* L., and for seed germination of *Poa annua* L. These allelochemicals in rice straw may affect germination and growth of susceptible weed and crop plants when straw is left in the field after harvesting.

Key words: rice, allelochemical, momilactone, oryzalexin

Introduction

Allelochemicals in rice plants (*Oryza sativa* L.) have been extensively studied in relation to their effects on the germination and growth of both crops and weeds (Olofsdotter *et al.*, 1995). Most of these chemicals are inhibitory, but some may promote the germination and growth of other plant species (Kawaguchi *et al.*, 1997). In Korea, rice straw is left in the fields after harvesting and allelochemicals released from the straw as well as shading by mulching may thus affect the germination and growth of successive crops and weeds. In the present study, we describe isolation and identification of allelochemicals in rice straw and their inhibitory effects on germination and growth of *Digitaria sanguinalis*(L.) Scop., *Amaranthus lividus* L. and *Poa annua* L.

Materials and Methods

Plant materials and isolation of allelochemicals

Rice plants (*Oryza sativa* L. cv. Haresugata) were grown in a paddy field, and their straw parts were harvested at full maturity. The samples were air-dried in the greenhouse for 2 weeks and cut into small pieces. The dried samples (20 kg) were extracted with 80% aqueous methanol for 24 hrs. After filtration, the methanol was evaporated at 40°C under reduced pressure. The aqueous residue, being adjusted to pH 7, was extracted with an equal volume of ethyl acetate (EtOAc) and then with butanol (BuOH). The most active EtOAc fraction (170 g) was purified by silica gel column chromatography (EtOAc in hexane, stepwise). The active fractions (30%–70% EtOAc in hexane) were combined and loaded on a silica gel column (chloroform–EtOAc). The strong activity was detected in the 20% EtOAc in chloroform fraction, which was further purified by ODS column chromatography (50–100% acetonitrile, stepwise). The active fractions (50–60% acetonitrile) were combined and purified by ODS column chromatography (50% acetonitrile) affording almost pure momilactone B and a mixture of momilactone A, oryzalexin A and oryzalexin C. After recrystallization from hexane–EtOAc, the mixture was further purified by silica gel column chromatography (chloroform–EtOAc). The purity of purified fractions was examined by high performance liquid chromatography (HPLC). Details of purification are shown in Fig. 1. The structures of isolated allelochemicals were confirmed by spectroscopic analyses (NMR and MS) and are shown in Fig 2.

Bioassays

One hundred μl of acetone solution of the crude or purified fraction was added to a glass vial (15 mm diameter, 45 mm high) containing a filter paper. The solvent was allowed to evaporate before adding 100 μl of distilled water. Then, seeds of *Poa annua* or *Amaranthus lividus*, 50 each, or germinating seeds of *Digitaria sanguinalis*, 10 each, were placed on the filter paper and kept in a growth chamber maintained at 25°C with a 12 hr photoperiod. Germination percentage of seeds was determined after 6 days. Hypocotyl and radicle lengths of *Digitaria sanguinalis* were measured after 3 days. All the experiments were done with 3 replications. Results are expressed as percent of controls.

Results and Discussion

In the present study, four inhibitory allelochemicals, momilactone A (M-A), momilactone B (M-B), oryzalexin A (O-A) and oryzalexin C (O-C), were identified in rice straw. Isolated yield of M-A, M-B, O-A and O-C were 37.1, 12.9, 12.1 and 3.4 mg, respectively, from 20 kg (dry weight) of rice straw. Although M-A and M-B were originally isolated from rice husks as growth and germination inhibitors (Kato *et al.* 1973; 1977), they along with other phytoalexins have been to shown to participate in the plant defense systems against pathogens (Cartwright *et al.*, 1981). The endogenous levels of these compounds are increased by both biotic and abiotic stresses (Kodama *et al.*, 1988ab; Tamogami *et al.*, 1995).

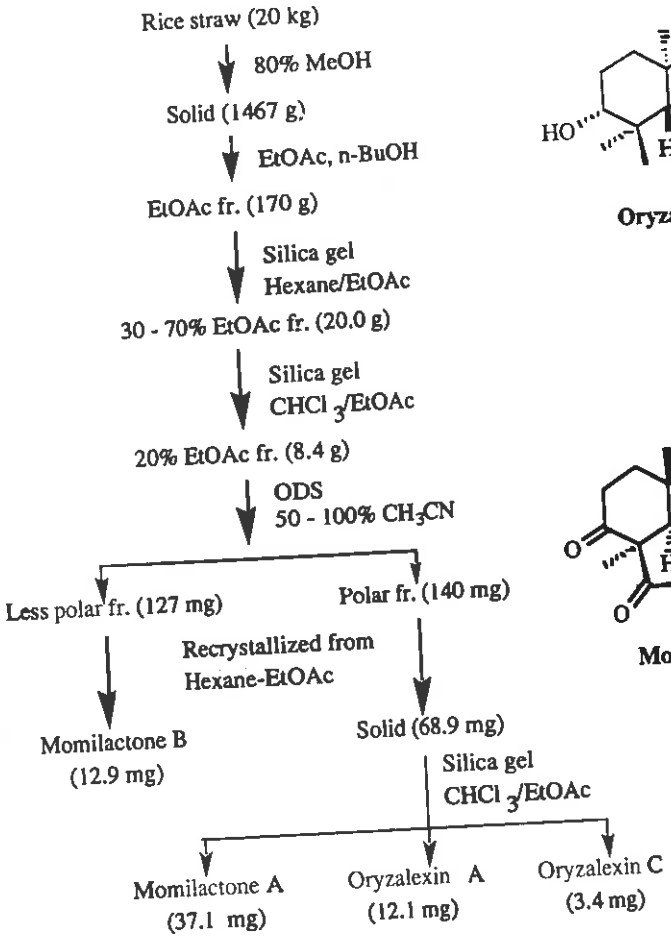

Fig. 1 Purification of allelopachemicals in rice straw

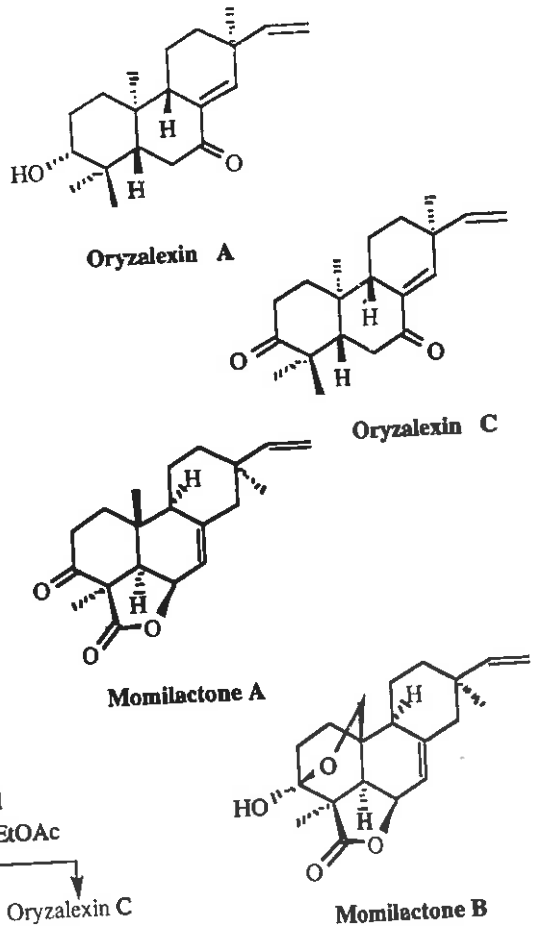

Fig. 2 Structures of allelochemicals isolated from rice straw

Among the isolated allelochemicals from rice straw, M-B exhibited the most pronounced inhibition on both germination and early growth of weeds tested (Figs. 3 & 4). For example, M-B at 5 μM decreased the germination of *Amaranthus lividus* L. to 50%, and at 50 μM more than 50% inhibition was observed for root and shoot growth of *Digitaria sanguinalis*, and for seed germination of *Poa annua*.

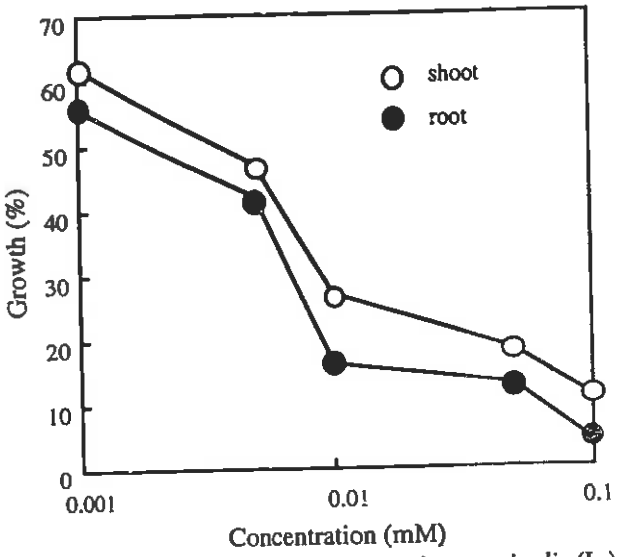

Fig. 3 Effect of momilactone B on the root growth of *Digitaria sanguinalis* (L.) Scop.

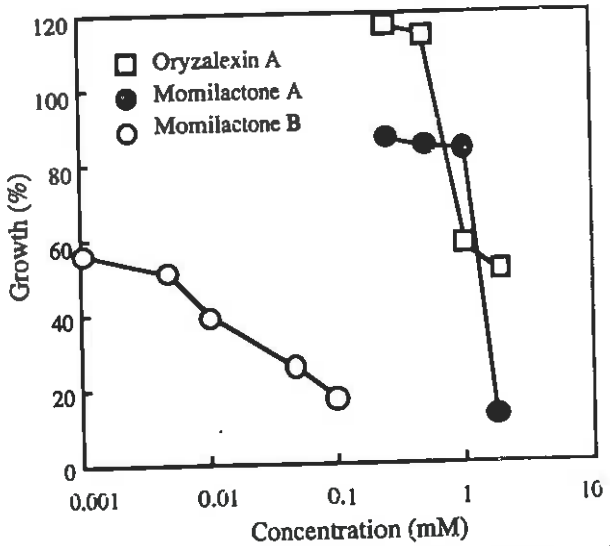

Fig. 4 Effect of isolated allelochemicals on the root growth of *Amaranthus lividus* L.

We also clarified the changes in the levels of M-A and M-B in rice straw harvested at different growth stages, and that these rather hydrophobic allelochemicals were relatively easily extracted with water (Lee *et al.*, 1999).

Consequently, these allelochemicals in rice straw may affect germination and growth of susceptible weed and crop plants when straw is left in the field after harvesting.

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APPLICATION OF THE NICOSULFURON FOR ITCHGRASS CONTROL IN MAIZE

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Abstract: The effect of nicosulfuron on phytotoxicity, growth and yield of 8 corn (*Zea mays*) varieties was studied when herbicides was applied at different stage of growth. It was found that Big 717 and DK 888 was tolerant and Pioneer 3012 were susceptible to nicosulfuron. Some plants were dead which were depending on stage of growth during application. Nicosulfuron at 40 g ai/ha applied at 3 weeks after emergence gave the best control of itchgrass (*Rottboellia cochinchinensis*). Adjuvant, Latron CS-7, APSA 8 and Sylgard 309 enhanced itchgrass control with nicosulfuron. However these adjuvants also reduced the tolerance of the corn and resulted in some leaf discolouration and occational stunting. The spray volumes of 62.5, 250 and 500 L/ha provided the same efficacy of nicosulfuron for itchgrass control and corn yields were not different.

Key words: adjuvant, corn, itchgrass, nicosulfuron, spray volume

Introduction

Itchgrass is an annual grass that spreads by seed. It is one of the 18 worst weeds in the world (Holm et al.,1977), including Thailand. Itchgrass infests many hectares of cropland in the Thailand. It can reduce crop yields, including irritate when touched. Ampom (1997) found that a itchgrass density of 80 shoots per square meter reduced corn yields an average of 60%. Visit (1995) reported that population of itchgrass at 5, 10, 20 and 40 shoots per square meter reduced corn yields 8, 14, 25 and 53 %, respectively.

There are several herbicides that control itchgrass. However, selective postemergence itchgrass control in corn has not been inavailable until recently. Nicosulfuron is a new highly selective, sulfonylurea herbicide use at low rates for selective control emerged annual and perennial grasses and some annual broadleaf weeds. Preliminary studies have shown excellent activity of nicosulfuron in controlling itchgrass in field corn (Visit, 1995).

This study was conducted to a) evaluate the timing of postemergence applications of nicosulfuron for itchgrass control and b) determine the effect of nicosulfuron on phytotoxicity of 8 corn varieties. c) determine the effect of adjuvants on nicosulfuron activity. d) determine the effect of spray volume on nicosulfuron activity for itchgrass control.

Materails and Methods

Field experiments were conducted in area which was infested by natural populations of itchgrass. The experiments were carried out at the Suwan farm, Pakchong district, Nakornratchsima in 1995 to 1997. The soil was Pakchong clay loam with 10.43% sand, 31.14% silt and 58.49 % clay (Pc,clayey kaolinitic, isohypertermic, oxic) (Visit,1995). The soil pH was 5-7. Seedbeds for the crop were prepare by no-tillage , glyphosate 1500 g ai/ha was applied postemergence at 7 days before planting for the control of weeds in all experiments. In all experiments, corn was planted that spacing was 75x25 cm. The plot size was 5x3 m (4 rows). The experiments were irrigated by

sprinkler system. Fertilyzers were applied at recommended rates. Herbicides were applied with knapsack sprayer in 500 L/ha spray volume. During herbicide application in 1995 and 1996, air temperature was 21-28 °C, and 17-28 °C with relative humidity of 70-80% and 75-85% respectively. The experimental design was a randommized complete block with four replications. Weed control efficacy and phytotoxicity were recorded at 2 and 4 weeks after application (WAA). The corn was harvested by hand at maturity. The center 3 m of each 2 center rows was harvested. Data were collected on ear weight and ear number per plant and total yield. All data were analyzed using appropriate analysis of variance with mean seperations computed by DMRT test at the 0.05 level of probability.

Timing of application and corn varieties studies

The corn was planted in the early of September 1995. This experiment was designed as a split-plot. Eigth field corn varieties, Hercules 40, Big 717, DK 888, Pioneer 3012, Tevada 77, Royal 90, KSX 3601 and KSX 3504 were main plots and timing of nicosulfuron applications (1, 2, 3 and 4 weeks after emergence ,WAE) were subplots. Herbicide uses applied at 40 g ai/ha. The corn was harvested in the middle of December 1995.

Adjuvants studies

KSX 3601 corn was planted in the early of November 1996 and harvested in the middle of Febuary 1997. Nicosulfuron at 30 and 40 g ai/ha were applied with 4 adjuvants, ammonium sulphate 1%, Latron CS-7 0.25%, APSA 80 0.2% and Sylgard 309 0.25 %v/v at 3 WAE.

Spray volumes studies

KSX 3601 corn was planted in the early of November 1996 and harvested in the middle of February 1997 Nicosulfuron at 40 g ai/ha was applied with CDA (controlled droplet application) sprayer in 62.5 L/ha, and knapsack sprayer in 250 and 500 L/ha spray volume at 3 WAE.

Results and Disscussion

Timing of application studies

Nicosulfuron at 40 g ai/ha was most effective in controlling itchgrass when it was applied to 3 WAE. However, all nicosulfuron treatments provided less control of itchgrass when compared with all hand weeding treatments for 4 WAA (Fig. 1).

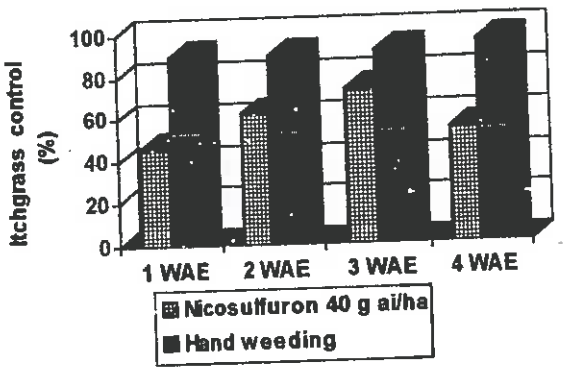

Fig. 1 Itchgrass control rating at 4 weeks after application of nicosulfuron at 1, 2, 3 and 4 WAE.

Corn varieties studies

Field corn varieties varied in tolerant to 40 g ai/ha nicosulfuron when applied at the 1, 2, 3 and 4 WAE.Corn injury consisted primarily of height reduction and some malformation of the plant. Height of corn was not different when later stage of growth (data not shown). Visual injury evaluation at 4 WAA found that Pioneer 3012 to be the least (approximately 14%), Hercules

40, Tevada 77, KSX 3601 and KSX 3504 were moderately tolerant (5%) and Big 717 and DK 888 highly tolerant (2%) (Table 1). Corn injury ranged from slight leaf chlorosis and leaf widening in tolerance varieties to severe plant stunting and death of susceptible varieties .

Table 1 Visual corn injury rating at 4 weeks after application of nicosulfuron at 1, 2, 3 and 4 WAE.

Treatment	Corn injury[1/] at 4 WAA (%)								mean
	Hercules	Big717	DK 888	Pioneer	Tevada77	DK818	KSX3601	KSX3504	
Applied at 1 WAE									
Nicosu. uron 40 g ai/ha	13 a[2/]	2 b	5 a	8 d	10 bc	3 cd	5 bc	5 bc	6
Hand weeding at 1 and 4 wks.	0 b	0 b	0 a	0 e	0 d	0 d	0 c	0 c	0
Applied at 2 WAE									
Nicosulfuron 40 g ai/ha	12 a	4 ab	5 a	38 b	10bc	8 bc	7 b	8 ab	12
Hand weeding at 2 and 5 wks.	0 b	0 b	0 a	0 e	0d	0 d	0 c	0 c	0
Applied at 3 WAE									
Nicosulfuron 40 g ai/ha	18 a	6 ab	5 a	28 c	19 a	17 a	10 b	13 a	15
Hand weeding at 3 and 6 wks.	0 b	0 b	0 a	0 e	0 d	0 d	0 c	0 c	0
Applied at 4 WAE									
Nicosulfuron 40 g ai/ha	17 a	10 a	3 a	50 a	12 b	13 ab	20 a	7 abc	16
Hand weeding at 4 and 7 wks.	0 b	0 b	0 a	0 e	3 cd	0 d	0 c	0 c	0
Non weeded	0 b	0 b	0 a	0 e	0 d	0 d	0 c	0 c	0
Mean	7	2	2	14	6	5	5	4	

1/ Visual injury rating : 0=no injury, 100=plant killed

2/ Mean in the same column followed by the same letters are not significantly different at the 5% level by DMRT.

The yield of 8 corn varieties treated with nicosulfuron were not different with non weeded treatment excepted Hercules 40, Pioneer 3012 and KSX 3504 were greater when applied at 4, 3, 3 and 4 WAE, respectively. The trend of nicosulfuron at 3 WAE application provided corn to producted highly yield compared to applied at 4, 1 and 2 WAE, respectively. These results indicate that the yield might be affected by nicosulfuron injury and incomplete control of weeds when it was applied at 40 g ai/ha (Table 2).

Table 2 Yield of corn after application of nicosulfuron at 1, 2, 3 and 4 WAE.

Treatment	Corn yield (kg/ha)								mean
	Hercules	Big717	DK 888	Pioneer	Tevada77	DK818	KSX3601	KSX3504	
Applied at 1 WAE									
Nicosulfuron 40 g ai/ha	1750 d[2/]	2256c	1950e	2675c	4106b	3094c	3275b	3656bc	2844d
Hand weeding at 1 and 4 wks.	3994ab	4206ab	5213ab	5138ab	3994b	4294abc	3975ab	3588bc	4300b
Applied at 2 WAE									
Nicosulfuron 40 g ai/ha	3156a-d	2394c	2256de	2719c	2931bc	3281bc	3313b	3156c	2900d
Hand weeding at 2 and 5 wks.	3606abc	2969c	2969cde	4275bc	4150b	4156abc	3963ab	4119abc	3775c
Applied at 3 WAE									
Nicosulfuron 40 g ai/ha	2681bcd	3406bc	4519abc	4913ab	3800b	4906ab	4488ab	5512a	4281b
Hand weeding at 3 and 6 wks.	4300a	4275ab	5769a	5900a	5788a	5263a	5306a	5706a	5313a
Applied at 4 WAE									
Nicosulfuron 40 g ai/ha	4481a	3619bc	3000cde	2606c	2206c	3850abc	4625ab	5350a	3719c
Hand weeding at 4 and 7 wks.	4463a	5394a	3781bcd	4644b	4075b	3856abc	4850ab	4863ab	4494b
Non weeded	2238	2350c	2025e	3881bc	4100b	4250abc	4088ab	3319bc	3281d
Mean	3406	3461	3500	4081	3931	4106	4206	4362	

1/ Mean in the same column followed by the same letters are not significantly different at the 5% level by DMRT.

Adjuvant studies

Nicosulfuron at 30 and 40 g ai/ha gave the same level of in itchgrass control. Adjuvant, Latron CS-7, APSA 80 or Sylgard 309 enhanced both rates of nicosulfuron. But ammonium sulphate apparently did not enhanced itchgrass control when compared with nicosulfuron alone (Table 3).

At 2 and 4 WAA, corn injury was observed with primirily of the height reduction and some malformation of the plants. Both rates of nicosulfuron plus Latron CS-7, APSA 80 and Sylgard 309 caused greater corn height reduction and corn injury than ammonium sulphate (Table 3).

Thus, yield of corn treated with both rates of nicosulfuron plus adjuvants were reduced. Corn yield in the nicosulfuron plus Sylgard 309 or ammonium sulphate was lower than Latron CS-7 and APSA 80 compared with nicosulfuron alone (Table 3).

Table 3 Effect of nicosulfuron at 30 and 40 g ai/ha on itchgrass control, visual injury, plant height and yield of corn when the herbicide was applied at 3 WAE.

Treatment	Itchgrass control [1/] (%)		Corn injury [2/] (%)		Corn height (cm)		Yield
	2 WAA	4 WAA	2 WAA	4 WAA	2 WAA	4 WAA	(kg/ha)
Nicosulfuron 30 g ai/ha	80 b[3/]	78 b	8 a	5 a	57 de	108 f	8481 de
Nicosulfuron 30 g ai/ha+as[4/] 1 %	84 bc	81 bc	9 a	7 a	54 cd	98 de	7744 b-e
Nicosulfuron 30 g ai/ha+Lt 0.25%	93 e	91 de	59 c	45 d	36 a	66 a	6638 a
Nicosulfuron 30 g ai/ha+Aps 0.2%	87 cd	87 cd	53 bc	33 c	39 ab	78 b	7125 abc
Nicosulfuron 30 g ai/ha+Sg 0.25%	90 de	87 cd	44 b	26 b	40 ab	80 b	7631 a-d
Nicosulfuron 40 g ai/ha	81 bn	83 bc	8 a	6 a	62 c	101 ef	8634 e
Nicosulfuron 40 g ai/ha+as 1 %	82 b	88 cd	9 a	7 a	49 bcd	92 de	8033 cde
Nicosulfuron 40 g ai/ha+Lt 0.25%	89 de	96 e	53 bc	33 c	43 abc	81 bc	7100 abc
Nicosulfuron 40 g ai/ha+Aps 0.2%	87 cd	90 de	54 bc	43 d	45 abc	72 ab	6825 ab
Nicosulfuron 40 g ai/ha+Sg 0.25%	89 de	92 de	47 bc	25 b	46 abc	90 cd	7763 b-e
Non weeded	0 a	0 a	0 a	0 a	58 de	100 ef	7263 abc

1/ Itchgrass control : 0=no control, 100=completely control
2/ Crop injury rating : 0=no injury, 100=plant killed
3/ Mean in the same column followed by the same letters are not significantly different at the 5% level by DMRT.
4/ Adjuvants: as=ammonium sulphate, Lt=Latron CS-7, Aps=APSA 80 and Sg=Sylgard 309

Spray volume studies

Nicosulfuron in the spray volume of 62.5, 250 and 500 l/ha gave the same exellent itchgrass control and better than glyphosate at 4 WAA. However, nicosulfuron was applied by mixing with adjuvant (Feser 0.25% v/v) therefore it caused highly corn injury. The spray volume of 250 and 500 l/ha caused corn injury more than 62.5 l/ha but corn height and corn yields were not affect (Table 4).

Table 4 Effect of spray volume to nicosulfuron application on itchgrass control, injury, height and yield of corn.

Treatment	Itchgrass control [1/] (%)		Corn injury [2/] (%)		Corn height (cm)		Yield
	2 WAA	4 WAA	2 WAA	4 WAA	2 WAA	4 WAA	(kg/ha)
Nicosulfuron 40 g ai/ 62.5 l/ha	92 bc[3/]	98 d	3 b	18 b	49 a	100 a	8063 abc
Nicosulfuron 40 g ai/ 250 l/ha	90 b	96 cd	18 c	25 c	47 a	98 a	8381 bc
Nicosulfuron 40 g ai/ 500 l/ha	89 b	92 c	18 c	30 c	42 a	92 a	7788 ab
Glyphosate 1500 g ai/ 250 l/ha	96 c	75 b	2 a	5 a	51 a	107 a	8375 bc
Hand weeding at 3 and 6 WAE	91 b	95 cd	0 a	0 a	50 a	95 a	9000 c
Non weeded	0 a	0 0	0 a	0 a	51 a	102 a	7225 a

1/ Itchgrass control . 0=no control, 100=complete control
2/ Crop injury rating : 0=no injury, 100=plant killed
3/ Mean in the same column followed by the same letters are not significantly different at the 5% level by DMRT.

Conclusion

These studies illustrate that nicosulfuron are currently available to provide early complete control of itchgrass in corn. The use of nicosulfuron alone would be limited primalily to field with annual grasses and certain broadleaf weeds. Most complete and consistent control of a wide spectrum of annual grass and broadleaf weeds would require one or more companion herbicides.

Field corn varieties vary in response to nicosulfuron with stage of application. Adjuvant, Latron CS-7, APSA 80 and sylgard 309 enhanced itchgrass control of nicosulfuron. But these adjuvants also reduced the tolerance of the corn and resulted in some leaf discolouration and occational stunting.

Spray volumes of 62.5, 250 and 500 L/ha provided the same efficacy of nicosulfuron for itchgrass control and corn yields were not different.

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EFFECT OF CROP ROTATIONS ON THE CONTROL OF WILD OAT AND OTHER WEEDS IN WHEAT IN MIDDLE EGYPT

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Abstract:

Two year and three year crop rotations were experimentally practiced in naturally soil infested with wild oat seeds at Mallawi research stations to study the effect of crop rotations with integration of some weed control treatments on both weeds and wheat productivity.

Results indicated that cutting berseem performed well against weeds and in consequence wheat grain yield was attained. Crops sequences viz. berseem / berseem / wheat gave 100%control of grassy weeds either wild oat or canary grass and gave highest grain yield as compared with the crop sequence of wheat / wheat / wheat which gave the lowest wheat grain yield,may be due to the high density of both grassy and broad leaved weeds. The integration between crop sequence berseem / wheat / wheat and using Grasp at 2.38 L/ha supported with hand weeding once (70 DAS) was excellent in weed control especially grassy weeds and improving wheat productivity.

Key words: Rotations, weed control, integration, cutting, yield.

Introduction

Wheat is an important cereal crop in Egypt . Weed control in such crop becomes a problem especially under heavy weed infestation in Middle as well as Upper Egypt.

The most prevailing weeds in wheat fields were wild oats (*Avena* spp.) and canary grass (*Phalaris* spp.) as annual grasses weeds, *Ammi majus, Beta vulgaris, Sonchus oleraceus, Rumex dentatus, Medicago spp.* and *Brassica nigro* as broadleaved weeds. The population of these weeds becomes very high and threaten the production of wheat. This fact makes it urgent to find an effective way to get rid of these weeds. Wilson and Phipps (1985) indicated that crop rotation system using cutting crops i.e. barley for silage exhausted the reserve of wild oat through three years. O'Donovan (1988) found that population of wild oats increased in wheat / wheat rotation (>200 plants / m^2) by the fourth year, whereas in canola / barley rotation, the population increased only by 40 plants / m^2 or less by fourth year.

Thus, the present study was starting in 1992 /93 winter season for three successive years to know the role of berseem, Faba bean and wheat crops and different weed control treatments in different sequences crops through integration of effective measures being, crop rotation, manual operation and herbicidal treatments on reducing the reserves of weed seeds in soil in order to keep the wheat plants out of weed competition and to gain consequent increases in wheat yield.

Materials and Methods

Three year crop rotation:

A proper experiment was undertaken in 1992/93 winter season as the first season in three-year crop rotation at Mallawi Agriculture Research Station. The trial is completed throughout 1994/95 winter season. The site of the trial was naturally infested with winter annual weeds. In summer 1992/93, 1993/94 and 1994/95 seasons the plots were handhoeing and planted with maize. It is to be noted that all the seasonal preparation before planting was done manually.

The experimental design was split plot design in nested classification with four replications. The winter crops were randomly assigned to the main plots, while the weed control treatments were randomized within each main plot as sub-plot, plot size was 10.5 m^2.(Table 1)

It is to be noted that all herbicidal rates were calculated on the basis of the commercial product. The sub-treatments were applied to the respective crop in the start and second season while at the third season (final season) no treatments were carried out neither mechanical nor chemical means in order to study the effect of the previous sequence crops in the rotation on weeds and wheat yield.

Two – year crop rotation experiment:

A proper experiment was undertaken in 1992 / 93 winter season as the first season of 2-year crop rotation and located at Mallawi Agricultural Research Station (Table 2). Randomized Complete Block Design with four replicates was followed, plot size was 10.5 m The experimental field was naturally infested with grasses and broadleaved weeds.

Wheat (CV Sakha 69), faba bean (CV Giza 2) and berseem (CV Meskawi) were sown at a seeding rate 143, 167 and 47 k/ha, respectively. Sowing data was the third week of November for the two types of crop rotations two year as well as three year crop rotation. The normal agricultural practices for each crop were carried out perfectly in the manner commonly followed in the Regional.

Obtained data were subjected to the proper statistical analysis of variance of split plot in nested design (Steel and Torrie 1960) and the least significant difference at 5 % level was used for comparison between means.

Results and discussion

Three – year crop rotation:

A- Effect of crop sequence on wheat and associated weeds

Data recorded in Table (1), clearly revealed that the crop sequence berseem / berseem / wheat (B / B / W) occupied the first category from the view point of weed control and wheat grain yield, whereas gave 100 % controlling on grassy weeds (wild oats as well as canary grass) and 52.62 % for broadleaved weeds. Such crop sequence gave the highest wheat grain yield (5.603 t/ha). This may be attributed to the frequent cutting of berseem for two successive winter seasons (1992/93 and 1993/94) as forage crop without using any treatment for controlling weeds neither mechanical nor herbicidal. In addition improving soil fertility which in turn overcome weed competition to some extent and increase grain yield as compared with the crop sequence of wheat / wheat / wheat (W / W/ W) whereas wild oats (*Avena spp.*) dominate over all other weed species in the three successive winter seasons. Moreover, the fresh weight of wild oats recorded in the final year of this crop sequence (W / W/ W) by 1797.74 g/m^2 versus 61.07 and 159.16 g/m2 for canary grass (*phalaris spp.*) and broad-leaved weeds, respectively. So, according to the high density of wild oats, it gave the lowest wheat grain yield (2.413 t/ha).

Concerning the crop sequence berseem / wheat / wheat (B / W / W), it occupied the second category from view point of grassy weed control and wheat grain yield, whereas it gave 86.82 % controlling of grassy weeds and wheat grain yield by 3.960 t/ha. These results indicated that crop rotation including berseem as forage crop for two successive seasons (B / B / W) is more better than that (B / W / W) especially in case of heavy infestatin with weed species mainly wild oats weed. Such result asserts the importance of frequent cutting of berseem plants for reducing existed weeds successively. Moreover frequent cutting intended to prevent certain weeds from further development in the following winter seasons.

On the contrary, the heaviest infestation with annual grassy and broadleaved weeds was found where the crop rotation included *Faba bean* as precedent crop for one winter season (1992/93) and followed by wheat for two successive seasons viz 1993/94 and 1994/95 (F / W / W). the respective value of the fresh weight of grassy and broadleaved weeds were 1573 and 165 g/m^2. So, such crop sequence gave the lowest wheat grain yield (2.468 t/ha) without any significant difference from that obtained in non rotated wheat (W / W / W) by 2.413 t/ha. These results are confirmed with those obtained by Wilson and Phipps (1985), O'Donovan (1988), Mekhail *et al,* (1994), Al-Marsafy *et al.* (1994) and Hassanien *et al.* (1994).

B- Effect of weed control treatments on wheat and associated weeds:

Data presented in Table (1) of 1994/95 winter season clearly, revealed that the wild oats was the dominant weed which recorded 3379.0 and 3036.0 g/m^2 for the unweeded check in (W / W/ W) and (F / W / W), respectively. On the contrary, the respective values for broadleaved weeds were 60.10 and 141.25 g/m^2. This may be due to the fact that wild oats was more competitive than either wheat or broadleaved weeds. Moreover, the former two treatments gave the lowest wheat grain yield which was estimated by 0.889 and 0.952 t/ha respectively.

In the case of the light infestation of grassy as well as broadleaved weeds in the crop sequence (B / W/ W) whereas the respective values of the fresh weight in the unweeded check were 455 and 208 g/m^2. Such phenomenon shows the importance of frequent cutting of berseem in reducing weed population existed in berseem in the second and third winter seasons. Wheat grain yield obtained from the former treatment was 2.318 t/ha.

Also data in Table (1) indicated that wheat grain yield took the same trend whereas the respective values were 4.508 and 4.381 t/ha compared with the unweeded check (2.318 t/ha).

Thus, we can conclude that the difference in wheat grain yield in different crop sequences are attributed mainly to the decrease of grassy weed densities especially wild oats. These results were in full agreement with those obtained by Bell and Nalewaja (1968), AL-Marsafy *et al.* (1993), Hassanein *et al* (1993); Mekhail *et ai.* (1994) and Hassanien *et al.* (1994).

Effect of Two-year crop rotation on weeds:

Results of 1992/93 as the start year, denoted that the crop rotation including berseem as a forage crop accounted for the significant reduction in the fresh weight of all weeds to a reasonable extent (81%) as a result of repeated cutting (Table 2).

Such result asserts the important of frequent cutting of berseem plants for reducing existed weed successively.

With the precedence of faba bean (1992-93 season), the effect on weeds in wheat (1993/94) could be noted as follows:

- Igran alone reduced the broadleaved weeds by 56 % which was found as comparable to the most effective treatment being Igran + one handhoeing (72 % reduction). (Tables 3 and 4).

Seemingly Igran alone (in faba bean 1992/93), surpassed the unweeded check with a significant difference in the respect of the effect against broadleaved weeds.

Taking into account the effect on grassy weeds (wild oats 82 % + Phalaris 18 %) in wheat (1993/94) followed by faba bean (1992/93), it is quite clear that all weeds management treatments (3) did not differ from each other and were significantly better than the unweeded check (Table 2).

Regarding wheat (in 1993/94 season) result in table (2), revealed that hand-removal (twice) as well as hand-removal (once) + Grasp (in wheat 1992/93), gave reduction in the fresh weight of broadleaved weeds of 29 % and 28 %, respectively. Grasp alone appeared with no effect in this trait as it's a gramincide affect in grasses only. Grasp applied either alone or in combination with handremoval (once) in wheat (1992/93) appeared with distinct superiority.

The situation in concern to annual grasses (wild oats 91 % + phalaris 9 %), all weed management treatments (in wheat 1992/93) did not differ significantly from each other and all were found with significant superiority to the unweeded check (Table 2).

Effect on wheat yield (1993/94):

Data in Table (2) indicated that wheat grain production was affected by weed reduction, where it was significant. The highest yield of wheat grains was obtained where wheat (1993/94) succeeded

berseem (1992/93). This could be attributed to the frequent cutting of berseem in that season. In addition improving soil fertility which in turn overcome weed competition to some extent and increase grain yield. the least grain yield (1.78 t/ha) (of 1993/94) was found where wheat was raised in both seasons.

With faba bean raised in the last season (1992/93) all treatments of weed management in such crop gave comparable yields and all over yielded significantly the unweed check which gave 2.19 t/ha (Table 2).

It might be mentioned in conclusion that berseem where it was included in the rotation before wheat appeared with significant worthiness.

These results were in good agreement with those obtained by Bell and Nelewaja (1968), Wilson and Phipps (1985), Rolaj (1987), Sutton (1987), Donvan (1988), AL-Marsafy *et al.* (1992) and Hasanein *et al.* (1992).

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Table 1 Effect of crop sequences and weed control treatments on the occurrence of weeds (broad-leaved and grasses) g/m² in wheat and wheat grain yield (t/ha).(Mallawi 1994/95 3rd year of three-year crop rotations).

Crop sequences and weed control methods			Broad leaved weeds g/m²					Grassy weeds g/m²			Wheat grain yield t/ha
1992/93	1993/94	1994/95	*Ammi majus*	*Medicago polymorpha*	*Beta vulgaris*	*Sonchus oleraceus*	Total	*Avena fatua*	*Phalaris Paradoxa*	Total	
Wheat:	Wheat:	Wheat:									
Unweeded check	Unweeded check	Unweeded	38	22	0	0	60	3380	123	3503	0.889
Handweeding twice	Handweeding twice	Unweeded	39	60	12	14	125	2170	79	2249	2.825
Grasp alone	Grasp alone	Unweeded	111	100	49	34	294	287	32	319	2.921
Grasp + handweeding once	Grasp + handweeding once	Unweeded	71	71	24	32	198	218	10	228	3.016
Berseem:	Berseem:	Wheat:									
Unweeded check	Unweeded check	Unweeded	30	30	17	8	85	0	0	0	5.603
Berseem:	Wheat:	Wheat:									
Unweeded check	Unweeded check	Unweeded	62	111	19	16	208	421	34	455	2.318
Unweeded check	Handweeding twice	Unweeded	57	100	15	17	189	301	22	323	4.381
Unweeded check	Grasp alone	Unweeded	69	86	16	11	182	101	17	118	4.508
Unweeded check	Grasp + handweeding once	Unweeded	49	48	24	9	130	84	0	84	4.635
Faba bean:	Wheat:	Wheat:									
Unweeded check	Unweeded check	Unweeded	19	12	79	31	141	3035	231	3266	0.952
Handweeding twice	Handweeding twice	Unweeded	20	33	16	14	83	2196	198	2394	2.857
Igran alone	Grasp alone	Unweeded	46	30	148	29	253	1059	103	1162	2.984
Igran + handweeding once	Grasp + handweeding once	Unweeded	22	36	113	14	185	900	106	1006	3.08
L.D.S.			31	40	44	23	31	112	N.S.	55	0.31

Table 2 Effect of 2-years rotations with different weed control management treatments on weeds (g/m^2) and wheat grain yield (t/ha). (Mallawi, 1993/94 2nd year of two year crop rotations).

	Preceding winter crop 1992/93 season	Preceding weed control method in 1992/93	Broad leaved weeds g/m^2						Grassy weeds g/m^2			Grain yield t/ha
			Ammi majus	*Beta vulgaris*	*Sonchus oleraceus*	*Rumex dentatus*	*Brassica nigra*	Total	*Avena fatua*	*Phalaris Minor*	Total	
1	Berseem	Unweeded	25.85	50.65	14.73	39.18	40.43	170.84	77.90	20.83	98.73	4.92
2	Wheat	Unweeded	136.50	88.68	31.38	80.28	107.25	444.09	812.68	81.80	894.48	1.78
3	Wheat	Handweeding (twice)	104.58	96.93	42.00	23.43	68.78	335 72	492.10	13.78	505.88	3.30
4	Wheat	Grasp	189.38	63.45	36.15	46.43	96.93	432.34	478.70	20.38	499.08	3.65
5	Wheat	Grasp + Handweeding (once)	99.70	91.00	47.13	20.93	62.20	320.96	446.20	39.95	486.15	3.87
6	Faba bean	Unweeded	198.78	234.83	41.20	130.63	33.85	639.29	668.30	147.60	815.90	2.19
7	Faba bean	Handweeding (twice)	100.90	188.75	22.30	95.05	71.58	478.58	520.10	220.88	740.98	3.74
8	Faba bean	Igran	224.90	50.33	32.18	33.18	60.95	401.54	701.30	81.05	782.35	3.43
9	Faba bean	Igran + Handweeding (once)	93.50	65.08	39.75	31.15	78.30	307.78	596.60	180.95	777.55	3.75
	L.D.S. 5 %		35.80	63.96	N.S.	N.S.	N.S.	43.42	123.40	119.49	42.31	0.30

Table 1 *Effect of crop sequences and weed control treatments on the occurrence of weeds (broad-leaved and grasses) g/m^2 in wheat and wheat grain yield (t/ha).(Mallawi 1994/95 3rd year of three-year crop rotations).*

EFFECT OF IRRIGATION ON THE PERFORMANCE AND LEACHING BEHAVIOUR OF ISOPROTURON AND METOXURON FOR THE CONTROL OF GULLI DANDA (*PHALARIS MINOR* RETZ.) IN WHEAT

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Abstract: Field studies were conducted from 1995-96 to 1997-98 at the Research Farm of Punjab Agricultural University, Ludhiana (India) on loamy sand soil having 76.4% sand, 12.5% silt and 11.0% clay with low organic matter (0.3%) and normal pH (7.9). The results reveal that first and second irrigation of 7.0 cm each resulted in significant reduction (24%) in the dry matter of *Phalaris minor* and thus increased the grain yield of wheat significantly over the irrigation application of 10.5 cm each. Post-emergence application of isoproturon at 1.41 kg and of metoxuron at 2.10 kg/ha registered significant increase in the grain yield of wheat compared to isoproturon 0.94 kg and metoxuron 1.40 kg/ha. The higher doses of isoproturon and metoxuron increased the grain yield by 5.3 and 4.7 per cent over their lower doses. The data pertaining to the leaching behaviour of isoproturon (0.94 kg/ha) and metoxuron (1.40 kg/ha) envisage that per cent loss in top 0-15 cm soil layer recorded 15 days after spray was 34.4 and 32.5 in 7.0 cm and 54.6 and 49.8 per cent in 10.5 cm application of first irrigation, respectively.

Key words: Isoproturon, Metoxuron, Leaching, Irrigation, Persistence

Introduction

Wheat (*Triticum aestivum* L.) occupies the prime position among the food crops in the world. In India, it is second important food crop next to rice. The major wheat growing states of India are Punjab, Haryana and Western parts of Uttar Pradesh and are contributing more than 90 per cent wheat grain in central pool (Malik and Singh, 1993). In this zone, rice-wheat is a common crop rotation adopted by the farmers. This cropping system has brought in its wake emergence of *Phalaris minor* Retz., as a serious and troublesome weed of wheat causing yield reduction to the extent of 40 per cent (Pandey *et al.*, *1980).* Isoproturon provided very effective control of this weed for about 12-15 years. However, complaints regarding poor efficacy of this herbicide were received since 5-7 years from certain pockets of Punjab state. The reasons for poor efficacy of isoproturon may be due to crop residue management and irrigation depths (w.r.t. herbicide adsorption and leaching respectively), rate and time of herbicide application, physical, chemical and biological properties of soil etc. might be involved in influencing the bioefficacy of isoproturon. So, present studies were planned to investigate the role of variable depths of first and second irrigation on the performance of isoproturon and metoxuron for the control of *P. minor* in wheat.

Materials and Methods

These investigations were carried out at the experimental farm of Punjab Agricultural University, Ludhiana from 1995-96 to 1997-98. The soil under the experimental field was low in available nitrogen, medium in available phosphorus and potassium and loamy sand in texture with 76.4, 12.1 and 11.0 per cent of sand, silt and clay respectively. Also, the field on which this trial was conducted (for 3 years) was under rice-wheat cropping system since last many years. Experiment was laid out in split plot design with depths of irrigation in main plots and weed control treatments in sub-plots. Four irrigation treatments were : first irrigation 7.0 cm + second irrigation 7.0 cm ($I_{7.0\text{-}7.0}$); first irrigation 7.0 cm + second irrigation 10.5 cm ($I_{7.0\text{-}10.5}$); first irrigation 10.5 cm + second irrigation 7.0 cm ($I_{10.5\text{-}7.0}$) and first irrigation 10.5 cm + second irrigation 10.5 cm ($I_{10.5\text{-}10.5}$). Five weed control treatments i.e. isoproturon 0.94 and 1.41 kg/ha and metoxuron

1.40 and 2.10 kg/ha and control (unweeded) were kept in sub plots. Sowing of HD 2329 variety of wheat was done on 16th Nov 1995 and 18th Nov 1996. During 1997-98 crop season, PBW 343 was sown on 18th Nov 1997. Sowing was done with single row cotton drill by keeping row to row spacing of 22.5 cm. A uniform seed rate per sub plot at the rate of 100 kg/ha was used. Spray of herbicide treatment was done on 15th, 18th and 23rd of Dec during 1995, 1996 and 1997 respectively with manually operated Knap Sack Sprayer. Main plot size was 16.0m x 7.5m whereas sub plot size was 7.5 m x 3.6 m during all the three years of study.

Crop was raised by applying 125 kg N and 62.5 kg P_2O_5 per ha. Whole of P_2O_5 and half of N was applied at the time of sowing and remaining half N was applied with first irrigation. Variable depths of first and second irrigation (as per treatment) were applied with parshall flume and later on uniform depth of irrigation was applied. First irrigation was applied 3 weeks after sowing the crop and second irrigation 3-4 weeks thereafter. Harvesting was done on 16th, 22nd and 24th of April during 1996, 1997 and 1998 respectively. Net sub plot harvested was 11.25 sq.m. during all the years. Population and dry matter of *Phalaris minor* was recorded from 2 spots per sub plot by using quadrate measuring 50 cm x 50 cm.

Results and Discussion

Effect on *P. minor*

Data pertaining to panicles and dry matter of *Phalaris minor* recorded at the time of harvest are presented in Table 1. Lowest number of panicles/ear head of *P. minor* were recorded in $I_{7.0\text{-}7.0}$ treatment which were significantly less than all other irrigation treatments during all the years. Application of first and second heavy irrigations ($I_{10.5\text{-}10.5}$) resulted in the production of significantly higher number of panicles of *P. minor* during 1995-96 and 1996-97 as compared to other irrigation treatments. Among the weed control treatments, all herbicide treatments significantly reduced number of panicles of *Phalaris/m²* during all the years as compared to control (unweeded) treatment (Table 1). Among the herbicide treatments, application of isoproturon at 0.94 kg/ha (recommended dose) produced significantly more panicles of *Phalaris* compared to isoproturon 1.41 kg, metoxuron 1.40 and 2.10 kg/ha during all the years of study. Moreover, higher rates of metoxuron (2.10 kg/ha) recorded significantly less number of panicles of *Phalaris* during 1995-96 and 1996-97 as compared to lower dose of metoxuron and both levels of isoproturon.

Dry matter accumulation by *Phalaris minor* recorded at the time of harvest was significantly low in the plot which received both first and second light irrigation ($I_{7.0\text{-}7.0}$) as compared to plot receiving heavy irrigations of 10.5 cm ($I_{10.5\text{-}10.5}$). These observations holds good from 1995-96 to 1997-98. On an average of three years application of both light irrigations (7.0 cm each) decreased dry matter accumulation by *P. minor* by 19.2 per cent as compared to where both irrigations were given heavy (10.5 cm). Similarly Kulshrestha (1983) reported half life of isoproturon 43, 25 and 11 days in air dry, moist (field capacity) and flooded soil respectively. All herbicide treatments significantly reduced dry matter accumulation by *P. minor* as compared to control (unweeded) during all the years of investigations. Recommended level of isoproturon (0.94 kg/ha) resulted in significantly more accumulation of *Phalaris* dry matter as compared to isoproturon 1.41 kg and metoxuron 1.40 kg and 2.10 kg/ha from 1995-96 to 1997-98 It was further observed that higher rates of isoproturon and metoxuron resulted in significant reduction in dry matter accumulation by *P. minor* as compared to their lower rates (recommended).

Effect on crop

Data pertaining to effective tillers and grain yield of wheat are presented in Table 2. The number of effective tillers/m row length of wheat recorded at the time of harvest were significantly low in $I_{10.5\text{-}10.5}$ as

compared to $I_{7.0\text{-}7.0}$ treatment during 1995-96 and 1996-97. However, during 1997-98, these differences being non-significant. Among sub plot treatments, unweeded (control) recorded significantly less number of effective tillers as compared to herbicide treatments during all the three years of study. Also, application of metoxuron at 1.40 and 2.10 kg/ha produced significantly more tillers as compared to isoproturon 0.94 kg/ha (recommended) from 1995-96 to 1997-98.

The differences in grain yield were found to be significant from 1995-96 to 1997-98 (Table 2). Data revealed that significantly more grain yield was produced by the crop receiving first as well as second irrigation of 7.0 cm depth (light) as compared to the crop receiving both heavy irrigations (10.5 cm each). These investigations holds good for all the three years of study. The higher grain yield in the former treatment may be due to significantly less number of panicles/sq.m., and dry matter of *Phalaris* and more effective tillers/m row length as compared to latter treatment. On an average of three years $I_{7.0\text{-}7.0}$ increased wheat grain yield by 6.5 per cent as compared to $I_{10.5\text{-}10.5}$ treatment. Sandhu and Sandhu (1987) reported that metoxuron at optimum soil moisture gave good weed control, however, under low soil moisture it gave poor weed control with reduction in grain yield.

Among the herbicides, all the treatments applied from 1995-96 to 1997-98 produced significantly more grain yield as compared to control (unweeded) treatment. It was also observed that higher rates of isoproturon (1.41 kg/ha) and metoxuron (2.10 kg/ha) significantly improved grain yield of wheat (excepting for metoxuron during 1997-98) as compared to their lower rates (recommended) during all the three years of investigations. On an average of three years, application of isoproturon at 1.41 kg and metoxuron at 2.10 kg/ha increased grain yield of wheat by 30.9 and 50.1 percent than control (unweeded) treatment respectively. Similarly Walia and Gill (1985) reported that post-emergence application of isoproturon 0.94 kg/ha and metoxuron 1.5 kg/ha provided good control of *Phalaris* and significantly improved grain yield than two hand hoeing treatment.

Effect on leaching:

Concentration of herbicides was estimated in soil samples collected from different depths at 0, 15, 30, 60, 90 and 120 days after herbicides spray and pertinent data for 1995-96 and 1996-97 on herbicide residues are presented in Table 3 and 4. In the present study concentration of herbicides (isoproturon and metoxuron), was presumed as 100 per cent. Data presented in Table 3 shows that on an average of two years isoproturon losses in the top 15 cm soil at 0.94 kg ha^{-1} and 1.41 kg ha^{-1} within 15 days was 32.5-31.8 per cent and 35.3-41.5 per cent respectively under first irrigation of 7 cm, whereas, under 10.5 cm irrigation the respective losses of herbicide was 48.7-60.5 per cent in isoproturon 0.94 kg ha^{-1} and 54.4-63.1 per cent in isoproturon 1.41 kg ha^{-1}. At 30 days after application of isoproturon 0.94 kg ha^{-1}, the 15 cm soil contained 61.9 and 20.9 per cent of herbicide during 1995-96 and 50.0 and 23.8 per cent during 1996-97 under second irrigation level of 7.0 and 10.5 cm respectively, whereas at these irrigation levels in isoproturon 1.41 kg ha^{-1} soil contained 44.9 and 16.2 per cent in 1995-96 and 35.4 and 16.9 per cent of herbicide in 1996-97.

The data on periodic concentration estimation of metoxuron at different depths under different irrigation levels are presented in Table 4. The data revealed that on an average of two years losses in the top 15 cm soil within 15 days in metoxuron 1.40 kg ha^{-1} and 2.1 kg ha^{-1} was 32.5-42.8 per cent under application of 7.0 cm water as first irrigation. Whereas first irrigation of 10.5 cm resulted in the loss of metoxuron upto 49.8 and 58.4 per cent in metoxuron 1.40 and 2.10 kg ha^{-1} respectively. Higher level of irrigation ($I_{10.5\text{-}10.5}$) caused losses of metoxuron 1.40 and 2.1 kg ha^{-1} to the tune of 71.6-76.3 per cent and 78.9-81.6 per cent, respectively, at 30 days, whereas the respective losses in $I_{7.0\text{-}7.0}$ was 45.7-51.7 and 50.5-52.8 per cent. Both the herbicides (isoproturon and metoxuron) were not detected in any of the soil layers at 120 days after spray.

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Table 1. Influence of depth of irrigation and weed control treatments on final panicles and dry matter accumulation by *P. minor*.

Treatments	Panicles of *P. minor*				Dry matter of *P. minor*			
	95-96	96-97	97-98	Mean	95-96	96-97	97-98	Mean
Irrigation depth (cm)								
I_1 7.0-7.0	67	73	11	50	7.7	8.4	2.7	6.3
I_2 7.0-10.5	70	76	16	53	7.9	8.6	3.7	6.7
I_3 10.5-7.0	70	76	19	55	8.0	8.7	3.5	6.7
I_4 10.5-10.5	77	87	17	60	8.8	9.9	4.8	7.8
CD (0.05)	2.6	2.3	3.2		0.37	0.42	0.53	
Herbicide (dose kg/ha)								
T_1 IPU 0.94	121	136	10	89	13.7	15.6	3.3	10.9
T_2 IPU 1.41	21	18	0	10	2.6	2.2	0.0	1.6
T_3 MTU 1.40	17	18	2	13	2.3	2.1	1.5	2.0
T_4 MTU 2.10	2	4	0	2	0.4	0.6	0.0	0.3
T_5 Control (unweeded)	193	215	65	158	21.4	24.1	13.3	19.6
CD (0.05)	2.5	2.1	4.4		0.31	0.36	1.1	
CD for interaction (0.05)	4.9	3.9	NS		0.61	0.73	2.3	

Table 2. Effective tillers and grain yield of wheat as influenced by irrigation depths and weed control treatments

Treatments	Effective tillers/m row length				Grain yield (q/ha)			
	95-96	96-97	97-98	Mean	95-96	96-97	97-98	Mean
Irrigation depth (cm)								
I_1 7.0-7.0	64.3	64.9	79.4	69.5	38.1	39.1	45.8	41.0
I_2 7.0-10.5	63.0	64.2	77.7	68.3	37.9	38.9	45.5	40.8
I_3 10.5-7.0	63.4	64.6	77.0	68.3	36.6	36.8	44.1	39.2
I_4 10.5-10.5	63.9	63.9	77.2	68.0	35.5	35.9	44.2	38.5
CD (0.05)	0.93	0.70	NS		2.10	1.98	1.20	
Herbicide (dose kg/ha)								
T_1 IPU 0.94	65.2	65.3	75.5	68.7	37.6	38.3	43.1	39.7
T_2 IPU 1.41	66.2	67.9	77.2	70.4	39.0	40.0	46.3	41.8
T_3 MTU 1.40	67.9	68.6	82.0	72.8	40.4	41.4	47.3	43.0
T_4 MTU 2.10	71.2	71.2	87.0	76.5	43.0	43.9	48.2	45.0
T_5 Control (unweeded)	47.7	49.8	67.4	55.0	25.1	24.9	39.8	29.9
CD (0.05)	1.62	1.56	2.4		1.95	1.84	2.2	
CD for interaction (0.05)	NS	NS	NS		NS	NS	NS	

IPU- Isoproturon
MTU- Metoxuron

Table 3. Effect of irrigation levels on the persistance of isoproturon (ppm) in soil(1995-96 and 1996-97).

Days after spray	Dose of isoproturon (Kg/ha)							
	0.94				1.41			
	Depth (cm)				Depth (cm)			
	0-15	15-30	30-60	60-90	0-15	15-30	30-60	60-90
Irrigation level ($I_{7\,0\text{-}7.0}$)								
0	0.042	*	*	*	0.069	*	*	*
	(0.044)	(*)	(*)	(*)	(0.065)	(*)	(*)	(*)
15	0.031	0.011	*	*	0.041	0.015	*	*
	(0.030)	(0.013)	(*)	(*)	(0.042)	(0.013)	(*)	(*)
30	0.026	0.008	*	*	0.031	0.010	0.001	
	(0.022)	(0.007)	(*)	(*)	(0.023)	(0.009)	(0.001)	
60	0.009	0.001	*	*	0.009	0.002	*	*
	(0.007)	(*)	(*)	(*)	(0.010)	(*)	(*)	(*)
90	0.004	*	*	*	0.005	*	*	*
	(0.001)	(*)	(*)	(*)	(0.003)	(*)	(*)	(*)
Irrigation level ($I_{10.5\text{-}10.5}$)								
0	0.043	*	*	*	0.068	*	*	*
	(0.042)	(*)	(*)	(*)	(0.065)	(*)	(*)	(*)
15	0.017	0.020	0.010	*	0.026	0.027	0.013	*
	(0.018)	(0.020)	(0.011)	(*)	(0.024)	(0.029)	(0.016)	(*)
30	0.009	0.020	0.010	0.001	0.011	0.021	0.011	0.002
	(0.010)	(0.018)	(0.009)	(0.001)	(0.011)	(0.019)	(0.009)	(0.001)
60	0.006	0.011	0.008	*	0.008	0.011	0.006	*
	(0.005)	(0.012)	(0.005)	(*)	(0.006)	(0.011)	(0.005)	(*)
90	*	0.003	*	*	*	0.002	*	*
	(*)	(0.001)	(*)	(*)	(*)	(0.001)	(*)	(*)

Table 4. Effect of irrigation levels on the persistance of metoxuron (ppm) in soil (1995-96 and 1996-97).

Days after spray	Dose of Metoxuron (Kg/ha)							
	1.40				2.10			
	Depth (cm)				Depth (cm)			
	0-15	15-30	30-60	60-90	0-15	15-30	30-60	60-90
Irrigation level ($I_{7.0\text{-}7.0}$)								
0	0.345	*	*	*	0.475	*	*	*
	(0.356)	(*)	(*)	(*)	(0.472)	(*)	(*)	(*)
15	0.228	0.116	*	*	0.294	0.169	*	*
	(0.206)	(0.117)	(*)	(*)	(0.282)	(0.157)	(*)	(*)
30	0.187	0.089	0.021	*	0.235	0.139	0.089	*
	(0.172)	(0.082)	(0.016)	(*)	(0.223)	(0.121)	(0.091)	(*)
60	0.098	0.023	0.006	*	0.096	0.028	0.016	*
	(0.089)	(0.021)	(0.003)	(*)	(0.087)	(0.031)	(0.014)	(*)
90	0.021	0.001	*	*	0.023	0.002	*	*
	(0.022)	(0.001)	(*)	(*)	(0.025)	(0.002)	(*)	(*)
Irrigation level ($I_{10.5\text{-}10.5}$)								
0	0.338	*	*	*	0.471	*	*	*
	(0.351)	(*)	(*)	(*)	(0..468)	(*)	(*)	(*)
15	0.182	0.117	*	*	0.206	0.206	*	*
	(0.176)	(0.114)	(*)	(*)	(0.196)	(0.212)	(*)	(*)
30	0.096	0.129	0.054	*	0.099	0.132	0.065	*
	(0.083)	(0.116)	(0.042)	(*)	(0.086)	(0.121)	(0.072)	(*)
60	0.087	0.052	0.018	*	0.082	0.072	0.011	*
	(0.067)	(0.049)	(0.012)	(*)	(0.079)	(0.059)	(0.009)	(*)
90	0.012	0.001	*	*	0.013	0.005	*	*
	(0.009)	(*)	(*)	(*)	(0.011)	(0.002)	(*)	(*)

* Not detected and Figures in parenthesis are the values for the year 1996-97.

EFFECT OF SOME HERBICIDES ON SMALL-WHITE FLOWER MORNINGGLORY (*Ipomoea obscura* (L.) Ker-Gawl) CONTROL IN PINEAPPLE

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Abstract : The study was conducted in two experiments, the first involving herbicide efficiency on small-white flower morningglory control and the second involving herbicide toxicity to pineapple. The first experiment, 23 treatments including herbicides applied alone and in combinations were applied to small-white flower morningglory in both seedling and flowering stages. Results revealed that quinclorac at 46.90 and 93.75 g.a.i./ha, bromacil at 3.60 kg.a.i./ha, and dicamba at 0.48 kg.a.i./ha provided great control of the weed, but in the second experiment, some of these herbicides caused phytotoxic to pineapple plant. Quinclorac and dicamba caused stunt, growth decrement, and abnormal leaves of the plant. However, at three months after application, the plant could regrown again. While bromacil caused yellow leaf for one month and the plant was recovered later on. Furthermore, all herbicides did not affect the yield and yield components.

Key Words : small-white flower morningglory, quinclorac, bromacil, dicamba, pineapple

Introduction

Some morningglory weeds, such as ivyleaf morningglory is generally not a problem in young cotton until later in the growing season but in irrigated cotton, ivyleaf morningglory usually appears after the first irrigation (Robert and Keeley, 1983). Taylor and Heathman (1977) reported that to achieve control of annual morningglory (*Ipomoea* spp.) multiple applications of herbicides are required, but under general farming practice, repeated application of herbicides are done until the cotton becomes too tall to treat easily and then the control of morningglory is forgotten. Ivyleaf morningglory does infest field after the last cultivation or when the last application of the herbicide has lost its effectiveness. Mathis and Oliver (1980) reported that entireleaf and ivyleaf morningglory may become greater problems than other moringglory species due to more herbicide tolerance. The experiment on herbicide application indicated that metribuzin controls some morningglory species, but it is ineffective on entireleaf and ivyleaf morningglory (Mathis and Oliver, 1980). Lee and Oliver (1982) reported that entireleaf and ivyleaf morningglory at four leaf stage showed tolerance to acifluorfen application. Loasinwattana (1994) reported that small-white flower morningglory (*Ipomoea obscura* (L.) Ker-Gawl) become greater problems in pineapple than other broadleaf and grass weeds because this specie showed tolerance to diuron, ametryn, bromacil, haloxyfop, fluazifop and quizaiofop. The objectives of this experiment were to determine the efficiency of some post-emergence herbicides on small-white flower morningglory control and to determine the impacts of herbicides on pineapple production.

Materials and Methods

The study was carried out in two experiments, the first involving herbicide efficiency on small-white flower morningglory control comprised of two growth stages, and the second involving herbicide toxicity to pineapple. In June 1997, greenhouse experiment was conducted at Department of Agronomy, Kasetsart University for the first experiment in order to evaluate an efficiency of some post-emergence herbicides on small-white flower morningglory control in both seedling (4-6 leaf stage, 20 days after germination) and flowering stage (45 days after germination). Treatments were herbicides applied alone and in combinations. The seedlings of small-white flower morningglory were planted in 15 cm. diameter and 15 cm. height pot. The soil was characterized as sandy clay loam which composed of sand 68%, silt 7%, clay 25% and 1.54% of organic matter. Both experiments were designed as a completely randomized design with 5 replications. Herbicides were applied by the laboratory sprayer with 500 l/ha spray volume. Efficiency of herbicides and herbicide combinations were evaluated on dry weight of the plant at 49 days

after application and weed control rating from 0 (no control) to 100 (complete control) according to Bryan (1977) was also reccorded by visual observation.

Field experiment at Siam Agro-Industry Co, Ltd. (Public) was conducted for the second experiment in December 1997. In this study, land preparation, plant spacing and population density, fertilization and other cultivation were managed by field maintenance division of the company, so they, as well as the pineapple plant, were uniform. The soil was characterized as sandy clay loam which composed of sand 65%, silt 9%, clay 26% and 1.87% of organic matter. At 6 months after planting, herbicides which showed high efficiency for small-white flower morningglory control in the first experiment were selected and applied to pineapple plants by knapsack sprayer in 500 l/ha of spray volume. Experiment was designed as randomized complete block design with 4 replications and the plot size was 7.0 x 7.0 meters. Visual injury rating of pineapple was recorded according to Bryan (1977). Plant height, 50% of flowering date, yield and yield components were also recorded.

Results and Discussion

Normally, the farmers applied bromacil and diuron + bromacil as pre-emergence and as post-emergence at 3 months after planting. These herbicides provided great control of this weed at early stage of pineapple growth. Small-white flower morningglory are usually found in pineapple plantation at 6 months after planting or later. Therefor, only post-emergence herbicides were applied in these experiments.

In the first experiment, efficiencies of post-emergence herbicides on small-white flower morningglory control were shown in Table 1 and Table 2. The results indicated that all herbicides had high efficiencies for the control of this weed, whereas the other herbicides, which their data were not shown in the Tables, did not meet the satisfying control due to they did not give complete control of the weed. Weed dry weight at 50 days after herbicide application was shown in the Table 2 in order to evaluate the efficiencies of herbicides. But it was not shown in the Table 1 because the weed had been naturally decayed. The results of the first experiment indicated that this weed could be controlled at both stages (completely died) by the effective herbicides such as bromacil at 3.6 kg. a.i/ha, dicamba at 0.24 and 0.48 kg. a.i/ha, quinclorac at 46.90 g.a.i./ha and 93.75 g.a.i./ha. Whereas bromacil at 1.8 kg.a.i./ha, diuron at 1.8 and 3.6 kg.a.i./ha and tank mixed of bromacil + haloxyfop methyl at 2.0 + 0.45 kg.a.i./ha, diuron + haloxyfop methyl at 2.0 + 0.45 kg.a.i/ha could control only at seedling stage of the weed. However, an invasion of small-white flower morningglory in pineapple production area was caused by seed dormancy and soil seed bank that protected it from herbicide, so, the weed could germinate at any time and it infested the crop at several growing stages. Moreover, the weed seedlings were also hindered from herbicide spray droplet by the canopies of pineapple plats. Therefore, the post-emergence herbicides such as bromacil at 3.6 kg.a.i./ha, dicamba at 0.24 and 0.48 kg.a.i./ha and quinclorac at 46.90 g.a.i./ha and 93.75 g.a.i./ha should be more profitable for the control.

Fig.1 showed the impacts of post-emergence herbicides on 6-months old pineapple plants in the second experiment. The most effective herbicides were selected from the first experiment in Table 1. and 2 were directly applied to the 6-months old pineapple plants, in order to study the responses of the plants to herbicides. Results indicated that dicamba at 0.48 kg.a.i./ha caused the highest injury and growth decrement of the plants, compared with bromacil and quinclorac. However, the results also revealed that quinclorac at 46.90 and 93.75 g.a.i./ha caused stunt of the plants at 49 to 98 days after application. But the injury symptom such as abnormal leaves was disappeared at 70 days after application (Fig.1) After that, the plant height became increasing. Therefore, there was not significantly difference in plant height at 161 days after application (Fig.1) Whereas bromacil caused yellow leaf of the plants for about 45 days after application, and it did not affect plant growth in comparison with the control.

In this experiment, ethephon was applied at 11 months after planting in order to induce their reproductive phase. The ethephon application was done twice on November 10 and 13, 1998 (140 and 143 days after herbicides application). The results indicated that there was slightly difference in 50% of flowering date of pineapple plants (Table 3.) So, they verified that the plants among the treatments were not different in growth, or they were not get any impact from herbicide application, even after the plants were induced to flowering by ethephon, As well as the percent of harvested plants, yield and yield components of the plants, shown in the Table 3, were not different among the herbicides. The study was concluded that the herbicides did not give any impact on yield and yield components of the 6 months old pineapple plants even though some herbicides such as dicamba at 0.48 kg.a.i./ha and quinclorac at 46.90

and 93.75 g.a.i./ha caused phytotoxic, stunt and growth decrement at the period of 2 or 3 months after application.

Table 1 Efficiency of post-emergence herbicides on small-white flower morningglory control at seedling stage.

Herbicides and rates	Control rating 1/ 2/			
	Days after application			
	4	7	14	21
Control (no herbicide)	0	0	0	0
Bromacil 2.0 kg.a.i./ha + haloxyfop 0.45 kg.a.i./ha	84	100	100	100
Bromacil 3.60 kg.a.i./ha	80	100	100	100
Diuron 2.0 kg.a.i./ha + haloxyfop 0.45 kg.a.i./ha	76	100	100	100
Bromacil 1.80 kg.a.i./ha	80	98	100	100
Diuron 3.60 kg.a.i./ha	80	98	100	100
Diuron 1.80 kg.a.i./ha	78	98	100	100
Dicamba 0.48 kg.a.i./ha	74	86	100	100
Quinclorac 93.75 g.a.i./ha	52	70	100	100
Quinclorac 46.90 g.a.i./ha	50	70	100	100
Dicamba 0.24 kg.a.i./ha	60	76	98	100

1/ = Control rating ; 0 = no control, 100 = complete control
2/ = The means of 5 replications

Table 2 Efficiency of post-emergence herbicides on small-white flower morningglory control at flowering stage.

Herbicides and rates	Control rating 1/ 2/								Dry weight 2/ (g./plant)
	Days after application								
	4	7	14	21	28	35	42	49	
Control (no herbicide)	0	0	0	0	0	0	0.	0	13.9 a
Bromacil 3.60 kg.a.i./ha	48	84	100	100	100	100	100	100	3.5 h
Dicamba 0.48 kg.a.i./ha	68	82	92	96	100	100	100	100	4.4 h
Quinclorac 93.75 g.a.i./ha	34	68	88	96	98	98	100	100	2.9 h
Quinclorac 46.90 g.a.i./ha	30	66	82	90	98	98	98	100	3.5 h
Dicamba 0.24 kg.a.i./ha	52	78	90	96	98	98	98	100	4.5 h

1/ = Control rating ; 0 = no control, 100 = complete control
2/ = The means of 5 replications
Means within a column followed by a common letters are not significantly different at the 5 % level by the LSD test

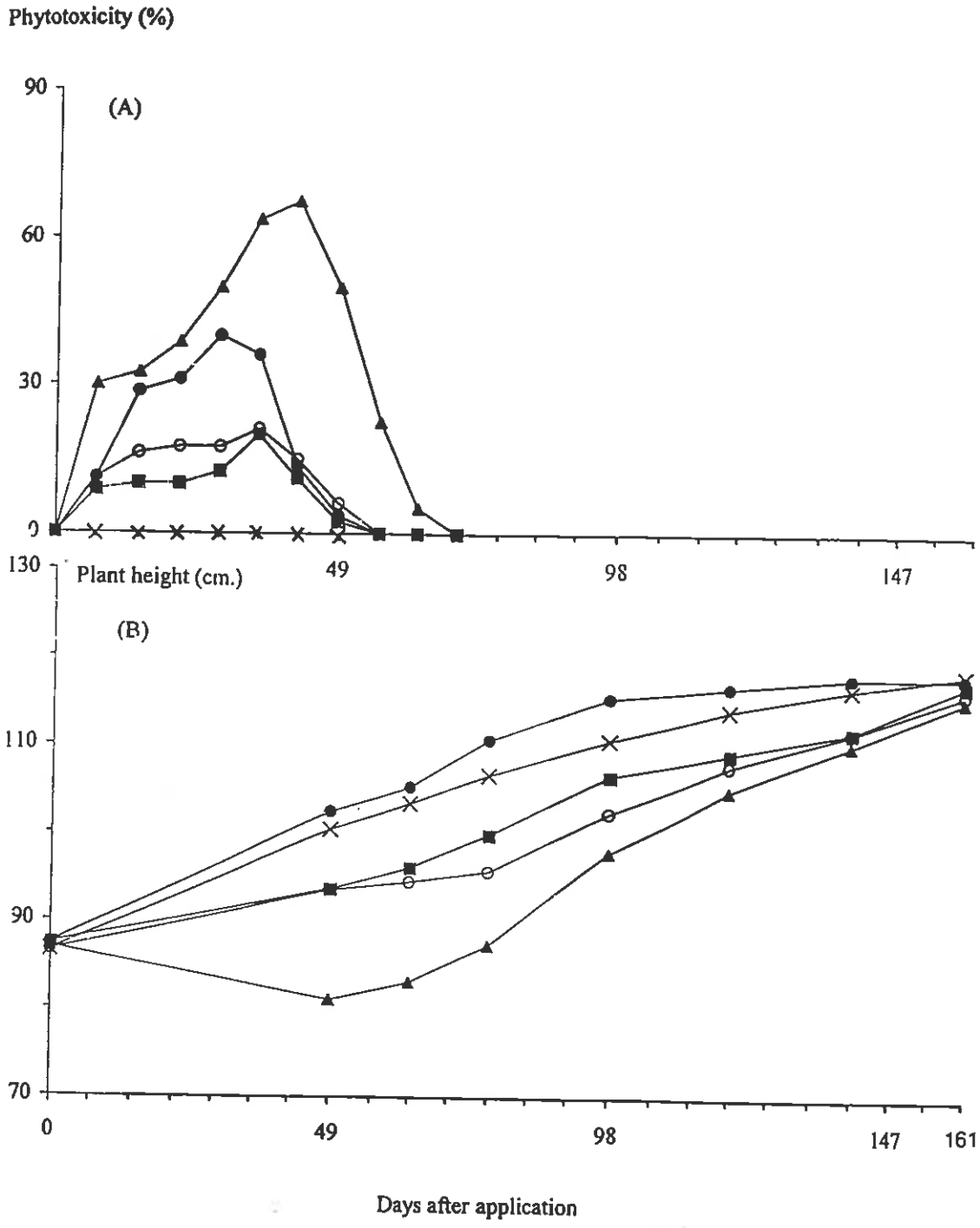

Fig. 1 Effects of bromacil 3.60 kg.a.i./ha (●-●), dicamba 0.48 kg.a.i./ha (▲-▲), quinclorac 46.90 g.a.i./ha (■-■), quinclorac 93.75 g.a.i./ha (O-O) on phytotoxicity (A) and plant height (B) of pineapple compared with control (x-x).

Table 3 Effect of post-emergence herbicides on days to 50 % of flowering, percent of plant harvested, yield and yield components of pineapple when the herbicide was applied at 6 months after planting [1/]

Herbicides and rates	Days to[2/] 50 % flowering (days)	Plant harvested (%)	Fruit sizes			Yield (t/ha)
			Diameter (cm.)	Length (cm.)	Weight (g/fruit)	
Control (no herbicide)	42.0 b	98.7 a	12.8 a	18.7 a	1,881.8 a	87.6 a
Bromacil 3.60 kg.a.i./ha	42.0 b	98.5 a	12.7 a	18.4 a	1,875.1 a	86.7 a
Dicamba 0.48 kg.a.i./ha	43.0 ab	97.2 a	12.7 a	18.4 a	1,870.4 a	86.5 a
Quinclorac 46.90 g.a.i./ha	43.7 a	98.3 a	12.7 a	18.5 a	1,871.8 a	87.5 a
Quinclorac 93.75 g.a.i./ha	43.2 a	98.1 a	12.7 a	18.5 a	1,869.7 a	86.4 a

1/ Means within a column followed by a common letters are not significantly different at the 5 % level by the LSD test

2/ Days after application of ethephon which was applied at 11 months after planting

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EVALUATION OF DINITROANILINE HERBICIDES AGAINST ISOPROTURON RESISTANT *Phalaris minor* IN WHEAT (*Triticum aestivum*) UNDER ZERO AND CONVENTIONAL TILLAGE

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Abstract: Two experiments were conducted during winter season of 1997-98 and 1998-99 to study efficiency of pendimethalin (Stomp 30%) and trifluralin (Treflan 48%) both applied pre-plant incorporation (PPI) using different seed rates both under zero and conventional tillage. The experiment were conducted at the Agronomy Research Farm, CCS Haryana Agricultural University, Hisar during first year in a field infested with susceptible population of *Phalaris minor* and at farmers field during second year in a field infested with resistant population of *Phalaris minor*. There was a significant reduction in population of *Phalaris minor* due to application of pendimethalin or trifluralin. Pendimethalin and trifluralin applied PPI allowed reduction in the population of resistant *Phalaris minor* both under zero and conventional tillage. Although these treatments increased the wheat yield by 10 to 12 per cent yet the increase in wheat yield and *Phalaris minor* control was not to the satisfaction of the farmers. Under farmers field situation the reduction in wheat yield due to *Phalaris minor* in the untreated check was more in the conventional tillage than in the zero tillage.

Key words : Pendimethalin, trifluralin, zero tillage, conventional tillage

Introduction

Littleseed canarygrass (*Phalaris minor*) is the most serious weed of wheat in the rice-wheat cropping system. With the introduction of high yielding varieties and subsequently greater use of fertilizer and irrigation in both rice and wheat, *Phalaris minor* started dominating over other weeds (Singh and Malik, 1992). Initially the weed flora dominated by broad leaf weeds was replaced by weed flora dominated by *Phalaris minor* in late seventies. Due to increased problem of *Phalaris minor*, the herbicide use became obligatory (Malik *et al.*, 1995). A number of phenylurea compounds were recommended in late seventies (Gill *et al.*, 1978). Most farmers accepted the herbicide use and eventually isoproturon was the only herbicide which dominated the market for about 10 years. The continuous use of isoproturon for more than 10 years in rice-wheat cropping system led to the development of resistance in littleseed canarygrass against isoproturon (Malik and Singh, 1993). The development of resistance again brought about a convergence of *Phalaris minor* problem with the density levels at same level or more than the density levels that existed in late seventies (Malik *et al.*, 1995). The weed control failures from last 4-5 years brought about a significant reduction in wheat yield and the farmers were in need of alternate herbicides to combat the yield losses in wheat grain yield due to the problem of resistance in *Phalaris minor*. The resistance reports for wheat from other countries have shown that the use of alternate herbicide is full of uncertainties. Reports available so far, indicate that the possibility of development of cross resistance of isoproturon resistant *Phalaris minor* against pendimethalin are less (Malik and Singh, 1995; Yaduraju and Ahuja, 1995). Therefore, pendimethalin and trifluralin were evaluated against resistant populations of *Phalaris minor* under field conditions in the present investigation.

Materials and Methods

Two experiments were conducted during winter season of 1997-98 and 1998-99 at the Agronomy Research Farm, CCS Haryana Agricultural University, Hisar in a field infested with susceptible population of *Phalaris minor* and at farmers field during second year in a field infested with resistant population of *Phalaris minor*. Pendimethalin and trifluralin were applied PPI at 0.75 and 1.0 kg a.i. ha^{-1} along with weedy and weed free check using

seed rates of 100, 125 and 150 kg ha^{-1} under zero and conventional tillage.

The 18 treatments were replicated thrice in a split plot design with a plot size of 6.0 m× 2.5 m. The soil of the experimental field was sandy loam, low in available nitrogen, medium in phosphorus and high in potassium. Sowing of wheat var. WH-542 was done on 24 and 2 December during 1997 and 1998, respectively.

Phalaris minor density and dry matter accumulation were recorded at 50, 75, 100 days after sowing (DAS) and at harvest. Crop studies were made regarding growth, yield attributing characters and yield of wheat. Under conventional sowing, field was prepared thoroughly (3 harrowing each followed by planking) before sowing, whereas, under zero tillage sowing, crop was sown directly into the soil using zero tillage seed cum fertilizer drill.

Results and Discussion

Effect on *Phalaris minor* density

During first year experiment was conducted against susceptible population of *Phalaris minor* at the experimental farm whereas during second year the experiment was conducted against resistant population at farmers field. The increase in seed rate brought about a significant reduction in population both against susceptible and resistant populations of *Phalaris minor* under zero tillage (Table 1 and 2). Similar effects have been reported by Singh *et al.* (1993).

Pendimethalin or triflurain each applied PPI at 0.75 kg a.i. ha^{-1} brought about an average population reduction by 32.7, 33.8 and 37.6, 38.7 per cent at 50 DAS and harvest respectively. Under zero tillage and 33.2, 31.5 and 55.6, 54.7 per cent at 50 DAS and harvest respectively under conventional tillage. Pendimethalin or trifluralin each applied PPI at 1.0 kg a.i. ha^{-1} brought about an average population reduction by 37.7, 41.9 and 36.7, 45.9 per cent at 50 DAS and harvest respectively under zero tillage and 36.4, 63.0 and 36.2, 60.3 per cent at 50 DAS and harvest respectively under conventional tillage (Table 1 and 2).

At 50 DAS the population of *Phalaris minor* under conventional tillage was more than zero tillage. But the differences were statistically not comparable. The effect of herbicides in two separate experiments under zero tillage and conventional tillage and both against susceptible and resistant population remained similar. The overall effect of these herbicides on both resistant and susceptible population of *Phalaris minor* ranged from 48.3 to 57.2 per cent respectively during both years. *Phalaris minor* control with pendimethalin has also been reported by Mustafee (1989, 1991), Malik and Singh (1995), Singh and Singh (1996) and Singh and Kolhe (1998).

Effect on yield

The increase in seed rate brought about a significant increase in grain yield up to 125 kg seed ha^{-1} under conventional tillage. However, further increase in seed rate increased the grain yield but the difference was non-significant (Table 3). Similar results have been reported by Panwar *et al.* (1989).

Pendimethalin or trifluralin each applied PPI at 0.75 kg a.i. ha^{-1} brought about an average yield increased by 9.7, 6.6 and 8.7, 9.2 per cent respectively under zero and conventional tillages. Pendimethalin or trifluralin each applied PPI at 1.0 kg a.i. ha^{-1} brought about an average yield increase by 10.9, 11.7 and 11.3, 10.4 per cent respectively under zero and conventional tillage. The overall effect of these herbicides on yield increase ranged from 11.3 to 10.8 per cent respectively under zero and conventional tillage, whereas, with respect to susceptible and resistant population it ranged from 13.5 to 8.6 per cent yield increase, respectively (Table 3). Similar results of Pendimethalin have been reported by Mustafee (1989), Phogat *et al.* (1991) and Yadav *et al.* (1996). Yield increase with trifluralin have been reported by Jain *et al.* (1985) and Malik *et al.*(1995).

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Table 1. Influence of seed rate and herbicides on the population (No./m²) of *Phalaris minor* at different stages (DAS) of wheat crop under conventional sowing during 1997-98 and 1998-99

Treatment	Dose (kg ha^{-1})	1997-98		1998-99	
		50	Harvest	50	Harvest
S1	100	9.00 (80.5)	5.16 (26.1)	11.79 (138.5)	7.44 (54.8)
S2	125	8.64 (74.1)	3.81 (14.0)	11.51 (133.0)	7.41 (54.4)
S3	150	8.54 (72.4)	3.53 (12.0)	10.60 (111.9)	7.45 (55.0)
S.Em(±)	-	0.29	0.44	0.29	0.34
C.D. at 5%	-	NS	NS	NS	NS
Pendimethalin	0.75 PPI	10.28 (105.3)	4.48 (19.6)	12.36 (152.3)	8.25 (67.6)
Pendimethalin	1.00 PPI	9.92 (98.0)	3.72 (13.3)	12.27 (150.0)	8.01 (63.7)
Trifluralin	0.75 PPI	10.12 (101.9)	4.47 (19.5)	13.05 (169.8)	8.41 (70.2)
Trifluralin	1.00 PPI	9.92 (97.9)	4.22 (17.3)	12.32 (151.3)	7.85 (61.1)
Weedy	-	11.39 (129.3)	7.41 (53.9)	17.10 (291.9)	11.39 (129.2)
Weed free	-	0.71	0.71	0.71	0.71
S.Em(±)		0.30	0.37	0.36	0.41
CD at 5%		0.86	1.07	1.05	1.18

Data is square root transformed ($\sqrt{x+0.5}$) and values given in the parenthesis are original values

Table 2. Influence of seed rate and herbicides on the population (No./m²) of *Phalaris minor* at different stages (DAS) of wheat crop under zero tillage sowing during 1997-98 and 1998-99

Treatment	Dose (kg ha^{-1})	1997-98		1998-99	
		50	Harvest	50	Harvest
S1	100	8.23 (67.2)	4.44 (19.2)	11.38 (129.0)	8.12 (65.4)
S2	125	7.49 (55.6)	3.63 (12.6)	11.08 (122.2)	7.65 (58.0)
S3	150	7.36 (53.7)	3.32 (10.5)	10.36 (106.8)	7.05 (49.2)
S.Em(±)	-	0.12	0.12	0.22	0.24
C.D. at 5%	-	0.49	0.48	0.89	0.96
Pendimethalin	0.75 PPI	8.21 (66.9)	4.41 (18.9)	12.52 (156.3)	8.47 (71.2)
Pendimethalin	1.00 PPI	7.78 (60.0)	4.26 (17.6)	12.25 (149.5)	8.17 (66.3)
Trifluralin	0.75 PPI	8.10 (65.1)	4.49 (19.7)	12.49 (155.5)	8.14 (65.8)
Trifluralin	1.00 PPI	8.07 (64.6)	4.28 (17.8)	11.95 (142.3)	7.56 (56.6)
Weedy	-	9.73 (94.1)	5.62 (31.0)	15.71 (246.3)	10.59 (111.6)
Weed free	-	0.71	0.71	0.71	0.71
S.Em(±)		0.49	0.29	0.23	0.33
CD at 5%		1.43	0.85	0.66	0.94

Data is square root transformed ($\sqrt{x+0.5}$) and values given in the parenthesis are original values

Table 3. Influence of seed rate and herbicides on the grain yield (q ha^{-1}) of wheat

Treatment	Dose (kg ha^{-1})	Conventional sowing		Zero tillage sowing	
		1997-98	1998-99	1997-98	1998-99
S1	100	24.67	37.40	26.27	37.74
S2	125	27.88	41.19	27.87	39.42
S3	150	28.47	40.05	28.44	39.56
S.Em(±)	-	0.72	0.96	1.03	0.69
C.D. at 5%	-	2.84	3.76	NS	NS
Pendimethalin	0.75 PPI	27.15	39.00	28.14	38.77
Pendimethalin	1.00 PPI	27.72	40.42	28.37	39.49
Trifluralin	0.75 PPI	27.10	39.52	26.46	38.45
Trifluralin	1.00 PPI	27.33	40.14	28.60	39.82
Weedy	-	24.00	36.70	24.43	36.33
Weed free	-	28.75	41.50	29.16	40.59
S.Em(±)		1.07	1.13	1.36	0.85
CD at 5%		3.09	3.27	3.92	2.46

HERBICIDAL EFFICACY OF BISPYRIBAC-SODIUM AGAINST RICE WEEDS

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Abstract: Bispyribac-sodium is a selective post-emergence herbicide for direct seeded rice (*Oryza sativa*) and was launched recently in South East Asian countries, and Central and South American countries under the trade name, "Nominee®". In the greenhouse trials, bispyribac-sodium at 15g a.i./ha showed excellent and stable efficacy against 2 to 8 leaf stage of *Echinochloa oryzicola, Echinochloa crus-galli, Ischaemum rugosum, Cyperus iria, Fimbristylis miliacea, Monochoria vaginalis, Sphenoclea zeylanica, Aeschynomene indica, Ludwigia epilobioides* and *Alisma canaliculatum,* and 2 to 6 leaf stage of *Cyperus difformis* and *Eclipta prostrata.* On the other hand, bispyribac-sodium at the recommended rate (20-40g a.i./ha) showed low efficacy against *Leptochloa chinensis*, one of the troublesome weeds in direct seeded rice, when applied at more than 4 leaf stage. To improve the efficacy of bispyribac-sodium against *Leptochloa* spp., tank-mixture treatment with other rice herbicides was evaluated in greenhouse. The result showed that bispyribac-sodium tank-mixed with fenoxaprop-ethyl can control 4 to 8 leaf stage of *Leptochloa chinensis* with good selectivity. The tank-mixture treatment of bispyribac-sodium and fenoxaprop-ethyl is now being evaluated under various rice field conditions in several rice growing countries to determine the proper application rate of fenoxaprop-ethyl to be mixed with bispyribac-sodium.

Key words: bispyribac-sodium, post-emergence herbicide, growth stage, *Leptochloa chinensis*

Introduction

Direct seeded rice is one of the most popular rice production systems in the world today. However, weed pressure in direct seeded rice is generally very high and weed control is difficult compared to transplanted rice. Some weeds are escaped from the application of existing rice herbicides, which is one of the main reasons for rice yield reduction. Rice growers are always looking for herbicides having excellent characteristics such as broad herbicidal spectrum, wide application window and high selectivity to establish well-designed weed control program for their rice fields.

Bispyribac-sodium (chemical name: sodium 2,6-bis [(4,6-dimethoxypyrimidin-2-yl)oxy] benzoate) is a new selective post-emergence herbicide for direct seeded rice discovered and being developed by Kumiai Chemical Industry Co., Ltd. (1, 2 and 3) This product was launched recently in South East Asian countries, and Central and South American countries under the trade name of Nominee. Bispyribac-sodium is being accepted rapidly by rice farmers in many countries due to its excellent characteristics.

This paper summarizes the results of our greenhouse trials carried out in Japan to evaluate the herbicidal efficacy of bispyribac-sodium against various growth stage of paddy weeds, and the efficacy of bispyribac-sodium in tank-mixture with other rice herbicides against *Leptochloa chinenses*, which was hardly controlled by bispyribac-sodium alone.

Materials and Methods

Herbicidal efficacy of bispyribac-sodium alone against various growth stage of weeds

Each test weed was grown until its target leaf stage for testing in a separate plastic pot (10.5cm×10.5cm)

containing clay loam soil in a greenhouse. Bispyribac-sodium, formulated to 100g a.i./liter Suspension Concentrate (SC), was applied to the test weeds at 2, 4, 6 and 8 leaf stage at 500 liter/ha spray volume with a non-ionic surfactant (0.1% V/V) utilizing a CO2-pressured sprayer. Herbicidal efficacy was evaluated visually at 30 days after application using a scale of 0 = no effect to 100 = plant death. All trials were conducted with 3 replications.

Herbicidal efficacy of bispyribac-sodium against *Leptochloa chinensis* and rice injury when applied in mixture with fenoxaprop-ethyl or cyhalofop-butyl

Leptochloa chinensis and rice (variety: RD-23) were planted into separate plastic pots (10.5cm×10.5cm) containing clay loam soil and grown in a greenhouse. Bispyribac-sodium mixed with fenoxaprop-ethyl (7.5EW), or cyhalofop-butyl (10EC) was applied with a non-ionic surfactant (0.1%V/V) and spray volume was 500 liter/ha. Herbicidal efficacy and rice injury were evaluated visually at 30 days after application using a scale of 0 = no effect to 100 = plant death.

Results and Discussion

Data on herbicidal efficacy of bispyribac-sodium against grass weeds are shown in Table 1-A. Bispyribac-sodium at 15g a.i./ha showed very high efficacy against 2 to 8 leaf stage of *Echinochloa oryzicola, Echinochloa crus-galli* and *Ischaemum rugosum*. Especially *Ischaemum rugosum* was the most susceptible grass weed to bispyribac-sodium. On the other hand, bispyribac-sodium at even 30g a.i./ha showed low efficacy against *Leptochloa chinensis* when applied at more than 4 leaf stage, although the herbicidal efficacy of bispyribac-sodium against 2 leaf stage of *Leptochloa chinensis* was sufficient. From this result, it was considered to be very difficult to control *Leptochloa chinensis* with bispyribac-sodium at its recommended commercial rate (20-40g a.i./ha). Tank-mixture treatment with other rice herbicides was considered to be necessary to improve the efficacy of bispyribac-sodium against *Leptochloa* spp.

Table 1-A Herbicidal efficacy of bispyribac-sodium against grass weeds

Test weeds	Rate	Leaf stage at application			
	(g a.i./ha)	2	4	6	8
Echinochloa oryzicoloa	30	100	100	100	98
	15	100	100	100	90
	7.5	100	100	77	60
	3.75	98	85	30	20
Echinochloa crus-galli	30	100	100	100	95
	15	100	100	92	93
	7.5	100	97	86	68
	3.75	93	88	23	13
Leptochloa chinensis	30	95	79	56	5
	15	93	65	30	0
	7.5	63	40	10	0
	3.75	30	5	0	0
Ischaemum rugosum	30	100	100	100	96
	15	100	100	100	92
	7.5	100	99	99	90
	3.75	100	95	97	67

Evaluation scale : 0 = no effect — 100 = complete death

Data on herbicidal efficacy of bispyribac-sodium against sedges are shown in Table 1-B. Bispyribac-sodium at 15g a.i./ha had very high efficacy against 2 to 6 leaf stage of *Cyperus difformis*. Also, 7.5g a.i./ha showed excellent efficacy against 2 to 8 leaf stage of *Cyperus iria* and *Fimbristylis miliacea*.

Table 1-B Herbicidal efficacy of bispyribac-sodium against sedges

Test weeds	Rate	Leaf stage at application			
	(g a.i./ha)	2	4	6	8
Cyperus difformis	30	97	100	100	82
	15	91	90	100	33
	7.5	77	47	65	16
	3.75	53	33	16	0
Cyperus iria	30	100	100	99	98
	15	100	100	98	98
	7.5	100	98	95	90
	3.75	93	66	65	60
Fimbristylis miliacea	30	100	100	100	92
	15	98	98	92	90
	7.5	98	95	92	90
	3.75	92	90	67	63

Evaluation scale : 0 = no effect — 100 = complete death

Data on herbicidal efficacy of bispyribac-sodium against broadleaf weeds are shown in Table 1-C. Bispyribac-sodium showed excellent efficacy against all broadleaf weeds in this test. Sufficient efficacy was observed when bispyribac-sodium was applied at 2 to 8 leaf stage of *Alisma canaliculatum* and *Sphenoclea zeylanica* at 3.75g a.i./ha, 2 to 8 leaf stage of *Aeschynomene indica* and *Ludwigia epilobioides* at 7.5g a.i./ha, 2 to 8 leaf stage of *Monochoria vaginalis* at 15g a.i./ha, and 2 to 6 leaf stage of *Eclipta prostrata* at 15g a.i./ha, respectively.

Table 1-C Herbicidal efficacy of bispyribac-sodium against broadleaf weeds

Test weeds	Rate	Leaf stage at application			
	(g a.i./ha)	2	4	6	8
Monochoria vaginalis	30	100	100	100	100
	15	100	100	100	100
	7.5	92	95	93	80
	3.75	33	73	67	60
Alisma canaliculatum	30	100	100	100	93
	15	96	90	93	90
	7.5	90	90	90	90
	3.75	90	88	90	90
Sphenoclea zeylanica	30	100	100	100	98
	15	100	99	99	95
	7.5	100	99	98	95
	3.75	100	95	92	91
Aeschynomene indica	30	100	100	100	100
	15	100	98	95	95
	7.5	100	95	91	94
	3.75	97	84	77	85
Ludwigia epilobioides	30	100	100	100	100
	15	96	93	100	99
	7.5	93	90	90	96
	3.75	80	90	90	70
Eclipta prostrata	30	100	97	96	86
	15	98	90	90	82
	7.5	88	70	67	60
	3.75	80	57	10	10

Evaluation scale : 0 = no effect — 100 = complete death

To improve the efficacy of bispyribac-sodium against *Leptochloa* spp., mixture treatments with other post-emergence rice herbicides were evaluated in a greenhouse. Data on herbicidal efficacy against *Leptochloa chinensis* and rice injury when bispyribac-sodium was applied in mixture with fenoxaprop-ethyl or cyhalofop-butyl are shown in Table 2. Fenoxaprop-ethyl and cyhalofop-butyl showed very high efficacy against *Leptochloa chinensis* even at one-fourth to one-eighth of their commercial rates. Tank-mixture treatment of bispyribac-sodium and fenoxaprop-ethyl showed very good efficacy, and the efficacy and rice injury of fenoxaprop-ethyl were not affected significantly by the addition of bispyribac-sodium. However, in the case of tank-mixture treatment of bispyribac-sodium and cyhalofop-butyl, very strong antagonism was observed and the efficacy of cyhalofop-butyl was decreased remarkably by mixing with bispyribac-sodium

From the above, the tank-mixture treatment of bispyribac-sodium and fenoxaprop-ethyl is considered to be recommendable, in the case that infestation of *Leptochloa* spp. is observed in rice field. The tank-mixture treatment is now being evaluated under various rice field conditions in several rice growing countries to determine the proper application rate of fenoxaprop-ethyl to be mixed with bispyribac-sodium.

Table 2 Herbicidal efficacy against *Leptochloa chinensis* and rice injury when bispyribac-sodium was applied in mixture with fenoxaprop-ethyl or cyhalofop-butyl

Treatment	Rate	*Leptochloa chinensis*			Rice	
	(g a.i./ha)	4L	6L	8L	4L	5L
Bispyribac-sodium	60	—	—	—	0	0
	30	70	40	20	0	0
	15	35	26	5	0	0
Fenoxaprop-ethyl	100	—	—	—	30	18
	50	—	—	—	16	8
	25	100	100	100	0	0
	12.5	100	100	100	0	0
	6.25	100	100	99	—	—
Bispyribac-sodium + Fenoxaprop-ethyl	15+100	—	—	—	30	20
	15+50	—	—	—	18	16
	15+25	100	100	100	0	0
	15+12.5	100	100	100	0	0
	15+6.25	98	98	98	—	—
Cyhalofop-butyl	100	—	—	—	0	0
	50	—	—	—	0	0
	25	100	100	—	0	0
	12.5	100	100	—	0	0
	6.25	100	96	—	—	—
Bispyribac-sodium + Cyhalofop-butyl	15+100	—	—	—	0	0
	15+50	—	—	—	0	0
	15+25	—	36	—	0	0
	15+12.5	50	50	—	0	0
	15+6.25	76	58	—	—	—

Evaluation scale : 0 = no effect — 100 = plant death

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IDENTIFICATION OF PHYTOTOXINS FROM MANILAGRASS (*Zoysia matrella* (L.) Merr.) PLANTS

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Abstract: Plant growth-inhibitory effects were found in the aqueous extract of manilagrass shoots. To isolate growth inhibitors, methanol extract of manilagrass shoots was partitioned into ethyl acetate soluble acidic (AE) and neutral (NE) fractions, and aqueous (AQ) fraction. AQ and NE fractions were inhibitory to seed germination and seedling growth of livid amaranth. AE fraction was also inhibitory but stimulatory at lower concentrations. Column chromatographies, HPLC and gas chromatography-mass spectrometry (GC-MS) were the principal methods used to identify the phytotoxins. The active fractions from ODS-HPLC were derivatized (Me/TMS), and then analyzed by GC-MS. The identities were finally confirmed by the comparison with authentic compounds by GC-MS. Eleven compounds, nine fatty acids (C_{10} to C_{18}) and two phenolic compounds (*p*-coumaric and *p*-hydroxybenzoic acids) were identified as phytotoxtns from manilagrass. Soil surrounding manilagrass root system also contained phytotoxins which would be released from manilagrass plants, and most of these phytotoxins are fatty acids and phenolic acids.

Key words: allelopathy, phytotoxins, manilagrass, identification

Introduction

Manilagrass (*Zoysia matrella* (L.) Merr.) is a persistent, drought-tolerant, and slow growing turfgrass that reduces the requirements of watering, mowing and fertilizing (Turgeon, 1980). The detrimental interference of manilagrass on germination and seedling growth of weed species have been demonstrated in the field. Weed density and biomass clearly demonstrated that much less weeds infested in manilagrass than in non-crop fields. These effect may be due to the aggressiveness of competition of manilagrass and also production of toxic substances. The allelopathic effects of manilagrass in the laboratory have been confirmed by aqueous extract from residues of manilagrass on germination and seedling growth of the weed and crop plants. Aqueous extract of the dried shoot tissue (50 mg DW/ml) showed stronger inhibitory effect on seed germination and seedling growth of radish (*Raphanus sativus*), livid amaranth (*Amaranthus lividus*), goose grass (*Eleusine indica*), tomato (*Lycopersicon eculentum*) and other plants than that of the fresh shoot tissue. Extracts of the shoots were more inhibitory than those of roots and seeds (Laosinwattana, 1997). Furthermore, soil bioassay and the activity of soil extracts suggest that inhibitory substances released from manilagrass are decomposed in a short period by soil microorganisms or various reactions with soil components (Laosinwattana, 1996).

The objectives of the present study was to identify the major active components in manilagrass shoot extracts. Investigations with individual chemicals would be useful in the overall understanding of the allelopathic effects of manilagrass.

Materials and Methods

Preparation and Collection of Samples

Manilagrass plants were grown in a turfgrass field at Weed Science Center, Utsunomiya University. A compound fertilizer ($N\text{-}P_2O_5\text{-}K_2O$ = 15-15-15) was applied at a rate of 400 kg ha^{-1} to the field in mid June. The turf field was regularly mowed so that the plant height would not exceed 15 cm. Cutting height was kept at about 1-2 cm. Weeds were removed by hands and thus no herbicides were sprayed to the field. The aboveground parts of manilagrass (about 10-15 cm in plant height) were harvested in August, 1996. The plant materials were air-dried in a greenhouse for 3 days and kept at room temperature.

Extraction and solvent partitioning of active compounds

Dried shoots of manilagrass (10 kg) were cut separately into 1-2 cm pieces, and extracted with methanol (100 L) for 48 h at room temperature. The extract was filtered and the methanol was removed slowly by vacuum evaporation at 40°C, leaving sticky residue (842 g). Details of the extraction method are shown in Fig. 1. The residue was diluted with 2 L of distilled water, and resulting aqueous solution was acidified to pH 2 with 6 N HCl and filtered to remove small amount of precipitates. The filtrate was extracted with ethyl acetate (2 L × 3). After adjusting the pH to neutral, the aqueous phase was concentrated *in vacuo* to afford aqueous residue (AQ, 588.08 g). The ethyl acetate solutions were combined, dried over anhydrous $MgSO_4$, concentrated to about 2 L and then extracted three time with saturated aqueous sodium bicarbonate (2 L × 3). The combined ethyl acetate phase was dried over $MgSO_4$ and concentrated *in vacuo*, affording ethyl acetate soluble neutral fraction (NE, 197.42 g). The sodium bicarbonate phase was evaporated to about 2 L, acidified, and then extracted with ethyl acetate (2 L × 3). The ethyl acetate solutions were combined, dried over $MgSO_4$, and then evaporated to afford the ethyl acetate soluble acidic fraction (AE, 30.00 g). The fractions which demonstrated sufficient activity in the bioassay were purified by appropriate chromatographic procedures. The choice of absorbents for column chromatography depended upon the nature of the active fractions. Each column chromatography was monitored by combination of thin layer chromatography (TLC) and bioassay.

Identification of phytotoxic compounds

Chromatography was used as the principal method for purification and identification of the phytotoxins. Silica gel column, ODS column, high performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) were employed. For the first column chromatography, the residues were chromatographed over silica gel (20 folds of samples, w/w) which were eluted with hexane - ethyl acetate (0, 10, 20, 30, 40, 50, 75 and 100%). For the second step purification, active fractions were separated using silica gel columns (200 folds of the samples, w/w) which were eluted with appropriate mixtures of hexane - ethyl acetate. The active fractions were then applied to ODS column chromatography using appropriate mixtures of water - methanol. The fractions showing sufficient activity were further purified by ODS-HPLC. The instrument used was a high pressure gradient system equipped with a diode array detector. The samples dissolved in methanol (100 µL) were applied to an ODS column (Merck, LiChrosorb RP-18-7 µm, 10 mm × 250 mm). The solvent was delivered in a linear gradient (for example, 50% aq. methanol to 100% methanol) at a flow rate of 3 mL/min and the eluent was monitored at 254 nm. The eluent was collected every 1.5 min. Each fraction was taken to dryness and bioassayed. The active fractions were further purified by an ODS analytical column (Merck, LiChrosorb RP-18-4 µm, 4 mm × 250 mm) using appropriate solvent mixtures of methanol and 0.1% acetic acid, at a flow rate of 1 ml/min. The eluent was collected every 1 min. The collected HPLC fractions showing bioactivity were dried at 45°C under gentle stream of dry nitrogen and then analyzed by GC-MS. For GC-MS, samples were first analyzed without derivatization. If necessary, samples were treated with diazomethane (CH_2N_2) and then with *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide (MSTFA). A Hewlett-Packard GC (Model 5980) combined to MS (Model 5970) were operated at an electron energy of 70 eV. A fused silica capillary column (J & W Scientific, DB-1, 15 m × 0.25 mm) was used. Helium was used as the carrier gas. Chromatographic conditions were as follows; injection port temperature, 260°C; detector temperature, 280°C; oven temperature, held at 100°C for 4 min, increased in a linear gradient from 100°C to 210°C (10°C/min), further increased in a linear gradient from 210°C to 310°C (8°C/min) and then held at 310°C for 7.5 min. The GC-MS spectral data were compared with the Wiley 138 and NBS75k Mass Spectra Data Base. The mass spectra and retention time were also verified by comparing with those of the authentic compounds. Chemicals used as standards in GC-MS analysis were purchased from commercial suppliers. Methyl esters and trimethylsilyl derivatives (Me/TMS) were prepared as described above.

Bioassay

Biological activities were determined with livid amaranth [*Amaranthus lividus* (L.)] seeds because of their high sensitivity to the inhibition by manilagrass aqueous extracts (Chamroon, 1997). Each sample dissolved in an appropriate solvent was added to a 5-cm Petri-dish and the solvent was evaporated. After the addition of 1.5 mL of distilled water, each twenty seeds of livid amaranth were placed in the Petri-dish, and then kept in an incubator maintained at 27°C and 80% relative humidity with a 12 h photoperiod. For HPLC fractions, 50 µL of each fraction was added to a 5-mL test tube and the solvent was evaporated. After adding 200 µL of distilled water, each ten seeds of livid amaranth were placed in the test tube and incubated as above. Three days after incubation the germination

percentage and seedling growth were determined. The authentic compounds, suberic acid, azelaic acid, *n*-tridecanoic acid, *n*-pentadecanoic acid, *n*-heptadecanoic acid, oleic acid, vaccenic acid, elaidic acid and petroselinic acid, were prepared in a series of aqueous solutions, 125, 250 and 500 ppm, and used for bioassay. The activities of *p*-hydroxybenzoic acid and *p*-coumaric acid were determined at 15.625, 31.25 and 62.5 ppm. All data were subjected to an analysis of variance followed by the separation of means by the LSD at .05%.

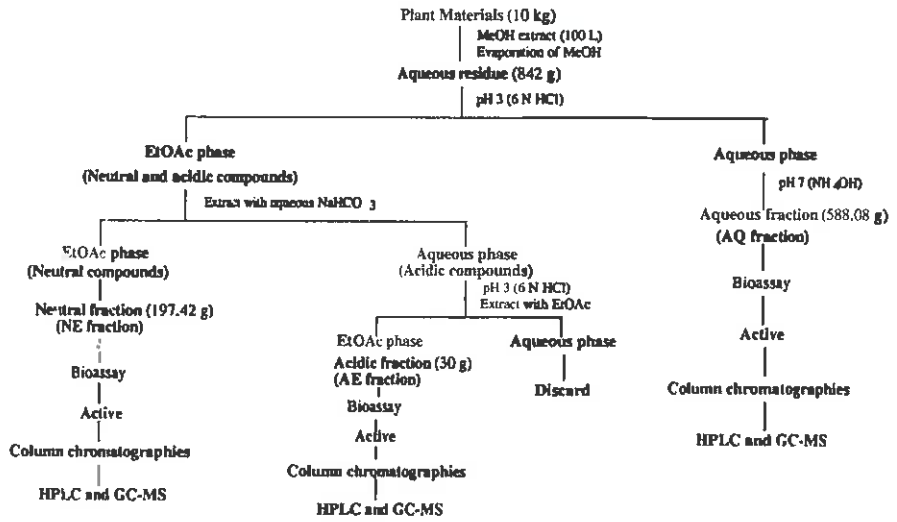

Fig. 1 Flow chart for extraction and partial purification of active compoun from manilagrass shoots.

Results and Discussion

Extraction and solvent partitioning of active compounds

The growth inhibitory activities of AQ, NE and AE fractions were compared with that of the original extract. The concentrations for bioassay varied from 50 to 1000 ppm for AQ, NE and AE fractions, and 6.25 mg DW/ml to 100 mg DW/ml for the original extract, all fractions inhibited germination and seedling growth of livid amaranth (Fig. 2). The results showed that inhibitory effects increased after the solvent partitioning. For example, the original extract completely inhibited germination and growth at 100 mg dw/ml which is corresponding to 8420 ppm (8.42 mg/ml). AQ, NE and AE fractions caused a complete inhibition of livid amaranth seed germination at 1000, 1000, and 500 ppm, respectively. AE fraction at lower concentrations slightly promoted germination and seedling growth of livid amaranth. As a rule, however, growth was inhibited at higher concentrations and stimulated at lower (Saggese et al., 1995). Many phytotoxic compounds produced by higher plants are phenolic compounds that would be present in AE and/or AQ fraction. In this study, it was interesting that all fractions after solvent partitioning were more phytotoxic than the original extract. Still more interesting, the finding that even NE fraction which would not appear to contain any phenolic acids was equally phytotoxic.

Purification of phytotoxic compounds

After solvent partitioning, all the fractions were first purified by silica gel column chromatography. The inhibitory effect of active fractions on germination of livid amaranth seeds tended to increase after the second chromatography; seed germination was strongly inhibited at 200 ppm by the active fractions after the second silica gel chromatography. The results from the seed germination tests indicated that inhibitory substances were separated into several fractions having different levels of inhibitory potencies (data not shown). Each active fraction was further purified by ODS-HPLC. Analysis of the active fractions by ODS-HPLC demonstrated that more than two compounds presented in these active fractions. The collected ODS-HPLC fractions showing bioactivity were analyzed by GC/MS without derivatization. The results indicated that most of the active compounds were fatty acids and phenolic acids, even for those from NE fraction. This clearly demonstrated that the solvent partitioning was not

successful probably due to that the original extract contained large amount of these lipophilic fatty acids and hydrophilic phenolic acids. Therefore, all the active fractions were then analyzed after treatment with diazomethane and/or MSTFA. These techniques permitted the GC separation of the mixtures without the excessive tailing characteristics of underivatized acids. For example, GC-MS analysis of fraction II (Me/TMS) showed 2 major peaks that were corresponding to Me/TMS derivatives of *p*-hydroxybenzoic acid and *n*-heptadecanoic acid. This was confirmed by comparing retention times and full-scan mass spectra with those of authentic samples. Other active compounds were identified in a similar manner. Analysis of peaks by mass spectroscopy revealed that the compounds were suberic acid, azelaic acid, *n*-tridecanoic acid, *n*-pentadecanoic acid, *n*-heptadecanoic acid, oleic acid, vaccenic acid, elaidic acid, petroselinic acid, *p*-hydroxybenzoic acid and *p*-coumaric acid. These results indicated that the main active compounds in manilagrass extracts were fatty acids and phenolic acids (Fig. 3).

The nine fatty acids and two phenolic compounds identified in the manilagrass extract are ubiquitous in the crop vegetation of the world (Wang et al., 1967b, Borner, 1971). It has been known for many years that the allelochemicals produced by an algae, *Chlorella*, are C_{16} and C_{18} fatty acids. (Spoehr et al., 1949). Proctor (1957) suggested that the inhibitors in *Chlamydomonas reinhardi* is a long-chain fatty acids or mixtures of such acids. This was confirmed by McCracken et al (1980), who identified fifteen fatty acids of 14 to 20. Nine long-chain fatty acids with 14-22 carbons were identified in *Polygonum aviculare* residues (Alsaadawi, 1983). Eight phenolic compounds including *p*-hydroxyphenolic and *p*-coumaric acids were identified from kikuyu grass (*Pennisetum clandestinum*). Wu and Guo (1997) reported that allelopathic compounds produced by *Buchloe dactyloides* were phenolic compounds such as *p*-coumaric, ferulic, gentistic, homoveratric, vanillic and *p*-hydroxybenzoic acids. Twelve phenolic compounds including chlorogenic, *p*-coumaric and cafeic acids were identified from *Lolium perenne* (Takahashi, 1992).

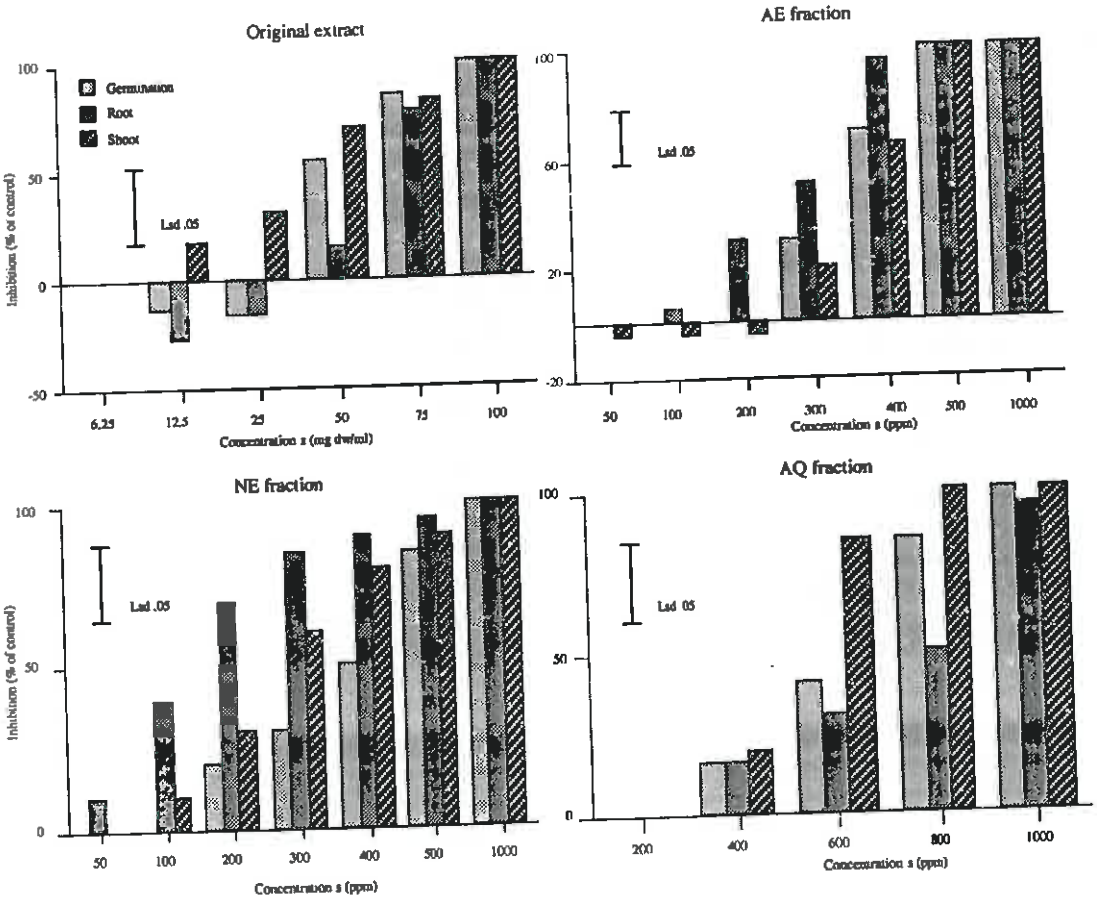

Fig. 2 Effect of original extract and different fractions obtained from manilagrass on germination and seedling growth of livid amaranth

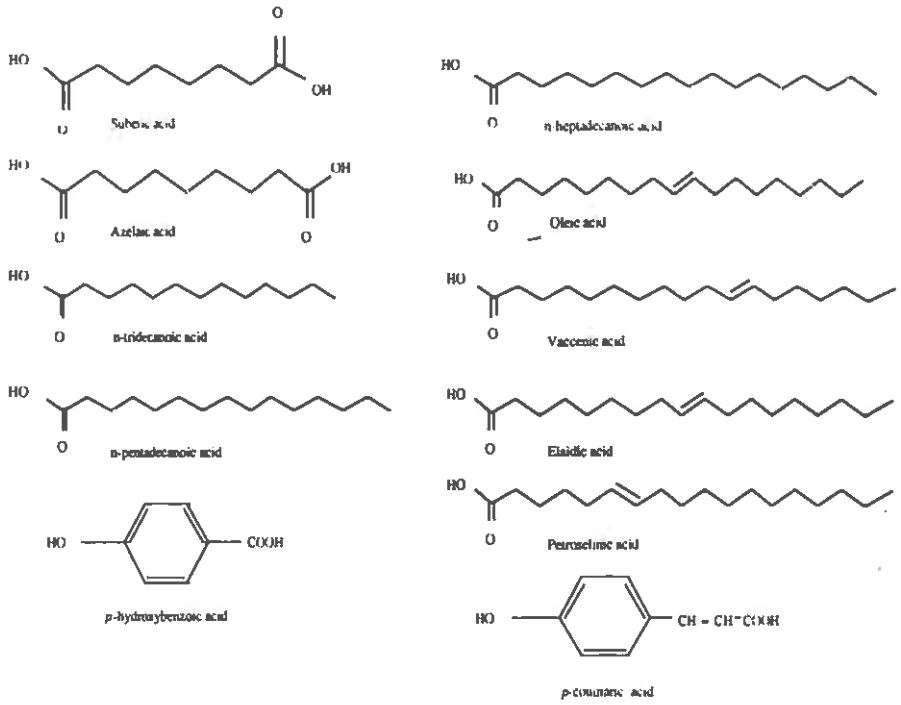

Fig. 3 Compounds identified from manilagrass (The identity of the plant-derived phytotoxins was based on comparison with retention time and bioassay results of known synthetic compounds).

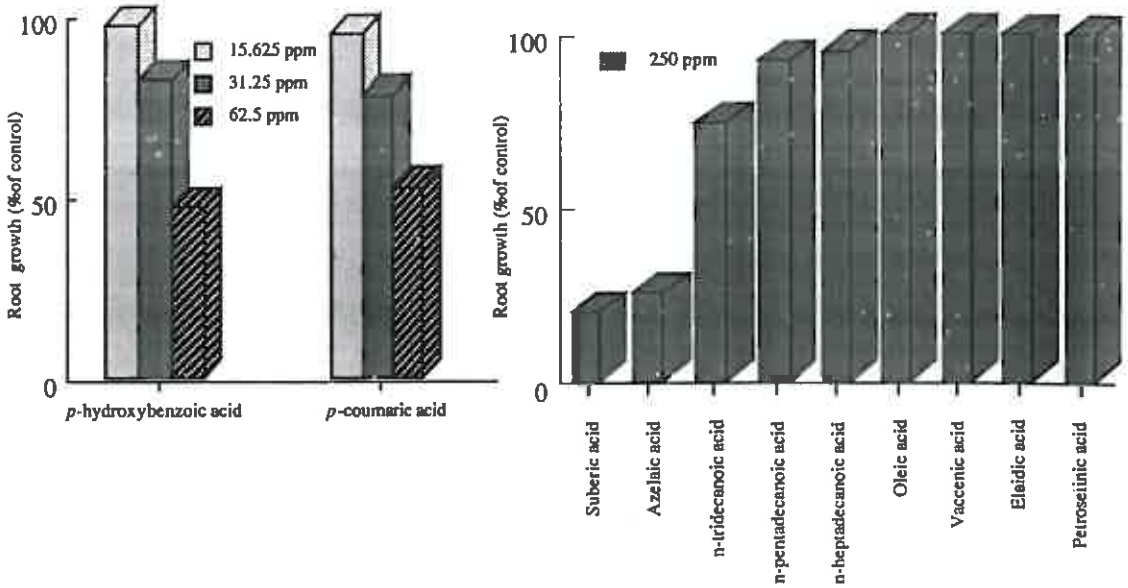

Fig. 4 Effects of authentic compounds on root growth of livid amranth after 72 hours at 27-28°C

Bioassay of authentic compounds

Since the identities of the phytotoxins in the manilagrass were established with GC-MS analyses, bioassays were also conducted using the authentic compounds to determine whether similar phytotoxic effects were produced. In the present study , 11 authentic compounds were bioassayed. The results (Fig.4) expressed as the percent root growth of the control. The results showed that *p*-hydroxybenzoic acid and *p*-coumaric acid were highly inhibitory to the growth of livid amaranth even at 62.5 ppm. The rest of test compounds reduced growth in different levels at 250 ppm. In general, the results with the

known authentic compounds were similar to those obtained with the plant derived phytotoxins, and identities of these phytotoxins were thus confirmed.

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INTEGRATED WEED MANAGEMENT IN PADDY FIELDS

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Abstract: Fifty years research and practice on paddy field weed control showed that the strategy of integrated weed management according to characteristics of distribution and tendency of succession of weed communities consisted of two aspects, restricting weeds or their seeds in field from the outside and controlling weed emergence or development. Based on agricultural and ecological measures, regional integrated weed management adopted chemical control as its major method, would solve weed problems effectively, ecologically and consistently, which had significance to high rice production.

Key words: paddy field, weed, integrated management

Introduction

China rice growing area, about 33 million hectares, is the largest in the world. There are more than 350 weed species in rice in China. Among of them, more than 20 species are important. There are rice fields of 15 million hectares damaged by weeds. Annual grain yields loss is as high as 10 million tons. About fifteen percent of the total grain output losses. According to a survey, 1.5—2.0 billion workdays and 500 million yuans of herbicides were annually used for control weeds in rice every year in recent years.

Jiangsu province locates the low reaches of Changjian, Huaihe, Yinhe and Shuhe rivers and covers central and northern Asia tropic and southern temperate zones. The south part of the province is the plain of the middle and low reach of Changjiang river, Where cropping system is single-or double-rice. The north part is the plain and hilly lands of Huanghe and Huaihe rivers. According to the geographical position, natural resource, cultivation sistem, cultivar type, and productive condition, the province is divided into six rice growing regions: Taihu, Ningzhenyang, Yanjiang, Lixiahe, Yanhai and Xuhuai. In recent years, total rice growing area remained about 2.4 million hectares.

1. Composition and Change Trend of Weed Community in Rice

There are 69 weed species, belonging to 27 families, in rice fields in Jiangsu province. Important weed species are barnyardgrass (*Echinochloa crus-galli*), small-flowered umbrella-plant (*Cyperus difformis*), monchoria (*Monochoria vaginalis*), low oldworld arrowhead (*Sagittaria pygmaea*), water flatsedge (*Juncellus paneeus*), carp intestines (*Eclipta prostrata*), Javan waterdropwort (*Oenanthe javanica*), flatstalk bulrush (*Scirpus planiculmis*), procumbent falsepimpernel (*Linderia procumbens*), climbing seedbox (*Ludwigia diffusa*), Chinese sprangletop (*Leptochloa chinensis*), Alligator Alteranthena (*Alternanthera philoxeroides*), and Indian rotala (*Rotala india*), which compose main eight weed communities. Barnyardgrass and small-flowered umbrella-plant are distributed province-wide.

Weed communities in paddy field are effected by ecological environment, crop-rotation method, temperature, water, and chemical herbiecide application. In the 70's widely used

herbicides were nitrofen and butachlor which were effctive to annual grasses and sedges such as barnyardgrass and small-flowered umbrella-plant. Due to continuel use of these herbicides for many years, the weed community of paddy fields changed into broadleaf weed such as climbing seedbox, indian rotala, and procumbent falsepimpernel, low oldworld arrowhead, and monochoria. In the early 90's, sulfonylurea herbicides such as bensulfuron-methyl and pyrazosulfuron-ethyl were used widely in the paddy field, which could control the most broadleaf and sedge weeds, but could not effectively control grasses of gramineae. Continuely using bensulfuron-methyl, pyrazosulfuron-ethyl, mixture of sulfometuron-methyl and bensulfuron-methyl caused grass weeds to become predominate. In recent years, one-shot mixtures such as butachlor plus bensulfuron-methyl or pyrazosulfuron-ethyl, acetochlor plus bensulfuron-methyl, and quinclorac plus bensulfuron-methyl have been widely used in China. These mixtures effectively control common weeds in paddy fields. However, difficultly controlled premuned weeds such as flatstalk bulrush, late juncellus, low oldworld arrowhead, and climbing seedbox increased slowly. The change of cultivation methods, such as drily raise vaising rice seedlings in the fertile and dry soil, divectly seeded rice broadcasting seedlings also caused the change of weed community. for example, in the beds of drily raising rice seedlings in the fentile soil upland weeds such as common crabgrass, goosegrass wiregrass, sping amaranth, and lambsquarters goosefoot are main weeds. In directly seeded rice Chinensis sprangletop have become dominant.

2. Weed seed bank in paddy field

Emergence, dynamics, and succession of weed communities in paddy fields are closely related to the their seed banks. Since 1996, we have studied weed seed bank in paddy fields in Yizheng city of Jiangsu province where the fields were of swamp paddy soil, lightly clay with about 18 cm plough layer, 6.5 pH, organic mather content 1.5~1.9%. No herbicides were applied in sampling areas from 1996 to 1998. Five samples were taken from each fields after field ploughing and before rice transplanting in 1996. The quadrat size was 0.1 m^2(0.5m×0.2m). The samples of four layers 0~5cm, 5~10cm, 10~15cm, 15~20cm were collected separately. The samples of the same layers was mixed and put in a box (0.1m^2). The boxes were placed outdoors and watered untill saturation everyday from June 10. Numbers of emergenced weeds were counted before weed removed every 5 days from the begining of weed emergence to no weed emergance. The weed emergances of the samples were continually examined in 1997 and 1998. Different weed emergances were observed in the fields from 1996 to 1998 still five samples were taken. The experimented results (Table 1) should that the weed seed bank was mainly composed of E. crus—galli, Difformed Galingale, Bacciferous Ammania, Prostrate Seedbox, Procumbent Falsepimpernel, and Prostrace Yerbadetajo in paddy field in Jiangsu province.

3. The technology of integrated weed management in paddy fields

Why does weed damage occur although herbicides are applied in paddy fields every year? The first reason is: herbicides can't control all weeds in paddy fields and survival weeds can reproduce seeds or remaining stolons or rhizomes. These reproductive organs will reproduce next year. The second one is: weed seeds spread with straw and farmyard manure, rice seeds, or from ridge or outside into paddy fields, weed seedlings can be transplanted with rice seedlings. Experimental results from Yizheng showed that: num-

bers of weed emergence increased when no herbicides was applied only in a year, and weed density went up to 141.5 plants/m^2, and the Coverage was 91.0%. They severely inhibited rice growth and reduced rice yield by above 20%. So the technology of integrated weed management should consider two aspects: preventing weed or their seeds from spreeding into paddy field from the outsides and controlling weed emergence or development. The technology of weed control in paddy field in Jiangsu province was presented in Fig. 1.

3.1 The first strategy of integrated weed management control weed emergence or development in paddy field. Apply herbicides with charcters of effectiveness, broad spectrum and fafety, such as acetochlor plus bensulfuron-methyl, butachlor plus bensulfuron-methyl, etc.; extend the cultural methods for weed control, such as selecting early growth and quick-development cultivaters, cultivating robust rice seedlings, planting high density, transplating in bigger rice seedling and promoting rice seedling developping early by fertilizer and water managements; adopt suitable cropping system such as rotating rice with wheat, oilseed, cotton, corn or bean; plow deeply to burry weed seeds in the depth of 15 ~20 cm to reduce their emergence; removing remaining weeds in fields by hands in the middle and late growth stages to reduce the seed bank.

3.2 The second strategy of integrated weed management-preventing weeds or their seeds from spreading into paddy fields from the outside, control weeds in seedling beds to reduce transplanted numbers of weeds in paddy fields; specially it needs to control barnyardgrass in rice seedling beds because the weed affects the crop yield greatly; decompose straws and dungs before they are applied; control weeds on ridges of paddy fields to reduce weed seed production which results in low input of the seed bank.

Table 1. Weeds plants in paddy fields (1996—1998)

Weed name	first year						second year						third year						total	field
	0~5 cm	5~10 cm	10~15 cm	15~20 cm	Total	field	0~5 cm	5~10 cm	10~15 cm	15~20 cm	Total	field	0~5 cm	5~10 cm	10~15 cm	15~20 cm	Total	field		
Echinochloa crusgalli	44	38	16	8	105	38	11	3	1		15	43	3	1	1		5	56	0~5cm	131
Leptochloa chinensis	5	2	1		9	2						5						8		15
Cyperus difformis	15	13	8		36	6	3				3	11	3	1			4	12	346	29
Cyperus fuscus	4	2	1		7														5~10cm	
Fimbristylis miliacea	3	2			5															
Fimbristylis dichotoma	2	1	1		4															
Eriocaulon sieboldianum	5	5	1		11															
Monochoria vaginalis	4	4	1		9	2						4						4	250	10
Polygonum Lapathifolium	3	3	3	1	10		2	2	2	1	7								10~15cm	
Alternanthera sessilis	6	4	3	2	14		3	1	1	1	6									
Aeschynomene indica	3	3	1	1	8	2	2	1	1	1	5	4						4		10
Ammannia baccifera	5	4	3	2	14	5	3	1			4	8	3	1			4	10	115	23
Ammannia arenaria	10	5	3		18	13	8	5	2		16	15						18		46
Ammannia multiflora	2	6	2	2	17	6	3	1			4	11						13	15~20cm	32
Rotala indica	55	50	13	8	126	42	11	8	8		27	55	5	3	1		9	61		158
Ludwigia prostrata	15	16	10	3	50	10	5	2			7	13	3				3	15	46	35
Lindernia procumbens	25	18	11	8	68	20	8	3	1		12	33	3	2	1		6	38		101
Lindernia antipoda	3	2			5														total	
Lindernia crustacea	5	1			6		1				1									
Veronica undullata	3	3	1		7															
Eclipta prostrata	21	20	15	8	64	23	10	3	2		15	25	5	3			8	13	757	61
Centipeda minima	2	4			6															
total	251	200	93	49	593	175	70	30	19	3	122	227	25	11	3		39	252		654

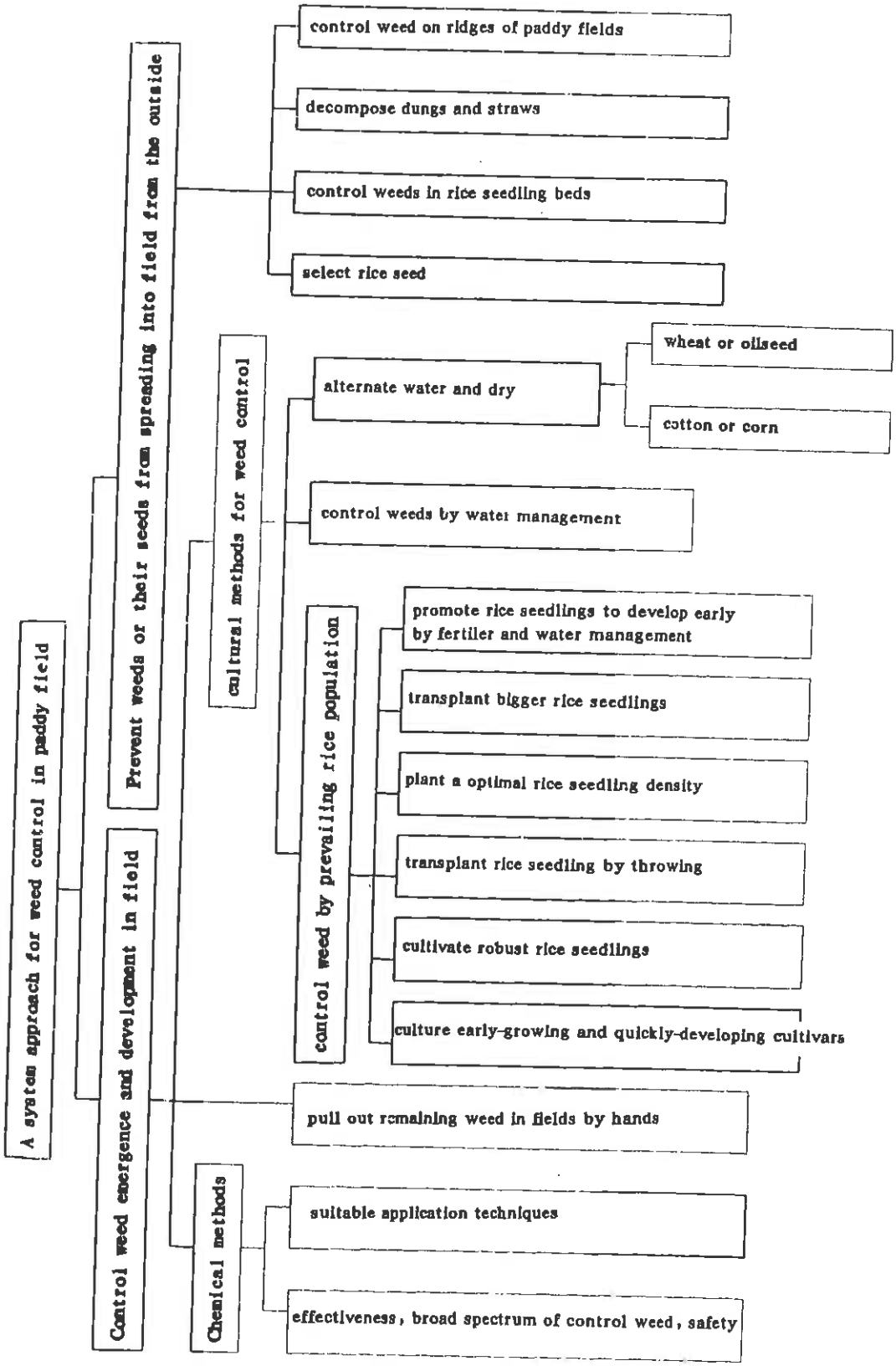

Figure 1. The technique of weed control in paddy field

ITCHGRASS CONTROL IN CORN WITH PRIMISULFURON

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Abstract: Two experiments were carried out to study the efficacy of primisulfuron for control of itchgrass (*Rottboellia cochinchinensis* (Lour.) W.D. Clayton) in corn (*Zea mays* L.) plantation at Suwan farm in 1995-1997. Pot experiment revealed that primisulfuron at the rate of 40 g ai/ha applied at 1 to 3 weeks after emergence (WAE) was the most effective mean to control itchgrass. Postemergence application of primisulfuron in pot experiment was more effective than in the field. Field experiments of 8 hybrid corn varieties found that application of primisulfuron at the rate of 40 g/ha at 1 - 3 WAE, 3-8 leaves stage of corn plants or 3-7 leaves of itchgrass was the most effective control. The response of corn varieties to primisulfuron revealed that Pioneer 3012 was the most susceptible while DK 888, Hercules 40, and Tevada 77 were tolerant which produced grain yield as high as in hand weeding. Adjuvants Latron CS-7 0.25%(v/v) and Sylgard 309 0.25%(v/v) enhance primisulfuron efficiency for control of itchgrass, but Latron CS-7 reduced corn KSX 3601 yield. Spray volume of 250 and 500 l/ha controlled itchgrass better than of 62.5 liters/ha but less effective than glyphosate at the spray volume of 250 l/ha.

Key words: itchgrass, corn, primisulfuron, adjuvants, spray volume

Introduction

Itchgrass is 1 of 18 worst weeds in the world (Holm *et al.*, 1977). It is an annual grass native to tropical Asia, has become a major weed problem in sugarcane, soybean, corn (Lencse and Griffin, 1991). It reduced corn yields 33-71% (Richards and Thomas, 1970). Itchgrass has the capacity for high photosynthetic rates which may explain its ability to compete with corn and sugarcane (Patterson, 1979). Mercado (1978) reported that population of itchgrass at 50 and 142 shoots/m^2 reduced corn yields about 50 and 70% respectively.

Generally, itchgrass is controlled in corn plantation in Thailand by preemergence herbicide, pendimethalin. However, selective postemergence herbicides control itchgrass in corn has not been available up to the present. Primisulfuron is a new selective, sulfonylurea herbicide use at low rates for selectively control annual and perennial grasses and some annual broad leaf weeds. Tweedy and Kapusta (1995) reported that primisulfuron at 40 g ai/ha controlled rhizome of johnsongrass 85-100%.

The objectives of these experiments were to determine a) the most effective timing of post emergence application of primisulfuron for itchgrass control, b) the effect of primisulfuron on phytotoxicity of 8 hybrid corn varieties, c) the effect of adjuvants on primisulfuron activity, d) the effect of spray volume on primisulfuron activity for itchgrass control.

Materials and Methods

Pot experiments were conducted in the grass house in 1995. Pot size is 15 cm diameter and 15 cm height with 5 itchgrass shoots/pot. The experimental design was a randomized complete block with four replications. Weed control efficacy was recorded at 1, 2, 3 and 4 WAA (week after application). The itchgrass harvested at 6 weeks after planting for dry matter and was dried by hot air oven at 105°c for 24 h.

Field experiments were conducted in area that was infested by itchgrass, at the National Corn and Sorghum Research Center, Pakchong district, Nakhonratchasima province dering 1995 to 1997. The soil pH was 5-7, clay loam with 10.4% sand, 31.1% silt, and 58.5 % clay. Seedbeds for the crop were prepared by no-tillage, glyphosate at 1500 g ai/ha was applied as postemergence at 7 days before planting for the control of weeds in all experiments. In all experiments, corn was planted at spacing 75x25 cm. The plot size was 5x3 m (4 rows). The experiments were irrigated by sprinkler system. Fertilizers were applied at recommended rate. Herbicides were applied with knapsack sprayer with 500 l/ha spray volume. During herbicide application in 1995 and 1996, air temperature was 21-28□c with relative humidity of 70-80% and 75-85% respectively. Weed control efficacy and phytotoxicty were recorded at 2 and 4 WAA. The corn was harvested by hand at 110 days, in the 2 middle rows. Data were collected on ear weight, ear number and yield. All data were analyzed using appropriate analysis of variance with mean separations computed by DMRT test at the 0.05 level of probability.

Timing of application and corn varieties studies

The corn was planted in the early of September 1995. This experiment was designed as a split-plot with 3 replications. Eight field hybrids corn varieties were Big 717, DK888, Hercules 40, Pioneer 3012, Tevada 77, Royal 90, KSX 3601 and KSX 3601 were the main plots and timing of primisulfuron applications 1, 2, 3 and 4 WAE were the subplots. Herbicide was applied at 40 g ai/ha. The corn was harvested in the middle December 1995.

Adjuvants studies

This experiment was designed as randomized complete block with 4 replications. KSX 3601 corn variety was planted in the early of November 1996 and harvested in the middle of February 1997. Primisulfuron at 30 and 40 g ai/ha were applied with 4 adjuvants, ammonium sulfate 1%, Latron CS-7 0.25%, APSA 80 0.20% and Sylgard 309 0.25%v/v at 3 WAE.

Spray volume studies

This experiment was designed as randomized complete block with 4 replications. KSX 3601 corn variety was planted in the early of November 1996 and harvested in the middle of February 1997. Primisulfuron at 30 and 40 g ai/ha were applied with controlled droplet application sprayer in 62.5l/ha spray volume, and knapsack sprayer in 250 and 500 l/ha spray volume at 3 WAE.

Results and Discussion

Timing of application

Primisulfuron at 40 g ai/ha was the most effective in controlling itchgrass when it was applied at 1 to 3 WAE both in pot experiment (Fig. 1) and field experiment (Table 2). Primisulfuron reduced the itchgrass height and dry matter when applied at 1-3 WAE greater than applied at 4 WAE (Table 1). However, all primisulfuron treatments provided less control of itchgrass when compared with all hand weeding treatment for 2 and 4 WAA. (Table 2).

Corn varieties studies

Primisulfuron reduced corn height in some corn varieties when applied at 3 and 4 WAE when evaluated at 4 WAA, but they were not difference when evaluated at harvesting stage (data not shown). Visual injury evaluation at 4 WAA found that primisulfuron caused approximately to 27% phytotoxicity to Pioneer 3012 when applied at 1 WAE (Table 3). However, Tweedy and Kapusta (1995) reported that primisulfuron at 40 g ai/ha was not affect to Pioneer 3156 hybrid corn variety that gave twice yields than untreated. Big 717, DK 888, Tavada 77, KSX 3504 were highly tolerant (<10%) and KSX 3601, Hercules 40 and Hercules 74 were moderately tolerant (10-20%) (Table 3). Corn injury ranged form slight leaf chlorosis and leaf widening in tolerance varieties to severe plant stunting and death of susceptible varieties.

Primisulfuron reduced the yield of Big 717, Hercules 74 , Pioneer 3012, KSX 3601 and KSX 3504 when applied at 2,3 and 4,1, 2 3 and 4 , 2, 3 and 4 and 2 WAE respectively (Table 4).

Adjuvant studies

Primisulfuron at 30 and 40 g ai/ha gave the same level of control. Adjuvant, ammonium sulfate, Latron CS-7, APSA 80 and Sylgard 309 were not enhanced both rates of primisulfuron at 4 WAA excepted Latron CS-7 and Sylgard 309 enhanced primisulfuron at 40 g ai/ha at 2 WAA (Table 5).

Corn injury was observed at 2 and 4 WAA. Both rates of primisulfuron plus ammonium sulfate, Latron CS-7, APSA 80 and Sylgard 309 were not reduced corn height and no corn injury was observed when compared with primisulfuron alone (Table 5).

Yield of corn treated with both rates of primisulfuron plus adjuvants were not reduced when compared with primisulfuron alone, but primisulfuron at 40 g ai/ha plus Latron CS-7 gave less yield than non weeded (Table 5).

Spray volume studies

Primisulfuron in the spray volume of the 62.5, 250 and 500 l/ha of water gave the same moderate itchgrass control and less effective than glyphosate at 4 WAA. The spray volume of 250 and 500 l/ha caused corn injury and reduced grain yields (table 6).

Conclusion

Pot experiment revealed that primisulfuron at the rate of 40 g ai/ha applied at 1 to 3 WAE was the most effective mean to control itchgrass. Primisulfuron application in pot was more effective than in the field.

Field corn varieties were varied in tolerant to primisulfuron which were depending on timming of application. Pioneer 3012 was the most susceptible while DK 888, Hercules 40, and Tevada 77 were tolerant which produced grain yield as high as hand weeding.

Adjuvant, Latron CS-7, and Sylgard 309 enhanced the activity of primisulfuron for itchgrass control but Latron CS-7 reduced corn yield.

Spray volume of 250 and 500 l/ha controlled itchgrass better than of 62.5 l/ha but less effective than glyphosate at the spray volume at 250 l/ha. However higher spray volume reduced corn yield.

Table 1 Effect of primisulfuron at 40 g ai/ha on itchgrass height and dry weight when the herbicide applied at 1, 2, 3 and 4 WAE in pot experiment.

Treatments	Applied at WAE[1/]	Itchgrass height (cm)					Dry weight	
		1WAA[2/]	2WAA	3WAA	4WAA	6WAA	(g)	(%)
Primisulfuron	1	4.0 e[3/]	4 2 e	4.0 e	4.0 e	4.0 e	0.4 d	10
Untreated	1	7.6 d	10.9 c	11.9 c	12.5 c	13.7 bc	4.1 b	100
Primisulfuron	2	7.2 d	6.6 d	7.2 d	7.2 d	7.2 d	0.5 d	13
Untreated	2	10.9 c	11.9 bc	12.6 bc	12.7 c	13.7 bc	3.9 b	100
Primisulfuron	3	10.9 c	12.0 bc	12.1 bc	12.1 c	12.1 c	1.9 c	47
Untreated	3	11.9 bc	12.5 b	12.7 b	13.7 bc	16.2 a	4.1 b	100
Primisulfuron	4	14.0 a	14.1 a	14.1 a	15.2 ab	15.5 ab	5.7 a	142
Untreated	4	13.5 a	13.7 a	14.7 a	16.2 a	17.4 a	4.0 a	100

1/ WAE = week after emergence
2/ WAA = week after application
3/ Mean in the same column followed by the same letters are not significantly different at the 5% level by MDRT.

Table 2 Effect of primisulfuron at 40 g ai/ha for itchgrass control average in 8 hybrid corn varieties when applied at 1, 2, 3 and 4 WAE.

Treatment	Applied at WAE[2/]	Itchgrass control rating 1/		
		2 WAA 3/	4 WAA	Harvested
Primisulfuron 40 g ai/ha	1	69 d 4/	88 c	78 ab
Hand weeding	1,4	98 a	94 ab	90 ab
Primisulfuron 40 g ai/ha	2	51 c	68 d	85 ab
Hand weeding	2,5	97 a	91 b	95 a
Primisulfuron 40 g ai/ha	3	51 c	75 c	80 b
Hand weeding	3,6	97 a	90 b	89 ab
Primisulfuron 40 g ai/ha	4	57 b	63 d	65 c
Hand weeding	4,8	96 a	100 a	88 ab
Non weeded	-	0 e	0 e	0 d
Mean		68	74	74

1/ Itchgrass control rating; 0 = no control, 100 complete control
2/ WAE = week after emergence
3/ WAA = week after application
4/ Mean in the same column followed by the same letters are not significantly different at the 5% level by MDRT.

Table 3 Visual corn injury rating on 8 hybrids corn varieties at 4 WAA after application of primisulfuron at 40 g ai/ha at 1, 2, 3 and 4 WA

Treatment	Applied WAE[2/]	Corn injury 1/								
		Hercules40	Big 717	DK 888	Pioneer 3012	Tevada77	Hercules 74	KSX 3601	KSX 3504	mean
Primisulfuron	1	13 b 3/	5 b	10 a	27 c	17 a	7 c	15 b	17 ab	14
Hand weeding	1,4	0 b	0 b	0 a	0 d	0 b	0 c	0 b	0 b	0
Primisulfuron	2	47 a	5 b	13 a	57 b	27 a	28 b	14 b	17 ab	26
Hand weeding	2,5	0 b	0 b	0 a	0 d	0 b	0 c	0 b	0 b	0
Primisulfuron	3	33 a	27 a	15 a	77 a	17 a	65 a	37 a	10 ab	35
Hand weeding	3,6	0 b	0 b	0 a	0 d	0 b	0 c	0 b	0 b	0
Primisulfuron	4	43 a	7 b	12 a	80 a	22 a	50 a	33 a	22 ab	34
Hand weeding	4,8	0 b	0 b	0 a	0 d	0 b	0 c	0 b	0 b	0
Non weeded	-	0 b	0 b	0 a	0 d	0 b	0 c	0 b	0 b	0
Mean		15	5	6	27	9	17	11	7	12

1/ Visual injury rating; 0 = no injury, 100 = plant killed
2/ WAE = week after emergence
3/ Mean in the same column followed by the same letters are not significantly different at the 5% level by DMRT

Table 4 Yield of 8 hybrids corn after application of primisulfuron at 40g ai/ha at 1, 2, 3 and 4 WAE

Treatment	Applied	Yield (kg/ha)								
	WAE[1/]	Hercules 40	Big 717	DK 888	Pioneer	Tevada77	Hercules 74	KSX 3601	KSX 3504	mean
Primisulfuron	1	1781 ab[2/]	4800 abc	2519 a	3300 c	1206 a	1869 ab	5056 bc	3163 ab	2963
Hand weeding	1,4	1544 ab	5738 ab	3369 a	7044 a	1500 a	1006 ab	6219 ab	3850 ab	3906
Primisulfuron	2	1288 b	4169 bc	2556 a	1606 d	1156 a	1106 ab	3788 cd	2613 b	2281
Hand weeding	2,5	2763 ab	6069 a	3938 a	5300 b	1969 a	2444 a	5994 ab	4350 a	4106
Primisulfuron	3	1300 b	4406 bc	2738 a	1988 cd	1438 a	781 b	3019 d	3100 ab	2344
Hand weeding	3,6	2619 ab	5288 ab	3168 a	6081 ab	1950 a	2438 a	6694 ab	4006 ab	4031
Primisulfuron	4	3000 a	4806 abc	3138 a	1419 d	1113 a	131 b	3388 d	2750 ab	2556
Hand weeding	4,8	1681 ab	6200 a	4181 a	5663 ab	1750 a	398 a	5469 ab	4088 ab	3938
Non weeded	-	2138 ab	5319 c	3281 a	6500 ab	1550 a	357 ab	5713 ab	3213 ab	3531
Mean		2012	5013	3213	4325	1513	288	5038	3456	3294

[1/] WAE = week after emergence

[2/] Mean in the same column followed by the same letters are not significantly different at the 5% level by DMRT.

Table 5 Effect of primisulfuron at 30 and 40 g ai/ha plus 4 adjuvants on itchgrass control, visual injury, plant height and yield of corn when the herbicide was applied at 3 WAE.

Treatment	Rate	Itchgrass control		Corn injury		Corn height (cm)		Yield
	(g ai/ha)+(%)	2 WAA[1/]	4 WAA	2 WAA	4 WAA	2 WAA	4 WAA	(kg/ha)
Primisulfuron	30	59 c[2/]	70 ab	10 c	9 bc	48.2 ab	88.4 abc	6263 abc
Primisulfuron + ammonium sulfate	30 + 1.0	60 bc	69 ab	12 bc	18 ab	43.3 bc	82.7 abc	5394 cd
Primisulfuron + Latron CS-7	30 + 0.25	61 bc	70 ab	16 abc	15 ab	42.6 bc	78.9 abc	5763 a-d
Primisulfuron + APSA 80	30 + 0 20	61 bc	73 ab	17 abc	19 ab	40.7 bc	76.0 bc	5544 bcd
Primisulfuron + Sylgard 309	30 + 0.25	66 ab	65 b	15 abc	7 bc	48.8 ab	89.4 ab	6806 a
Primisulfuron	40	63 bc	69 ab	17 abc	19 ab	45.2 abc	85.3 abc	6081 a-d
Primisulfuron + ammonium sulfate	40 + 1.0	65 abc	72 ab	15 abc	19 ab	42.6 bc	79.4 abc	5275 cd
Primisulfuron + Latron CS-7	40 + 0.25	70 a	77 a	21 a	19 ab	45.2 abc	84.5 abc	4938 d
Primisulfuron + APSA 80	40 + 0.20	64 abc	73 ab	19 ab	28 a	37.2 c	72.4 c	5231 cd
Primisulfuron + Sylgard 309	40 + 0.25	70 a	78 a	15 abc	17 ab	41.9 bc	78.0 bc	5675 a-d
Non weeded	-	0 d	0 c	0 d	0 c	51.6 a	94.6 a	6712 abc
Mean		58.1	65.1	14.3	15.5	44.3	82.7	5789

[1/] WAA = week after application

[2/] Mean in the same column followed by the same letters are not significantly different at the 5% level by DMRT.

Table 6 Effect of spray volume at 62.5, 250 and 500 l/ha on primisulfuron activity for itchgrass control, crop injury, plant height and yield of corn.

Treatment	Spray volume (l/ha)	Itchgrass control [1/]		Corn injury [2/]		Corn height (cm)		Yield (kg/ha)
		2 WAA	4 WAA	2 WAA	4 WAA	2 WAA	4 WAA[3/]	
Primisulfuron 40 g ai/ha	62.5	74 c [4/]	73 bc	12 c	20 b	45.9 a	92.9 a	6594 cd
Primisulfuron 40 g ai/ha	250	78 c	71 c	15 b	29 a	42.7 a	86.6 a	6563 cd
Primisulfuron 40 g ai/ha	500	74 c	66 c	20 a	35 a	41.5 a	90.4 a	6256 d
Glyphosate 1,500 g ai/ha	250	100 a	80 b	11 c	9 c	47.9 a	103.5 a	7788 b
Hand weeding	-	90 b	90 a	0 d	0 d	60.4 a	88.8 a	9163 a
Non weeded	-	0 d	0 d	0 d	0 d	51.7 a	102.6 a	7256 bc
Mean		69	63	10	16	48.3	94.1	7270

[1/] Itchgrass control rating; 0 = no control, 100 complete control

[2/] Corn injury rating; 0 = no injury, 100 = plant killed

[3/] WAA = week after application

[4/] Mean in the same column followed by the same letters are not significantly different at the 5% level by DMRT.

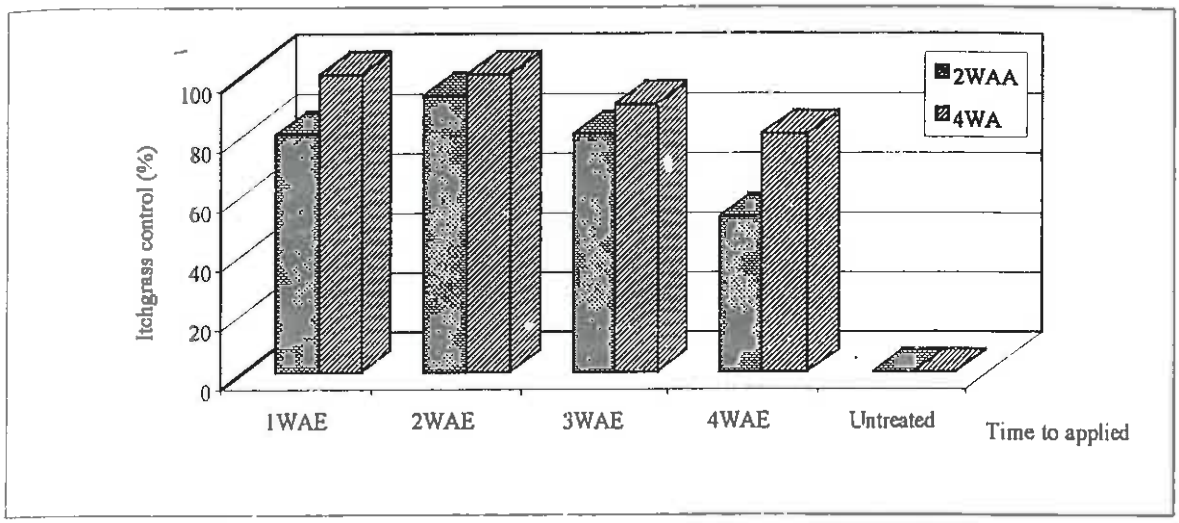

Fig.1 Itchgrass control with primisulfuron 40 g ai/ha at 1, 2, 3 and 4 WAE in the pot.

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MANAGEMENT OF *CYNODON DACTYLON* IN SUGAR CANE

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Summary: In field trials at Kenana, Sudan, the effects of integrated fallow and post planting cultural and chemical treatments on *C. dactylon* control and the yield of sugar cane was evaluated, Excellent control of *C. dactylon*, in plant cane was achieved by post-planting application of glyphosate preceded by pre-planting application of glyphosate, pre-planting irrigation or combining both in sequential manner during the fallow period. Such integration of cultural and glyphosate treatments kept *C. dactylon* ground cover below 20 % over 18 weeks period after planting, compared to 80 % in the untreated control and resulted in high cane yields in both plant and second ratoon crops.

*Keywords: Cynodon dactylon,*sugar cane, pre-planting irrigatiom, ploughing , fallowing, glyphosate.

INTRODUCTION

Cynodon dactylon is a persistent and troublesome weed in most of the irrigated agricultural schemes of the Sudan (1,2). It is spreading fast in sugarcane fields of Kenana Sugar Estate, resulting in severe reductions in cane yields (3), and the need to re-plant before an acceptable number of ratoon crops has been achieved. It may also lead to abandoning of some badly affected fields. The rapid increase in infestation in cane field of Kenana by *C. dactylon* is due to a combination of factors. The most important was the early practice of growing *C.dactylon* on canal banks in order to protect them from erosion and collapse. Other factors include, the use of pre-emergence herbicides that have no activity on *C.dactylon,* and dispersal of the rhizomes as a result of the movement of cultivation and harvesting machines from field to field, cane monoculture with no fallow periods, and the perennial nature of the sugar cane crop.

The current adopted land preparation practice coupled with the absence of a fallow period encourages and helps the spread of the weed instead of eliminating it. At present there is no selective pre- or post-emergence herbicide to control *C.dactylon* within the sugar cane crop (4).Various methods have been adopted to control *C. dactylon* elsewhere. These include, crop rotation,summer fallow tillage to expose the rhizomes to hot dry summer atmosphere (5) and non-selective herbicides usually for pre-plant and spot applications. However, these methods independently, failed to sufficiently control this weed because of its intensive rhizome system. A weed management system combining cultural and chemical methods is apparently needed for effective control of *C. dactylon*. Therefore an experiment was carried out to evaluate the effects of integrated fallow and post-planting cultural and chemical treatments on *C. dactylon* control and the yield of sugar cane.

MATERIALS AND METHODS

The field selected for this experiment was in the second ratoon crop and was heavily infested with *C. dactylon,* before being used for this experiment in 1993. It was harvested in Feb., 1993 and was intended to be left as fallow for 2 - 3 years due the failure of the previous crop as a result of heavy infestation of *C. dactylon.*

Immediately after harvesting the experimental plots were clearly marked out on a part of this field and each plot designated for a specific set of treatments. In mid March 1993 the area selected was disc ploughed and harrowed, according to the standard practice adopted in Kenana. This consists of two deep ploughings to a depth of 20 - 25 cm and one disc harrowing.

With the onset of the rainy season in mid June, *C.dactylon* resumed growth and by late July, it was evenly present in all plots. During the second week of August one half of the experimental plots, was treated with glyphosate at 4.0 kg ai/ha. In late Sept.1993, about 3 weeks after the last rain, the whole experimental area was disc harrowed again and ridged ready for cane planting. Immediately

afterwards, a pre-planting irrigation was given to half of the plots treated with glyphosate and those not treated. It was intended here to encourage and ensure regrowth of *C. dactylon* to obtain a good green cover of the weed. One month later (late Oct.), the cane setts were planted and covered. Planting was immediately followed by an application of pendimethalin plus atrazine as the standard herbicide treatment to all plots. Ten days later, just before cane emergence, half of the plots were sprayed with glyphosate at 3.0 kg ai/ha as full cover. The individual sets of treatments are shown with the results in Tables 1 -3

The effect of treatments on *C. dactylon* was evaluated by visual estimation of the weed percent ground cover on a 0 to 100% scale, where 0 = no effect and 100 = complete kill. Assessment of treatments effects on *C.dactylon* was made in mid August (before application of glyphosate in the fallow), late Sept. (1.5 month after glyphosate application), one week before planting (3 weeks after pre-planting irrigation) and 6, 12 and 18 weeks after planting.

One day before harvest, the cane was burnt, and stalk height was measured on ten randomly selected stalks from the inner two rows of each plot. The sugar cane was then harvested manually and all cane stalks in the inner four rows of each plot were counted, arranged in bundles and weighed and cane yield per ha. was calculated. A 10 stalk sample from each plot was taken to the Laboratory, crushed and analysed for estimated recoverable sucrose (ERSc%). Tonnes of sugar was then calculated by multiplying TC/ha by ERSc%.The residual effect of these treatments on cane yield (TC/ha) was also determined on the second ratoon crop.

The experiment was laid down in a randomized complete block design with 4 replications. Plot size was 30m by 6-cane rows 1.55m apart. All data were subjected to analysis of variance .

RESULTS

a) Effect on C.dactylon: Visual evaluations of *C.dactylon* ground cover in mid August before any fallow treatment application, indicated that the experimental area was uniformally covered by *C. dactylon* (48 -60%) ground cover (Table 1)

C.dactylon ground cover ratings recorded in late September and one week before planting corresponds to the effects of the fallow treatments that had been applied during the fallow period prior to cane planting. Evaluations at 6, 12 and 18 weeks after planting (wap) showed the effects resulting from each complete set of fallow plus post-planting treatments (Table 1).

Evaluation made in late Sept. showed that glyphosate reduced *C.dactylon* ground cover to a maximum of 10% compared to about 75% in untreated plots. One week before planting (wbp) *C.dactylon* percent ground cover was only 0 - 18% in plots which had received glyphosate alone or glyphosate followed by pre- planting irrigation. Those plots which received a per-planting irrigation treatment only had ground cover of 58 - 70%. Six weeks after planting *C.dactylon* percent ground cover ranged from 0 to 15% in all plots received glyphosate at 10 days after planting (dap), regardless of the earlier fallow treatments (Table 1)

At 18 weeks after planting, the visual evaluation quantified the season-long effect of various treatment combinations on *C. dactylon* percent ground cover. Post planting glyphosate preceded by a fallow glyphosate, pre-planting irrigation or combination of both, maintained a low ground cover (0-28%) through 18 weeks after planting (Table 1). Glyphosate alone in the fallow period or followed by a pre -planting irrigation during the fallow, with no subsequent treatments after cane planting, were ineffective on *C.dactylon* growth and resulted in 48 to 58% ground cover at 18 weeks after planting (Table 1). *C. dactylon* ground cover in plots that received only a pre - planting irrigation during the fallow period with no subsequent treatments after cane planting did not differ significantly ($P<0.05$) from that of the standard check treatment (Table 1).

b) Effect on cane yield: Cane stalk height was increased significantly ($P<0.05$) when fallow and post-planting herbicide treatments were given along with a pre-planting irrigation (Table 2). Pre-planting irrigation without fallow herbicide, but with post-planting treatment with glyphosate also gave quite tall cane stalks (Table 2). Cane stalk populations were also greatest in these same treatments (86-96 x 10^3)/ ha and much decreased in all other treatments (Table 2).

Estimated recoverable sucrose (ERSc%) was not significantly affected ($P<0.05$) by any of the

treatments. Maximum cane and sugar yields (in plant and second ratoon crops) were obtained from glyphosate treatments given after planting when they were combined with either a pre-planting irrigation or a fallow glyphosate application or the combination of both fallow treatments. The latter had the largest cane yields (Table 3).

DISCUSSION

The main purpose of any weed management system is to improve the competitive capacity of the crop in association with weeds. Weed control methods available for any crop production are cultivation, crop rotation, herbicides and combination of these (6). Timely decision and adequate selection of control measures to build up an adverse environment for the weed growth and development is necessary to obtain high crop yields.

From the present study, it is clearly evident that the use of traditional ploughing and fallowing only to subject the weed rhizomes to desiccation by the hot dry weather had failed to check the regrowth of *C.dactylon*, raising doubts as to the assumption of (7) that, the potential advantage from ploughing for *C. dactylon* control comes from prolonged exposure of rhizomes to desiccation in hot dry season and as to the effectiveness of this method to control this weed. There is always the question of using dry conditions to control *C. dactylon*. Working in the Sudan, (8) found no buds survived on *C. dactylon* rhizome stored for seven days in a light sandy soil which was air dry. However, he stated that, rhizomes were able to remain viable after suffering a considerable water loss. In the present study, the experimental area was ploughed and subjected to the hottest part of the dry summer season for about three months. However, the ability of the heavy clay soils (> 60 % clay) to retain a high water content and the humid conditions prevailing in the field around the experimental area during the nights may have kept the rhizomes with sufficient water content and prevented the desiccation of the rhizomes.

Excellent reductions on the further regrowth of *C. dactylon* without injury to cane was maintained with post planting glyphosate when preceded by a pre-planting irrigation, fallow glyphosate or combination of both fallow treatments, indicating that even with ploughing and fallowing, use of other cultural methods and herbicides are necessary to achieve good control of this weed. The superiority of the combined effect of cultural and chemical control measures over ploughing and following alone in checking regrowth of *C. dactylon* and expectation of good crop establishment and yield had been reported elsewhere (9,10).

The ability of these combined cultural and chemical treatments to control *C. dactylon*, resulted in reduced competition between the weed and the sugar cane crop and significantly increased the yield of cane even in the third year crop (second ratoon). Attainment of such high cane yield in the second ratoon crop suggested a long and strong residual effect from such integration of chemical and cultural control measures on the grass regrowth.

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Table 1 : Percentage ground cover of *Cynodon dactylon* as influenced by fallow glyphosate and irrigation treatments followed by post-planting herbicide applications

Treatments			*C. dactylon* ground cover(%)					
During fallow period		After cane planting	In fallow			After cane planting		
Mid August	Late September	10 dap	mid August	late Sept	1 wbp	6 wap	12 wap	18wap
Glyphosate	Pre-planting irrigation		52	8	18	35	42	58
Glyphosate	Pre-planting irrigation	Glyphosate	48	6	12	0	5	5
Glyphosate			53	10	3	30	40	48
Glyphosate		Glyphosate	58	5	0	15	15	20
	Pre-planting irrigation		60	69	70	78	90	85
	Pre-planting irrigation	Glyphosate	51	68	58	5	10	28
		Glyphosate	49	60	10	25	15	63
Control*			55	75	8	55	88	80
LSD (P<0.05)			ns	7	7	8	10	13

wbp = weeks before planting * standard treatment wap= weeks after planting

Table 2: Sugar cane stalk height and population as influenced fallow glyphosate and irrigation treatments followed by post-planting herbicide applications

Treatments				
During fallow period		After cane planting		
Mid August	Late September	10 dap	Cane heigh - (cm)	Cane population (no.x1000)/ha
Glyphosate	Pre-planting irrigation		203	47.10
Glyphosate	Pre-planting irrigation	Glyphosate	278	96.18
Glyphosate			210	55.65
Glyphosate		Glyphosate	230	85.63
	Pre-planting irrigation		150	32.18
	Pre-planting irrigation	Glyphosate	210	88.78
		Glyphosate	170	43.00
Control*			167	39.60
LSD (P=0.05)			27	10.59

dap = days after planting

Table 3: Estimated recoverable sucrose (ERsc%) and cane and sugar yields as influenced by fallow glyphosate and irrigation treatments followed by post-planting herbicide applications.

Treatments			Cane and sugar yield			
During fallow		After cane planting	Cane and sugar yields			
Mid August	Late September	(10 dap)	TC/ha (PC)	ERsc% (PC)	TS/ha (PC)	TCha (2R)
Glyphosate	Pre-planting irrigation		33.0	11.0	3.73	24.0
Glyphosate	Pre-planting irrigation	Glyphosate	83.7	10.8	9.03	85.4
Glyphosate			40.9	10.8	4.39	32.3
Glyphosate		Glyphosate	76.1	10.7	8.14	70.0
	Pre-planting irrigation		22.8	11.1	2.54	10.4
	Pre-planting irrigation	Glyphosate	73.4	10.8	8.12	60.3
		Glyphosate	29.4	10.9	3.00	11.3
Control*			25.4	11.2	2.86	10.1
LSD (P>0.05)			10.4	ns	1.21	12.7

dap = Days after planting
* = Standard treatment
ERsc% = Estimated recoverable sucrose
TC/ha = Tonnes cane /ha
TS/ha = Tonnes sugar/ha
PC= plant cane
2R= second ratoon crop

No-Till Irrigated Corn Production in Thailand

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Abstract: Eight hybrids selected commercial hybrids were compared for yield and other performance factors when grown in irrigated paddy fields under no-till and conventional tillage. On-farm trials were conducted on silty clay loam at Phitsanulok, Thailand, from November, 1998 to April, 1999. The no-tillage treatment displayed a 56% increase in average grain yield over the conventional tillage treatment. The increased yield is attributed to better moisture conservation and less weed competition. No-till was also superior to conventional tillage in plant growth and development, as expressed in terms of biomass, kernel number and kernel weight.

Introduction

No-tillage cultivation is practice that can be effective not only increasing soil and water conservation, but also in reducing labor requirement and time (Unger and McCalla, 1980). Maize grown under the no-tillage system usually results in the higher yields. Successful results have been reported in Thailand, particularly when applied to rainfed environments (Na Nagara *et al.*, 1984). However, there is very little information available in Thailand about no-tillage systems for maize grown under irrigated conditions in paddy fields.

The objective of this study was to evaluate yield and other performance factors of maize grown in paddy fields under no-till and conventional tillage systems.

Materials and Methods

Eight commercial maize hybrids were evaluated under no-till and conventional tillage. Two fields were selected, one for no-till and other for conventional tillage. Each plot was approximately 1.6 hectares and was divided into eight subplots. Twelve rows of each hybrid were planted on an area of 9 x 200 meters in each subplot.

To assure uniform crop emergence, irrigation water was uniformly applied by flooding to the experimental area approximately 30 days after rice harvest. After soil moisture was suitable for planting and crop emergence, the conventional tillage plot was plowed once and then harrowed before planting.

On the no-till plot, glyphosate 48% EC (N-(phosphonomethyl) glycine, isopropylamine), a pre-planting herbicide, was sprayed at 3 kg of active ingredient per hectare in two applications. The first application was seven days before planting and the second application was at planting.

Each hybrid in both the no-till and conventional tillage plots was planted at approximately 0.75 x 0.20 meter spacing, with a population objective of 65,625 seeds per hectare. Planting was done with a four-row White-New Idea planter equipped with no-till coulters.

Fertilizer treatments, based on soil test results, were the same on both plots. Lime was broadcast at a rate of 625 kg per hectare immediately after the pre-planting irrigation. DAP (18-46-0) and KCL (0-0-60) fertilizers were mixed and applied at a rate of 187.5 kg per hectare and 125 kg per hectare, respectively. Urea (45-0-0) was also applied at 188 kg per hectare, with top-dressing done by hand at 30 days after planting and again at flowering.

On the conventional tillage plot, weeds were controlled with metolachlor (40% EC) at 1.5 kg of active ingredient per hectare followed by one cultivation 30 days after planting. There was no weed control on the no-till plot. Carbosulfan and furadan were used for insect control om both plots.

The conventional tillage plot was irrigated three times in addition to the pre-plant irrigation. The no-till plot was irrigated only two times in addition to the pre-plant irrigation. Both plots also received 19.3 mm of rainfall during the late growing season.

Results and Discussion

Plant Growth

As shown in Table 1, plant growth factors, except for plant height, were superior in the no-till plot. No-till treatments showed an average 15% increase in biomass over the conventional tillage

Weed Competition and Soil Moisture Conservation

The results in Table 1 also indicate significantly greater water storage in the top of 0.2 m of soil sampling at 1 month in the no-till plot as compared to the conventional tillage plot. Better weed control, as indicated in Table 1 by weed dry weight, is also associated with the no-till practice.

Yield Components

Kernel number and kernel weight were the most important of yield components in this trial. As displayed in Table 2, the no-till plot produced an average of 12% more kernels per ear and 5% greater weight per 100 seeds than the conventional tillage plot. They were also significant difference between the two plots in terms of days to 50% silking and shelling percentage(Table 1). However, there was no significant difference in ear number and plant population between the two plots (Table 2).

Grain Yield

As shown in Table 2, the no-till plot produced an average 56% greater grain yield than the conventional tillage plot. This superior yield is attributed mainly to greater soil moisture conservation and less weed competition.

Conclusions

These results indicate that the yield of maize following of rice may be improved by using no-till practices. The increase in yield under the no-till system results from conservation of soil water and reduction of weed competition. No-till could be applied in areas with limited irrigation water, as well as rainfed environments, when weeds can be effectively controlled using pre-plant herbicides. More research is needed, however, to determine the effectiveness of no-till practices in areas of low fertility and coarse textured soils.

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Table 1 Some agronomic characters of maize hybrids grown under different tillage at PSL FCES during 1998-1999.

Treatment	Biomass (kg/ hectare)	Plant height (cm)	Days to 50% silking	Shelling (%)	Vol. Soil moisture content (%)	Weed dry Weight (g/m^2) at harvesting
No-till	13,125	228.7	59.3	84.0	27.2	122
Tillage	11,456	229.0	62.1	79.6	18.4	292

Table 2 Mean grain yield at 15% moisture content and yield components of maize hybrids grown under different tillage at PSL FCES during 1998-1999.

Treatment	Grain yield (kg/hectare)	Ear number per hectare	Kernel Number per ear	100 kernel weight (g)	Plant population per hectare
No-till	4955.0	37,975	555	29.6	37,708
Tillage	3162.5	39,055	497	28.1	36,958

RICE-WEED COMPETITIVENESS

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Abstract: Weed is one of the major problem for upland rice. This experiment was conducted to testing empirical methodologies for quantifying rice competitiveness by different seed rates; 0 50 100 250 500 and 1000 plants per square meter of 2 rice varieties; Khao Daeng Hawm and Sew Mae Jan with 2 weeding practices; weeding at 10 days after planting and continuous weeding (weeding every week). The result of first season study showed that seed rates affected leaves area and dry weight at seedling and tillering stages. Rice with continuous weeding resulted in higher leaves area and dry weight than weeding at 10 DAS. For yield, result showed significant interaction between seed rates, varieties and weeding practices. Khao Daeng Hawm var., seed rate had no effect on yield and continuous weeding treatment provided higher yield than weeding 10 DAS. For Sew Mae Jan, the result revealed that there was no significance in yield between seed rates in treatment weeding at 10 days after planting but significance in continuous weeding, treatment of seed rate- 50 plants per square meter produced the highest yield 307 g./m^2. The major weeds found were goatweed (*Ageratum conyzoides* L.), slender amaranth (*Amaranthus viridis* Linn.), goosegrass (*Eleusine indica* (L.) Gaertn.) and giant sensitive plant (*Mimosa invisa* Mart.). Further study has been investigated.

Key words: rice-weed competitiveness, seed rates, weeding practices

Introduction

Nearly 100 million people depend on upland rice as their daily staple food. Many of the world's poorest farmers live in the uplands of Asia, Africa, and Latin America, where farming systems are highly diverse. More than 19 million hectares of upland rice grown worldwide around 63 percent of them in Asia (IRRI1998-1999). Enhancing upland rice productivity provides an entry point to reducing the interrelated problems of subsistence agriculture; low productivity, weed infestations, nutritional imbalance, soil erosion, inadequate cultural practices, inefficient control of insect pests and diseases, and lack of suitable varieties that create severe poverty in the fragile uplands. Arraudeau (1994) reported that the major abiotic contains were drought, P deficiency, and soil acidity, and major biotic constrains were weed, blast, brown spot and stem borers. Roder *et al* (1995) reported the farmers in Lao PDR considered weed, rodents, insufficient rainfall, the lack of land availability, and insects to be the major constraints to upland rice production.

Weed suppression by vigorously growing rice is an important trait for upland rice cultivars since weeds constitute one of the major constraints to yield in the uplands. Suppressive characteristics include early vegetative growth, rapid development of leaf area and plant morphological form and reflect "competitiveness". However providing breeders with a simple index for rice competitiveness is a complex, if not impossible, task as many traits contribute to differential resource acquisition during the growth and development of a weed-crop community. Time of species emergence, relative growth rates, density and availability of limiting resources are just some of the factors that interact in determining inter- and intra-specific interactions. Past work suggests that breeders tend to use accumulated biomass as an index of "competitiveness" and quantifying competition at the individual plant, population and community level is complex (Grace and Tilman,1995). Two alternate generic approaches have been used in assessing competitiveness : a) empirical studies manipulating species frequency in mixture and the timing of the start and duration of competition in relation to resources and b) mechanistic simulation models to describe the course of competitive interactions in relation to the dynamics of resource availability (Kropff and van Larr, 1993). The second approach provides a rigorous quantification of the dynamics of competition but as yet cannot be routinely applied for screening purposes as it is data intensive and requires parameterisation for specific environments. Competitive outcomes between plant species can be described on a continuum from exploitation competition to interference competition. In the former, one species acquires the majority of a

limiting resource outright, possibly as a consequence of relative time of emergence and rapid growth rate, largely to the complete detriment of others in mixture. In the latter, competition occurs over a longer time frame and represents a contest for limiting resources. In agriculture plant communities, competitive interactions tends to be of the interference type. Two definitions of empirical competitive effects are widely used. The first is the total competitive effect of a species or entire community on another species. This is based upon the measured difference between the biomass that a target plant attains in the absence of neighbors compared to its biomass in presence of neighbors. The additional biomass attained after the removal of competitors is the total competitive effect of the competitors. The second definition extends the first and recognizes that competitive effects should take into account the relative abundance of competing neighbors or the absolute amount of biomass removed in the measurement of competition. The total competitive effect of neighbors is considered per unit biomass removed. This experiment was conducted to testing empirical models describing rice yield and weed biomass in relation to rice density for quantifying rice competitiveness.

Materials and Methods

Rice varieties

V1 = Khao Daeng Hawm
V2 = Sew Mae Jan

Seed rates

S0 = 0 plants per square meter
S1 = 50 plants per square meter
S2 = 100 plants per square meter
S3 = 250 plants per square meter
S4 = 500 plants per square meter
S5 = 1000 plants per square meter

Weeding practices

T1 = weeding at 10 days after sowing
T2 = continuous weeding (weeding every week)

Design

Experiment design was factorial randomized complete block with 4 replication. Plot size was 3X3 m^2. Rice were planted in rows which spacing was 20 cm.

Results and Discussion

A summary of upland rice growth in terms of leaves area, dry weight, yield and major weed biomass were presented in Table1-13.

Plant establishment

Plant establishment was not good due to drought spell at early growth stage. Germination of Khao Daeng Hawm var. was lower than 50% almost of seed rate treatment except S3 and S4 treatments (Table 1).

Leaf area and dry weight

At seedling and tillering stage (27 DAS, 54 DAS), higher seed rates as S3, S4, and S5 affected leaves area and dry weight significantly (Table 2-3). For V1, the highest leaves area at 27 DAS obtained from S4 was 14162 cm2/m2 where V2 was 19450 cm2/m2 from S5 treatment compared to V1S1 produced 12499 cm2/m2 and V2S1 as 2885 cm2/m2 with weeding at 10 DAS. For continuos weeding, both V1 and V2, the S4 and S5 treatments produced the highest leaf area as 12177, 13487 and 16736, 23058 cm2/m2 respectively. Similarly, at 27 DAS, V1S4 produced significantly highest dry weight of 10.37 g/0.2 m2. S1 and S2 with weeding at 10 DAS gave lower dry weight as 2.42 and 2.91 g/0.2 m2. For weed control treatment, V1S4 and V2S5 with continuous weeding resulted in higher dry weight as 11.33 and 19.54 g/0.2 m2 respectively. After stem elongation stage, it was found that seed rates did not affect leaves area and dry weight.

Upland rice yield

For Khao Daeng Hawm, seed rates had no effect on yield under both weeding practices. Continuous weeding treatment provided an average of 1.27 fold yield for V1 and 2 fold yield for V2 higher than weeding 10 DAS treatment. For Sew Mae Jan , result revealed that there was no significance in yield between seed rates under weeding 10 DAS but significance under continuous weeding.V2 S1 treatment (50 plants per square meter) with continuous weeding produced highest yield as 307 g/m2 (Table 12)

Weed biomass

Both V1 and V2, the treatments of S3 and S4 produced lower weed biomass than S1 and S2 treatments The major weed found at vegetative stage were goatweed (*Ageratum conyzoides* L.), slender amaranth (*Amaranthus viridis* Linn.), goosegrass (*Eleusine indica* (L.) Gaertn.) and at reproductive stage were giant sensitive plant (*Mimosa invisa* Mart.), goosegrass (*Eleusine indica* (L.) Gaertn.) and itchgrass (*Rottboellia exaltata* Linn. f.). Further study has been investigated.

Table 1 Plant establishment (plants/m^2)

	Weeding 10 DAS			Continuous weeding		
Seed rate	V1	V2	Diff.	V1	V2	Diff.
S1	27.3 d	37.0 d	-9.8 ns	30.5 d	43.0 d	-12.5 ns
S2	47.5 d	73.5 d	-26.0ns	49.3 d	79.8 d	-30.5 ns
S3	155.5 c	193.8 c	-38.3ns	145.8 c	196.0 c	-50.3 ns
S4	258.8 b	381.3 b	-122.5**	280.5 b	381.8 b	-101.3**
S5	382.3 a	823.8 a	-441.5**	449.3 a	802.5 a	-353.3**

CV = 19.0%

Table 2 Leaf area at 27 DAS (cm^2/m^2)

	Weeding 10 DAS			Continuous weeding		
Seed rate	V1	V2	Diff.	V1	V2	Diff.
S1	2499 b	2785 c	-286 ns	2819 b	3152 c	-333 ns
S2	5302 b	5615 bc	-313 ns	3301 b	4268 c	-967 ns
S3	9325 ab	11107 b	-1782 ns	7031 ab	11207 b	-4176 ns
S4	14262 a	19017 a	-4755 ns	12177 a	16736 ab	-4559 ns
S5	8347 ab	19450 a	-11103**	13487 a	23058 a	-9571**

CV = 47.3%

Table 3 Leaf area at 54 DAS (cm^2/m^2)

	Weeding 10 DAS			Continuous weeding		
Seed rate	V1	V2	Diff.	V1	V2	Diff.
S1	10723 b	18784 b	-8061 ns	14492 b	17539 b	-3047 ns
S2	16372 b	21800 b	-5428 ns	18565 b	17074 b	1491 ns
S3	25149 ab	23242 b	1907 ns	27153 ab	34163 a	-7010 ns
S4	38477 a	34645 a	3832 ns	29410 ab	42072 a	-12662 ns
S5	24745 ab	42244 a	-17499**	41732 a	41133 a	598 ns

CV = 42.1%

Table 4 Leaf area at 75 DAS (cm^2/m^2)

Seed rate	Weeding 10 DAS V1	V2	Diff.	Continuous weeding V1	V2	Diff.
S1	30979 a	31028 b	-49 ns	49091 a	57104 b	-8013 ns
S2	38298 a	52570 ab	-14272 ns	54196 a	61855 ab	-7659 ns
S3	49040 a	46803 b	2237 ns	50454 a	91005 a	-40551**
S4	55936 a	65764 a	-9828 ns	76765 a	68398 ab	8368 ns
S5	36606 a	69868 a	-33262**	53382 a	76865 a	-23482 ns

CV = 34.2%

Table 5 Leaf area at 96 DAS (cm^2/m^2)

c

Seed rate	Weeding 10 DAS V1	V2	Diff.	Continuous weeding V1	V2	Diff.
S1	21151 a	33274 a	-12123 ns	52261 a	70528 ab	-18266 ns
S2	23827 a	25370 a	-1543 ns	45159 a	53629 b	-8740 ns
S3	27058 a	23600 a	3458 ns	37340 a	60819 b	-23480 ns
S4	31706 a	34273 a	-2567 ns	43205 a	90815 a	-47611**
S5	18628 a	36413 a	-17785 ns	52695 a	48700 b	3995 ns

CV = 46.0%

Table 6 Leaf area at 131 DAS (cm^2/m^2)

Seed rate	Weeding 10 DAS V1	V2	Diff.	Continuous weeding V1	V2	Diff.
S1	19848 a	26146 a	-6298 ns	31188 a	56056 ab	-24868 *
S2	15233 a	30908 a	-15676 ns	25567 a	42128 b-	-16561 ns
S3	21546 a	36558 a	-15012 ns	27713 a	63359 a	-35646 **
S4	26176 a	40791 a	-14616 ns	35538 a	40006 b	-4468 ns
S5	21059 a	38386 a	-17327 ns	23485 a	37494 b	-14009 ns

CV = 40.5%

Table 7 Rice dry weight at 27 DAS (g./0.2 m^2)

Seed rate	Weeding 10 DAS V1	V2	Diff.	Continuous weeding V1	V2	Diff.
S1	2.42 b	3.21 c	-0.79 ns	3.79 a	4.25 d	-0.46 ns
S2	2.91 b	5.23 c	-2.32 ns	4.38 a	3.97 d	0.41 ns
S3	7.46 a	10.57 b	-3.11 ns	7.67 a	9.51 c	-1.84 ns
S4	10.37 a	11.89 b	-1.52 ns	11.33 a	15.52 b	-4.19 *
S5	6.56 ab	19.95 a	-13.39 **	6.73 a	19.54 a	-12.81**

CV = 33.2%

Table 8 Upland rice dry weight at 54 DAS (g./0.2 m^2)

Seed rate	Weeding 10 DAS V1	V2	Diff.	Continuous weeding V1	V2	Diff.
S1	11.28 b	23.57 c	-12.29 ns	23.67	b 18.43 b	5.24

ns

S2	22.85 ab	31.89 c	-9.04 ns	28.97 b	38.57 b	-9.60 ns
S3	45.45 a	42.45 bc	3.00 ns	46.50 ab	65.05 a	-18.55 ns
S4	44.84 a	56.76 b	-11.92 ns	67.77 a	61.22 a	6.55 ns
S5	24.55 ab	85.71 a	-61.16 **	33.39 b	71.75 a	-38.36 **

CV = 37.6%

Table 9 Upland rice dry weight at 75 DAS (g./0.2 m^2)

Seed rate	Weeding 10 DAS V1	V2	Diff.	Continuous weeding V1	V2	Diff.
S1	44.67 b	55.72 b	-11.05 ns	81.13 a	91.84 a	-10.71 ns
S2	63.97 ab	58.60 b	5.37 ns	67.49 a	91.66 a	-24.17 ns
S3	62.74 ab	52.20 b	10.54 ns	82.16 a	116.07 a	-33.92 *
S4	89.13 a	100.60 a	-11.47 ns	92.04 a	105.17 a	-13.13 ns
S5	64.07 ab	101.00 a	-36.93 **	62.49 a	117.31 a	-54.82 **

CV = 26.7%

Table 10 Upland rice dry weight at 96 DAS (m./0.2 m^2)

Seed rate	Weeding 10 DAS V1	V2	Diff.	Continuous weeding V1	V2	Diff.
S1	66.26 a	65.10 b	1.16 ns	125.67 a	156.97 ab	-31.30 ns
S2	63.32 a	63.23 b	0.09 ns	141.02 a	142.45 b	-1.43 ns
S3	98.94 a	132.57 a	-33.63 ns	133.17 a	157.14 ab	-23.97 ns
S4	88.55 a	126.06 a	-37.51 ns	165.11 a	196.50 a	-31.38 ns
S5	72.98 a	142.20 a	-69.22 **	145.17 a	169.43 a	-24.26 ns

CV = 24.9%

Table 11 Upland rice dry weight at 117 DAS (g./0.2 m^2)

Seed rate	Weeding 10 DAS V1	V2	Diff.	Continuous weeding V1	V2	Diff.
S1	70.33 a	73.57 b	-3.24 ns	154.35 a	232.97 a	-78.62 *
S2	79.68 a	118.93 ab	-39.26 ns	130.96 a	225.41 a	-94.45 **
S3	96.77 a	164.49 a	-67.72 *	141.84 a	226.70 a	-84.86 **
S4	101.18 a	165.72 a	-64.54 *	182.10 a	212.82 a	-30.72 ns
S5	80.87 a	185.65 a	-104.78 **	158.95 a	168.31 a	-9.36 ns

CV = 29.8%

Table 12 Upland rice yield (g./m^2)

Seed rate	Weeding 10 DAS V1	V2	Diff.	Continuous weeding V1	V2	Diff.
S1	81.06 a	55.38 a	25.68 ns	137.64 a	306.74 a	-169.10 **
S2	37.98 a	72.77 a	-34.79 ns	189.76 a	204.13 b	-14.37 ns
S3	77.04 a	100.07 a	-23.03 ns	128.45 a	173.28 bc	-44.83 ns
S4	66.07 a	98.48 a	-32.41 ns	146.32 a	186.31 bc	-39.92 ns
S5	63.22 a	90.88 a	-27.66 ns	140.98 a	130.78 c	10.21 ns

CV = 26.7%

Table 13 Weed biomass (g./m^2)

Seed rate	V1	V2
S1	801.40	435.23
S2	650.83	946.45
S3	369.68	327.63
S4	236.83	288.05
S5	666.05	239.60

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STUDIES ON RESISTANCE OF *Solanum phoreinocarpum* AND *Bidens pilosa* TO HERBICIDE METSULFURON-METHYL[1]

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Abstract: In the survey plot applied with metsulfuron-methyl, the weed resistant biotypes were surveyed by following their tracks, Resistance levels of shiningfruit nightshade (*Solanum photeinocarpum* Nakai et Odash.) and railway beggarticks (*Bidens pilosa* L.), the weed species showing resistance, were deter.nined by bioassay and biochemical analysis, The result has shown that with 4 times of the metsulfuron-methyl application in 2 successive years only the shiningfruit nightshade showed resistance (resistancefactor=2.19; controlling effect at a given same concentration reduced by 29%; susceptibility ratio=1.58; activity ratio of acetolactate synthase (ALS)=1.61); differences of resistance in railway beggarticks being unapparent.

Key words: metsulfuron-methy, shiningfruit nightshade(*Solanum photerinocarpum* Nakai et Odash.); railway beggarticks(*Bidens pilosa* L.), resistance biotype, acetolactate synthase

Introduction

Sulfuronylurea herbicides are high in activity (He kang et al. 1993) and selectivity, used for a wide range of weeds and easily handled; consequently, becoming the main development of herbicide products in the past decade. These herbicides are simple in target (effecting ALS, acetolactate synthase, only.) (Thill D.C.C.A et al. 1991), therefore, readily for weeds to produce resistance, In order to prolong their longevity of use and ensure safety and rational application, a research on the resistance has been surveyed in plot science 1996, choosing metsulfuron-methyl, the most active herbicide, as the testing material, two resistance biotypes of the weeds tested were found, i.e, shiningfruit nightshade (*Solanum photeinocarpum* Nakai et Odash.) and railway beggarticks (*Bidens pilosa* L.), and , then, their resistance levels were determined by bioassay and biochemical analysis.

Materials and Methods

Materials

Herbicide used 10%metsulfuron-methy WP produced by 4th Trail Factory of Jiangsu Hormone Institute Weed seeds of 15 species collected from the field, with their germination rates over 90%. They are: railway beggarticks, glandularstal st paulswort(*Siegesbeckia pubescens* Makino.), nepal dock. (*Rumex nepalensis* Spreng.), dockleaved knotweed (*Polygonum lapathifolium* L.), toothed fruit dock (*Rumex dentatus* L.), small goosefoot (*Chenopodium serotinum* L.), belvedere (*Kochia scopria* (L.) Schrad.), redroot amaranth (*Amarantus retroflexus* L.), shiningfruit nightshade, shepherdspurse (*Capsella bursa-pastoris* (L.) Medic.) chickweed (*Stellaria media* (L.) Cyr.), apple peru (*Nicandra physoloides* (L.) Gaerth.), jimsonweed (*Datura stramonium* L.), cluster mallow (*Malva verticiliata* L.), and hispid barnyyardgrass *(Echinochloa crusgalli var hispidula* (Retz.) Honda.).

Determination by acetalactate synthase (ALS) biochemical analysis showed that all the reagents were of AR grade.

Field observation trail of the resistance of the weeds

Plots were set in the observation trail fields of the plant protection Institute in autumn of 1996, 40m^2/plot, sown with each species at the same seeding rate, herbicide application taking place in spring and autumn of 1997 and 1998, 4 applications in all, 5 treatments of the chemical mount: 1.5, 3.0, 6.0, 12.0g.a.i./ha of metsulfuron-methyl and the CK, 2 replicates, 10 plots in all, sprayed with the MATBI surper Green Type-16 plastic Knapsack sprayer, with the solution, amounting to 750kg/ha. 3 spots in each plot which species and plants were surveyed, spot area=1m2, amount and method used were the same in

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every time of application following the survey, quantification of the 4 parameters was made according to uniformity, density, frequency and relative numerousness of the species and plants in the field(Zhang Chaoxian et al. 1998), seeds of the resistant biotypes were collected in autumn in 1998.

Bioassay and biochemical analysis

Bioassay

Dosage-reaction-curve method employed: Plastic pots (diameter=11.5cm) in the greenhouse, with 250g of perlite soil (perlite:soil=1:2)in each pot, were sown with shiningfruit nightshade and railway beggarticks (the 2 resistant biotypes) seeds, retaining 10 stronger seedling in each pot and removing the rest at 2 leaves stage. The mesulfuron-methy solution of 10, 20, 40, 80, 160 and 320ppb in concentration were prepared, with clean water as the CK, treated in sequence, from lower to higher concentration, 4 replicats per treatment trickled simultaneously for same concentration, 50ml/pot; 30 days after the treatment, the plants were pulled and their height and weight measured and the inhibition rates calculated. The logarithms and probabilities of the concentrations and the inhibition rates were also calculated respectively, and, then, the toxicity regression equation (LD-Dline), the inhibition mid-concentration and the correlation coefficient evaluated, and the dosage reaction curve F test carried out to evaluate the Ic_{50} 95% confidence limits and the resistance factors.

Comparison of controlling effects at the same concentration level

$6\times1m^2$ concrete pond in the greenhouse were filled with water to get rid of the original weed seeds and other impurities, divided into 15 plots after the water dried out, with each plot area = $0.4\times1m^2$, 5 treatments; seeds of the resistance biotypes were sown in each plot, 3 replicates for each treatment. The 3-leaf seedlings were treated with 8ppm and 10ppm metsulfuron-methyl solution with clean water as CK. 30 days after the culture under moist condition, the controlling effects were surveyed.

ALS activity determination

In the $6\times1m^2$ concrete pond, seeds that had been treated 4 times in 2 years were sown, 4g of shoot sample pulled out from each treatment at 2-leaf stage, ready to test.

ALS extraction: Put the shoot sample into the extracting medium (containing 1mmol/L of Sodium pyrurate, 0.5mmol/L TPP, 1mmol/L DTT, 5mmol/K $MgCl_2$, pH 7.5). In the ice bath, it was grinded, with small amount of Silica sand. At temperature of 4□, it was centrifuged for 20 minutes at rate of 2500r/min. The supernatant taken is the crude enzyme solution.

ALS activity determination: 0.1ml of metsulfuron-methyl (320ppb) and 0.1ml crude enzyme solution were added to 0.5ml of the reaction medium (50mmol/L K_3PO_4 buffer solution containing 1mmol/L of $MgCl_2$, 200mmol/L Sodium pyrurate, 1mmol/L DPT, PH 7.0). Kept at 37□ for 1h, then, 50 □l 3mol/L H_2SO_4 added, decarboxylatd reaction at 60□for 15 minutes; then, sequentially added 0.5ml of 0.083% creatine and 0.5ml 0.83% methyl naphthol (solved in 2.5mol/L of NaOH); colour reaction lasted 5 minutes at 60□ centrifugation at rate of 2000r/min. for 10 minutes. The light absorption level determined at the 530 nm position (A530). The ALS activity was directly showed with ΔA530nm/mg(pro.)h.

Protein contents determined by method of coomassie brilliant blue G250

Results and Analysis

Results from the resistance survey polt

Shinigfruit nightshade and railway beggarticks appeared to be of resistant biotype. The seeds of the 2-years, 4-time-tested shiningfruit nightshade and railway beggarticks were collected for bioassay and biochemical analysis in the next step to determine their resistance levels.

Results from the bioassay

The results (Table 1) shows that the shiningfruit nightshade toxicity Ic_{50} is 62.00~136.45ppb, the resistance factor up to 2.19, it trends to increase with increase of herbicide concentration. No difference is found between treatments in the railway beggarticks.

Table 1 Shiningfruit nightshade resistance to metsulfuron-methyl in different concentration treatments

Treatment g.a.i./ha	LD-pline r	correlation coefficient	Inhibition mid-cone Ic_{50}(ppb)	95%confidence limit of Ic_{50}	resistance factor
CK	y = 3.1434 + 0.4495x	0.9989	62.00	66.00~ 62.44	-
1.5	y = 3.5283 + 0.3477x	0.9977	69.36	69.24 ~ 69.47	1.12
3.0	y = 3.4038 + 0.2429x	0.9897	103.96	103.94~103.98	1.67
6.0	y = 2.1912 + 0.5214x	0.9391	121.31	119.83~122.97	1.95
12.0	y = 3.8696 + 0.2093x	0.942?	136.46	136.10~136.82	2.19

$$\text{Resistance factors (times)} = \frac{Ic_{50} \text{ of weed in trail with different concentration of herbicide application}}{Ic_{50} \text{ of weed in trail without herbicide application}}$$

Controlling effects of metsulfuron-methyl to shiningfruit nightshade and railway beggarticks

Plant-number controlling effect

In the herbicide (10ppm) application at 3-leaf stage, a negative correlation between plant-number controlling effect and the concentration, i.e. the lower the concentration, the higher the controlling effect is found in the shiningfruit nightshade, no difference between concentrations in the railway beggarticks the controlling effect 100% (Fig.1).

Fresh-weight controlling effects

The fresh-weight inhibition rate correlated with concentration of metsulfuron-methyl in the shiningfruit nightshade, when the herbicide 8ppm was applied, the higher the concentration, the lower the controlling effect; no difference between concentration treatments in the railway beggarticks, the fresh-weight inhibition being 100%(Table2).

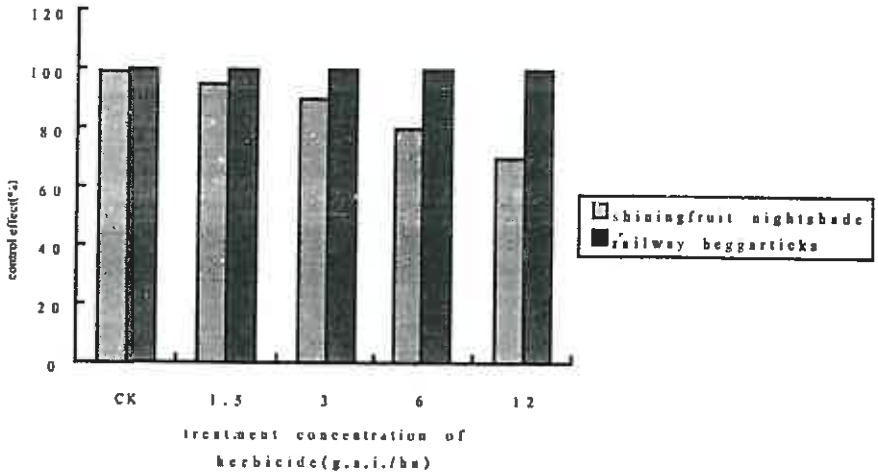

Fig.1.Control effect of equal concentration (10ppm) of metsulfuron-methyl on the shiningfruit nightshade and railway beggarticks

Table 2 8ppm Metsulfuron-methyl fresh-weight controlling effects to the shiningfruit nightshade

Treatment g.a.i./ha	CK mg/plant	sprayed plant mg/plant	Inhibition ratio %	Susceptibility ratio
CK	36	7.5	79.17	-
1.5	38	11.5	69.74	1.14
3.0	38	15.5	59.21	1.34
6.0	34	16.5	51.47	1.54
12.0	37	18.5	50.00	1.58

$$\text{Inhibition rate} = \frac{\text{Fresh weight (CK) - the fresh weight (sprayed)}}{\text{Fresh weight (CK)}}$$

$$\text{Susceptibility ratio} = \frac{\text{Inhibition rate of weed in trail without using herbicide (CK)}}{\text{Inhibition rate of weed in trail with different conc. of herbicide application}}$$

Metsulfuron-methyl effect on ALS in the shiningfruit nightshade

The results (Table 3) shows that metsulfuron-methyl's ALS inhibition rate in the shiningfruit nightshade correlates with the treatment's concentration, the CK's inhibition rate is 86.7% and 12.0g.a.i./ha treatment's only 53.80%.

Table 3 Metsulfuron-methyl inhibition rate to ALS in the shiningfruit nightshade

Treatment g.a.i./ha	metsulfuron-methyl (ppb)	ALS activity (A530nm/mg(pro.)h)	ALS inhibition rate (%)	ALS activity ratio
CK	0	0.88	86.7	1
	400	0.12		
1.5	0	1.22	58.3	1.49
	400	0.51		
3.0	0	1.90	54.8	1.58
	400	0.86		
6.0	0	2.80	54.3	1.60
	400	1.28		
12.0	0	1.27	53.8	1.61
	400	0.59		

Discussion

The problem of herbicide resistance in weeds has already threatened the use of herbicide with its extensive and lasting application, which will impact the crops lasting, stable and higher yielding. The simpler the target acted of the herbicide, the easier the development of resistance. Weed spices resistant to the ALS inhibitors, maining the sulgurinylurea herbicides, have increased from 18% to 27% (Li Yongfeng etal. 1999). The high speed of the resistance development and the wide range of the weed spices have become the main problem in the increasing severity of the resistance of weed in the past decade. Therefore it is really an urgent task to carry out study on weeds' resistance to the sulfuronylurea herbicides. In this paper several resistant biotypes of the weeds were discovered by starting from establishment of the resistance survey plot and subsequently carrying out bioassay and biochemical analysis of shiningfruit nightshade and railway beggarticks, results proved that the shiningfruit nightshade appealed to be resistant but the railway beggarticks did not. It might be the reason why the latter did not that herbicide had not yet, sufficiently got into the plant (railway beggarticks have not yet emerged in the spring and the weed plants have grown too large to sufficiently show the controlling effect). This remains to be further studies.

Metssulfuron-methyl is known as a new-type, super-high effect herbicide for its low dosage high activity and other good paints, so it is extensively used. However, it is easy to cause development of resistance of the weed due to its simplicity in target. As a result, measures must be studied in time to slow the resistance by integrated means including use of metsulfuron-methyl in mixture of varied herbicides possessing different functioning mechanisms or alternate application with these herbicides in turns, limited times in applying the herbicide on the same crops weeds, practice of rotation, tillage, well handling of synergists and the safety agents, reasonable use of the herbicides should be combined with ecological and biological controls to prevent resistant weed spices from producing and control them at their lower level of resistance.

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STUDY ON RELATIONSHIPS BETWEEN *BECKMANNIA SYZIGACHNE* (STUED.) FERNALD AND OTHER WEEDS IN RAPE FIELD AND FALLOW FOLLOWING RICE

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Abstract The species association between *Beckmannia syzigachne* (Stued.) Fernald and other weeds in rape field with no-herbicide application and fallow following rice as well as comparing Frequency, Relative Abundance and Average Dominance Rate of populations were studied. Results obtained showed that in rape fields under normal cultivation and no-herbicide application *Beckmannia syzigachne* (Stued.) Fernald had poor adaptability and competition ability compared with *Alopecurus acquallis* Sobol., *Malachium aquaticum* (L.) Fries and *Stellaria alsine* Grimm., but would become dominant species in water logged winter fallow , leading to yield loss of rape and wheat after a few years.

Key Words: *Beckmannia syzigachne* (Stued.) Fernald; Species assotiation

1 Introduction

Beckmannia syzigachne, an annual weed belong to *Gramineae* growing by water or moist area, was a good fodder in China[2]. In recent years, this weed had infected rape and wheat field following rice rapidly and become the dominant malignant weed in many fields of Yangtzi River valley[1,2]. Besides, infection of this weed to cropland had been reported in Japan[4,6]. Herbicides used currently are hard to control it so that this new weed problem has been attracting people's attention increasingly. Study on species association between *Beckmannia syzigachne* and other weeds could explain the cause that lead to this new weed problem from the vantage point of ecology, and would be beneficial to integrated management over this weed.

2 Materials and methods

An investigation was conducted in May 1998 in Zhejiang province. Objects studied were 10 rape fields with no-herbicide application in history and 10 fallow following rice (3 samples per field) respectively. Relative Abundance (RA), Frequency (F), Relative Coverage (C), Relative Height (H) and Average Dominance Rate (S') were determined,

and data obtained was dealt with as follows:

Weed Dominance (S): $S = H \times C$

Average Dominance ($\bar{S}$): $\bar{S} = \frac{\sum_{i=1}^{n} S_i}{n}$

Weed Dominance Rate (S'): $S' = \frac{S_{N-1}}{\sum_{i=1}^{N} S_i} \times 100\%$

Average Weed Dominance Rate ($\bar{S'}$): $\bar{S'} = \frac{\sum_{i=1}^{n} S_i}{n} \times 100\%$

(n: sample number, 30 in the investigation; N: weed population number)

Relationship between two weed species were calculated by Point correlation coefficient[5]:

$$r = \frac{ad - bc}{\sqrt{(a+b)(a+c)(b+d)(c+d)}}$$

(a: Sample number both specie A and specie B existed; b: Sample number specie B existed and specie A not; c: Sample number specie A exist and specie B not ; d: Sample number both species not .)

In order to form a judgment for Relationship between weed species, x^2 test was carried out[3,7]:

$$x^2 = \frac{n\left[|ad - bc| - \frac{1}{2}n\right]^2}{(a+b)(c+d)(a+c)(b+d)}$$

3 Results

Frequency, Relative Abundance and Average Dominance Rate of weed populations in rape field and fallow following rice obtained are as figure 1, 2, and 3. (1: *Alopecurus acquallis* Sobol.; 2: *Beckmannia syzigachne* (Steud.) Fernald); 3: *Poa annua* L.); 4: *Polygonum persicaria* L.; 5: *Malachium aquaticum* (L.) Fries); 6: *Stellaria alsine* Grimm.); 7: *Ranunculus sceleratus* L.; 8: *Cardamine hirsuta* L.; 9: *Vicia sativa* L.; 10: *Mazus japonicus* (Thunb.) O. Kuntze; 11: *Galium aparine* L.; 12: *Lapsana apogonoides* Maxim)

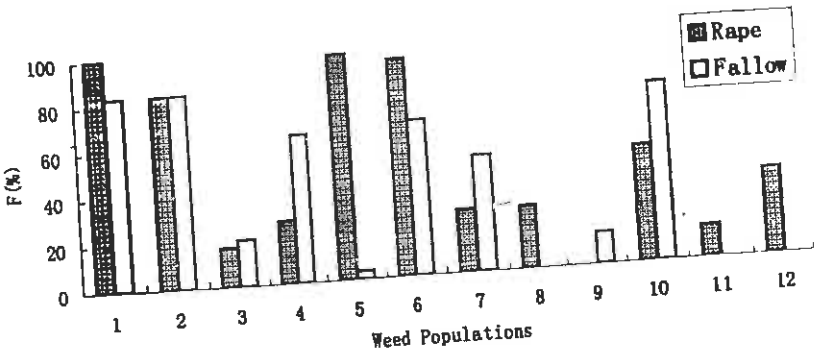

Fig.1 Frequency of weed populations in rape and fallow following rice

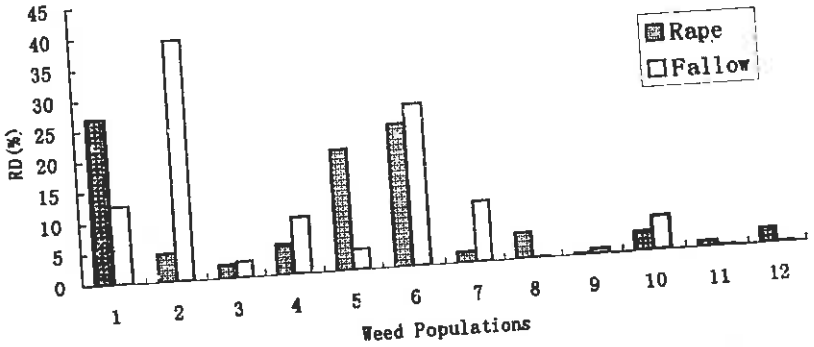

Fig.2 Relative Abundance of weed populations in rape and fallow following rice



Fig. 3 Average Dominance Rate of weed populations in rape and fallow following rice

Above figures showed that rape fields with no-herbicide application in history are apparently different from fallow following rice in 3 fundamental characteristics of weed community. Of 12 main weed populations, *Beckmannia syzigachne* varied prominently on RA and S', although it's F was similar in the above two weed communities. In rape

fields, RA of this weed was less than 5% while about 40% in fallow, 8 times as much as the former; S' were 8.8% in rape as against 62.7% in fallow. Thus showed clearly that fallow following rice was advantageous to the infection of *Beckmannia syzigachne*--in fact, *Beckmannia syzigachne* had become the dominant specie instead of *Alopecurus acquallis* in winter fallow. Of the other weed populations, *Malachium aquaticum* varied apparently in the two weed communities also: F, RA and S' were all decreased sharply in the fallow compared with in rape field. Otherwise, FA and S' of *Alopecurus acquallis* (the dominant specie in the rape field) was declined notably in fallow compared with in rape field. Meanwhile, population numbers had a marked declining tendency in the fallow compared with in rape field. Thus the analysis of the species association between *Beckmannia syzigachne* and other weeds can be based on the above characteristics.

Association coefficients among main weed populations in rape field (AC_1) and in winter fallow following rice (AC_2) are illustrated in matrix:

AC_1=

	1	2	3	4	5	6	7	8	10	12
1	1	*	*	*	*	*	*	*	*	*
2	*	1	-0.447	-0.135	-0.083	-0.083	0.27	0.067	0.268	0.155
3	*	-0.447	1	-0.113	-0.111	0.149	-0.113	-0.113	0	0.311
4	*	-0.135	-0.113	1	0.161	0.112	0.318	0.138	0.151	-0.442
5	*	-0.083	-0.111	0.161	1	0.035	-0.308	*	-0.186	-0.244
6	*	-0.083	0.149	0.112	0.035	1	0.112	0.112	-3.93	-0.244
7	*	0.27	-0.113	0.318	-0.308	0.112	1	0.148	0.151	0.146
8	*	0.674	-0.113	0.318	*	0.112	0.148	1	0.302	0.52
10	*	0.268	0	0.151	-0.186	-3.93	0.151	0.302	1	0.069
12	*	0.155	0.311	-0.442	-0.244	-0.244	0.146	0.52	0.069	1

AC_2=

	1	2	4	6	7	9	10
1	1	0.042	-0.34	0.064	0.451	0.182	0.182
2	0.042	1	-0.151	-0.135	0.27	0.182	0.182
4	-0.34	-0.151	1	0.056	-0.351	-0.31	-0.419
6	0.064	-0.135	0.056	1	-0.419	-0.35	0.11
7	0.451	0.27	-0.351	-0.42	1	0.39	0.078
9	0.182	0.182	-0.31	-0.35	0.39	1	0.22
10	0.182	0.182	-0.42	0.11	0.078	0.22	1

As the above association coefficients matrix shown, associations of varying degrees existed in the main weed populations in rape field and winter fallow. In rape existed 34 pairs of such weeds, including 20 pairs with positive association and 14 pairs with

negative association, 1 pair without any association, and 10 pairs without calculating results, while in the fallow, 21 pairs, taking in 13 pairs with positive association and 8 pairs with negative association.

In rape, no association coefficients were obtained between *Beckmannia syzigachne* and the dominant specie *Alopecurus acquallis*, but *Beckmannia syzigachne* had association with the rest weed populations,--negative association with *Poa annua*, *Polygonum persicaria*, *Malachium aquaticum*, and *Stellaria alsine*, and positive association with *Ranunculus sceleratus*, *Cardamine hirsuta*, *Mazus japonicus*, and *Lapsana apogonoides*, etc.. But in fallow, *Beckmannia syzigachne*, the dominant specie, had positive association with *Alopecurus acquallis*, the subdominant specie. Thus the associations between *Beckmannia syzigachne* and other weed populations in fallow were similar to those in rape, but the association with *Polygonum persicaria* turned to be positive, appositive to that in rape. x^2 of coefficients among main weed populations in rape and in fallow are illustrated by matrix x^2_1 and matrix x^2_2 (whether x^2 is positive or negative is determined by ad-bc).

$X^2_1=$

	1	2	3	4	5	6	7	8	10	12
1	*	*	*	*	*	*	*	*	*	*
2	*	23.2	-0.375	-1.01	-0.823	-0.828	0.852	0.341	0.96	0.155
3	*	-3.375	24.1	-0.011	-0.023	0.003	-0.011	-0.011	0.208	1.52
4	*	-0.034	-0.011	25.1	0.003	0.288	1.63	1.63	0.17	-4.037
5	*	-0.828	-0.023	0.003	6.99	6.59	-0.288	*	0	-0.079
6	*	-0.828	0.023	0.288	6.99	6.99	0.288	0.288	0	-0.079
7	*	0.852	-0.011	1.63	-0.788	0.288	25.1	0.117	0.17	0.138
8	*	0.034	-0.011	1.63	*	0.288	0.117	25.1	1.53	0.236
10	*	0.96	0.208	0.17	0	0	0.17	1.53	26.1	0
12	*	0.155	1.52	-4.037	-0.079	-0.079	0.138	0.236	0	25.8

$X^2_2=$

	1	2	4	6	7	9	10
1	23.2	0.192	-1.841	0.032	3.84	0.058	0.153
2	0.192	23.2	-0.11	-0.032	0.955	0.058	0.153
4	-1.841	-0.11	25.9	0.02	-2.302	-1.327	-3.433
6	0.032	0.032	0.02	25.7	-3.75	-1.768	0.02
7	3.84	0.955	-2.302	-3.75	26.1	2.603	0
9	0.058	0.058	-1.327	-1.768	2.603	22	0.303
10	0.153	0.153	-3.433	0.02	0	0.303	24.7

Comparing x^2 of each associated pair with $x^2_{(0.05)} = 3.84$, it could be found that the

associations between *Beckmannia syzigachne* and other populations did not reach top level, no matter in rape following rice or in fallow. In other words, *Beckmannia syzigachne* is greatly independent among weed populations, which maintain loose associations with other populations. Consider other specie pairs, *Malachium aquaticum* and *Stellaria alsine* in rape have strong positive association, while *Polygonum persicaria* and *Lapsana apogonoides* have strong negative association; *Stellaria alsine* and *Mazus japonicus* in fallow have strong negative associatior

4 Discussion

Whittaker categorized relationships between species into 5: 1. Existing of specie A relies on that of specie B only; 2. Existing of specie A relies on that of specie B which could be substituted by other species; 3. Existing of A has no relationship with specie B; 4. Existing probability of specie A would be declined by the existing of specie B; 5. Specie A would not be able to exist when specie B exists.[3] Investigation in this paper showed that in rape and winter fallow following rice the relationships between *Beckmannia syzigachne* and other weed populations belong to the 3types of No. 2, No. 3, and No. 4.

From results obtained, not only in rape field with no-herbicide application but also in winter fallow following, *Beckmannia syzigachne* had negative association with *Poa annua*, *Malachium aquaticum* and *Stellaria alsine*, and had positive association with *Ranunculus sceleratus* and *Mazus japonicus*, etc.. Thus show clearly that *Beckmannia syzigachne* is characterized by the adaptation to moist. In rape field with relative lower water content, *Malachium aquaticum* and *Stellaria alsine* had their tremendous individuals, but *Beckmannia syzigachne* had its lower RA and S' although it's F was higher. Thus imply *Beckmannia syzigachne* had poor adaptability and competition ability in rape field with no-herbicide application in history. And specie *Alopecurus acquallis*, *Malachium aquaticum* and *Stellaria alsine*, with higher seed production compared with *Beckmannia syzigachne*, were dominant or sub-dominant weeds.

In Yangtzi River valley, fallow following rice of many regions often accumulated water in winter, so that *Malachium aquaticum*, *Stellaria alsine* and *Alopecurus acquallis* were hard to emergent or develop that would lead to niche vacancy, and then lead to the infection of *Beckmannia syzigachne*. Without strong competition, *Beckmannia syzigachne* would produce tremendous seeds and enlarge its population increasingly. When these fields were planted with rape or wheat, weed problem from *Beckmannia syzigachne* would occur. Otherwise, seeds of *Beckmannia syzigachne* produced in fallow would float into other fields in early summer when rice are planted because of the

lower weight of the weed seeds, leading to the harmfulness to rape or wheat in winter.

It can be concluded through the study on relationships among species that water accumulation in winter fallow is one of the most important causes of the new weed problem from *Beckmannia syzigachne*.

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STUDY ON THE OCCURRENCE OF *Sclerochloa kengiana* AND IT'S CHEMICAL CONTROL IN WHEAT

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Abstract: Field trials were conducted during 1994-1996 in Dazhong farm of Jiangsu province to determine the occurrence period of *Sclerochloa kengiana* and chlorotoluron, butachlor, trifuralin, napropamide, isoproturon, fenoxarop (Puma) for wheat (cv. Yangmai5#) tolerance and control of *Sclerochloa kengiana.* The investigation showed that *Sclerochloa kengiana* turned up from 5 days to 25 days after seeding, 85.7% of them turned up in 15-25 days after seeding. Mixing chlorotoluron at 0.75 kg ai ha^{-1} with trifuralin at 0.576 kg ai ha^{-1}, the weed control was 94.4% (30 DAT) and 97.2% (60 DAT) respectively, the yield of mixing chlorotoluron at 0.75 kg ai ha^{-1} and trifuralin at 0.576 kg ai ha^{-1} ranked the first in the tests. It was not any significant compared with weed free plots. Ranking the second of the weed control and the yield in the trials were mixing chlorotoluron at 0.75 kg ai ha^{-1} with napropamide at 0.24 kg ai ha^{-1}, mixing chlorotoluron at 0.75 kg ai ha^{-1} with butachlor at 0.72 kg ai ha^{-1}, isoproturon 1.35 kg ai ha^{-1}. Successful weed control achieved with the mixture of chlorotoluron at 0.75 kg ai ha^{-1} and trifuralin at 0.576 kg ai ha^{-1} to control *Sclerochloa kengiana* covering 4000 ha. from 1996 to 1998 in Dazhong Farm.

Key words: *Sclerochloa kengiana*, wheat, chemical control

Introduction

Sclerochloa kengiana is the most important weed in rice-wheat cropping system in northeast part of Jiangsu province. Almost 140 thousand ha is severely infested with *Sclerochloa kengiana*. The density of the weed ranges 1000-6000 plant per m^2 in some wheat field. 11.4%-34.3% of wheat yields loss has been caused by the damage of the weed (Yuan). When the density of this weed increased 1000 plant per m^2, the loss of wheat yield up 342kg ha^{-1}. Chlosulfuron failed to control *Sclerochloa kengiana*. This study aims to determine the occurrence of *Sclerochloa kengiana* and the best herbicide for control *Sclerochloa kengiana.*

Materials and Methods

The occurrence of *Sclerochloa kengiana:* Field observation were conducted in the rice-wheat cropping field where were located on a sandy over loam, not any herbicide applying in 3 years in Dazhong farm in 1994-1995. The main weed was *Sclerochloa kengiana.* With 1.1% of organic matter, water contain of soil is 23%-24%. The observation for the occurrence of *Sclerochloa kengiana* was done every 10 days: The first time began from 5 days after wheat sowing.

***Sclerochloa kengiana* chemical control:** Field experiments were conducted in 1994-1996 at Dafeng farm, Jiangsu province. The soil in the trial area was sand with pH 7.9 and 1.1% organic matter content. Five PRE herbicides were applied with a Changjiang-10# backpack hand pump sprayer set to deliver 200L/ha at 5 days after wheat sowing. Three POST herbicides were also applied with a Changjiang-10# backpack hand pump sprayer set to deliver 200L/ha at 35 days after wheat sowing. Plots were 3.6×5 m. The wheat cultivar was Yangmei5#. *Sclerochloa kengiana* control was evaluated 30 days and 60days after treatment. Wheat injury by POST herbicide was evaluated at 7 DAT,14 DAT and 28 DAT. Wheat yield were collected from the experimental plots. Herbicide treatments were arranged in a randomized complete block design with 3 replications. The results were analyzed using Duncan's Multiple Range Test.

Result and Discussion

The occurrence of *Sclerochloa kengiana*: *Sclerochloa kengiana* turned up at the 5th days after wheat sowing. It rapidly came out of earth at 15th days after wheat sowing. 85.7% of total *Sclerochloa kengiana* came out of earth during 15-25 days after wheat sowing (Fig. 1).

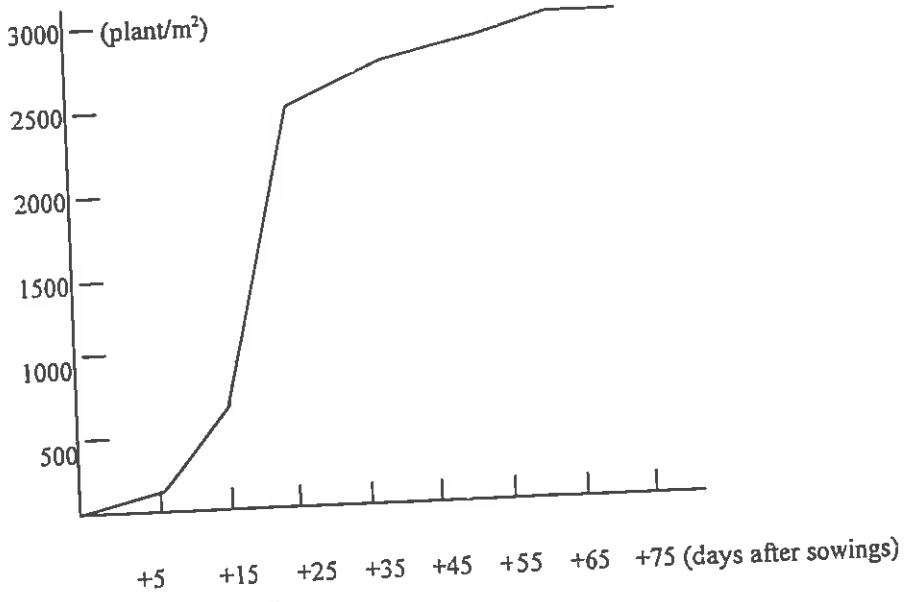

Fig 1. The occurrence of *Sclerochloa kengiana* in wheat in Dazhong farm ,Jiangsu province 1994-1995

***Sclerochloa kengiana* chemical control:** All pre- herbicides were not effect on the seedling growing and tillers. Except isoproturon at 1.35 kg ha $^{-1}$,the post- herbicides were not influence on the tillers of yangmai 5#. Mixing chlorotoluron at 0.75 kg ai ha^{-1} with trifuralin at 0.576 kg ai ha^{-1}, the weed control was 94.4% (30 DAT) and 97.2% (60 DAT) respectively, the yield of mixing chlorotoluron at 0.75 kg ai ha^{-1} and trifuralin at 0.576 kg ai ha^{-1} ranked the first in the tests. Mixing chlorotoluron at 0.75 kg ai ha^{-1} with butachlor at 0.72 kg ai ha^{-1}, the weed control was 88.2% (30 DAT) and 90.6% (60 DAT) respectively, the yield of this treatment ranked the third in the tests. Mixing chlorotoluron at 0.75 kg ai ha^{-1} with napropamide at 0.24 kg ai ha , the weed control was 88.3% (30 DAT) and 87.5% (60 DAT) respectively,

the yield of the plots ranked the second in the tests. The weed control by fenoxaorop at 0.052.kg ha^{-1} was only 62.1-64.3%, the yield of the treatment was not high (Table 1).

Table 1 *Sclerochloa kengiana* control by chemicals and affect on the yield of Yangmai 5#

Chemical	Rate Kg ai ha^{-1}	Weed control (%)		Tiller of yangmai5# +7	+14	+28	Yield of yangmai 5#kg ha^{-1}	
PRE-								
Chlorotoluron	1.313	77.7*	77.1**				5122.5	b
chlorotoluron+butachlor	0.75+0.72	88.2	90.6				5421.0	a
chlorotoluron+ trifuralin	0.75+0.576	94.4	97.2				5500.5	a
chlorotoluron+ napropamide	0.75+0.24	88.3	87.5				5427.0	a
chlorotoluron+ trifuralin	0.563+0.36	68.7	70.3				5230.5	b
POST-								
isoproturon	0.9	65.3	60.7	57.8	68.2	101.3	4834.5	c
isoproturon	1.125	72.6	71.1	56.8	67.3	100.3	5116.5	b
isoproturon	1.35	87.8	86.8	54.6	62.6	87.3	5403.0	a
Chlorotoluron	1.313	31.6	33.7	51.6	57.8	108.5	4659.0	d
Fenoxarop	0.052	64.3	62.1	58.5	71.8	115.5	4863.0	c
Weed free				63.5	83.8	119.8	5530.5	a
Untreated		(4755.5)	(4589.1)	62.6	76.6	115.6	4536.0	d

Note:1.the number in the brackets are weed number per m^2,* 30 days after treatment; ** 60 days after treatment.

2. Means within a trial followed by the same letter are not significantly different according to Fisher's Protected LSD Test at P=0.05

3. +7=7 days after treatment; +14=14 days after treatment; +28= days after treatment

According to the field trial, successful weed control achieved with the mixture of chlorotoluron at 0.75 kg ai ha^{-1} and trifuralin at 0.576 kg ai ha^{-1} to control *Sclerochloa kengiana* covering 4000 ha. From 1996 to 1998 in Dazhong Farm.

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THE INTEGRATED WEED MANAGEMENT SYSTEM (IWMS) IN SOYBEAN FIELD

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Abstract: this paper discusses some parts of the Integrated Weed Management System (IWMS) in soybean field. Crop rotation, tillage and herbicide application are most common and most effective methods in the present stage. Some problems in weed chemical control had been discussed.

Key words: IWMS, Soybean

In 1970s, the Integrated Weed Management System (IWMS) was put forward by American weed research It means that the people should restrict the negative of weed and utilize its positive to the maximum extent by making full use of the self-regulative of the ecological system and the human factors. So, we should control the weed within acceptable iever, which is under the lever of ecological economy, and make crops high yield and high quality on the basis of weed biological and ecological characteristics, of varied advanced techniques, of safety agricultural production and of benefit to agriculture. Obviously, IWMS is a systems method that alleviates the disturbance of weed to crop, combining plant breeding, fertilizer, crop rotation, competition, sustained management with weed chemical and mechanical control. Therefore, IWMS should be in conformity with the economy, society and environment.

In soybean field, weed growth, dynamic relation of weed and crop and competition are often influenced by varied factors, as in other crop fields. Because any single control method wouldn't satisfy the needs of production. It is extremely important to comply with the ecological rules and to establish the good IWMS by utilizing the botany, pedology, crop management, chemistry, agricultural mechanism, and weed management comprehensively. Presently, we should use crop rotation, tillage and proper herbicide application, combining with biological control and herbicide-resistant breeding, to control the weed comprehensively in soybean field.

1. Crop rotation favors the control over some weed species, especially over coexistent weeds.

It had been proved that crop rotation and herbicide application have special efficacy in controlling perennial weeds and some annual broadleaf weeds in soybean field. For example, many annual broadleaf weeds could be controlled in cornfield by herbicides in rotation of soybean and corn. In winter wheat succeeding soybean, perennial weeds and some summer annual weeds those are common in soybean field exactly could be controlled, even after gathering wheat. Other rotations are soybean and rice, cotton or peanut. It is a good rotation to plant soybean after planting corn for two years successively. They play a positive role in weeding in soybean field.

Till-less comes into fashion more and more, which mean to plant soybean directly in fields on which winter crops have been harvested without preparing seedbed. This method could control most weeds in whole grow stage of soybean by applying proper herbicide only once.

2. Successful cultivation controls the weeds in soybean field effectively.

Cultivation before sowing could control the perennial weeds in successive till-less soybean field because tillage buries the weed seed in deeper soil and lowers the weed occurrence. 2-4 times tillage can remove the remained weeds after herbicide application, reduce the produce of weed seed and lower the amount of seed bank in soil. So, people often use these methods to weed while herbicides are applied widely in more than 90 percent of soybean region. But the most effective cultivation should avoid to damaging the soybean root system to the maximum, to disturbing the seed in deeper soil and should turn up the soil to cover the small weed.

3. Application of herbicide is an important way by which soybean yield could be high, together with crop rotation, cultivation and other successful management.

It is about 50 years that herbicides be applied in soybean field. In 1970s, some pre- emergence herbicides were developed and applied vigorously, for example 2,4-D, dinoseb, chloramben, vernolate, triflualin and metolachlor etc. A series of after-emergence herbicides, such as sethoxydim, fluazifop, haloxyfop, quizalofop, fomesafen, acifluocfen, lactofen and so on, were developed and their applied techniques were perfected from the end of 1970s to 1980s. Until 1990s, many researched and developed herbicides were characterized by superhigh efficiency. We developed some sulfouylurea herbicides and imidazolinone herbicides, such as chlorimuron, thifensulfuron, imazethapyr and imazaquin. Presently, it is main trend to synthesize superhigh herbicides by using ALS and Accase as target. In home, herbicides had been applied in soybean field from 1960. At the beginning of 1970s. linuron was screened out in Heilongjiang province. Lately, we imported some herbicides in soybean field. Now, the main herbicides applied in soybean field are clomazone, fomesafen, imazethapyr and haloxyfop etc. From 1980s, we have been researching on weed and its management and have made considerable headway in soybean field.

In fact, there are some troubles in weed chemical control in soybean field because of complicated and varied environment. 1) Herbicides have some limitations in production because of weed phase, weeding scope, harm to crop and residue in soil. Only one herbicide cannot control the weeds in soybean field effectively.2) Weed community succession and development of herbicide-resistant mutant add to the difficulties in weed chemical control. So, in the first IWCC, weed researchers pointed out that new herbicides should be applied at lower dosage not only, but also their application techniques should be improved in order to alleviate their pollution. Recently, there are some focuses in weed research. They are mixture of herbicides, delay of herbicide-resistance and application of after-emergence herbicide.

4. Biological control and herbicide-resistant breeding are important content in IWMS.

Research on weed biological control had made for some years and reports about this are rich and varied in abroad, but at the beginning in home. *Echinochloa crusgalli* L., *Digitaria sanguinalis* L., *Eclipta prostrata* L., *Setaria viridis* L. and *Celosia argentea* L. could be selected firstly as target plant. The study on herbicide-resistant characteristics more early in abroad than in home too. There is a great deal of difference in resistance to many herbicides between soybean variety. The gene that resists obviously to some herbicides had been screened out. But, many thorough research works have yet to be made in these two aspects.

Summarily, IWMS in soybean field should lead the research on weed biology and ecology to weed control. It is a tendency that weeds chemical control, mechanical control and biological control combine with agricultural measures in the future. The problems that we should pay attention to in establishing IWMS as follows: 1) we should make full use of the weeds and control the weeds under the lever of ecological economy on the basis of complying with agricultural ecological system. 2) Measures should be

taken in the competition critical period of weed and crop. 3) Tillage, planting type and cultivation should be in favor of crop in its competition with weed.

(112 references are omitted)

VEGETATION ANALYSIS TO STUDY RANGELAND QUALITY AND SOURCES OF POTENTIAL NOXIOUS WEEDS

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Summary: In Selangor State, West Malaysia, vegetation analysis was used to study quality and quantity of forage at four different types of rangeland and to locate which types of rangeland have potential risks to develop specific weeds noxious for rice or plantation crops. Palatability indices of different weeds gives an indication to what extent goats can be used as biological control agents. Contrary to common believe, goats are no lawn-mowers, selecting only 3-12% of the total species available for their main diet. Non-palatable shrubs were *Chromolaena odoratum*, *Melastoma malabathricum*, *Lantana aculeata* and *Urena lobata*, which occurred mainly in the ex-tin mine area and roadside. *Imperata cylindrica* and sedges were also found to be non-palatable. As biological control with herbivores is not applicable for non-palatable species, other control methods need to be applied to improve rangeland quality and reduce the source of potential noxious weeds in areas bordering agricultural lands. *Mimosa pudica*, known to be unpalatable and hazardous to herbivores such as cattle and sheep was most highly prefered by goats.

Keywords: goats, range grazing, biological control, floristic composition, nutrition, palatability, Malaysia.

INTRODUCTION

Agricultural land, whether used for rice cultivation, food crops or plantation crops always hosts non-crop plants, which, if unwanted are commonly called weeds. These weeds origin from the soil seed bank, or are introduced from other localities such as roadsides and waste lands. Natural agents for spatial dispersal of weed seeds are water, wind, animals and humans (1). Seeds of many Asteraceae weeds, such as *Chromolaena odorata*, are dispersed by wind, or by clinging with their hook to fur and clothing. This weed is a major problem in plantation crops in many countries in Africa and Asia and is forecasted to spread rapidly to other countries (31). In a similar way spikelets of *Imperata cylindrica* are spread, while sections of their rhizomes are also scattered by humans or animals. Fruits of *Mimosa pudica* are also dispersed by humans and animals. Studying vegetation of rangelands can as such yield interesting information about potential sources of infestation of agricultural land by noxious weeds.

In Malaysia, some efforts have been undertaken to analyse distribution of weeds in places other than agricultural fields. Geographical distribution of *M. pudica* and *M. invisa* has been recently studied (3) as they are considered serious weeds in agricultural, recreational and residential areas. For a much wider variety of plant species, roadside vegetation of major roads passing through agricultural areas has been assessed (16). Vegetation under plantation crops has been extensively studied both in terms of distribution of weed species and classical control measures (5, 6), as in terms of biological control using herbivores (8, 25).

Vegetation analysis can also yield interesting information about the quality of rangelands for free-grazing animals. Native rangelands all over the world have been studied extensively, mostly in relation to forage availability and dietary preferences of specific plants for different types of herbivores (15, 22, 24, 27). In a broader context, vegetation analysis is considered as an important component when applying the systems approach to animal production (4). Few studies, however, concern rangelands in the humid tropics.

In Peninsular Malaysia, the majority of ruminants rely on natural vegetation available at waste lands, along roadsides, irrigation canals and under plantation crops. In the early 1990s there were about 288,500 heads of goat including the indigenous Kambing Kacang mostly reared by smallholder farmers. Economic viability of livestock production based on improved pastures proved doubtful (11) increasing the importance to analyse quality and quantity of native vegetation. The botanical composition of diets selected by goats have been described for different types of rangeland (28, 29), as well as for forage under plantation crops (7, 8).

In this paper we will focus on the use of vegetation analysis to quantify forage availability at four different rangelands in Malaysia. This analysis formed the basis for calculating palatability and preference indices of different weeds. Combined with nutritional analysis, quality of rangelands could be assessed. Implications for weed management in rice and plantation crops are discussed both in relation to biological control by herbivores as to infestation by noxious weeds.

MATERIALS AND METHODS

The study was carried out from October 1993 until April 1994. Four representative smallholdings were selected with different vegetation types as goat grazing areas. These farms were located in Beranang (3) and Ulu Langat (1), Selangor State, Malaysia, located in the humid tropics in the agro-ecological zone with a 1-month or no prominent dry season (21).

In October-November, a preliminary study of the rangelands was carried out by inventorying the plant species. For easy identification a reference collection of herbarium specimens was made. Vegetation at the old tin mine area was sampled in January 1994, the month with less rainfall. The area was characterised by a variety of vegetation types: vast scarcely covered sandplains, *Imperata* grassland, bushwood, pondside vegetation, etc. The other rangelands were sampled from February until April, 1994. The fallow (about 10 years) paddy field consisted mainly of grasses, sedges and some leguminous plants. This grazing area was very rich in plant species. The roadside under study consisted mainly of grasses and shrubs, the former being mown frequently. The grazing area in Ulu Langat was a traditional Malay orchard, consisting mainly of *Musa* cv., *Artocarpus heterophyllus, Artocarpus integer, Nephelium lappaceum* and *Durio zibethinus.* The vegetation was homogeneous and disturbed. Relatively smaller groups of plant communities but dominating were found under such multi-strata canopies. Herbaceous weeds such as the shade-tolerant, invasive *Asystasia intrusa* was one of the typical examples of native forage under orchard.

Within each grazing area the vegetation was sampled according to the Braun-Blanquet analytical approach by means of representative plots (13, 19). At the four different grazing sites, 62 plots were sampled. Depending on the vegetation type plots were used measuring 2 x 2 m for herbaceous communities, and 10 x 10 m for bushy communities. Vegetation composition was assessed by recording the cover of each individual species, using the decimal scale of Londo (14). Vegetation at the ex tin mine area, characterised by sandy soils, was changing fast during the dryer month (January 1994). As the major aim of the study was to quantify forage availability rather than making a phytosociological study, information on plant availability had to be gathered fast and the approach of Tansley (26) was used in combination with the Londo-scale. At the other grazing areas changes due to the short dryer period were not noticed. Cover data were entered in a spreadsheet and hierarchical classification of the species was accomplished with Twinspan (12).

To extrapolate cover data of species within samples to availability of each species within the rangeland, each rangeland was divided into different vegetation types of which the area was measured with topographic instruments. The total cover value of each species within a rangeland was calculated as the weighted sum of the cover values of that species within the different vegetation types.

In December 1993, feed preference studies were carried out through direct observations of grazing goats. At each rangeland, one herd was observed for 5 consecutive days. The plants and the time (rounded to 5 s) spent feeding on a particular plant species were recorded for each animal during its grazing journey. Full details of this technique were described by Van Mele *et al.* (29). From the records of feed selection and plant availability, preference indices were calculated for plant species and plant groups. The preference index (PI) was calculated as:

$$PI = \frac{Plant\ consumed}{Plant\ availability}$$

where plant consumed is the feeding time on a particular species as a percentage of the total feeding time and plant availability is the percentage cover of plant material not exceeding a height of 2 m. On the basis of preference indices plant species were ranked in palatability classes (Table 1).

Table 1. Palatability based on preference index.

Palatability	Preference index	Code
Highly palatable	> 5.00	+++
Very palatable	1.51 – 5.00	++
Palatable	0.51 – 1.50	+
Not palatable	0.00 – 0.50	-

Samples of major plant species were taken immediately after each observation period. Plant material was harvested by hand clipping, which simulates grazing (17). Collected samples were weighed fresh, dried at 55-60 °C for 48 h and ground to pass through a 1-mm sieve. After dry matter determination they were mixed in equal proportions to give composite samples for each period. Collected samples were weighed fresh, dried in the oven at 55-60°C and ground to pass through a 1-mm sieve. Contents of dry matter were determined by standard procedures and ash, crude protein (CP), crude fiber (CF) and fat according to Weende Analyses. The neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by the procedures of Goering and Van Soest (10). Gross energy (GE) was determined in a Parr oxygen bomb calorimeter.

RESULTS AND DISCUSSION

Within the plots and surroundings 162 species from 42 families of herbs, creepers and shrubs were recorded. Poaceae was the dominant family with 34 species. From the Cyperaceae 20, Leguminosae 14, Euphorbiaceae 13, and Rubiaceae and Asteraceae 8 species were recorded, respectively. *Ottochloa nodosa* was the dominant grass species covering about 12 % of the total study area. The frequency of *O. nodosa* and other species such as *Axonopus compressus*, *Paspalum conjugatum*, *Mikania cordata*, *Asystasia intrusa* and *Melastoma malabathricum* is high indicating these species were widely distributed in the area.

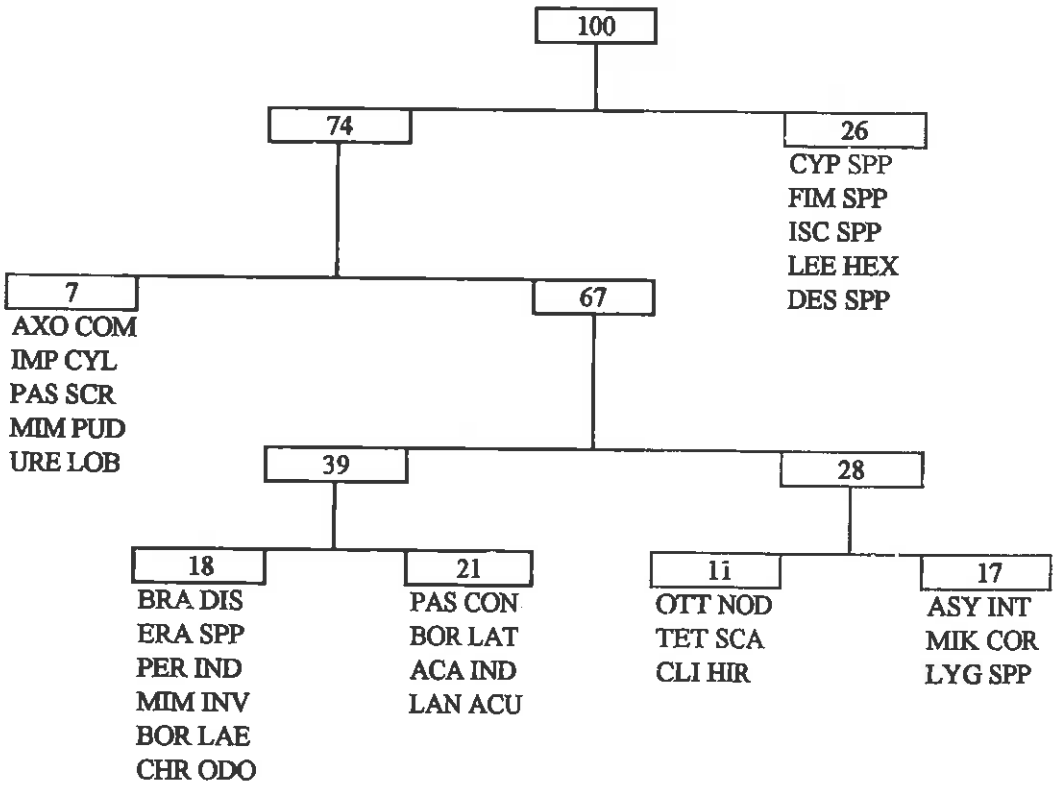

Figure 1. Hierarchical classification of species with Twinspan. Numbers indicate number of species within each group. Explanation of abbreviations of scientific names can be found in text.

Hierarchical classification of all species recorded within plots resulted in 6 different plant groupings (Figure 1). Group 1 was characterised by species which are typical rice weeds, including the sedges *Cyperus* spp. and *Fimbristylis* spp., the grasses *Ischaemum* spp. and *Leersia hexandra* and the leguminous *Desmodium* spp.

Seemingly even 10 years after being abandoned as rice field, the weed flora is still determined by its history. Abandoned rice fields therefore continue to be a potential source of infestation for rice producing areas. Grazing of these lands reduces chance of some weeds getting in their reproductive phase.

Group 2 and 3 include species which occurred at the ex-mine area, and which have great potential for infesting upland crops and planations. Major weeds were the grasses *Axonopus compressus*, *Imperata cylindrica* and *Paspalum scrobiculatum*, the leguminous *Mimosa pudica* and the shrubby *Urena lobata* in group 2, while *Brachiaria distachya*, *Eragrostis* spp., *Perotis indica*, *Mimosa invisa*, *Borreria laevicaulis* and *Chromolaena odoratum* characterised group 3.

Latter species was aslo very abundant at roadsides, of which the vegetation was characterised by species of group 4, namely *Paspalum conjugatum*, *Borreria latifolia*, *Acalypha indica* and *Lantana aculeata*.

Species of group 5 and 6 are typical weeds for more shaded environments, namely *Ottochloa nodosa*, *Tetracera scandens* and *Clidemia hirta* in group 5 and the even more shade tolerant species *Asystasia intrusa*, *Mikania cordata* and the ferns *Lygodium* spp. in group 6. All these weeds have great potential in orchards and plantation crops. The shrub *Melastoma malabathricum* occurred at all sampling sites except for the fallow paddy field.

Vegetation covered at least 100% of the total sample area except at the tin mine area (51%). In the other cases the sum of the cover of groups of plants exceeded 100%, which indicates overlapping of species and hence giving an idea about available plant biomass at the different rangelands (Figure 2). The fallow paddy field had the highest number of species (96), whereas at the roadside only 44 species were recorded. Due to the more scarce vegetation, goats had to walk longer distances and hence spent less time feeding at the tin mine area.

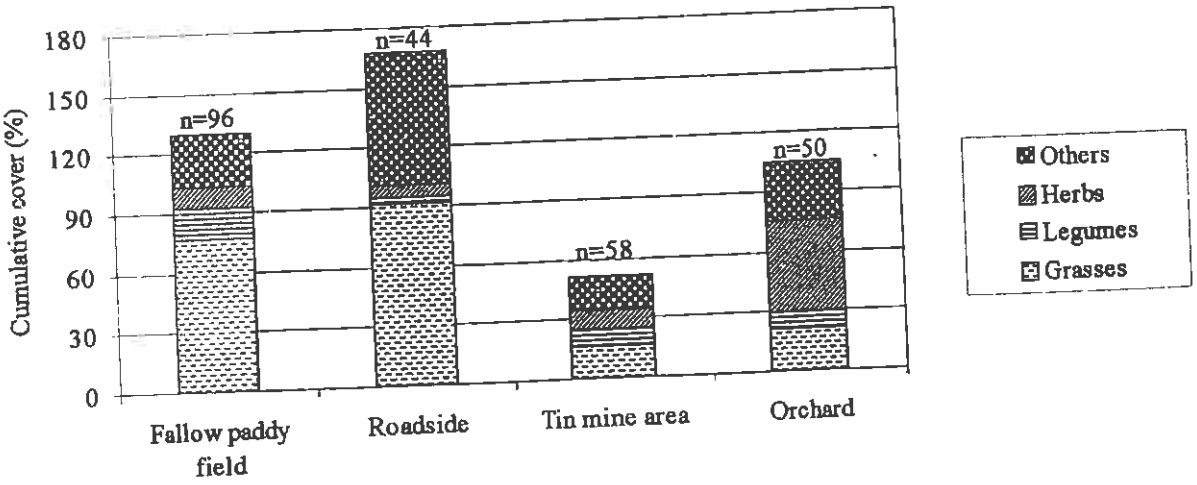

Figure 2. Cumulative cover of major plant groups at different vegetation types. Frequencies higher than 100% indicate that different species overlap each other, giving an idea about the vertical distribution of the vegetation. n= number of plant species, excluding trees.

Different plant groups were selected according to the rangeland vegetation (Figure 3), emphasizing the mixed and opportunistic feeding habits of goats. The herd grazing under orchard had a significant larger proportion of grazing time on dicotyledonous weeds (of higher nutrient content) than on grasses (of lower nutrient value) compared to the herd grazing along the roadside. Dicotyledonous weeds were prefered to grasses in all cases except along the roadside, where palatability of plants in general was low. This might have some implications for managing rice weeds. In Malaysia, direct-seeded rice culture since the mid-1980s and the continuous use of phenoxy herbicides have resulted in species shift in weed community structure favouring the more competitive grassses such as *Echinochloa* spp, *Leptochloa chinensis*, *Ischaemum rugosum* and *Paspalum distichum* (2). Grazing on fallow rice fields might avoid these grasses reaching maturity and hence contribute to the depletion of the soil seed bank. Problem rests with the sedges which are generally non-palatable (Table 3) and have to be controlled by other means.

Goats are well-known to be browsers (15, 22, 23, 24, 27). This has mainly been described for semi-humid and semi-arid environments, where availability of plant biomass is generally lower than in the humid tropics. In our study, the general low palatability of the group 'others' is due to the occurence of mainly non-palatable shrubs

such as *Chromolaena odoratum, Melastoma malabathricum* and *Lantana aculeata*. Nutritional analysis revealed that hand clipped samples of *C. odoratum* had a lower crude protein content (11%) than even that of grasses (13-18%) (Table 2) and could be one of the reasons why goats did not select this plant species. *L. aculeata* is known to be toxic to grazing animals. Nutritional values of native rangelands are of higher value than those of improved pastures due to a wider choice of different plant species. Hand clipped samples of an improved pasture of MARDI Digit (*Digitaria setivalva*) had crude protein contents of 15-17% and gross energy contents of 17-18 MJ/kg DM (30), comparable to those of native grasses. Nutritional values of the highly selected *Mimosa pudica* was comparable to those of leaves of the leguminous fodder crop *Leucaena leucocephala*, which is often supplemented to animals in intensive production systems. Besides, ash content of broadleaves such as *Mikania cordata* and *Asystasia intrusa* had very high ash contents, indicating their high mineral content. The large availability of different nutritious weeds can offer a very balanced and nutritious diet to grazing animals. However, quality of rangelands has to be maintained as selective grazing by animals lead to the building up of woody shrubs and noxious weeds.

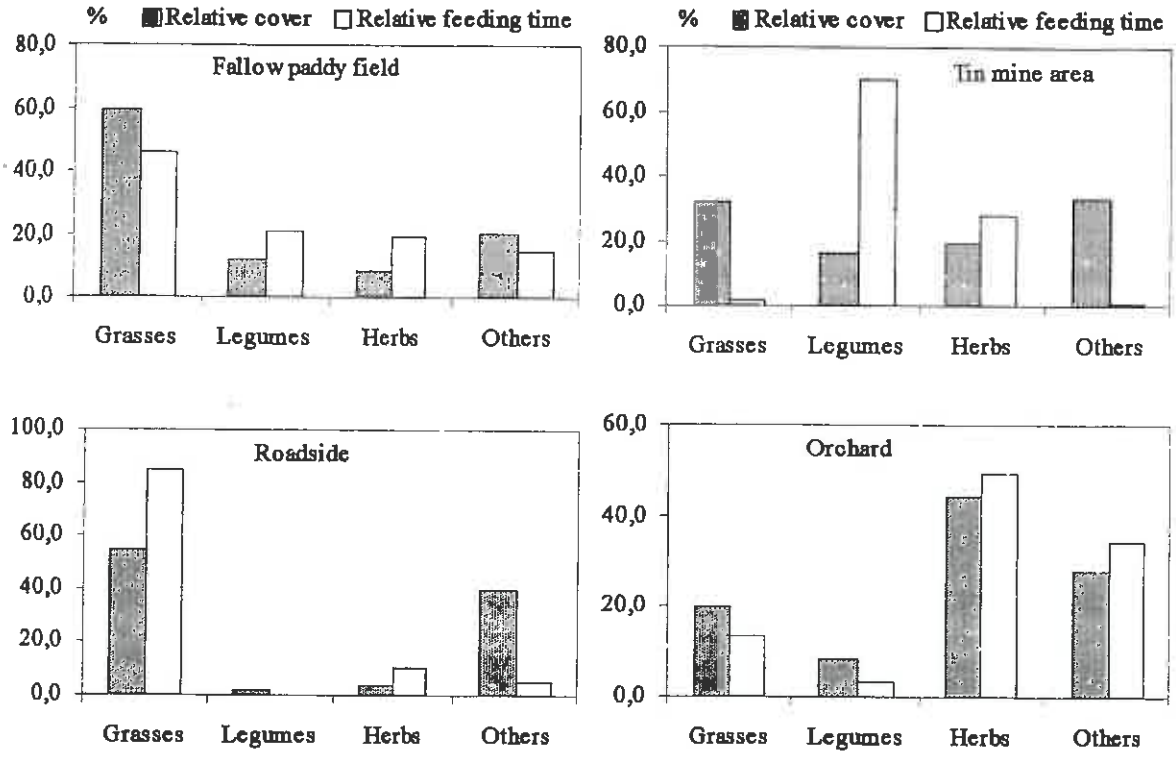

Figure 3. Relative cover and relative feeding time of goats on major plant groups in different vegetation types.

Rangelands such as roadsides and old tin mine areas often have a high occurrence of noxious weeds (29). The time goats spent feeding was therefore highly correlated (r^2=0.88) with the availability of non-woody vegetation. The amount of unpalatable species not only indicates rangeland quality, but also the potential danger as a source of noxious weeds for agricultural lands. These shrubs are indeed considered among the most noxious weeds in plantation crops (8). Biological control of weeds using herbivores is no solution for non-palatable species and hence grazing has to be integrated with other weed management techniques. Both smallholder farmers with ruminants, as owners of large plantation crops could benefit from occasional weed management in these often neglected sites.

At the orchard, legumes had a low preference index. Seemingly glabrous leaves of other species were prefered to the hairy leaves of *Pueraria phaseoloides* and *Calopogonium mucunoides*. Both these species are used as leguminous cover crops in plantations, but they are reported not to persist under grazing (8). Similarely, the very shade tolerant *Asystasia intrusa*, which has a very high protein content of 24% (Table 2), was readily consumed by goats, and persisted under the given circumstances contrary to reports by others (11). The fact that goats at our study site had a choice between fifty different plant species made that these beneficial plants – *A. intrusa* is also important in attracting insect pollinators - were not exhausted within a limited time. Questions

can be raised whether diversity poor ground covers are the most economical and sustainable solution for tree crops.

On the fallow paddy field, roadside, tin mine area and orchard there were 23, 11, 14 and 15 different species respectively grazed by the goats. Although they consumed a large variety of species, most of their diet consisted of just 8, 4, 2 and 6 species (28). These results are consistent with previous findings (20, 23) stating that although goats may consume a large variety of species, most of their diet consists of 3-8 main species. *Mimosa pudica*, which contains the toxic agent mimosine, was highly selected by goats. Species diversity of a rangeland is an important factor as it allows grazing animals to select a greater variety of plant species. A mixed diet is not only better balanced but also prevents the animal's detoxification mechanism from being confronted with a large dose of one single toxin (18).

The main species selected were *Mimosa pudica, Ottochloa nodosa, Asystasia intrusa, Mikania cordata, Paspalum conjugatum, Axonopus compressus* and *Ischaemum* spp.

Table 2. Nutritional characteristics of some major weed species at different rangelands.

Species	D.M. (%)	Ash	CP	CF	Fat	NDF	ADF	Gross E (kJ/g DM)
		(g/100 g DM)						
Fallow paddy field								
Commelina	17,7	12,4	17,0	16,4	3,1	60,7	25,7	18,4
Mimosa	30,5	5,0	21,6	15,9	2,5	38,4	21,1	19,8
Axonopus	23,5	9,4	13,6	24,9	0,8	68,3	34,7	17,8
Ischaemum	23,1	9,6	13,7	23,4	2,6	65,7	34,6	17,5
Leersia	33,0	12,3	13,1	29,3	2,6	64,7	40,3	17,5
Mix	15,3	10,3	18,4	19,8	2,9	48,1	27,4	17,8
Roadside								
Ottochloa	23,8	10,0	16,3	22,8	0,9	69,7	30,0	17,9
Axonopus	21,9	7,7	12,8	24,8	1,8	72,3	33,4	18,1
Paspalum	18,5	9,3	16,6	24,3	2,5	66,4	31,2	18,4
Ficus	35,4	5,6	11,8	24,0	2,7	45,6	31,3	18,1
Eupatorium	22,8	8,5	11,4	20,2	1,3	33,1	18,8	19,8
Calopogonium	25,0	5,2	24,3	12,3	3,8	51,3	29,3	19,7
Mix	15,9	9,1	15,5	23,3	4,4	52,4	30,0	17,9
Tin mine area								
Mimosa	30,8	4,2	23,2	12,5	1,0	51,9	27,0	20,3
Mikania	10,8	12,1	19,1	11,3	3,7	34,8	26,6	18,1
Mix	18,0	9,1	23,1	17,7	2,0	42,6	26,0	18,2
Orchard								
Asystasia	12,0	13,8	24,4	13,1	2,0	49,5	31,9	18,0
Ottochloa	23,5	8,4	18,3	22,8	0,8	74,2	30,8	18,9
Heliconia	19,0	8,1	16,8	25,1	2,7	80,5	34,2	19,1
Mikania	11,0	12,4	19,2	12,3	4,6	37,9	29.3	18,2
Mix	17,4	8,3	20,9	20,5	1,9	61,5	34,2	18,8

From the recordings of cover and relative feeding time, preference indices were calculated and species were ranked in palatability classes (Table 3). Leaves from the crab-claw (*Heliconia hybrid*) and leaves from the legume *Mimosa pudica* were highly palatable. The climber *Mikania cordata*, the herbs *Borreria latifolia* and *Commelina nudiflora*, leaves from the palm *Caryota mitis*, and the fern *Lygodium flexuosum* were very palatable. The swamp grasses *Leersia hexandra* and *Panicum amplexicaule* had a higher palatability than the other grasses.

Attention has to be paid in that palatability is also determined by the structure of the vegetation, *i.e.* accessability of plant species. The twining herb *Merremia umbellata* was very palatable at the tin mine area, where vegetation was less dense, while it was found unpalatable at the orchard, where it was overgrown by other species. Vegetation constraints become important because they alter rates of encounter of preferred forages (9). The

availability of the different sward components can indeed limit preference expression. This suggests that studies of comparitive palatability has to be evaluated carefully when transfered to other vegetation types.

Table 3. Palatability of different plant species for goats at different rangelands, Malaysia, 1993-1994.

	Fallow paddy field	Roadside	Tin mine area	Orchard
Mimosa pudica	++	0	+++	0
Heliconia hybrid	0	0	0	+++
Mikania cordata	++	0	++	++
Merremia umbellata	0	0	++	+
Borreria spp.	0	+	++	+
Commelina nudiflora	++	0	0	0
Leersia hexandra	++	0	0	0
Panicum amplexicaule	++	0	0	0
Lygodium spp.	++	0	0	+
Caryota mitis	0	++	0	0
Paspalum conjugatum	+	+	0	+
Ottochloa nodosa	+	+	+	+
Asystasia intrusa	+	0	0	+
Ischaemum spp.	+	0	0	0
Axonopus compressus	-	+	0	+
Aneilema nudiflorum	+	0	0	0
Centrosema pubescens	0	0	+	0
Mimosa invisa	0	0	+	0
Imperata cylindrica	-	-	-	0
Cyperus spp.	-	0	-	-
Pueraria phaseoloides	0	0	0	-
Calopogonium mucunoides	0	0	0	-
Lantana aculeata	0	0	-	0
Urena lobata	-	0	0	0
Melastoma malabathricum	0	-	-	-
Chromolaena odorata	-	-	-	0

0 = absent data, - = non-palatable, + = palatable, ++ = very palatable, +++ = highly palatable.

CONCLUSIONS

Vegetation analysis was succesfully used (1) to study quality and quantity of forage at rangelands and (2) to locate which types of rangeland are potentially risky to develop large amounts of weeds noxious for rice or plantation crops. Palatability indices of different weeds gives an indication to what extent goats could be used as biological control agents. Contrary to common believe, goats are no lawn-mowers, selecting only 3-12% of the total species available for their main diet. *Mimosa pudica*, known to be unpalatable and hazardous to herbivores such as cattle and sheep was most highly prefered by goats.

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WEED CONTROL AND MANAGEMENT OF RESISTANT GOOSEGRASS (*Eleusine indica*) IN MALAYSIA

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Abstract: Tolerant goosegrass had been reported and identified in several locations in Peninsular Malaysia. They are normally found in abundance in orchards, vegetable areas, nurseries and in young oil palm where watermelon had been cultivated. Among the locations where resistant goosegrass was identified included Bidor, Telok Intan and Ipoh in northern region; Temerloh in eastern region; Rawang, Tanjung Malim, Broga and Semenyih in central region; and Yong Peng and Chaah in southern region. Glyphosate was evaluated at 1.08, 2.16, 3.24 and 4.32 kg ae/ha applied at mature growth stage for resistant goosegrass control. Results showed that resistant goosegrass can survive significantly higher glyphosate rates of more than 4x the commercial label recommended rate at spray volume of 450 liters/ha. At lower spray volume of 60 liters/ha and applied using Turbo CDA, the effective glyphosate rate was about 3x to 4x the commercial label recommended rate of 1.08 kg ae/ha. Contact herbicides like paraquat and glufosinate-ammonium gave poor control. However, mixtures of glyphosate + sethoxydim and fluozifob showed promising results. Mixtures of glyphosate + sethoxydim at 2.16 + 1.40 kg ae (ai)/ha and glyphosate + fluozifob at 2.16 + 0.56 kg ae (ai)/ha gave complete control of resistant goosegrass in 30 DAT. Evaluations on other options including tillage; pre-emergent herbicide and other control measures are in progress to manage the resistant goosegrass. It can be summarised from the results todate that the best management plan for resistant goosegrass is to obtain maximum weed control every time glyphosate is applied before seeding. This accompanied by scouting, monitoring and good agronomic practice will ensure sustainable management of the resistant weed.

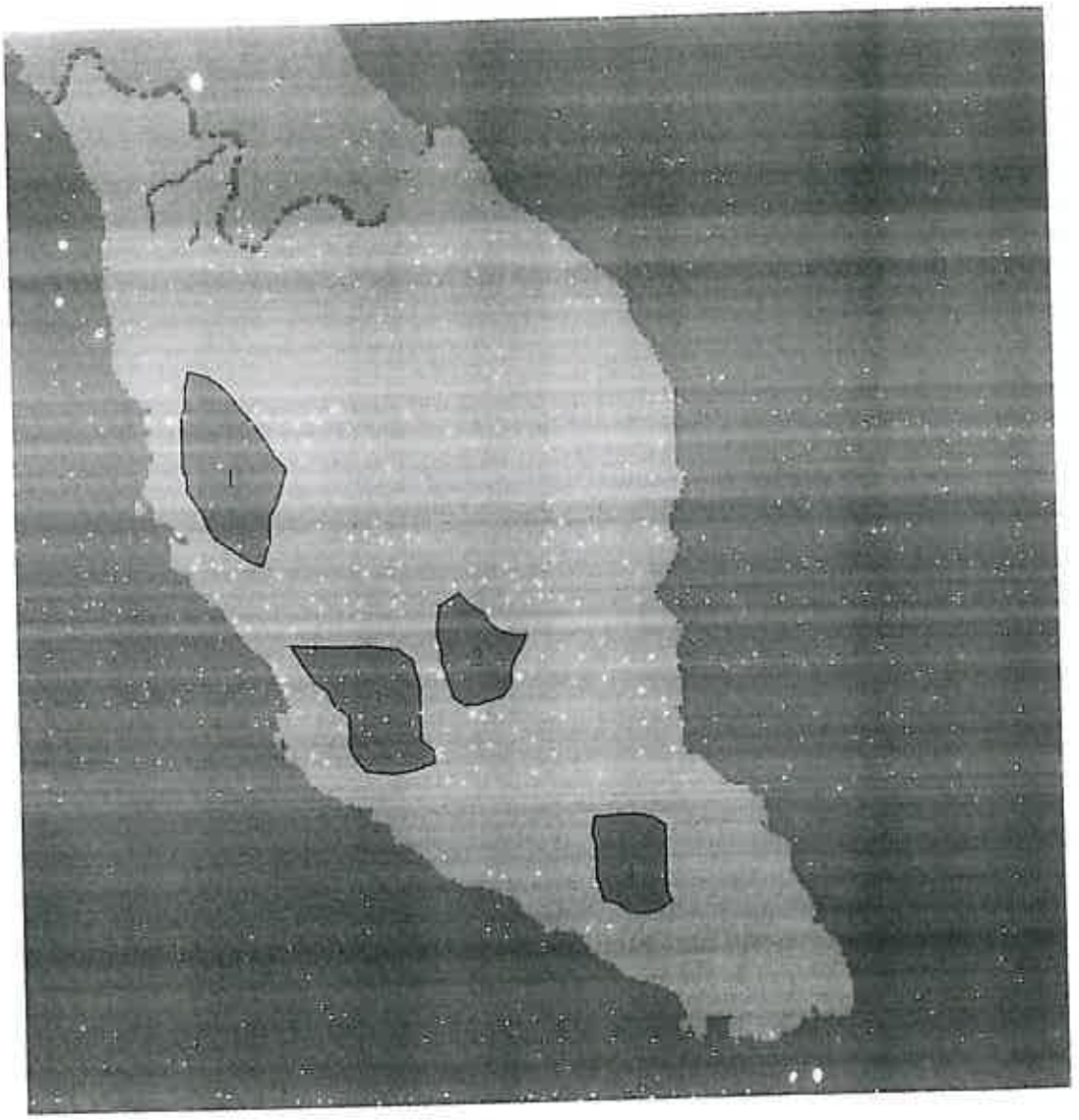
Key words: Resistant, *Eleusine indica*, glyphosate, weed control, management

Introduction

Eleusine indica (Linn.) Caertn, which is commonly known as goosegrass or crow's foot grass are commonly found sporadically in waste grounds and road sides in Peninsular Malaysia (Gould, 1986; Holm *et al.*, 1977). It is an annual weed with compressed, turfted stem of about 5 to 60 cm in height and are often branched. The leaf blades are narrow and long of about 5 to 20 cm; and are often folded and shiny below. It has a few scattered hairs on the upper surface and the leaf sheath is keeled. Its ligule is short and membranous with a jagged edge and is hairy on the margins about the junction of blade and sheath. The inflorescence is made up of one to many spikes, unbranched, and usually three or more radiating at the top of the culm with one attached below. The axis is flat. The spikelets are without stalks, flattened, three-to-five flowered. The spikelets ware all bisexual, jointed below the florets which fall off and leave the 2 basal glumes. The floret is single with minute teeth on the upper keels of glumes. The floret is bisexual and after the flumes are removed, it shows 3 stamens and a feathery bifid purple stigma. The seeds are transversely ridged.

In recent years, more and more farmers in vegetable areas, orchards and nurseries have encountered the widespread of resistance *Eleusine indica* in their farms (Teng, 1998). Investigations have confirmed that resistance goosegrass are abundantly found in vegetable areas and orchards of small farms in Bidor, Simpang Pulai, Temerloh , Rawang, Tanjong Malim, Broga, Yong Peng and Chaah (See Figure 1). For the successful cultivation of these vegetable and orchard crops, a sustainable management of the resistant goosegrass is important. Results of several trials on the use of glyphosate to manage the resistant weed are presented and discussed.

Figure 1. Areas of Tolerant *Eleusine indica* Identified in Peninsular Malaysia



1- Northern Region = Bidor (240 Ha), Simpang Pulai (150 Ha)
2. East Coast = Temerloh (25 Ha)
3. Central Region = Rawang (15 Ha), Tanjung Malim (15 Ha), Broga (85 Ha)
4. Southern Region = Yong Peng (10 Ha), Chaah (45 Ha)

Materials and Methods

Several trials were conducted in Peninsular Malaysia to evaluate the efficacy of glyphosate for the control of resistant goosegrass. The initial trial was conducted at Batu 12, Bidor, Perak in 1998 in a small farm of mature ciku trees. Long strips of 2 m by 20 m were plotted in the crop inter-rows. Each strip was sprayed with glyphosate at the rates from 1.08 kg ae per hectare to a maximum of 8.64 kg ae per hectare. The herbicide was applied at a spray volume of 225 liters per hectare using a SOLO 425 pump with a Yamaho low-drift nozzle. Observations were made upto 30 days after treatment (DAT) on the control of the resistant goosegrass by the various treatments.

Another two trials were established from March 1999 in various farms at the same location in Batu 12, Bidor, In these trials, the objectives were to refine the glyphosate rates and to identify suitable glyphosate mixtures with fluazifob-butyl or sethoxydim and their rates to control the resistant goosegrass. Spray volume at 60 liters per hectare using the Turbo controlled droplet applicator (Turbo CDA) was used for all glyphosate treatments. In the third trial, comparison was also made on the efficacy of various glyphosate treatments to that of paraquat and glufosinate-ammonium. The last two treatments were sprayed using CKS with flat fan nozzle at spray volume of 450 liters per hectare. Long strip of 2 m by 20 m and non-replicated was used for each treatment. Observations on control of resistant goosegrass by the various treatments were done upto 30 to 60 DAT.

All the goosegrass were actively growing and had seeded at time of herbicide application for all the above trials.

Results and Discussion

Preliminary investigations on the distribution of resistant goosegrass in Peninsular Malaysia had shown that they were found mainly in vegetable and orchard farms, nurseries and young oil palm areas where inter-cropping with watermelons was practised. In Peninsular Malaysia, about 585 hectares of farm lands had been confirmed to be infested with resistant goosegrass (Figure 1). In the northern region, about 390 hectares of farm lands were confirmed to be infested with tolerant goosegrass in small farms of about 2 hectares each. These farms were located in Bidor and Simpang Pulai areas of Perak and were cultivated with orchard crops like starfruit, guava, ciku, etc. and vegetables like chilly, okra, etc. In the eastern region, another 25 hectares of resistant goosegrass had been confirmed in the Temerloh areas of Pahang. This was a smallholding of young oil palm which was previously cultivated with vegetables. In the central region, about 15 hectares of resistance goosegrass was confirmed in papaya farms in Rawang and in about 15 hectares of starfruit farm in the Tanjung Malim areas of Selangor. In addition, one oil palm plantation where inter-cropping with watermelon was practised, about 85 hectares of the plantation were infested with tolerant goosegrass. In the southern region, another 55 hectares of resistant goosegrass had been confirmed in an ornamental plants nursery in Yong Peng and in an ex-vegetable oil palm smallholding in Chaah, Johor. However, there may be more areas with resistant goosegrass infestations that had yet to be identified and confirmed.

Results of the initial trials in Bidor, Perak showed glyphosate rate of about 4.32 kg ae per hectare was needed to control at least 90% of the resistant goosegrass at 30 DAT (Table 1). At lower rates, only about 50 to 60% of the resistant weeds were controlled. Only at glyphosate rates of 6.84 kg ae per hectare and above were the resistant goosegrass were totally killed without any regeneration. At sub-lethal dosages of glyphosate, the treated resistant weeds regenerated by 30 DAT and more branches were produced. The regenerated weeds had shorter branches, leaves and inflorescences.

Table 1: Control of resistant goosegrass by glyphosate

No.	Treatments	Rate (kg ae/ha)	Percent Control (DAT)	
			15	30
01	Check	-	-	-
02	Glyphosate	1.08	60	50
03	Glyphosate	2.16	70	60
04	Glyphosate	4.32	90	90
05	Glyphosate	6.48	100	100
06	Glyphosate	8.64	100	100

The second trial initiated in March 1999 to evaluate the efficacy of glyphosate and other potential herbicides for control of resistant goosegrass under very low spray volume of 60 liters per hectare confirmed glyphosate at 4.32 kg ae per hectare was needed to give acceptable control of the weed in 30 DAT (Table 2). Fluazifob-butyl and sethoxydim at their highest rates of 0.56 kg ai and 1.40 kg ai per hectare respectively gave about 90% control at the same period. However, glyphosate + fluazifob-butyl at 2.16 kg ae + 0.56 kg ai per hectare and glyphosate + sethoxydim at 2.16 kg ae + 1.40 kg ae per hectare provided the same control of 99% as glyphosate at 4.32 kg ae per hectare.

Table 2: Control of resistant goosegrass by glyphosate, fluazifob-butyl and sethoxydim.

No.	Treatment	Rate (kg ae/ai per ha)	Percent Control (DAT)		
			7	15	30
01	Check	-	-	-	-
02	Glyphosate	1.08	10	30	40
03	Glyphosate	2.16	30	60	70
04	Glyphosate	4.32	60	90	99
05	Fluazifob-butyl	0.28	20	60	80
06	Fluazifob-butyl	0.56	40	70	90
07	Sethoxydim	0.70	30	65	80
08	Sethoxydim	1.40	40	75	90
09	Glyphosate + fluazifob-butyl	1.08 + 0.28	60	70	90
10	Glyphosate + fluazifob-butyl	1.08 + 0.56	60	80	85
11	Glyphosate + fluazifob-butyl	2.16 + 0.28	70	85	95
12	Glyphosate + fluozifob-butyl	2.16 + 0.56	70	90	99
13	Glyphosate + sethoxydim	1.08 + 0.70	50	75	90
14	Glyphosate + sethoxydim	1.08 + 1.40	60	90	95
15	Glyphosate + sethoxydim	2.16 + 0.70	50	95	95
16	Glyphosate + sethoxydim	2.16 + 1.40	60	95	99

Fluazifob-butyl and sethoxydim were selective grass herbicides. They were effective on certain grasses including goosegrass only. This necessitated the farmers to spray another round to eradicate the remaining broadleaf and other grass weeds and thus increased the application costs.

The third trial was to confirm the effective glyphosate rate and its mixtures with fluazifob-butyl for the control of resistant goosegrass in another location. As shown in Table 3, the effective glyphosate rate was 4.32 kg ae per hectare and the control was 98% at 60 DAT. All the glyphosate + fluazifob-butyl mixtures also gave similar control of 99% at 60 DAT. The lower rates of glyphosate at 2.16 kg ae per hectare and below did not provide complete kill and regeneration of the treated weeds was observed. Similarly paraquat at 0.56 kg ai per hectare and glufosinate-ammonium at 0.50 kg ai per hectare did not provide satisfactory control.

Table 3: Control of resistant goosegrass by glyphosate, paraquat and glufosinate-ammonium

No.	Treatments	Rate (kg ae/ai per hectare)	Percent Control (DAT)		
			15	30	60
01	Check	-	-	-	-
02	Glyphosate	1.08	40	50	60
03	Glyphosate	2.16	50	60	65
04	Glyphosate	4.32	70	90	99
05	Glyphosate + fluazifob-butyl	1.08 + 0.56	50	80	98
06	Glyphosate + fluazifob-butyl	1.08 + 1.12	60	90	99
07	Glyphosate + fluazifob-butyl	2.16 + 0.56	60	90	99
08	Glyphosate + fluazifob-butyl	2.16 + 1.12	70	95	99
09	Paraquat	0.56	60	30	10
10	Glufosinate-ammonium	0.50	70	40	15

Based on the results of the above trials, it can be summarised that a minimum rate of 4.32 kg ae glyphosate per hectare is needed for the complete control of the resistant goosegrass when treated at very low spray volume of 60 liters per hectare. Under such circumstances, the glyphosate rate used is about 4x to 6x the normal recommended glyphosate rate for general weed control. As for the mixture, glyphosate + fluozifob-butyl at 2.16 kg ae + 0.56 kg ai per hectare consistently gave complete control.

For sustainable management of the resistant goosegrass, it is imperative that the weeds should be eradicated before seeding. This is to prevent the multiplication and spread of resistant goosegrass weeds through seeds. The correct effective glyphosate rate and spray volume should be used so that the weeds are eradicated during first time, at all time of spraying. Any missed or under-dose treated weeds should be removed manually.

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WEED POPULATION DYNAMICS IN COFFEE PLANTATION MANAGED BY DIFFERENT SOIL CONSERVATION TECHNIQUES

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Abstract: A long-term field experiment was conducted to evaluate the loss and benefits of vegetative cover by using weeds in order to reduce soil erosion in coffee plantation located in the western mountainous Lampung areas, Sumatra, Indonesia. Main plots consist of three treatments with weed-free soil surface by manual weeding once a month (T-1); soil surface covered with *Paspalum conjugatum* (T-2); and soil surface covered with natural vegetation (T-3). The most diverse weeds were found in natural vegetation plot (T-3) with maximum of 23 weed species in March 1997 and minimum of 8 species in October 1997. The cover with *Paspalum* resulted in a decrease in total number of emerged weed species. In manually weeded plot (T-1), *Ageratum conyzoides* was the species present all year long. In natural vegetation plot (T-3) , succession of weed community was occurred. Namely *Imperata cylindrica* was dominant species before setting up in November, 1995, however, within one year after them, *Clibadia surinamense* (woody species) replaced as the dominant species, reaching mean plant height of 136 cm. *Paspalum* covering was a better way to reduce soil erosion compared to natural vegetation covering, even though both vegetation dramatically reduced soil erosion compared to the manually weeded plot. However , the growth of coffee was also affected by the techniques used with the best growth in manually weeded plot.

Key words: ***Paspalum conjugatum*, succession of weed vegetaion, coffee plantation, soil erosion, vegetative cover**

Introduction

About a half percent of Indonesia's coffee is planted in the Southern Coffee Belt of Sumatra, and the mountain zone of Lampung Province is located in its southern section. Due to the high coffee prices during the late 1970's, coffee cultivation exhibited a real boom, and the coffee cultivation area increased and increased[1)]. This has resulted in the destruction of huge areas of mountain forest, and undoubtedly there is the threat of erosion in the coffee cultivation area. However as long as there are a few data on erosion over extended periods, all comments remain speculation.

Coffee farmers have to apply fertilizer and plant protection. Weeding has to be done 3 times a year. Weeding is commonly done with a hoe or by hand, however, recently the application of herbicides such as Glyphosate has been gradually popular. If the application of herbicides is repeated several times per one year, the soil surface of coffee plantation might be always keeping bare. Under such a condition, these steep slopes of coffee plantation suffer high erosion rates.

On the other hand, complete control of weeds may not be necessary or under certain conditions even undesirable. There are indications that weeds can play a beneficial role such as cover crops. Reliable soil conservation technologies reduce erosion rates by maintaining a protective vegetative cover over the soil.

In this study, therefor, we examine weed population dynamics in coffee plantation managed by different soil conservation techniques, and assess the interactive processes between weed vegetation and soil with erosion.

Materials and Methods

Experimental site

The expeerimental main plots were established on slope area where is locatied at the University farm in Sunmberjaya, Lampung, Sumatra, Indonesia in November, 1995. The slope where the main plots are located faces toward the north, and the grade of main plots is about 15 degree. The upper soil coior over a depth of 15 cm is dark brown while the lower one is red brown.

Description of main plots

Before setting up the main plots, we sprayed the herbicide (Glyphosate) in all planned area. Prior to its application, the vegetational survey was done by a quadrat method. As shown in Fig.1, Main plots consist of three treatments which has an area of 100 m^2 (5 (W) m x 20 (L) m) as follows: Treatment-1 (T-1) ; Soil surface was always kept bare by hand-weeding once a month. This management is a general practice in these coffee plantation areas so that this treatment is regarded as a control. Treatment-2 (T-2); Soil surface was covered with *Paspalum conjugatum* grass, which is used as a cover plant on slope area in spite of a serious weed. Young plants were reproduced at the campus of Lampung University, and after that, *Paspalum conjugatum* was transplanted to the experimental plot twice uniformly, in November 1995 and in February, 1996. At the initial time, their coverage is 16 % in T-2 plot. Treatment -3 (T-3); This plot was maintained under natural weed vegetation.

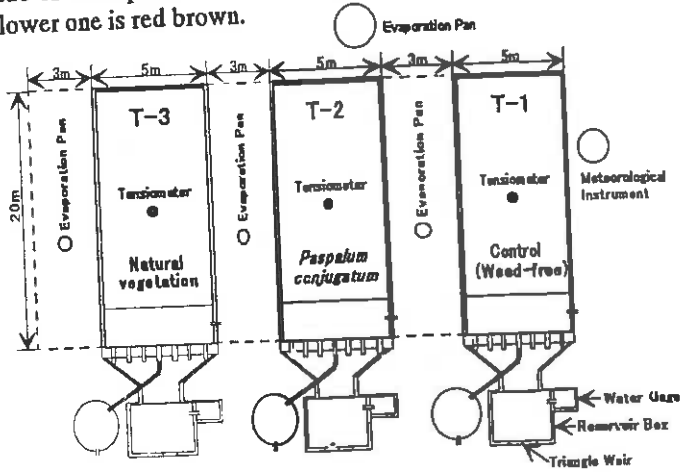

Fig. 1. Layout of experimental plots

Six-month old seedlings of Arabica coffee were transplanted in November 1995 with 2 m x 1.25 m planting space. In order to avoid the competition between coffee plants and weeds, the spot hand-weeding in area of 1 m diameter around the coffee plant, has been conducted in all treatments once a month. Also the *P. conjugatum* mats in T-2 have been mowed at a height of 15 cm before and after rainy season, and natural weed vegetation in T-3 have been mowed by the same practice. The spray of pesticides and the application of fertilizer have been adopted the standard usual practice.

Measurement items

①**Meteorological data:** Meteorological data such as rainfall wind velosity, sunshine, air temperature and humidity are self-recorded by using Weather Survey System which is set up near T-1 area.

②**Surface runoff:** Reservoir boxes with triangle weir attached water gauge were constructed at the downstream end of three treatments (Fig.1). Surface runoff discharge hydrograph was obtained by measuring the overflow water depth over the triangle weir of reservoir box. Daily surface discharge volume is obtained by measuring the water volume sotred in the sedimentation tank, which placed beside the reservoir box.

③**Erosion:** Distribution of erosion in soil surface , Cumulative erosion and sedimentation in the tank and ditch were measured.

④**Soil survey:** Physical properties of soil, such as soil moisture content, soil color, hardness, soil texture, density, three phases of soil, saturated permeability and soil characteristics curve, pH, EC, organic matter were investigated twice per year.

⑤Succession of weed vegetation and their biomass: The coverage and plant height of weeds grown in each treatment were measured by a quadrat method (50 x 50 cm), laid randomly with 10 replications at one month intervals. From these data, succession of weed vegetation was estimated. Regarding shoot and root biomass , it was measured by sampling of a quadrar (50 x 50 cm) laid at the upper and the lower site of the area beside each treatment where is maintained under the same conditions of each treatment twice per one year. The inside of a quadrat was digging up to the 1 m depth, and each soil layer of every 10 cm depth in a soil profile was colleted.

⑥**Coffee plant growth and their yield:** Plant height, number of branches, diameter of trunk and canopy area of coffee plants were measured from 8 sample plants in each treatment at a month intervals. And total production of coffee beans (yield) was also measured in order to assess crop losses due to weed competition at every harvest time.

Results and Discussion

Succession of weed vegetation

Before setting up these main plots, we sprayed the herbicide of Glyphosate in all planned area. Prior to its application, the vegetational survey was done by a quadrat method. Dominant species were *Imperata cylindrica* and *Melastoma affine*, *Chromolaena odorata*,*Paspalum conjugatum* and *Borreria alata* followed. *Paspalum conjugatum* grasses were transplanted twice in the Treatment-2 (T-2), in November, 1995 and in February, 1996. After planting, their coverage was maintained almost 100 %, and their mean plant height ranged from 38 cm to 77 cm by mowing at twice per year intervals.

Table 1 indicates that weed species present over 3 years period from setting up the main plots, and most dominant weed species present in each treatment . *Ageratum conyzoides*, *Bidens pilosa*, *Borreria alata*, *B. repens*, *Clibadia surinamense*, *Cyrtococcum acrecens*, *Hedyotis auricularia*, *Imperata cylindrica*, *Melastoma affine*, *Oxalis barrelieri*, *Paspalum conjugatum*, *Phylanthus niruri* and *Polygala paniculata* were frequently found in all treatments. However most dominant species were different among treatments as shown in Table 1.

In the Treatment-1 (T-1) that were kept clean by hand-weeding at one month intervals, small seedlings of *Ageratum conyzoides* was the species present all year long beside other species that was frequently seen such as *Borreria repens*, *Erechtites valerianifolia* , *Imperata cylindrica* and *Oxalis barrelieri*. *Ageratum conyzoides* is annual herb, stems erect with ascending branches, up to 80cm tall. Seeds are transported by wind or by clinging to the clothing or hairs of passing people or animals. They have a wide distribution on dry to moist, fertile to moderately fertile soils, in the open or lightly shaded area. They can use as a medicine in various ways. Though this species is reported that regular weeding and short fallow periods will diminish the occurrence, we recognized this species as a dominant species in manual weeding plot (T-1).

On the other hand, soil surface is covered with *Paspalum conjugatum* in the Treatment-2 (T-2). This species is perennial and stoloniferous grass. It is propagated by seeds and stolons, and widely distributed in open places, orchards, coffee and tea plantation. Though they are serious weeds, they are also used as a cover plant on slope area experientially in Indonesia. The dominant species were *Polygala paniculata*, *Hedyotis auricularia* and *Ageratum conyzoides* in T-2.

In the Treatment-3 (T-3) that were kept natural weed vegetation, succession of weed community was occurred. Namely *Imperata cylindrica* was dominant species before setting up in November, 1995, however, within one year after them, *Clibadia surinamense*, (woody species) replaced as the dominant species, reaching mean plant height of 136 cm even though other species were also frequently present such as *Clidemia hirta*, *Chromolaena odorata*, *Melastoma affine* and *Imperata cylindrica*. *Imperata cylindrica* is perennial and rhizomatous grass. It's reproduced by seeds and extensive rhizomes. It is undoubtedly taht the most troublesome weed found in orchards, coffee and tea plantation, and newly open cultivated lands, also in non-cultivated land such as old clearings, abandoned lands after shifting cultivation. From these informations, we understand easily that *Imperata cylindrica* hold the position of next dominant

Table 1 Weed species present over 3 years period in coffee plantation managed by different soil conservation techniques.

Weed Species	No-cover (T-1)	*Paspalum* Covered (T-2)	Natural Vegetation Covered (T-3)
Ageratum conyzoides	★ ★	★ ★	★
Axonopus compressus		★	★
Bidens pilosa	★	★	★
Borreria alata	★	★	★
Borreria leavis	★		
Borreria repens	★ ★	★ ★	★
Centella asiatica	★		★
Celosia argentea	★		★
Chromolaena odorata	★	★	★ ★
Clibadia surinamense	★	★	★ ★
Clidemia hirta		★	★ ★
Commelina nudiflora	★	★	
Cyperus kyllingia	★		
Cyrtococcum oxyphylu	★	★	★
Dicranopteris linearis	★		★
Digitaria sanguinalis	★		★
Digitaria nuda			★
Digitaria ciliaris			★
Emilia sonchifolia	★		
Erechtites valerianifo	★ ★	★	
Erigeron sumatrensis			★
Fimbristylis dichotom	★	★	★
Galinsoga sp.			★
Hedyotis auricularia	★	★ ★	★
Hyptis brevipes		★	★
Imperata cylindrica	★ ★	★ ★	★ ★
Lantana camara			★
Melastoma affine	★	★	★ ★
Mikania micrantha		★	★ ★
Oxalis barrelieri	★ ★	★	★
Paspalum conjugatun	★	★ ★	★ ★
Phylanthus niruri	★	★	★
Physalis angulata			★
Polygala paniculata	★	★ ★	★
Sacciolepsis indica		★	★
Scleria sumatrensis		★	
Total Number	24	23	30

★ ★ : Dominant weed species

species in all treatments.

The most diverse weeds were found in natural vegetation plot (T-3) with maximum of 23 weed species in March, 1997 and minimum of 8 species in October, 1997, while the maximum number of species was 15 and 13 in *Paspalum* covered and manual weeding plots (T-2 and T-1), respectively(Fig.2). The cover with *Paspalum* resulted in a decrease in total number of emerged species. It was not apparent whether the phenomenon such a decline in weed numbers may be due to allelopatibic potential of *Paspalum* or only resource competition. The conspicuous decline in weed numbers in September and October 1997 in all treatments may be due to a long drought. At that time, it became clear that both *Imperata cylindrica* and *Paspalum conjugatum* have high drought tolerance.

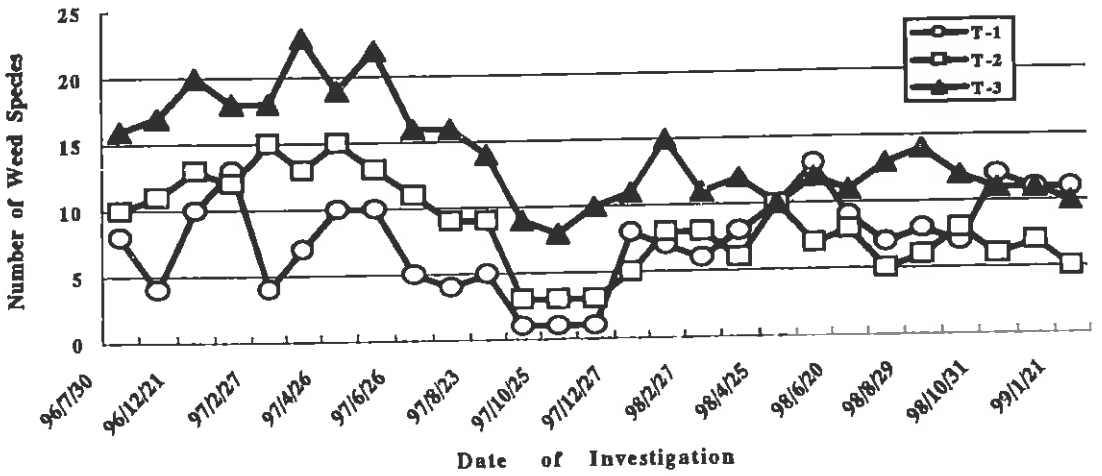

Fig. 2 Change in number of weed species present in coffee plantation managed by different soil conservation techniques.

Biomass, root system and soil erosion

Fig.3 indicates the comparison of root biomass among thee treatments. The total biomass of the aboveground part (top) in T-2 was relatively constant in 1997 compared to those in T-3, which fluctuated markedly. The total biomass of root in T-2 for 2 years showed a similar tendency which produced the constant biomass of about 2 kg DW/m^3, and total root biomass in T-1 decreased by half that of before initial time in accordance with the hand-weeding. On the other hand, regarding the proportion of root biomass in each layer of soil depth, both T-1 and T-2 had a tendency that most of the roots distributed in the 0 - 20 cm soil depth, in spite of the fact that the amount of root biomass was different obviously.

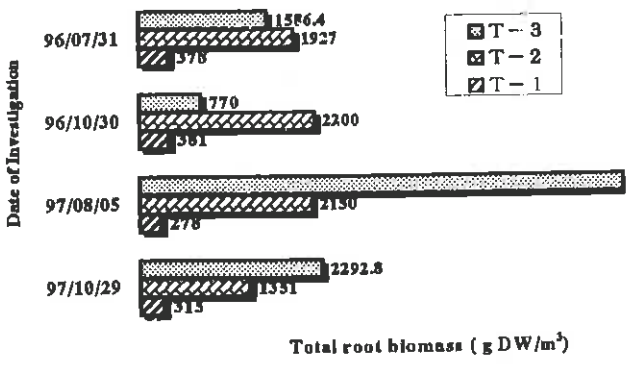

Fig. 3 Comparison of total root biomass.

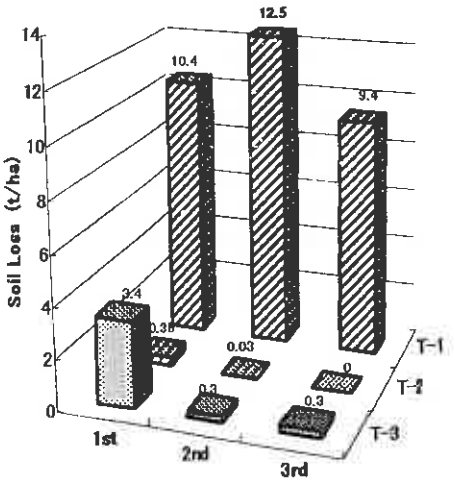

Fig. 4 Total soil loss of each treatment.

However weeds grown in T-3 penetrated their roots more deeply. Since it is considered that these root systems in both T-2 and T-3 may allow weed vegetation to protect against soil erosion, soil loss among three treatments were shown in Fig. 4. The cover with *Paspalum* was a better way to reduce soil erosion compared to the cover with natural vegetation, even though both techniques dramatically reduced soil erosion compared to the manual weeding treatment.

Growth of coffee plants

As expected, there are significant reductions in both height and number of branches of individual coffee plants in T-2 and T-3. The reason is considered the reduction in light intensity from full sunlight to the level under a coffee plant canopy by around taller weeds, and the competition for water and nutrients has also occurred. Over a period of 8 months after transplanting coffee plants, the growth of coffee plants grown in T-2 was suppressed due to competition between *Paspalum conjugatum* and coffee plants, while those in T-3 was not inhibited remarkably. However after then, as shown in Fig.5, over a period of 2 years, competition between *Paspalum conjugatum* or natural weed community and coffee plants had become serious. Total production of coffee beans (yield) was measured in order to assess crop losses due to weed competition at every harvest time. The yield loss of coffee plants in T-2 and T-3 was estimated by severe competition of weed vegetation.

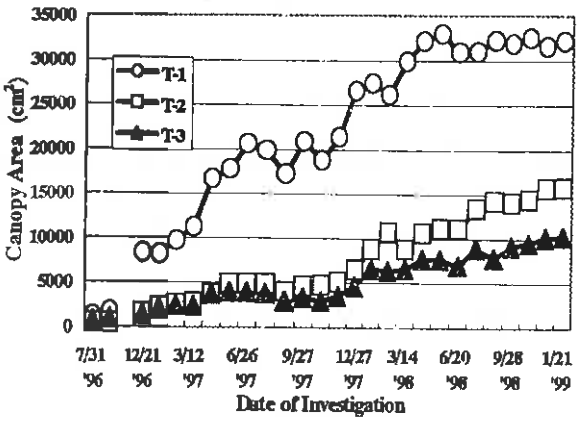

Fig. 5 Canopy area of coffee plants affected by Different soil conservation techniques.

Conclusion

Reliable and proven soil conservation technologies include ridge-planting, no-till cultivation, crop rotations, strip cropping, grass strips, mulches, living mulches, agro-forestry, terracing, contour planting, cover crops etc. Although the specific processes vary, all conservation methods reduce erosion rates by maintaining a protective vegetative cover over the soil. There are many interactive processes between a plant and its soil that affect erosion[2]. Some of these processes include the following: (1) The physical binding of soil by plant stems and roots, (2) Detention of runoff by stalks and organic litter, (3) Improved infiltration along root channels, (4) Greater incorporation of organic matter into the soil, resulting in better structural and water-holding qualities, (5) Increased faunal and biological activity, leading to better soil structure.

From our results , we declare that the vegetation consisted of *Paspalum conjugatum* contribute to reduce soil erosion effectively. And among the above-mentioned interactive processes between plants and soils, some of them were determined, but some points still remain. However, the growth of coffee plants was greatly affected by the cover with *Paspalum* on soil surface. The condition demands further research to synchronize the benefit of reduced soil erosion without inhibition of coffee growth. Therefore it is needed to determine how to manage the *Paspalum* vegetation without competition for a long period. To determine the setting time of the *Paspalum* (stage of coffee plants), their appropriate cutting frequency and utilization of its biomass must be considered.

Acknowledgements:

The authors are grateful to Ir. Affandi, Ir. Bustomi Rosadi and Ir. Tumiar K. Manik from Faculty of Agriculture, The University of Lampung, Indonesia, for their support and shares.

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WEED SEEDBANK RESPONSE TO HERBICIDES USE IN PADDY RICE FIELDS

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Abstract: More than 30 years have passed since herbicides effective against annual weeds were first used in Japan. Currently, there are few annual weeds in the paddy rice fields and the number of weed seeds in soil is decreasing under successive herbicide applications. Research was conducted to estimate the seed density in the soil based on the number of seedlings emerging from paddy soil samples collected from 21 paddy fields. Initially, we attempted to categorize changes in the seedbank of rice fields. In the case of an increasing seedbank in the soil, the seedbank of the surface soil layer is larger than that of the middle soil layer before tillage. It is, however, difficult to predict seedbank dynamics in the case of increasing seed in the soil , but we can judge that more effective herbicide application should be made to the paddy rice field. In the case of decreasing seed in the soil, the seedbank of the surface soil layer is smaller than that of the middle soil layer before tillage. I concluded that the reduction rate of the seed were 3.5 to 18.5 % per year in the rice fields where herbicides were applied effectively.

Key words : seedbank in soil, paddy field, weed seed, organic cultivation

Introduction

More than 30 years have passed since herbicides effective against annual weeds were first used in Japan. The dominant weed species in paddy fields are influenced by the kind of herbicides applied. The application of effective herbicides for three years eradicated the perennial weeds, but not the annual weed. Many studies have been carried out on the analysis of vegetation in paddy fields treated with herbicides. Currently, there are few annual weeds in the paddy rice fields and the number of weed seeds in soil is decreasing under successive herbicide applications. Thus, it is very important to analyze the seed bank in the soil, because seed density estimation is useful for predicting weed infestation and enables a minimum use of herbicides(Buhler,1996).

Schweizer et al.(1984) have reported that the number of seeds in the seedbank in continuous corn in Colorado dropped by approximately 70% after 3yr of atrazine application plus inter row cultivation. In a similar study, Burnside et al. (1986) reported that broadleaf and grass seed density in the soil declined 95% after a 5-yr weed-free period in Nebraska. Roberts(1981) also reported a steady decline in total seedbank densities in plots receiving repeated herbicide application. We have reported the changes in the weed seed density in soils of paddy fields during a period of 12 consecutive years of herbicide application (Sago,1995.1997.). I conducted the current study in order to categorize changes in the seed density in paddy fields and to examine the effects of herbicides or without herbicide application practices on weed seedbanks.

Materials and Methods

Investigated fields

A survey was conducted in 21 rice fields in Japan. Table 1 shows the weed management systems and tillage systems in each paddy field from 1982 to 1996. The fields of Chiba E, G, Ibaraki A, B and Akita A were no-tillage cultivation. For five years from 1991, Chiba A and D fields were cultivated organically and no agrochemicals including herbicides were applied. Other fields were treated with herbicides which were selected by the farmer.

Table 1 Investigated fields, year and characteristic of fields

Prefecture	Field	Distribution of soil seedbank	Dynamics of soil seedbank	Management of paddy field	
		Investigated year	Investigated year	Tillage	Methods of weed control
Kanagawa	A	1982	1982,83,94	Conv. tillage	Herbicides
	B	1982			
	C	1982			
	D	1982	1982,83,94	Conv. tillage	Herbicides
	E		1982,83,94	Conv. tillage	Herbicides
	F		1982,83,94	Conv. tillage	Herbicides
	G	1983		Conv. tillage	Herbicides
	H	1983		Conv. tillage	Herbicides
	I	1983		Conv. tillage	Herbicides
	J	1983		Conv. tillage	Herbicides
Chiba	A		1994,96	Conv. tillage	Organic
	B		1994,96	Conv. tillage	Herbicides
	C		1994,96	Conv. tillage	Herbicides
	D		1994,96	Conv. tillage	Organic
	E	1996		No-tillage	Herbicides
	F	1996		Conv. tillage	Herbicides
	G	1996		No-tillage	Herbicides
Ibaraki	A	1997		No-tillage	Herbicides
	B	1997		No-tillage	Herbicides
Akita	A	1997		No-tillage	Herbicides
	B	1997		Conv. tillage	Herbicides

Note: Vacant column indicates no investigation

The fields of KanagawaA,B and C were combined with one field in 1984.

Method for estimating seed numbers in the soil

Studies on seedbanks in arable soil have led to the development of many techniques for estimating seed density from soil samples. We reported previously that the glasshouse tray method enabled us to estimate the seedbank in paddy fields based on the sampling date, tray size and conditions of emergence or dormancy breaking(Sago,1995). This report viewed the number of seedlings in paddy soil during one season as the number of seeds in soil . To determine the number of viable seeds in soil, soil samples were taken from each field after tillage in January. Thirty random samples were taken from each field at the level of the cultivated soil layer. Samples were stored at 5C for more than 3 months, each sample was thoroughly mixed in May and kept in a plastic container 100 cm square and 6cm deep, then placed in a plastic greenhouse where the temperature was not controlled, thus allowing viable seeds to germinate. Soil samples containing seeds were watered as needed and maintained with water at a depth of 1 cm.

Seedlings were identified, counted and removed at the seed-leaf stage or 1-leaf stage to avoid the periodic interaction of weeds, throughout about 100 days of the germination period beginning in May and through September. Seeds of 6 annual weed species were identified, *Lindernia* spp. including *Lindernia procumbens* (Krock.) Borbas and *Lindernia dubia* (L.) Penn., *Dopatrium junceum* (Roxb.) Hamilt. (DOPJU), *Elatine triandra* Schk. var. *pedicellata* Krylov (ELATR), *Rotala indica* (Wild.) Koehne var. *uliginosa* (Miq.) Koehne (ROTIN), *Cyperus* spp. including *Cyperus difformis* L. and *Cyperus flaccidus* R.Br. and *Monochoria vaginalis* (Burm. f.) Kunth (MOOVA). The seeds of *Lindernia* spp. were combined due to the difficulty in differentiating *L. procumbens* from *L. dubia* at the seed-leaf stage. *Cyperus* spp. seeds were also combined because of the difficulty in differentiating *C. difformis* from *C. flaccidus* at this stage.

Distribution of weed seed in the soil and dynamics of soil seedbank

It is difficult to make it beneficial to model seedbank dynamics system to gain a better conceptual understanding of the contributing processes, substantially. Ball (1992) explained a model of the different life stages of a typical annual weed species reproducing from seed. I changed a part of Ball's model and, as illustrated in Fig. 1, the proportion of individuals progressing through each life stage is dependent on several environmental and genetic factors.

To measure the vertical distribution of weed seed to a 15cm depth in the soil, the soil was collected at surface(A1:0-5cm), middle(A2:5-10cm) and lower(A3:10-15cm) of cultivated soil layer in 15 fields before tillage (Table 1).

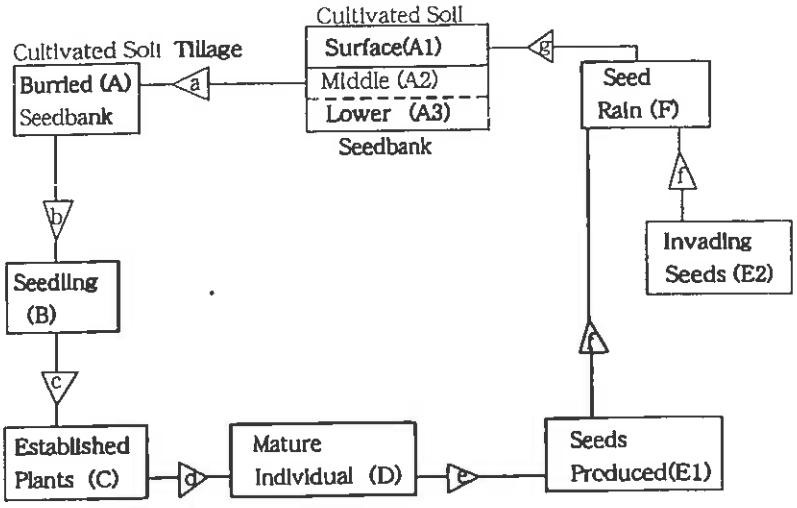

Fig. 1 Scheme of seed reproduction

A through F are six intermediate life stages.
Small letters represent interphase multiplier variables of seed survival.
Changed D. A. Ball (1992)

I regarded the number of seed in Kanagawa soil in 1981,1982 and Chiba soil in 1994 as the initial density of seeds of each field after tillage in this survey. The rate of change (r) was calculated by an expression (1). $S_n = S_0(1+r)^n$ (1)

Where S_n is the seed density after n year, S_0 is the seed density in initial year, r is the rate of change per one year , ri shows that the tendency is increasing (ri>0) and rd indicates decrease (rd<0).

Results

1.Distribution of weed seed in the soil

Initially , I attempted to categorize changes in the seedbank of rice field. Fig. 2 shows the typical fields, that is , the seedbank of the surface soil layer(A1) in Akita B was smaller than that of the middle soil layer(A2), but the seedbank of surface soil layer(A1) in Kanagawa J was larger than that of the middle soil layer(A2). It was Kanagawa G, H, I, J, Chiba E, G, Ibaraki A, B and Akita A that the seedbank density of A1 was larger than that of A2. But the seedbank of lower soil layer was smallest at all of the soil.

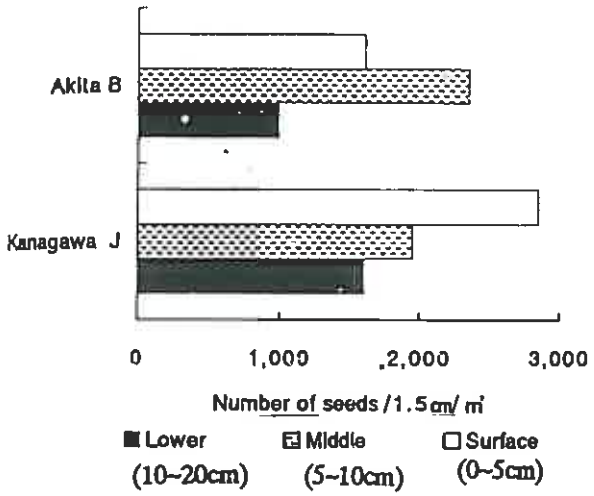

Fig. 2 Seed density in each soil layer

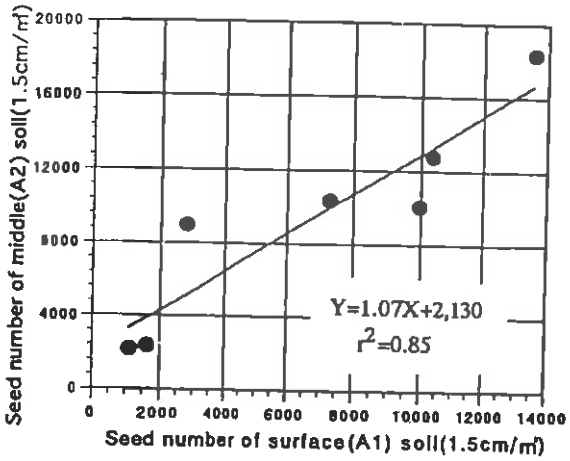

Fig. 3 The relation of seed density between the surface soil layer(A1) and the middle soil layer(A2) in case of A1<A2

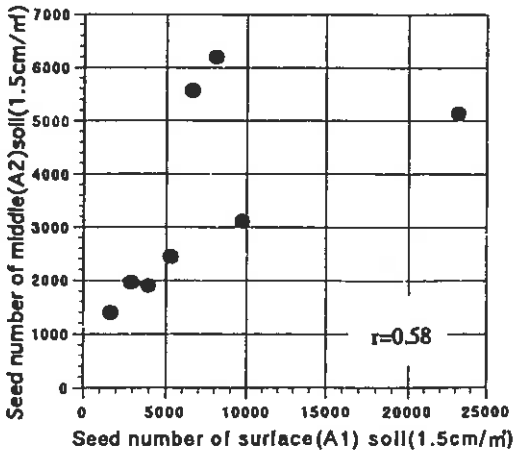

Fig. 4 The relation of seed density between the surface soil layer(A1)and the middle soil layer(A2) in case of A1>A2

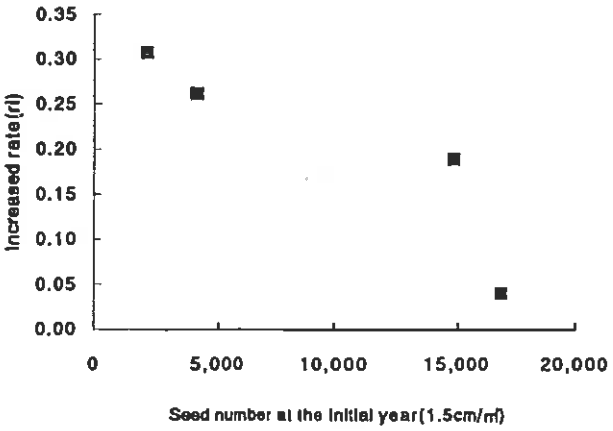

Fig.5 The increased rate of seedbank in soil that rained the weed seed

It was observed that if good weed control was obtained, the seedbank of A1 layer was smaller than that of A2 layer and the seed density declined. And the seed density of A2 layer correlated to the seed density of A1 layer (Fig.3).

But, in the case of an increasing seed density in the soil, the seedbank density of A1 layer is larger than that of A2 layer. And the seed density of A2 layer was no relate to the seed density of A1layer(Fig.4).

2. Dynamics of soil seedbank

The total number of seeds in the soil sampled from the 8 rice fields in 1982 to 1996 were counted. *Lindernia* spp., *Dopatrium junceum*, *Elatine triandra*, *Rotala indica* and *Cyperus* spp. were dominant in all the fields sampled. The number of seeds in the soil increased between 1994 and 1996 in Chiba A, B, C, D fields and the increase was most evident in fields A and D which were not treated with herbicides. The total number of seeds in field A soil and field D soil increased from 14,817/㎡ to 20,980/㎡ and from 4,126/㎡ to 6,570/㎡ respectively. This increasing rate of the seedbank were 4 to 31% per year (Fig 5).

However, The number of seeds in the soil decreased between the 12 or 11 years in Kanagawa A, B, C, D, E and F. The fields of Kanagawa A ,B and C were combined with one field in 1984, and the decrease was most evident in fields A ,B, C and E. The reduction rate of the seedbank were 3.4 to 18.5% per year in the rice fields(Fig 6).

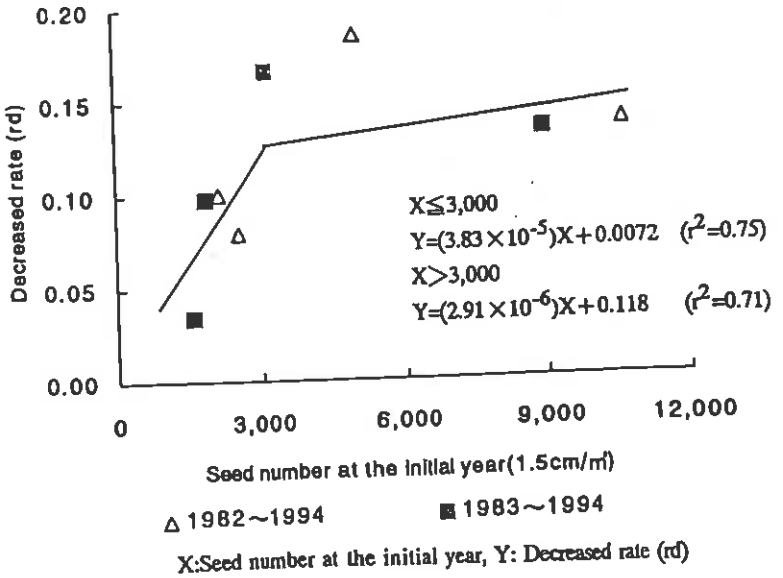

Fig.6 The decreased rate in the soil that treated herbicides continuously

Discussion

In many paddy fields in Japan the number of residual weeds is very small because of consecutive herbicide applications. It is, therefore, very important to analyze the seed bank in the soil, because the seed density estimation is useful for predicting weed infestation and enables a minimum use of herbicides. Initially I attempted to categorize changes in the seedbank of rice field. I tried to estimate the seedbank dynamics at the surface soil seedbank (A1) and middle soil seedbank(A2) before tillage. In the case of an decreasing seedbank in the soil, the number of established weed(D) was very few because of effective

herbicide applications consecutively. And the seedbank of the surface soil(A1) was smaller than that of the middle soil(A2). The decreasing rate were affected by the efficacy of herbicides(c), number of emerged weed (b) and lost seed viability in the buried seedbank. We can predict the rate of decreasing seedbank in paddy fields. This rate depends on the number of seed in the soil with the number affecting the decreasing rate being 2000~3000 seeds in the soil. The decreasing rate is 12.2~18.5% per year at more than 3,000 seeds in the soil and 12.2~3.4%per year at less than 3,000 seeds in the soil.

The decreasing rate of seedbank in paddy rice field under successive herbicide applications was smaller than that of seedbank in upland field). More detailed studies, including one on the percent of seed emergence, the number of established plants, seed production and seeds supplied for irrigation is needed to address the questions raised in the present study.

In the case of an increasing seedbank in the soil, it is necessary to measure the number of new seeds added to the population. It is difficult to measure the number of seeds which was produced by the mature individuals(D) and invading seeds(E2). If the seedbank of surface soil(A1) was larger than that of middle layer(A2), We can suggest that more effective herbicide application should be made to the rice field. The increasing rate of smaller seedbank in the soil was larger than that of larger seedbank. But we cannot predict the increasing rate of the seedbank, because the number of seeds added to the seedbank depends on the efficacy of herbicidal effect, but not the seedbank of the previous year.

Acknowledgements: This research is based upon work cooperated with Zen-noh Agr. Tech. Center, Chiba prefectural Agricultural Experiment Station , Akita Prefectural University and Ibaraki University. I am grateful to the co-researcher of those organizations.The advice of H. Chisaka is greatly appreciated.

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YIELD AND COST OF PRODUCTION IN REDUCED – TILLAGE SUGARCANE

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Abstract : The studies on the different planting methods in sugarcane production; no-tillage, minimum tillage and conventional tillage have been carried out in the farmer's field at Kamphaeng saen, Nakhon Pathom during March 1997 – February 1999. The results indicated that the growth of sugarcane, yield components and cane quality were not significantly different among different planting methods. The weed species and density of grasses and sedges in reduced – tillage were less than that of conventional tillage. The cost of production for the first year of no-tillage, minimum tillage and conventional tillage were 6210.41, 6544.01 and 6567.16 bath/rais, respectively. Soil organic matter in no-tillage was increased whereas conventional tillage was decreased year by year. Soil moisture content in no-tillage showed most the consistency the whole year round.

Key words: sugarcane, cost of production, reduced-tillage, no-tillage, minimum tillage, conventional tillage.

Introduction

Sugarcane is one of the most important economical crops in Thailand. It occupies almost 6 million rai and produces about 52.6 million tons of cane annually, the average yield is still low at about 8.7 tons per rai. Normally at every 3 year the farmers have to replant the sugarcane by trash burning before land preparation by disc plow twice and followed by a single rotary plow which costs about 600-700 baht per rai (Chinawong and Cheosamutr, 1998). Although no-tillage in crops production have been studied for along time, but the idea of no-tillage sugarcane had only began in 1975 after invention of glyphosate. The studies on no-tillage sugarcane by applied glyphosate at the rate of 2.0-3.5 kg ai/ha for got rid of the old ratoon and weeds before growing new plants between the old rows which have been reported by a number of researchers (Iggo and Moberly, 1976; Hadlow and Millard, 1981). The previous reports showed that there were no differences in cane yield between conventional tillage and no-tillage (Hunsigi,1993; Chinawong and Cheosamutr, 1998). However, Moberly and Turner (1978) reported that in light soil structure no-tillage sugarcane might increase yield more than by conventional tillage of 10% in new crop and 5% in first ratoon. The objectives of this research were to determine the yield and cost of sugarcane production in no-tillage compared with minimum tillage and conventional tillage.

Materials and Methods

The experiment was carried out in the farmer's field at Kamphaeng saen, Nakhon Pathom province, during March 1997 to February 1999. Using the randomized complete block design with 3 replications of 1 rai plots size. The sugarcanes were planted with 3 different planting methods as following ; no-tillage, the ratoons were got rid of by applied glyphosate at the rate of 2.0 kg ae/ha. The trash were cut by coulter between the old rows prior to planting by sugarcane planter. Minimum tillage, the ratoons were also applied by glyphosate and then trash slashing before plowing by three disc plow one time prior to planting by planter. Conventional tillage, trash slashing before land preparation by three disc plow twice and followed by a single rotary plow prior to planting by planter. All plots were planted with the same planter.

The herbicides atrazine + ametryn were applied at the rate of 3 kg ai/ha at 2 weeks after planting, and paraquat + diuron at 1+2 kg ai/ha were applied at 3 months after planting for weed control in each plot. The fertilizers were applied twice with 15-15-15 formulation at the rate of 25 kg/rai at planting and 46-0-0 formulation at the same rate at 3-4 months after planting. The measurement of plant height, tillering, yield components, cane quality, weed infestation, soil moisture content, and soil chemical analysis were determined in the first year and first ratoon.

Results and discussion

Plant growth

The height of sugarcane was not significantly different among the different planting methods in both first year and first ratoon. The number of shoots per tiller from no-tillage, minimum tillage and conventional tillage were 5.6, 4.8 and 4.4, respectively. However, The yield and C.C.S.of sugarcane were not much different among the different planting methods. (Table 1)

Table 1 Plant height and the number of shoots/tiller at 8 months, yield and C.C.S. from different planting methods.

Planting methods	Height (cm.)		Shoots/tiller		Yield (tons/ha.)		C.C.S.	
	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year
NT.[1/]	229.2 a	259.2 a	5.6 b	9.3 a	122.2 a	149.5 a	12.7 a	14.4 a
MT.	230.6 a	255.9 a	4.8 a	8.9 a	122.7 a	147.0 a	13.0 a	15.0 a
CT.	236.2 a	251.6 a	4.4 a	8.5 a	121.1 a	145.8 a	13.1 a	15.0 a
CV.(%)	3.4	3.7	7.5	8.4	7.7	1.9	3.4	3.0

Means in column followed by the same letter are not significantly different at 5 % level by DMRT.
[1/] No-tillage(NT), minimum tillage(MT) and conventional tillage(CT).

Weed infestation

It was found that weed number and weeds dry weight per m^2 at 4 months after planting showed a shift of weed species, the annual broadleaves and annual grasses were dorminant in conventional and minimum tillage whereas perennial broadleaves (vine) such as *Ipomoae gracillis* was found in no-tillage plots. However, *Cyperus rotundus* was almost the same density in different planting methods. (Table 2). It was also reported that the annual grass density increased in no-tillage compared to conventional tillage in corn-soybean rotation, but no change in annual broadleaf species (Wrucke and Arnold, 1985).

Cost of production

When comparing the cost of production among the different planting methods, no-tillage could reduce the cost of land preparation in the first year since the cost was for glyphosate, herbicide application and trash cutting which less than land preparation in minimum tillage and conventional tillage about 320 and 386.67 baht per rai, respectively. The other costs were all the same. The total cost of sugarcane production in the first year of no-tillage, minimum tillage and conventional tillage were 6,210.41, 6,544.01 and 6,567.16 baht per rai, respectively. The cost of production in first ratoon showed a little higher in no-tillage because of higher yield which caused a higher cost for harvesting and transportation. (Table 3,4)

Table 2 Weed number and weeds dry weight per m^2 at 4 months after planting from different planting methods.

Planting methods	Weeds number (plants/ m^2)			Weeds dry weight (g/m^2)		
	boardleaves	grasses	sedges	Boardleaves	Grasses	sedges
NT.[1/]	15.0	297.0	2.0	108.3	507.6	1.8
MT.	7.0	279.0	3.0	36.7	496.2	2.4
CT.	6.0	321.0	3.0	41.1	633.8	2.4

[1/] No-tillage(NT), minimum tillage(MT) and conventional tillage(CT).

Table 3 The cost of sugarcane production form different planting methods in first year.

Item	Cost (Baht/rai)		
	No-tillage	Minimum tillage	Conventional tillage
1. Tillage and planting			
Knockdown herbicide (glyphosate)	180.00	180.00	-
Herbicide application	53.33	53.33	-
Trash cutting before planting	40.00	-	-
Trash slashing before land preparation	-	210.00	210.00
Land preparation	-	150.00	450.00
Planting materials preparation	80.00	80.00	80.00
Planting materials	650.00	650.00	650.00
Planter	100.00	100.00	100.00
Labors for planting	35.56	35.56	35.56
2. Crop management			
Fertilizers			
The 1st applied (15-15-15)	133.33	133.33	133.33
The 2nd, 3rd applied (46-0-0)	305.56	305.56	305.56
Fertilizers application	80.00	80.00	80.00
Replanting	142.22	142.22	142.22
Herbicides			
The 1st applied (atrazine+ametryn)	117.00	117.00	117.00
The 2nd applied (paraquat+diuron)	151.80	151.80	151.80
Hand weeding (3 times)	222.22	222.22	222.22
Herbicides application	62.22	62.22	62.22
Labors for irrigation	533.33	533.33	533.33
3. Harvesting and Transportation			
Cane harvesting (90 baht/ton)	1,759.68	1,766.88	1,743.84
Transportation (80 baht/ton)	1,564.16	1,570.56	1,550.08
Total cost in first year	**6,210.41**	**6,544.01**	**6,567.16**

Organic matter and soil moisture content

Soil organic matter in no-tillage showed an increasement, whereas the minimum tillage and conventional tillage was decreased year by year. (Figure 1). These results were agreed with the previous reports which indicated that the soil organic matter increased with time in no-tillage and reduced tillage could improve soil properties (Follett et al., 1998; Powell and Unger, 1998; Bordousky et al., 1999). It was found also that the soil moisture content in whole year round of no-tillage was more consistent than that of minimum tillage and conventional tillage. This is because of in no-tillage the soil was not turned over and exposed to the sun's radiation, plant residue shade the soil, causing lower soil temperature and less evaporation. (Figure 2)

Table 4 The cost of sugarcane production form different planting methods in second year (first ratoon).

Item	Cost (Baht/rai)		
	No-tillage	Minimum tillage	Conventional tillage
1. Crop **management**			
Fertilizers			
The 1st and 2nd applied 46-0-0	333.33	333.33	333.33
Fertilizers application	66.67	66.67	66.67
Herbicides			
The 1st applied (atrazine+ametryn)	144.00	144.00	144.00
The 2nd applied (paraquat)	75.36	75.36	75.36
Herbicides application	111.11	111.11	111.11
Hand weeding (2 times)	333.33	333.33	333.33
Labors for irrigation (3 times)	166.67	166.67	166.67
2. Harvesting and transportation			
Cane harvesting (90 baths/ton)	2,152.80	2,116.80	2,099.52
Transportation (80 baths/ton)	1,913.60	1,881.60	1,866.24
Total cost in second year	5,185.76	5,117.76	5,085.12
Total cost in 2 years	11,396.17	11,661.77	11,652.28

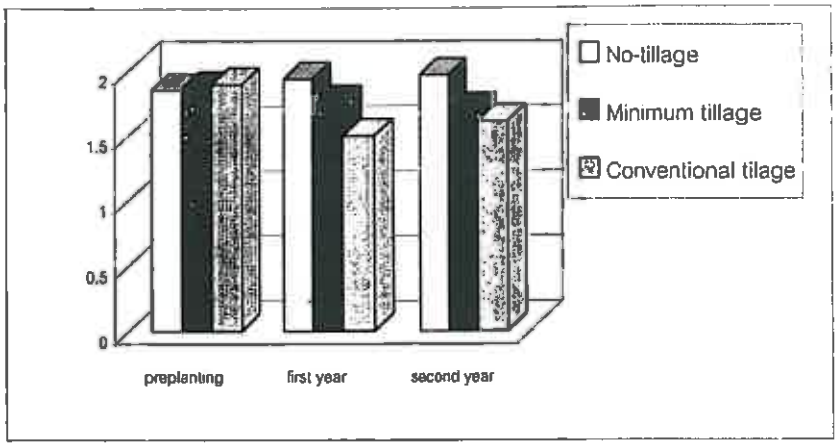

Figure 1 Organic matter (%) quantity from different planting methods

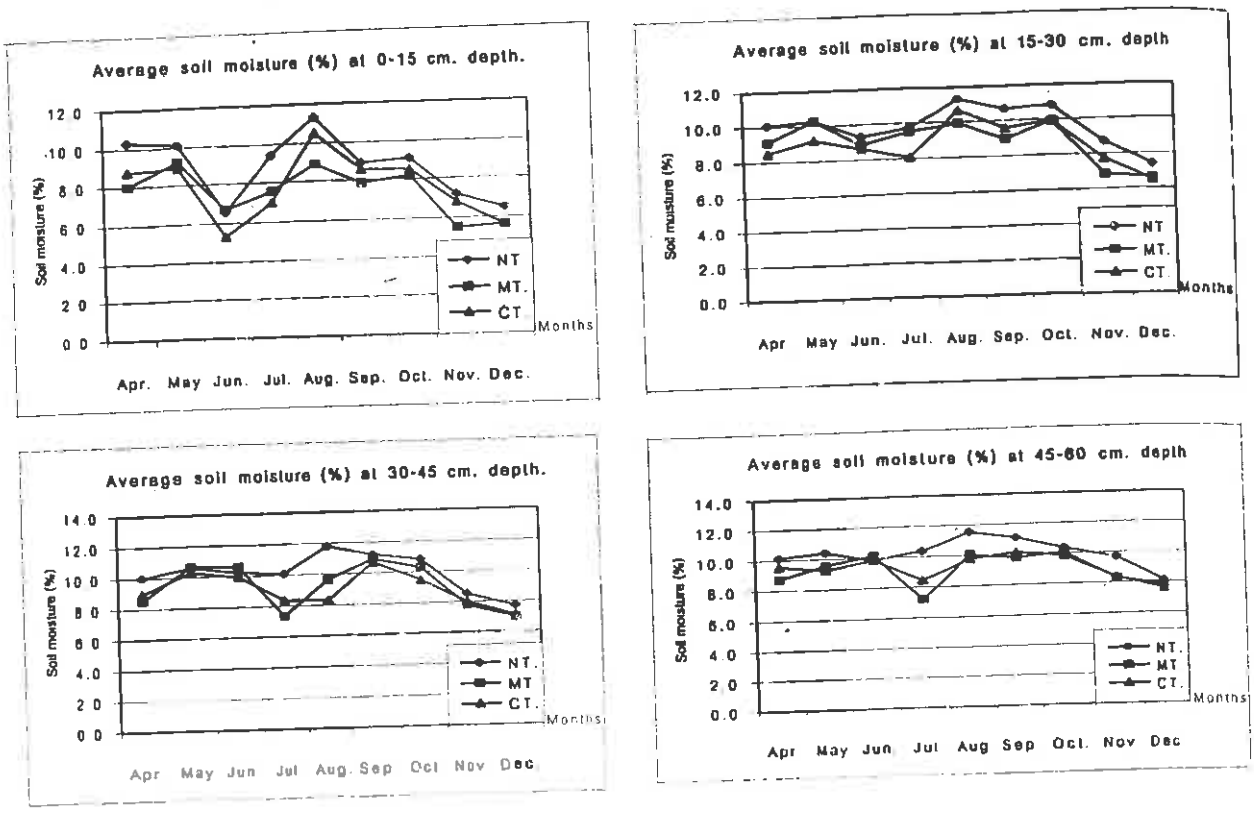

Figure 2 Soil moisture content (%) at 0-15, 15-30, 30-45 and 45-60 cm. level from different planting method.

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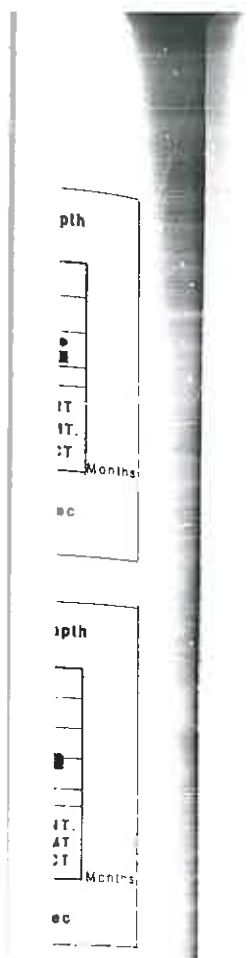

3. Herbicides

3.1 Application Techniques

EFFECTS OF SPRAY VOLUMES AND DOSAGES ON PROPANIL/2,4-D TOXICITY AND WEED CONTROL IN PRE-GERMINATED DIRECT-SEEDED RICE

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Abstract: The effects of spray volumes and dosages on propanil/2,4-D(30% / 6%EC) on toxicity and weed control were conducted in pre-germinated direct-seeded rice. CDA sprayer was evaluated to involve with treatment combinations of spray volume at 75 and 150 L/ha and propanil/2,4-D at 2.0, 1.8, 1.6, 1.4, 1.2 and 1.0 kg a.i./ha applied at 15 DAS. The results showed that spray volume at 75 L/ha was more phytotoxic than the other, and propanil/2,4-D at 2.0 kg a.i./ha was more phytotoxic than dosages at 1.8 and 1.6 kg a.i./ha. But, dosages of propanil/2,4-D at 1.4, 1.2 and 1.0 kg a.i./ha were non-toxic to rice. Propanil/2,4-D was highly effective in controlling annual grasses when sprayed at 2.0, 1.8 and 1.6 kg a.i./ha , broad-leaved weeds when sprayed at 2.0, 1.8 and 1.6 kg a.i./ha to spray volume at 150 L/ha and sedges when sprayed at 2.0 and 1.8 kg a.i./ha, which were no significant difference to propanil/2,4-D at 2.0 kg a.i./ha and spray volume at 500 L/ha were sprayed by knapsack sprayer. Spray volume at 150 L/ha was better control of weeds than the other. The use of knapsack sprayer was higher height of rice than the other treatments. Spray volume at 75 L/ha combined to propanil/2,4-D at 2.0 and 1.8 kg a.i./ha and spray volume at 150 L/ha combined to propanil/2,4-D at 2.0, 1.8, 1.6 and 1.4 kg a.i./ha gave rice grain yield there was no significant difference to knapsack sprayer.

Key words: propanil/2,4-D, spray volume, dosage, toxicity, weed control

Introduction

Environmental and economic concerns have created renewed research interest in methods of improving efficiency of weed management. The objectivity of any spray application must be to get an effective deposit onto the target of weedy species without detrimental environmental and ecological effects and user safety (Nicholls *et al.* ,1993 ; Thomas *et al.*, 1992). When sprays are applied at high volume as it is produced a very wide range of droplet sizes. The part of droplets will be retained on the plant surface then penetrate through crops but other droplets are loss due either to drift or run-off or both that drift and run-off could be reduced by controlling the sizes of the droplets in the sprays (Matthews, 1979). Droplet size and spray volume interact inevitably (McKinley *et al.*, 1974). The effects of spray volume and dosage on the toxicity of 2,4-D ((2,4-dichlorophenoxy) acetic acid) were discussed by McKinley *et al.* (1972) that the effective dosage could be increased equally well by increasing the concentration or by increasing the number of droplets per unit area when spray volume was applied at the same rate. Nakornsri (1996) resulted that the use of CDA sprayer was applied to 2,4-D, when applied spray volume at 0.4 L/min was equal to *Sphenoclea zeylanica* Gaertn. control at 0.8 L/min and used propanil (3′,4′-dichloropropionanilide) to *Echinochloa crus-galli* (L.) Beauv. when applied spray volume at 0.8 L/ha was better weed control than the spray volume at 0.4 L/ha in the same dosage rate. McKinley *et al.* (1974) concluded that, at the same dosage rate of paraquat (1,1′-dimethyl-4,4′ -bipyridinium ion) , low volume applications were more phytotoxic on test plants than high volume applications. Therefore, if the same amount of dosage is applied in a higher volume, then the spray must be less concentrated and vice versa. The dosage may penetrate more rapidly when smaller droplets are used so that less dosage would be needed. Thus, experiments were conducted to determine the use of CDA sprayer with spray volume and dosage on the phytotoxicity weed control and yield of rice of propanil/2,4-D.

Materials and Methods

In order to evaluate efficiency in the CDA sprayer , the field trials were undertaken to examine the effect of spray volume and dosage of propanil/2,4-D on the phytotoxicity and weed control in pre-germinated direct-seeded rice. The treatments were applied in combinations between propanil/2,4-D at 2.0, 1.8, 1.6, 1.4, 1.2 and 1.0 kg a.i./ha and spray volume at 75 and 150 L/ha. The CDA sprayer was by the Micron Herbi when used 75 L/ha spray volume, flow rate and swath width were 0.4 L/min and 1.2 m , respectively and 150 L/ha spray volume, flow rate and swath width were 0.8 L/min and 1.5m, respectively. The knapsack sprayer fitted with a flat fan nozzle was by the Osatu, the propanil/2,4-D was applied the recommended dosage rate at 2.0 kg a.i./ha, spray volume at 500 L/ha, flow rate and swath width were 0.9 L/min and 1.0m, respectively. The timing of application was done at 15 days after seeding. At 15 days after herbicide application, visual phytotoxicity scoring were measured, where 0 no crop injury, 1-3 slight crop stunting, 4-6 moderate injury, crop usually recovers, 7-9 heavy crop injury and stand loss and 10 complete crop destruction. At 30 days after herbicide application, dry weight of weed species from 0.5 m^2 per plot were measured to calculate weed control value. Plant height was measured on 30 days after seeding and rice yield was assessed at 14% moisture content.

A factorial arrangement was used to assess the effects of all combinations of six dosage rates of propanil/2,4-D and two volumes of spray per unit area and were compared with knapsack sprayer and untreated check. The trials were done in 4 replications.

Results and Discussion

The CDA sprayer was applied with dosages of propanil/2,4-D at 2.0 , 1.8 and 1.6 kg a.i./ha were toxic to rice (Table 1). The knapsack sprayer was applied with propanil/2,4-D at 2.0 kg a.i./ha and spray volume at 500 L/ha were more phytotoxic than the CDA sprayer. When the phytotoxic is considered, spray volume of 75 L/ha was more phytotoxic than spray volume of 150 L/ha. For example, applying at 2.0 kg a.i./ha of propanil/2,4-D, spray volume at 75 L/ha resulted in an average damage score per plot of 2.80. AT the same dosage rate, applying spray volume at 150 L/ha gave an average damage score of only 1.50. Result of phytotoxicity in this experiment was similar to McKinley *et al.* (1974) used paraquat to sunflower seedling when applied in 5.5 L/ha of spray volume was more phytotoxic than the spray volume at 22.0 L/ha in the same dosage rate.

Table 1 Effects of spray volumes and dosages of propanil/2,4-D on toxicity to rice at 15 days after herbicide application

Spray volumes (L/ha)	Dosages (kg a.i./ha)						Average
	2.0	1.8	1.6	1.4	1.2	1.0	
75	2.80	2.00	0.75	0.00	0.00	0.00	0.93
150	1.50	0.75	0.50	0.00	0.00	0.00	0.46
Average	2.15	1.38	0.63	0.00	0.00	0.00	0.70

Knapsack sprayer applying at spray volume 500 L/ha and dosage of propanil/2,4-D at 2.0 kg a.i./ha gave an average toxicity score of 2.95.

* An arbitrary scoring system was used where 0=no crop injury, 1-3 = slight crop stunting, 4-6 = moderate injury, crop usually recovers, 7-9 = heavy crop injury and stand loss and 10 =complete crop destruction.

The effectiveness of different treatments on weed control in annual grasses (*Leptochloa chinensis* (L.) Nees , *Echinochloa colona* (L.) Link. and *Ischaemum rogosum* Salisb.) is presented in Table 2 . It was found that dosages of propanil/2,4-D at 2.0 , 1.8 and 1.6 kg a.i./ha , spray volume at 75 L/ha , and propanil/2,4-D at 2.0 , 1.8 , 1.6 , 1.4 , 1.2 and 1.0 kg a.i./ha , spray volume at 150 L/ha , gave total dry weight of annual grasses there were no significant difference to propanil/2,4-D at 2.0 kg a.i./ha and spray volume at 500 L/ha were applied by knapsack sprayer. However, total dry weight of annual grasses when used the CDA sprayer with dosages of propanil/2,4-D at 2.0 , 1.8 , 1.6 , 1.4 , 1.2 and 1.0 kg a.i./ha and spray volume at 75 and 150 L/ha were not significantly different. The higher dosage rate of propanil/2,4-D and spray volume gave better weed control in annual grasses than the lower. This experiment showed

that increasing spray volume increased efficiency to control of propanil/2,4-D on annual grasses. The data in this experiment was similar to Nakornsri (1996) used the CDA sprayer was applied to propanil on *Echinochloa crus-galli* (L.) Beauv. when applied spray volume at 0.8 L/min was better weed control than the spray volume at 0.4 L/min in the same dosage rate.

Table 2 Effects of spray volumes and dosages of propanil/2,4-D on dry weight (g/0.5m^2) of annual grasses at 30 days after herbicide application

Spray volumes (L/ha)	Dosages (kg a.i./ha)						Average[1]
	2.0	1.8	1.6	1.4	1.2	1.0	
75	1.63	1.46	1.43	3.18	3.11	3.15	2.33
150	1.06	1.19	1.78	2.36	2.00	2.32	1.79
Average[1]	1.35	1.33	1.61	2.77	2.56	2.74	2.06

Knapsack sprayer applying at spray volume 500 L/ha and dosage of propanil/2,4-D at 2.0 kg a.i./ha gave an average dry weight of annual grasses of 1.43 g/0.5m^2.
Untreated check gave an average dry weight of annual grasses of 6.36 g/0.5m^2.
CV (%) = 44.05
$LSD_{.05}$ of 1.03 g/0.5m^2 was used to compare an average dry weight of annual grasses between treatment combinations, knapsack sprayer and untreated check.
[1]Means of dry weight of annual grasses of dosages and spray volumes are not significantly different.

Total dry weight of broad-leaved weeds (*Sphenoclea zeylanica* Gaertn. , *Ludwigia hyssopifolia* (G. Don) Excell and *Lindernia anagallis* (Burm. f.) Pennell) were not significantly different when used the CDA sprayer with dosages of propanil/2,4-D at 2.0 , 1.8 and 1.6 kg a.i./ha and spray volume at 150 L/ha compared with the knapsack sprayer at 2.0 kg a.i./ha of propanil/2,4-D and spray volume at 500 L/ha which gave the lowest total dry weight of broad-leaved weeds (Table 3) . The dosages of propanil/2,4-D and spray volume gave total dry weight of broad-leaved weeds there were significant difference . The efficacy of propanil/2,4-D in 2.0 and 1.8 kg a.i./ha were not significantly different while spray volume in 150 L/ha gave the lowest total dry weight. This experiment showed that increasing the dosage of propanil/2,4-D and spray volume increased efficiency to control on broad-leaved weeds. When the dosages of propanil/2,4-D are considered , if spray volume was fixed , the effective dosage could be increased by increasing the concentration (McKinlay *et al.*, 1972). Nakornsri (1996) resulted that the

Table 3 Effects of spray volumes and dosages of propanil/2,4-D on dry weight (g/0.5m^2) of broad-leaved weeds at 30 days after herbicide application

Spray volumes (L/ha)	Dosages (kg a.i./ha)						Average[1]
	2.0	1.8	1.6	1.4	1.2	1.0	
75	2.16	2.49	3.95	3.95	4.04	5.07	3.61 b
150	1.16	1.81	1.87	2.29	2.16	5.21	2.42 a
Average[1]	1.66 a	2.15 ab	2.91bc	3.12 c	3.10 c	5.14 d	3.01

Knapsack sprayer applying at spray volume 500 L/ha and dosage of propanil/2,4-D at 2.0 kg a.i./ha gave an average dry weight of broad-leaved weeds of 0.89 g/0.5m^2.
Untreated check gave an average dry weight of broad-leaved weeds of 9.30 g/0.5m^2.
CV (%) = 39.23
$LSD_{.05}$ of 1.06 g/0.5m^2 was used to compare an average dry weight of broad-leaved weeds between treatment combinations, knapsack sprayer and untreated check.
[1] Means followed by the same letter are not significantly different at 5 % level by DMRT.

CDA sprayer was used to 2,4-D in the same dosage rate when applied spray volume at 0.4 L/min was equal to *Sphenoclea zeylanica* Gaertn. control at 0.8 L/min.

Total dry weight of sedges (*Cyperus difformis* Linn. , *C. iria* Linn. and *Fimbristylis miliacea* (L.) Valh) were not significantly different when used the CDA sprayer with dosages of propanil/2,4-D at 2.0, 1.8 kg a.i./ha , spray volume at 75 L/ha , and propanil/2,4-D at 2.0 , 1.8 and 1.6 kg a.i./ha , spray volume at 150 L/ha , compared with the knapsack sprayer was applied to propanil/2,4-D at 2.0 kg a.i./ha and spray volume at 500 L/ha which gave the lowest total dry weight of sedges (Table 4). The CDA sprayer was applied to propanil/2,4-D at 2.0 , 1.8 , 1.6 and 1.4 kg a.i./ha and spray volume at 75 and 150 L/ha were not significantly different but the higher dosage rate of propanil/2,4-D and spray volume gave better weed control of sedges than the lower.

Table 4 Effects of spray volumes and dosages of propanil/2,4-D on dry weight (g/0.5m^2) of sedges at 30 days after herbicide application

Spray volumes (L/ha)	Dosages (kg a.i./ha)						Average[1]
	2.0	1.8	1.6	1.4	1.2	1.0	
75	5.93	7.38	9.75	8.10	10.55	9.60	8.55 a
150	3.40	4.03	8.18	9.63	9.68	10.10	7.50 a
Average[1]	4.67 a	5.71 a	8.97 ab	8.87 ab	10.12 b	9.85 b	8.03

Knapsack sprayer applying at spray volume 500 L/ha and dosage of propanil/2,4-D at 2.0 kg a.i./ha gave an average dry weight of sedges of 2.78 g/0.5m^2.
Untreated check gave an average dry weight of sedges of 10.48 g/0.5m^2.
CV (%) = 60.52
$LSD_{.05}$ of 6.78 g/0.5m^2 was used to compare an average dry weight of sedges between treatment combinations, knapsack sprayer and untreated check.
[1] Means followed by the same letter are not significantly different at 5 % level by DMRT.

The higher efficiency of propanil/2,4-D to annual grasses , broad-leaved weeds and sedges control when the higher spray volume was applied (Table 2,3,4). Previous work on the relationship between spray volume and the performance of herbicides has produced conflicting to this experiment. In the same dosage rate , Douglas and Orvin (1983) resulted that glyphosate was more effective control in low spray volume on barnyardgrass and similar to fluazifop-butyl , haloxyfop-methyl and sethoxydim to forage sorghum and yellow foxtail (Douglas and Orvin , 1984) and 2,4-D to seedling of sunflower (McKinley

Table 5 Effects of spray volumes and dosages of propanil/2,4-D on plant height (cm) at 30 days after seeding

Spray volumes (L/ha)	Dosages (kg a.i./ha)						Average[1]
	2.0	1.8	1.6	1.4	1.2	1.0	
75	35.03	35.28	36.85	35.53	36.78	36.10	35.89
150	36.30	36.75	35.45	37.10	36.70	35.08	36.23
Average[1]	35.54	36.02	36.15	36.32	36.74	35.59	36.06

Knapsack sprayer applying at spray volume 500 L/ha and dosage of propanil/2,4-D at 2.0 kg a.i./ha gave an average plant height of 37.68 cm
Untreated check gave an average plant height of 36.55 cm
CV (%) = 5.12
[1] Means of treatment combinations, knapsack sprayer and untreated check, dosages and spray volumes are not significantly different.

et al. , 1972). They are foliar-absorbed and translocated herbicides (Anonymous, 1989). There are possible explanations for increased propanil/2,4-D control when spray volume is increased. Propanil is a contact herbicide (Yih *et al.*, 1968). The active ingredient of the propanil in mixture between propanil and 2,4-D is more than 2,4-D. When spray volume was increased the propanil/2,4-D herbicide in the spray solution may give better distribution and retention over the leaf surface. At a dosage rate of 0.14 kg/ha of paraquat as contact herbicide, McKinley *et al.* (1974) resulted that the spray volume was applied at 22 L/ha gave more effective than the spray volume at 5.5 L/ha. Nakornsri (1996) resulted that, at the same dosage rate of propanil, spray volume at 0.8 L/min was better control to *Echinochloa crus-galli* (L.) Beauv. weed than spray volume at 0.4 L/min.

Plant height at 30 days after seeding were not significantly different (Table 5) is probably due to less phytotoxicity of propapnil/2,4-D (Table 1). The use of knapsack sprayer was higher height of rice than the treatment combinations by the CDA sprayer and untreated check.

Table 6 Effects of spray volumes and dosages of propanil/2,4-D on grain yield of rice (t/ha) at 14 % moisture content

Spray volumes (L/ha)	Dosages (kg a.i./ha)						Average[1]
	2.0	1.8	1.6	1.4	1.2	1.0	
75	3.48	3.57	2.96	2.98	2.68	2.43	3.02 a
150	3.56	3.31	3.08	3.10	3.02	2.58	3.11 a
Average[1]	3.52 a	3.44 a	3.02 b	3.04 b	2.85 b	2.50 c	3.06

Knapsack sprayer applying at spray volume 500 L/ha and dosage of propanil/2,4-D at 2.0 kg a.i./ha gave an average grain yield of rice of 3.47 t/ha

Untreated check gave an average grain yield of rice of 2.31 t/ha

CV (%) = 10.02

$LSD_{.05}$ of 0.43 t/ha was used to compare an average grain yield of rice between treatment combinations, knapsack sprayer and untreated check.

[1] Means followed by the same letter are not significantly different at 5 % level by DMRT.

The CDA sprayer with dosage of propanil/2,4-D at 2.0 and 1.8 kg a.i./ha, spray volume at 75 L/ha and propanil/2,4-D at 2.0 , 1.8 , 1.6 and 1.4 kg a.i./ha, spray volume at 150 L/ha gave grain yield there was no significant difference to knapsack sprayer in 2.0 kg a.i./ha of propanil/2,4-D and spray volume at 500 L/ha (Table 6). Grain yield was significantly different when the CDA sprayer was applied to propanil/2,4-D. The dosage rates of propanil/2,4-D at 2.0 and 1.8 kg a.i./ha gave higher grain yield. Grain yield in 75 and 150 L/ha of spray volume were not significantly different.

Results of this indicate that the increased efficiency of the mixture between propanil and 2,4-D, propanil is a contact herbicide, were applied by the CDA sprayer was due to higher spray volume. Both propanil and 2,4-D are postemergence herbicides. Further research needs to be conducted to assess in the use of CDA sprayer with early-postemergence herbicides on weed control.

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POSTEMERGENCE APPLICATION OF BROADLEAF HERBICIDES ON GROWTH OF ONION (*Allium cepa* L.) AND GARLIC (*A. sativum* L.)

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Abstract: Effect of herbicides namely; lactofen, fomesafen, chlorimuron ethyl, metsulfuron methyl, imazethapyr and 2,4-D were foliar applied at low and high rate on onion seedling at 20 days after transplanting. Garlic at 20 days after planting by cloves were treated with the same herbicides at low rate, including oxyfluorfen and oxadiazon at 150 and 500 g ai./ha. Greenhouse study in a completely randomized design with 3 replication were conducted at Weed Science Group to determine the effect of herbicides on growth of onion and garlic. Phytotoxicity of herbicides to onion was in the order at lactofen = fomesafen < imazethapyr = chlorimuron ethyl < metsulfuron methyl < 2,4-D. Herbicides at high rate were more toxic to onion than at low rate. Onion were recovered after treated with fomesafen and lactofen for 20 days. Plant number and plant height at 40 days indicated significant difference among treated plots. The best result was obtained from lactofen at 90 and 120 g ai./ha and fomesafen at 250 and 312.5 g ai./ha. They provided hightly significant level of bulb fresh weight and bulb diameter difference from other treated onion and untreated check. Phytotoxicity of herbicides to garlic was in order at imazethapyr < lactofen < oxadiazon < oxyfluorfen < fomesafen <metsulfuron methyl < chlorimuron ethyl < 2,4-D. Toxic symptom were showed in all treated except imazethapyr at 5 days after treated. Imazethapyr was slighly toxic to garlic. Lactofen, oxadiazon, oxyfluorfen and fomesafen showed the same bleaching symptom. Onion is more tolerance to herbicides than garlic. Garlic in imazethapyr treatement was provided the most highest growth and yield. Lactofen, oxyfluorfen, untreated check, fomesafen respectively garlic yield were not different.

Key words: bleaching herbicides, sulfonylurea, imdazolinone, postemergence, amaryllis crops

Introduction

Onion (*Allium cepa* L.) is in amaryllidaceae family. In Thailand, onion seed is expensive. Usually commercial grower prepared onion seedling and transplanted at 45 - 60 days. Weeds are serious problem in onion as a small, hard seeded crop. It is difficult to germinate, plus a very little canopy during the germination and early growth stages. It can not shade the soil to suppress weeds growth. (Kurtz, 1985) This is a good time for control weeds by using postemergence herbicides.

Garlic (*Allium sativum* L.) is the same family as onion. It is biennial herb normally grown for its bulb. Bulb separated into small bulb, or clove formed axillaly bud. Farmer grown garlic from cloves. (Tindall, 1983) Garlic cloves produce vigorously growing plants, but nevertheless, garlic is susceptible after emergence to plant stunting from weed competition. (Kurtz, 1985) Hand weeding was the main method to control broadleaf weeds in garlic.

Lactofen, fomesafen, oxyfluorfen and oxadiazon are the photobleaching herbicides. They can be applied as postemergence. (Cobb, 1992) Oxyfluorfen is a selective postemergence herbicide in onion at > 2 - 3 leaf stage. While lactofen in cotton and soybean, where as fomesafen in soybean. (Ashton and Monaco, 1991) In Thailand, Sermsiri and Kleopan (1997) reported that oxyfluorfen at 150 g ai./ha and oxadiazon at 500 g ai./ha were used as postemergence on onion at 2 to 3 weeks after transplanting. They controled broadleaf weeds especially common purslane (*Portulaca oleracea* L.) and horse purslane (*Trianthema portulacastrum* L.). In China, Guang (1995) reported that oxyfluorfen can be applied on garlic to control broadleaf weeds during preemergence to 1 - leaf stages or 2.5 - 3.5 leaf stages. It was safe to garlic. Chlorimuron ethyl and mesulfuron methyl are selective postemergence herbicides in soybean. However, imazethapyr is broad spectrum herbicide use in soybean and other leguminous crops. It can applied preemergence and early postemergence and provided exellent weed

control. Since 2,4-D is certain broadleaf herbicide. (Ashton and Monaco, 1991) It was used to observe injury symptom.

Materials and Methods

Two experiments were conducted in greenhouse at Weed Science Group during November 1996 to March 1997. Treatments were arranged in a completely randomized design with three replications. Herbicides were applied by knapsack sprayer with a spray volume of 333 L/ha. Crop injury were determined visual rating 0 - 10: whereas 0 = no crop injury, 10 = crop death. Plant growth: plant number, leaf number, plant height were mesured at 40 days after treated

Postemergence herbicides application on growth of onion.

Onion seeds were sown in seedbed and transplanted into the 26 - cm diameter pot at 60 days after sowing nine plants per pot. Foliar herbicide application were treated at 20 days after transplanting. Herbicides namely; lactofen (Cobra 24 % EC) at 90 and 120 g ai./ha, fomesafen (Flex 25 %EC) at 250 and 312.5 g ai./ha, chlorimuron ethyl (Classic 25 %WP) at 91.6 and 106.6 g ai./ha, metsulfuron methyl (Ally 20 %WP) at 24.9 and 33.3 g ai./ha, imazethapyr (Pursuit 5 %AS) at 100 and 125 g ai./ha and 2,4-D ester (Deshomone-ET 72 %EC) at 750 and 1000 g ai./ha compared with untreated check. The study were rated for crop injury at 1, 5, 20 and 30 days after treated. Harvesting was done at 100 days after transplanting. Onion bulb weight and diameter were mesured.

Postemergence herbicides application on growth of garlic.

Garlic cloves were planted in a 34 by 46 cm pot thirty cloves per pot. Foliar herbicide application were treated at 20 days after planting. Eight herbicides, they were lactofen at 90 g ai./ha, fomesafen at 250 g ai./ha, oxyfluorfen (Goal 23.5%EC) at 150 g ai./ha, oxadiazon (Ronstar 25 EC 25%EC) at 500 g ai./ha, chlorimuron ethyl at 91.6 g ai./ha, metsulfuron methyl at 24.9 g ai./ha, imazethapyr at 100 g ai./ha and 2,4-D ester at 750 g ai./ha compared with untreated check. Crop injury were rated at 5, 20, 30 and 40 days after treated. Garlic were harvested to mesure weight at 70 days after planting

Results and Discussion

Postemergence herbicides application on growth of onion.

Table 1 showed phytotoxicity rating of herbicides. At one day after applied herbicides, treated onion at low and high rate of all treated herbicides except 2,4-D showed slighly phytotoxic, where as 2,4-D treated onion provided moderatedly phytotoxic. After that the injury symptom of all treated onion were increased at 5 to 20 dsys Lactofen and fomesafen caused the same symptom that onion leaves occured bleaching spot. Area of injury due to fomesafen was showed more phytotoxic than lactofen. The symptom was found only on the leaves those exposed at herbicide application time, the new leaves were normal.

Chlorimuron ethyl, metsulfluron methyl and imazethapyr treated onions injury symptom slighly increase to moderately after applied for 20 days, after that plants recovered to normal. These results corresponded with Cobb (1992) that sulfonylurea treated plant growth is retarded or inhibited within houre of foliar treatment but physical symptoms may take days to develop.The young plants were more susceptible to herbicides than the old one. 2,4-D was severely toxic to onion. Furthermore, high rate of herbicides were more toxic to the plants than low rate.

Table 1 Visual injury rating of herbicides on onion. Herbicides were applied at 20 days after transplanting.

Herbicides	Rate (g ai./ha)	Onion visual injury rating (days after application)			
		1	5	20	30
Lactofen	90	1.5	2	1.5	0
Lactofen	120	1.5	3	1.5	0
Fomesafen	250	1.5	5	3	0
Fomesafen	312.5	1.7	7	3	0
Chlorimuron ethyl	91.6	1.5	1.5	3	1
Chlorimuron ethyl	106.6	1.5	3	4	1
Metsulfuron methyl	24.9	1	1.5	3	1
Metsulfuron methyl	33.3	1.5	2	4	1
Imazethapyr	100	0	1.5	3	1
Imazethapyr	125	0	2	4	1
2,4-D ester	750	3.5	4	7	2
2,4-D ester	1,000	4	5	7	2.5
Untreated check		0	0	0	0

Rating were taken on a scale from 0 - 10 ; 0 = no crop injury, 10 = crop death.

Table 2 recorded onion growth and yield. The results showed that growth of onion did not affect by lactofen at 90 and 120 g ai./ha and fomesafen at 250 and 312.5 g ai./ha.Leaf number, plant number and plant height at 40 days after application, bulb weight and diameter at harvest (100 days after trnsplanting) all indicted the same high level. There at, they provided the highest yield significant different from other treatments. Chlorimuron ethyl and metsulfuron methyl at low and high rate and imazethapyr at low rate provided the same level growth as untreated check. 2,4-D gave the lowest yield.

Table 2 Effect of herbicides on plant number, leaf number and plant height at 40 days after application, and onion yield at harvest. (100 days after transplanting)

Herbicides	Rate (g ai./ha)	Plant no.	Leaf no.	Plant ht. (cm)	Bulb wt. (g/pot)	Bulb diameter (cm)
Lactofen	90	7.0 a-d	4.8 a	35 a	173 a	3.12 a
Lactofen	120	6.8 a-d	4.4 a	31 a	143 a	2.94 a
Fomesafen	250	7.0 a-d	4.4 a	32 a	160 a	3.04 a
Fomesafen	312.5	7.3 a-d	4.6 a	32 a	180 a	3.18 a
Chlorimuron ethyl	91.6	6.5 a-d	4.3 ab	23 b	60 b	2.10 b
Chlorimuron ethyl	106.6	7.0 a-d	3.7 ab	19 b	58 b	2.07 b
Metsulfuron methyl	24.9	4.8 d	4.0 ab	17 b	34 b	1.94 b
Metsulfuron methyl	33.3	6.0 bcd	3.7 ab	17 b	38 b	1.92 b
Imazethapyr	100	6.0 bcd	4.1 ab	21 b	52 b	2.19 b
Imazethapyr	125	6.3 bcd	3.7 ab	19 b	43 b	1.88 b
2,4-D ester	750	5.5 cd	3.3 ab	21 b	32 b	1.70 b
2,4-D ester	1,000	5.3 cd	2.5 c	19 b	34 b	1.76 b
Untreated check		8.3 ab	3.6 ab	23 b	72 b	2.10 b
CV (%)		16.1	17.5	20.4	59.2	22.9

Means followed by the same letter are not significantly different at 5 % level by DMRT.

Postemergence herbicides application on growth of garlic.

Table 3 showed phytotoxic rating of herbicides on garlic. All herbicides treated garlics except imazethapyr provided various crop injury after application. Garlic seemed to tolerance to imazethapyr. At five days after applied lactofen, fomesafen, oxyfluorfen and oxadiazon, photobleaching area on garlic leaves were no frontier. The symptom was different from onion which were restricted. This might be due to more wax covered on leaf surface of onion than garlic. Furthermore, onion leaves are cylindrical, hollow and uplight. Where as garlic leaves are flattened and solid, shoot is the part of leaf sheath conected with leaves blade. At leaf collar more spray droplet could be retained on garlic than onion. Since diphenyl ethers and oxadiazole are contact herbicides. (Cobb, 1992) So that garlic was susceptible to these herbicides than onion.

Table 3 Visual injury rating of herbicides on garlic. Herbicides were applied at 20 days after planting.

Herbicides	Rate (g ai./ha)	Garlic visual injury rating (days after application)			
		5	20	30	40
Lactofen	90	4	2	1.5	1
Fomesafen	250	6	5	4	3
Oxyfluorfen	150	4	4	3	3
Oxadiazon	500	2.5	3	3	3
Chlorimuron ethyl	91.6	3	5	6	7
Metsulfuron methyl	24.9	2	2	4	6
Imazethapyr	100	0	2	2	0
2,4-D ester	750	6	7	7	8
Untreated check		0	0	0	0

Rating were taken on a scale from 0 - 10 ; 0 = no crop injury, 10 = crop death.

Lactofen treated garlic were recovered faster than the other. While the other still occured injury more than 40 days after application. Chlorimuron ethyl and metsulfuron methyl after applied caused slighly toxic to garlic and slowly increase to severely toxic. 2,4-D was severely toxic to garlic, it caused yellow curl leaves and root also curl. New leaves development would continue. Late germinated garlic were more susceptible to herbicides than the early germinated garlic.

Table 4 Effect of herbicides on plant number, plant height and leaf number at 40 days after application. Garlic yield were harvested at 70 days after planting.

Herbicides	Rate (g ai./ha)	Leaf no.	Plant no.	Plant ht. (cm)	Yield (g/pot)
Lactofen	90	4.7 abc	29 a	36 ab	20.1 ab
Fomesafen	250	4.1 bc	19 b	35 abc	15.3 ab
Oxyfluorfen	150	5.4 a	30 a	33 abc	17.3 ab
Oxadiazon	500	5.5 a	27 a	32 abc	14.6 b
Chlorimuron ethyl	91.6	3.8 cd	2 c	28 c	3.2 c
Metsulfuron methyl	24.9	4.1 bc	10 c	37 a	7.5 c
Imazethapyr	100	4.3 bc	29 a	29 bc	21.9 a
2,4-D ester	750	3.1 d	5 c	33 abc	2.2 c
Untreated check		4.9 ab	28 a	29 abc	16 ab
CV (%)		8.8	17	9.3	20.1

Means followed by the same letter are not significantly different at 5 % level by DMRT

Garlic growth were recorded at 40 days after application. Oxyfluorfen, oxadiazon and lactofen treated garlic gave the same high leaf number as untreated check. Where as chlorimuron ethyl and 2,4-D gave low leaf number. The result of garlic plant number and yield were lowest, it correlated to phytotoxic symptom, because majority of garlic plants treated with chlorimuron ethyl, 2,4-D and metsulfuron methyl were died.

Garlic yield were harvested at 70 days after planting. The highest yield was obtained from garlic treated with imazethapyr. The same high yield was in order at lactofen > oxyfluorfen> untreated check > fomesafen .

In conclution, our finding suggested that :

1. Onion : Lactofen at 90 and 120 g ai./ha and fomesafen at 250 and 312.5 g ai./ha applied as postemergence at 20 days after transplanting the 60 days onion seedling were gave the good result on growth of onion. They ocurred slighly toxic after treated untill 20 days, these did not effect on onion yield.

2. Garlic : Postemergence application of herbicides at 20 days after planting garlic cloves, imazethapyr at 100 g ai./ha and lactofen at 90 g ai./ha were provided satisfactory on garlic growth and yield.

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3.2 Physiology and Biochemistry

ISOLATION AND CHARACTERIZATION OF GLUFOSINATE TOLERANCE IN SOYBEAN CELL LINES

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Abstract: Selection of soybean (*Glycine max* L. cv. SJ 4) cell lines tolerant to glufosinate was attempted using cell suspension cultures induced from hypocotyls of young seedlings. The cell suspension was cultured on MS basic medium supplemented with B5 vitamins, 0.3% sucrose, and 10 mg/l NAA. Using a stepwise selection with increasing concentration of herbicide, a soybean cell line tolerant to 10^{-7} M glufosinate was obtained through direct selection of diploid cells in the suspension culture. It is referred to as 10^{-7} M glufosinate-tolerant soybean cell line. Determination of ammonia concentration indicated that the normal (non-tolerant) cells accumulated the amount of ammonia up to 15 folds more than the herbicide-treated tolerant cells. This information suggested that lower ammonia accumulation in the tolerant cells can be used as an indicator to select for glufosinate-tolerant soybean cell lines.

Key words: ammonia accumulation, cell suspension culture, glufosinate, soybean

Introduction

Glufosinate is a potent inhibitor of the biosynthetic enzyme glutamine synthetase (GS), which involves in general nitrogen metabolism in plants, including the assimilation of ammonia accumulated as a result of photorespiration and nitrate reduction (Devine *et al.*, 1993). However, the accumulation of ammonia caused by GS inhibitors was thought to be the principal cause of phytotoxicity in the plants (William, 1994).

We have obtained glufosinate-tolerant soybean cell lines from suspension cultures. So far no information is available on derivation of glufosinate tolerance from selected cell lines. Properties of the ammonia accumulation in plant cells are important factors affecting differential response to the herbicide between normal (non-tolerant) and tolerant cells. The availability of glufosinate-tolerant soybean cell lines should be of great agronomic interest and expand the use of the herbicide, which is now limited due to its non-selective mode of action. In the present study, we report the results of the selection of soybean cells tolerant to glufosinate, and their ammonia accumulation as an indicator of the tolerance.

Materials and Methods

Herbicide treatment

Technical-grade glufosinate {2-amino-4-(hydroxymethylphosphinyl) butanoic acid, purity 94.4%}, the ammonium salt from, was dissolved in ethanol and sterilized with a membrane filter. The herbicide as selective agent was added to the sterilized culture medium immediately before subculturing the cells with a final ethanol concentration in the medium at the maximum of 0.1%(v/v).

Cell suspension culture

Soybean cultivar "SJ 4" was used in the experiment. Callus was induced from hypocotyls of young seedlings. A friable callus was selected from compact organogenetic calli and subcultured on the fresh medium every 2 weeks. After 4 subcultures, about 10 g of fast growing callus was transferred into the MS (Murashige and Skoog, 1962) liquid medium supplemented with B5 vitamins, 0.3% sucrose, and 10 mg/l NAA (pH 5.8) on a gyrating shaker (120 rpm) at 28 °C. Subculturing was done at 10-day intervals by transferring 1 ml of pack cell volume (PCV) cells to fresh medium (50 ml). Cell growth reached a maximum at the 10th day. A 10-fold increase in PCV during each 10 day period was repeatedly obtained.

Selection of herbicide-tolerant cells

At first, the lowest concentration of the herbicide was used to treat the cells. Surviving cells were subcultured at 10 day intervals by transferring them to fresh medium with the same concentration of the herbicide. When the cells had reached the same growth rates as that of non-treated cells, they were transferred into a medium containing a progressively higher concentration of the herbicide. This process was repeated for several passages until extremely tolerant cells were obtained.

Ammonia accumulation test

The ammonia accumulation and its inhibition by glufosinate were compared between the normal and tolerant cells according to the modified procedures of Weatherburn (1967). Both types of cells were treated with glufosinate at the concentration of 10^{-10}-10^{-7} M. After subculturing, the cells obtained from day 0, 3, 5, 7, and 10 were used for the assay. All extraction procedures were carried out according to Desmaison *et al.* (1984). Ammonia accumulation was expressed as micrograms of ammoniacal nitrogen per gram of fresh weight and converted to percentages of control. Data were analyzed using analysis of variance, and means were separated by least significant difference at $P=0.05$.

Results and Discussion

Selection of herbicide-tolerant cells

Initial attempts to select for glufosinate-tolerant cell lines was performed on soybean cell suspension cultures. Growth of the cells were inhibited in accordance with the concentration of the herbicide. The growth was completely stopped by 10^{-5} M glufosinate. When the cells were treated with 10^{-9} M glufosinate, their growth rates were slightly reduced. This concentration was used as the initial selection pressure. The tolerant cell lines were isolated by sequencial transfers into media with the same or higher concentration of the herbicide. The cells grown in 10^{-7} M glufosinate were successfully isolated. The period required for selection was 8 months (data not shown). It is referred to as 10^{-7} M glufosinate-tolerant soybean cell lines.

The growth response of glufosinate tolerant cells was determined at different concentrations ranging from 10^{-9} to 10^{-5} M (Fig. 1). In normal cells, 50% growth inhibition occurred at $5x10^{-8}$ M, whereas the tolerant cells grew successively in the presence of 10^{-6} M glufosinate in which the normal cells did not survive. This indicated that the tolerant cells were 20-folds more tolerant to the herbicide than the non-selected normal cells (as measured by the ratio of tolerant I_{50} to normal I_{50}, Table 1). These results were consistent with those of the previous report, in which tolerant soybean cells had been selected from the cells or tissue cultures subjected to clomazone (Norman *et al.*, 1990), atrazine (Wrather and Freytag, 1991), fluazifop-P-butyl, imazaquin, and oxyfluorfen (Pornprom *et al.*, 1994b).

Ammonia accumulation test

In this study, the glufosinate-tolerant soybean cell lines were obtained through direct selection of diploid cells in the suspension cultures. As more glufosinate-tolerant lines became available, the mechanism of herbicide tolerance in relation to ammonia accumulation, can be compared between the cell lines (Fig. 2). Accumulation of ammonia from both cell lines increased with the increasing concentration of glufosinate herbicide throughout the time course of the experiment. However, the tolerant cells was less sensitive than the normal cells. The I_{50} concentrations of glufosinate on the ammonia accumulation from the normal and tolerant cells were $5x10^{-9}$ and $7.5x10^{-8}$ M glufosinate, respectively (Table 1). This indicates that the accumulation of ammonia differed by a factor of 15 folds between the normal and tolerant cells. Its accumulation in the herbicide treated tolerant cells was less sensitive than that from the normal cells. It is reasonable to suggest that a less sensitive to glufosinate confers the tolerance to these cells, particularly the tolerant cells.

Variation in ammonia accumulation between the cells contributed to alteration in the target site on the more tolerant cell lines. These effects may be directly related to accumulation of ammonia by glufosinate. The results were consistent with the reports on several inhibitors-tolerant crops, including soybean cells tolerant to clomazone (Norman *et al.*, 1990), and oxyfluorfen (Pornprom *et al.*, 1994a). In addition, there were several reports on the mechanisms of herbicide tolerance both in the whole plants (Botterman *et al.*, 1991; and Sankula *et al.*, 1998) and culture cells (Racchi *et al.*,

1995). In these studies, the tolerance apparently was due to an alteration in the target site, which correlated with a physiological and biochemical basis of the tolerance. This finding supported the view that accumulation of ammonia caused by GS-inhibitors was thought to be the principle cause of cytotoxicity in the plant cells. This suggests that one of the mechanisms for glufosinate-tolerant soybean cell lines is reduced ammonia accumulation in the tolerant cells. However, there may be other possible mechanisms as well.

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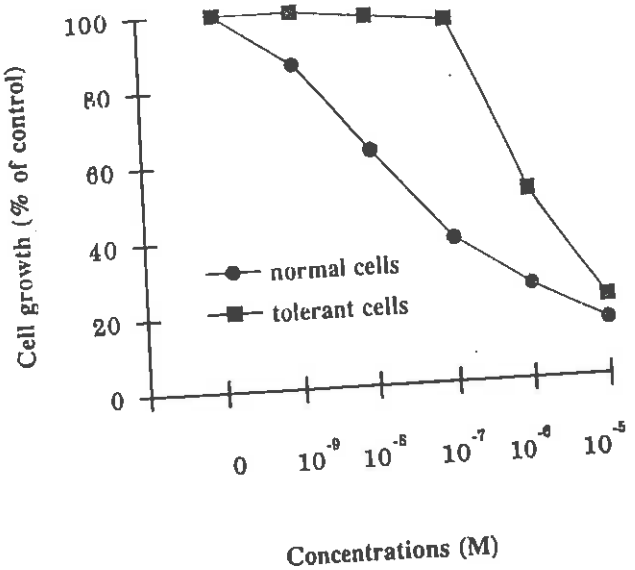

Fig. 1 Growth response to glufosinate of normal and tolerant soybean cells determined 10 days after herbicide treatment.

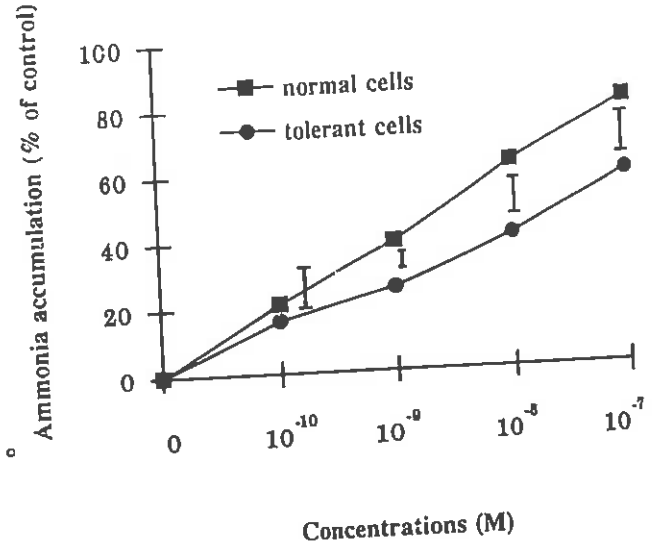

Fig. 2 Effect of glufosinate on ammonia accumulation in the normal and tolerant soybean cells. Each data point represents the average of 3 replicates. The vertical bars represent $LSD_{.05}$ at each concentration.

Table 1 I_{50} for glufosinate determined from the growth response and ammonia accumulation of the normal and tolerant soybean cells.

Cells	I_{50} (M)[b/]	
	Growth response	Ammonia accumulation
Normal	$5x10^{-8} \pm 0.75$	$5x10^{-9} \pm 0.55$
Tolerant	$10^{-6} \pm 1.05$	$7.5x10^{-8} \pm 0.90$
T/S[a/]	20	15

[a/] T/S = I_{50} tolerant/I_{50} normal was calculated to indicate the degree of tolerance.
[b/] Values represent the I_{50} mean ± SEM of three independent experiments.

TOLERANCE MECHANISM OF PEPPER CULTIVARS TO BENTAZON

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Abstract: Hyangchon was selected as susceptible cultivar and Singsinghouse was selected as tolerant cultivar to bentazon among 44 pepper cultivars. Singsinghouse was about 2-fold more tolerant to bentazon than Hyangchon. The difference in inhibition of quantum yield in PSII between cultivars was being rapidly increased with time and Singsinghouse was recovered completely at below 1.2kg ai/ha and was recovered considerably at the higher concentrations, but Hyangchon was still greatly inhibited at lower concentrations 1 week after bentazon treatment. Minor differences in absorption and translocation occurred between cultivars which were not correlated with tolerance, and limited amount of absorbed bentazon was mainly translocated to shoots. Singsinghouse metabolized most of the absorbed bentazon within 3 days and the metabolic rates of it were generally more rapid than those of susceptible cultivar. These results suggest that the tolerance mechanism of pepper cultivars to bentazon mainly relates to differential rates of metabolism of bentazon to nonphytotoxic compounds.

Key words: bentazon, pepper cultivar, tolerance, absorption, translocation, metabolism.

Introduction

Bentazon inhibits photosynthesis by binding to the Q_B-binding niche on the D1 protein of PSII complex in chloroplast thylakoid membranes, thus blocking electron transport and producing a lipid radical and initiating a chain reaction of lipid membrane peroxidation. It primarily controls annual broadleaf weeds in soybeans, peanuts, rice, corn and sorghum (Ahrens, 1994)). The main reason for the considerable success of modern herbicides such as bentazon is their selective phytotoxic action. But selectivity is a very relative term and totally dependent on dose, such that an application rate alone may determine crop tolerance and weed susceptibility. Connelly *et al.* (1988) reported that tolerance is clearly differentiated among species and even cultivars.

The objectives of this study were to determine the differential tolerance mechanism of pepper cultivars to bentazon by examining absorption, translocation and metabolism.

Materials and Methods

Selection

Plants were grown up to the six-leaf stage in pots (4x5 cm) containing loam soil before experiments. A preliminary experiment was conducted to assess the degree of variability in bentazon tolerance among 44 Korean pepper cultivars in greenhouse (35/25 ± 5°C, day/night) in mid-summer. Bentazon was treated at rates of 0, 0.6, 1.2, 3.4 and 6.7kg ai/ha. Plant height and shoot fresh weight were measured at 15 days after treatment. Injury of pepper was determined by visual observation at 5 days

interval.

The second experiment was conducted to compare the bentazon tolerance levels of selected pepper cultivars, Hyangchon and Singsinghouse in the growth chamber (30/20°C, day/night). Bentazon rates were 0.1, 0.3, 0.6, 1.2, 2.3, 3.4, 4.5, 5.6, 6.7 and 7.8kg ai/ha. Shoot fresh weight was measured at 15 days after treatment. Bentazon rates required to give I_{50} were estimated using linear regression analysis.

Chlorophyll fluorescence assay

Selected pepper cultivars were placed in greenhouse (30/20°C, 460 μmol/m²/sec) after being treated with 0.3, 0.6, 1.2, 2.3, 3.4, 4.5 and 5.6 kg ai/ha bentazon. Photosynthetic fluorescence parameters such as yield (the total photochemical quantum yield in PS II), Fm' (the maximal fluorescence yield of a preilluminated sample induced by a saturation pulse), and Ft (the fluorescence yield given at a time t) were measured with a portable chlorophyll fluorometer (PAM-2000, Walz) at 3 hours, 1 day, 2 days and 1 week after spraying bentazon. Quantum yield of PSII was calculated as following formula, yield = (Fm'-Ft)/Fm'.

Absorption and translocation

Plants (six-leaf stage) grown in sand were sprayed with 0.3 kg ai/ha bentazon prior to ^{14}C-treatment. The ^{14}C-bentazon(0.1% Tween 20) was applied in 10 droplets (specific activity: 0.05 μCi/plant) to the centers of the 3rd leaf of plants. Plants were placed in the growth chamber for 6, 12, 24, 48, 72 and 96 hours. The treated leaf was excised and the remaining plant part was sectioned into three parts, above and below the treated leaf and root. The treated leaf was sequentially rinsed with 10% acetone and distilled water. The washed solutions were combined for determination of radioactivity by liquid scintillation counter (Packard Tricarb 2100TR, LSC). Oven-dried plant material was weighed and oxidized in sample oxidizer (Packard Tricarb model 307) and then determined ^{14}C-activity by LSC.

Metabolism

Plants were treated with bentazon and ^{14}C-bentazon to the 3rd and 4th leaves (specific activity: 0.1 μCi/plant). The treated leaves were excised and rinsed 24 and 72 hours after treatment as described above. ^{14}C-treated leaves were extracted in 10㎖ of 90% methanol with liguid nitrogen gas in pestle and mortar. The extract was centrifuged for 20 min at 10,000g and was decanted a volumetric test tube and brought up to 10㎖. An aliquot (0.5㎖) of the supernatant was removed for determination of radioactivity by LSC. The remainder of supernatant evaporated with nitrogen gas, and then the aqueous sample was filtered (0.2 μm pore size), loaded on C_{18} catridge. The eluate was re-evaporated and dissolved in 200μl of 100% methanol. The sample was analyzed with HPLC and FSA (Flow scintillation analyzer). An aliquot was injected onto C_{18} column (3.9 x 300 mm, Nova-pak). Solvent (acetonitrile, 0.1% acetic acid, 20:80 to 60:40 in 30 min, v/v) was delivered at 1㎖/min.

Results and Discussion

Susceptible cultivars showed damages such as desiccation, chlorosis and necrosis, but tolerant cultivars showed only the reduction of leaf number and inhibition of growth, whereas most of other cultivars were dead. Reduction of fresh weights was greater than that of plant heights in many cultivars. As the I_{50} of Singsinghouse was 5.85 kg ai/ha and that of Hyangchon was 2.66 kg ai/ha, Singsinghouse was about 2-fold more tolerant than Hyangchon. Therefore, Singsinghouse was selected as tolerant cultivar and Hyangchon as susceptible cultivar for bentazon tolerance study.

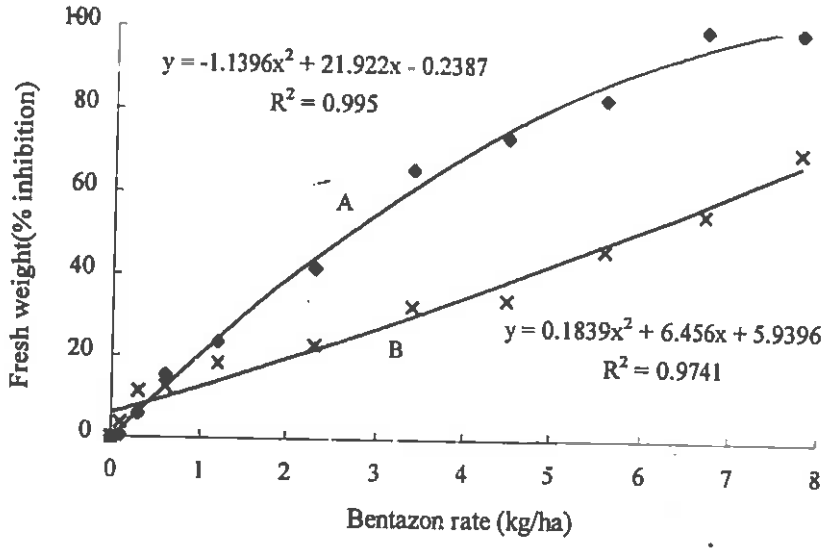

Fig. 1 Regression of percent inhibition of fresh weight of Hyangchon, susceptible cultivar(A, I_{50} = 2.66 kg ai/ha), or Singsinghouse, tolerant cultivar(B, I_{50} = 5.85 kg ai/ha) and application rate of bentazon

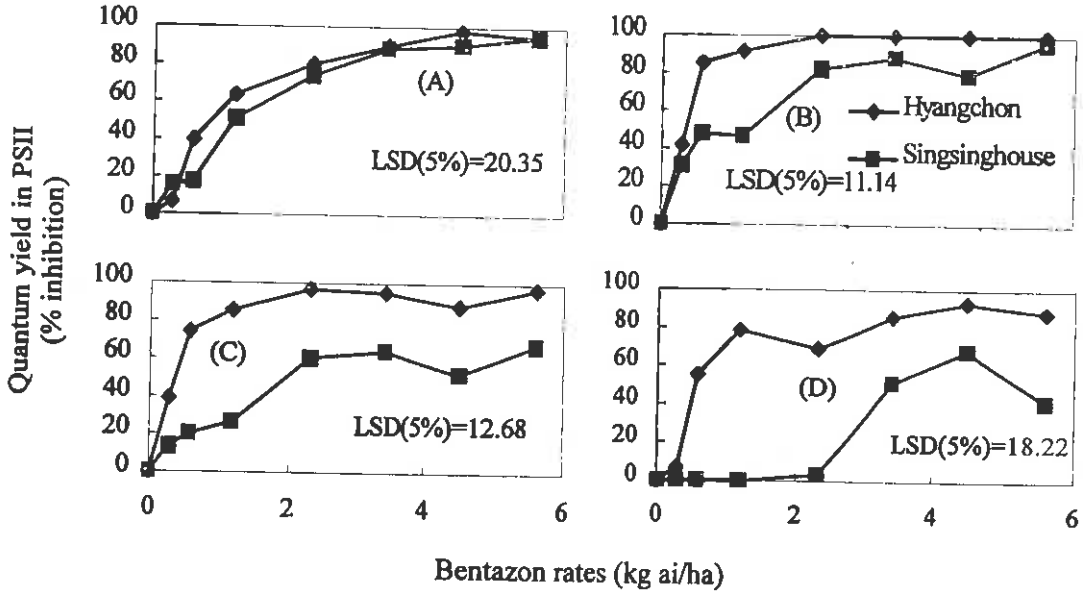

Fig. 2 The effect of bentazon rates on inhibition of quantum yield in PSII in pepper cultivars over times (A: 3 hours, B: 1 day, C: 2 day, D: 1 week).

The difference in quantum yield inhibition of PSII measured by chlorophyll fluorescence meter was not appeared apparently until 24 hours after bentazon treatment. The inhibition rates of both cultivars were more increased at 24 hours after treatment, but that of Singsinghouse was lower than that of Hyangchon. While the inhibition rate of Singsinghouse was reduced by the extent of 15-30% at all concentrations, Hyangchon was still significantly inhibited at 48 hours. One week after treatment, Singsinghouse was completely recovered at below 1.2kg ai/ha and recovered considerably at the higher

concentrations. Although the susceptible cultivar was being recovered, its degree was apparently lower compared to the tolerant cultivar, Singsinghouse.

Both species absorbed ^{14}C-bentazon in the treated leaves at the similar extent. The amount of ^{14}C-bentazon moved from the treated leaves into other plant parts also increased with time and was very similar at both species. Most of the ^{14}C-activity absorbed remained in the treated leaf of both species and was hardly translocated into the roots. This was similar to the study of Baltazar and Monaco (1984) in pepper cultivars. Similar trends in ^{14}C-uptake and translocation in both cultivars suggest that absorption and distribution of bentazon cannot account for the observed tolerance of pepper to bentazon.

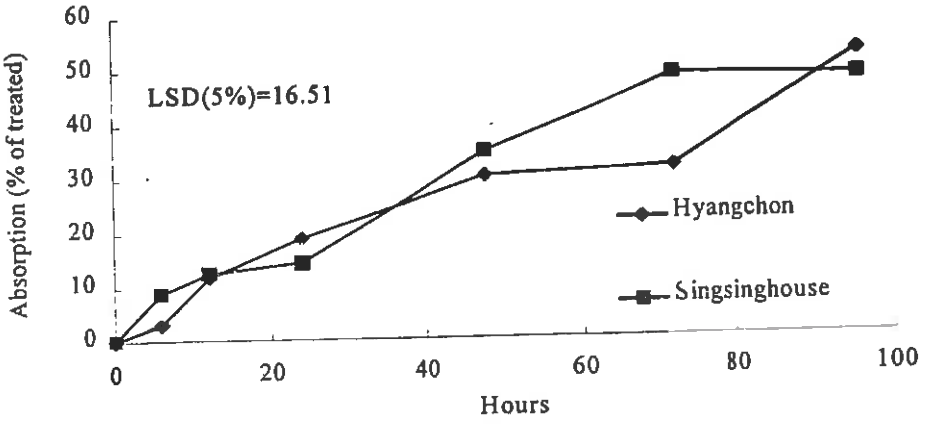

Fig. 3 Absorption of foliar-applied ^{14}C-bentazon at tolerant and susceptible cultivars over times.

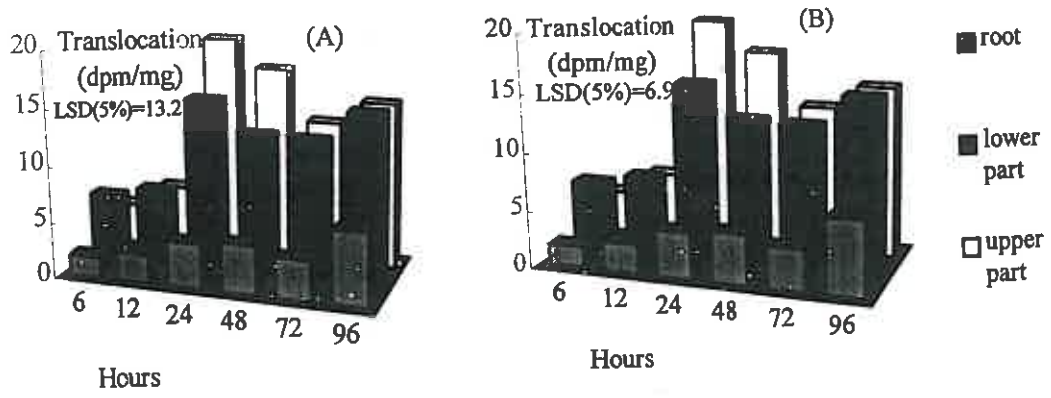

Fig. 4 Translocation of ^{14}C-bentazon out of the treated-leaf to other parts of Hyangchon(A) and Singsinghouse(B) cultivars.

Seventy three percent of the ^{14}C-bentazon was found in the treated leaves of Singsinghouse as metabolites, whereas 56.5% was found in the treated leaves of Hyangchon 24 hours after ^{14}C-bentazon application. Singsinghouse metabolized 93% of absorbed bentazon into detoxified compounds in comparison with 73% in Hyangchon 72 hours after application. These values indicate a faster rate of ^{14}C-bentazon metabolism in tolerant cultivar than in susceptible cultivar similar to the studies on pepper, rice

and soybean (Baltazar and Monaco, 1984; Clay and Oelke, 1988; Connelly *et al.* 1988). This suggests that tolerance mechanism between the two cultivars may be mainly based on differential rates of metabolism of bentazon which may detoxified by possibly cytochrome P-450.

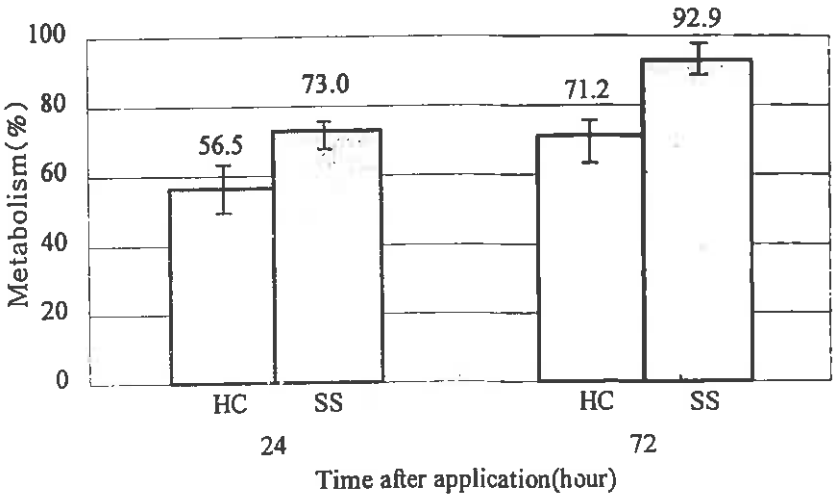

Fig. 5 The metabolic rate of bentazon in leaves of pepper cultivars.
HC; Hyangchon, SS; Singsinghouse

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3.3 Newly Developing Herbicides

DIMETHENAMID-A NEW HERBICIDE FOR THE USE IN VARIOUS SEEDED AND TRANSPLANTED CROPS IN ASIA

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Abstract: Dimethenamid 900 g a.i./l, EC (FRONTIER®) exhibited good to excellent selectivity to corn, sweet corn, peanut, soybean, pea, bean and radish when applied post-seeding as well as to cabbage, pepper, tomato, cucumber and eggplant when applied pre- or post-transplanting. Heavy rainfall directly after application of the pre-emergence herbicides increased the risk of phytotoxicity, especially on soil types with low cation exchange capacity (CEC). This can be avoided by applying dimethenamid post-planting in transplanted crops. Rates ranging from 1125 to 1350 g a.i./ha dimethenamid provided good selectivity and broad-spectrum weed control under the conditions of Southern Taiwan. At these rates dimethenamid controlled *Digitaria violascens, Eleusine indica, Setaria viridis, Echinochloa crus-galli, Leptochloa chinensis, Cyperus iria, Amaranthus spinosus and Portulaca oleracea* excellently and provided good control of *Acalypha australis, Ageratum conyzoides, Chenopodium ficifolium, Eclipta alba, Lindernia anagalis, Physalis angulata* and *Rorippa atrovirens.* Comparable performance was achieved with the resolved herbicidally active optical isomer dimethenamid-P at rates of 540 to 720 g a.i./ha.

Keywords: dimethenamid, pre- to post-emergence herbicide, seeded and transplanted crops

Introduction

Dimethenamid, the active ingredient of FRONTIER® (900 g/l r/s-dimethenamid, EC), belongs to the chemical class of chloroacetamides (mode of action: lipid biosynthesis inhibition). Dimethenamid is a broad-spectrum herbicide for the control of grasses, broadleaf weeds and sedges in a wide range of crops. Dimethenamid is characterized by lower use rates in comparison to other chloroacetamides and a favourable toxicological and eco-toxicological profile (Harr *et al.*, 1991). It offers a high degree of application timing flexibility, allowing applications to be made during an extended period from pre-emergence up to early post-emergence. Dimethenamid-P, the resolved herbicidally active optical isomer of r/s-dimethenamid, was included in some of the trials since it is under development. This paper summarises the results of field tests conducted during 1997-99 in Taiwan and describes the recommendations of use for dimethenamid under conditions which are with respect to climatic and growing conditions similar to those of Taiwan.

Material and Methods

Field trials for selectivity and herbicidal activity were designed as single crop or as multiple crop experiments. Randomised block design with three replicates was used in all trials and plot sizes ranged from 2-4 m^2. Treatments were applied using a knapsack sprayer equipped with a spray boom with 4 flat fan nozzles at a water volume equivalent to 300 litres/ha. Crop cultivation and fertilisation followed local standard practice. The trials were mostly located in Southern Taiwan with the majority of the tests conducted between September and May of the following year. The trial sites showed the following soil characteristics: pH-values from 7.4-8.7; soil types from sandy loam to sandy clay loam (texture 36-49 % sand, 16-30 % silt, 32-39 % clay); CEC (cation exchange capacity) from 10-20 M.E./100gm; organic matter from 0.9-1.5 %. Growth stages of crops and weeds were determined according to the BBCH code system (Stauss, 1990). Crop selectivity and herbicidal efficacy on weeds were assessed according EPPO guidelines (OEPP/EPPO, 1998). The following herbicides were used: Dimethenamid (900 g a.i./l, EC, „Frontier",), dimethenamid-P (720 g a.i./l, EC), pendimethalin (400 g a.i./l, EC, „Stomp"), alachlor (480 g a.i./l, EC, „Lasso").

Results

In Taiwanese agriculture, peanut, soybean, beans, pea, corn and sweet corn are among the most important seeded crops for upland conditions. A series of field experiments investigating the aspects of crop safety of post-sowing herbicide treatments in these crops was initiated. Table 1 summarizes the observed crop injury in percent for assessments made at about 1, 3 and 6 weeks after application of the herbicides. In about 50 % of the tests significant rainfall greater 30 mm was recorded within 2-5 days after application.

Table 1 Selectivity of dimethenamid (900 g a.i./l, EC) in different seeded crops (Pingtung, Southern Taiwan 1997-99)

Treatment		Dimethenamid			Pendimethalin	Alachlor
Rate: g a.i./ha		900	1125	1350	1200-1600	1440-1920
Crop	DAA	% injury (no. of trials)				
Corn	7-10	1 (7)	3 (4)	2 (7)	2 (7)	4 (4)
	20-23	2 (7)	2 (4)	3 (7)	11 (7)	3 (4)
	35-42	1 (6)	1 (3)	1 (6)	1 (6)	1 (3)
Sweet corn	7-10	0 (3)	0 (3)	1 (3)	0 (3)	0 (3)
	20-23	2 (3)	2 (3)	3 (3)	29 (3)	3 (3)
	35-42	0 (2)	0 (2)	0 (2)	0 (2)	0 (2)
Soybean	7-10	1 (4)	4 (4)	7 (4)	5 (4)	6 (3)
	20-23	1 (4)	2 (4)	3 (4)	2 (4)	2 (3)
	35-42	0 (4)	1 (4)	2 (4)	3 (4)	2 (3)
Peanut	7-10	6 (5)	8 (4)	9 (5)	4 (5)	7 (3)
	20-23	3 (5)	4 (4)	6 (5)	2 (5)	3 (3)
	35-42	3 (5)	2 (4)	6 (5)	3 (5)	2 (3)
Bean	7-10	1 (6)	3 (3)	4 (6)	1 (6)	4 (2)
	20-23	5 (6)	7 (3)	17 (6)	13 (6)	2 (2)
	35-42	8 (6)	4 (3)	18 (6)	17 (6)	2 (2)
Pea	7-10	2 (4)	4 (3)	3 (4)	1 (4)	1 (3)
	20-23	2 (4)	2 (3)	3 (4)	5 (4)	1 (3)
	35-42	6 (4)	1 (3)	9 (4)	5 (4)	1 (3)
Radish	7-10	0 (6)	0 (3)	0 (6)	1 (6)	1 (3)
	20-23	1 (6)	0 (3)	1 (6)	30 (6)	0 (3)
	35-42	2 (5)	0 (3)	3 (5)	18 (4)	0 (3)

DAA = days after application

At rates of 900 –1350 g a.i./ha, dimethenamid exhibited very good selectivity in corn. At their respective recommended rates, the reference product alachlor was comparably selective, whilst pendimethalin caused more symptoms of crop injury in corn at 3 weeks after application. Dimethenamid also showed a good safety margin for the use in sweet corn, which was comparable to the reference herbicide alachlor. The dinitroanilide herbicide pendimethalin was more phytotoxic to this crop. Crop injury ratings for dimethenamid were generally slightly higher in peanuts, beans and peas, in particular when heavy rainfall occurred within few days after application of the herbicides. The high rate of dimethenamid as well as the reference products caused clear symptoms of crop injury in these crops under such conditions. Therefore, a rate of 1125 g a.i./ha dimethenamid is considered the maximum tolerable rate in these crops, providing excellent weed control and acceptable to good crop selectivity.

Among the transplanted crops, cabbage, tomato and cucurbits are of major importance in Taiwanese agriculture. Dimethenamid was tested for pre-planting application with the results obtained from this trial series (tomato, cabbage, pepper, sweet pepper, cucumber and eggplant) compiled across all trials in Table 2. In all tested crops, dimethenamid showed acceptable selectivity at rates up to 1350 g a.i./ha. Slight growth inhibition was observed for the reference herbicide as well as for the highest tested rate of dimethenamid in some of the crops, but this was of temporary, transient nature and usually outgrown by the crop without affecting yields.

Table 2 Selectivity of dimethenamid (900 g a.i./l, EC) , applied pre-planting, in different transplanted crops (Pingtung, Southern Taiwan 1997-99)

Treatment		Dimethenamid			Pendimethalin
Rate: g a.i./ha		900	1125	1350	1200-1600
Crop	DAA	% injury (no.of trials)			
Cabbage	7-9	0 (4)	2 (2)	2 (4)	0 (5)
	20-23	2 (4)	6 (2)	5 (4)	1 (5)
	40-43	0 (4)	0 (2)	1 (4)	1 (5)
Tomato	7-9	0 (3)	0 (1)	0 (2)	0 (3)
	20-23	1 (3)	0 (1)	2 (3)	0 (3)
	40-43	0 (3)	0 (1)	8 (2)	4 (3)
Cucumber	7-9	1 (2)	0 (1)	2 (2)	1 (2)
	20-23	4 (2)	6 (1)	6 (2)	3 (2)
	40-43	0 (1)	0 (1)	0 (1)	1 (1)
Pepper	7-9	0 (1)	-	0 (1)	0 (1)
	20-23	1 (1)	-	1 (1)	1 (1)
	40-43	0 (1)	-	3 (1)	2 (1)
Eggplant	7-9	0 (1)	-	0 (1)	0 (1)
	20-23	1 (1)	-	3 (1)	0 (1)
	40-43	1 (1)	-	1 (1)	0 (1)

DAA = days after application; (-) = not tested

In additional experiments, the effects of post-planting applications in tomato, cabbage and cucumber were evaluated. This application method prevents direct contact of the seedling roots with the herbicide-treated soil during the transplanting procedure. Dimethenamid caused slight initial crop damage (table 3). Assessments made at 3 and 6 weeks after application revealed better selectivity of dimethenamid in post-planting applications in comparison to respective pre-planting treatments. The initial damage observed at 7 days after application eventually grew out completely. The reference products were less suitable for this application method. Apart from higher initial crop injury with alachlor and pendimethalin, the latter product damaged the tested crops over an extended period and to a high degree. In one trial on cabbage, heavy rainfall (60 mm) occurred immediately after herbicide use, thus creating a high risk for crop injury (figure 2). Under such conditions the crop injury caused by pre-planting herbicide application was much higher than the injury observed in post-planting application of dimethenamid-P.

Table 3 Influence of pre- and post-transplanting application on selectivity of dimethenamid in transplanted crops (Pingtung, Southern Taiwan 1997-99)

Treatment	g a.i./ha	Application timing	% injury (days after application)								
			Cabbage			Cucumber			Tomato		
			(7)	(21)	(35)	(7)	(21)	(35)	(7)	(21)	(35)
Dimethenamid	900	Pre-plant	0	0	0	0	4	0	0	0	0
Dimethenamid	1125	Pre-plant	0	1	0	0	6	0	0	1	0
Dimethenamid	1350	Pre-plant	0	3	0	0	6	0	0	2	0
Pendimethalin	1600	Pre-plant	0	5	0	0	23	22	0	3	0
Alachlor	1920	Pre-plant	0	0	0	0	4	0	0	0	0
Dimethenamid	900	Post-plant	4	0	0	4	0	0	0	0	0
Dimethenamid	1125	Post-plant	5	0	0	4	0	0	1	0	0
Dimethenamid	1350	Post-plant	8	0	0	6	0	0	3	0	0
Pendimethalin	1600	Post-plant	7	30	20	34	43	37	13	8	0
Alachlor	1920	Post-plant	4	0	0	18	3	0	18	0	0

The herbicidal activity of dimethenamid in pre-emergence applications is shown in Figure 1. Control of grass weeds including *Digitaria violascens*, *Eleusine indica*, *Echinochloa crus-galli*, *Setaria viridis and Leptochloa chinensis* at 1125 to 1350 g a.i./ha dimethenamid was excellent and superior to 1920 g a.i./ha alachlor. Pendimethalin (1600 g a.i./ha) achieved comparable grass control, except for the important grass weed *Eleusine indica*, which was better controlled with dimethenamid. *Cyperus iria* was well controlled by dimethenamid at all rates, superior to pendimethalin. Additionally, dimethenamid controlled *Amaranthus spinosus*, *Portulaca oleracea*, *Physalis angulata* and *Acalypha australis* very effectively, but only moderate activity on *Chenopodium album* and *Rorippa atrovirens* was recorded. The reference product pendimethalin was less effective in controlling *Physalis angulata, Acalypha australis* and *Rorippa atrovirens*, but provided very good control of *Chenopodium ficifolium*.

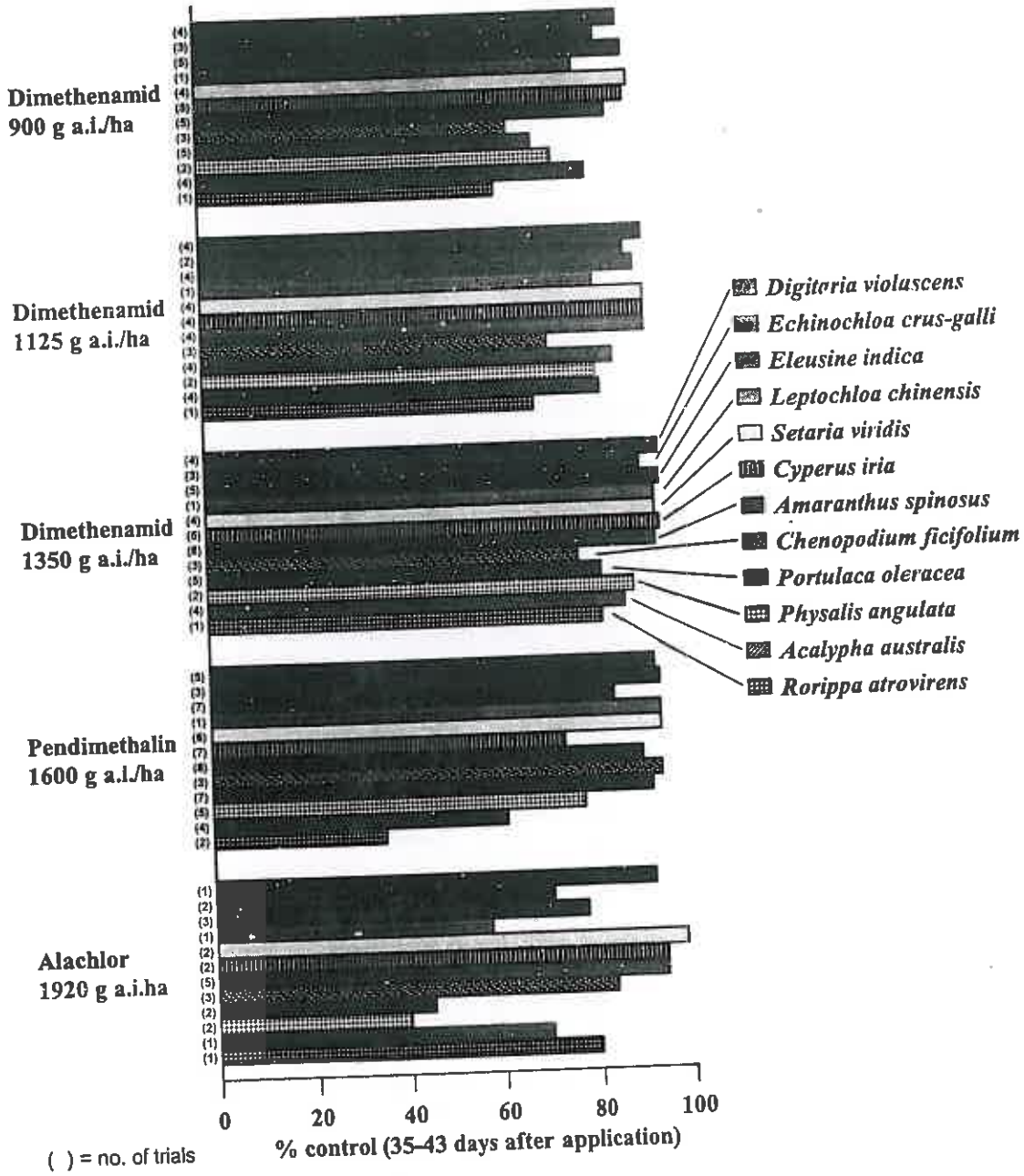

Fig. 1 Herbicidal activity of dimethenamid in pre-emergence applications in seeded and transplanted crops (Pingtung, Southern Taiwan, 1997-99)

In some of the trials the resolved herbicidally active optical isomer dimethenamid-P was compared to the racemic mixture r/s-dimethenamid (Table 4). The activity per unit active ingredient of dimethenamid-P in comparison to r/s-dimethenamid was about 2-fold (table 4). This means, dimethenamid-P provided comparable weed control at rates of only 540 to 720 g a.i./ha. No difference in selectivity was observed between dimethenamid-P and the racemic dimethenamid (data not shown).

Table 4 Herbicidal activity of racemic dimethenamid (900 g a.i./l, EC) in comparison with the optical isomer dimethenamid-P (720 g a.i./l, EC) after pre-emergence application in seeded and transplanted crops (Pingtung, Southern Taiwan 1997-99)

Treatment		Dimethenamid		Dimethenamid-P		Pendimethalin
Product/ha (l)		1.25	1.5	0.75	0.9	4.0
a.i./ha (g)		1125	1350	540	648	1600
Weed	No. of trials	% control (35-43 days after application)				
Digitaria violascens	4	93	95	93	95	93
Eleusine indica	6	93	96	93	95	87
Setaria viridis	6	94	96	94	96	94
Cyperus iria	6	95	97	95	97	72
Amaranthus spinosus	6	93	95	93	93	89
Acalypha australis	3	79	84	78	84	51
Ageratum conycoides	3	70	81	72	79	14
Eclipta alba	1	73	83	70	82	23
Lindernia anagalis	1	67	85	73	87	13
Physalis angulata	5	66	74	66	72	73
Potulaca oleracea	4	90	92	89	93	92
Rorippa atrovirens	2	70	83	72	80	36

DAA = days after application

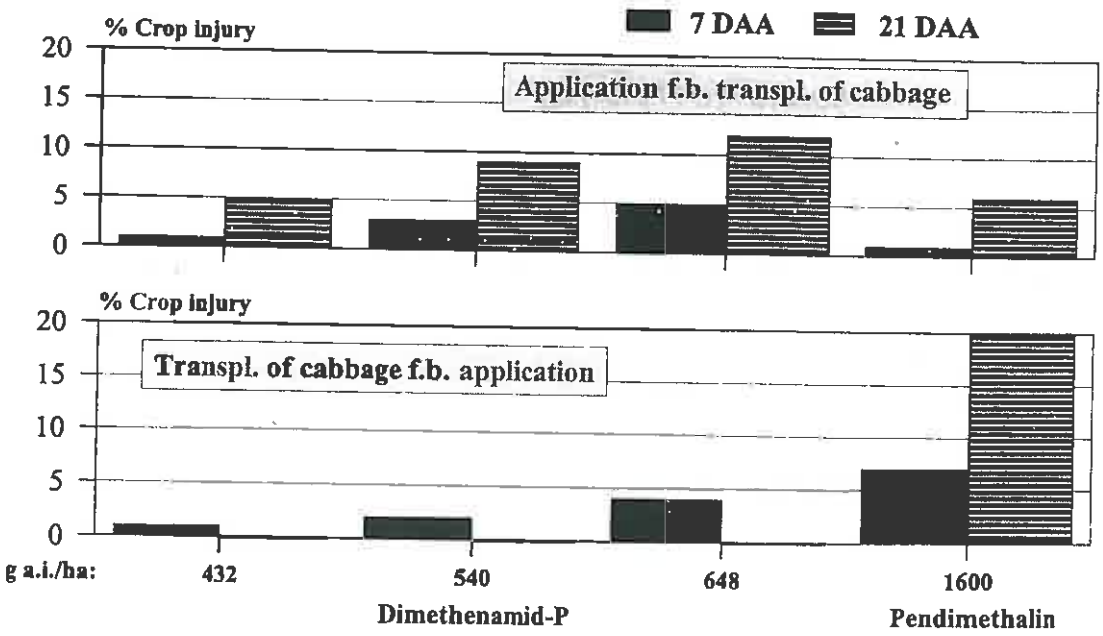

Fig. 2 Effect pre- and post-planting application of dimethenamid-P on selectivity in cabbage followed by heavy rain (60 mm) at 2-5 days thereafter

Discussion

Dimethenamid and dimethenamid-P proved to be highly effective pre-emergence herbicides for applications made post-seeding as well as pre- or post-planting in a wide range of upland crops under the conditions of Taiwan. A major factor affecting crop selectivity of herbicides in upland crops is the occurrence of rainfall after application. This is of particular importance for weed control measures during the rainy season. Heavy rainfall of greater than 20 mm within one week after treatment created a high risk of crop injury for all pre-emergence herbicides tested. This was also the case when herbicides were applied on water saturated soils after intensive rainfall or irrigation. Crops grown in soils with a low CEC (cation exchange capacity) below 12 due to low contents of organic matter, silt and clay were also at higher risk to suffer crop injury from herbicide applications. However, the excellent crop safety of dimethenamid applied as post-planting spray can minimise the risk of damage in transplanted upland crops like cabbage, tomatoes or cucurbits. Dimethenamid and dimethenamid-P provide control of a broad spectrum of weeds including important grasses, broadleaf weeds and sedges at rates of 900 – 1350 and 540 – 720 g a.i./ha, respectively. Comparing the herbicidal activity spectrum of the products tested revealed that complementary weed control can be achieved by tank mixtures of pendimethalin with dimethenamid in cases where a wide range of weeds must be controlled. The performance of the resolved herbicidally active optical isomer dimethenamid-P, which is currently under development, showed that in the future a product will be available, which needs considerably lower amounts of active ingredient per hectare in comparison to other products used in these segments.

Table 6 FRONTIER® (900 g/l dimethenamid, EC): Recommendation for the use on different crops in Taiwan

Crop	Dosage (litres/ha)	Timing
Corn	1.5	2-5 days after seeding
Sweet corn	1.25-1.5	2-5 days after seeding
Soybean, peanut, bean, pea	1.0-1.25	2-5 days after seeding
Cabbage, tomato, cucumber	1.0-1.25	Pre-planting
	1.25-1.5	Post-planting
Eggplant, pepper	1.0-1.25	Pre-planting
	1.25-1.5	Post-planting

- In case of water saturated soil after planting: delay application by a few days
- In case of expected rainfall: delay application by a few days
- Use the lower rates in areas of frequent high rainfall
- Use the lower rates on soil types with low CEC (O. M.< 1%; sandy soil)
- In case of frequent rain: change from pre- to post-planting application in transplanted crops

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4. Environment

INFLUENCE OF CHLORSULFURON RESIDUE ON GROWTH AND DEVELOPMENT OF TRANSPLANTED RICE

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Abstract: Residue of chlorsulfuron applied to wheat fields at a certain concentration in soils had effect on growth and development of after-crop rice variety "Xiushui 04". When residue of chlorsulfuron was above 0.16 ppb, it decreased yield of rice significantly. The herbicide mainly reduced the number of effective panicles of rice, but showed little effect on the number of grains per panicle and 1000-grain weight of rice.

Key words: Chlorsulfuron,Residue,Rice

Introduction

Chlorsulfuron, a sulfonylurea herbicide, is high herbicidal activity, a broad weed spectrum, extremely persistent and low price. Due to its persistence in soils, it showed injury to rice following wheat when it was applied to wheat fields irregularly. In recent years, chlorsulfuron and herbicide mixtures containing chlorsulfuron were repeatly applied to wheat fields in rice-wheat cropping systems in Jiangsu Province, resulting in tillering delay of rice seedlings. The residual injury to japonica rice was greater than that of indica ones. The effect of chlorsulfuron residual dose in soils on growth and development of rice were examined in laboratory. Safety to rice in soils where chlorsulfuron was applied in Jiangsu Province was tested.

Materials and Methods

The test rice variety in this study was japonica rice variety "Xiushui 04"

1. Pot experiments

The paddy soil (pH 6.8-7) where chlorsulfuron was never applied from Nanjing, Jiangsu Province was selected as the test soil. On June 23, 35-day seedlings of rice variety "Xiushui 04" were transferred to pots (20 cm in diameter, 20 cm in height) containing the soil treated with 0, 0.08, 0.16, 0.32, 0.64, 1.28, 2.56 or 5.12 ppb chlorsulfuron. Each treatment was replicated four times with randomized block arrangement. Three holes of rice seedlings were planted in each pot, 3 rice seedlings were in each hole. Fertilizer application, irrigation, disease and insect management in pots was as ususal as in rice fields. At the 15th,30th, 45th or 60th day, plant height,the number of tillers and other agronomic characters of rice were investigated. On October 13, main agronomic characters of rice were examined. Theorectical yield of rice was evaluated according to data from investigations.

Data from investigations were input to computer.

2. The soils from Baoying and Jiangpu County of Jiangsu Province where chlorsulfuron had been applied to wheat fields for long time were selected as the test soils after wheat harvesting. Seedlings of rice were transplanted in pots containing the soils from Baoying, Jiangpu County. Residues of chlorsulfuron in the soils were measured. Effect of chlorsulfuron residue on growth of rice was tested.

Result s

1. .Effect of different concentration of chlorsulfuron on growth and development of rice

At the 15th day after transplanting, leaf colour of rice varied from dark green to pale yellowish green when concentration of chlorsulfuron residue in soil increased progressively. When concentration of chlorsulfuron in soil was below 0.16 ppb, it had little influence on leaf colour of rice. It had significant effect on leaf colour of rice at high concentration. Residue of chlorsulfuron also reduced root growth. When it increased progressively, the later roots became few and thin, root systems became short, colour of roots was from white to brown. It indicated that both leaf colour and growth of root in rice were affected by residue of chlorsulfuron in soils.

The plant height of rice was tested at 15th day after transplanting (Table 1). The plant height was significantly reduced at high concentration of the herbicide. When chlorsulfuron concentration in soils was at 2.56 ppb and 5.12 ppb, the plant height was 85.2% and 73.5% of the control's, respectively. There was no apparent effect on plant height at low chlorsulfuron concentration. On August 3 (the 45th day after transplanting), the results showed that plant height was inhibited at 0.16 ppb chlorsulfuron, its plant height was 94.43% of the control's. In the growth period of rice, growth of roots was markedly inhibited, thereby growth of aerial parts of rice was affected, and plant height was apparently reduced. Final plant height of each treatment ranged from 90.3 cm to 101.3 cm which was 87.7% - 97.7% of the control's (101.6 cm).

In this experiment, the percentage of inhibition of plant height of rice was increased progressively with chlorsulfuron concentrations in soils, there was correlation between plant height of rice and chlorsulfuron concentration in soils. Regression equations between plant height and chlorsulfuron concentration were as follows:

$Y1=50.4054-2.3099x+0.0814x2$ $r=0.9784^{**}$

$Y2=62.6200-6.3772x+2.4588x2+0.2974x3$ $r=0.9266^{**}$

$Y3=100.877-4.9974x+1.1908x2-0.1208x3$ $r=0.9929^{**}$

(Y means plant height of rice (cm), x means chlorsulfuron concentration (ppb))

From dynamics of rice tillers, the percentage of tillers of rice was inhibited by residue of chlorsulfuron in soils was higher than that of plant height of rice. The results of investigation on July 3 (Table 2) indicated that the number of tillers per hole at seven concentrations of chlorsulfuron residue was 10.4, 9.3, 9.1, 8.6, 8.5, 6.3 and 4.4, respectively, which was 91.23-38.6% of the control's (11.4). The inhibition percentage of rice tillers at 5.25 ppb chlorsulfuron in soil was as high as 61.4%.

Table 1 Effects of different chlorsulfuron residues on plant hight of rice

Chlorsulfuron (ppb)	7/3		8/3		10/3	
	Plant height (cm)	Inhibition (%)	Plant height (cm)	Inhibition (%)	Plant height (cm)	Inhibition (%)
0.08	49.8	0.26	62.2	3.86	101.25	2.27
0.16	49.2	1.46	61.1	5.56	100.00	3.47
0.32	48.5	2.86	59.6	7.88	98.65	4.78
0.64	47.4	5.06	59.3	8.35	98.1	5.31
1.28	46.4	7.07	59.0	8.8	96.68	6.68
2.56	42.5	14.88	57.1	11.75	93.7	9.56
5.12	36.7	26.5	54.9	15.15	90.3	12.84
CK	49.93		64.7		103.6	

Regression equation between the number of rice tillers per hole and concentration of chlorsulfuron residue in soils was as follows:

$$Y=10.5076-3.0062x+0.7792x^2 \qquad r=0.9960^{**}$$

Due to decrease in the number of rice tillers, the number of effective panicles in rice was also reduced. At 0.08-5.12 ppb chlorsulfuron residues of seven treatments, the number of effective panicles per hole was 11.6, 11.3, 10.3, 10.0, 9.7, 9.2 and 9.0, respectively, which was 95.08-76.23% of the control's.

Table 2 Effects of different chlorsulfuron residues on tillers of rice

Chlorsulfuron (ppb)	7/3		8/3		10/3	
	Tiller	of the control's (%)	Tiller	of the control's (%)	Tiller	of the control (%)
0.08	10.4	91.3	17.8	93.68	11.6	95.08
0.16	9.3	81.58	17.5	92.11	11.3	92.62
0.32	9.1	79.83	17.25	90.79	10.3	84.43
0.64	8.6	74.44	16.5	86.84	10.0	81.97
1.28	8.5	74.56	16.25	85.53	9.7	79.51
2.56	6.3	55.26	16.0	84.21	9.2	75.41
5.12	4.4	38.60	13.0	68.42	9.0	73.77
CK	11.4		19.0		12.2	

Tiller in table 2 means the number of tillers per hole rice seedlings.

2. .Effect of chlorsulfuron residue in soil characters related to spike and yield of rice

Yield components of rice is composed of the number of effective panicles, the number of grains per panicle and 1000-grain weight and so on. The number of effective panicles in rice affected by the environment was greatest among these characters, but both the number of grains per panicle and grain weight of rice were controlled by environment and genes. In this experiment, clorsulfuron residues in soils had little effect on the number of grains per panicle in rice (Table 3), it also had a little influence on grain weight of rice, these results in the experiment was slight different from the predecessors'. The theorectical yield of rice based on data from the experiment was evaluated, chlorsulfuron residues in soils had significant effect on yield of tested rice variety "Xiushui 04". Yield loss of rice was not remarkable when chlorsulfuron residue in soil was 0.08 ppb. When chlorsulfuron residue in soils reached to 0.16 ppb, yield of rice decreased by 10.18%, it had remarkable effect on yield of rice.

3. Monitoring of chlorsulfuron residues in wheat fields of Jiangsu Province and its safety to rice

After wheat harvesting, the soils were selected from wheat fields where chlorsulfuron had been applied for successive four years in Baoying and Jiangpu County of Jiangsu Province. Chlorsulfuron residues in the soils were from 0.32 to 0.64 ppb, the residual concentratiom of the herbicide in soils was very high. The yield of rice variety "Xiushui 04" was decreased by 20-23.3% at the concentrations of chlorsulfuron residue in soil.

Table 3 Effects of chlorsulfuron residues in soils on yield of spikelets in rice

Chlorsulfuron (ppb)	No. of Spike /mu (ten thousands)	Kernel/spike	1000-grain weight (g)	theorectical yield (kg/mu)	yield loss (%)
0.08	20.92	62.5	16.6	217.02	6.71
0.16	20.38	62.9	16.3	208.96	10.18
0.32	18.58	62.9	16.2	189.27	18.64
0.64	18.03	62.7	16.3	184.27	20.79
1.28	17.49	62.8	16.3	179.06	23.03
2.56	16.59	62.4	15.8	163.56	29.70
5.12	16.22	61.8	15.9	149.44	35.76
CK	22	63.7	16.6	232.64	0

Discussion

1. .According to the results from this experiment, residues of chlorsulfuron in soils had effects on root growth, plant height, leaf colour, tiller of rice, it also had effects on ear characters of rice at high concentration. When residue of chlorsulfuron in soil was above 0.16 ppb, it had significant effect on yield of transplanted japonica rice variety "Xiushui 04". When residue of chlorsulfuron in soil was below 5.12 ppb, it had effect on the number of effective panicles of rice, but had a little

influence on grain number per panicle and 1000-grain weight of rice.

2. Accordint to the results from Baoying and Jiangpu County of Jiangsu Province, residues of chlorsulfuron in the soils had been as high as 0.32-0.64 ppb, it resulted in great residual injury to rice variety "Xiushui 04" and decreased yield of rice signicantly. It should be paid attention to.

THE MOBILITY AND DEGRADATION OF GLUFOSINATE-AMMONIUM IN TROPICAL SOILS

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Abstract: Studies on the adsorption and degradation of glufosinate-ammonium in Sungai Buluh (sandy loam) and Lating soil series (clay loam) and the influence of organic matter including the rainfall on herbicide mobility in the soil columns were carried out under greenhouse and field conditions using a bioassay technique. Cucumber (*Cucumis sativus* L.) was used as a bioassay indicator. Adsorption of glufosinate-ammonium in Sungai Buluh series was observed to be slightly higher than Lating soil series. Degradation of glufosinate-ammonium in Sungai Buluh and Lating soil series at a depth of 0 – 15 cm showed no significant difference as compared to the degradation in both soil series at a depth of 15 – 30 cm. The least half-life (2.1 days) was observed in Sungai Buluh series soil taken at the 0-15 cm and incubated at 30°C with soil moisture of 90% field capacity. Herbicide was also seemed to move to a deeper layer of the soil corresponding to the increasing amount of rainfall. The downward movement of glufosinate-ammonium under natural conditions during the second month was higher than the first month where herbicide was detected in 20-25 cm zone at a total rainfall of 156.5 mm.

Key words: adsorption, glufosinate-ammonium, degradation, persistence, mobility

Introduction

The fate of herbicides in soils is greatly influenced by their interaction with the soil environment. It is now well established that the behaviour of most herbicides is influenced by the organic matter content of the soil (Blumhorst *et al.*, 1990). Generally, adsorption and decomposition are the two major factors that influence the bioactivity of most herbicides (Aldrich, 1984). It has been suggested that variation in the phytotoxicity of the herbicides in soil is caused by differences in the adsorptive capacity of the soil (Okafor *et al.*, 1983). In general, adsorption is the most important factor affecting the leachability of herbicides in soil. Herbicides that are adsorbed into soil particles do not leach, unless the soil particles themselves move with the flow of water.

Herbicides dissipate in the soil through a combination of microbiological and chemical processes. Microbial degradation of herbicides increases with increasing soil temperature (Bouchard *et al.*, 1982) and soil moisture levels (Hurle and Walker, 1980). Temperature can, therefore, be an important factor affecting the persistence of herbicides in the soil, especially in the tropics. Herbicide degradation in soil generally increases with increasing moisture levels up to field capacity, probably reflecting increased microbial activity (Hurle and Walker, 1980).

Glufosinate-ammonium (ammonium DL-homoalanin-a-[methyl] phosphinate) is a non-selective herbicide which is registered for control of most annual grasses and broadleaf weeds in rubber, oil palm and cocoa plantations, in orchads, in vegetable gardens and on non-crop land. The herbicide was introduced into the Malaysian market in the 1980s. While much is known about its physiological activity, efficacy, and mode of action, little quantitative information is available concerning the adsorption, degradation and mobility in tropical soils.

Materials and Methods

Soil samples

Two different soils were used: Sungai Buluh Series and Lating Series. Sungai Buluh soil was collected near the RRIM Research Station, Sungai Buluh, while Lating Series soil sample was obtained from the experimental plot, Faculty of Science & Technology, Universiti Kebangsaan Malaysia, Bangi. Soil characteristics are shown in Table 1. All soils were collected from the 0-10 cm depth, air-dried and screened through a 1.0-cm sieve prior to use.

Table 1 Physical and chemical properties of the soils.

Soils	pH	% N	% organic matter	% sand	% silt	% clay	CEC (Meq $100g^{-1}$)
Sungai Buluh	4.8	0.1	0.45	89.2	4.2	7.6	3.3
Lating	4.9	0.2	0.39	25	36.5	38.5	7.5

Herbicide

The herbicide used in the study was glufosinate-ammonium (Basta®) containing 150 g/l aqueous solution.

Adsorption study

Five g samples of each soil series were weighed into 250 ml conical flasks, to which 20 ml of 10 ppm (w/w) glufosinate-ammonium were then added. Control samples were prepared without adding any soil. The flasks were sealed immediately with aluminium foil. The samples were allowed to equilibrate on an orbital shaker at 180 rpm for 0.5, 2, 4, 6, 8, 10 and 12 hours (preliminary studies had shown that adsorption plateaued at 12 hrs) and then were centrifuged for 15 minutes at 3000 rpm to obtain a clear solution. Then 5 ml of the supernatant was transferred to 9 -cm diameter petri dishes, each lined with one sheet of Whatman No. 3 filter paper. Ten cucumber seeds were placed in each petri dish and incubated at 28°C for seven days (Ismail and Teoh, 1994). After incubation, the radicle length of the seedlings was measured and expressed as a percentage of the untreated control value. The concentration of herbicide in the supernatant was estimated by referring to a dose-response curve developed concurrently.

To prepare a standard curve, 5 ml of glufosinate-ammonium at 0, 0.1, 0.25, 0.5, 1.0, 2.0, 5.0, 8.0 and 10 ppm (w/w) was placed in 9-cm petri dishes lined with two layers of filter paper. Ten cucumber seeds were placed in each petri dish. There were three replicates for each concentration. Radicle length of 5 seedlings of each petri dish of each concentration was measured. A standard curve was obtained by plotting the average radicle length of each replicate as a percent of untreated control against log herbicide concentration.

Degradation study

The soil used for this study was taken from two different depth, viz. 0-15 cm and 15-30 cm. About 126 kg of air-dried soil of each series from each depth were treated to obtain final concentrations of 10 ppm (w/w) for glufosinate-ammonium. For the control treatment, soils were not treated with the herbicide. After mixing, 3.5 kg samples of treated soil were kept in each of 36 polyethylene bags. The bags were then divided into two groups (18 bags) for incubation at either 20°C or 30°C. The moisture levels of the soil was maintained at 50%, 70% and 90% of field capacity for each of temperature treatment. The soil moisture level was maintained by watering after weekly weighing. One hundred gm of soil were taken from each bag on days 0, 4, 8, 12, 16 and 20. All soil samples were bioassayed immediately after sampling. The soil sample was then placed in a pot into which 10 cucumber seeds were then planted at a depth of 0.5 cm. The pots were kept in the greenhouse under natural conditions. The soils were watered twice a day to maintain the moisture level at about 90% of field capacity. After emergence, plants were thinned to four per pot. Eleven days after emergence, the plants were cut at soil level; fresh weights were recorded and expressed as a percentage of the control value. The concentration of herbicide in the soil was estimated by referring to a dose-response curve developed concurrently. The herbicide concentrations calculated for each data point using the dose-response curve were plotted against time, from which the half-life was calculated by assuming first-order kinetic behaviour.

For the degradation study, a standard curve of each soil series was plotted using cucumber for bioassay in order to define the corresponding concentration range of the herbicide in the soils used for the greenhouse studies. The required volume of the herbicide was thoroughly mixed with air-dried soil to obtain required concentrations (w/w). The concentrations used were 0, 0.1, 0.25, 0.5, 1.0, 2.0, 5.0, 8.0 and 10 ppm (w/w). Treated soil was transferred into pots, each containing 100 g of either soil. Ten cucumber seeds for bioassay were planted in each pot at a depth of 1 cm. After emergence, plants were thinned to four per pot. Eleven days after emergence, the plants were cut at soil level and fresh weights of the four seedlings were recorded. A standard curve was obtained by plotting the average plant fresh weight of five replicates as a percent of the untreated control against log herbicide concentration.

Mobility study in greenhouse and natural conditions

PVC columns (12 cm diameter) were uniformly packed to a depth of 25 cm with 0.74 g/cm^3 Sungai Buluh series soil at 60% field capacity. After equilibrating, a 5-cm layer of soil treated with glufosinate-ammonium at 70 ppm (w/w) was placed on top of the column which was lined with Whatman No. 3 filter paper. Twelve hours after adding treated soil, the soil column was watered with 40 ml water daily (equivalent to 4.2 mm of rain). On Day 1, 7, 14 and 28, the distribution of herbicide in each soil segment was determined using the bioassay method as described.

Another set of the columns were kept outside the greenhouse under natural conditions. The distribution of the herbicide in each soil segment was determined after 1 and 2 months using the bioassay method as described.

Statistical analysis

The design of adsorption, mobility and persistence experiments was a randomized block with four replications except for the mobility study which had three replicates. Where else complete randomize design with four replications was used for bioassay studies All data were subjected to analysis of variance and means were compared with an LSD test at the 5% level of significance.

Results and Discussion

Adsorption study

A response curve, percentage radicle length (expressed as percentage of the control value) against log herbicide concentration was plotted with correlation coefficient of 0.94. Based on the response curve, percentage radicle length of seedlings in supernatant containing unknown concentrations of the herbicide were converted to herbicide concentration in the supernatant.

An increase of incubation time elevated the adsorption of glufosinate-ammonium onto soil particles which reduced the level of the herbicide in the supernatant (Fig. 1). Adsorption of glufosinate-ammonium onto Sungai Buluh series soil was slightly higher than in Lating series soil. Significant reduction in the supernatant was observed after 10 min incubation. After 30 min incubation, about 89% and 77% of the glufosinate-ammonium was adsorbed onto Sungai Buluh and Lating series soil, respectively. After 1 hour, more than 90% of the herbicide was adsorbed onto both soils.

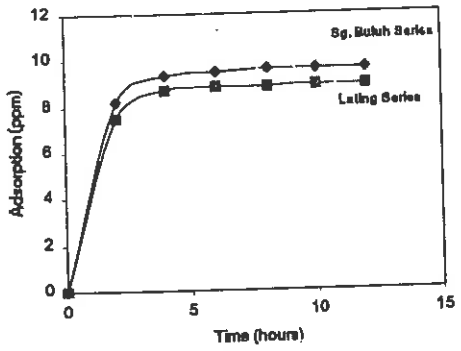

Fig. 1 Adsorption of glufosinate ammonium in two soils.

The results of the experiments clearly shown that glufosinate-ammonium could be adsorbed onto soil particles. Our results showed that no significant difference were observed in the adsorption of glufosinate-ammonium in Sungai Buluh and Lating series soil. However, the adsorption of the herbicide was slightly higher in Sungai Buluh than Lating series soil. This may due to the content of organic carbon which was slight higher in Sungai Buluh than Lating series. This results are in line with other previous reports which showed that organic matter had an influence on the adsorption of most herbicides in soils (Ismail and Teoh, 1994; Oppong and Sagar, 1992).

Degradation study

A plot of fresh weight (expressed as a percentage of the control value) against log herbicide concentration gave a first-order linear relationship with correlation coefficients of 0.8 and 0.97 for Sungai Buluh and Lating series, respectively. Based on the response curve, percentage fresh weight of seedlings grown in soil samples containing unknown concentrations of the herbicide were converted to herbicide concentration remaining in the soil.

The half-life of glufosinate-ammonium at different moisture levels and temperatures in Sungai Buluh series is shown in Tables 2. No significant difference in the half-life of glufosinate-ammonium in soil taken from 0-15 cm depth at 20°C, irrespective of any moisture level. The half-life of glufosinate-ammonium was the least (2.1 days) in this Sungai Buluh series soil incubated at 30°C with soil moisture of 90% field capacity. The half-life of glufosinate-ammonium in soil taken from 15-30 cm depth was slightly longer than in soil at 0-15 cm depth. The half-life of glufosinate-ammonium in soil from 15-30 cm depth ranged between 8.7 to 13.7 days.

Table 2 Half-lives of glufosinate-ammonium in Sungai Buluh series at different temperatures and moisture levels.

Temperature (°C)	Moisture content (% field capacity)	Half-life (days)	Correlation coefficient (r)
	0 – 15 cm depth		
20	50	10.3	0.65
20	70	10.3	0.83
20	90	10.2	0.71
30	50	10.2	0.61
30	70	10.1	0.84
30	90	2.10	0.65
	15 – 30 cm depth		
20	50	12.8	0.88
20	70	13.7	0.62
20	90	8.9	0.79
30	50	11.1	0.62
30	70	10.9	0.71
30	90	8.7	0.65

Table 3 shows the half-life of glufosinate-ammonium in Lating series soil taken from 0-15 cm and 15-30 cm depth. There was no significant differences in the half-life of glufosinate-ammonium in soil taken from 0-15 cm depth at 20°C irrespective of moisture levels. However, the least half-life of glufosinate-ammonium was 5.2, in soil incubated at 30°C and 90% field capacity. Soil taken from 15-30 cm depth showed no significant difference in the half-life of the herbicide.

Table 3 Half-lives of glufosinate-ammonium in Lating series at different temperatures and moisture levels.

Temperature (°C)	Moisture content (% field capacity)	Half-life (days)	Correlation coefficient (r)
	0 – 15 cm depth		
20	50	10.2	- 0.82
20	70	10.5	- 0.89
20	90	10.3	- 0.75
30	50	10.4	- 0.72
30	70	9.0	- 0.72
30	90	5.2	- 0.89
	15 – 30 cm depth		
20	50	10.0	- 0.83
20	70	10.1	- 0.79
20	90	10.2	- 0.92
30	50	10.1	- 0.82
30	70	9.8	- 0.81
30	90	9.7	- 0.87

Temperature and soil moistures are among the two factors which determine the persistence of the herbicide in soil. Increased degradation rates of many herbicides at increased temperature have been confirmed in numerous field studies (Ismail and Teoh, 1994; Zimdahl and Gwynn, 1977). There is evidence that herbicide degradation rates are increased at higher temperatures, which in most instances, probably reflects increased microbial activity. It was reported that the degradation of glufosinate-ammonium is very rapid in a microbiologically active environment (WSSA, 1989). Our results showed that the shortest half-life of glufosinate-ammonium was at 30°C and 90% field capacity. An increase in herbicide degradation at higher soil moisture levels is expected since a high moisture levels hinder herbicide adsorption onto soil particles, making herbicide molecules more readily available for degradation by soil microbes.

Mobility study under greenhouse and field conditions

Twenty-four hours after application, the herbicide was located primarily in the first top 0-5 cm of the profile, but after 7 days significant level of the herbicide were detected in the 5-10 cm zone (Fig. 2). After 14 days of watering, the herbicide residue was detected at 20-25 cm zone and after 28 days significant reduction in fresh weight were recorded for plant grown in soil taken from 20-25 cm depth. This demonstrates that after four weeks of daily application of simulated rain, glufosinate-ammonium had leached to a depth of 20-25 cm.

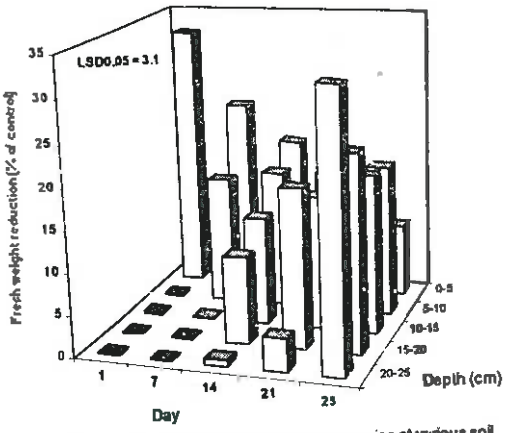

Fig. 2 Fresh weight reduction of the bioassay species at various soil depths under greenhouse conditions.

One month after application, phytotoxicity was detected at a depth of 15-25 cm (Fig. 3). Fresh weight of the bioassay species did not much affected when grown in soil from 0-5 cm layer. After two months under natural conditions, no reduction in fresh weight was detected for plant grown in soil taken from 0-5 cm, 5-10 cm and 10-15 cm depths. While soils taken from 15-20 cm and 20-25 cm depth showed significant in growth reduction of the bioassay species. This demonstrates that herbicide residue moved downward and accumulated at deeper soil layer where greater reduction in fresh weight was observed.

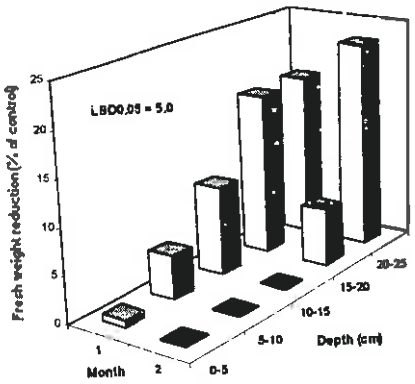

Fig. 3 Fresh weight reduction of the bioassay species at various soil depths under natural conditions.

Under conditions of high rainfall, the herbicide has potential to leach downward. Application of a total 118 mm of simulated rain (4.2 mm/day) over 28 days following the addition of glufosinate-ammonium to the soil surface, caused the phytotoxic levels of the herbicide to leach into the 20-25 cm zone of the column. Glufosinate-ammonium leached to a depth of at least 25 cm over one month when the soil columns were exposed to a total of rainfall of 156.5 mm. The importance of the amount of rainfall in moving glufosinate-ammonium down the soil profile was proven in this experiment. The present study suggests that the weak binding of glufosinate-ammonium in the soil leads to an increase in leaching through the soil column. Eventhough in the earlier experiment have shown that glufosinate-ammonium could be adsorbed onto soil but how strong the molecules attached to the soil particles is not known since the desorption study had not been carried out.

It is known that the results from chemical analysis may not be comparable to bioassays, because the chemical analysis goes to the specific compounds, whereas bioassays estimate the level of phytotoxicity due to the manifestation of the effect. Nevertheless there are numerous papers that reported a positive correlation between the two methods (Gomez *et al.*, 1991; Menges and Hubbard, 1970). Gunther *et al.* (1989) reported that the bioassay methods could represent the chemical analysis in estimating the herbicide disappearance rates quantitatively under field conditions.

In conclusion, the results of this study clearly demonstrate that in warm and moist soil, the breakdown of glufosinate-ammonium is more rapid than in cooler and drier soils. This herbicide was found to be mobile in the soils studied and probably could contaminate underground water. However, further research need to be pursued to prove the hyphothesis.

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5. Others

A BIOTYPE OF *Limnocharis flava* MULTIPLE-RESISTANT TO 2,4-D AND BENSULFURON-METHYL IN MALAYSIA AND ITS CONTROL

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Abstract: Susceptibility to 2,4-D dimethylamine and bensulfuron-methyl in fourteen collections of *Limnocharis flava* (L.) Buchenau from peninsular Malaysia was evaluated. For 2,4-D, an approximately 30-time-difference in I_{50} value was observed between the resistant biotype (the collection from MARDI S.P.) and the susceptible biotype. For bensulfuron-methyl, the difference between the resistant biotype (the collection from MARDI S.P.) and the susceptible biotype was more than 28 times. Treating approx. 20-cm plants with bensulfuron-methyl at 10 to 100 times the recommended rate was useful for a diagnosis for bensulfuron-methyl resistant biotype in *L.flava*. Besides MARDI S.P. collection, Seberang Perak Rice Estate collection was confirmed to have developed 2,4-D resistant biotype. Bentazone controlled the herbicide-resistant *L.flava* effectively, and adequate rice seedling density was also effective in suppressing the weed.

Key Words: *Limnocharis flava*, 2,4-D, bensulfuron-methyl, multiple-resistance, Malaysia

Introduction

2,4-D and sulfonylurea herbicides such as bensulfuron-methyl are widely used by farmers in Malaysia to control broad-leaved weeds in paddy fields. The former, first used from early 1960's is still popular, the latter was introduced to Malaysian farmers in the late 1980's.

Acetolactate-synthase (ALS)-inhibitor-herbicides including sulfonylurea compounds are widely used throughout the world. In recent years, however, ALS-inhibitor-resistant biotypes have been reported in many weed species in many countries (Heap, 1997). In Malaysia, too, recently many farmers reported that sulfonylurea herbicides provided insufficient efficacy in controlling some broad-leaved weed species such as *Bacopa rotundifolia* (Michx.) Wettst. , *Lindernia* spp.

On the other hand, compared to ALS-inhibitors, only a small number of 2,4-D-resistant weeds has been reported in spite of its long history of usage in the world (Heap, 1997). In Malaysia, development of 2,4-D-resistant biotypes was confirmed to *Fimbristylis miliacea* (L.) Vahl (Watanabe *et al.*, 1997). Regarding *Limnocharis flava* (L.) Buchenau, which is the focus of this study, the presence of its 2,4-D-resistant biotype was confirmed in Indonesia (Edison, 1996).

In MARDI Seberang Perai Station (MARDI S.P.), a rice breeder observed that *L.flava* seedlings in his experimental field were not killed by applications of 2,4-D or bensulfuron-methyl. The purpose of this study was (1) to evaluate the susceptibility to 2,4-D and bensulfuron-methyl in the *L.flava* collection from MARDI S.P. and other collections from the northwest part of Peninsular Malaysia, and (2) to establish effective control methods against the herbicide-resistant weed.

Materials and Methods

L.flava plants were collected from fourteen locations in the northwest part of Peninsular Malaysia (Fig.1). Plants of each collection were transplanted into a cement or plastic pot respectively and were vegetatively propagated in MARDI S.P. In Experiment 1 and 2, Coll.1 and Coll.2 were used; Coll.1 from a paddy field in MARDI S.P., suspected to be herbicide resistant, and Coll.2 from an abandoned paddy field in Lahar Ikan Mati, 6km away from MARDI S.P., expected to be susceptible. After transplanting, the pot where Coll.1 was propagated was treated with 2,4-D butylester at the recommended rate to kill

2,4-D-susceptible plants in Coll.1.

Experiment 1

Pot studies using plastic pots measuring 20-22 cm in diameter were carried out in MARDI S.P., Pulau Pinang to evaluate the susceptibility of Coll.1 and Coll.2 to 2,4-D dimethylamine and bensulfuron-methyl.

Four ramets of both collections that were developed on flowering shoots were transplanted into each pot filled with paddy soil from Bumbung Lima field of MARDI S.P. 2,4-D dimethylamine (commercial name: 2,4-D amine 720, 60% a.i.) solution was sprayed at the rate of 50 ml/m^2 on the pots containing the ramet plants on 2nd February 1999 when the plants were approx. 20 cm in height. The spray solution contained 1.3×10^{-3} mol/l (1/8x the recommended rate: the recommended rate = 1.1 kg a.i./ha) to 8.0×10^{-2} mol/l (8x) of 2,4-D for Coll.1 and 7.8×10^{-5} mol/l (1/128x) to 5.0×10^{-3} mol/l (1/2x) of 2,4-D for Coll.2. Non-treated control pots were also maintained for both collections. Three replications were prepared for each treatment. Efficacy of the herbicide treatments was determined based on the dry weight of the surviving plants 21 days after the herbicide treatment.

For bensulfuron-methyl (commercial name: Londax, 10% a.i.), pots were filled with Bumbung Lima paddy soil containing seeds of Coll.1 and Coll.2. The trial pots were puddled and levelled on 20th November 1998. Ten days after puddling, 1.63×10^{-3} mol/l bensulfuron-methyl solution was applied to the trial pots ranging from 1.5 ml/m^2 (1/4x the recommended rate: the recommended rate = 0.04 kg a.i./ha) to 6144 ml/m^2 (1024x the recommended rate) for Coll.1 and 6.0 ml/m^2 (the recommended rate) for Coll.2. Non-treated control pots were also prepared. Three replications were made for each treatment. The efficacy of the herbicide treatments was determined based on the dry weight of the surviving plants 30 days after the herbicide treatment.

To determine I_{50} (50% Inhibition dose) values, a logistic curve modified by Brain and Cousens (1989) was fitted to the observed data:

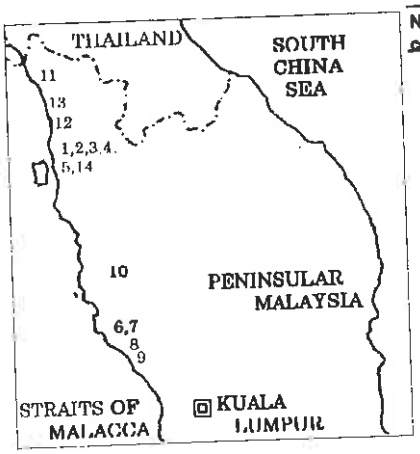
$$y = \frac{k + fx}{1 + e^{bg} x^{b}} + d \qquad , \qquad (1)$$

where y denotes top dry weight in each pot, x denotes herbicide dose and b, d, f, g, k are parameters. The curve fitting was done by non-linear least square method based on Seiden *et al.* (1998) using Microsoft Excel 97.

Four ramets of Coll.1 and Coll.2 were transplanted into each pot with 20-22cm in diameter filled with Bumbung Lima paddy soil. 1.63×10^{-3} mol/l bensulfuron-methyl solution was applied to the trial pots ranging from the recommended rate to 1000 times the recommended rate on 23rd October 1998 when the ramet plants had grown to approx. 20 cm in height. Non-treated control pots were also prepared. Two replications were made for each treatment. Efficacy of the herbicide treatments was determined by the dry weight of the surviving plants 36 days after the herbicide treatment.

Experiment 2

80 *L.flava* seedlings, emerging in the source-paddy-field of Coll.1, were transplanted into each plastic container measuring 1.0x2.5m filled with Bumbung Lima paddy soil. Five ramet plants of Coll.2



No. of collection	Collection site	Land category of site
1	MARDI Seberang Perai, Pulau Pinang	Paddy field
2	Lahar Ikan Mati, Pulau Pinang	Abandoned paddy field
3	Kepala Batas, Pulau Pinang	Domestic drain
4	Kubang Menerong, Pulau Pinang	Paddy field
5	Lahar Kepar, Pulau Pinang	Irrigation channel
6	Sungai Besar Rice Estate, Selangor	Paddy field
7	Sungai Besar, Selangor	Abandoned paddy field
8	Sekinchan, Selangor	Paddy field
9	Tanjong Karang, Selangor	Drain
10	Seberang Perak Rice Estate, Perak	Paddy field
11	CII MADA, Kedah	Paddy field
12	FIV MADA, Kedah	Paddy field
13	AIV MADA, Kedah	Paddy field
14	MARDI Seberang Perai, Pulau Pinang	Pot for weed display

Collection No.1 was treated with 2,4-D beforehand.

Fig.1 Location of collection sites of L.flava.

were also transplanted into each trial container for comparison. The trial containers were treated with 2,4-D dimethylamine at the recommended rate, bensulfuron-methyl at 10 times the recommended rate, or sequential application of bensulfuron-methyl at 10 times the recommended rate and 2,4-D dimethylamine at the recommended rate. A non-treated container was also prepared as control. Bensulfuron-methyl was applied on 5th February 1999 when the plants were approx. 20 cm in height. 2,4-D dimethylamine was sprayed on 10th February 1999. The number of surviving plants and dead plants in each container were counted on 26th February 1999.

Experiment 3

Four ramet plants of the fourteen *L. flava* collections (Fig. 1) were transplanted into each pot filled with Bumbung Lima paddy soil respectively. The trial pots were treated with 2,4-D dimethylamine at the recommended rate or bensulfuron-methyl at 10 times the recommended rate when the plants were approx. 20 cm in height. Non-treated pots were maintained as control for each collection and for each herbicide. Three replications were prepared. 2,4-D dimethylamine was applied on 22nd September 1998. The number and dry weight of surviving plants in each pot were determined 34 days after the treatment. Bensulfuron-methyl was applied on 4th February 1999. The number of surviving plants in each pot was determined 22 days after the treatment.

Experiment 4

To develop effective control methods against *L.flava* population that has multiple herbicide resistance to 2,4-D and bensulfuron-methyl, field experiments were carried out during the second cropping season of 1997 (II/1997) and I/1998 at the source-paddy-field of Coll.1 in MARDI S.P. A randomized block design with five replications was adopted in both seasons. Each plot size was 6.5x7.5m. Treatments in each cropping season are shown in Table 1 and Table 2 respectively.

Cultivated rice MR84 was sown on 18th October in II/1987 and on 21st May in I/1998. Seeding rate in II/1997 was 100 kg/ha. Efficacy of treatments was determined based on the weed dry weight in four 50x50-cm quadrats for each plot on 26th-31st December in II/1997 and on 24th-31st July in I/1998. Only in I/1998, the number of established rice plants was counted in four 25x25-cm quadrats for each plot on 16th June. To control grass weed, molinate was applied according to the recommendation. Other cultivation methods were done following the standard practice in the station.

Table 1 Treatments in II/1997

No.	Herbicide	Dose*1	Time of treatment*2	Wetting agent
1	2,4-D	1x	+33	-
2	2,4-D	1x	+33	+
3	2,4-D	2x	+33	-
4	2,4-D	2x	+33	+
5	2,4-D	3x	+33	-
6	2,4-D	3x	+33	+
7	2,4-D	4x	+33	-
8	2,4-D	4x	+33	+
9	Non treated control			

*1: times the recommended rate

*2: days after sowing

Table 2 Treatments in I/1998

No.	Herbicide	Dose*1	Time of treatment*2	Seeding rate
1	BSM	1x	+7	60kg/ha
2	BSM	1x	+14	60kg/ha
3	2,4-D	1x	+14	0, 20,60,100,180kg/ha
4	2,4-D /2,4-D	1x/1x	+14/+30	60kg/ha
5	2,4-D /2,4-D	1x/4x	+14/+30	60kg/ha
6	2,4-D /bentazone	1x/1x	+14/+30	60kg/ha
7	Non treated control			60kg/ha

*1: times the recommended rate

*2: days after sowing

Results

Experiment 1

Plants of Coll.1 were not killed by 2,4-D at the recommended rate. Its I_{50} value was 1.30 times the recommended rate (Fig. 2). On the other hand most of the plants of Coll.2 were killed by 2,4-D, even at 1/16th of the recommended rate. I_{50} value in Coll.2 was 0.04 times the recommended rate (Fig. 2).

On the average, 25 plants/pot of Coll.1 and 40 plants/pot of Coll.2 emerged in the non-treated pots. Plants of Coll.1 were not killed by bensulfuron-methyl at the recommended rate and its I_{50} value was 28.1 times the recommended rate (Fig. 3). On the other hand plants of Coll.2 were completely killed by bensulfuron-methyl at the recommended rate (Fig. 3).

Approx. 20-cm plants of Coll.1 were not affected by bensulfuron-methyl, ranging from the recommended rate to 100 times the recommended rate. Only at 1000 times the recommended rate, their growth was considerably inhibited, nevertheless the plants still survived (Fig. 4). Plants of Coll.2 were

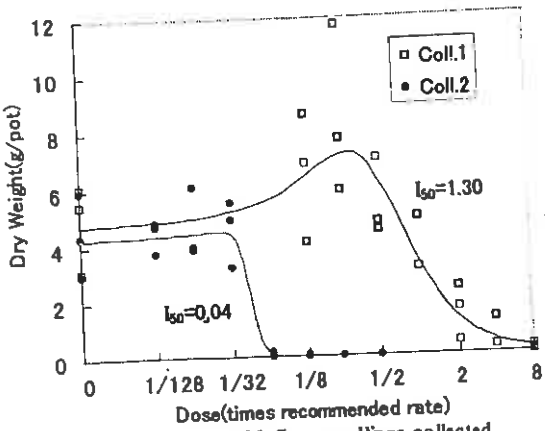


Fig. 2 Dosage response of *L.flava* seedlings collected at a paddy field in MARDI Seberang Perai (Coll. 1) and an abandoned paddy field in Lahar Ikan Mati (Coll. 2) to 2,4-D dimethylamine: The recommended rate was 1.1 kga.i./ha.

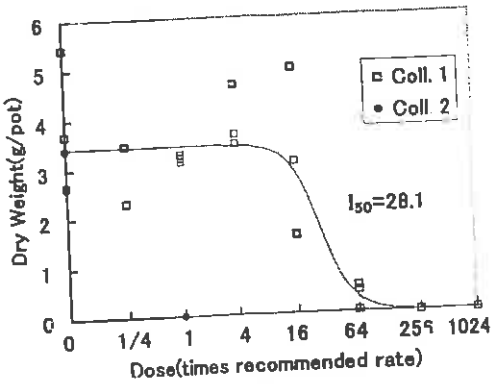


Fig. 3 Dosage response of *L.flava* from a paddy field in MARDI Seberang Perai (Coll. 1) and an abandoned paddy field in Lahar Ikan Mati (Coll. 2) to bensulfuron-methyl: The recommended rate was 0.04 kga.i./ha.

completely killed by bensulfuron-methyl at 10 times the recommended rate or more and even at the recommended rate its growth was considerably inhibited (Fig. 4).

Experiment 2

While all ramet plants of Coll.2 in the herbicide-treated plots were killed, 86.6%, 86.1% and 88.3% of Coll.1 plants survived 2,4-D dimethylamine at the recommended rate, bensulfuron-methyl at 10 times the recommended rate, and sequential application of 2,4-D and bensulfuron-methyl respectively (Table 3). No significant (p=0.05) difference was detected between the ratios of surviving plants in the herbicide-treated plots and that of the non-treated control by the χ^2-test.

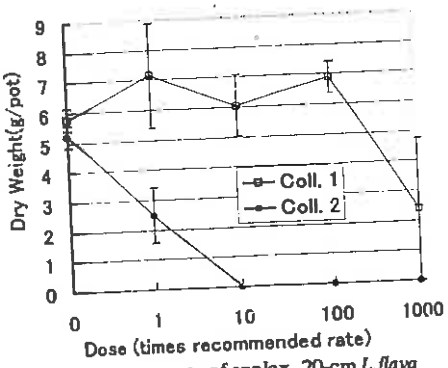


Fig. 4 Dosage response of applox. 20-cm *L.flava* plants from a paddy field in MARDI Seberang Perai (Coll. 1) and an abandoned paddy field in Lahar Ikan Mati (Coll. 2)

Table 3 Effect of 2,4-D at the recommended rate and bensulfuron-methyl (BSM) at 10 times the recommended rate against *L.flava* from paddy field in MARDI Seberang Perai (Coll. 1).

Treatment	Number of plants		Ratio of surviving plants
	Surviving	Dead	
Non	65	3	95.7
2,4-D	51	8	86.6
BSM	56	9	86.1
2,4-D+BSM	67	9	88.3

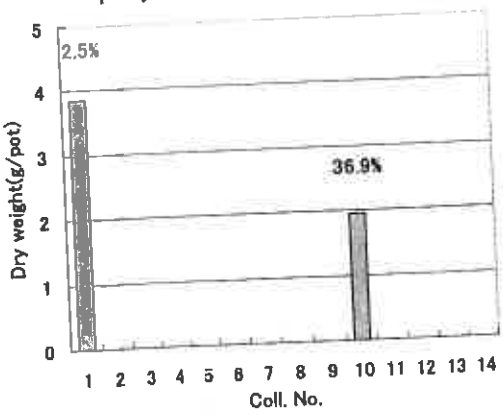


Fig. 5 Efficacy of 2,4-D dimethylamine against 14 collections of *L.flava* : Values on bars show percentage of control.

Experiment 3

Except for Coll.1, only plants of Coll.10 from Seberang Perak Rice Estate survived the 2,4-D treatment at the recommended rate (Fig. 5). All plants of the other collections were killed and 8 out of 12 plants of Coll.10 were also killed by the 2,4-D treatment. All plants except Co.ll.1 plants were killed by bensulfuron-methyl at 10 times the recommended rate.

Experiment 4

For the II/1997, even at four times the recommended rate, which provided high efficacy against herbicide-resistant *L.flava* in pot studies, the control efficacy of 2,4-D dimethylamine was unsatisfactory, its percentage was 77.5% and 76.5% with/without wetting agent respectively (Treatment No. 7 and 8 in

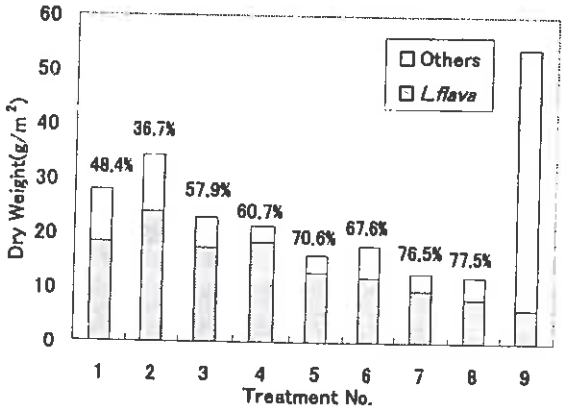


Fig. 6 Efficacy of 2,4-D dimethylamine at different dosages with /without wetting agent in paddy field infested by *L.flava* resistant to 2,4-D dimethylamine and bensulfuron-methyl: Values on bars show percentage of control in total dry weight of weeds.

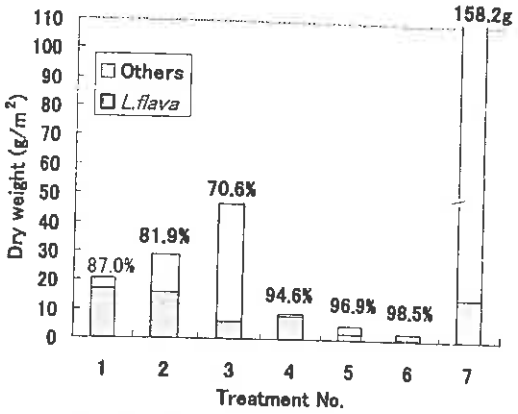


Fig. 7 Efficacy of some herbicide treatments in paddy field infested with *L.flava* resistant to 2,4-D and bensulfuron-methyl at seeding rate of 60 kg/ha: Values on bars show percentage of control in total dry weight of weeds.

Fig. 6). The effect of wetting agent on control efficacy was not detected (Fig. 6).

In the I/1998, single application of bensulfuron-methyl at seven days after sowing (DAS) and 14 DAS and 2,4-D dimethylamine at 14 DAS did not provide satisfactory results in plots where the seeding rate was 60 kg/ha (Treatment No. 1,2 and 3 in Fig. 7). Sequential application of 2,4-D dimethylamine at 14 DAS followed by 2,4-D dimethylamine at the recommended rate, 2,4-D dimethylamine at four times the recommended rate and bentazone at 30 DAS provided good control effects (Treatment No. 4, 5 and 6 in Fig. 7). For Treatment No. 4, however, *L.flava* made up a high percentage of total weed weight.

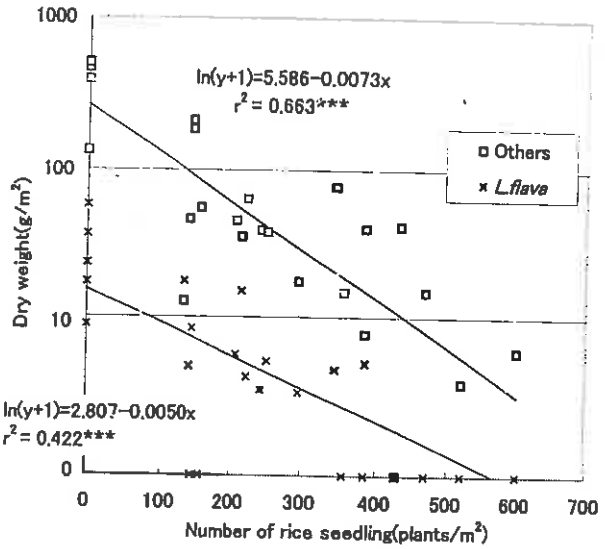


Fig. 8 Relationship between seedling density of cultivated rice and weed growth in paddy field infested with *L.flava* resistant to 2,4-D and bensulfuron-methyl: Plots were treated with molinate and 2,4-D following the recommendation.

Under the condition of single application of 2,4-D dimethylamine, the density of rice seedling strongly affected weed growth, establishing a linear relationship between density of rice seedling and dry weight of *L.flava* or other weeds in logarithmic scale (Fig. 8). No significant difference (p=0.05) was detected between the slopes of lines for *L.flava* and other weeds (Fig. 8).

Discussion

For 2,4-D dimethylamine, an approximate difference of 32 times in value of I_{50} was observed between two collections (Fig. 2). Although the I_{50} values of the collections in this study were about 10 times lower than Edison's LD_{50} values (1996) on *L.flava* in Indonesia, the 32-time-difference in I_{50}, between resistant biotype and susceptible biotype in this study was similar to the 40-time-difference in LD_{50} in Edison's study (1996). For bensulfuron-methyl, the I_{50} value of Coll.1 was 28.1 times the recommended rate (Fig. 3). Although I_{50} value of Coll.2 was not determined in this study, it should be less than the recommended rate. Therefore at least a 28.1-time-difference in I_{50} existed between seedlings of Coll.1 and Coll.2 for soil application of bensulfuron-methyl.

The difference in susceptibility to bensulfuron-methyl between the resistant biotype (Coll.1) and the susceptible biotype (Coll.2) was detectable using approx. 20-cm plants (Fig.4). Although this method is

different from the application method of farmers, it can be used for a simple diagnosis of resistant biotypes in *L.flava*. That is, plants collected from farmers' field are treated with bensulfuron-methyl with the rate between 10 to 100 times the recommended rate after a few weeks of rearing.

The result of Experiment 2 (Table 3) indicated that most of the *L.flava* plants in the source-field of Coll.1 were multiple-resistant to both 2,4-D dimethylamine and bensulfuron-methyl. The multiple-herbicide-resistant *L.flava* reported here is the first confirmation of multiple-herbicide-resistance and also ALS-inhibitor-resistance in Malaysia and probably in tropical Asia. As pointed out by Heap (1997), herbicide-resistant weeds have serious economic impact on crop production when there are few or no alternative herbicides to control the resistant biotypes. In this context, *L.flava* that has multiple-resistance to 2,4-D and bensulfuron-methyl is of greater concern to farmers in Malaysia, where these two herbicides are used extensively.

In this study, only 2,4-D resistant biotype was found in Seberang Perak Rice Estate and MARDI S.P. (Fig. 5), and no bensulfuron-methyl resistant biotype or multiple resistant biotype was found except for Coll.1. The field in MARDI S.P. infested with multiple-herbicide-resistant *L.flava* was used as experiment plots for rice breeding until I/1997. The history of herbicide usage was not recorded exactly, but according to a research assistant in MARDI S.P., bensulfuron-methyl was used for two seasons or more and 2,4-D was used for many seasons before that. Although the field was used for the special objective of rice breeding, where some sections of the field were left empty a long time after herbicide application, vacant spots caused by poor rice germination were often observed in farmers' fields in Malaysia. Such a condition, where no selection pressure except the herbicide is effective, is favorable for development of herbicide-resistant biotypes. Moreover in Malaysia many herbicides, such as sulfonylurea, are used in single-formulations. There is possibility that repeated use of particular single-formulation herbicides hastens development of herbicide-resistant biotypes. Moreover, double cropping of rice is adopted extensively in the country, thus resistant biotype can be selected twice a year.

As shown in Fig7, bentazone controled the resistant *L.flava* effectively and certainly 2,4-D and bensulfuron-methyl are still useful for other broad-leaved weeds. The use of a single type of herbicide, however, may give rise to the risk of another herbicide resistance. Actually farmers have already reported some weed species that appeared to develop herbicide-resistant biotypes. In this study, the density of rice plants strongly affected growth of the herbicide-resistant *L.flava* as well as other weeds (Fig. 8). Therefore adequate density of rice seedlings can pose another selection pressure. As pointed out by Watanabe *et al.* (1997), continuous monitoring is necessary to control herbicide resistant weed readily. Moreover importance of integrated weed management must be stressed much more in the management of herbicide-resistant weed in Malaysia.

In this study, the efficacy of other ALS-inhibitors and other auxin-type herbicides to the resistant biotype of *L.flava* was not examined. The responses of the resistant biotype to those herbicides and the mechanism of the resistance expected to provide valuable information to improve the management of herbicide-resistant weed.

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ALLELOPATHIC EFFECTS OF ACETONE EXTRACTION OF *WEDELIA CHINESE*

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Abstract: *Wedelia chinese* is an aggressive garden herb in south China and usually forms pure community. The acetone extraction from its leaves, stems and roots significantly inhibited the radicle and seedling growth of *Leptochloa chinese*, *Digitaria sanguinalis*, *Portulaca oleracea* and *Chenopodium album*, seed germination of *L. chinese*, *D. Sanguinalis* was obviously inhibited, but *P. oleracea* and *C. album* germinared well. The allelopathic potential of *W. chinese* was in the order: leaf > rotted leaf > root > stem.

Keywords: Allelopathic effect, *Wedelic chinese*

Introduction

Wedelia chines is common garden herb and usually forms pure community in south China. Zeng Rensen et al (1994,1996) reported the allelopathic effect of *W. chinese* on *Raphanus sativus*, *Cucumis sativus*, *Oryza sativa* and *Echinoloa crusgalli*, and separated two allelochemicals from methylene chloride extraction of leaves. We now study the allelopathic potential of acetone extraction on other four receptor plants .

Materials and Methods

Plants materials

W. chinese was harvested from garden everywhere in south China in April. Seeds of four receptor plants: *Leptochloa chinese*, *Digitaria sanguinalis*, *Portulaca oleracea* and *Chenopodium album* were collected from the field of Guangdong Academy of Agricultural Sciences.

Acetone extraction

One hundred gram of newly harvested leaves, stems or roots of *W. chinese* were homogenized, 1000 ml acetone was added to this homogenization and kept for 12 hours, the homogenization was filtered and then decolored with 20-g active carbon two times, filtered again, the acetone phase was condensed with rotary vacuum evaporator at 60 °C to the volume of 100 ml, the concentration of this extract was 1 g FW / ml.

Bioassay

Two piece of filter paper (ϕ 7 cm) were placed in ϕ9 cm petric dish and then added 0.5 ml extracts and 3 ml tap water, 100 enough water-absorbed receptor seeds were scattered on paper. The petric dishes were kept at 30 °C with 8-h photoperiod of natural radiation for seed germination and seedling growth. The experiment was repeated with 4 replications, data presented were the means of 4 replication.

Results and Discussion

Allelopathy is a common phenomenon in nature. Some plants inhibit other plants by physical, chemical or biological, especially allelopathic effects and tend to form pure populations. *W. chinese* is a garden herb in south China, there is little weed within its community. The acetone acetone extraction from different part of W. chinese, especially from leaves and rotted leaves, inhibited the seed germination of *L. chinese D. Sanguinalis,* but had no obviously inhibitory effects on the germination of *P. oleracea* and *C. album* (Table 1).

After germination, the radicle growth of four receptor plants was significantly inhibited, the root apex of *P. oleracea* and *C. album* were necrosis. If the cotyledon of *P. oleracea* contacted the acetone extracts, it was necrosis too. The growth of coleoptile of *L. Chinese* and *D. Sanguinalis* or hypocotyl of *P. oleracea* and *C. album* was also obviously inhibited (Table 2). The allelopathic potential of *W. chinese* was in the order: leaf > rotted leaf > root > stem. It can be conferred that *W. chinese* inhibited other weeds by allelopathic effects and forms pure community.

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Table1 Percentage of seed germination of four receptor plants treated with acetone extraction from different part of *W. chinese.*

Donator	*L. chinese*	*D. Sanguinalis*	*P. oleracea*	*C. album*
Leaves	19.2c	18.3c	62.1	70.6
Stems	27.6b	26.6b	65.9	75.1
Roots	22.9bc	24.1b	66.2	73.6
Rotted leaves	17.1c	17.8c	64.5	75.3
Control	78.6a	96.3a	69.8	76.2

Note: • Means followed by the same letter are not significantly different according to Fisher's Protected LSD test (α=0.05)

• Data are the results of 5-d culture

Table 2 Effects of acetone extraction from different part of *W. chinese* .on seedling growth of four receptor plants

Donator	*L. chinese*		*D. sanguinalis*		*P. oleracea*		*C. album*	
	Seedling Height	Root length	Seedling height	Root length	Seedling Height	Root length	Seedling height	Root length
Leaves	0.26c	0.18d	0.55b	0.21d	0.59b	0.19d	0.69b	0.23d
Stems	0.44b	0.51b	0.63b	0.68b	0.72b	0.52c	0.81b	0.60c
Roots	0.41b	0.32cd	0.61b	0.38c	0.68b	0.46c	0.70b	0.52c
Rotted leaves	0.30c	0.21d	0.57b	0.25d	1.38a	0.87b	1.57a	0.98b
Control	1.22a	1.30a	2.61a	1.83a	1.56a	1.49a	1.66a	1.52a

Note: • Means followed by the same letter are not significantly different according to Fisher's Protected LSD test (α=0.05)

• Data are the results of 8-d culture

DEVELOPMENT OF IMMUNOASSAY FOR DETECTION OF THE HERBICIDE THIOBENCARB RESIDUES IN WATER

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Abstract: A direct competitive enzyme-linked immunosorbent assay (direct C-ELISA) was developed using the monoclonal antibody TBC7-2. The direct C-ELISA determined the thiobencarb at the concentration range from 0.4 ng/ml to 4.0 ng/ml. While the other popular herbicides applied to paddy fields, such as bensulfuron-methyl, pyrazosulfuron-ethyl, esprocarb, mefenacet, 2,4-D etc were almost no reactions on this condition. The direct C-ELISA could determine the thiobencarb fortified in paddy waters, a river water and a pond water without any influence of their ingredients. For the surface water samples collected from the paddy field to which the formulation was applied, the correlation between the direct C-ELISA and gas chromatography (GC) analysis was good (r=0.964). These results suggest that the direct C-ELISA can use practically to determine thiobencarb residue in water samples.

Key Words: thiobencarb, immunoassay, C-ELISA, monoclonal antibody, residue analysis

Introduction

Thiobencarb, S-4-chlorobenzyl N,N-diethylthiocarbamate, is a herbicide that is widely used for the control of monocotyledonous weeds including a barnyard grass (*Echinochloa crus-galli*) and annual broad-leaved weeds in paddy fields (Tomlin, 1997). Since the formulation is generally used for the rice-planting season, May and June in Japan, it is important to monitor the thiobencarb residue flowed out from the paddy field to river or pond of the area. The residue is generally analysed by gas chromatography (GC) (Au and Fung, 1988). GC analysis can sensitively and precisely determine thiobencarb, but it is time-consuming and labor-intensive since the large volume of sample and the complicated pre-treatment is necessary, as described in Fig.1.

On the other hand, a direct competitive enzyme-linked immunosorbent assay (direct C-ELISA) is also known as a sensitive and convenient method to determine herbicides (Aga and Thurman, 1997). Recently, we reported that the monoclonal antibody TBC7-2 toward the thiobencarb was prepared and the direct C-ELISA based on the TBC7-2 showed the working range from 5 ng/ml to 100 ng/ml (Miyake et al., 1998). While the range was not sufficiently sensitive to determine the residue in water since the quality standard values for public, tap and ground water were 0.02mg/ml. Then, we attempted the improvement of the sensitivity. This proceeding describes the applicability of the improved direct C-ELISA to the

monitoring of thiobencarb residues in water.

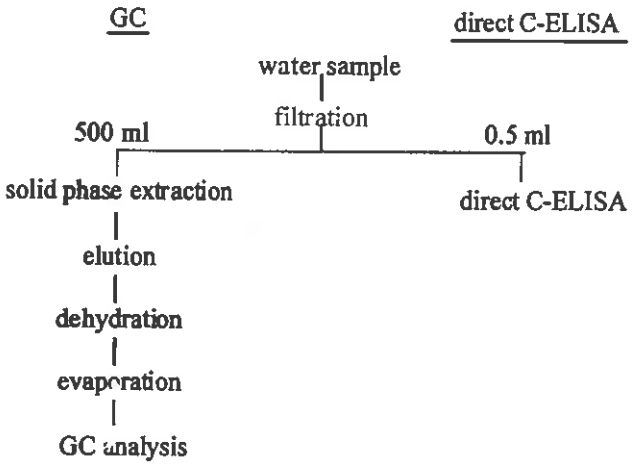

Fig. 1 Comparison of typical steps in GC and direct C-ELISA for thiobencarb analysis in water

Materials and Methods

Chemicals and Biochemicals

Thiobencarb, alachlor, atrazine, bensulfuron-methyl, cyanazine, daimuron, esprocarb, imazosulfuron, metolachlor, pendimethalin, prometryn, pyrazosulfuron-ethyl and trifluralin were purchased from Wako Pure Chemical Industry Co. Ltd (Osaka, Japan). Chlorpropham, 2,4-D, dimepiperate, MCPB, mefenacet, molinate, monuron and pyriminobac-methyl were purchased from Riedel-de Haën (Germany). Microtiter plate with 96-wells was purchased Sumitomo bakelite Co., Ltd (Tokyo, Japan) and 3,3',5,5'-tetramethylbenzidine (TMBZ) was from Wako Pure Chemical Industry Co. Ltd. All other chemicals and reagents were of analytical grade.

Direct C-ELISA

The direct C-ELISA was constituted on the following condition. A hundred µl of the purified monoclonal antibody solution (2µg/ml) was added to each well of microtiter plate with 96-wells to saturate the available sites on the wells and then the wells were blocked with 1% BSA dissolved in phosphate buffered saline (PBS; pH 7.0).

On the other hand, methanol solution of thiobencarb was diluted to 1% methanol with PBS supplemented with 10mM EDTA (dilution buffer). Then, 500 µl of the diluent or a water sample was mixed with 500 µl of the thiobencarb-hapten horseradish peroxidase (HRP) conjugate solution diluted to 2-folds higher concentration than that of 50% binding ability to the coating antibody with the dilution buffer supplemented with 0.3% BSA, from the results of preliminary examinations to determination the conjugate concentration.

Each of 100 µl of the mixture was added to the above wells and incubated for 1.0 hr at 25°C. After the wells were washed 3 times with PBS supplemented with 0.02% Tween-20, 100 µl of the substrate (100 µg/ml TMBZ and 0.006% H_2O_2) solution of HRP was added to the wells and the plate was incubated for 10 min at room temperature. Color reaction developed is stopped by adding an equal volume of 1N H_2SO_4 and the absorbance at 450 nm was measured with an automated microplate reader (Bio-tek Instruments, USA).

Collection of water samples

Surface water samples were collected from a paddy field to which the formulation including thiobencarb as active ingredient (Wolf-Ace) was applied. Those of the other paddy fields before herbicides were applied, a river (Tone-river) and a pond (Inba-numa) in winter season were also collected. These water samples were filtered through a conventional filter paper and used to determine thiobencarb.

Gas chromatography analysis

C_{18} SPE cartridge was used for the concentration of thiobencarb from water samples. After pre-washing the cartridge, 500 ml of water samples were applied at the rate of 20 ml/min. The column was dried by ventilation of compressed air for 30 sec. Thiobencarb trapped was eluted with 10 ml of dichloromethane and, after evaporated to dryness, was reconstituted with a fixed volume of acetone.

The solution was employed to GC analysis using Shimazu GC-17A gas chromatograph equipped with a DB5 column (30 m x 0.25 mm) with a film thickness of 0.25 μm and a flame thermionic detector. The column temperature was programmed from 60°C to 160°C at 25°C/min, from 160°C to 230°C at 10°C/min and from 230°C to 270°C at 5°C/min with a final hold of 3 min. The gas was conditioned at 25 KPa hydrogen (1 ml/min), 75 KPa air (230 ml/min) and 80 KPa make-up helium gas (30 ml/min). The injector temperature was 270 °C and that of the detection was 290°C. The recovery from the water sample fortified with thiobencarb (100 ng/ml) was amounted to 94.6%.

Results and Discussion

Sensitivity

Fig.2 shows a typical standard curve in the direct C-ELISA. The working range was 0.4 ng/ml to 4.0 ng/ml and IC_{50} value was 1.7 ng/ml. This result suggests that the direct C-ELISA is sufficiently sensitive to determine thiobencarb residue in water since the quality standard value is 0.02 mg/ml.

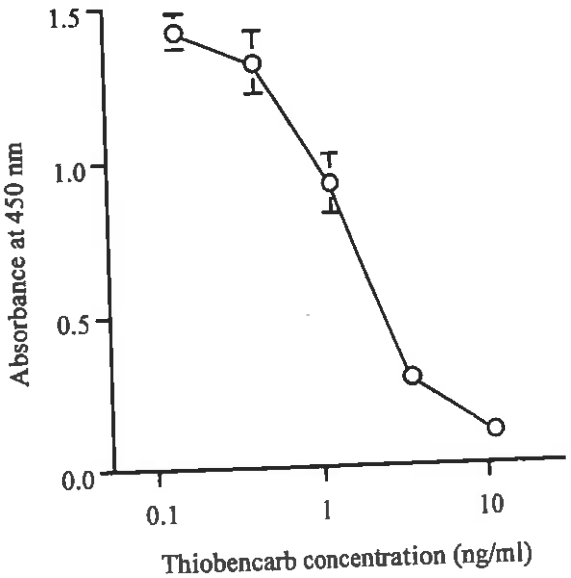

Fig. 2 Standard curve for thiobencarb determination in the direct C-ELISAs. Results are represented as means±standard deviation (n=3).

Specificity

The cross-reactivities of the monoclonal antibody TBC7-2 with the major herbicides applied in paddy fields are described in Table 1. TBC7-2 didn't quite recognize the herbicides examined excepting mefenacet was slightly reacted (1.0%).

The result suggests that the TBC7-2 based direct C-ELISA possesses the good specificity to thiobencarb and the herbicides included in water samples don't basically interfere the thiobencarb determination. But the paddy water, which the formulation including mefenacet has been applied just before the sampling, may give the influence to the assay.

Table 1 Cross reactivity of TBC7-2 with herbicides for the direct C-ELISA

Herbicide	IC_{50} (ng/ml)	Herbicide	IC_{50} (ng/ml)
Thiobencarb	1.7	MCPB	>10,000
Alachlor	>10,000	Mefenacet	170
Atrazine	>10,000	Metolachlor	>10,000
Bensulfuron-methyl	>10,000	Molinate	>10,000
Chlorpropham	>10,000	Monuron	>10,000
Cyanazine	>10,000	Pendimethalin	>10,000
2,4-D	>10,000	Prometryn	>10,000
Daimuron	>10,000	Pyrazosulfuron-ethyl	>10,000
Dimepiperate	>10,000	Pyriminobac-methyl	>10,000
Esprocarb	>10,000	Trifluralin	>10,000
Imazosulfuron	>10,000		

Assay of thiobencarb fortified water

Since the direct C-ELISA showed the good sensitivity and specificity, we collected water samples from paddy fields in two different area, a river and a pond. The assay of water samples fortified with thiobencarb was examined.

As shown in Fig. 3, each of the reaction curves was agreed well with standard curve. Although the results are preliminary, it is considered that the direct C-ELISA can use to determine thiobencarb residues in water. Further comparisons will need to be carried out using many water samples from different area.

Comparison of the data between the direct C-ELISA and GC

Thiobencarb residues were analysed by the direct C-ELISA and GC using the water samples collected from the paddy field applying the formulation including thiobencarb. As shown in Fig.4, the data of the direct C-ELISA were agreed well with those of the GC analysis. This result suggests that the values determined by the direct C-ELISA is reliable as well as those of GC analysis.

Thus, the direct C-ELISA developed is appeared to be useful to simplify the analysis of real water samples. It is needed to take more reliability by examining many water samples.

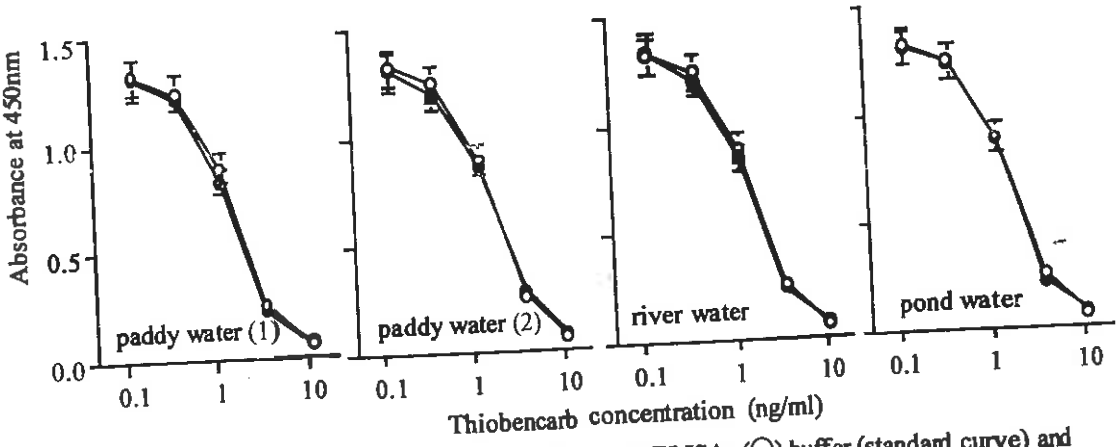

Fig. 3 Influence of various samples in the direct C-ELISA: (○) buffer (standard curve) and (●) water sample. Results are represented as means ± standard deviation (n = 3).

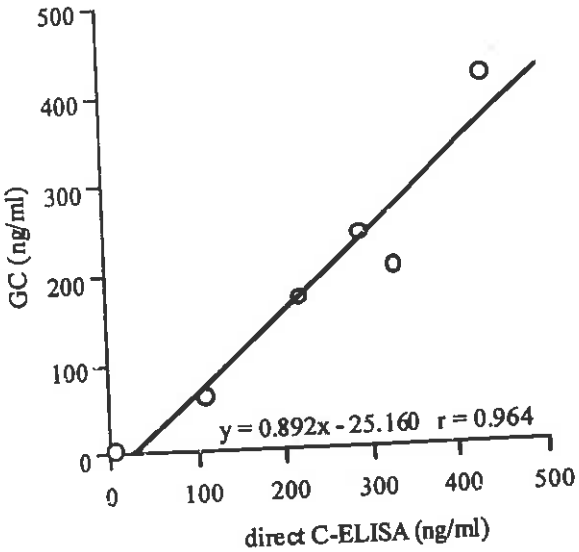

Fig. 4 Comparison of the direct C-ELISA and GC analysis

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EFFECT OF CYANOBACTERIA EXTRACT ON GERMINATION AND GROWTH OF SOME PLANTS

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Abstract: Tris-HCl buffer extract from *Scytonema* sp. and *Hapalosiphon* sp. at the concentration of 0.1 g (fresh weight)/ml reduced seed germination of popping pod (*Ruellia tuberosa* Linn.) but had no effect on germination of mung bean (*Vigna radiata* (L.) Wilczek.) 's seeds. However, seedlings of both plants were abnormal. Cultured medium of *Hapalosiphon* sp. also showed a significantly decreased in germination of popping pod.

Key words: cyanobacteria, germination, growth, plants

Introduction

The cyanobacteria (blue-green algae) was found to inhibit the growth of other algae (Mason *et al.*, 1982) and some angiosperms including lemna (*Lemna gibba*), pea (*Pisum sativum*), corn (*Zea mays*), curled dock (*Rumex crisous*), wild buckwheat (*Polygonium convolvulus*), wild oats (*Avena fatua*) and green foxtail (*Setaria virids*) (Gleson and Case, 1986). This inhibition was due to the production and excretion of a secondary metabolite by *Sytonema hofmanni* (Mason *et al.*, 1982). The active metabolite has been isolated and chemically characterized. This metabolite, cyanobacterin, is a diaryl-substituted gamma lactone with a choline substituent on one of the aromatic rings (Pignatells *et al.*, 1983). Cyanobacterin inhibits O_2 evolving photosynthetic electron transport in all plants and the most probable site of action is in photosystem II. Cyanobacterin had no effect on electron transport in photosystem I.

Hapalindole, an indole alkaloid, is responsible for most of the antibacteria and antimycotic activity associated with *Hapalosiphon fontinalis* (Moore *et al.*, 1987).

This paper shows the effect of cyanobacteria on germination and growth of popping pod (*Ruellia tuberosa* Linn.) and mung bean (*Vigna radiata* (L.) Wilczek.).

Materials and Methods

Growth of the cyanobacteria

Scytonema sp. and *Hapalosiphon* sp., collected from water, soil and rock surface, were purified by streak plate method and cultured in liquid BG-11_0 medium (Richmond, 1986) at 30 °C under 60 µmole (photon)/m^2/s.

Effect of cyanobacteria extract on germination and growth of some plants

Cell cultures, grown in liquid medium for 3 weeks, were centrifuged at 10,000 xg for 10 minutes. Supernatant (cultured medium) was collected for seed germination and growth test of popping pod (*Ruellia tuberosa* Linn.) and mung bean (*Vigna radiata* (L.) Wilczek.).

Cells were resuspended in 10 mM tris-HCl buffer pH 7.1 (0.1 g fresh weight/ml), and were broken by utrasonic. The suspension was separated to two parts. The first one was directly used for seed germination and growth test as crude extract and the second one was then centrifuged at 10,000 xg for 10 minutes. The supernatant was assayed for seed germination test.

Results

Tris-HCl buffer extract from *Scytonema* sp. reduced seed germination of popping pods. The percentages of germination were 76.8 and 72.8 when treated with supernatant and crude extract, respectively. The percentage of germination, treated with cultured medium, was 85.6 which was not

significantly difference from the control. In case of mung bean, both tris-HCl buffer extract and cultured medium had no effect on its germination. However, seedling of both plants were abnormal by short roots, hypocotyls and epicotyls (Table 1).

The percentages of germination of popping pod's seed were 57.6 and 43.2 when treated with supernatant and crude extract of *Hapalosiphon* sp. Germination of popping pod's seed treated with cultured medium was 43.2% which was very low when compared to its control. Both tris-HCl buffer extract and cultured medium had no effect on mung bean's seed germination (Table 2)

Table 1 Effect of tris-HCl buffer extract and cultured medium of *Scytonema* sp. on germination of popping pod and mung bean's seed

Extractant	Germination of popping pod's seed (%)	Germination of mung bean's seed (%)	Symptom
Water	88.8c	100	Normal
Tris-HCl buffer	84.8bc	100	Normal
Supernatant	76.8ab	100	Short roots, hypocotyls and epicotyls
Crude extract	72.8a	100	Short roots, hypocotyls and epicotyls
Culture medium	82.4bc	100	Normal
3 weeks-cultured medium	85.6bc	100	Normal

Table 2 Effect of tris-HCl buffer extract and cultured medium of *Hapalosiphon* sp. on germination of popping pod and mung bean's seeds

Extractant	Germination of popping pod's seed (%)	Germination of mung bean's seed (%)	Symptom
Water	92.8d	100	Normal
Tris-HCl buffer	66.4c	100	Normal
Supernatant	57.6bc	100	Brown roots, Short hypocotyls and epicotyls Densely roots
Crude extract	43.2b	100	Brown roots, Short hypocotyls and epicotyls Densely roots
Culture medium	96.0d	100	Normal
3 weeks-cultured medium	18.4a	100	Brown roots, Short hypocotyls and epicotyls Densely roots

Discussion

Tris-HCl buffer extract and cultured medium of both *Scytonema* sp and *Hapalosiphon* sp. had nc effect on germination of mung bean' s seeds, but affected on germination of popping pod's seeds. It might be possible that the cotyledon of mung bean's seed was so thick that the extract and cultured medium could not imbibe through the embryo. The percentage of popping pod's seeds germination was significantly decreased with supernatant and crude extract. Although the percentage of its germination decreased when treated with cultured medium, it was not significantly difference from the control. This

showed that tris-HCl buffer could extract some substance from these cyanobacteria that can inhibit seed germination and seedling growth, and *Scytonema* sp. did not secrete this substance to the culture medium.

Cultured medium of *Hapalosiphon* sp. had strongly effect on germination of popping pod's seeds. The percentage of germination was drastically decreased. This showed that *Hapalosiphon* sp. excrete some substance to the medium and inhibit growth and development of another plant associated with it. This finding is the same as Moore *et al.* (1987) reported about hapalindole that could inhibit bacteria and mycotic activity associated with *Hapalosiphon fontinalis*.

The supernatant and crude extract which was extracted from *Hapalosiphon* sp. by tris-HCl buffer had effect on germination of popping pod's seeds. The percentage of germination was significantly difference from the control. This also showed that tris-HCl buffer could extract some substance from these cyanobacteria that can inhibit seed germination and seedling growth.

The percentage of seed germination treated with the extract and cultured medium of *Hapalosiphon* sp. was lower than that of *Scytonema* sp., especially cultured medium of *Hapalosiphon* sp.. This results might be showed that the metabolite in *Hapalosiphon* sp. affected on seed germination and seedling growth stronger than *Scytonema* sp.

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EFFECTS OF SOIL ENVIRONMENTAL CONDITIONS ON DECOMPOSITION RATE OF HERBICIDE PRETILACHLOR IN SOILS

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Abstract:. Pretilachlor is a herbicide for control of annual weeds in rice paddy fields. The decomposition of herbicide pretilachlor in soils was carried out under different environmental conditions. Pretilachlor degradation in soils followed first-order reaction kinetics. In the laboratory incubations the half-life pretilachlor in flooded soil varied from 17 days at 30 °C to 45 days at 15 °C. Degradation rate of pretilachlor was rapider in moist soil conditions than in submerged soil conditions The decomposition rate was faster in silty clay soil (Chonju soil) than in sandy clay loam soil (Kunsan soil). Pretilachlor in the surface water of flooded soil was lost rapidly. In the field experiment half-life of pretilachlor was shorter than 3 days.

Key words: pretilachlor, decomposition, soil, environment

Introduction

Pretilachlor (2-chloro-2',6'-diethyl-N-(2-propoxylethyl) acetanilide) is a herbicide for control of annual grasses, broad-leaved weeds and sedges in rice paddy fields. It is primarily applied pre-emergence and hence behavior in soil is of particular importance. In general, persistence and mobility of herbicides in soil are greatly influenced by environmental conditions such as temperature, soil moisture and soil characteristics (Walker, 1987 and 1989, Alexander and Scow, 1989). In the experiments of Ma *et al.* (1987) and Moon *et al.* (1988), the period of residual activity of pretilachlor in the soils was about 50 days. The faster degradation occurred under 30 °C soil temperature by comparison under the 20 °C. The degradation of pretilachlor was effected by soil characteristics. The mobility of pretilachlor in the soils was 6 cm in sandy clay loam soil and 10 cm in sandy loam soil.

The present experiment was made to gain further information on the degradation of pretilachlor in soil under Korean conditions. The detailed studies were made of the effects of temperature and soil moisture on decomposition rates of pretilachlor in laboratory. Persistence of residue in the soils was also measured under natural conditions in the field.

Materials and Methods

Soil and herbicide

The soils used were from the farm of college of agriculture, Chonbuk National University at Chonmi, Chonju, Chonbuk, South Korea (Chonju soil) and a farm at Hoehyon, Kunsan, Chonbuk (Kunsan soil). The properties of soils were as follow: Chonju soil; silty clay soil, pH 4.9, organic matter content 2.84 %, CEC 9.78 me/100 g and Kunsan soil; sandy clay loam soil, pH 5.4, organic matter content 2.52 %, CEC 12.12 me/100 g. Soil samples were collected from the 0-15 cm horizon of the Chonju farm and Kunsan farm at March 1999 and passed through a 5 mm sieve for laboratory experiments and then kept in laboratory conditions until the incubation experiments.

The herbicide used was a commercial 2 % granule and analytical grade pretilachlor from Novartis (Ciba-Geigy) Korea Limited.

Laboratory studies

Each 20 g of Kunsan soil was put in test tubes (3 cm id, 18 cm long). The analytical grade of pretilachlor in acetone was added to each soil in the test tubes and mixed well. The soil was flooded up to 2 cm deep. For the effect of soil moisture contents on degradation of pretilachlor, water was added to adjust soil moisture content to 21.9 % (near field moisture capacity), 37.6 % and 45.3 % (near maximum water holding capacity). The test tubes were covered with aluminum foil and then incubated at 30 °C. For the effect of soil temperatures the flooded soil was incubated at 15 °C, 22 °C and 30 °C. The soil water contents were maintained throughout the experiments by addition of water. The soils were sampled at designated intervals and frozen until analyzed.

Field experiments

Plots (4.5 X 9.2 m) in Chonju and Kunsan Farm were prepared and paddy rice (Cultivar: Geihwa) were transplanted on May 26 and May 27, 1992, respectively. Pretilachlor 2 % granule at 0.08 kg ai/10a was treated to the soil surface of the plots on 5 and 15 days after the transplanting. Two plots were used for the control. Immediately after the second application, 10 cores (5 X 10 cm) of soil were taken from each plot at random positions for the residual analysis of field. The cores from each plot were bulked together. The samples were air-dried under laboratory conditions within one day from the collection. The air-dried soil was mixed well using a 5-mm sieve. The samples were kept frozen until analysis. Further soil samples were taken at appropriate intervals.

For the loss study in flooding water, Chonju soil was put in a pot (23 cm id, 25 cm height). The soil was flooded up to 3 cm. Pretilachlor 2 % granule at 0.06 kg ai/10a was applied to the soil surface of the pots in a green house. The 30 ml of water samples was taken from each pot at 1 hr after the application. Further the water samples were taken at appropriate intervals after the application.

Herbicide analysis

The duplicate amounts of soil (20 g) from the laboratory and field samples were shaken with 75 ml

acetone for 1 hr. The extracts were filtered and the residue soil was re-extracted with 50 ml acetone. The acetone extracts were combined and then evaporated to about 30 ml. The evaporated extracts were transferred to a separating funnel and placed in 100 ml of 5 % NaCl solution, and 30 ml of n-hexane. The mixture was shaken and the n-hexane was removed. The aqueous residue was extracted with 30 ml of n-hexane. The hexane extracts were combined, dried with sodium sulfate anhydrous, and evaporated to nearly dryness and then dried by nitrogen gas. The residue was dissolved in 10 ml n-hexane and the concentration of pretilachlor was measured by GLC. The water sample (30 ml) was shaken with 30 ml of n-hexane for 10 min. The hexane extract was dried with sodium sulfate anhydrous and evaporated. The residue was dissolved in 1-5 ml of n-hexane and the concentration of pretilachlor was measured by GLC. All samples were analyzed using a Shimadzu model GC-14AP with a NP-FID. A glass column (3.5 mm id, 1 m long) packed with 3 % Silicone SE-30 (Chromosorb WHP) was used. The operating temperatures of injection port, detector and column oven were 260, 300 and 250 °C, respectively. The gas flow rate of helium carrier gas, hydrogen and air was 30, 4 and 150 ml/min, respectively. The recovery of pretilachlor from soil and water samples fortified with analytical grade herbicide in the range 0.1 to 5 ppm was always greater than 80 %.

Results and Discussion

The results from the incubation studies at constant temperature are summarized in Table 1. The rate constants and half-lives were derived by linear regression analysis of residual concentrations on a logarithmic scale against time of incubation for each treatment. The data indicate close correspondence to first-order degradation kinetics ($r > 0.951$ for all treatment).

Table 1. Degradation rate constant (K_{deg}), first-order half-lives (HL) and determination coefficient(r) for pretilachlor degradation in soil at different temperature, soil moisture content and soil types.

Soil	Incubation temperature (℃)	Soil moisture content(%)	r	K_{deg} (day^{-1})	HL (days)
Kunsan	30	21.9	0.986	0.0709	9.8
	30	37.6	0.976	0.0517	13.4
	30	45.3	0.951	0.0436	15.9
	15	flooded	0.965	0.0155	44.7
	22	flooded	0.993	0.0320	21.7
	30	flooded	0.984	0.0410	16.9
Chonju	30	flooded	0.985	0.0518	13.4

There was a marked effect of temperature on decomposition rate with a variation in half-life from 16.9 days at 30 °C to 44.7 days at 15 °C. Many studies indicated that herbicides in soils were rapidly degraded in high temperature conditions more than in low temperature conditions. Temperature effects on degradation of herbicides can often be characterized using the Arrhenius equation (Hurle & Walker, 1980, Moon & Walker, 1991). The fit of the present data to this relationship was determined by regression analysis of the logarithm of the half-lives against the reciprocal of the absolute temperature as shown in Fig. 1. The regression coefficient was 0.96 and the activation energy derived from the slope of the line was 45.2 KJ/molecules. Walker *et al.* reported that the activation energy of alachlor was 68.3-79.1 KJ/molecules.

Fig. 1 Fit of the degradation rate of pretilachlor at different temperature to the Arrhenius relationship.

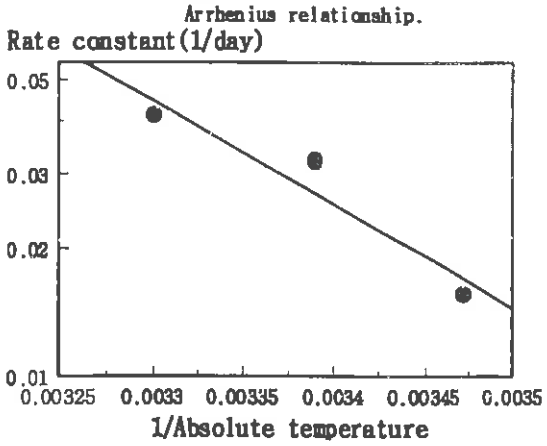
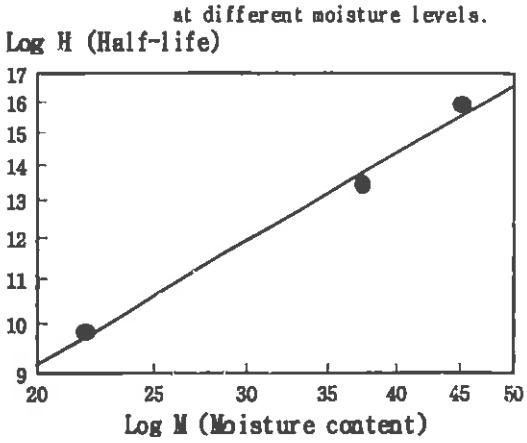


Fig. 2 Empirical plots for the degradation rate of pretilachlor in Kunsan soil at different moisture levels.



The results from the incubations with varying soil moisture levels also gave good approximations to first-order reaction kinetics and the rate constants and half-lives derived from the data are listed in Table 1. The half-lives varied from 9.8 days at 21.9% moisture (near field capacity) to 15.9 days at 45.3 % moisture (near maximum water holding capacity) and 16.9 days at flooded conditions. It is suggested that pretilachlor in soil is rapidly degraded in aerobic conditions more than anaerobic conditions. In previous experiments the effect of soil moisture on herbicide degradation rate was characterized using an empirical equation: $H=AM^{-B}$ in which H is the half-life at moisture content M, and A and B are constants. The fit of the present data to this relationship was determined by linear regression analysis of the logarithm of the half-life against the logarithm of soil moisture as shown in Fig. 2. The slope of the line (B) gives a measure of the moisture dependence of degradation. The value of B derived from the present data was 1.32. In the experiments of Moon & Walker (1991) and Walker *et al.* (1992) the value of B on moisture effect of alachlor degradation 1.33 - 2.07.

With different soil characteristics, decomposition rate of pretilachlor was faster in Chonju soil than Kunsan soil as shown in Table 1. The half-life of pretilachlor was 16.9 days in Kunsan soil and 13.4

days in Chonju soil.

The loss rate of pretilachlor in the surface water of flooded pot soil is shown in Fig. 3. The concentration of pretilachlor in the water was 7.7 ppm at 1 hr after the treatment and 10.3 ppm at 1 day after the treatment. However the concentration was 1.5 ppm at 5 days after the application and 0.1 ppm at 14 days after the application. This study was carried out in the non-leaching conditions. Therefore, loss of pretilachlor in surface water of flooded soils may be more rapid under a leaching conditions such as a paddy field.

Fig. 3 Loss of pretilachlor in the surface water of flooded pot soil.

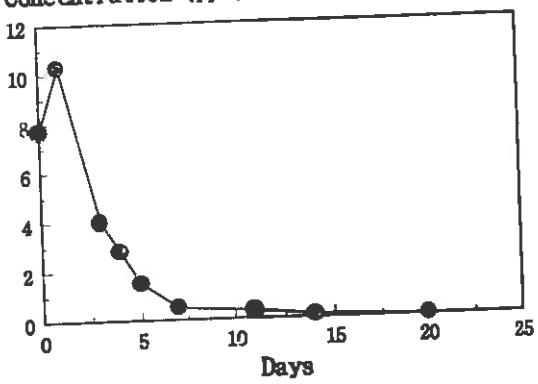
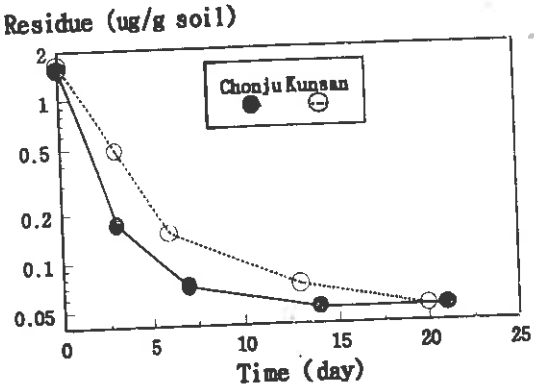


Fig. 4 Decomposition of pretilachlor in the field soils.



Results from the field residue experiments are sown in Fig. 4. They are presented as residual concentrations on a logarithmic scale against time after the treatment. At the 0 days after the treatment the concentration of pretilachlor was 1.36 and 1.63 ppm in the Chonju and Kunsan soil, respectively. The pretilachlor in the field soils was rapidly degraded and the half-life in the both soils was shorter than 3 days. The rate of degradation was faster in Chonju soil than in Kunsan soil as like laboratory study. The decomposition rate of pretilachlor was faster in the field experiment than in the laboratory experiment such as many studies (Shaner, 1994).

Acknowledgements

We are grateful to Novartis (Ciba-Geigy) Korea Ltd. for their support in this study.

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FAUNA AND ACTIVITY OF INSECTS VISITING *LINDERNIA* FLOWERS IN PADDY FIELDS IN NORTHERN JAPAN

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Abstract: To know dispersal process of gene of paddy weeds of *Lindernia* (Scrophulariaceae) by insects, the species composition and activity of insects visiting flower were studied at fallow rice fields in Kawanishi, Yamagata Prefecture and Nangai, Akita Prefecture, northern Japan. Among seven species, five bees and two hover-flies collected on flowers, *Lasiogloum scitulum* (Halictidae, Hymenoptera) and *Sphaerophoria macrogaster* (Syrphidae, Diptera) were major visiters in number of individuals. Observation of flower-visiting behavior proved that *L. scitulum* is the most important pollinator to *Lindernia* flowers. Comparison between two *Lindernia* flowers showed that *L. micrantha* is cross-pollination type and *L. dubia* var. *major* self-pollination type.

Keywords: ***Lindernia*, pollinator, *Lasiogloum scitulum*, flower-visiting behavior, flower structure**

Introduction

Resistance to sulfonylurea herbicides was discovered in natural populations of *Lindernia* spp. in paddy fields, which had been treated with sulfonylurea-based herbicides for several consecutive years (Itoh, 1998, Itoh et al, 1999). In order to understand dispersion process of gene, the survey was conducted to clarify the insect fauna visiting *Lindernia* flowers, to determine key species as pollinator and to observe its behavior on flowers. We will prelimiarily present some notes from our observation in this short-term survey.

Areas surveyed and methods

Insects visiting *Lindernia* flowers were collected by nets in fallow fields, Kawanishi in Yamagata Prefecture, Ohmagari and Nangai in Akita Prefecture (Fig. 1), and Tagajoh in Miyagi Prefecture. Ethological survey regarding flower-visiting behavior of bees and floral character of weeds was carried out in Kawanishi late July and in Nangai mid August in 1999.

Results and Discussion

1. Insect fauna visiting *Lindernia* flowers

Seven species of insects, five bees and two hover-flies, were observed to visit *Lindernia*

flowers, showen in Table 1. Major visiters were two species, *Lasioglossum scitulum* and *Sphaerophoria macrogaster*. The former seemed to be most important pollinator to these flowers in dominant number of individuals and in its behavior to visit one flowers to another very freguently for gathering nectar and pollen for brood. However, the latter seemed not to be so much important pollinator because of its inactive behavior in visiting flower only for its own nutrition.

Small and black or yellow thrips was discovered in the inside of *Lindernia* flowers at high rate. It was identified by Mr. M. Masumoto to be *Frankliniella intonsa* widely distributed in Palaearctic region and a most common agricultual pest prefering various host plants. The small insect may possibly help to pollinate in *Lindernia* flolwers.

2. Behavior of *Lasioglossum scitulum* visiting *Lindernia* flowers

L. scitulum is the small-sized halictine bee about 8 mm in body lenght, common everywhere in Japan. This species is bivoltine and basically solitary bee digging nest in the soil (Miyanaga et al, 1999). For this bee was most frequently observed to visit *Lindernia* flowers, their behavior visiting flowers was examined in fallow rice fields.

Bees usually showed uniform behavior patterns on flowers as shown in below:

A: Arrival on flower.

B: Inserting head and thrax into flower for sucking nectar or gather pollen (Fig. 2).

C: After drawing out head and thorax, staying for a few seconds to clean up and gather pollen dusted on the body surface and store in the space among hairs of corbicula (Fig.3).

D: Flying up for searching next flower.

Female bees behaved in complete pattern, A → B → C → D. Our observation was unable to confirm whether bees suck nectar or gather pollen in C. However male bee behaved in imcomplete pattern, A → B → D. It shows females are more effective pollinator than males. In the fallow rice fields mixed with three species of weed flowers, *L. micrantha, L.dibia* var. *major* and *L. pyxidaria*, some bees selected to visit a species of flowers selectively but others did mixed species. Female stayed on a flower longer than male, i.e. the duration of a flower-visit ranged from 3.1 to 12.4 sec. (mean=7.9 ± 2.6 sec. n=9) in female and from 2.7 to 5.2 sec.(4.4 ± 1.4 sec. n=10) in male.

For blooming plants are likely to be very poor around rice fields in mid-summer season, *Lindernia* flowers are very attractive to this bee and important resource of nectar and pollen for brood. There is no doubt that *L. scitulum* plays important role as pollinator of *Lindernia* flowers. However it is incomprehensive that other similar-sized bee species which occurs simultaneously was rarely seen to visit *Lindernia* flowers. Because nesting site was not discovered, flight range of this bee could not be determined.

3. Comparison of structure and nectar secretion between two *Lindernia* flowers

Each floral structure of two species of *Lindernia*, *L. micrantha* (Fig. 4) and *L. dubia* var. *major* (Fig. 5), is comparatively shown in Table 2. As far as their floral structure is concerned, the former seemed to be cross-pollination type, however the latter self-pollination type (Fig. 6).

When bees insert though narrow opening of flowers in *L. micrantha*, they cannot suck nectar without receiving pollen dust on their back surface from outer stamena. Judging from structural difference, bees seems to be able to harvest nectar and pollen in *L. dubia* var. *major* with atrophied outer anthers and small nectar secretion lesser than in L. micrantha with complete outer anthers and large nectar secretion. It proves by comparison of amount of nectar secretion among *Lindernia* flowers, i.e. *L. micrantha* secrets more amount of nectar than *L. dubia* var. *major*, shown in Table 3.

Table 1. Insect fauna visiting *Lindernia* flowers

Locality Prefecture Date Flower visited *	Ohmagari Akita 20.Ⅷ.1997 MC	Nangai Akita 20.Ⅶ.1998 MC	Kawanishi Yamagata 29.Ⅶ.1999 MC	Nangai Akita 10.Ⅷ.1999 DM	Nangai Akita 11.Ⅷ.1999 PY	Tagajoh Miyagi 19.Ⅷ.1999 MC	Total number
HYMENOPTERA (Apoidea)							3/22
1. *Lasioglossum scitulum*	0/ 1		0/ 8	1/13	2/ 0		2/ 4
2. *Lg. (Evylaeus) pallilomum*	0/ 3				0/ 1	2/ 0	0/ 3
3. *Halictus aerarius*	0/ 1		0/ 1			0/ 1	1/ 1
4. *Sphecodes japonica*				1/ 1			0/ 1
5. *Apis mellifera*			0/ 1				
DIPTERA (Syrphidae)							11/ 8
6. *Sphaerophoria macrogaster*	5/ 5	4/ 3	2/ 0				0/ 1
7. *Episyrphus balteatus*	0/ 1						
Total number	5/11	4/ 3	2/10	2/14	2/ 1	2/ 1	17/40

*) MC: *Lindernia micranth*, DM: *L. dubia* subsp. *major*, PY: *L. pyxidaria*. **) Number represents male/female.

Table 2. Comparison of structure between two *Lindernia* flowers

Flower species	*Lindernia micrantha*	*Lindernia dubia* subsp. *major*
Nectar glands	on surface under ovary, large	ringlike in underpart of ovary, small
Amount of nectar secretion	large	small
Stamens	both outer and inner stamens	only inner stamens, outer degenerated
Opening of corolla	narrow	wide
Nectar mark	yellow mark in entrance of corolla	degenerated, outer stamen substituting it

Table 3. **Comparison of amount of nectar secretion among three *Lindernia* flowers**

Weed species	Amount of nectar	SD	Number examined
L. micrantha	0.185 μl	0.116	10
L. dubia subsp. *major*	0.106	0.064	11

As far as the period for our survey in hot mid-summer season, most bees visits *Lindernia* flowers exclusively only between 9:00 and 12:00 p.m. Time of visit by insects must be restricted in nectar-secretion and pollen-release of anthers in flowers. Further study is necessary to clarify complicate relationship of coexistence between plants and insects.

Acknowledgements

We would like to express our sincere gratitude to Mr. H. Ito, Kawanishi Branch, JA Okitama, and Mr. H. Watanabe, Chief of Weed Control Laboratory, Tohoku Natioal Agricultural Experiment Station, for their kind guidance to areas studied and for giving us their valid informations.

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Explanation of Figures

Fig. 1. Survey at patchs of *Lindernia. dubia* var. *major* in fallow rice field in Nangai village

Fig. 2. Female *Lasioglossum scitulum* inserting head and throax into flower (behavior B)

Fig. 3. Female *Lasioglossum scitulum* cleaning and gathering pollen dusted on his head and back (behavior C)

Fig. 4. Flower of *Lindernia micrantha*

Fig. 5. Flower of *Lindernia. dubia* var. *major*

Fig. 6. Longitudinal section of both flowers of *Lindernia micrantha* (A) and *Lindernia. dubia* var. *major* (B), and cross section in base of ovary of the former flower (C)


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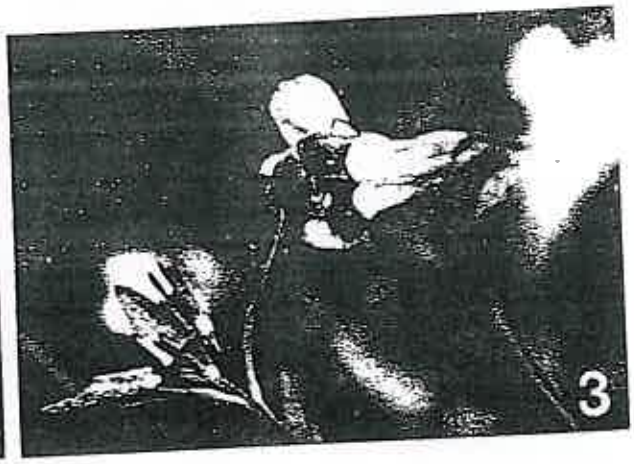
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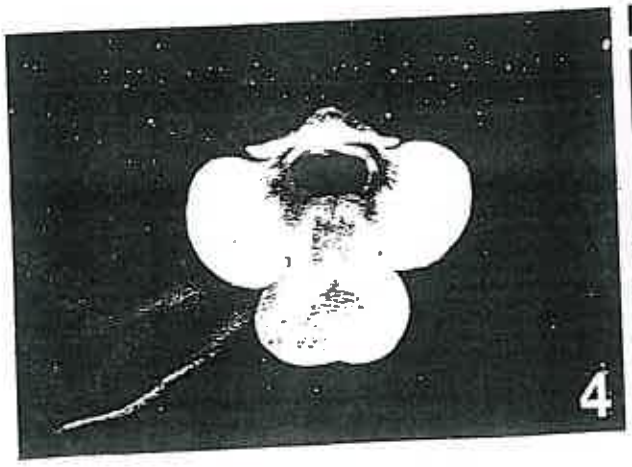
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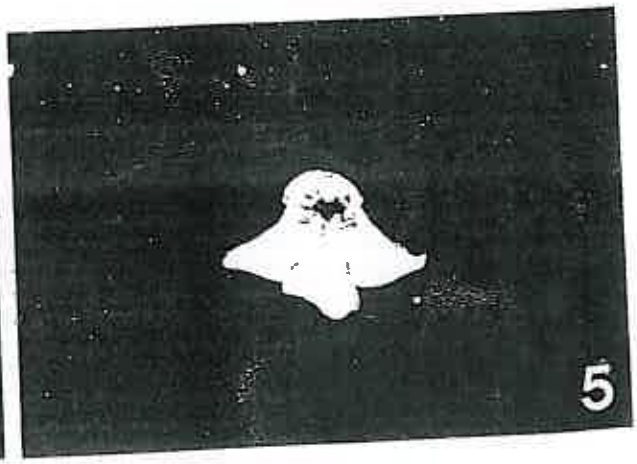
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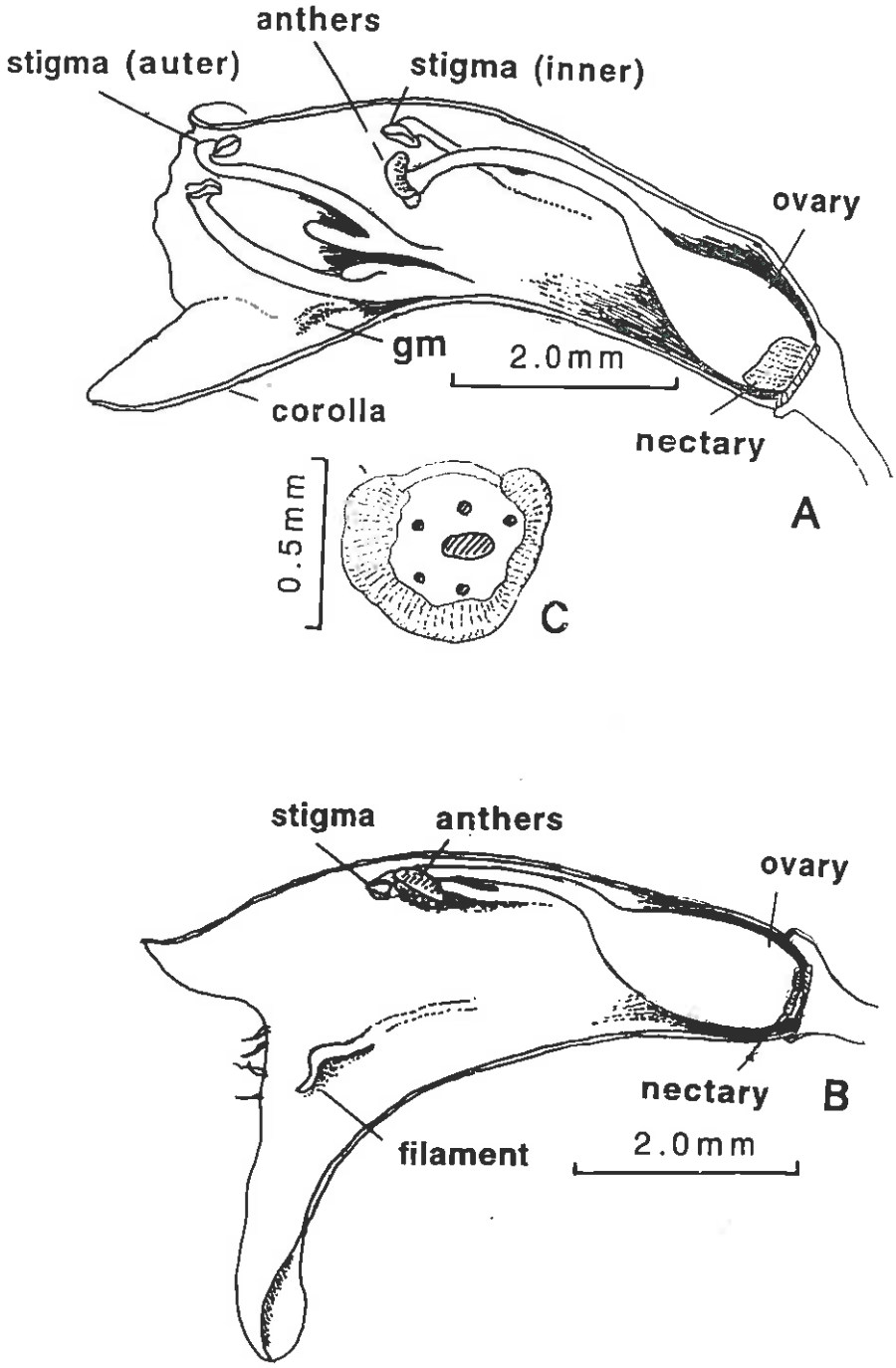

Fig. 6. Longitudinal section of both flowers of *Lindernia micrantha* (A) and *Lindernia. dubia* var. *major* (B), and cross section in base of ovary of the former flower (C)

IN VITRO SELECTION AND PROPAGATION OF SALT TOLERANT *Sesbania rostrata* Brem. & Oberm.

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Abstract: Seeds of *Sesbania rostrata* Brem. & Oberm. were selected and propagated by imbibing in water with 0, 0.25, 0.75 and 1.0 percent NaCl for one night and then transferred to MS with 11 mg/l BA at the same concentrations of NaCl for 14 days. It was found that the number of seedlings from 1.0 percent NaCl medium decreased more than 50 percent of the control. Cotyledonary nodes which were excised and placed on MS with 1 mg/l BA and 0.05 mg/l NAA for 30 days produced 10.2, 8.4, 7.2 and 4.2 shoots/explant, respectively. Then shoots were cultured on MS with 5 mg/l IBA within 30 days for root induction.

Plantlets, from salt-free medium and medium supplemented with 0, 0.5, 0.75 and 1.0 percent NaCl, were propagated and then tested by cultured on the MS medium with NaCl at the same concentrations for 60 days. The result showed that the percentage of plantlets from salt-medium were 100, 93.3, 53.3 and 26.7 percent of the control which were higher than plantlets from salt-free medium which were 86.7, 33.3 and 20 percent of the control, respectively.

Key word: ***Sesbania rostrata*** **Brem. & Oberm., sodium chloride,** ***in vitro*** **selection, salt tolerance, propagation**

Introduction

Saline soil was estimated to be 17 percent of the total area in the Northeast Thailand. The dominant salt in this area is sodium chloride. *Sesbania rostrata* Brem. & Oberm. is one of the halophyte that is reccommended to use as a highly effective manure for improving saline soil. *S. rostrata* is a salt-tolerant leguminous plant that can grow on 0.4-0.5 percent NaCl (Anulaxtipun, 1987) and has a lot of nodules on both root and stem. Stem nodules are a special structure that salt can not affect on nitrogen fixation of *Rhizobium* sp. (Roger and Watanabe, 1986). However, *S. rostrata* can grow in the limit of sodium chloride. The purpose of this study is to select and propagate *S. rostrata* tolerance to higher salinity by using tissue culture technique.

Materials and Methods

Salt tolerance selection and propagation

Seed of *S. rostrata* were soaked in 0, 0.5, 0.75 and 1.0 percent NaCl for one night. After surface sterization, these seeds were cultured on MS with 11 mg/l BA and supplemented with NaCl at the same concentrations for 14 days. Cotyledonary nodes were excised and tranferred to MS with 1 mg/l BA and 0.05 mg/l NAA for 30 days for multiple propagation. Then shoots were cultured on MS with 5 mg/l IBA for 30 days for root induction.

Salt tolerance test

Plantlets, from 0, 0.5, 0.75 and 1.0 percent NaCl as mention above, were tranferred to MS medium with the same concentration for 60 days and plantlets from salt-free medium were used for comparison.

Results

Salt tolerance selection and propagation

Effect of NaCl on germination and survival of seedling of *S. rostrata* showed that the number of survival seedling was 3.1, 2.64, 2.4, 0.8 and the percentage of control were 85.3, 77.1 and 26.2 at 0.5, 0.75 and 1.0 percent NaCl, respectivly (table 1)

Table 1 Effect of NaCl on germination and survival of seedling 14 days after treatment.

NaCl (%)	Number of survival seedling	Percentage of the control
0	3.1	100
0.5	2.64	85.3
0.75	2.4	77.1
1.0	0.8	26.2
F-test	**	
CV (%)	34.4	
LSD (1%)	0.63	

Numbers of shoot propagated from cotyledonary nodes of seedling from 0, 0.5, 0.75 and 1.0 percent NaCl were 7.2, 5.6, 5.2 and 3.5 shoots. However, shoot length was not significantly difference (table 2). Shoots from salt-free and from 0.5 percent NaCl produced numbers and good characteristic of roots better than the others (table 3).

Table 2 Shoot number and shoot lenght from cotydonary nodes cultured on MS with 1 mg/l BA and 0.05 mg/l NAA for 30 days.

Source of explants NaCl (%)	Shoots number/explant	Shoots lenght/explant (cm.)
0	7.2	2.2
0.5	5.6	2.5
0.75	5.2	2.4
1.0	3.5	2.3
F-test	**	ns
CV (%)	17.0	19.8
LSD (1%)	1.1	

Table 3 Root induction from shoots were cultured on MS with 5 mg/l IBA for 30 days.

Source of explants NaCl (%)	Root formed
0	2
0.5	2
0.75	1
1.0	1

1 Few roots formed
2 Many roots formed

Salt tolerance test

The percentages of survival of plantlets from 0.5, 0.75 and 1.0 percent after treating with the same concentrations of NaCl were 93.3, 53.3 and 26.7, respectively. They were higher than that of plantlets from salt-free medium which were 86.7, 33.3 and 20 % (table 4). Plantlets, that from 0.75

percent NaCl, could grow normally on the medium containing with 0.75 percent NaCl while growth of the plantlets from salt-free medium was inhibited (table 4).

Table 4 Survival of selected and non-selected plantlets on MS with NaCl at the same concentrations for 60 days.

NaCl (%)	Total plantlets	Survival of plantlets		Survival (percent of the control)		Characteristic of plantlets	
		A	B	A	B	A	B
0	15	15	15	100	100	Normal	Normal
0.5	15	13	14	86.7	93.3	Normal	Normal
0.75	15	5	8	33.3	53.3	Growth Inhibited and leaves fall	Normal
1.0	15	3	4	20	26.7	Growth Inhibited and leaves fall	Growth inhibited

A : plantlets from salt-free medium
B : plantlets from salt medium

Discussion

NaCl usually inhibits germination and growth of glycophyte. However, halophyte such as *S. rostrata* was also inhibited at high concentration. Wongwattana et al. (1998) reported that seedling growth of *S. rostrata* was enhanced at 0.125 to 0.5 percent NaCl but was inhibited at higher concentration. This result comfirmed by Phupaibul (1990) that *S. rostrata* could grow normally at 0.5 percent NaCl and the inhibition showed at 0.9-1.0 percent NaCl. In this experiment, the number of survival seedling decreased with the concentration of NaCl increased. The number of seedling from 1.0 percent NaCl decreased more than 50 percent compared to the control. Soaking seed in NaCl solution for one night is the first step of the selection because the only salt tolerance could grow normally after soaking (Kumlung, 1982). The high concentrations of NaCl reduced water potential of the medium compared to plant cells so seedling could not absorb water for their growth and development. In addition, high concentration of Na^+ and Cl^- might be toxic to plant cells.

Cotyledonary nodes from high NaCl concentration (0.75 and 1.0 percent) produced shoots and roots very slowly in salt medium which were 1.3 and 0.4 shoots/explant (the result was not showed). Cotydonary node from medium containing NaCl produced shoots less than that from salt-free medium and the number of shoots decreased with the concentration of NaCl increased. This results showed that NaCl supplemented in the germination medium affected the propagation of these explants. NaCl did not affect only multiple shoot propagation but also root induction. The data showed that shoots propagated from cotyledonary node from 0.75 and 1.0 percent NaCl produced few roots.

Plantlets from NaCl-treated cotyledonary node were more tolerant than that from salt-free medium. It was showed that the survival percentage of them was 93.3 and 53.3 percent of control at 0.5 and 0.75 percent NaCl while plantlets from salt-free medium were 86.7 and 33.3 percent, respectively. This indicated that selected plantlets could tolerate 1.07 and 1.6 fold of non-selected planlets at 0.5 and 0.75 percent NaCl. It may be possible that selected cells could survive in higher concentration of NaCl by some adaptation which mechanisms should be clearified.

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TRADITIONAL USE OF COGONGRASS (*Imperata cylindrica*) IN JAPAN AND FUTURE PERSPECTIVE ON ITS UTILIZATION

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Abstract: Cogongrass (*Imperata cylindrica* (L.) Beauv.) is the world worst weed. It is distributed widely in Japan and one of the noxious weed in the south and western parts, where it grows vigorously. The aboveground standing crop of cogongrass reaches 772 g/m^2 in dry weight and that of rhizome is 653 g/m^2. Water extract from leaves of cogongrass suppresses the growth of some crops and weeds. Cogongrass seems to have potential as a useful plant. How will cogongrass be able to be utilized? Ethnobotanical survey on the traditional use of cogongrass in Japan was made to discuss future perspective of its utilization. Cogongrass has been used as a thatching material, forage for livestock, a mulching material in vegetable gardens, a folk medicine as a diuretic, a soil binder to avoid soil erosion, and so on. Children sometimes chew immature panicles and rhizomes. There is also old religious beliefs that cogongrass protects man from evils or epidemics. As a result, cogongrass seems to be very useful as a soil binder to avoid soil erosion.

Key words: cogongrass, forage grass, *Imperata cylindrica*, soil binder, utilization

Introduction

Cogongrass (*Imperata cylindrica* (L.) Beauv.) is the world worst rhizomatous perennial weed (Holm *et al.*, 1977). It is distributed widely in Japan and one of the noxious weed in the south and western parts, where it grows vigorously (Fig. 1). Its shoots sprout all over the year except for the coldest season from December to February. It flowers in mid-May and seeds mature in late June of the rainy season. The seeds germinate quickly under the moist condition at 25 to 30 C, for the seed has no dormancy (Matumura *et al*, 1983). The aboveground standing crop of cogongrass reaches 772 g/m^2 in dry weight and that of rhizome is 653 g/m^2 (Tominaga *et al.*, 1989).

Water extract from leaves of cogongrass suppresses the growth of some crops and weeds (Eussen and Soerjani, 1978, Tominaga and Watanabe, 1997).

As cogongrass produces much biomass, has many long rhizomes and have an allelopathic effects on some weeds, it seems to have potential as a useful plant. How will cogongrass be able to be utilized? It is very important to compile the information on the traditional use of cogongrass for its future utilization.

Ethnobotanical survey on the subject in Japan was made to discuss future perspective of its utilization.



Fig. 1. Cogongrass population in Wakayama Prefecture, Japan.

Materials and Methods

To know the traditional use of cogongrass in Japan, information on the subject was compiled at about 450 sites from Hokkaido to Okinawa Prefecture, Japan and the results were summarized.

Results and Discussion

Cogongrass is a noxious weed in Japan, but it has been sometimes utilized in many ways from quite old times. The results of the ethnobotanical survey on the traditional use of cogongrass were summarized as follows.

1) a thatching material; in Ryukyu Islands, south parts of Kyushu and Izu Islands, cogongrass has been used as a thatching material and there are still many houses which roofs are thatched by the grass leaves in these area (Fig. 2). The thatched roof stands about 30 years.

2) forage for livestock; in old times, cogongrass was very useful forage grass for the cow, horse, sheep and goat. In a part of Ryukyu Islands, the goat is still feeding on this grass.

3) a mulching material in vegetable gardens; cut cogongrass leaves are used as a mulching material. Farmers told that the leaves suppressed weed growth. This information was experimentally proved by

Eussen and Soerjani (1978), and Tominaga and Watanabe (1997).



Fig. 2. Houses which roofs are thatched by cogongrass leaves in Okinawa Prefecture, Japan.

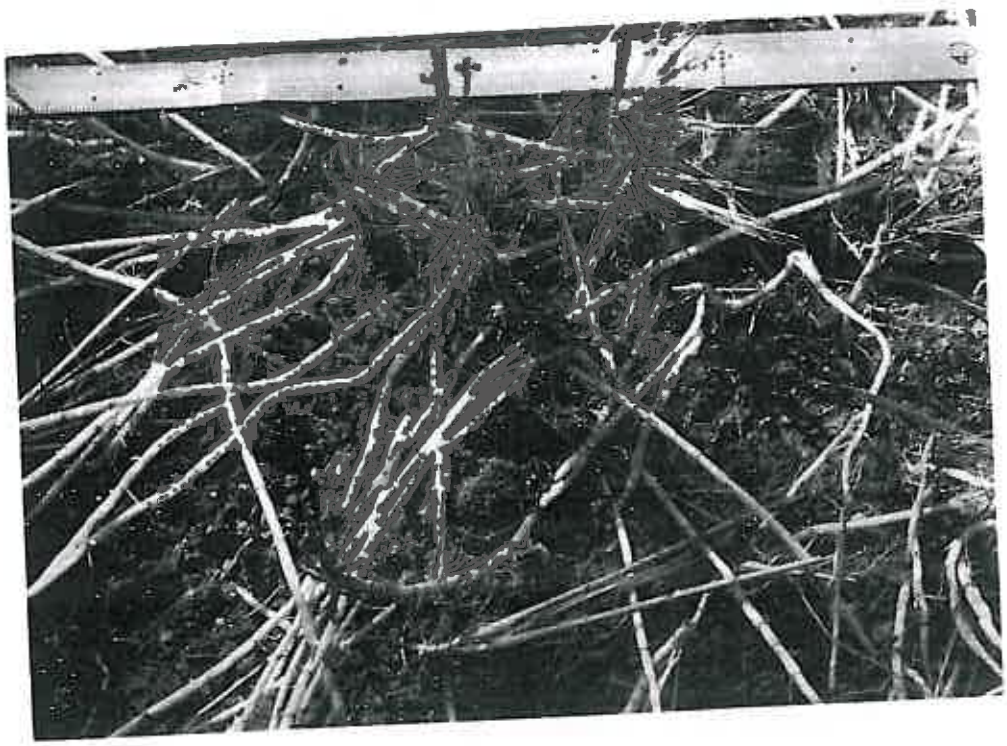

Fig. 3. Rhizomes of cogongrass.

4) a folk medicine as a diuretic; the rhizomes of cogongrass were dug and dried, then boiled. Effective components are arundoin, cylindrin, fernenol, simiarenol and isoarborinol.

5) a soil binder to avoid soil erosion; cogongrass has many long rhizomes (Fig. 3). These rhizomes bind soil tightly and that avoids soil erosion especially in paddy levees.

6) religious uses; there is also old religious beliefs that cogongrass protects man from evils or epidemics and at the end of June, the religious ceremony that people go inter the ring that made from cogongrass is taken (Fig. 4). In the southern part of Wakayama Prefecture, at the full moon ceremony in September, a kind of mat is made from cogongrass leaves, and offerings such as rice-cakes and some vegetables are put on the mat.

7) other uses; some kinds of basket are made from cogongrass leaves. Children sometimes chew immature panicles and rhizomes of the grass.



Fig. 4. Ring made from cogongrass leaves in Nachi Shrine, Wakayama Prefecture, Japan.

Cogongrass seems to be very useful as a soil binder to avoid soil erosion.

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Author Index

Abdullah, H.	347	Boonsrirat, C.	322
Abeysekera, A.S.K.	130, 174	Bose, A.	235
Adachi, T.	759	Bowran, D.	164
Adkins, S.W.	51	Brar, H.S.	674
Adlis, G.	219	Brar, L.S.	193, 674
Ahn, B.S.	797	Brinker, R.	527
Alif, A.F.M.	368	Cabanilla, H.	379
Allard, J.L.	449, 455	Casagranda, L.	527
Allen, J.	449	Casimero, M.C.	65, 134, 308
Al-Marsafy, H.T.	152	Castro, E.	226
Amarasinghe, L.	519	Caton, B.P.	116
Amartalingam, R.	389	Chaitep, W.	721
Antameng, F.	205	Chandakul, S.	583
Armes, N.	480	Changfu, L.	414
Aspuria, J.	379, 447	Changsaluk, S.	706
Azmi, M.	199	Chantarasa-ard, S.	205,322,480
Azwir,	219	Cheam, A.	164
Baerson, S.	527	Che, I.	143
Bakar, B.B.	105	Chen, Y.	333
Bakar, M.A.	105	Cheng-mei, Z.	739
Balasubramaniam, R.	487	Cheosamutr, C.	770
Baltazar, A.M.	65, 134, 308	Chin, D.V.	443, 562
Baohong, S.	344, 476	Chinawong, S.	770
Bariuan, F.V.	65, 308	Ching, L.	317
Beeckman, H.	745	Chiong, T.K.	753
Bell, M.A.	226	Cho, K.Y	403
Bhowmik, P.C.	446	Chun, J.C.	361
Bieboand, C.	650	Chunghong, L.	650
Boonpradub, S.	719	Chunyan, H.	344, 476

Crabtree, G.D. 83, 99
Cunyue, X. 140
Das, T.K. 242
De Datta, S.K. 65, 134, 308
Dill, G. 527
Dizon, M.A. 146
Dupo, H. 379, 447
Ekanayake, E.M.M.S. 130
El-Haroun, M. 505
Eng, O.K. 389
Estores, R.A. 460, 499
Faletti, M. 527
Feng, Y. 527
Finley, C. 431, 805
Fischer, A.J. 116
Foin, T.C. 116
Fu, Z.W. 168
Fujii, Y 577, 594, 614, 627
Fursch, H. 437
Gamage, A. 353
Gang-yue, L. 833
Garcia, C.O. 226
Ghani, R.A. 827
Gibson, K.D. 116
Gingya, W. 732
Gohbara, M. 547
Gonai. T. 604
Goukon, K. 850
Gressel, J. 21
Guan, C.H. 338
Guang, X. 472, 739
Gui, L. 742
Halim, M.R.A. 389
Harada, J. 9
Hashem, A. 164
Hashiba, T. 604
Hassanein, E.E. 152, 668
Heling, T. 619
Hill, J. 146
Hill, J.E. 116, 226
Hiradate, S. 577, 614
Ho, L.S. 493
Hong, M. 727
Hongfu, W. 288
Hongsbhanich, N. 395
Hosoda, A. 455
Hu, X.E. 168, 276
Hwang, I.T. 403
Hyakutake, H. 639
Ibrahim, H.M. 189, 668
Ibrahim, M.M. 152
Ishii, Y. 836
Ito, S. 836
Itoh, K. 537, 850
James, T.K. 255, 513
Janes, A.J.V. 213
Janiya, J.D. 146, 226
Jeong, B.R. 303
Jianjun, C. 732
Jianqiang, Y. 813
Jianrong, W. 650
Jian-xia, M. 472

Jinglun, W. 701
Julakasewee, A. 583, 635, 680
Jungklang, J. 856
Juntakool, S. 639
Kagawa, Y. 77
Kanai, S. 77
Karim, S.M.R. 71
Kawabata, O. 261
Kawakami, G. 317
Kawamoto, A. 691
Kawata, M. 77
Kazemi, H. 552
Khawtepawan, P. 374
Kim, C.S. 251
Kim, D.W. 403
Kim, K.U. 251
Kim, K.Y. 361
Kim, L.J. 480, 487
Kim, M.H. 361
Kim, S.E. 361
Kim, S.T. 455
Kim, T.J. 403
Kim, Y.S. 844
Kim, Y.W. 455
Kitamura, S. 691
Kiyono, A. 317
Ko, Y.K. 403
Kobayashi, K. 15, 93
Koga, C. 317
Komai, K. 594
Kon, K.F. 449, 455
Kongsaengdao, S. 785
Konnai, M. 659, 695
Kow-in, P. 721
Krishnamoorthy, R. 186
Kumar, N. 674
Kuppuswamy, G. 186, 232
Kwon, S.L. 493
Lamid, Z. 219
Lan, T.T.P. 292
Landes, A. 431,493,805
Landes, M. 431
Laosinwattana, C. 695
Lee, C.W. 659
Lee, S. 164
Lee, Y.D. 303
Li, F. 833
Li, S. 143
Liew, C.Y. 205, 487
Lim, J.K. 205
Lin, W. 727
Llabores, E. 379, 447
Lu, E. 805
Lubis, A.T. 759
Lueang-a-papong, P. 374,395,424
Lüthy, C. 449
Mahajan, G. 193
Makkawi, A.A. 712
Malik, R.K. 180, 685
Man, A. 827
Man, L.H. 562
Mansheng, Y. 288

Mansor, M. 347
Manuel, J.S. 134
Maramba, B. 130, 383, 519
Martin, E.C. 65, 308
Martin, L.D. 213
Matsumoto, H. 39
Matsumura, T. 850
Matsuo, K. 537
McDaniel, S.G. 499
Medina, J.B. 460
Medina, M.J.B. 499
Mekhail, G.M. 505, 668
Mele, P.V. 296, 745
Mellsop, J. 513
Miyake, S. 836
Moenandir, J. 419
Mongkolbunjong, P. 639
Moolsri, S. 721
Moon, Y.H. 844
Morimoto, M. 594
Morimune, K. 77
Morishima, Y. 455
Morita, H. 89
Morita, T. 594
Mortimer, A.M. 116, 199
Mortimer, M. 45, 146, 721
Motooka, P. 317
Murakami, S. 455
Murniningtias, E. 419
Nagai, G. 317
Nakajima, N. 577, 614
Nakatani, K. 627
Nakayama, S. 827
Nakornsri, K. 779
Namuco, O.S. 645
Namwongprom,K 841, 856
Nanakorn, M. 856
Nanakorn, W. 123
Nanevas, N. 852
Naylor, R.E.L. 71
Nemeth, M. 527
Ni, H. 333
Nimruksa, Y. 635
Nishimoto, R.K. 261
Noopradit, W. 322
Norsangsri, M. 123
Nur-Razana, M. 818
Nuyken, W. 431
Obien, S.R. 65, 134, 308
Ogasawara, M. 659, 695
Ogawa, A. 547
Oh, Y.K. 251
Ohde, K. 836
Omar, D. 389
Ono, Y. 691
Palis, F. 379, 447
Panyafoo, S. 567
Park, J.P. 251
Park, T.S. 251
Park, Y.S. 361

Phen, T.V. 296
Pieris, H.S.N. 353
Piggin, C. 146, 645
Piggin, C.M. 226
Pimol, B. 493
Pingyi, G. 288
Policello, G. 327
Pongpisutta, R. 600
Pornprom, T. 600, 793
Powley, J. 317
Prakongvongs, C. 588
Prematilaka, K.G. 353
Price, C.E. 712
Punia, S.S. 180
Pyon, J.Y. 797
Qian, Y.X. 168, 276
Qiang, S. 556
Qingyun, G. 140
Rahman, A. 255, 513
Raman, R. 186, 232, 239
Ranasinghe, L.L. 83, 99
Ranjit, J.D. 246
Razote, E. 226
Reynolds, T. 527
Rodriguez, D. 527
Ronglong, J. 650
Ros, L. 205
Ryu, E.K. 797
Sago, R. 764
Sahid, I.B. 818
Suwanketnikom, R. 583,635, 663, 680, 706
Sanevas, N. 841
Salim, A.A. 189, 505
Sardana, V. 193
Sasipreyajun, L. 480
Satoh, S. 604
Schafer, D. 527
Schöfl, U. 431, 493, 805
Seema, 242
Senge, M. 759
Sharma, M. 487
Shebl, S.M. 152
Shibaike, H. 537
Shibayama, H. 547
Shimi, P. 552
Shishido, G. 317
Sievernich, B. 431
Singhara Na Ayudhaya, S. 424
Sirohi, R.M. 685
Sirvejabandhu, K. 600
Smitamana, P. 567
Soetedjo, P. 213
Song, S.D. 303
Srinives, P. 793
Sriyani, N. 282, 759
Stalker, D. 527
Summerell, B. 556
Sun, J.S. 327
Suprapto, H. 759
Surawattananon,S 793, 841, 856
Susanto, H. 759
Welsh, T. 719

Suwanrak, K. 785
Tadakitisarn, A. 395
Takeuchi, Y. 659, 695
Tamaki, S. 594
Taylor, N. 527
Teng, Y. 527
Tewari, A.N. 266
Thai, T.Y. 753
Thi, H.L. 562
Tiebao, C. 344, 476
Tominaga, T. 860
Tongma, S. 93
Tran, M. 527
Umemoto, S. 610
Unglaub, W. 431
Urakawa, M. 594
Usui, K. 93
Vasquez, F. 379, 447
Vongsaroj, P. 779
Walia, U.S. 674
Wanchai, T. 493
Wang, G. 850
Wang, G.X. 537
Wang, X.F. 338
Wang, Z.Y. 276
Waramit, N. 663
Watanabe, K. 77
Watson, A.K. 567
Wei, D. 288
Whytock, G.P. 71
Xing-shan, T. 833
Xuelin, Q. 140
Yadav, A. 685
Yaduraju, N.T. 242
Yamaguchi, Y. 836
Yanagisawa, K. 691
Yanghan, L. 732
Yao, D. 632
Ye, G.B. 408
Yoneyama, K. 659, 695
Yoshioka, T. 604
Yu, W. 344, 476
Yuanlai, L. 813
Yuasa, Y. 836
Yuting, Y. 414
Zahran, M.K. 668
Zhang, C.X. 168
Zhang, L. 333
Zhang, Z.P. 158, 276, 408
Zhu, N. 727
Zimdahl, R.L. 3
Zongjian, Z. 414
Zungsontiporn, S. 577