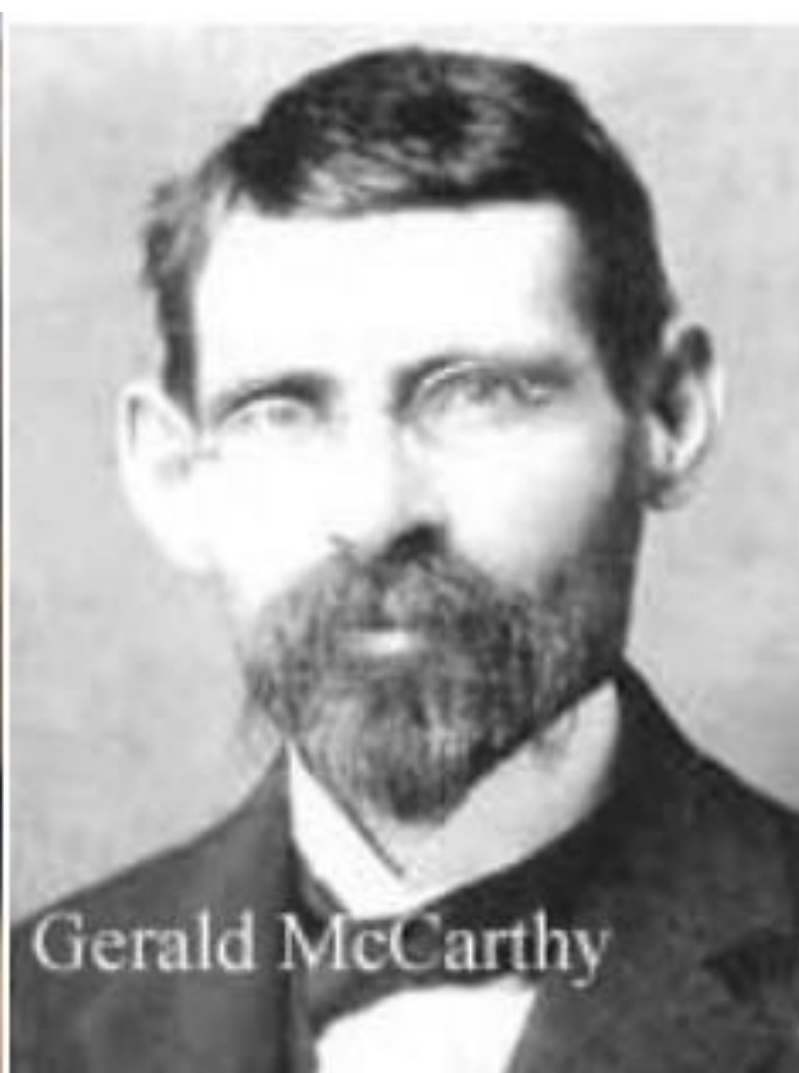
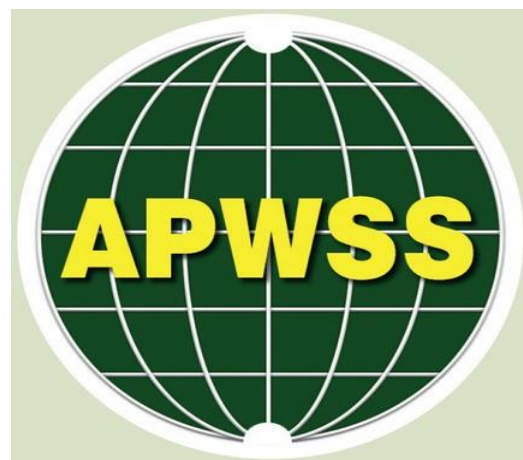


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‘Seeing Weeds with New Eyes’ Part II– Some Historical Perspectives and ‘Proto Weeds’

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“...What is a weed? A weed is a plant whose virtues have not yet been discovered...”

Ralph Waldo Emerson (1863)

Emerson, a renowned American philosopher, and poet, who led the transcendentalist movement of the mid-19th century in the USA, had an enlightened view of weeds. Those words, spoken in a famous speech, ‘*Fortune of the Republic*’, in December 1863, against the backdrop of the American Civil War (1860-65), are often quoted in *Weed Science* textbooks. Their deeper meaning is: Weeds *do* have admirable virtues, and one would see them if one looked closely.

As I said previously (Chandrasena, 2019), the incessant slandering of colonizing plants (weeds) by some people is a critical issue for *Weed Science*. It has inhibited the emerging generation of weed scientists from appreciating the utilitarian values and other redeeming qualities of weeds, as well as their ecological roles. It also prevents weed research from operating under a different paradigm and proving the worth of colonizing taxa, while controlling them to the extent necessary with sustainable approaches.

Weeds are plants with colonizing attributes, which thrive on habitat disturbed by man (such as agricultural fields), or by natural phenomena. As they are ‘pioneers of secondary succession’ (Baker, 1965; Bunting, 1965), ‘disturbances’ are the key. They grow where someone does not want them, and often that is in areas that have been disturbed or altered intentionally. Weeds grow especially well in gardens, cropped fields, golf courses, and similar places.

As Zimdahl (2007, p. 20) wrote, the ability of weeds to grow in habitats that have been disturbed by man makes them a kind of ecological ‘Red Cross’: They rush right into disturbed places to occupy those places and then, restore the land.

Weeds, important in crop competition, are often present in the earliest ecological successional stages (the ‘ecological Red Cross’; Zimdahl, 2007, p. 256) following abandonment of crop lands because there is an absence of competition and a large weed seed bank in the soil that still has abundant nutrients.

‘*War with Weeds*’, a common slogan bandied around in popular media, is the wrong choice of words to describe how we should manage weeds. Some weed scientists and agriculturists still live with the delusion that we can win a ‘war-with-weeds’ using herbicides as ‘weapons of mass destruction’ (WMDs). These views need to be challenged, as they mislead the public and are also unsustainable and counter-productive (see Low and Peric, 2011; Dwyer, 2012).

Continuing the theme ‘*Seeing Weeds with New eyes*’ (Chandrasena, 2019), in this issue of *Weeds*, I expand on some historical perspectives on matters related to colonizing taxa that have not received much attention within our discipline. My hope is that the emerging generation of weed scientists may benefit from deeper insights about the discipline’s history and how our attitudes towards weeds have changed and evolved with time.

I also provide a brief account of some recent archaeo-botanical findings from the Levant, that push the record of first-known weeds back 23,000 years to a time well before settled agriculture.

Human attitudes towards ‘weeds’ appear to slowly change over time, through the 1st and 2nd Millennia A.D. Reviewing the history of *Weed Science*, Timmons (1970) reported that: “*available literature indicates that relatively few agricultural leaders and farmers became interested in weeds as a problem before 1200 A.D. or even before 1500 A.D.*”

The general attitude seemed to be that: “weeds were a curse which must be endured, and about which little could be done except by methods which were incidental to crop production, and by laborious supplemental hand methods” (Timmons, 1970). Much of the time, it appeared that weeds were ‘manageable with some effort’. Farmers, who made a living by growing crops, considered weed control only as an ‘incidental’ activity to land preparation and other cultural practices. The early agriculturists were more concerned with crop damages and losses caused by insects and plant pathogens, which were spectacular, compared with negative effects of weeds.

During the last three centuries, discoveries, such as steam power gave rise to industrial-scale agriculture. As the industrial revolution transformed agricultural societies into manufacturing societies, attitudes changed. Over time, intolerance of any obstacles to productivity and profits crept in, as humans flourished across many parts of the world.

The 18th century could well be considered as the most transformative for agriculture. It saw a revolution in large-scale food production, due to both efficient land preparation and monoculture cropping. This period also saw major efforts to control agricultural weeds with human and animal labour. The negative attitudes towards weeds hardened in the USA during the 19th century as agriculture expanded on a large scale (Timmons, 1970).

Jethro Tull’s musings

In the middle of the 18th century, an English inventor, Jethro Tull (1674-1740) revolutionized tillage practices in agriculture in Britain. His seminal book - *The New Horse-Hoeing Husbandry*, written in 1731, was among the first to extensively use the word ‘weeds’ in its present meaning. Tull’s horse-drawn seed drill invention was one of the first that began the mechanical era of agriculture.

Tull mistakenly believed that particles of the earth were the ‘food’ of plants and that pulverization of soil particles through tillage made it easier for plants to absorb these nutrients. He advocated cultivation as a substitute for crop rotation, fertilizer, and fallow. Although Inter-row cultivation would have accomplished weed control, weeds were not an important part of his hypothesis about plant nutrition. Zimdahl (2010, p. 30) points out that:

“...Tillage surely accomplished weed control but weeds were not an important part of his hypothesis about plant nutrition. Plant nutrition was derived from what Tull called infinitely divisible particles of earth. Tillage made the particles small and thereby plants were nourished. Tull’s hypothesis was false in all respects but he deserves credit for promoting the new practice of cultivation even though he ignored its benefits for weed control...”

The ‘hoe’ probably was better adapted for weed control than the ploughing, even though its intended function was pulverizing the surface soil so that “the needed soil elements could be absorbed more readily by plant roots” (see Timmons, 1970, quoting Tull).

Herbae inutiles and Herbae noxiae

My reading of Tull is that while he may not have promoted tillage for weed control, he certainly implied it. Writing a full chapter and more on weeds, he appreciated the strengths of weeds, while detesting them as constraints to farming. Tull likened weeds in farmer's fields to ‘*muscae*’, a reference to domestic houseflies (*Musca domestica* L.), who are ‘*uninvited guests*’ along with other domestic pests. At the start of Chapter VII - “Of Weeds”, Tull muses as follows:

“....Plants that come up in any land, of a different kind from the sown, or planted crop, are weeds....That there are in Nature any such things as *inutiles Herbae*, the Botanists deny; and justly too, according to their meaning. But the farmer, who expects to make profit of his land from what he sows or plants in it, finds not only *Herbae inutiles*¹, but also *noxiae*, unprofitable and hurtful Weeds; which come like *Muscae* or *uninvited guests*, that always hurt, and often spoil his crop, by devouring what he has, by his labour in digging and tilling...”

“...All weeds are pernicious, but some much more than others; some do more injury and are more easily destroyed; some do less injury, and are harder to kill; others there are, which have both these bad qualities. The harder to kill will propagate by their seed, and also by every piece of their roots, as couch-grass, coltsfoot, melilot, fern...”

“...Some are hurtful only by robbing legitimate (or sown) plants of their nourishment, as all weeds do; others both lessen a legitimate

¹ The Latin term: *Herbae* refers to ‘herbs’ or ‘grass’.
‘*Herbae inutiles*’ refers to ‘useless, unusable’ the

opposite of *utilis* = useful. *Herbae noxiae* (from the Latin word ‘*noxia*’, meaning ‘harmful plants’.

crop by robbing it, and also spoil that crop, which escapes their rapine, when they infect it with their nauseous scent and relish, as melilot, wild Garlick..."

Tull's book is full of sketches that show a degree of respect for weeds. By categorizing some plants as useless and unusable, while others may be more injurious and harmful, his writings gave rise to the popular modern-day adjective 'noxious', attached to some weeds. Yet, he said that some botanists might disagree with this viewpoint, which mirrored the way farmers looked at these problematic plants.

In his book, Tull also discussed the growth and reproductive strategies (such as the production of seed and underground vegetative structures) of many weeds. He also had some harsh words for farmers, whom he told to pay greater attention to weeds and annihilate them as a 'whole race' in a manner like how the '*much more innocent and less rapacious*' wolves (*Canis lupus* L.) were eradicated from Britain ².

"...It is needless to go about to compute the value of the damage weeds do, since all experienced husbandmen know it to be very great, and would unanimously agree to extirpate their whole race as entirely as in England they have done the wolves, though much more innocent and less rapacious than weeds..."

As Timmons (1970) says, Jethro Tull, therefore, must be judged as a crusader against weeds urging their extermination from Britain. Notions of the possibilities of 'weed eradication', perhaps, arose out of such writings. With many global examples, we now know that, once established, colonizing taxa are hard to 'exterminate' or 'eradicate, unless they are small populations detected early and subject to control.

However, Tull's efforts were primarily aimed at selling his invention - horse-drawn hoeing - as a new

tillage practice in Britain and Europe, where he had travelled widely making observations on how farming was done. As the sub-title indicates, the book was:

"Designed to introduce a new method of culture; whereby the produce of land will be increased, and the usual expense lessened".

It would be fair to assume that his invention allowed the 18th century British farmers who adopted the horse-drawn hoe to grow crops in rows and attain better growth conditions in the fields. The tillage practices would have simultaneously achieved a high degree of weed control in the row-sown crops.

William Darlington's *American Weeds and Useful Plants*, 1859

In tracing how attitudes towards weeds evolved over time, a particularly fascinating account comes in the introduction of William Darlington's book on '*American Weeds and Useful Plants*', published in 1859 (Darlington, 1859, pages xv-xvi). The book had been first published in 1847 under the title *Agricultural Botany* and was later reissued as with a new title and illustrations (see cover, Figure 1).

Dr. Darlington (1782-1863) was a famous American medical doctor, a physician who had travelled extensively when young, and in later years, a US congressman for Pennsylvania ³. He was also a highly-respected, amateur botanist (Nickerson, 1936; Flannery, 2017) who maintained close contact with several world-renowned botanists. Botanists with whom he enjoyed '*an eminent degree of friendship*' (Nickerson, 1936) included the Swiss botanist Augustin de Candolle ⁴ in Geneva, and Asa Gray ⁵ and John Torrey ^{6 7} in the USA.

² Wolves (grey wolf) were once abundant in the British Isles but were hunted from Roman times (>2000 years ago). The dates when last wolf in the British isles was killed are disputed - in 1680 or 1743 and they may have survived until the early-19th century. (source: https://en.wikipedia.org/wiki/Wolves_in_Great_Britain)

³ William Darlington – Wikipedia – Source: https://en.wikipedia.org/wiki/William_Darlington

⁴ Augustin Pyramus de Candolle (1778-1841) – a Swiss Botanist originated the idea of "Nature's War", which influenced Charles Darwin and the principle of natural selection as the primary driver of evolution.

⁵ Asa Gray (1810-1888) is one of the most important American Botanist of the 19th century. His *Darwiniana*

– a collection of essays, responding to Charles Darwin's *Origin of Species* (1859) attempted to show how religion and science were not mutually exclusive. Gray was adamant that a genetic connection must exist between all members of a species.

⁶ John Torrey – A New York Botanist of high reputation. He gave William Darlington, perhaps, his greatest homage in 1853, by naming a newly discovered Californian plant, found in 1841 – the Californian pitcher plant or cobra plant – originally named *Darlingtonia californica* Torr. (1853).

⁷ Nickerson (1936) records that it was de Candolle who first honoured Darlington with the naming of the genus in 1825, but due to a question of priority, the name was not accepted. Dr. Torrey, then, described the species again in 1953, dedicating it to honour Darlington.

Darlington was a keen advocate for applying scientific knowledge of plants to improve 'old agriculture'. Presumably, what he meant was more extensive knowledge about the life cycles, and factors, which contribute to the growth of both crops and weeds in farmers' fields. The author's dedication in the book emphasized his motivations:

*"..To The Young Farmers of the United States, this humble attempt - to aid and persuade them to cultivate a Department of Science- essential to an enlightened Agriculture and indispensable to an accomplished yeomanry..."*⁸



Figure 1- Cover of William Darlington's *American Weeds and Useful Plants*, 1859

'Plants Out of Place' and unwelcome intruders

The history of *Weed Science*, reviewed by others (Timmons, 1970; Evans, 2002; Falck, 2010; Zimdahl, 2010) shows that weed scientists, for more 100 years, accepted the notion that weeds should not be tolerated and that they are unwanted. *How did this notion arise?* In my reading of history, Darlington's book, written with noble intentions, popularized this idea in the mid-19th century.

⁸ 'Yeomanry' is a term applied to the body of small landed proprietors of the middle class, anxious to live self-sufficiently by cultivating their land.

The definition of a weed as 'A *Plant Out of Place*', which arose in the USA, can be traced back to his writings. However, Darlington clearly states that the notion was an 'old one' (see quote below) and had evolved before his time. However, his book may have popularized the notion among the agricultural communities in the USA and elsewhere⁹. The quotes below open his six-page introduction to weeds in the 1859 Edition:

"...In popular language any homely plant which is not noticeable for the beauty of its flowers, not entitled to respect by a reputation for medicinal or other useful qualities, is designated by the epithet weed. In an agricultural sense, the term is used with a more restricted meaning, and is applied to those intrusive and unwelcome individuals that will persist in growing where they are not wanted – in short, the best definition that has yet been given of a weed is the old one. "a plant out of place..." (p. xiii)

"...Most of the weeds troublesome in our agriculture are immigrants, either from the Old World, or the warmer portions of this continent. The number of plants indigenous to our country, entitled to rank as pernicious weeds, is comparatively small..." (p. xiii)

Writing from his resident state, Pennsylvania, which had previously seen the ravages of war during the European colonization, Darlington invoked the notion that nearly all 'pernicious' weeds were immigrants from the Old World (Europe). To him, weeds were 'unwelcome intruders', with no value and weeds also persist in growing where they are not wanted. Interestingly, from his viewpoint, only a small number of indigenous plants of the USA qualified as pernicious weeds.

Darlington's writings clearly depicted the close relationship between human immigration and plant immigration: wherever humans go, some plants will follow them. Such ideas, written so unambiguously as advice, may have influenced the 'next generation of US agriculturists, 'the young farmers', Darlington was keen to address. Perhaps, these thoughts, penned at a time when the USA was heading towards the Civil War (1860-65), in some way changed the attitudes of farmers. It was evident to him that farmers need to have considerable respect for weeds, something they did not have at that time.

⁹ A chronological summary of definitions of weeds can be found in Zimdahl's *Fundamentals in Weed Science* (3rd Edition, 2007. P. 17).



Figure 2. A portrait of William Darlington (Source: Wikipedia: https://en.wikipedia.org/wiki/William_Darlington)¹⁰

Darlington's style included the use of powerful metaphors to stress a point. Other sections of the Introduction show a degree of indifference towards Indigenous Americans, whom he used as metaphor:

"...As the aborigines disappeared with the advance of the whites, so do the native plants generally yield their possessions as cultivation extends, and the majority of the plants to be met along the lanes and streets of villages, and upon farms, are naturalized strangers, who appear to be quite at home, and are with difficulty to be persuaded or driven away..." (p. xiii)

The reference to the retreat of the Indigenous Americans as the 'whites' advanced, brings up images of violent conquests, which took place during the European colonization of the Americas. A much more accurate description would be 'pushed out of the away' or 'decimated' rather than 'retreat'.

The history of the USA, recorded elsewhere, shows that Indigenous Americans did not entirely 'disappear'. Against the wishes of the 'whites', they do exist, centuries later, but as marginalized people, just as other dispossessed and relegated Indigenous peoples exist elsewhere.

It is also important to note that even as an amateur botanist, it was clear to him that colonization by 'human immigrants' arriving from the Old World would be followed by plant immigrants. He highlighted that the new immigrants would soon become 'naturalized' in their new environments, and some would be hard to be 'persuaded to leave' or '*be driven away*'. Persistence of weeds after establishment is an ecological fact and a major theme in ecology. I agree with Zimdahl's judgement (*pers. comm.*, June 2020) that, unfortunately, it has never been a central theme in Weed Science since the time the discipline was formed ca. 100 years later.

"...In agriculture, as in morals, idleness is the mother of vice, and if the ground be not occupied with something good, there will be plenty of the opposite character to take its place. Possession is a great advantage in other matters than those of the law, and a plant, whether useful or troublesome, when once fully established is not disposed to yield without an argument..." (p. xiii)

These astute observations on the nature of weeds show that even as an amateur agriculturist and botanist, Darlington understood weeds well. It was clear to him that weedy plants would be the first colonizers, who 'take possession' of a vacant and disturbed area, such as 'agricultural fields', 'lanes and streets of villages'. In such areas, the 'naturalized stranger' may thrive. And once established these persistent plants will not leave '*without an argument*'. Indeed! The clarity of thought is evident.

As a discipline, ecology had not quite developed in the mid-19th century. Therefore, such ideas, written more than 170 years ago, in 1847, are predictors of our understanding of how and why weeds behave as they do. Giving good agronomic and scientific advice, describing '*idleness as the mother of vice*', Darlington also emphasized that: *farmers would do well to not be idle in dealing with weeds*.

Correspondences with de Candolle

The correspondences Darlington had with de Candolle are particularly interesting for students of *Weed Science*. The account (see below) relates to de Candolle's concept of all plants being perpetually at war with each other (i.e. 'Nature's War'), which initiated the concept of allelopathy (Rice, 1984).

¹⁰ Portrait of William Darlington, painted by John Neagle, about 1825. West Chester University, West Chester, Pennsylvania (Source:

https://en.wikipedia.org/w/index.php?title=William_Darlington&oldid=927853133

"...That learned and sagacious observer of Nature – the late Professor De Candolle remarks, that,

"...all the plants of a country, all those of any given place, are in a state of war, in relation to each other. All are endowed with means, more or less efficacious, of reproduction and nutrition. Those which first establish themselves accidentally, in a given locality, have a tendency, from the mere fact that they already occupy the space, to exclude other species from it; the largest ones smother the smallest ones; the longest lived ones supersede those of shorter duration; the most fruitful gradually take possession of the space which would otherwise have been occupied by those which multiply more slowly..."

"...The farmer, therefore, should avail himself of this principle, and aid the more valuable plants in their struggle to choke down or expel the worthless..." (p. xiv)

In 1805, de Candolle had written about a 'soil sickness' as part of a 'Nature's war', reporting that some plants excreted substances from roots that were harmful to other plants. He noted the specific inhibition of oat (*Avena sativa* L.) by thistles (*Cirsium* sp. L.) and of wheat (*Triticum aestivum* L.) by ryegrass (*Lolium* sp. L.). He reasoned that in the natural environment, such interactions have potential applications in agriculture and that rotation of crops could alleviate the problem (Willis, 2012). De Candolle's early writings about excreted substances from plant roots were an essential part of the history of 'allelopathy', which developed as a sub-discipline within *Weed Science* (Rice, 1984).

Interestingly, the correspondence with de Candolle, referred to by Darlington in 1847, contains no reference to any excreted substances. Instead, de Candolle only invoked what ecologists and weed scientists refer to as 'inter-specific' and 'intra-specific' competition, which are based on jostling for physical space. De Candolle also refers to '*all plants being endowed with the means to efficiently reproduce and obtain nutrition*'.

De Candolle pointed out to Darlington that some plants, taking possession first and occupying the space will lead to physically excluding others; the largest ones will smother the smallest ones; the longest lived ones will supplant or 'supersede' those of shorter duration; the most fruitful (meaning, both fast-growing and more fecund) gradually take possession of the space which would otherwise have been occupied by those, 'which multiply more slowly'.

Darlington saw agriculture as a constant struggle (quote below). He advised young farmers to learn '*something about the nature and character*' and '*peculiar habits of the individuals with which he has to contend*' referring to both the crop and non-crop (weeds). This is important to dissuade the non-crop plants to be ousted and make the others (crops) grow and produce to their 'utmost capacity'.

"...The labours of the agriculturist is a constant struggle as he endeavors to make certain plants grow and produce to their utmost capacity; on the other hand, he has to prevent the growth of certain other plants that are ready to avail themselves of these favourable conditions..."

"...The farmer is interested in two points concerning weeds: how they get into his grounds, and how to get them out. As cultivation is all the more profitably carried out if the farmer knows something of the nature and character of the plants he would raise, so, if he would successfully operate in the other direction, and stop plants from growing, he can do so all the better if he knew what are the peculiar habits of the individuals with which he has to contend..." (p. xiii)

As early as in mid-1800s, Darlington stressed the importance of studying agronomic requirements of crops, to make them grow better. At the same time, he wanted farmers to understand why and how weeds get into their fields, so that the pathways could be avoided (i.e. preventative weed control).

On annual seed-producers

Darlington (1859, pp. xiv-xv) writes about various aspects of the biology of weeds that are relevant to their control. His primary objective was to educate the young farmers that they should understand weeds better, along with the botany of the crops they are trying to grow. These are some of the earliest writings of the discipline, which evolved to be *Weed Science*. The attitude for more than 150 years was simple 'weed control'. However, ideas that later developed into the more holistic approach of 'weed management' can be gleaned from the following:

"...Weeds are introduced upon a farm in a variety of ways. Many have their seeds sown with those of the crops; this is particularly the case where the seeds of the weeds and of the grain are so much alike in size that their separation is difficult. Proper care in procuring and preserving clean seed will often save much future trouble and vexation..."

"...The observing farmer will notice the means which nature has provided for the scattering of seeds; he will find that the most pernicious weeds seem to have been especially furnished with contrivances to facilitate their dispersion. The Clot-bur¹¹, Beggar's lices¹², and others, have barbs and hooks by which they adhere to clothing and coats of animals and are widely distributed through this agency. All of the Thistles have a tuft of fine silky hair attached to seeds, more properly, fruit, by which they are buoyed upon the air and wafted from place to place..."

"...So numerous are the ways by which seeds are distributed, that, however careful a farmer may be upon his own premises, a slovenly and neglectful neighbour may cause him infinite annoyance by furnishing his lands with an abundant supply..." (p. xiv-xv)

"...The vitality of seeds, particularly, if buried in the earth below the influences which cause germination, in some cases endures through many years; hence, an old field, after deep plowing, has often a fine crop of weeds from the seeds thus brought to the surface..."

"...Weeds that have been cut or pulled after they have flowered, should not be thrown into the barnyard or hog-stye, unless the farmer wishes to have the work to do over again with their progeny, as the seeds will be thoroughly distributed in the manuring of the land..."

"...In all weeding, it is of the greatest importance that it should be done before the plants have formed seed. This should be regarded equally with annual and perennial weeds. The prolific character of some weeds is astonishing; each head of an Ox-eye daisy¹³ or White weed¹⁴ is not a simple flower, but a collection of great many flowers, each of which produces a seed; and, as a single plant bears a great many heads, the number of seeds that a single individual is capable of supplying in a season amounts to several hundreds..." (p. xiv-xv)

On perennial weeds

Observations on species, such as thistles with deep tap roots and grasses, such as couch grass¹⁵, with rhizomatous underground stems, are particularly pertinent to describing the life cycle of perennial weeds with special attributes. As Nickerson (1936) noted, Darlington was writing at a time when so little had been written on agriculture or weeds.

"...A perennial weed, like Canada thistle or Couch grass, is, during early stage of its existence, easily destroyed; but later in the season it makes strong underground stems, or roots, as they are commonly but incorrectly called, which have great tenacity of life, and which have within them an accumulation of nourishment which enables them to throw up successive crops of herbage; ploughing such weeds generally aggravates the trouble, for unless every fragment be removed from the ground, a thing very difficult to accomplish, each piece that is left makes a separate plant..."

"...In the case of weeds of this description, the necessity of early eradicating them is apparent, for, if once well established. An underground provision depot formed, the farmer and the plant are placed in the condition of being besieging and the besieged forces – as long as the provisions hold out the latter can maintain its ground..."

"...It becomes a question of endurance, for the underground supply must be eventually exhausted in the attempt to produce new stems and leaves, and if the farmer, by persistently cutting these away, prevents any new accession to the stock of provisions, the enemy must at length succumb..."

"...Often, repeated cutting will at length exhaust the underground portion of its vitality. In some cases, salt has been used with success, especially upon Thistles, applied immediately after mowing..." (p. xv-xvi)

¹¹ Clot-bur or common cocklebur: *Xanthium strumarium* L. (Asteraceae)

¹² Beggar's lices or stickseed: *Hackelia virginiana* (L.) I.M. Johnston (Boraginaceae)

¹³ Ox-eye daisy: *Leucanthemum vulgare* Lam. (syn. *Chrysanthemum leucanthemum* L.) (Asteraceae)

¹⁴ White weed: *Ageratum* L.; (Asteraceae)

¹⁵ Darlington did not quite name the species he

called 'couch grass' here. In the USA, Bermuda grass (*Cynodon dactylon* (L.) Kuntze), which he described on p. 377, is sometimes called 'couch grass'. But he was probably referring to the English/European couch grass - *Elytrigia repens* Desv. Ex Nevski (syn. *Agropyron repens* (L.) Beauv.; *Elymus repens* (L.) Gould).

The use of war imagery to describe the subterranean reserves of perennial weeds, is particularly noteworthy. It reflected the time in which he lived, just before the American Civil War, which broke out only one year later (1860-65). "Provisions depot", 'besieging (farmer) or the besieged (weeds) forces', 'holding out the ground', 'stocks of provisions' and the 'enemy must at length succumb' describe, through jargon associated with wars, what the farmers must do. Strong metaphors indeed to make a point that the young farmers, many of whom had already returned to agriculture after serving in the army or would be doing so at a future date (the word 'yeomanry' in the sub-title also appears deliberate).

In pages xv-xvi of the introduction, there is a paragraph that is particularly striking. Darlington calls weeds as 'evil' and advises the agriculturists to have a 'zero tolerance' attitude towards weeds. To equate weed control to Native American Indians killing women and children of enemies to stop the latter from producing offspring who might seek revenge is an extraordinarily strong and offensive imagery indeed! He picked the wrong metaphor.

"...In weeds, evil should be emphatically, nipped in the bud. In this respect, the farmer should act in the spirit of the Western savages who kill the women and children of the enemies, as a tolerably sure way of preventing the multiplication of warriors..." (page xv)

*"...The farmer will do well to keep in mind two rules. **Do not let weeds flower, and do not let them breathe**, for the leaves may be considered the lungs of the plant, and without the aid of these it cannot long maintain itself..."*

Darlington also wrote strongly on the need for correctly identifying plants, highlighting the mis-identification of Canada Thistle (*Cirsium arvense* (L.) Scop.) with clot-bur (*Xanthium strumarium*). Canada Thistle, a native of Europe and Northern Africa, had been introduced to North America soon after the arrival of European settlers. Its invasiveness was soon recognized. It is historically known as one of the first plants to have noxious weed laws enacted requiring its control: first in Vermont in 1795, followed by New York in 1831 (Timmons, 1970).

On pages 179-80, Darlington described clot-bur (*Xanthium strumarium*) and was scathing in his criticism of law-makers wrongly identifying this species for Canada Thistle. Referring to *Xanthium strumarium*, his observations were:

"...This execrable weed believed to have originated in tropical America, and now widely diffused through various parts of the old world, becoming naturalized in many portions of our country,—particularly in the Southern States. It may be frequently seen along the side-walks, and waste places, in the suburbs of our northern sea-port towns, and is a vile nuisance wherever found..."

While stressing the mis-identification of clot-bur with Canada Thistle, he acknowledged that the misnomer did not harm the enactment of laws across many States to prevent its spread:

"...I have understood that the authorities of one of our cities, a few years since, enacted an Ordinance against the plant, in which enactment it was denounced by the name of the Canada Thistle ! The misnomer probably "did not" impair the efficacy of the Ordinance: yet I cannot help thinking it would be decidedly preferable that both law givers and farmers should avoid confounding objects which are essentially distinct, and learn to designate even weeds by their proper names..." (p. 179-180)

Perhaps, Darlington's writing in the first edition of the book, in 1847 influenced the US law-makers and agricultural advisors to make a correction. As Hartzler (undated) noted, Iowa's first noxious weed law was subsequently written in 1868 by the 16th General Assembly and stated:

"...Be it enacted by the General Assembly of Iowa, that if any resident owner of any land in this state after having been notified in writing of the presence of Canada thistles on his or her premises, shall permit them or any part of the root to blossom or mature, he or she shall be liable to a fine of five dollars and cost of collection for each offense..."

Darlington's contribution to the development of our discipline is significant, especially, his dedication to promoting agriculture based on science. He was probably the first to write and publish accounts that argue strongly for obtaining:

'...An accurate knowledge of the distinctive characteristics, and economic properties, together with a precise nomenclature of those plants that interest the cultivator of the soil..."

The point Darlington raised in 1847 about correctly identifying weeds is also a historical first that has also not received much attention from historians writing about botany, weeds, or agriculture in the USA

or elsewhere. Weed researchers, nowadays, know how important it is to correctly identify weeds in planning their management.

As opposed to common names, scientific names have a universal meaning. Those who know scientific names will be able to verify a plant's identity by reference to standard texts or will immediately know the plant in question when the scientific name is used. Those who do not share the same native language can make use of Latin, an unchanging language, to share information about plants (Zimdahl, 2010, p. 47).

Darlington's book contains more than 400 pages of accounts on crops, weeds, and other plants that were of interest to him. These botanical descriptions and personal observations on individual species, along with keys to plant families, genera, and species, must rank among the very first published material in the corpus of knowledge in *Weed Science*.

McCarthy vs. Halsted

In a late-19th century Letter-to-the Editor in *Science*, Gerald McCarthy (Figure 3), a botanist from North Carolina (McCarthy, 1892), took exception to New Jersey Professor, Brian Halsted's listing of 750 plants as 'American Weeds' (Halsted, 1889).

This dialogue occurred between 1889-1892 and is worthy of re-recording as it too has gone largely unnoticed in the *Weed Science* literature. Annoyed by Halsted producing a long list of plants, which included many useful and beneficial species among America's 'worst weeds', McCarthy wrote:

"Well may the long-suffering farmers turn up the whites of his eyes at this formidable list".

Continuing, McCarthy explained that he had indeed tried to clarify with various professionals how they related to weeds. His narrative reads as follows:

"...all plants are born free and equal; the distinguishing of plants as weeds and not weeds is purely human and artificial. The popular idea of a weed seems to be a repulsive, or hurtful, wild plant. But few persons give exactly the same definition..."

I have taken some trouble to secure the definitions of a number of intelligent persons and give below a few examples: -

"A plant where you don't want it – Director, Experiment Station.

"A noxious or useless plant" – Curator of Museum.

"A troublesome plant" – Chemist.

"An obnoxious plant of many species not fit for food or medicinal purposes" – Clerk.

"A plant not edible, so far as known, nor medicinal, or otherwise serviceable to man, and which always thrives where not wanted" – Inspector of Fertilizers.

"A plant for which we have no use so far as we know" – Meteorologist.

"(1) Underbrush or bushes; (2) a useless or troublesome plant" – Webster (Dictionary).

My own definition: Any plant which from its situation or inherent properties is hurtful to human interests; a vegetable malefactor..."

As reported by Troyer (1999) and McCormick (2011), before the turn of the 19th century, two institutional herbaria existed in Raleigh, North Carolina. The oldest was initiated by the first State Botanist, Gerald McCarthy (1858-1915). He was highly respected as a botanist and for his botanical collections and contributions. By 1890, he had presented more than 4000 specimens to the USA's National Museum (Smithsonian Institution).

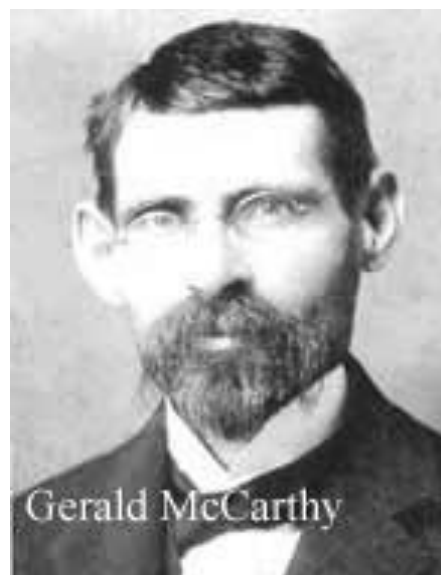


Figure 3. A portrait of Gerald McCarthy (Source: James R. Troyer's 1999 article)¹⁶

¹⁶ (Available at: http://www.herbarium.unc.edu/Collectors/McCarthy_Gerald.htm)

http://www.herbarium.unc.edu/Collectors/McCarthy_Gerald.htm

McCarthy was deaf because of childhood meningitis¹⁷ but was an active member of scientific circles at the time. For someone to write with such a deep appreciation of weeds, in 1892, is important because McCarthy objected strongly to Halsted's perfunctory listing species, such as clovers (*Trifolium* spp.), medics (*Medicago sativa*), vetches (*Vicia* spp.), and grasses, as 'wildlings of nature' for which 'we have as yet found no important use'. Calling this attitude foolish, he berated Halsted:

"...justice requires, in the case of plants and persons, everyone shall be innocent until they are proven guilty of wrong..."

McCarthy was drawing on the famous 'innocent until proven guilty' legal principle that entered the legal system in the USA in the mid-19th century¹⁸.

His writing preceded the better-known reference, which established the principle in 1895¹⁹. However, as Pennington (2003) explained, the principle is much older and can be traced back to the 13th century, used in defense of marginalized defendants, including heretics and witches. It is such an important legal maxim that the United Nations incorporated the principle in its *Declaration of Human Rights* in 1948 under Article 11, Section 1 (UN, 1948). The article reads as follows:

"...Everyone charged with a penal offence has the **right** to be presumed innocent until proved guilty according to law in a public trial at which he has had all the guarantees necessary for his defence..."

The maxim also found a place in the European Convention for the Protection of Human Rights in 1953 as Article 6, Section 2 (ECHR, 1953). It was then additionally incorporated into the United Nations International Covenant on Civil and Political Rights as Article 14, Section 2 (CCPR, 1966).

In many countries, nowadays, the presumption of innocence is a legal right of the accused in a criminal trial. Under the presumption of innocence, the legal burden of proof is on the prosecution, which must present compelling evidence to a judge or a jury) to prove that the accused is guilty beyond reasonable doubt. If reasonable doubt remains, the accused must be acquitted.

Regrettably, this supreme legal principle has been reversed when it comes to colonizing taxa (weeds) and is used to summarily condemn and brand them as 'invasives'. Some commentators have taken this phrase to unjustified depths, maligning weeds as 'guilty, until proven innocent' (see SOC, 2007). However, this viewpoint, taken by invasion biologists, along with the appropriateness of using fear-invoking terminology (viz. 'aliens', 'invaders', 'invasions') in public discourses on weeds has been questioned with vigour (see Davis and Thompson, 2001; Sagoff, 2005; Davis et al., 2011; Guiaşu and Tindale, 2018).

The reversal of the esteemed phrase of universal importance, so clearly enunciated for public good, is unwarranted, intellectually dishonest, and a form of populism at its worst. It is driven by the self-interest of the proponents in their push for one side of the argument (i.e., negative impacts of weeds, presented as a world at the cusp of an imminent 'invasion'). I doubt whether it has anything to do with a genuine interest in saving the world from marauding invaders, who, it is alleged, commit mass murder across continents, and crimes against nature!

As a botanist, Gerald McCarthy recognized two essential aspects of weeds: 'situations' (interpreted as the occupation of vacant spaces) and 'inherent properties' (heritable characteristics) of some taxa that could be hurtful to human interests. Perhaps, this writing inspired our discipline's founding fathers, such

¹⁷ Dr. James R. Troyer's article on Gerald McCarthy (1999), summarized by Carol Ann McCormick (2011), records that he was sacked from his job in 1897 as a result of departmental mergers and politics. The termination of his services has also been attributed to a claim that the 'physical infirmity prevented his being a teacher', although McCarthy had delivered numerous oral presentations and had interacted well with hearing persons. Troyer notes that McCarthy was not a research scientist despite holding many equivalent positions. For his enormous contributions to Botany, the Gallaudet University, a federally chartered private university in Washington D.C. for the education of the deaf and hard of hearing conferred upon McCarthy a D.Sc. in 1904.

¹⁸ According to Gary Martin (The Phrase Finder), the phrase 'innocent until proven guilty' was first cited as a legal principle in the *Law Reports of the Supreme Court of Ohio*, 1835. "The law presumes all innocent of crime until proven guilty" (see: <https://www.phrases.org.uk/meanings/innocent-until-proven-guilty.html>).

¹⁹ Coffin v. United States, 156 U.S. 432 (1895), was an appellate case of the US Supreme Court. In this case, F.A. Coffin and P.B. Coffin were charged with aiding and abetting the President of the Indianapolis National Bank, Theodore P. Haughey, in misdemeanor and bank fraud. The Supreme Court's commentary led to the establishment of this legal principle (Source: https://en.wikipedia.org/wiki/Presumption_of_innocence).

as Herbert George Baker and Arthur Hugh Bunting, to describe 'situations' (viz. 'disturbed' environments and man-modified habitat) and 'characteristics and attributes' that define weedy taxa (Bunting, 1960; Baker, 1965 - see Baker's 'Ideal Weed').

McCarthy's reference to weeds as 'vegetable malefactors' was unfortunate, as these taxa do not commit a crime; nor do they intend to cause harm to anyone. Nevertheless, McCarthy, a much-underrated individual for his varied botanical accomplishments (see Troyer, 1999), is amongst the more enlightened biologists of the late-19th century, who saw weeds differently from farmers and agriculturists who disliked weeds intensely.

Along with Emerson, McCarthy must be recognized for challenging the intolerant views on weeds, which were prevalent at that time in the USA. The reasons why such alternative viewpoints did not get much traction and stalled in the 20th century also need further discussions within our discipline.

Weeds and 'Proto-weeds'

An understanding of the 'origins' of weeds must define what they are. Such an understanding comes from archaeo-botanical investigations of prehistoric sites where nomadic hunter-gatherers first trialled the growing of food crops. The area where systematic cultivation (viz. settled agriculture) first occurred is the 'fertile crescent'. It is a crescent-shaped region in the Middle East, which spans south-western Iraq (ancient Mesopotamia, between the rivers, Euphrates, and Tigris), south-eastern parts of Turkey (Anatolia) and the western fringes of Iran, Syria, Lebanon, Israel, Palestine, Jordan, and Egypt (Zeder, 2011).

The Middle East was home to some of the earliest known human civilizations. Archaeology shows that significant human populations roamed the region from around the last ice age (ca. 23,000 years ago), mostly as hunter-gatherers. The Neolithic period (the 'new stone age') is thought to have begun around 11,000 years ago in the Middle East. This period is marked by evidence of domestication of both animals and plants (i.e., settled agriculture), construction of shelters, and the manufacture of pottery and textiles. Thriving in this 'cradle of civilization', Neolithic people were both nomadic and hunter-gatherers before they settled.

Until now, the consensus of researchers has been that farming was 'invented' in the Neolithic period, possibly around 12,000-11,000 years BCE in the fertile crescent region. This view is challenged by some new findings by an international collaboration

of researchers from Tel Aviv University, Harvard University, Bar-Ilan University, and the University of Haifa. This research discovered the first evidence that 'trial' plant cultivation began far earlier - some 23,000 years ago. The study (Nadel et al., 2004) described the discovery of the first weed species, named 'proto-weeds', at the site of a sedentary human camp on the shore of the Sea of Galilee.

The researches from the University of Haifa excavated Ohalo II, in 1989, during a drought that caused a drop in water levels in the Sea of Galilee (Lake Kinneret, Israel). However, when the drought abated and waters of the Sea of Galilee rose, the site became inaccessible, and work at Ohalo was halted for the next 10 years. When the water receded again, following several years of drought and intensive water pumping in the Jordan River, in 1999, the work recommenced. The two main excavations at Ohalo II, located on the south-western shore of the Sea of Galilee, occurred during six seasons from 1989 to 1991 and from 1998 to 2001 (Nadel et al., 2004).

Because weeds thrive in cultivated fields and disturbed soils, a significant presence of weeds in archaeo-botanical assemblages at neolithic sites of a later age, could serve as an indicator of some form of systematic cultivation. The well-preserved material from the Ohalo II site, which had been submerged for millennia, has provided evidence for the first appearance of weeds, much earlier than the presumed dates of the beginning of agriculture. Below is an excerpt from Snir et al. (2015).

"...Weeds are currently present in a wide range of ecosystems worldwide. Although the beginning of their evolution is largely unknown, researchers assume that they developed in tandem with cultivation since the appearance of agricultural habitats some 12,000 years ago. These rapidly-evolving plants invaded the human disturbed areas and thrived in the new habitat..."

"...Here we present unprecedented new findings of the presence of "proto-weeds" and small-scale trial cultivation in Ohalo II, a 23,000-year-old hunter-gatherers' sedentary camp on the shore of the Sea of Galilee, Israel. We examined the plant remains retrieved from the site (ca. 150,000 specimens), placing particular emphasis on the search for evidence of plant cultivation by Ohalo II people and the presence of weed species..."

"...The archaeo-botanically-rich plant assemblage demonstrates extensive human gathering of over 140 plant species and food preparation by grinding wild wheat and barley. Among these, we identified 13 well-known current weeds mixed with numerous seeds of wild emmer, barley, and oat. This collection provides the earliest evidence of a human-disturbed environment, at least 11 millennia before the onset of agriculture, that provided the conditions for the development of "proto-weeds", a prerequisite for weed evolution..."

The Ohalo site was inhabited by hunter-gatherers during the Last Glacial Maximum (LGM) - 27,000 to 21,000 years ago when world-wide, glacial ice sheets reached a maximum, at ca. 23,000 years ago. The Ohalo findings support the view that the species we brand 'weeds' did not necessarily arise out of agriculture. These colonizing taxa evolved and existed millions of years before humans, and well before settled agriculture.

Weeds are typically regarded as synchronous with the domestication of plants and animals. Weeds are also considered as the unwanted, unconsciously selected reciprocals of intensive agriculture. The 'no man-no weed' rhetoric is a much repeated theme within contemporary Weed Science (Young and Evans, 1976). The recent Ohalo II findings can be interpreted as indicative of agriculture not being a necessity for weeds to evolve. Agriculture, characterized by marked disturbances, would have expedited the successional species, who have the capacity to take possession quickly of vacant niches.

Species, branded as 'weeds' are simply colonizing taxa, which evolved well before humans to colonize vacant habitat wherever it existed (Chandrasena, 2019). Many such species then rapidly evolved to inhabit habitat associated with and disturbed by man. Given that the 2000 m² Ohalo 'camp' site is dated back to 23,000 years ago, the evidence suggests that today's weeds, or their ancestors, were present in the region, at least 10,000 years before settled agriculture (Snir et al., 2015).

That several colonizing species may have been thriving around the ancient human settlements is no surprise. The study authors suggested that the species identified in the archaeo-botany studies were, perhaps, the fore-runners of the present day 'weedy' counterparts. My view is that agriculture was not a prerequisite for most weeds to evolve, although, there may be some exceptions. Associations with humans (selection pressure) may have influenced some

colonizing taxa to evolve. These were most likely the species we find associated with agriculture today ('agrestal' weeds). The evolution of such species was expedited by the disturbances caused by agriculture.

The Ohalo excavations unearthed well-preserved plant matter amongst the remains of several small dwelling huts. There were also hearths outside the huts, human burial sites, as well as stone tools. The thousands of years old plant material offers clues as to how people lived during one of the coldest periods in recent human history - the last glacial period. These include material that had been used for building the huts and bedding. The plant material, initially preserved by charring and the sedimentation of silts, had been sealed in the low-oxygen conditions under the lake water. These conditions were ideal for preserving the organic material (Snir et al., 2015).

The species used for building the huts were thick branches of tamarisk (*Tamarix* sp.), willow (*Salix* sp.), and Mount Tabor oak (*Quercus ithaburensis*). These had been covered by smaller branches and leaves of other woody species, such as orach (*Atriplex* sp.) sedlitzia (*Sedlitzia* sp.) and mesquite (*Prosopis* sp.).

Apart from such woody colonizers, seeds of 13 current weed species were found among the ca. 150,000 identified charred seeds and fruits (Table 2). The weed seeds were mixed with grains of cereals, such as wild emmer wheat (*Triticum dicoccoides*; syn. *Triticum dicoccocum* Schrank), wild barley (*Hordeum spontaneum* (K. Koch) Thell.), and wild oat (*Avena barbata* Pott ex Link or *Avena sterilis* L.).

The high-frequency occurrence of weed seeds among the preserved seeds (~15,726 or 10.5%) reflects their common presence. Were they precursors of the modern-day weeds? Almost all the seeds (93.2%) belong to two important, current crop weeds: corn cleavers (*Galium tricornutum*), and dandelion (*Lolium temulentum*).

Until now, the original habitat of these plants was unknown, as they are rare outside agricultural environments in the region. Ohalo II, therefore, provides the oldest known indication of their origin, as well as the time of their entrance into the human-made habitat. Some other species found at the site – common lambsquarters (*Chenopodium album*), mallow (*Malva parviflora*), Syrian thistle (*Notobasis syriaca*), and milkthistle (*Silybum marianum*) - are well-known weeds. They occur in the region, typically in disturbed areas or waste sites. However, some of their parts are edible and would have been eaten.

Table 1 Earliest weeds or 'proto-weeds' identified from archaeological studies of seeds at the Ohalo II pre-historic site (Source: Snir et al., 2015)

Species & Family	Common names and observations
<i>Adonis dentata</i> or <i>Adonis microcarpa</i> (Ranunculaceae)	pheasant's eye; red chamomile; Eurasian weeds; now cosmopolitan.
<i>Chenopodium album</i> (Chenopodiaceae)	Fathen; common lamb's quarter; Eurasian weed; cultivated for millenia; now cosmopolitan.
<i>Fumaria densiflora</i> or <i>Fumaria parviflora</i> or <i>Fumaria macrocarpa</i> (Fumariaceae)	Fumitory; several species; common Eurasian weeds; now cosmopolitan; known for medicinal uses.
<i>Galium tricorntum</i> (Rubiaceae)	Rough corn-cleavers; Eurasian weed; now cosmopolitan.
<i>Lolium rigidum</i> or <i>Lolium multiflorum</i> or <i>Lolium temulentum</i> (Poaceae)	Ryegrass; many species; Eurasian weeds; now cosmopolitan; naturalized all over the world.
<i>Malva parviflora</i> or <i>Malva aegyptiaca</i> (Malvaceae)	small-flowered mallow, cheeseweed; or Egyptian mallow; Eurasian and North African weeds; now cosmopolitan.
<i>Melilotus indicus</i> (Fabaceae)	Sweet clover; sour clover; Eurasia and North African weeds; now cosmopolitan
<i>Neslia apiculata</i> (Berassicaceae)	Ball mustard; Eurasia and North African weeds; now cosmopolitan
<i>Notobasis syriaca</i> (Asteraceae)	Syrian thistle; Eurasian Weed; now cosmopolitan.
<i>Silybum marianum</i> (Asteraceae)	Milkthistle; European weed, now cosmopolitan; known for medicinal uses.

The presence of such a wide variety of weeds, particularly corn cleavers, indicate that these species might have been growing together with the wild cereals. It is possible that the inhabitants engaged in small-scale trial plot cultivation of cereals for food. It is also possible that the 'proto-weeds' may have been gathered in the wild or from a local dump area where they grew (Snir et al., 2015). Since these wild cereals and weeds currently grow in both cultivated fields, waste dumps and uncultivated regions of the Jordan Valley, both ideas are plausible.

Archaeological evidence from several locations appear to indicate that some nomadic hunter-gatherer human groups, who lived ca. 23,000 years ago, may have tried out a more sedentary life. Staying in one place, they might have engaged in elementary, cereal cultivation. Overall, the fortuitous findings at Ohalo II provide the earliest botanical evidence of a disturbed environment of an ancient permanent camp, around which today's weeds proliferated.

Research of the Paleolithic period has already demonstrated that humans may have caused significant modifications to their environments. This would have been long before the Neolithic revolution ca. 23,000 years ago. Ancient humans set fire to vegetation, hunted, and trapped preferred species of mammals, birds, reptiles, and fish. They also cut down trees for shelters, and to produce tools and objects. Small human populations of the past, conducting such activities, on a small-scale, cannot be considered as deforestation, in the sense the term is used today; it was merely part of their survival strategy. However, even small populations of humans would create waste, as well as waste dumping areas, in and around their habitations.

Later, while attempting to cultivate coarse and large-grained grasses, hunter-gatherers, transitioning to a more sedentary lifestyle, would have cleared areas near their dwellings for some basic planting. The disturbance of environments around camps would have led to the proliferation of species that follow humans and thrive alongside the obliging human ally (these are called synanthropic plants).

These plant species, both annuals, and perennials exhibit functional and adaptive traits that enable them to withstand the stresses of the disturbed habitats. By being successful, they would have increased their biological fitness in natural plant communities, altered by their ally, or natural forces.

Concluding comments

What the research confirms is that the relationship between weeds and men is an old one; weeds are *shadows of men*, as well as *shadows of man's history and manipulations of his environment*.

As Young and Evans (1976) foretold several decades ago, "*The introduction of colonizing species to new environments may be one of the greatest manipulations that the human agency is responsible for. The total consequences of such actions will be determined in the future*".

The most damaging impacts humans have on other organisms (biodiversity, including colonizing taxa) come from the large-scale land clearing to grow monocultures of crops, deforestation for timber, land reclamation and drainage of wetlands for uses, such as agriculture, mining, and urban growth. The relentless mining for coal, minerals, oil, and gas, and large infrastructure projects, such as the oil and gas pipelines, also cause damages to landscapes on a scale hitherto unknown to the planet.

In the meantime, a deeper ecological and historical understanding of how, why, and where weeds have come about would help modify our attitudes, allowing us to avoid creating conflicts with potentially useful plant taxa and getting into situations from which we cannot win.

As photosynthetic organisms, colonizing taxa are critical biological resources. We may have to depend on them in times to come. As part of Nature's rich biodiversity, all that weeds are doing is to take the opportunity, when presented, to grow, survive, and reproduce. In 1859, Charles Darwin called this a 'struggle for existence' which is the title of Chapter 3 (p. 66) of his '*On the Origin of Species*'.

Darwin mentioned weeds in Chapter 3 and stressed the vital role of competition among organisms in driving forward natural selection and biological evolution. Colonizing taxa (weeds) will often win in 'struggles for existence' with other species because they are adapted by millions of years of evolution to do so. In so doing, they are perpetually engaged in the biological conservation of their identity and kind. *Isn't that what all successful organisms are supposed to do?*

Finally, to conclude this Editorial, I wish to highlight some sentiments expressed by Robert Zimdahl, which I echo (Zimdahl, 2010, preface, p. xi):

"...Understanding the past and knowing where we came from is essential to interpretation of the present and exploration of routes to the future..."

"...How I evaluate that history, however, reflects my judgments based on years of thought and study. I have tried to think like others and have listened to the stories of many concerning the development of weed science..."

Although not a trained historian, I am interested in the historical communications, past events, and occurrences that defined *Weed Science*, before it became the formidable scientific discipline it is today. Interpreting and analyzing history, drawing out actual

or potential, explicit, or implicit meanings, is a worthwhile endeavour. However, interpretations and the likely conclusions need to be logical, well-informed, and supported by chronicles, documents, diaries, letters, official archives, all of which constitute proper research.

As George Santayana (1852-1953), a Spanish-born, US philosopher (Santayana, 1906, p. 284) said:

"...Those who cannot remember the past are condemned to repeat it..."

In my view, the new generation of weed scientists would benefit from rigorous examination of past documents, which record meaningful and worthy activities of our science's founders, related to botany, weeds, and agriculture.

I emphasize that knowledge of history is intelligible only to those who are prepared by education, technique, and attitude to ask the right questions and listen for the answers.

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I also wish to state that some of the material used in this Editorial, is freely available in the public domain, such as Wikipedia (www.wikipedia.org) and other on-line sources. I declare that this material has been solely used for non-commercial, educational purposes in this 'open access' journal.

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Smooth Cordgrass (*Spartina alterniflora* Loisel.): The Case for Utilization of a Colonizing Plant Species

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Abstract

The status of utilization of colonizing plants (weeds) must be regarded as being in infancy. With the increasing need for alternative feedstocks to replace petroleum, which is used to produce energy, chemicals, and other products, attention is now on alternative sources of biomass, such as agricultural and forestry residues. However, weeds represent a considerable amount of biomass which remains a largely untapped resource. Smooth cordgrass (*Spartina alterniflora* Loisel.) is an example of such a species that has yet to be exploited to its full potential. This coastal, salt-tolerant plant has proliferated in many areas, especially in China, forming extensive stands. The species was introduced to China from North America, about 40 years ago, for coastal erosion protection. Since introduction, smooth cordgrass has colonized the coastal areas forming extensive stands along China's eastern seaboard. These infestations have become a severe problem at many locations in the eastern coastal regions.

The present report is a case study and perspective concerning the utilization of smooth cordgrass in China. I describe examples of the economical and efficient utilization of the plant's biomass to form a variety of practical products on a commercial scale. These show that it is possible to find new and effective ways to achieve large-scale usage of otherwise waste biomass from this species and others, which are similar. Further comprehensive research and development towards full valorization of smooth cordgrass with innovative utilization are required. The future will hopefully see increasing utilization of weeds to meet the increasing demand for resources that are sustainable and renewable.

Keywords: *Spartina alterniflora*; cord grass; bioresources; biomass utilization; renewability; sustainability

Introduction

There is globally an increasing interest in the search for renewable, and environmentally-benign resources to replace petroleum as the source to produce energy and raw materials for manufacturing of a wide range of products. The interest is primarily driven by increased energy demands, rising costs and depleting supplies of petroleum, and environmental concerns over the use of petroleum and petroleum-based products. Concurrently, there is an increasing interest in renewable and sustainable biological resources

(bioresources) as industrial raw materials. Such bioresources, particularly those based on lignocellulosic biomass from plants, have seen rapidly increased utilization for biomass applications. These sources include waste materials such as agricultural, forestry, and aquatic or fisheries wastes. The large amounts of these materials, generated annually, in almost any country of the world, make them ideal candidates as bioresources. Biological resources meet many criteria of the "Green Movement" i.e. they are, in general, renewable, sustainable, environmentally-safe, non-toxic, and their use contributes to overall greenhouse gas reduction (Walker, 2015).

The main components of lignocellulosic biomass are cellulose, hemicellulose and lignin, together with small amounts of proteins and other compounds. Such material is sometimes referred to as plant biomass as plant cell walls are mainly composed of these compounds. In general, many sources of lignocellulosic bioresources are abundant, sustainable, and renewable, being composed mostly of carbohydrates produced by plants as products of photosynthesis. With adequate sunlight, water, nutrients, and soil, plant-based bioresources are accordingly renewable (Tursi, 2019).

In utilizing plant biomass, the whole biomass may be utilized, such as burning it for heat or fuel in its most simplistic form, or only a few components may be utilized, e.g. carbohydrates for bioethanol. It generally involves converting it to another form (biomass conversion), which involves the conversion of biomass into its various constituent components (e.g. cellulose, hemicellulose, and lignin).

These components can then be further converted into other products by a variety of methods such as chemical, mechanical (pressure, agitation, grinding) and/or biological processes (enzymes, microbes) to produce many possible products. These products include bioenergy/biofuels (bioethanol, biomethanol, biodiesel, biogas), cellulose fibres, lignin, carbohydrates, proteins, phytochemicals, as well as smaller chemical building blocks to synthesize other, larger chemicals that would otherwise be obtained from petroleum refining (Stevens and Verhé, 2004; Tursi, 2019).

In addition to traditional and extraneous sources of lignocellulosic biomass from agriculture, forestry and fisheries and their wastes, another potential source exists in the large numbers of colonizing plants that exist in most parts of the world. Utilization of colonizing plants represents a vast pool of available lignocellulosic biomass, which can be an economically and environmentally advantageous alternative to the use of fossil fuels as a resource.

There are many potential applications for colonizing taxa, although, for the most part, they remain a vast untapped and unrealized pool of available biomass (Burry et al., 2104; Sharma and Pant, 2018). The issue of some of these taxa becoming 'invasive species' is a common theme worldwide. There is a general perception that some colonizing taxa can crowd out desirable, native species both on land and in waterways and coastal areas. They may also have detrimental effects on local habitats, environments, and economies. These plants, if dealt with at all, are commonly removed

and then buried or burned, creating an additional environmental pollution problem (DiTomaso et al., 2006; Duns and Chen, 2009). Utilization of these otherwise problematic species would accordingly be a way to not only reduce pollution but to help local economies as well by providing raw materials to produce energy or other products (Chandrasena, 2008; 2014; 2019; Duns and Chen, 2009).

Smooth cordgrass (*Spartina alterniflora* Loisel.) is one such example of a colonizer that can produce extensive biomass. The vast growths of smooth cordgrass in some parts of the world, such as the west coast of North America and the eastern coast of China, represent a vast amount of biomass produced every year. The species has proven to be problematic in places where it has established. Despite considerable attention towards its management and control, it remains a problem at many locations. The significance of smooth cordgrass is indicated by the fact that there is an international conference solely dedicated to it, with the first one held in 1990 in Seattle, Washington, U.S.A. (Mumford et al. 1991).

Smooth cordgrass is presented here as an example of a potentially problematic species, but one that can be extremely useful for a variety of uses. The species was selected to illustrate the case for the utilization of colonizing taxa because of many years of personal experience, studying its utilization, primarily in China. Seeing it from all stages to being a locally abundant weed, to being utilized on a commercial scale, has been a rewarding experience, while also teaching some valuable lessons as to how to deal with such plant species more broadly.

Colonizing Species as Bioresources

Colonizing plants (weeds), some of which can become 'invasive', generally grow quickly, have high fecundity, and many can tolerate a wide variety of growing conditions. Many are stress-tolerant and can grow where other species may not grow easily in different environments. They often produce large stands, displacing others. However, this same prolific growth also represents the production of large amounts of biomass that may be utilized.

There has been limited interest in the utilization of weeds for beneficial purposes, mainly because the discipline of Weed Science has been so focused on controlling weeds. (It should be clarified that 'utilization' here, in general, refers to processing the biomass of the dead plant biomass and not the

utilizing of living plants for purposes, such as erosion control or as ornamental purposes. The latter applications are also valid and should be noted, as such). While there has been a considerable increase in the use of biomass from various sources including forestry, agricultural, aquatic and fisheries as raw materials for energy and manufacturing, this interest has not been extended to the utilization of biomass from weeds to nearly the same extent.

Despite the ever-expanding plethora of journals, trade magazines and textbooks dealing with various aspects of biomass and bioresources, only a small percentage of the publications deal with the commercial utilization of weeds in some form or other. This lack of attention indicates the under-appreciation of weeds as a viable source of biomass. The reason could be that weeds are, traditionally, considered a nuisance, and as such to be dealt with by control or eradication, rather than considered as a credible source of utilizable biomass; this theme will be revisited in subsequent sections.

In addition to smooth cordgrass, various aspects of the utilization of other weeds have been undertaken (see Catallo et al., 2008; Liao et al., 2013; Brouwer et al., 2019; Sharma and Pant, 2019) in recent years. Other studies have been more specific, focusing on the utilization of well-known weeds, such as water hyacinth (*Eichhornia crassipes* (Mart.) Solms), reviewed by Malik et al., (2007), Guna et al., 2017, and Yan et al., 2017); common reed (*Phragmites australis* (Cav.) Trin. ex. Steud.) reviewed by Burry et al. (2014, 2017), and parthenium weed (*Parthenium hysterophorus* L.) (Chandrasena and Rao, 2019). A more general account of the utilization potential of colonizing taxa has been provided by Chandrasena (2008) with an appeal to consider a closer integration of beneficial aspects of weeds into human societies (Chandrasena, 2014; 2019).

To date, most of the investigations into the possible utilization of weed biomass have remained at the research study and assessment stage. Only a few have resulted in further development or viable commercialization. The consensus appears to be that weeds will remain a much under-utilized and neglected resource for some time, which is the viewpoint shared by this author. There are, of course, both advantages and disadvantages in the use of weeds as bioresources, and utilization is not the “*be-all and end-all*” for the problems they cause. These advantages and disadvantages, particularly concerning the exemplar I am using, will be further expanded on in the Discussion section.

***Spartina alterniflora*: General characteristics and habitat**

Smooth cordgrass is a rhizomatous perennial herbaceous C4 grass plant that generally ranges from 1-3 m in height, with leaf blades that are around 30 to 50 cm long and are 6 to 15 cm wide. The leaves lack auricles and have ligules that consist of a fringe of hairs. The plant stems are hollow and hairless, while the rhizomes are long and hollow. A dense stand of smooth cordgrass is somewhat like a small forest of dark green plants, with minimal light penetration to the mud or soil beneath the stand. The plant is deciduous; its stems die back at the end of each growing season. These thick, extensive stands represent a large pool of biomass, going to waste when nothing is done with it.

Smooth cordgrass is hexaploid and can undergo both vegetative and sexual reproduction. The latter contributes little to the maintenance of established stands of growth but may play a more critical role in the establishment of large disturbance-generated patches of plant growth. During September and October, seed heads are normally present that are approximately 30 cm in length and can carry spikes containing 12-15 spikelet seeds and have flowers that are generally inconspicuous and are normally 5 to 8 cm long (Landin, 1991; Li et al., 2020). Its seeds are dispersed primarily via water which may facilitate its rapid spread over considerable distances, which plays a significant role in its tendency to be invasive (Thompson, 1991; Chelaifa et al., 2010).

Smooth cordgrass is a typical, strongly salt-tolerant species (halophyte). This physiological adaptation to high salt content allows the species to grow abundantly in coastal or marsh habitats as a warm-season grass. High salt tolerance, prolific reproduction and efficient C4 photosynthesis have combined to give the species the capacity to produce large biomasses in a typical growth cycle.

The species tends to thrive in anoxic, marsh habitats (Figure 1) due to its ability to oxygenate its roots and rhizosphere (Thompson, 1991; Simenstad and Thom, 1995). In its native range, it exhibits varying forms of growth in different salt marsh zones, depending on the local habitat or environment. Plants growing under optimum conditions can reach a maximum height of above 2 m while those growing in highly salt marshes may be stunted to a height under 1.0 m, including inflorescences. Unlike most

other marsh plants, the salt-tolerance of smooth cordgrass is directly proportional to water depth, forming dense monospecific stands in salt and brackish marshes with mid to high tide levels (Landin, 1991; Thompson, 1991; Ayres et al., 2004; Chelaifa et al., 2010).



Figure 1. Growth of smooth cordgrass in a coastal salt marsh in eastern China (from Qin, 2013)

Biogeographical Distribution, Native range and Spread

As noted previously, the seeds of smooth cordgrass are dispersed mainly by water, facilitating their spread over considerable distances, playing a major role in its spread. Biogeographical patterns suggest that the genus *Spartina* originates from the Atlantic and Gulf Coasts of North America. The genus *Spartina* contains 14 to 17 species, most of which are native to North America, while only *Spartina maritima* is native to Europe (Landin, 1991; Chelaifa et al., 2010).

Smooth cordgrass made its way across the North American continent to the west coast where it is now found along the Pacific coast in the Washington State, where it has become a significant problem (Simenstad and Thom, 1995). It has also spread to California, especially in the San Francisco Bay area, and north, to British Columbia in Canada.

Smooth cordgrass was introduced to Europe and East Asia for coastal protection, and eventually even spread to the coasts of South Africa, Australia, and New Zealand (Ayres et al., 2004; Grevstad et al., 2007; Patten et al., 2017). The species was also introduced to China in 1979, for coastal protection, erosion control and sediment stabilization. It must be said that the species did play a decisive role in these

aspects but also has had a significantly detrimental impact on coastal ecosystems.

Since its introduction, smooth cordgrass has grown out of control to become a problematic species, to the point where, in 2003, it was among the first plants included in the official list of *Invasive Alien Species* (IAS) in China. It has flourished over the past 40 years and rapidly expanded along China's eastern coastline. China now has the world's largest area of this species, which is presently approximately 55,000 ha (Xie, Han et al., 2019).

The area of spread increased by 10,000 ha between 1985 and 2015 with the fastest expansion rate of 463.64 ha occurring between 1995 and 2005. In 2004, smooth cordgrass salt marsh areas in Jiangsu Province alone reached a total of about 150 km² (Li et al., 2020). Significantly, from a utilization point of view, it is estimated that the total dry matter production of its above-ground parts ranges from 7.5 x 10⁵ to 1.15 x 10⁶ tons per year. This represents a considerable, yearly pool of utilizable plant biomass (Xie et al., 2019; Li et al., 2020).

Despite controlling erosion and stabilizing sediments, the adverse effects resulting from smooth cordgrass infestations along the coast are considered to outweigh any positive environmental effects of its intended introduction. Smooth cordgrass, as a typical halophyte, has readily adapted to high salt content in coastal habitats, occupying bare flats as well as often replacing native C3 plants, such as common reed and seepweed (*Suaeda salsa* (L.) Pall.), to become one of the dominant plants in China's coastal wetlands, and the most abundant halophyte in tidal flats in China.

Smooth cordgrass infestations have had significant negative impacts on coastal ecosystems and economies. Many native species have been displaced as smooth cordgrass occupies ecological niches of food molluscs, plants, fish, and endangered birds. In addition, it causes fast sediment deposition blocking harbours to shipping and fishing. Infestations have even invaded fishponds and mangrove swamps (Wang et al., 2008; Li and Qiu, 2011; Li et al., 2020).

The management of Smooth Cordgrass

While smooth cordgrass continues to flourish, considerable effort has been directed at methods to effectively manage these infestations. The traditional, time-honoured way was to mechanically

remove and then burn the biomass. In some cases, the residue is added to the soil as a fertilizer or soil amendment. The physical control methods are widely practiced in China, normally after the stems have died (Figure 2). This burning represents a vast amount of potentially useful biomass, and the smoke from the burning of large stands of plants can be a source of air pollution presenting a significant health risk to those with respiratory problems.



Figure 2. Residual smooth cordgrass stalks being collected by hand (upper photo) and burning of gathered stalks (lower photo) in eastern China

Other methods of control for smooth cordgrass include mechanical removal using machinery (mowing/waterlogging and mowing/tilling) (Xie et al., 2019), and effective herbicides, such as Haloxypop-R-methyl (Xie et al., 2019; Zhao et al., 2020), and Imazapyr (Patten et al., 2017). In addition, biological control has also been attempted with the application of a delphacid plant hopper -*Prokelesia marginiata* (Van Duzee, 1897; Grevstad et al., 2007) and the fungal pathogen *Fusarium subglutinans* (Wollenw. & Reinking, 1983; Gong et al., 2012). Added to the above efforts are ecological manipulations and restoration attempts to substitute and displace smooth cordgrass with common reed (*Phragmites australis*) (Wang et al., 2008) and a fast-growing mangrove pioneer species - *Sonneratia apetala* (Buch. -Ham). (Chen et al., 2014).

Utilization of Smooth Cordgrass

Smooth cordgrass has been utilized for some traditional applications over the years, and other possible utilizations of a more sophisticated technical level have also been investigated. In the sections below, I describe what they are, what has been done, and what could be done in the future.

Ecological roles and environmental benefits

As previously stated, smooth cordgrass was initially introduced from North America to China for erosion control and estuary reclamation. It has certain fisheries and wildlife uses in its native range; in these native habitats, some waterfowl and wetland mammals are known to eat its roots and shoots. The species is also palatable to livestock (Simenstad and Thom, 1995). Stands of smooth cordgrass may also serve as a nursery area for estuarine fishes and shellfishes, and mangroves.

In the Pacific Northwest estuaries in the USA, species that can utilize smooth cordgrass marshes, include juvenile Chinook salmon (*Oncorhynchus tshawytscha* Wlbaum). Such species may benefit from the spread of salt marsh vegetation (Landin, 1991; Simenstad and Thom 1995). In addition, the species has also been recognized as having strong carbon sequestration capabilities and could be significant for the carbon cycle of the coastal and ocean ecosystems (Lu and Zhang, 2013).

Biomass utilization

As smooth cordgrass is deciduous, its stems die back at the end of each growing season. These dead or dying plants represent a tremendous amount of plant biomass that may normally go to waste at the end of each season. In many instances, in China, and elsewhere, it is simply left to decay and disintegrate or burned, as previously noted.

The most common non-food utilization of lignocellulosic plant biomass, by far, is for bioenergy and biofuels (Stevens and Verhé, 2004; Sharma and Pant, 2018). The increasing awareness of climate change and greenhouse gas effects, depleting petroleum resources and the pollution their use creates, together with increasing petroleum prices over the last couple of decades, has led to the search for safer and renewable alternative energy

sources. This is where the option of the utilization of smooth cordgrass biomass becomes relevant.

A study by Lu and Zhang (2013) established the potential of this plant as a biofuel source in China. They estimated that the total annual biomass can reach 2.53 Mt, producing 39 PJ of energy. This amount of energy is equivalent to the energy produced by 1.33 Mt of standard coal. The annual biomass of the above-ground parts of the plant alone is 1.12 Mt, producing 18 PJ of energy, equivalent to that produced by 0.61 Mt of standard coal.

These figures represent a potential significant production of energy and substituting smooth cordgrass biomass for just a part of the massive amounts of coal burned in China would reduce air pollution caused by the burning of coal. This usage of smooth cordgrass biomass may occur in different forms, including incineration or pyrolysis, pelletizing, and combusting in electricity-producing facilities in place of coal, or as biogas production from anaerobic digestion of the biomass (Li and Qiu, 2011).

A recent review (Xie et al., 2019) has divided Chinese research on smooth cordgrass into various sectors, as shown in Figure 3. The data confirms that the central area of research for smooth cordgrass utilization is the bioenergy/biofuels sector, with 43% of the total studies, the majority using stems or straw from the plants, aimed at this area. "Traditional" uses of the species, as feed for livestock and other animals and aquatic species, is the second-highest area of research. This is followed by medicinal applications, for extractive medicinal compounds, followed closely by raw materials for paper and other products (15%), with other lesser applications, such as fertilizers and pollution remediation, forming the remainder of the studies. The authors of the study conclude that the medicinal applications are the most likely viable, high-value utilization of the species.

Some additional research for combined ecological control and utilization management of the species has been undertaken in China. One of these is a combined ecological and integral utilization, whereby stands of smooth cordgrass are replaced by common reed (*Phragmites australis*), while also utilizing its biomass from other applications, such as fodder for dairy cattle, and as a source of extractives for feed and nutrient additives (Wang et al., 2008).

Another is a 7-step ecological engineering system for smooth cordgrass utilization, which involves a low waste, clean production process to make use of as much biomass as possible. This process firstly involves using the biomass as feedstock for the extraction of a bio-mineral liquid,

which can be encapsulated and used as a health supplement which has various purported uses (Lu et al., 2020). The residues left after extraction are used as a medium for mushroom cultivation, and then for growing earthworms.

As a final part of the process, the remaining residues are the main component of a microbe-enriched organic fertilizer that is returned to the soil. An analytical evaluation of this system indicated that, if fully implemented, it could result in a potential economic output greater than 2% of the national GDP, illustrating the significance of the use of biomass from weeds (Lu et al., 2020).

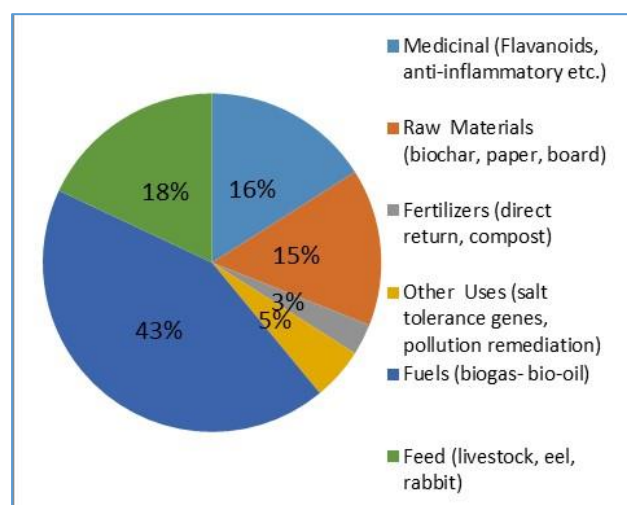


Figure 3. Proportions of studies on different utilization modes of smooth cordgrass in China (Source: Xie et al., 2019)

Commercialization of weed biomass utilization: a success story

An area of potential utilization of materials containing lignocellulosic biomass is as alternatives for wood fibres in the making paper and related products. This type of application has become increasingly important globally due mainly to the need to preserve and maintain the world's forests for conservation and carbon capture purposes (Stevens and Verhé, 2004; Ayoub and Lucia, 2018). With the advent of the internet and the increasing disappearance of many printed newspapers and other materials, the need for newsprint has decreased, but there are still plenty of applications for pulped materials, including packaging materials (Orts, 2002; Cao and Zhang, 2006).

The vast excesses of smooth cordgrass biomass in China make this plant an obvious candidate as a potential candidate as a source of fibres for such pulping applications. Fibre from straw or stems from various agriculture and forestry wastes, as well as other weeds, have been successfully converted into pulps (Orts, 2002; Ververis and Pereira, 2002; Burry et al., 2017). Accordingly, due attention was directed towards this type of utilization, with which I had personal involvement from 2008 to 2014.

The initial stage of this project, which commenced in 2008, involved a detailed study of smooth cordgrass biomass and its physico-chemical characteristics. Plant samples were harvested in October at the end of the growing season from the upper intertidal area of a muddy salt marsh, located in Dafeng District Port on the eastern coast of Jiangsu Province, China (Lat: 33.217809°; Long: 120.815462°). The dried samples were firstly cut into 1-2 cm pieces, which were then sieved. Samples from the 40–60 mesh fractions were selected to determine their chemical composition and for further use. Based on analysis by scanning electron microscopy, it was observed that smooth cordgrass stems, leaves, leaf sheath xylem and phloem tissue consisted of a considerable amount of fibre.

The fibres were examined microscopically (Figure 4), and their lengths determined biometrically by optical microscope. While the most abundant fibres were in the 0.50-0.60 mm length range, the average fibre were 1.19 mm (Table 1).

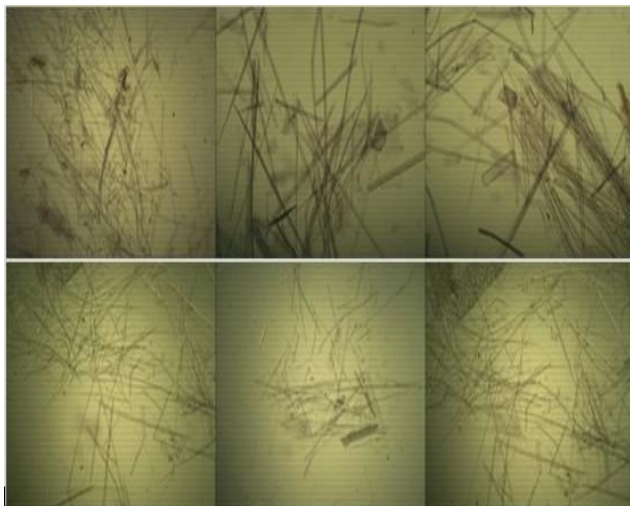


Figure 4. Smooth cordgrass fibres viewed by light microscopy at different magnifications (Source: Wu et al., 2011)

Smooth cordgrass stem samples were characterized chemically using standard methods for lignin, holocellulose, cellulose and hemicellulose

contents. The cellulose content of smooth cordgrass is lower than those of the other materials listed, but the lignin content is similar to that of sorghum stalks, and lower than those of common reed, rice straw, and wheat straw (Table 2). A lower lignin content is an advantage for the pulping process (Ververis and Pereira, 2002; Stevens and Verhé, 2004). The analysis of this study suggested that smooth cordgrass straw fibres provide an effective raw material for making pulp, containing on average of 70.1% holocellulose, 35.9% cellulose and 15.9% lignin, and suitable fibre size (Wu et al., 2011).

The results show that the average length of the smooth cordgrass fibres is similar to that of common reed, but greater than that of cotton straw, and lesser than those of wheat and rice straw (Table 1). In general, they are in the range suitable for the preparation of pulps (Ververis and Pereira, 2002; Stevens and Verhé, 2004).

Table 1. Fibre lengths of various lignocellulosic biomass materials (Source: Wu et al., 2011)

Biomass material	Average Fibre Length (mm)
Rice straw	1.29
Wheat straw	1.39
Cotton Straw	1.03
Common reed	1.16
Smooth cordgrass	1.19

Table 2. Chemical characterization of various lignocellulosic biomass based raw materials (Source: Wu et al., 2011)

Material	Holo-cellulose (%)	Cellulose (%)	Hemi-cellulose (%)	Lignin (%)
Smooth cordgrass	70.1	35.9	34.2	15.9
Wheat straw	76.2	39.7	36.5	17.3
Rice straw	60.7	41.2	19.5	21.9
Common reed	64.2	39.8	24.4	23.7
Sorghum stalks	65.9	41.5	24.4	15.6

Further studies indicated that the smooth cordgrass fibre pulp could be mixed with other pulps, such as obtained from bamboo (*Phyllostachys bambusoides* Siebold & Zucc.) pulp, cellulose fibre-residues from paper making, printing factories, corrugated cardboard, and other residues from

various paper product manufacturing facilities. Such an approach allows these otherwise waste materials to be recycled. Results indicated that the proportion of smooth cordgrass pulp in these mixtures could reach more than 50% (Chen et al., 2011).

The final step in the utilization process was to produce products from the smooth cordgrass fibre-pulp in a clean pulp production line. Following the process of Cao and Zhang (2006), the fibres were firstly sprayed to soften and swell them, with accompanying tension reduction, to facilitate breakdown and introduction to a pulping machine. Secondly, fibre pulp from the straw bundles was prepared through a straightforward process that saved energy and was environmentally-safe, minimizing pollution. This process can produce tons of fibre with a yield of 80% of 40 mesh fibre pulp. Thirdly, a metal mould for the product to be manufactured was submerged in the pulp slurry, and the slurry pulled into the mould by vacuum to form the shaped product. Finally, the products were ejected out of the mould and deposited on a conveyor, moving through a drying oven.

Using this process with composite fibre bundles and chemical pulps of smooth cordgrass fibres as one of the main components, various moulded products were made (Figure 5). These included industrial packaging products, such as fruit trays, lightweight shipping pallets, environmental protection products, decorative wall panels and automotive wheel containers (Wu et al., 2011; Qin et al., 2014).

A factory was built near the coastal city of Dafeng, Jiangsu Province, on the east coast of China, to establish a production line for the fibre-based packaging materials and other products in 2011. With an annual output of 5,000 tons, this facility realizes the industrial utilization of smooth cordgrass and represents the first of its kind in the moulded pulp industry. Dafeng has long been the site of severe smooth cordgrass infestations and therefore, a sensible location to place such a facility.

Although located in a remote location, this facility employs approximately 50 people including production line workers, engineers, office staff and management in addition to those seasonal workers hired to harvest the plant biomass. Various moulded products are sold nationally, and some, such as egg and fruit trays, sold to buyers in Germany and Switzerland, among other countries.

In a modification to the production, smooth cordgrass fibre bundles were prepared together with various additives and utilized in a hydraulic vulcanizing press moulding machine using flat plate

moulds and bowl mould plates to produce 'green' dishes and 'green' eating bowls (Qin, 2013). Product testing results met the FDA requirements for product quality standards, and the price for these products has reached more than 20,000 RMB (approximately USD 2,800) per ton. Such pulp-based products, which are made from plant fibres, possibly mixed with other organic pulps, as raw materials, are green technologies, which are environmentally superior and safer than those of many other industries. The potential for broader applications and uses are confirmed sales and interest in the products.



Figure 5. Wheel hub packing container (top) and back of fruit tray (bottom) made of mixed pulps containing smooth cordgrass fibres

Our studies indicated that smooth cordgrass biomass has the potential to be used in various other commercial applications as well. These include: (a) preparation of biochar in various forms, as the sole component, or part of a mixture (Liao et al., 2013; Sharma and Pant, 2018), (b) as a filler for improving the properties of concrete (Mello et al., 2014) and (c) fibres to partially or totally replace wood fibres in wood-plastic composites (WPCs), which can be used to produce a variety of building materials, including flooring, wall panels and furniture (Ayoub and Lucia, 2018). The production of WPCs with weed biomass was recently demonstrated using Canadian goldenrod (*Solidago canadensis* L.), another well-known colonizing plant in China (Liu et al., 2017).

Discussion

Based on fundamental chemical and biometric studies of smooth cordgrass and the moulded pulp products produced from it, it is apparent that pulp moulding provides a commercially effective means of utilization of the species, as a source of non-wood fibres. Using this species as an industrial raw material to make packaging products provides a basis for further utilization of other weeds and straw materials in the future. Fibres can be used as raw material to manufacture a variety of products on an industrial scale. These products, such as packaging materials and shipping containers, have been profitable, providing a boon to the local economy.

This type of raw material is mostly free, and the expenses involved in employing local workers to harvest and dry the plant stalks are minimal. From a personal perspective, it is rewarding to see the research and development come to fruition with the commercialization of such ventures. Not only does such an application provide an alternate means of dealing with a problematic plant, but it also doubles its environmental and ecological effectiveness by substituting for another limited resource, namely, wood fibres. Thus, the use of smooth cordgrass biomass serves two purposes: firstly, a free raw material for industrial manufacture, and secondly, a degree of biodegradability and recyclability.

As discussed previously, while several utilization options of smooth cordgrass biomass have been investigated, only a few have been adopted at the industrial scale. Most studies have been confined to research without commercial development. The exception, as presented here, utilizing fibres from the stems as raw material, pulped, and moulded to produce several products, is an example of what can be achieved using weeds as bioresources.

This lack of utilization of weeds may be due to several factors, including a general reluctance to work with weeds as they have associated stigma and negative connotations (Chandrasena, 2008; Sharma and Pant, 2018). Weeds are generally considered “unwanted”. The advantages of using weeds as raw materials are related to their robust and fast growth, tolerating a range of ecological conditions. They are also very tolerant and can be repeatedly harvested, leaving behind sufficient rootstock and plant parts (stems) that may regrow and are amenable for continual, sustainable harvesting.

Many weeds are ideally suited as bioresources, as they are generally not utilized for other purposes. This material can substitute for the biomass of

traditional crops, such as corn (*Zea mays* L.), sorghum (*Sorghum bicolor* (L.) Moench), wheat (*Triticum aestivum* L.), canola (*Brassica napus* L.) and others, which are used as feedstock for biofuels. Such use can alleviate the tension in the “food vs fuel” arguments, as well as land-use conflict issues (Stevens and Verhé, 2004; Piotrowski et al., 2015; Sharma and Pant, 2018). For coastal species, such as smooth cordgrass, these arguments do not hold, as they do not compete with crops for growing space. Many landowners, where large stands exist, would no doubt gladly let someone harvest them for free, or perhaps, impose a modest licensing fee.

On the negative side, there are certain disadvantages in the use of weeds as bioresources. These include the unpredictability of growth of any plant species and seasonal or yearly variations of growth. The abundance and harvesting can also sometimes be constrained by circumstances. If the raw material for production becomes unpredictable, manufacturers or users of those products may have to adjust production, taking account of these variations. There is also the possibility of seasonal changes, locational variations, or ageing, which may cause changes in biomass quality, energy content or chemical composition of smooth cordgrass, as has been reported in an early paper (Squiers and Good, 1974). Such variations may require obtaining alternative sources of biomass (Nordfjell, 2007), which could affect the corresponding uses of the substitute raw materials in specific applications (Bekele et al., 2017). In such cases, careful quality control and raw material analyses are required to take these possibilities into account.

Another important factor that must be considered with the utilization of weeds is the risk of introducing colonizing taxa into new locations. This concern has been raised for certain well-known bioenergy/biofuel crops. Examples are grasses – *Miscanthus* (*Miscanthus x giganteus* (Greef & Deuter ex Hodkinson & Renvoize), switchgrass (*Panicum virgatum* L.) and reed canary grass (*Phalaris arundinacea* L.) (Raghu et al., 2006; CAST, 2007). Thus, effective, and efficient utilization of colonizing taxa may require them to be managed appropriately, grown in isolated and controlled areas so that they do not spread to areas where they are undesired.

As an additional factor, there may be difficulties in harvesting stands of specific taxa, simply because of extent and access. Even in the case of smooth cordgrass, harvesting is a constraint as it is mostly done manually from coastal marshy or tidal areas by labourers. Such areas are not easily accessed mechanically harvesters. In my view, these problems

are, for the most part, not insurmountable and can be overcome to promote the utilization of colonizing taxa for profitable, commercially viable industries.

Despite the common problem created by weeds, there are differences in the way they are treated or dealt with according to countries or continents. Some jurisdictions, such as the Dafeng District in Jiangsu Province, China, have had local and provincial government support and successfully taken advantage of their smooth cordgrass infestations and are utilizing this source of biomass for profit. However, this is a clear exception to the norm. Most other areas that also have large-scale infestations have tended to either neglect them, ignore them, or attempt to eradicate them.

A tendency to utilize such sources of biomass depends mostly on the available local resources and whether possible alternatives exist. For a vast country like China, which still lacks many natural resources, there is an incentive to look for such alternatives. This may explain the more positive attitude toward their use compared to other places where there are plentiful resources, overall. In North America, for example, there are sufficient forests to sustainably provide for wood fibres to produce paper pulp and associated products.

The general attitude towards weeds, over many decades, has been a negative mindset. Some countries or societies have long made use of certain weeds and come to terms with them as useful resources. Under such circumstances, they generally are not considered as undesirable species and therefore not considered “weeds” as such (Chandrasena, 2008; Duns and Chen, 2009).

Conclusions and Outlook

Smooth cordgrass biomass is an example of what can be done in terms of the utilization of weeds. They can be turned into useful products that help the environment and be profitable at the same time. The ever-increasing need for environmentally safe, and renewable resources to replace petroleum-based materials for energy and manufacturing has led to the continual search for such materials and will undoubtedly continue in the future.

While there are certain disadvantages to utilizing colonizing plants as bioresources for industrial scale applications, as noted above, their advantages, especially for the environment and socio-economic reasons, outweigh these disadvantages. When considering the vast yearly amounts of biomass produced by smooth cordgrass,

combined with those of just a few of the other prolific pioneering plants, such as water hyacinth and common reed, the opportunity to do something constructive with it, while at the same time, reducing associated environmental problems, is an opportunity that should not be missed. Thus, there should be a common consensus, if not a strategy, towards utilization of colonizing taxa (weeds) on a global basis, if possible, and not just left for local jurisdictions to deal with as they see fit.

With the world's population estimated to reach 9.6 billion people by 2050 (Piotrowski et al., 2015) there will be increasing demands for food, and the diversion of agricultural resources for non-food uses. Thus, there will increasingly be a need for alternative sources of raw materials for energy and manufacturing, and weeds can certainly play a role in this regard, as an environmentally sound option in this supply versus demand issue. While the focus herein has been smooth cordgrass, similar approaches towards utilization should readily apply to other colonizing taxa, whether terrestrial or aquatic. If there is sufficient available biomass that can be readily harvested and reasonable logistics available for storage, shipping and processing, there are many possibilities to exploit these plants.

Suffice to say that not all weeds are suitable for industrial-scale applications. Their inherent physico-chemical properties, as well as fecundity and aggressive growth at specific locations, has caused apprehension as to the potential harmful effects they may have. Such misconceptions continue to inhibit or prevent their use. However, there is no shortage of weeds, and the opportunities to investigate those suitable for utilization seem endless.

For the effective use of a bioresource, whether it is a weed, crop plant, residues from agriculture or forestry, we need to understand the characteristics of the biomass through comprehensive physico-chemical analyses of its properties. (Vaz Jr., 2014; Tursi, 2019). This knowledge of the material is vitally important to develop suitable technology for processing, and then, move to develop commercial applications (Raguskas et al., 2006; Walker, 2015).

The best possible scenario from my perspective is to establish biorefineries for proper integral valorization of biomass from taxa, such as smooth cordgrass. In this way, several products could be made at the same location from local feedstock consisting of the weed's biomass, analogous to the different products resulting from the various fractions produced by a petroleum refinery (MacLachlan and Pye, 2007; Vaz Jr., 2015; Walker, 2015).

The utilization of an aggressive species, such as smooth cordgrass, is of course not the complete solution to the problems caused by it or other similar colonizing plants. There may be plenty of situations where utilization is not possible, or the population of such plants has grown out of control on a scale that solutions to control them may be required. In these instances, the most ecologically safe control solutions need to be applied, and utilization can be a part of an integrated approach in such solutions. An integrated management strategy would be ideal if it consists of a combination of judicious control and utilization methods that are environmentally responsible. If the approach provides some local economic benefit at the same time, it may prove most useful in dealing with species, such as smooth cordgrass (Wang et al., 2008).

I personally find that the quest for new, utilizable sources of biomass represents exciting times and limitless opportunities, bringing together scientists, engineers and entrepreneurs from various disciplines and backgrounds. I am optimistic that in the future, the utilization of weeds will become more common. To achieve this goal, further research, development and, importantly, education, are required regarding the useful properties of weeds.

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Weed biology – A required foundation for effective weed management

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Abstract

Understanding of the biology of weeds (characteristics of seed production, seed dormancy, seedling emergence, plant growth, reproduction, and seed retention, as well as other physiological and morphological traits) is a prerequisite for the development of effective and sustainable weed management systems. Weeds are a persistent problem in agriculture, as they pose a direct threat to farmers' profitability. Farmers currently rely heavily on herbicides for weed control; however, the development of herbicide-resistance and mechanisms of phenotypic, as well as genetic plasticity, in weeds amount to significant challenges in weed management. These are in addition to the underlying issue of environmental pollution as an outcome of excessive herbicide use. The results of weed biology studies are essential to reducing or eliminating the abundance of weeds and the development of herbicide-resistant weeds. Integrated weed management strategies, IWM (e.g. narrow row spacing, competitive cultivars, optimum sowing time and planting density, and harvest weed seed control) for effective weed control can be linked to currently available information on weed biology. The integration of management techniques based on biological knowledge of individual weeds could provide for sustainable weed control and the mitigation of herbicide resistance under both current and projected conditions.

Keywords: crop husbandry, harvest weed-seed tactics, seed ecology, weed phenology, seed bank, weed seed reproduction

Introduction

Weed biology examines the establishment, growth, reproduction, and life cycles of weeds. Weed science uses primarily chemically and physiologically-based research to develop weed management systems. The underlying foundation of effective weed management is the practical research outcomes of the knowledge of multiple aspects of plants' seed germination, recruitment biology, and phenology to ensure sustainable management of individual weeds (Jordan et al., 2002; Westwood et al. 2018).

Knowledge of weed biology is essential for sustainable weed control (Harper, 1960). In-depth understanding of crop-weed interactions is dependent on knowledge of biology, genetics, environmental response, and the responses of both crops and weeds to management practices. Cropping system designs are based on the unique characteristics of weed populations and available weed management options. (Buhler, 2008). Current knowledge of weed biology should be incorporated with the study of ecological interactions, technological innovations, and decision-making algorithms to effectively contribute to the further development of integrated weed management strategies (Zimdahl, 2018).

Weeds are highly prolific seed producers, especially annual species, which can produce an abundant flush of seeds. A single plant of junglerice [*Echinochloa colona* (L.) Link], feather fingergrass (*Chloris virgata* Sw.), and African mustard (*Brassica tournefortii* Gouan) can produce >4,000 seeds per plant (Mahajan et al. 2020a, 2020b). Seed setting in a single mature weed plant presents the potential for the infestation of subsequent years of cropping.

Therefore, it is essential to break the cycle of seed bank replenishment. Generally, most weed seeds possess some level of dormancy; therefore, all seeds produced by a weed in a single year do not germinate the following year, instead, they survive in the soil according to their specific dormancy characteristics (Chauhan and Johnson, 2010; Mahajan et al., 2020b). While it has been observed that nearly all buried seeds of common groundsel (*Senecio vulgaris* L.) germinated or died over the course of two years of burial in soil (Figueroa, 2003), seeds of some other species [creeping buttercup (*Ranunculus repens* L.), common lambsquarters (*Chenopodium album* L.), and dock (*Rumex crispus* L.)] survived after 20 years because of high levels of dormancy in their seeds (Lewis, 1973).

Historically, the availability of information on weed biology greatly informed and improved weed management practices. The problem of perennial weeds, for example, has been reduced by cultivating weeds before the development of carbohydrate reserves in their root systems (Norris, 1992). Many weed management strategies have been developed based on the available biological information for the wild oat (*Avena fatua* L.) species. Wild oat management in Great Britain is now a classic example of the application of weed biology to improve control strategies (Fryer, 1981). This occurred because of the decision to collect more comprehensive information on the population dynamics of wild oat in the early 1970s and the ensuing interdisciplinary research into its biology and ecology. As a result, several aspects of control were modified, such as delaying post-harvest cultivation (to permit predation of seed) and the development of multiple herbicide control programs, rather than reliance on single herbicide applications. Such tactics resulted in a significant decline in the wild oat populations (Fryer, 1981).

In modern industrial agricultural systems, weed management relies heavily on the use of herbicides. The concept of weed biology has been largely neglected in the era of chemical weed control; however, with the development of herbicide-resistant weeds, it has become imperative to generate more information on the fitness penalty and increased seed

dormancy in herbicide-resistant weeds (Navas, 1991). Knowledge of delayed germination, increased seed dormancy, and fitness penalty is especially important for the management of herbicide-resistant populations in weeds (Owen et al., 2015; Kumar and Jha 2017). Furthermore, the development of integrated weed management practices against herbicide-resistant weeds relies heavily on knowledge of weed biology for the generation of information on weed threshold levels, weed seed dynamics, and weed seed retention levels.

Knowledge of weed biology (e.g., phenology, competitive ability, seed production potential) is also required for assessing the impact of different weeds on revenue loss. It has been estimated that annual losses caused by weeds in Australian grain cropping systems are around AU\$ 3.3 billion (Llewellyn et al., 2016). This figure is probably an underestimate, as it does not include the cost of soil erosion resulting from the cultivation required for weed control; nor does it account for the spread of weeds into nature reserves. It could be argued that costs incurred because of pollen allergens produced by weeds should also be accounted for.

While herbicide-based programs have helped in solving many issues of weed control, Weed Science has subsequently suffered from a lack of research into basic weed biology in the era of herbicides. With the increasing problem of herbicide-resistant weeds, researchers once again need an improved understanding of weeds to design effective integrated weed management systems that could delay the further evolution of herbicide-resistant weeds.

Concepts of Weed Biology

1. Identification of weeds and their biological traits

Accurate identification of weeds is essential to the implementation of effective weed management strategies. Without proper identification of the target species and the availability of information on the weed's taxonomy, management is reduced to a 'shot in the dark' approach (Chauhan et al., 2017). One example of improper identification is the case of *Oryza sativa* (weedy/red rice) in India where this weed species cannot be distinguished from volunteer rice plants. Information on biological traits of weeds, such as the morphology of plant canopies; root types, and their architecture; variation in leaf size, shape, and orientation could all help in assessing the competitive ability of weeds.

2. Physiology, biochemistry, and reproductive biology of weeds

Knowledge of the physiology of seed dormancy is essential for mitigating seed persistence in soil (Wesson and Wareing, 1969). The concept of night tillage was introduced based on varied germination behaviour of weed seeds under light and dark environments (Hartmann and Nezedal, 1990). For example, seeds of eclipta (*Eclipta prostrata* L.) did not germinate in the dark but germinated 76-93% in light (Chauhan and Jonson, 2008). Information on the photosynthetic rate of weeds in response to varying light interception levels has helped to form a better understanding of assessing growth and competition at a mechanistic level (Murphy et al., 2017). The mechanism of plant competition can be better understood through the early detection of weeds according to changes in light quality in the red: far-red ratio (Rajcan and Swanton, 2001).

Understanding of the mechanisms whereby weeds respond to moisture and nutrient stress could help in developing precise cultivation strategies to reduce competition. Physiological information on the flowering of weeds could aid in designing better weed management practices such as timely weed control and harvest weed seed control tactics (Norris, 1992; Chauhan et al., 2017). This remains a little-explored aspect of weed physiology that presents great potential for new approaches to managing weeds.

Much work has been done on the effect of weed density and the duration of weed competition on crop yield losses; however, little information has been generated in understanding the mechanism through which crop and weed plants interact with each other and provide signals to each other (Tilman, 1987; Campbell et al., 1991; Grime et al., 1991; Westwood et al., 2018). There is a need to generate information on the mechanism of competition, rather than just quantifying the magnitude of losses. Specifically, what physiological and heritable changes may occur in crop plants in response to competition created by neighbouring weed seedlings and how this knowledge could be useful in assessing crop yield losses.

3. Dynamics of weed seed banks

Knowledge of weed seed banks is critical to the implementation of effective weed management (Chauhan and Johnson, 2010). Much of the existing weed seed longevity data are overestimations due to varying protocols amongst researchers, often excluding predation (Roberts, 1981; Cavers, 1989;

Chauhan and Johnson, 2010). Most weed seed longevity studies have been conducted on bare soil, indicating the possibility of highly variable outcomes under conservation tillage systems.

There is the possibility that crop residues in the field affect weed seed germination by releasing allelochemicals. It has been observed that soil amended with residues of sunflower (*Helianthus annuus* L.) and sorghum [*Sorghum bicolor* (L.) Moench.] resulted in reduced biomass of *E. colona* due to allelochemicals released by either crop (Khaliq et al., 2011). Therefore, there is a need for weed seed longevity studies in conservation agriculture systems that incorporate residue retention in their fields. In-depth knowledge of seed banks could provide an important contribution to weed management.

It is also essential to understand the mechanism of seed decay rather than to limit the outcome of these studies to decayed seed numbers. The regulatory mechanism of decayed seeds could help in designing optimum management strategies. Information is limited regarding the mechanism of spread and invasion of weeds such as mimosa (*Mimosa invisa* Mart.); kochia (*Kochia scoparia* L. Schrad.) gorse (*Ulex europaeus* L.); common milkweed (*Asclepias syriaca* L.) in different geographical regions.

Information on the phenology of weeds under different environments could provide valuable contributions to weed management practices, particularly in the wake of climate change. For example, early flowering in weeds in response to temperature increases may result in the shattering of weed seeds before crop maturity, and thereby replenish weed populations. Information on the development of an economic threshold level for weeds is particularly crucial in optimizing weed control strategies. The magnitude of seed rain produced by weeds is not well known. Information on the seed retention behaviour of weeds could help in optimizing harvest weed seed control strategies (Mahajan et al., 2020b; Walsh et al., 2013). Most farmers in Australia and the USA now attempt to decrease weed seed production in their fields to minimize future problems. They are successfully using harvest weed seed control practices for certain weeds because of greater biological knowledge.

4. Evolutionary changes in weeds

The genetics of herbicide-resistant biotypes and their ability to hybridize is a new principle research area for weed scientists. Knowledge of the biology and ecology of weeds, as well as factors that affect

biological traits, is essential to the development of better-integrated weed management strategies (Chauhan et al., 2017). However, the significance of this research has been under-estimated as its effects are indirect. Weed biology studies do not create new management products (e.g., herbicides). However, they do provide a concept for weed management that can be successfully used in making informed decisions in the selection of control tactics for researchers, growers, and industry. Identification of weeds resulting from a population shift or having adapted traits for different mechanisms of spread could prove valuable in the near future. This knowledge-base provides the opportunity for a screening program for the prediction of future weed problems. For example, a screening program to identify evolutionary forces responsible for target and nontarget site herbicide resistance.

5. Weed biology and effective weed management

Advanced knowledge of weed biology could aid in improved planning and projection for new molecular sites of herbicide action and the development of herbicides that block specific metabolic pathways. It could also aid in determining the best time to apply herbicides due to the accurate determination of a weed's sensitive physiological stages. Knowledge of weed biology could aid in developing effective weed control programs based on the integrated use of crop production methods, tillage practices, and herbicide selection. It may also assist in developing new techniques, such as robotic weed control.

Weed prescription maps can be prepared by utilizing weed phenology information to forecast weed losses. Modelling the hydrothermal, population-dynamics, and crop-weed interaction of various weeds, can be achieved thanks to weed biological information in order to assess the impact of weeds in advance. Crop-weed competition studies help in strengthening an integrated weed management program by suggesting agronomic techniques (sowing time, row spacing, planting density) that could make the crop more competitive. No doubt, an integrated weed management system is a technically sound program, however, for proper implementation of these techniques, the social, environmental, and economic advantages and disadvantages associated with any agronomic practices need to be ascertained. If growers are not convinced by the economic viability of an integrated weed management system, then implementation is inevitably hampered. In this regard, information on weed biology could help in developing viable integrated weed management practices.

Conclusions

Weed will always be present in agricultural fields and elsewhere, so long as disturbances occur. Knowledge of weed biology could provide a practical solution to improve weed management and to lessen dependence upon herbicides to manage the negative impacts of weeds. With more biological information on weeds, farmers and weed managers could effectively control weeds and save money. Weed scientists should seek to integrate their research with ecologists and biologists to augment the significance of results for practical and successful weed management.

Specifically, knowledge of weed dormancy and germination behaviour could be used to predict field emergence patterns. Information on their phenological and reproductive behaviour could improve timely weed management. Growing degree-day models based on the phenological stages of weeds could help managers make decisions for cultural practices and timely herbicide control. Weed seed retention knowledge could aid in the development of harvest weed seed control practices. Without the advancement and implementation of weed biological information, any of these advances in weed management remain limited due to serious gaps in understanding of those species directly impacting cropping and ecological systems.

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Weed control strategies for wheat (*Triticum aestivum* L.) in a cereal-legume cropping system on the Old Brahmaputra Floodplain, Bangladesh

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Abstract

Strip planting is a promising establishment method for wheat (*Triticum aestivum* L.); however, wheat yields can sharply decline if weeds in the fields are not effectively managed. Therefore, to obtain an adequate and economically-viable weed control strategy for strip-planted wheat, we conducted a study, over two years (2013-14 and 2014-15) with commercially available herbicide. Our study was in Mymensingh, in the Eastern Gangetic Plains (EGP) in Bangladesh. In the study, we used pre-emergence (pendimethalin, pretilachlor and triasulfuron), early post- (ethoxysulfuron and pyrazosulfuron-ethyl) and late post-emergence (carfentrazone-ethyl, carfentrazone-ethyl plus isoproturon and 2,4-D amine) herbicides, following a sequential application approach. Sixteen treatment combinations with these herbicides were tested in wheat, and the trials included one 'weedy check' and one 'weed-free check'. The study field was predominantly infested with three grass weeds [*Cynodon dactylon* (L.) Pers., *Digitaria sanguinalis* (L.) Scop. and *Echinochloa colona* (L.) Link], one sedge (*Cyperus rotundus* L.) and five broadleaf weeds [*Polygonum lapathifolium* L., *Physalis heterophylla* (L.) Nees, *Lepidium didymum* (L.), *Chenopodium album* L. and *Vicia sativa* L.]. Another broadleaf weed species - ragweed (*Senecio vulgaris* L.) - was also in the field as a minor weed. *Polygonum lapathifolium* was the most dominant weed species in both years. All herbicide treatments fully controlled this species during both years, except the treatments - pretilachlor followed by (fb) hand weeding at 25 days after sowing fb pretilachlor and pretilachlor fb 2,4-D amine.

The herbicide treatments reduced the total weed biomass of strip-planted wheat by 66-95% in the first year and 71-100% in the second year. With regard to the weed control efficacy, six herbicide treatments: (1) pendimethalin followed by (fb) carfentrazone-ethyl plus isoproturon; (2) pendimethalin fb ethoxysulfuron fb carfentrazone-ethyl; (3) pendimethalin fb pyrazosulfuron-ethyl fb 2,4-D amine; (4) pretilachlor fb pyrazosulfuron-ethyl fb 2,4-D amine; (5) pendimethalin fb carfentrazone-ethyl; and (6) pretilachlor fb ethoxysulfuron fb carfentrazone-ethyl were the best performing combinations. These treatments provided more grain yield than the 'weed-free check' by 2-19% with the economic returns increasing by 30 to 164%. Additionally, bioassay testing of the soil in the treated fields indicated that the succeeding mungbean crop was not adversely affected by the residues of herbicides applied in the previous strip-planted wheat. Overall, the study suggests that the sequential application of pendimethalin followed by carfentrazone-ethyl plus isoproturon, pendimethalin/ pretilachlor followed by ethoxysulfuron with 2,4-D amine or pendimethalin/ pretilachlor followed by pyrazosulfuron-ethyl followed by carfentrazone-ethyl would be the most effective combinations for highly effective weed control in strip-planted wheat in the EGP. Given that the wheat fields are usually rotated with rice (*Oryza sativa*) and mungbeans (*Vigna radiata*), we contend that year-wise rotational application of those herbicide treatments in strip-planted wheat might minimize the risk of herbicide resistant weed development in those crop rotations as well as in the cropping pattern.

Keywords: Herbicides; Productivity; Strip planted wheat; Weed management, Pendimethalin; Pretilachlor; Triasulfuron; Pyrazosulfuron-ethyl; Ethoxysulfuron; Carfentrazone-ethyl; Carfentrazone-ethyl plus isoproturon; 2,4-D amine

Introduction

In the sub-tropics of South Asia, farmers commonly grow wheat in the winter season after harvest of rainy season rice (Sarker et al., 2014). The rice-wheat-mungbean is one of the popular cropping patterns practiced in the Eastern Gangetic Plains (EGP) in the northern and north-western regions of Bangladesh (Bari and Islam, 2009).

This pattern can contribute to a nutritionally-balanced diet for farming families besides providing high economic returns and improving the soil health (Naresh et al., 2013). The adoption of strip planting of wheat (Hossain et al., 2014), mungbean (Bell et al., 2018) and rice (Haque et al., 2016) in a rotation help to conserve soil resources. However, the residue retention from the previous crop may influence weed population dynamics through various factors (Christoffoleti et al., 2007; Chauhan et al., 2012). Heavy weed infestations in strip-planted wheat causes up to 68% yield loss (Zahan et al., 2016), which demands an effective and affordable weed management strategy.

In Bangladesh, the use of pendimethalin as a pre-emergence (PRE) and carfentrazone-ethyl plus isoproturon as a late post-emergence herbicide (LPOST) is common for weed control in wheat (WRC, 2016). Apart from these, no other herbicide is usually applied in wheat. Generally, the continuous use of any herbicide in the same paddock, or even different herbicides belonging from the same group, may accelerate the development of herbicide resistant weeds (Owen and Powels, 2009). In 67 countries, 478 weed biotypes of 252 weed species are now reported as herbicide-resistant (Vrbničanin et al., 2017). Managing of herbicide resistant weeds is quite difficult, but resistance development could be delayed by selecting and applying herbicides rotationally from different groups or with different modes of action (Norsworthy et al., 2012).

Some weed species can escape the spray of pre-emergence (PRE) herbicide in conservation agriculture systems due to the presence of crop residues (Chauhan and Abugho, 2012). It is one of the reasons why sequential application of PRE and post-emergence (POST) herbicides may ensure effective weed control. On the other hand, despite

controlling weeds effectively, persistence of herbicide in soil is a major concern that could adversely affect the subsequently grown crops in a rotation (Hernández-Sevillano et al., 2001).

The primary objective of our study was to investigate how to achieve adequate weed control in wheat with combinations of PRE and POST herbicides, while avoiding undesirable residual effects for a subsequent mungbean crop. At the same time, a second objective was to evaluate the economic returns – whether the herbicide treatments and other inputs and increased weed control would result in increased profits for farmers. In addition, to slow down the development of herbicide resistant weed populations, our aim was to identify a range of efficient and economic herbicides for strip-planted wheat grown in rice-wheat-mungbean cropping pattern in the EGP that can be applied on a rotational basis, year after year, instead of repeated use of the same herbicide(s).

Materials and Methods

The Site and Experimental Design

The study was conducted at the Bangladesh Agricultural University, Mymensingh (24°75' N latitude and 90°50' E longitude), Bangladesh, on a rice-wheat-mungbean cropping system for two consecutively years (2013-14 and 2014-15). The experimental field was well drained medium-high land. The soil was a sandy clay loam in texture; with a pH of 6.8 and low organic matter content (1.74%).

The total amount of rainfall and monthly average of maximum and minimum air temperatures of the experimental site during the studied period are presented in Figure 1.

Cultural Practices

The experimental fields were fertilized with phosphorus (P), potassium (K) and sulphur (S) at 64, 24 and 13 kg ha⁻¹ in the form of triple super phosphate, muriate of potash and gypsum, respectively. These fertilizers were broadcast just before the strip planting of wheat (Figure 2).

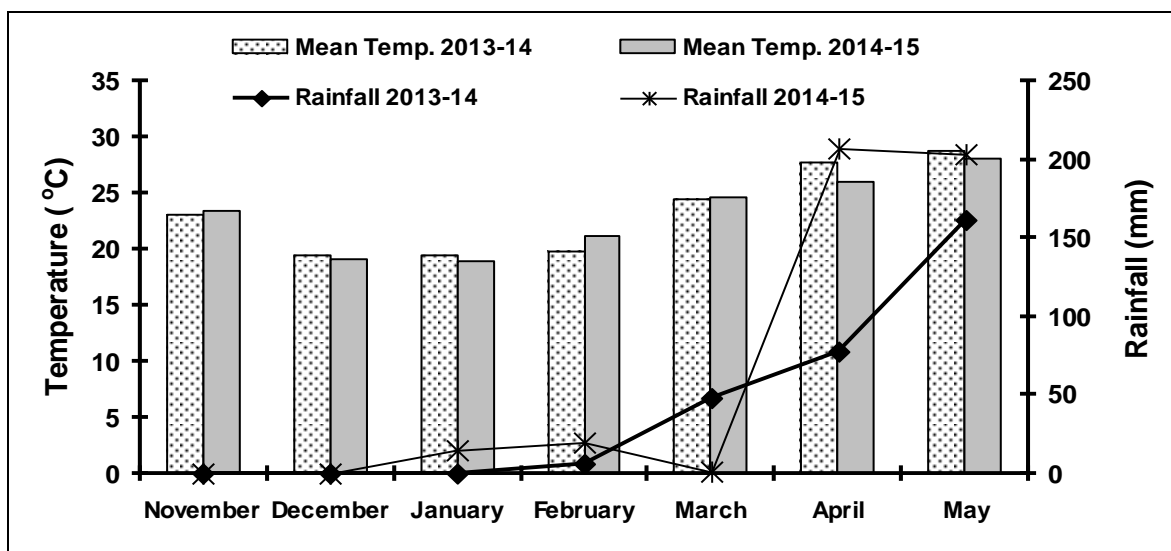


Figure 1. A schematic presentation of prevailing monthly mean air temperature and monthly total rainfall during the life cycle of wheat and mungbean in 2013-14 and 2014-15 at Mymensingh, Bangladesh. (Source: Weather Yard, Department of Irrigation and Water Management, BAU, Mymensingh).

Cow dung was also added and spread over the field at 3.5 t ha^{-1} three days before the strip planting. Nitrogen (N) was applied at 84 kg ha^{-1} as urea in two split applications at 7 and 35 days after sowing (DAS). The experimental fields were lightly irrigated at 20, 50 and 75 DAS. To avoid insect infestations, chlorpyrifos, at 1 L ha^{-1} was applied at 45 DAS and 65 DAS of wheat.

The first crop, strip-planted non-puddled rainy-season rice, was harvested from the fields retaining behind 20 cm crop residues. To prepare the field for wheat, pre-plant applications of glyphosate were applied twice at $1.54 \text{ kg a.i. ha}^{-1}$ to kill the standing weeds before growing wheat.

One week after the second glyphosate application, wheat (cv. BARI Gom-26) seeds were sown at 120 kg ha^{-1} on 22 November 2013, and 20 November 2014, within the strips 20 cm apart by a Versatile Multi-crop Planter (VMP) powered by two-wheel tractor (Haque et al., 2017).

The crop was harvested at maturity on 19 March 2014, and in the following year, on 15 March 2015, retaining 20 cm of standing residue. In each year, after the harvest of wheat, the rotational crop - mungbean cv. BARI mung-6 was planted in the same field plots. Mungbean was sown at 35 kg ha^{-1} by strip planting with the VMP on 01 April 2014 and 30 March 2015. Figure 3 shows a strip-planted field in the trials.



Figure 2. Field trial site – planting and fertilizing of wheat with Versatile Multi-crop planter in Mymensingh, Bangladesh



Figure 3. Field trial site – strip-planted wheat field in Mymensingh, Bangladesh

Weed Flora

The strip-planted wheat fields were infested by ten weed species (Table 1). grass weeds [*Cynodon dactylon* (L.) Pers., *Digitaria sanguinalis* (L.) Scop. and *Echinochloa colona* (L.) Link], one sedge (*Cyperus rotundus* L.) and five broadleaf weeds [*Polygonum lapathifolium* L., *Physalis heterophylla* (L.) Nees, *Lepidium didymum* (L.), *Chenopodium album* L. and *Vicia sativa* L.]. Another broadleaf weed species - ragweed (*Senecio vulgaris* L.) - was also in the field as a minor weed. *Polygonum lapathifolium* was the most dominant weed species in both years (Figure 4).



Figure 4. Field trial site – infestation of *Polygonum lapathifolium* in strip-planted wheat field, Mymensingh, Bangladesh

Herbicide Treatments and Applications

Eight commercially-available herbicides were selected for the study, drawn from different herbicide groups with different modes of action (MOA). Among those herbicides, three were pre-emergence (pendimethalin, pretilachlor and triasulfuron); two were early post- (ethoxysulfuron and pyrazosulfuron-ethyl) and three were late post-emergence (carfentrazone-ethyl, carfentrazone-ethyl plus isoproturon and 2,4-D amine) in action.

These herbicides were evaluated in the two consecutive years in 16 treatment combinations and their performance was tested against one 'weedy check' (unweeded) and one 'weed-free check' (manually weeded at 20, 35, 45 and 55 days after sowing). The experimental design was randomized complete block (RCB) with three replications. In each year, herbicide treatments were differentially randomized and allocated. This ensured that the individual plots (3 m x 4 m) did not receive the same treatment twice during the two-year study period.

The residual effect study of applied wheat herbicides was carried out in the following season on mungbean by using a micro-plot bio-assay technique as described by Hernández-Sevillano et al. (2001).

Pre-emergence (PRE) herbicides were applied three days after sowing wheat (DAS) and early post-emergence (EPOST) and late post-emergence (LPOST) herbicides were applied at 10 DAS and 25 DAS, respectively. Herbicides were applied as treatments only in wheat but not in mungbean; herbicides had also not been previously applied to the rice crops under the rice-wheat-mungbean cropping pattern. Manual weeding was done to control weeds in mungbean, which followed wheat, and, in the previous rice crop, before wheat.

The rates of herbicides (active ingredients, a.i.) applied in wheat in the trials were as follows: pendimethalin (PEND) 1.0 kg ha⁻¹; pretilachlor (PRETI) 0.5 kg ha⁻¹; triasulfuron (TRIA) 0.75 kg ha⁻¹; ethoxysulfuron (ETHOX) 15 g ha⁻¹; pyrazosulfuron-ethyl (PYRAZ) 1.5 g ha⁻¹; carfentrazone-ethyl (CARF) 24.96 g ha⁻¹; carfentrazone-ethyl plus isoproturon (CARF+ISO) 25.51 kg ha⁻¹; and 2,4-D amine (2,4-D) 1.01 kg ha⁻¹.

A hand operated knapsack sprayer (plastic bodied) with a flat-fan nozzle was used to apply the herbicides, delivering a spray volume of 300 L ha⁻¹ with 0.3 MPa spray pressure.

Weed Control Evaluation and Measurements

Data on weed densities and biomass were recorded from three randomly selected quadrats of 0.25 m² (50 x 50 cm) in each plot at 35 and 50 DAS of wheat. Weeds were counted species-wise per m² and then oven dried at 70° C for 72 hours. The weed biomass was expressed as g m⁻².

Data on wheat yield contributing characters were taken from 1 m² of each plot. Yield data was recorded from the central 3.75 m² (1.5x2.5 m) area of each plot and converted into t ha⁻¹ at 12% moisture content. Data on emergence, leaf chlorophyll content, shoot and root length and crop biomass at 25 DAS of mungbean were recorded following the procedure of Zahan et al. (2018).

Data were subjected to one-way analysis of variance (ANOVA) and means were compared by Tukey's Honestly Significant Difference (HSD) using the 'R' statistical package program, Version 3.3.3.

To determine the cost-effectiveness of herbicide treatments economic analysis was done according to Parvez et al. (2013) and the results presented in Table 4. Agronomic indices and sum dominance ratio (SDR) were calculated following the formula of Janiya and Moody (1989) and the weed control index (WCI) according to Devasenpathy et al. (2008).

Results

Effect of herbicides on weed species

The dominant weed species of 2013-14 were in the order of *Digitaria sanguinalis* > *Polygonum lapathifolium* > *Cynodon dactylon* > *Vicia sativa* > *Echinochloa colona* > *Cyperus rotundus* > *Physalis heterophylla* > *Lepidium didymum* > *Senecio vulgaris* > *Chenopodium album*, at 35 DAS.

The results of sum dominance ratio (Table 1) showed that grasses were dominant over other weeds at the early crop growth stages; however, subsequently, broadleaf weeds became the more dominant component. In 2014-15, the most dominant weed species were *P. lapathifolium* > *L. didymum* > *Chenopodium album* at 35 DAS and *L. didymum* > *P. lapathifolium* > *P. heterophylla* at 50 DAS. The most suppressed species was *E. colona* at 35 DAS and *S. vulgaris* at 50 DAS.

During both years, *Polygonum lapathifolium* was the most extensive weed species. Additionally, the study recorded that *C. album* and *L. didymum*, previously, minor weed species in 2013-14, emerged as major species in the weed community in the 'weedy check' plots during 2014-15.

Table 1. Summed dominance ratio (\pm standard error) of weeds at 35 and 50 days after sowing (DAS) of wheat in weedy plots during 2013-14 and 2014-15 under strip planting

Weed species	Family	Life cycle	Summed dominance ratio			
			2013-14		2014-15	
			35 DAS	50 DAS	35 DAS	50 DAS
Grass weeds						
<i>Cynodon dactylon</i>	Poaceae	Perennial	18.0 \pm 0.6	12.4 \pm 0.3	7.3 \pm 0.3	8.0 \pm 0.7
<i>Digitaria sanguinalis</i>	Poaceae	Annual	25.3 \pm 0.1	13.2 \pm 0.6	5.5 \pm 0.9	3.9 \pm 0.6
<i>Echinochloa colona</i>	Poaceae	Annual	10.3 \pm 0.5	12.2 \pm 0.1	0.8 \pm 0.2	3.2 \pm 0.2
Sedge weeds						
<i>Cyperus rotundus</i>	Cyperaceae	Perennial	7.8 \pm 0.6	7.7 \pm 0.5	5.4 \pm 0.2	3.5 \pm 0.0
Broadleaf weeds						
<i>Polygonum lapathifolium</i>	Polygonaceae	Annual	21.6 \pm 0.7	21.0 \pm 1.0	26.8 \pm 1.9	24.9 \pm 1.4
<i>Vicia sativa</i>	Fabaceae	Annual	12.8 \pm 0.2	11.6 \pm 0.6	10.1 \pm 0.8	6.0 \pm 0.4
<i>Physalis heterophylla</i>	Solanaceae	Perennial	3.1 \pm 0.3	19.5 \pm 0.4	4.1 \pm 0.7	12.4 \pm 1.8
<i>Lepidium didymum</i>	Brassicaceae	Annual/ Biennial	0.5 \pm 0.2	2.7 \pm 1.8	25.8 \pm 1.7	29.5 \pm 0.9
<i>Chenopodium album</i>	Amaranthaceae	Annual	0.2 \pm 0.2	0.8 \pm 0.8	12.7 \pm 1.2	7.2 \pm 0.3
<i>Senecio vulgaris</i>	Asteraceae	Annual	0.4 \pm 0.4	1.2 \pm 1.2	1.6 \pm 0.8	1.5 \pm 0.5

Effect of herbicides on weed species

The highest biomass (g m⁻²) of all weed species was recorded from 'weedy' plots in all trials. The herbicide treatments reduced the biomass of all weed species both at 35 and 50 DAS of the strip-planted wheat to varying degree compared with the 'weedy check' (Figure 5 and Figure 6; Table 2).

Grass weeds

The herbicide treatments reduced biomass of *C. dactylon*, *D. sanguinalis* and *E. colona* by 17-81%, 82-100% and 39-100 % in 2013-14 and by 29-100%, 50-100% and 100% in 2014-15, respectively, compared to the weedy check (Figure 5). Both *D. sanguinalis* and *E. colona* were fully controlled by PEND followed by (fb) CARF, PEND fb PYRAZ fb

2,4-D and PEND fb CARF+ISO in 2013-14 and by all the treatments with PEND during 2014-15.

Additionally, during both years, the PRETI fb PYRAZ fb 2,4-D treatment provided 100% control of *D. sanguinalis* and *E. colona*. The grasses were also fully controlled by TRIA fb CARF+ISO treatment. In 2014-15, all treatments with PRETI also achieved the full control of *E. colona*.

No herbicide treatment ensured the complete control of *C. dactylon*, except PEND fb ETHOX fb CARF in 2014-15 (Figure 5). Poor control of *C. dactylon* was also observed in PRETI fb CARF and PRETI fb 2,4-D treatments during 2013-14 and PRETI fb CARF+ISO treatment during 2014-15.

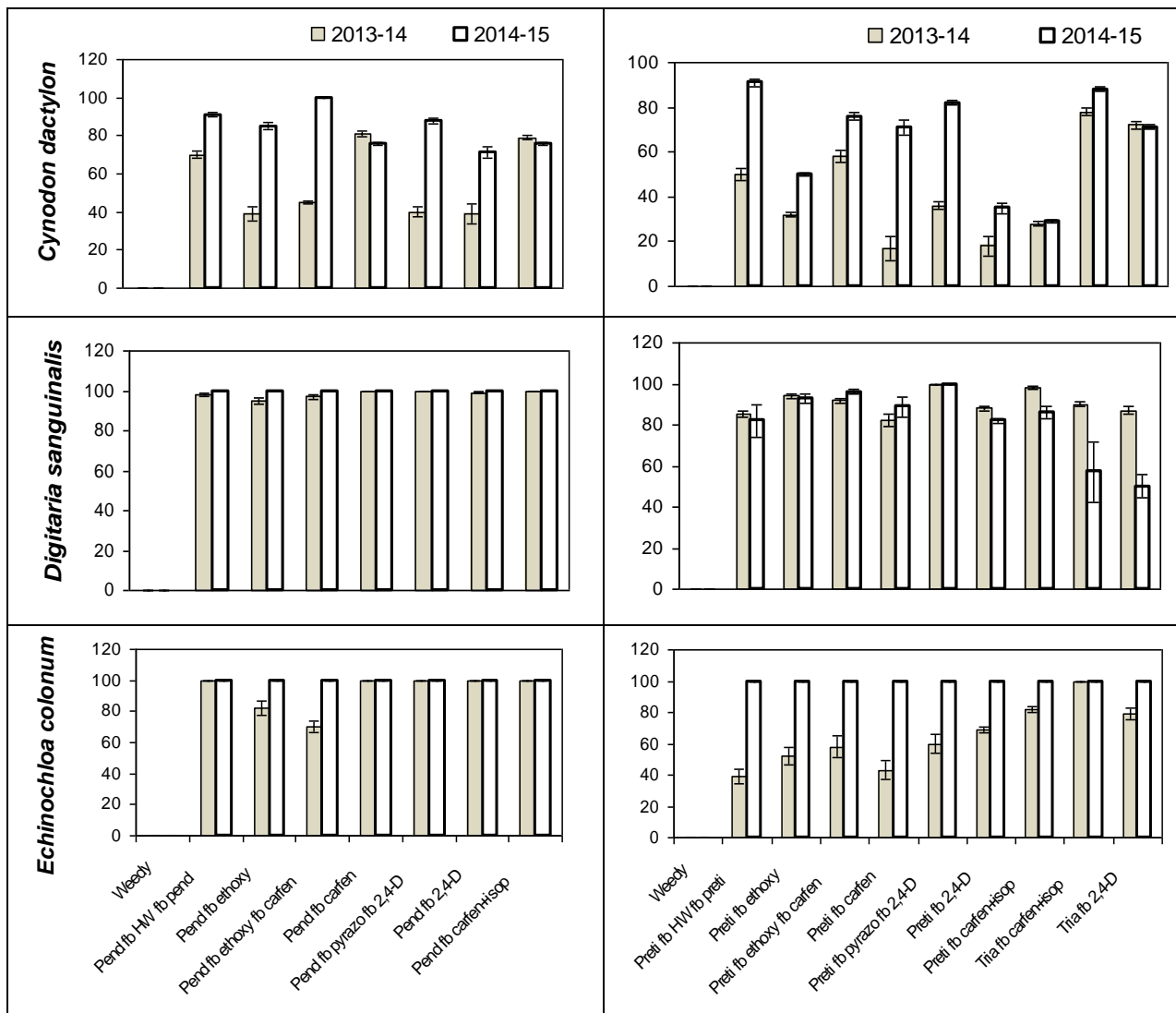


Figure 5. Control (% biomass reduction relative to weedy check) of grasses by herbicide treatments (left - treatments with PEND and other herbicides; right- treatments with PRETI and TRIA and other herbicides) at 35 DAS of wheat during 2013-14 and 2014-15. Vertical bars represent mean±standard errors.

Sedge weeds

The biomass of *C. rotundus* was significantly reduced by some herbicide treatments at 35 DAS compared to the weedy check (Figure 6). Treatments with PEND provided 38-100% biomass suppression of this sedge weed in 2013-14 and 32-100% in 2014-15. Complete control of *C. rotundus* was achieved by

PEND fb PYRAZ fb 2,4-D treatment during both the years. PEND fb ETHOX fb CARF also controlled this weed completely during 2014-15.

Among the treatments, applications of PRETI, PRETI fb ETHOX fb CARF offered the most complete control on this sedge by reducing 95% of its biomass in 2013-14, whereas PRETI fb CARF

gave the lowest control with only 5% biomass reduction. In 2014-15, applications of PRETI fb ETHOX, PRETI fb ETHOX fb CARF, PRETI fb PYRAZ fb 2,4-D or PRETI fb 2,4-D fully controlled the sedge compared with the weedy plots.

Treatments with TRIA reduced the sedge biomass by 38-45% in 2013-14 and 21-29% in 2014-15, which was considered inadequate

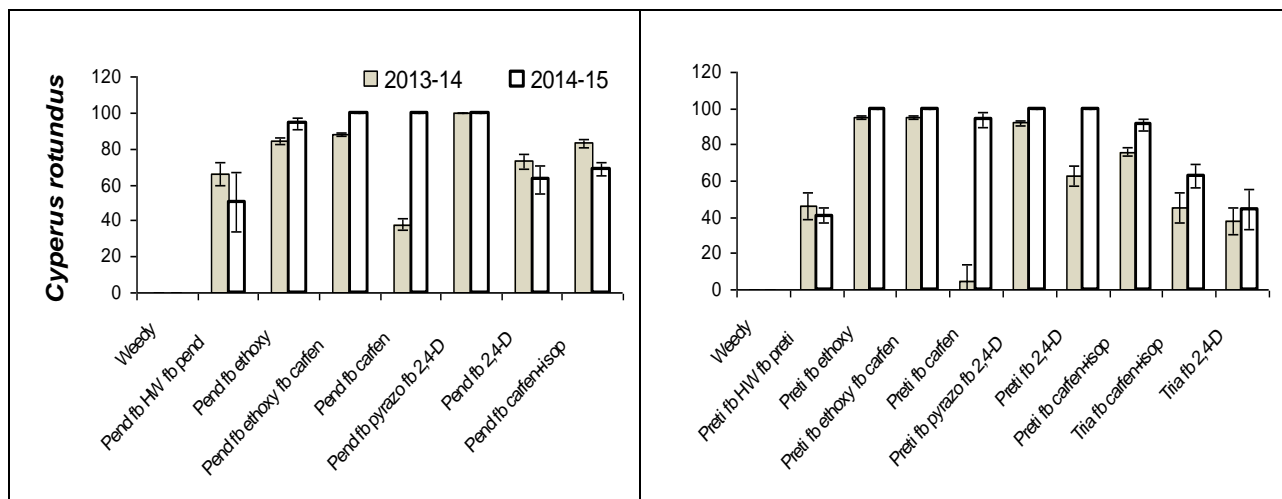


Figure 6. Control (% biomass reduction relative to weedy check) of sedge, *Cyperus rotundus*, by herbicide treatments (left - treatments with PENDING and other herbicides; right- treatments with PRETI and TRIA and other herbicides) at 35 DAS of wheat during 2013-14 and 2014-15; Vertical bars represent mean±standard errors.

Broadleaf weeds

The herbicide treatments reduced biomass of *P. lapathifolium*, *V. sativa*, *P. heterophylla* and some other species (*L. didymum*, *C. album* and *S. vulgaris*) by 100%, 98-100%, 82-100% and 100% at 35 DAS, respectively during 2013-14 (Table 2). In 2014-15, biomass reduction of *P. lapathifolium*, *V. sativa*, *C. didymus*, *C. album*, *P. heterophylla* and *S. vulgaris* by herbicide treatments ranged between 72-100%, 33-100%, 99-100%, 60-100%, 55-100% and 100%, respectively (Table 2).

All herbicide treatments except PRETI fb 2,4-D and PRETI fb CARF+ISO ensured complete control of all broadleaf weed species during 2013-14. Moreover, PRETI fb 2,4-D was unable to fully control *P. heterophylla*. In 2014-15, treatments supplying PENDING provided complete control of all broadleaf weed species, except *V. sativa*.

This broadleaf weed was fully controlled only by PENDING fb ETHOX fb CARF, PENDING fb CARF and PRETI fb ETHOX fb CARF. The study also demonstrated that PRETI fb hand weeding fb PRETI gave the lowest control of *P. lapathifolium*, *V. sativa*, *C. album* and *P. heterophylla* (Table 2).

Effect of herbicides on total weed biomass

The highest total weed biomass at 35 DAS was recorded from the weedy check during both years and herbicide treatments offered a significant reduction ($p < 0.001$) in total weed biomass compared to that of the weedy check (Figure 7).

During 2013-14, herbicide treated plots had 75-95% lower weed biomass than the weedy plots and PENDING fb CARF+ISO treated plots had the lowest amount of total weed biomass at 35 DAS. All herbicide treatments except PRETI fb CARF, PRETI fb 2,4-D and PRETI fb hand weeding fb PRETI ensured a weed control index (WCI) above 80% during 2013-14.

In 2014-15, PENDING fb ETHOX fb CARF was the most effective treatment having 100% WCI (Figure 7). Moreover, almost all herbicide treatments had >90% WCI both at 35 and 50 DAS, except for PRETI fb hand weeding fb PRETI and PRETI fb 2,4-D (WCI <90%).

Table 2. Control (% decrease in weed biomass relative to the weedy check) on broadleaf weed species by herbicide treatments in strip-planted wheat at 35 days after sowing in 2013-14 and 2014-15

Treatment	2013-14				2014-15					
	PL	VS	PH	Others	PL	VS	LD	CA	PH	SV
T ₁ = Weedy check	0 (8.3)	0 (4.7)	0 (1.0)	0 (0.5)	0 (16.9)	0 (5.2)	0 (7.4)	0 (4.7)	0 (1.1)	0 (0.7)
T ₃ = Pendimethalin fb HW fb pendimethalin	100	100	100	100	100	44	100	100	100	100
T ₅ = Pendimethalin fb ethoxysul	100	100	100	100	100	96	100	100	100	100
T ₇ = Pendimethalin fb ethoxysul fb carfentra	100	100	100	100	100	100	100	100	100	100
T ₉ = Pendimethalin fb carfentra	100	100	100	100	100	100	100	100	100	100
T ₁₁ = Pendimethalin fb pyrazosul fb 2,4-D	100	100	100	100	100	98	100	100	100	100
T ₁₃ = Pendimethalin fb 2,4-D	100	100	100	100	100	90	100	100	100	100
T ₁₅ = Pendimethalin fb carfentra + isoprot	100	100	100	100	100	98	100	100	100	100
T ₄ = Pretilachlor fb HW fb pretilachlor	100	100	100	100	72	33	100	60	55	100
T ₆ = Pretilachlor fb ethoxysul	100	100	100	100	100	92	100	100	100	100
T ₈ = Pretilachlor fb ethoxysul fb carfentra	100	100	100	100	100	100	100	100	100	100
T ₁₀ = Pretilachlor fb carfentra	100	100	100	100	100	65	100	100	100	100
T ₁₂ = Pretilachlor fb pyrazosul fb 2,4-D	100	100	100	100	100	98	100	100	100	100
T ₁₄ = Pretilachlor fb 2,4-D	100	98	82	100	95	88	100	83	100	100
T ₁₆ = Pretilachlor fb carfentra + isoprot	100	98	100	100	100	90	100	100	100	100
T ₁₇ = Triasulfuron fb carfentra + isoprot	100	100	100	100	100	98	100	100	100	100
T ₁₈ = Triasulfuron fb 2,4-D	100	100	100	100	99	92	99	100	100	100

Figures within the parenthesis are the weed dry matter (g m^{-2})

PL = *Polygonum lapathifolium*, VS = *Vicia sativa*, LD = *Lepidium didymum*, CA = *Chenopodium album*, PH = *Physalis heterophylla*, SV = *Senecio vulgaris*

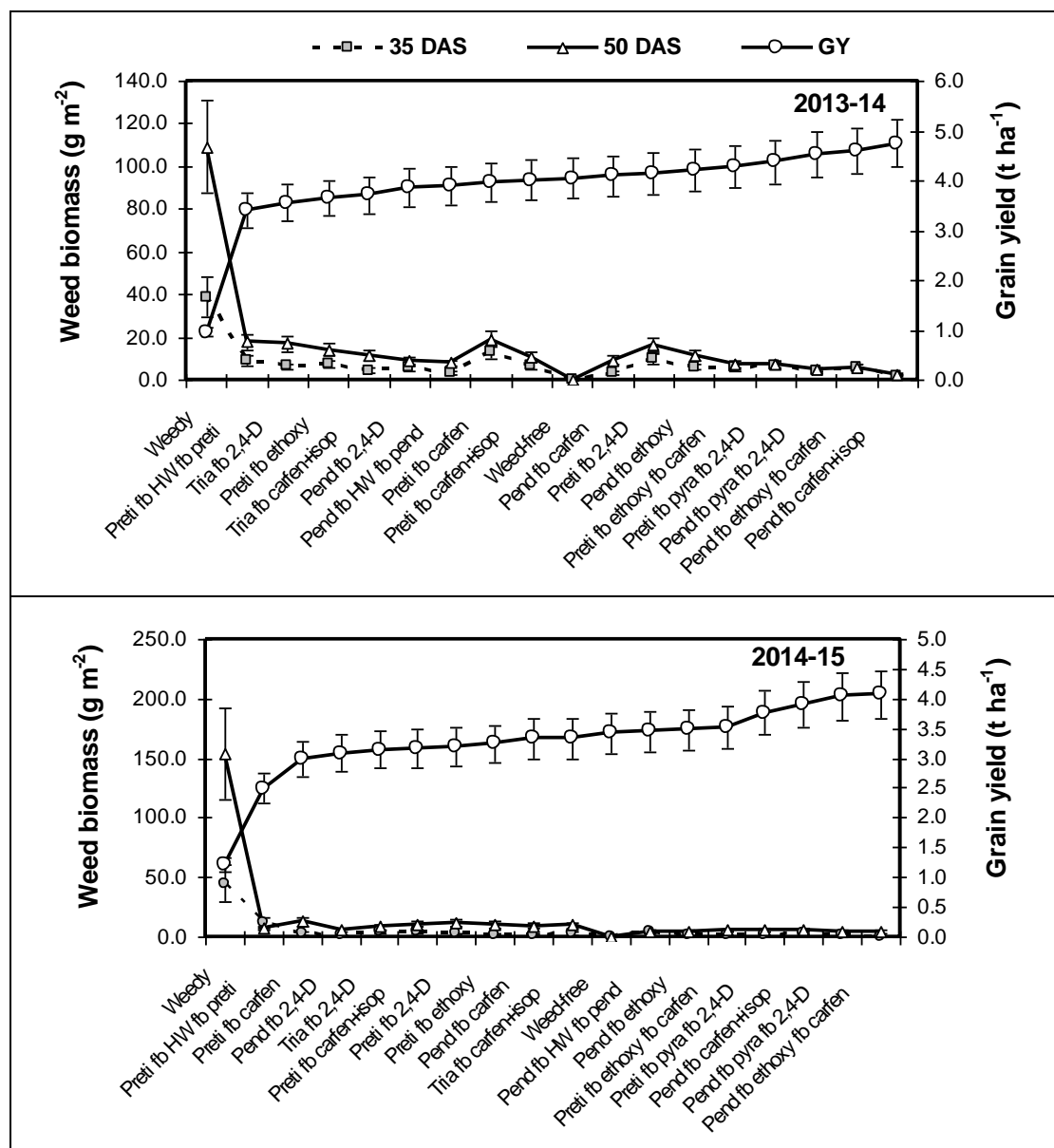


Figure 7. Effect of herbicide treatments on total weed biomass at 35 and 50 days after sowing (DAS) and grain yield (GY) of strip-planted wheat during 2013-14 and 2014-15 [vertical bar represents \pm standard errors]

Impact of herbicides on wheat yield

The grain yield of strip-planted wheat varied significantly with herbicide treatments during both years (Figure 7). The lowest wheat grain yields (0.96 and 1.22 t ha⁻¹) were in the 'weedy' check plots and the highest yield was in PEND fb CARF+ISO and PEND fb ETHOX fb CARF treated plots during 2013-14 and 2014-15, respectively. Treatments that produced higher grain yield over the weed-free control in 2013-14 were PEND fb CARF+ISO > PEND fb ETHOX fb CARF > PEND fb PYRAZ fb 2,4-D > PRETI fb PYRAZ fb 2,4-D > PRETI fb ETHOX fb

CARF > PEND fb ETHOX > PRETI fb 2,4-D and PEND fb CARF.

During 2014-15, compared to the 'weed-free' check, higher grain yield producing treatments were PEND fb ETHOX fb CARF > PEND fb PYRAZ fb 2,4-D > CARF+ISO > PRETI fb PYRAZ fb 2,4-D > PRETI fb ETHOX fb CARF > PRETI fb ETHOX. Among the herbicide treatments, PRETI fb hand weeding fb PRETI gave the lowest grain yield during both years. The strip-planted wheat yields under 'weed-free' conditions were 4.43-4.67 and 3.55-3.56 t ha⁻¹ in 2013-14 and 2014-15, respectively.

As expected, the wheat grain yields were negatively correlated with weed biomass obtained both at 35 and 50 DAS (in 2013-14, $R^2 = 0.81$ and 0.89 and in 2014-15, $R^2 = 0.80$ and 0.72) (Table 3).

From the regression equation, a 1% increase in weed biomass at 35 DAS decreased grain yield by 5.5-9.2% and at 50 DAS by 1.8-3.3%.

Table 3. Relationships of wheat grain yield (kg ha⁻¹) with weed biomass (kg ha⁻¹) at 35 and 50 DAS

y	x	2013-14		2014-15	
		Regression equation	R ²	Regression equation	R ²
Grain yield	Weed biomass at 35 DAS	$y = 4671 - 9.221x$	0.81 ^{***}	$y = 3561 - 5.469x$	0.80 ^{***}
	Weed biomass at 50 DAS	$y = 4431 - 3.272x$	0.89 ^{***}	$y = 3549 - 1.827x$	0.72 ^{***}

Here, *** means 0.1% level of significance

Economic cost evaluation

The 'weedy' check had the lowest gross return during both years with economic loss of US\$258 ha⁻¹ in 2013-14 and US \$171 ha⁻¹ in 2014-15 (Table 4). The highest gross return and net benefit were obtained, not by the weed-free control, but by PEND fb CARF+ISO during 2013-14 and by PEND fb ETHOX fb CARF in 2014-15.

The other treatments which showed higher gross return than the 'weed-free' check were PEND fb ETHOX fb CARF, PEND fb PYRAZ fb 2,4-D, PRETI fb PYRAZ fb 2,4-D, PRETI fb ETHOX fb CARF, PEND fb ETHOX and PEND fb CARF. Additionally, PRETI fb 2,4-D had also higher gross return over 'weed-free' check in 2013-14 and PEND fb hand weeding fb PEND in 2014-15 (Table 4).

Residual effects of herbicides on the succeeding mungbean crop

The results of the residual effects of the herbicide regime used in wheat on the subsequent mungbean crop are presented in Table 5 and Figure 8. The emergence percentages (Figure 8), leaf greenness (SPAD values) (Figure 8), seedlings shoot and root lengths (Table 5) and crop biomass (Table 5) of mungbean at 25 DAS were not significantly different among the treatments during both growing seasons in 2014 and 2015.

However, the emergence percentages were higher in 2014 (85.7-92.0%) than in 2015 (70.7-84.0%), as well as mungbean crop biomasses (1.26-1.59 g plant⁻¹ in 2014 and 7.8-9.3 g plant⁻¹ in 2015). We attribute this to reduced competition from weeds that have already been controlled well in the previous wheat crop.

Discussion

The sole application of pre-emergence (PRE) herbicide failed to achieve effective control of the perennial weeds with extensive stolons, tubers and rhizome systems (mainly, the grasses and the sedge). As discussed by Zahran et al. (2016), sequential application of herbicides effectively controls a diversity of weeds in wheat under minimum tillage. In the present study, the sequential application of PRE herbicides, followed by late post-emergence (LPOST) herbicides with or without an early post-emergence herbicide (EPOST), provided better control of the weeds, which occurred in our trial plots.

Treatments of PEND/TRIA as PRE, with or without one EPOST (either ETHOX or PYRAZ) and with one LPOSTs (CARF/CARF+ISO/2,4-D), were effective in controlling weeds, especially the grass weeds. Previous studies also reported excellent grass weed control in wheat by PEND (Alshallash, 2014) and by TRIA (Islam et al., 2011) under conventional tillage systems. In the present study, TRIA was less effective than PEND in the strip-planting system and the reason might be related to the absence of loose soil particles in strip-planted field. In conventionally-tilled soil, TRIA can easily be mixed with loose soil particles to enhance weed controlling efficiency through better uptake.

PRE application of PEND provides effective control of *E. colona* in zero-till rice (Mishra and Singh, 2012) and wheat (Singh et al., 2016). PEND and PRETI were very much effective against *C. rotundus* if applied with ETHOX or PYRAZ (an EPOST) followed by CARF or 2,4-D (an LPOST).

Table 4. Economic performance (US \$ ha⁻¹) of herbicide treatments in the trials during 2013-14 and 2014-15

Treatment	2013-14			2014-15		
	WM cost	Gross return	Net benefit	WM cost	Gross return	Net benefit
Weedy check	0	287	-258	0	373	-171
Weed-free check	313	1186	329	313	1019	162
Pend fb HW fb pend	154	1133	434	154	1026	328
Pend fb ethoxy	55	1228	628	55	1028	428
Pend fb ethoxy fb carfen	71	1340	725	71	1201	586
Pend fb carfen	53	1194	597	53	992	395
Pend fb pyrazo fb 2,4-D	75	1324	705	75	1190	571
Pend fb 2,4-D	64	1130	522	64	920	312
Pend fb carfen + isop	80	1381	756	80	1153	528
Preti fb HW fb preti	110	1000	346	110	750	95
Preti fb ethoxy	34	1069	491	34	957	379
Preti fb ethoxy fb carfen	49	1247	654	49	1038	444
Preti fb carfen	31	1156	580	31	893	317
Preti fb pyrazo fb 2,4-D	53	1275	678	53	1111	513
Preti fb 2,4-D	42	1215	629	42	945	358
Preti fb carfen+ isop	54	1168	570	54	942	344
Tria fb carfen+ isop	50	1082	488	50	988	394
Tria fb 2,4-D	34	1039	460	34	933	355

WM cost = weed management cost, fb = followed by, HW = hand weeding at 25 days after sowing, Pend = pendimethalin, Preti = pretilachlor, ethoxy = ethoxysulfuron, carfen = carfentrazone-ethyl, pyrazo = pyrazosulfuron-ethyl, 2,4-D = 2,4-D amine, carfen + isop = carfentrazone-ethyl + isoproturon

Market price of commercial herbicides: Pendimethalin = 31.56 US\$ ha⁻¹, Pretilachlor = 9.88 US\$ ha⁻¹, Triasulfuron = 1.56 US\$ ha⁻¹, Ethoxysulfuron = 11.25 US\$ ha⁻¹, Pyrazosulfuron-ethyl = 5.0 US\$ ha⁻¹, Carfentrazone-ethyl = 8.94 US\$ ha⁻¹, Carfentrazone-ethyl + isoproturon = 36.19 US\$ ha⁻¹ and 2,4-D amine = 20.19 US\$ ha⁻¹.

Manual weeding cost: 100 labourers ha⁻¹ for 4 weeding events (season-long weed free) @ 3.13 US\$ labour⁻¹ day⁻¹, Herbicide application cost: 2 labourers ha⁻¹ round⁻¹ @ 3.13 US\$ labour⁻¹ day⁻¹, Market price of grain: 275 US\$ ton⁻¹, Market price of straw: 15 US\$ ton⁻¹, Gross income = {grain yield (t ha⁻¹) × market price (US\$ t ha⁻¹)} + {straw yield (t ha⁻¹) × market price (US\$ t ha⁻¹)}, Net benefit = Gross income – Total cost.

Brecke et al. (2005) also reported that sequential application of PRE-, EPOST- and LPOST herbicides effectively controlled shoots and tubers of *C. rotundus*. PRE herbicides only provide temporary inhibition of tuber sprouting; however, they have no control on new tuber formation at different growth stages. The application of EPOST (ETHOX or PYRAZ) and LPOST (CARF or CARF+ISO) herbicides after the PRE spray was effective against newly sprouted or emerged *C. rotundus* plants.

Shyam and Singh (2015) and Singh et al. (2014) reported ETHOX to be the most effective herbicide on *C. rotundus* in their studies. El-Zanaty (2015) evaluating the weed control efficacy of some PRE and POST herbicides on *C. rotundus* in sandy and

clay soil, found that the efficacy of PRE herbicides significantly varied with soil types, while soil type did not play a significant role in the efficiency of POST herbicides. Their study confirmed that PYRAZ was highly effective against *C. rotundus* in sandy soil, and to a lesser extent, in clay soil. On the other hand, Raj et al. (2013) had earlier reported that CARF could achieve the effective control of *C. rotundus* in any type of soil.

The weed control efficacy of PRETI fb hand weeding at 25 DAS fb PRETI and PRETI fb 2,4-D for *P. laphathifolium* and *Vicia sativa* was not satisfactory in the second year. In our study, PRETI was used and tested with an aim to obtaining a substitute of commonly used wheat herbicide, PEND. PRETI is not a registered PRE herbicide for wheat. On the

other hand, PEND is the only available PRE herbicide widely used in many crops, such as rice, wheat, potato (*Solanum tuberosum* L.), and onion (*Allium cepa* L.), in Bangladesh.

Therefore, the risk of developing PEND resistant weeds in the country is an issue currently raising considerable concern.

Table 5. Residual effect of herbicides applied in strip-planted wheat on shoot and root length and crop biomass at 25 days after sowing of the succeeding mungbean during 2014 and 2015

Treatment	2014			2015		
	Shoot length (cm)	Root length (cm)	Crop biomass (g plant ⁻¹)	Shoot length (cm)	Root length (cm)	Crop biomass (g plant ⁻¹)
T ₁ = Weedy check	22.6±0.3	5.6±0.2	1.26	19.9±1.8	6.89±0.0	0.78
T ₂ = Weed-free check	24.1±1.1	5.7±0.3	1.27	21.1±0.9	5.94±0.3	0.86
T ₃ = Pendimethalin fb HW fb pendimethalin	26.2±1.1	6.0±0.2	1.29	20.4±1.5	6.04±0.2	0.81
T ₅ = Pendimethalin fb ethoxy	25.4±0.6	5.6±0.1	1.44	21.0±0.4	6.86±0.5	0.88
T ₇ = Pendimethalin fb ethoxy fb carfentra	27.0±1.1	5.7±0.6	1.29	22.7±1.2	5.96±0.1	0.90
T ₉ = Pendimethalin fb carfentra	25.6±1.2	5.6±0.2	1.36	24.7±1.4	6.34±0.3	0.84
T ₁₁ = Pendimethalin fb pyrazosul fb 2,4-D	24.3±0.6	5.6±0.1	1.41	23.2±1.4	6.63±0.2	0.89
T ₁₃ = Pendimethalin fb 2,4-D	25.4±0.3	5.8±0.1	1.50	24.6±1.1	5.83±0.1	0.87
T ₁₅ = Pendimethalin fb carfentra + isoprot	24.3±1.2	5.9±0.3	1.50	20.9±1.5	6.08±0.1	0.93
T ₄ = Pretilachlor fb HW fb pretilachlor	25.3±0.7	5.6±0.1	1.42	23.2±0.5	6.15±0.4	0.96
T ₆ = Pretilachlor fb ethoxy	23.7±1.2	6.0±0.7	1.35	23.2±0.8	6.22±0.2	0.81
T ₈ = Pretilachlor fb ethoxy fb carfentra	26.1±1.2	6.0±0.3	1.59	21.0±0.9	6.11±0.1	0.83
T ₁₀ = Pretilachlor fb carfentra	22.7±1.7	5.6±0.1	1.54	24.3±1.4	6.15±0.2	0.81
T ₁₂ = Pretilachlor fb pyrazosul fb 2,4-D	26.8±0.6	5.8±0.1	1.32	22.3±0.3	6.46±0.1	0.90
T ₁₄ = Pretilachlor fb 2,4-D	25.6±1.9	5.9±0.3	1.32	20.7±0.7	6.13±0.3	0.78
T ₁₆ = Pretilachlor fb carfentra + isoprot	23.5±1.5	5.7±0.2	1.36	19.8±0.6	5.79±0.2	0.84
T ₁₇ = Triasulfuron fb carfentra + isoprot	22.8±0.7	5.6±0.2	1.27	22.3±1.1	5.97±0.2	0.82
T ₁₈ = Triasulfuron fb 2,4-D	24.4±1.3	5.6±0.1	1.29	21.7±1.3	6.59±0.5	0.88
Level of significance	ns	ns	ns	ns	ns	ns
CV (%)	7.79	9.01	10.04	8.92	7.32	13.38

For shoot and root length, mean values are presented ± standard errors; fb = followed by, HW = hand weeding at 25 DAS. ethoxy = ethoxysulfuron, carfentra = carfentrazone-ethyl, pyrazosul = pyrazosulfuron-ethyl, 2,4-D = 2,4-D amine, carfentra + isoprot = carfentrazone-ethyl + isoproturon; CV = Co-efficient of variance, ns = non-significant

Our study recorded that two minor weed species (*Chenopodium album* and *Lepidium didymum*) of the first year of strip-planted wheat became major species in the next year. However, all herbicide treatments, except PRETI fb hand weeding at 25 DAS fb PRETI, fully controlled those species. These weeds emerged in several flushes and most of them escaped the hand weeding operations. Similarly, PRETI had no effect on *Chenopodium album* and *Lepidium didymum*.

It is important to note that in our study, treatments - PEND fb CARF+ISO, PEND fb ETHOX fb CARF, PEND fb PYRAZ fb 2,4-D, PRETI fb PYRAZ fb 2,4-D, PRETI fb ETHOX fb CARF and PEND fb ETHOX - produced higher grain yields than the weed-free control plots. One plausible explanation is that in the 'weed-free' control plots, the wheat plants experienced some degree of shock and disturbance, due to the manual hand weeding.

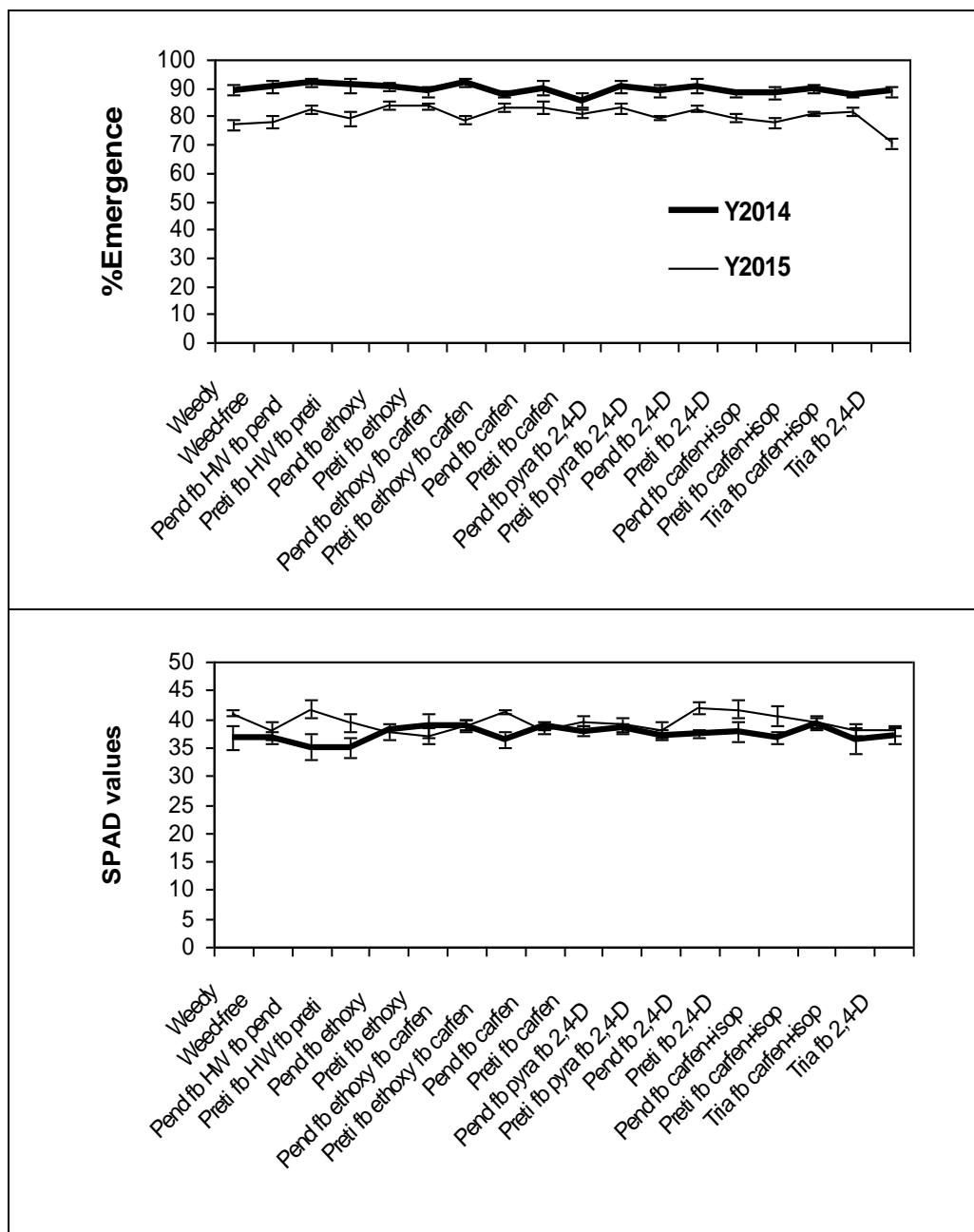


Figure 8. Residual effect of herbicides applied in strip-planted wheat on emergence percentage and leaf chlorophyll content (SPAD value) of the succeeding mungbean during 2014 and 2015

Such an effect may have temporarily caused a set back and slight retardation of the crop growth, while this type of shock was absent in the herbicide-treated plants. Many plant studies have also reported that sequential herbicide application could promote wheat yields (Mukherjee et al., 2011; Khalil et al., 2013; Hamouz et al., 2015) because the control mechanism of an herbicide is target-specific, whereas manual weeding is unable to offer effective control of weeds, which typically emerge as several flushes. Khaliq et al. (2014) also reported significant

improvement in wheat growth and grain yield by various herbicidal weed control treatment in comparison to manual weed control.

Our results also show that the wheat grain yield and weed biomass were negatively correlated. The rate of yield reduction with weed biomass accumulation was much higher at 35 DAS than at 50 DAS indicating that weed control early in the growth of the crop is more important than at later the stages. Our results agree with others who have reported that higher weed pressure in the first 30-50 day period of

the crop growth cycle causes significant wheat yield reductions (Awan et al., 2015; Fahad et al., 2015).

The economic analysis demonstrated that all herbicide treatments resulted in higher net returns over the weed-free control treatments in both years, except PRETI fb hand weeding fb PRETI. The use of herbicides eliminated the high cost of manual weeding, as has been previously reported in West Bengal by Mukherjee et al. (2011).

Importantly, the herbicides applied in the strip-planted wheat did not show any adverse residual effects on the emergence; shoot and root lengths or crop biomass of the succeeding mungbean as a rotational crop. This result indicates that herbicides applied in wheat might have limited persistence in soil, and any remaining residue (not extracted and/or analyzed), may not adversely affect the next crop. Herbicide persistency depends much on soil type and climatic condition (Curran, 2001).

Usually, in Bangladesh, seasonal rainfall starts after harvest of wheat and this could be a reason why herbicide residues from field applications of this scale may not remain in soil. Moreover, phytotoxic effects from any persistent herbicide residues also depend on the exposed crop species and cultivar and time duration of exposure. However, as our study did not extend to examining herbicide residues extractable from soil in the treated plots, there is scope for further research on this aspect, prior to a broader herbicide recommendation applicable for wheat farmers in the Eastern Gangetic Plains.

Conclusions

The sequential application of PEND or PRETI with or without ETHOX or PYRAZ, followed by CARF+ISO/CARF/2,4-D, would be effective in managing a diversity of weeds in strip-planted wheat, which was grown in trial plots with 20% previously-grown rice residues. Our study indicates that the application of any of these sequential herbicide treatments can increase the wheat yield by 2-16% and can provide an increased revenue for farmers by 21-127% compared to the 'weed-free' check.

Therefore, our study suggests applying the above-mentioned, effective PRE, EPOST or LPOST herbicides in a sequence that can be rotated in an intensive rice-wheat-mungbean cropping pattern in the Eastern Gangetic Plains. Such an approach would not only increase profits from growing wheat, but also slow down the development of herbicide resistance in the weeds encountered in the EGP

region. Further research should also be focused on the behaviour and efficacy of the herbicide combinations and regime at higher crop residue levels than used in the present study.

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The authors also wish to state that this work was conducted some time back (2013-2015) but it was not published in any other journal.

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Management of a herbicide-resistant ryegrass (*Lolium rigidum*) population in a crop rotation using alternative herbicides, row spacing, strategic nitrogen application and RR[®] canola (*Brassica napus*)

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Abstract

Rigid ryegrass (*Lolium rigidum* Gaud., henceforth, called ryegrass) is the most significant herbicide-resistant weed in Australian grain cropping. Failure to adequately control ryegrass causes grain yield losses of about 36%. Therefore, new approaches for the control of ryegrass are needed in diverse crop rotations. We studied the options for managing a high-density Acetyl CoA Carboxylase (ACCase)-resistant ryegrass population in a lupin (*Lupinus angustifolius* L.) - wheat (*Triticum aestivum* L.) - canola (*Brassica napus* L.) rotation, under dryland conditions, at Cunderdin (31.650908 ° S, 117.238906 ° E), Western Australia (WA). Field trials were conducted during 2012 to 2014.

In the 2012 lupin, and 2013 wheat crops, conventional herbicides (simazine in lupin, and trifluralin in wheat) and an alternative herbicide (dimethenamid-p in lupin, and pyroxasulfone in wheat) were tested. In 2014, Roundup Ready[®] (RR[®]) canola received two applications of glyphosate to control ryegrass. Three treatments of nitrogen (N) ((N₁) 25 kg N ha⁻¹ as urea; (N₂) 50 kg N ha⁻¹ as urea; and (N₃) 50 kg N ha⁻¹ as urea ammonium nitrate (UAN)) were applied to the 2013 wheat, and the 2014 RR[®] canola. Each crop was grown at two row spacings (22 cm, or 44 cm). None of the management factors except the herbicides significantly decreased the ryegrass density. Indeed, N₃ (UAN) increased the emergence of ryegrass (more in 44 cm than 22 cm rows) compared to N₁ and N₂. Compared to urea N₁, N₃ reduced canola establishment by 28% and generally increased the grain yield of RR[®] canola by 11% but increased the density of ryegrass rather than controlling it. Dimethenamid-p, the alternative herbicide, decreased the ryegrass density in lupin and increased grain yield of lupin by 53%.

While pyroxasulfone, the alternative herbicide, had no significant effect on the ryegrass density compared, to trifluralin in wheat, it increased the wheat grain yield by 25%. However, the 99% reduction in ryegrass by two applications of glyphosate in RR[®] canola was by far the most effective weed control. The inclusion of RR[®] canola technology in the rotation was the most effective approach to control the ACCase-resistant ryegrass, under dryland conditions of Western Australia.

Keywords: Herbicides; crop rotation, *Lolium rigidum*, resistant rigid ryegrass, urea ammonium nitrate (Flexi N), Roundup Ready[®] (RR[®]) canola, trifluralin, simazine, dimethenamid-p, pyroxasulfone

Introduction

In Australia, the overall cost of weed management and grain yield losses due to weeds is estimated to be \$3 billion, equivalent to \$146 ha⁻¹. Rigid ryegrass (*Lolium rigidum* Gaud.; henceforth called ryegrass) accounts for 36% of the overall losses in revenue, and 28% of the losses in grain production in Australia (Llewellyn et al., 2016). Competition from ryegrass can reduce wheat (*Triticum aestivum* L.) grain yield by 42% under dryland cropping conditions (Hashem et al., 1998). Broadly, in North America, the annual losses in crop yields due to competition from weeds are estimated to be US \$28 billion in corn (*Zea mays* L.) (Soltani et al., 2016), and US\$16 billion in soybean (*Glycine max* L.) (Soltani et al., 2017).

Although the use of herbicides has greatly improved crop grain yields in Australia, increased reliance on herbicides for weed control has led to a significant increase in herbicide resistance in various weeds (Owen et al., 2007; Walsh et al., 2007; D'Emden et al., 2008). Ryegrass has evolved widespread resistance to various herbicide modes of action in Western Australia (WA) (Owen et al., 2014) and other parts of Australia (Boutsalis et al., 2012). In WA, 96% of the ryegrass populations were equally resistant to the Acetyl CoA Carboxylase (ACCase)-inhibiting herbicides, such as diclofop-methyl and Acetolactate Synthase (ALS)-inhibiting herbicides, such as sulfometuron, with cross-resistance in these two modes of action in 95% of the ryegrass populations tested (Owen et al., 2014).

However, resistance to other herbicides with different modes of action was significantly lower, with only 27% of the ryegrass populations showing resistance to other herbicides, including glyphosate (Owen et al., 2014). The adoption of integrated weed management (IWM) practices has increased in WA in response to the increase in herbicide resistant weeds (Llewellyn, 2016). Practices, such as increased competition by the crop (i.e., manipulation of row spacing, seed rate, competitive cultivars, etc.) (GRDC, 2014), windrow burning (Pannell et al., 2004), harvest weed seed control (Walsh et al., 2013) and the use of alternative herbicides, have become more common on WA farms.

Compared to wide row spacing, narrow row spacing is likely to facilitate the growth of crop plants with greater competitive ability than weeds (Minkey et al., 2000). Crops sown in wide rows are considered less competitive with weeds and are at

an increased risk of seedling damage from close fertilizer placement. In addition, crops sown in wide rows reduced plant populations compared with those sown in narrower rows, even when fertilizer and seed were placed separately (Scott et al., 2013). However, the advantages of wide rows in Australian wheat, barley (*Hordeum vulgare* L.), canola (*Brassica napus* L.) and lupin (*Lupinus angustifolius* L.) may include improved stubble clearance, reduced fuel consumption, needs fewer ground-engaging components, increased speed of the sowing operation, and improved harvestability, seed size and grain quality but limited improvement of grain yield (Scott et al., 2013).

Some weed species are more efficient than crops in capturing nutrients from added fertilizers (Di Tomaso, 1995; Hashem et al., 2000; Blackshaw et al., 2003). Therefore, the addition of fertilizer can sometimes reduce crop grain yields by increasing weed growth. For example, Italian ryegrass (*Lolium multiflorum* L.) was two times more efficient than wheat plants at producing biomass and specific leaf area per unit of nitrogen (N) absorbed in a mixture of crop and weed (Hashem et al., 2000).

However, the placement and timing of applied fertilizers can increase access to nutrients by crops rather than weeds (Blackshaw et al., 2002; Dhima and Eleftherohorinos, 2001; Jørnsgard et al., 1996). For example, while weeds may have easy access to the N applied on the soil surface at sowing time, strategic N placement may maximise the access of crop plants to N compared to weeds. The widespread use of urea ammonium nitrate (UAN, henceforth, called N₃), applied as a liquid for in-season N application (Nelson, 2019), is a possible tool to direct N to the crop and decrease the access weeds may have to the N fertilizer.

Growers can improve production and monetary benefits from rotation with canola (GRDC, 2000). Despite known resistance to glyphosate in some weed species, glyphosate-resistant (GR) crops represent more than 80% of the 120 million ha of transgenic crops grown annually world-wide. This is attributed to the simple and superior weed control that GR crops deliver (Duke and Powles, 2009). In Australia, the genetically-modified (GM) canola was permitted for commercial production in Queensland (QLD) in 2003, New South Wales (NSW) and Victoria (VIC) in 2008, WA in 2010 (Office of the Gene Technology Regulator, OGTR, 2018). In South Australia, the State government lifted the moratorium on GM-canola in August 2019 (Heard, 2019).

The GM canola currently grown in Australia is resistant to glyphosate and can only be grown with the approval of the Office of the Gene Technology Regulator (OGTR), which carries out a science-based risk assessment before the crop is approved for release. In Australia, about 20% of the national canola crop is genetically modified (OGTR, 2018).

Since 2010, the area sown to glyphosate-resistant, Roundup Ready (RR[®]) canola in WA has grown to 34% of the total canola area, demonstrating an increasing growers' demand for this technology (DPIRD, 2019). Already in the USA, about 93% of the canola crop is genetically-modified (Nestle, 2020) due to added benefits, such as ryegrass-free cropping for up to five years, control of nematodes, and disease break for cereals. In WA, a comparison of RR[®], Clearfield[®] (CL) and Triazine-tolerant[®] (TT) canola by Zhang et al. (2014) found that RR[®] canola produced the highest grain yield at both the low (Cunderdin) and high (Kojonup) rainfall areas.

In a five-year-rotation study, Stanton et al. (2010) found that glyphosate-tolerant (i.e., RR[®]) and TT canola achieved high levels of ryegrass control and attained higher yields than the conventional system. They also found that glyphosate-tolerant canola provided extra control of broadleaf weeds and also achieved better seed oil levels when compared with the other canola systems. Based on the responses of 92 Australian farmers in a survey after 2008 growing season, Neilsen (2009) found that RR[®] technology increased canola yield by 20% and oil contents by 2% over CL and TT canola systems). Neilsen (2009) also noted that the level of weed control achieved using RR[®] canola was also superior to other herbicide-tolerant canola systems.

It, thus, appears that RR[®] canola technology can effectively be used to control herbicide-resistant ryegrass populations. However, diverse weed control methods are needed for ACCase-resistant ryegrass in crop rotations of legume, cereal, and canola.

Therefore, we conducted this study to assess the potential to manage a high density of a highly ACCase-resistant ryegrass population by: (a) application of alternative herbicides, (b) strategic management of N, and (c) the inclusion of RR[®] canola under normal and wide row spacing, in a lupin-wheat-RR[®] canola rotation.

Materials and Methods

Field site

Our rotation trial (lupin-wheat-RR[®] canola) was conducted within the dryland cropping systems of WA, on a sandy loam soil at Cunderdin, WA (31.5847843 S, 117.258432 E) during 2012 to 2014. The trial site had been cropped to wheat in 2011 and had a high density (1000 plants m⁻²) of ACCase-resistant ryegrass in 2012. The resistance status of the site was confirmed in a glasshouse dose response experiment, reported below.

The site received an annual rainfall of 225 mm in 2012, 304 mm in 2013 and 360 mm in 2014 cropping years, while the long-term average annual rainfall at this site was 307 mm (Figure 1). During the study period, the daily mean minimum temperature was 14.4 °C in July and the daily mean maximum growing season temperature was 32.4 °C in October. The mean daily temperature did not vary markedly among years. A frost was recorded in the 4th week of July 2012, the first week of July 2013 and in the last week of June 2014. A mild frost was also noted in the middle of September 2014.

Field Study - Seed Bank Size and Density of Ryegrass

The trial site was 90 m wide in the east-west direction and 100 m long in the north-south direction and was fully fenced out for the duration of the trial, before the lupin crop was sown in 2012. The initial density of ryegrass at the field site was determined from five randomly selected locations within the untreated buffer zone of the trial site, using a 50 cm x 50 cm quadrat. The unit plot size was 20 m x 2 m.

All the unit plots were oriented in the in the north-south direction. Block 1 and 2 (a 'block' is the whole set of treatments of one replication, grouped together into one homogeneous block of land to minimise experimental error, this is also the replicate 1 and 2) were laid out next to each other in the east-west direction with a four (4) m gap in between the blocks. All plots of one replication were laid out within one block without any gap in between plots.

Blocks 3 and 4 were laid out on the north side of the trial area with a gap of 20 m from block 1 and 2. So, there was a buffer zone of 15 m between the south end of block 1 and 2 and the fence on the south side of the trial area and a 20-m buffer

between north end of block 3 and 4, and the fence on the north side of the trial area.

The buffer zone used for plant count in the untreated buffer zone was 5 m x 90 m along the south side of the block 1 and 2 (buffer zone 1) within the fenced area of the field site.

A strip of 2 m x 90 m on the north side of the buffer zone and the south side of block 1 and 2 (buffer zone 2) was sprayed with 1 L ha⁻¹ of Roundup Ultra® Max (glyphosate 570 g L⁻¹) using a Ute-mounted boom sprayer. The buffer zone 1 (5 m x 90 m) was not sprayed with any grass herbicide, which allowed ryegrass to grow in this area.

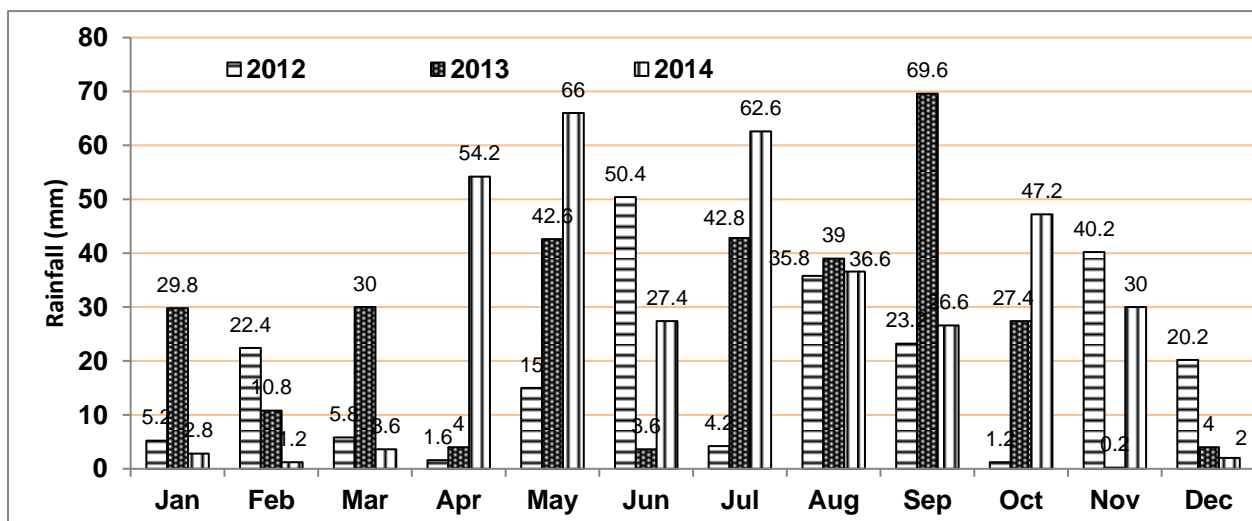


Figure 1. Monthly rainfall during 2012, 2013, and 2014 at Cunderdin Airfield (Station number: 10286), WA)

Ryegrass plants in the buffer zone were allowed to produce seed to mimic any failures of the herbicides in controlling the weeds. In each year, all treated plots were harvested using a 2-m wide plot harvester. The ryegrass (and canola crop in 2014) were harvested in the buffer zone using a 10-m wide header. The plant residues from the buffer were spread evenly within the harvested area and the ryegrass seed in the bin removed from the trial site.

To estimate the soil seed bank of the ryegrass population in 2012, soil samples were collected to a depth of 5 cm from 10 randomly selected locations within the trial area, using a 25 cm x 25 cm quadrat, before sowing of the crop. The existing emergence of ryegrass was recorded from each quadrat just before soil collection. Soil samples were transported to the glasshouse of DPIRD at Northam, WA and spread in a 3-cm thick layer on plastic trays (30 cm x 14 cm x 5 cm) and irrigated daily to keep the soil moist. The emergence of ryegrass was recorded at monthly interval for 15 months (from June 2012 to September 2013). After each counting, emerged seedlings were removed from the trays to prevent seed production. The seed bank size (viable seed number m⁻²) was calculated by adding total field

emergence before soil collection in 2012 to the total emergence of ryegrass in the glasshouse.

In the buffer zone 1, ryegrass plants were counted in a quadrat of 30 cm x 30 cm at five weeks after emergence (WAE) of the crop in 2012 and 2013. However, in 2014, ryegrass density was recorded 3 WAE in the untreated buffer zone of the study site sown to RR® canola. The density of ryegrass in the treated plots were also recorded at the same time as the buffer zone in each year.

Glasshouse Study to Confirm Resistance

To confirm and characterize the resistance in the ryegrass population at the study site (presumed herbicide-resistant, designated as 'R'), a dose response test was conducted under glasshouse conditions at Northam with diclofop-methyl, clethodim and glyphosate. In late June 2012, seedlings of ryegrass were collected from the trial site at 1- to 2-leaf stage. Roots and leaves were trimmed to 4 to 5 cm, and the seedlings then transplanted at 15 seedlings pot⁻¹ to 5-L pots filled with a soil potting mix.

A susceptible population of commercially-available ryegrass (cv. Safeguard) (designated as 'S') was included in the test for comparison. Two weeks after transplanting, when seedlings had developed two to three fully expanded leaves, they were treated with 1/4x, 1/2x, 1x (label rate) and 2x rates of diclofop-methyl (Hoegrass[®], 500 g diclofop methyl L⁻¹), clethodim (Select[®], 240 g clethodim L⁻¹) and glyphosate (Roundup PowerMAX[®], 540 g glyphosate L⁻¹) at 96 L of spray volume ha⁻¹.

The herbicides were applied using a laboratory closed-door belt-moving boom sprayer, equipped with three flat-fan nozzles at 200 kPa pressure moving at nine km hr⁻¹. The survival of the R and S biotypes of ryegrass seedlings was assessed at 24 days after herbicide application.

Field Study - Treatments in the Rotation Trial

Table 1 shows the herbicides, row spacing, and rates and sources of N used in the trials. Lupin (cv. Gunyidi) in 2012 was followed by wheat (cv. Mace) in 2013 and then by RR[®] canola (cv. 43Y23) in 2014. All herbicide treatments were applied in the plots using a Ute-mounted boom sprayer.

Lupin in 2012

The lupin crop was sown at 100 kg of seed ha⁻¹ at two row spacings (22 or 44 cm) with fertilizer applied at 100 kg of Double Phos[®] ha⁻¹ (17.7 P, 3.6 S, 16 Ca kg ha⁻¹) in mid-May.

To control ryegrass, the conventional herbicide simazine (H1) (simazine 500 g L⁻¹) at 1 kg ai ha⁻¹ and an alternative herbicide Outlook[®] (dimethenamid-p 63.9% (H2)) at 720 g ai ha⁻¹ were applied before sowing and were incorporated by the sowing operation. Subsequently, a commercial mixture of diflufenican (50 g ai ha⁻¹) and Metribuzin[®] 750 WG (metribuzin 750 g ai kg⁻¹) was applied at 112 g ai ha⁻¹ at the seven-leaf stage of the lupin crop to control broadleaf weeds, such as wild radish (*Raphanus raphanistrum* L.) and capeweed (*Arctotheca calendula* L.) in each plot.

Photo 1 shows the lupin field, heavily infested with ryegrass and other weeds. The different degrees of weed control achieved by the herbicides are shown in Photo 2 (conventional herbicide - simazine) and Photo 3 (alternative herbicide - dimethenamid-p).



Photo 1. The lupin 2012 experimental site heavily infested with ryegrass, Cunderdin, WA

Table 1 Row spacing, conventional (H1) and alternative herbicides (H2), and nitrogen rates applied as treatments in each crop during 2012, 2013 and 2014 seasons at Cunderdin, Western Australia.

Year	2012	2013	2014
Crops	Lupin (cv. Gunyidi)	Wheat (cv. Mace)	RR [®] Canola (cv 43Y23)
Row spacing (cm)	22, 44	22, 44	22, 44
Herbicides	Simazine (H1) Dimethenamid-p (H2)	Trifluralin (H1) Pyroxasulfone (H2)	Glyphosate
Nitrogen (kg N ha ⁻¹)	Nil	N1 25 kg as urea N2 50 kg as urea N3 50 kg as UAN	N1 25 kg as urea N2 50 kg as urea N3 50 kg as UAN



Photo 2. The lupin plot treated with simazine that controlled ryegrass by 21% in the 2012 lupin crop at Cunderdin, WA



Photo 3. The lupin plot treated with dimethenamid-p that controlled ryegrass by 61% in the 2012 lupin crop at Cunderdin, WA.

Wheat in 2013

Wheat seeds (75 kg ha^{-1}) was sown at two row spacings (22 or 44 cm) with 100 kg of Double Phos[®] fertilizer ha^{-1} (17.7 P, 3.6 S, 16 Ca (%)) applied at sowing time. The conventional herbicide (H1) Triflur Xcel[®] 500 ($500 \text{ g trifluralin L}^{-1}$) at 960 g ai ha^{-1} and an alternative herbicide (H2) Sakura[®] ($850 \text{ g pyroxasulfone kg}^{-1}$) at 118 g ai ha^{-1} were applied to the soil surface four hours before sowing and incorporated by the sowing operation.

Herbicide 1 and 2 in the wheat crop were applied in the same plots as Herbicides 1 and 2 in the 2012 lupin crop plots. The objective here was to compare the cumulative effect of conventional herbicides (H1) against the cumulative effect of alternative herbicide (H2).

Three treatments of nitrogen (N) namely, (N_1) (25 kg N ha^{-1} as urea), (N_2) (50 kg N ha^{-1} as urea) and (N_3) (50 kg N ha^{-1} as urea ammonium nitrate, UAN) were applied to the wheat crop. Urea granules (N_1 and N_2) were drilled over the crop rows just in front of the tines while N_3 was injected 4 to 5 cm below the crop seed at the time of sowing.

A commercial mixture of bromoxynil ($200 \text{ g bromoxynil L}^{-1}$) and MCPA ($200 \text{ g MCPA L}^{-1}$) was applied at 400 g ai ha^{-1} to all plots when wheat was at Z14 stage to control broadleaf weeds.

RR[®] Canola Crop in 2014

RR[®] Canola was sown in 2014 across all the plots of the 2013 wheat crop at 3 kg of seed ha^{-1} with two row spacings (22 cm or 44 cm). A compound fertilizer, Agras[®] (16.1 N, 9.1 P, 14.1 S, 0.5 Ca, 0.06 Zn kg ha^{-1}) at 100 kg ha^{-1} mixed with an extra 40 kg K ha^{-1} and $16.5 \text{ kg S ha}^{-1}$ (as potassium sulphate) was applied across all the plots of RR[®] canola.

The same three treatments of N were re-applied to RR[®] canola in the same plots as the wheat crop in 2013. As the compound fertilizer supplied some N, the amount of N applied in Agras[®] was deducted from each N treatment so the total N applied was the same as listed in the N treatments (Table 1). Roundup Attack[®] ($570 \text{ g glyphosate L}^{-1}$) was applied at 900 g ai ha^{-1} in RR[®] canola at 2- and 5-leaf stages to control ryegrass.

Measurements

In the field trials, densities of lupin, wheat and ryegrass were recorded 5 WAE while in RR[®] canola, the density of ryegrass was recorded at 3 and 12 WAE. The density of ryegrass in the treated field plots was compared with the density of ryegrass in the buffer zone in each crop and expressed as a percentage of the density of the buffer zone 1 in each year. In the 2014 RR[®] canola crop, crop vigour was visually assessed in every plot at five-leaf stage, assuming the vigour as 100% in the buffer zone, where no N was applied.

Crop vigour of all the plots were assessed as per cent of the reference plot (buffer zone 1). Crop establishment of the 2014 RR[®] canola crop was also assessed visually considering the plot 4 with N₂ and 22 cm row spacing as a reference plot, where more than 90% canola plants emerged uniformly, and the crop establishment in all other plots was assessed as per cent relative to the reference plot.

Each crop (wheat, lupin, and RR[®] canola) was harvested by a plot harvester and the weight of clean grains per plot was recorded and then converted to grain yield per ha. The moisture content of grains was determined by moisture meter and grain yield obtained at 12% moisture content.

Design and Analyses

The glasshouse experiments were conducted in a completely randomised design with three replications. To determine the LD₅₀ rate (lethal dose 50, a dose that would kill the 50% of the treated population), plant survival was analysed by probit analysis (GENSTAT 18th Edition) and then the LD₅₀ ratio of the field-collected population (R) relative to the susceptible (S) biotype was determined to explain the degree of resistance.

The experimental design for the field study was a split-split-plot design with four blocks using a unit plot of 20 m by 2 m in each year. Row spacing was assigned to the main plots, herbicides to the sub-plots, and N treatments (in wheat and canola only) to the sub-sub-plots.

The data on lupin, wheat and ryegrass were separately subjected to two- or three-way analysis of variance by GENSTAT 18th Edition (VSN, 2015). The data on canola were analysed using the background herbicides (H1 and H2) applied in the previous lupin and wheat crops and, row spacing, and N rates applied in RR[®] canola. Means were separated by Fischer protected LSD at P = 0.05.

Results

Resistance, Seed Bank Size and Density of Ryegrass

In the glasshouse resistance experiment, 90% plants from the field (R) population survived at 1x (label rate) and 2x rates of diclofop-methyl and 80% survived at 1x and 2x rates of clethodim, while no plants survived at 1x or 2x rates of glyphosate (Figure 2). All the plants of the susceptible (S)

population died at the 1x (label) rate of each of these herbicides.

The LD₅₀ ratio of the R to the S populations was 36 for diclofop-methyl, 19 for clethodim and 1.0 for glyphosate, demonstrating that the R population was 36 times more resistant to diclofop-methyl and 19 times more resistant to clethodim but was highly susceptible to glyphosate. In the untreated buffer zone, the average density of ryegrass was 1000 ± 64.9 plants m⁻² in 2012, 525 ± 44.1 in 2013, and 901 ± 84.7 in the 2014 season. The soil seed bank size of ryegrass, determined before sowing the lupin crop in 2012, was 6518 ± 291 viable seed m⁻² to a soil depth of 5 cm.

Ryegrass Control by Herbicides and RR[®] Canola Technology

Photo 4 shows RR[®] canola plots of blocks 3 and 4 in 2014. The levels of significance of each management factor and their interactions are presented in Table 2.



Photo 4. RR[®] canola plots of blocks 3 and 4 in 2014. On the left is the buffer plot, which was sprayed with Spray.Seed[®] (paraquat 125 g L⁻¹ + diquat 125 g L⁻¹) at 1 L ha⁻¹ to ease in the harvest of RR[®] canola plots at the maturity.

In the 2012 lupin crop, simazine (Herbicide 1) reduced ryegrass density from 1000 plants m⁻² to 794 plants m⁻², a 21% reduction in weed density (Table 3). The herbicide dimethenamid-p (Herbicide 2), applied in lupin, reduced ryegrass density from 1000 plants m⁻² in the buffer zone 1 to 391 plants m⁻² in the treated plots (Table 3), a 61% reduction.

In the 2012 lupin and 2013 wheat crops, there was a significant interaction effect of herbicides and row spacing on the density of ryegrass (Table 2).

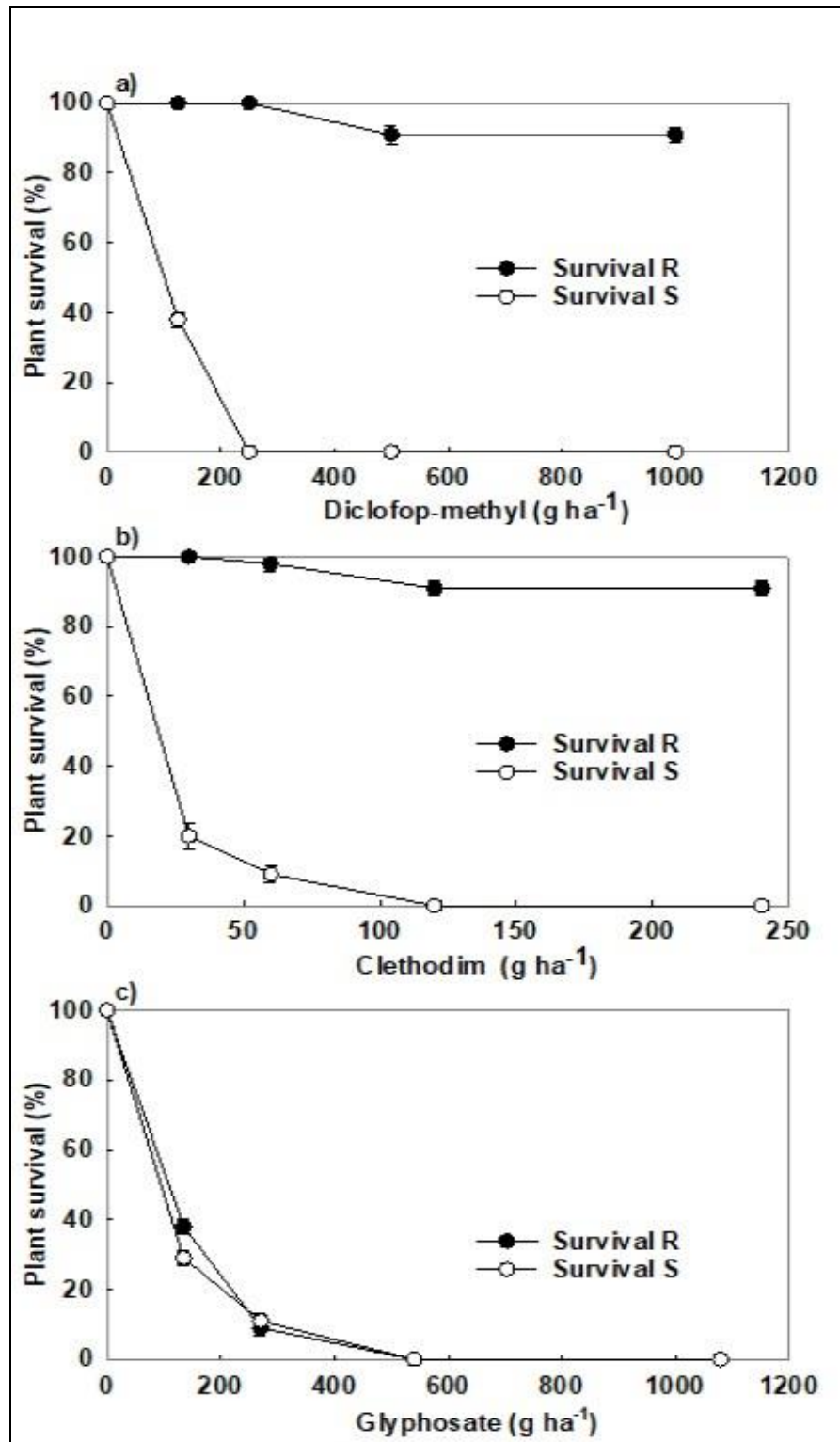


Figure 2 Plant survival (%) of the field-collected (R) population of ryegrass from the experimental site at Cunderdin, WA and the susceptible (S) population (*cv.* Safeguard) when treated with different rates of a) diclofop-methyl, b) clethodim or c) glyphosate at Northam in 2012. Where visible, vertical error bars in the graphs represent the standard errors (SE).

Table 2 Significance levels for the effect of row spacing (RS), herbicide (H) and nitrogen (N) and their interactions in the 2012 lupin, 2013 wheat and 2014 canola crops on ryegrass density, crop density and grain yield in a lupin-wheat-RR[®] canola rotation at Cunderdin, WA¹.

Treatments	Ryegrass density			Crop density			Crop grain yield		
	2012 lupin	2013 wheat	2014 canola	2012 lupin	2013 wheat	2014 canola	2012 lupin	2013 wheat	2014 canola
RS	ns	ns	ns	0.001	<0.001	<0.001	ns	<0.001	ns
H	<0.001	0.01	ns	ns	0.037	ns	<0.01	<0.001	ns
N	-	ns	ns	-	ns	<0.01	-	ns	0.061
RS*H	0.026	0.029	ns	ns	ns	ns	ns	ns	ns
RS*N	-	ns	ns	-	ns	ns	-	ns	ns
H*N	-	ns	ns	-	ns	ns	-	ns	ns
RS*H*N	-	0.05	0.001	-	ns	ns	-	ns	0.016

¹ns = Not significant; "-" indicates N was not applied in the lupin crop.

Table 3 The effect of row spacing and herbicide treatments on the initial density of ryegrass in the 2012 lupin at Cunderdin, WA

Herbicide	Row spacing (cm)	Density of ryegrass (plants m ⁻²)
Simazine	22	753
	44	835
Dimethenamid-p	22	445
	44	338
P-value		0.02

The overall density of ryegrass was halved with Herbicide 2 (dimethenamid-p) relative to Herbicide 1 (simazine) in the lupin crop (Table 3). However, under Herbicide 1 (simazine), the ryegrass density in the lupin crop was higher in 44 cm row spacing than 22 cm row spacing. In contrast, under the herbicide 2 (dimethenamid-p) ryegrass density was lower in 44 cm than 22 cm crop row spacing (Table 3).

In the 2013 wheat crop, there was a three way interaction between row spacing x herbicide x nitrogen. This interaction was due to the increase in ryegrass at the 44 cm row spacing with trifluralin when N₃ was applied (Table 4).

In the 2014 RR[®] canola, the average ryegrass density was 481 plants m⁻² at 3 WAE (i.e. before the first application of glyphosate) and unaffected by row spacing, N fertilizer or prior herbicide treatments. Ryegrass density declined sharply to 7 plants m⁻² (98.5% reduction) at 12 WAE, five weeks after second application of glyphosate in RR[®] canola (Table 4). The initial density of ryegrass in the buffer zone was 901 plants m⁻² at 3 WAE in 2014.

Herbicide, Row Spacing and N Effects on Crop Density and Grain Yield

The herbicides did not affect the emergence (density) of lupin (Table 2, Table 5). On the other hand, wide row spacing decreased the lupin density from 63 to 49 plants m⁻² (a reduction of 22%) but not its grain yield. Lupin grain yields were 53% greater with Herbicide 2 compared with Herbicide 1, an effect attributed to better ryegrass control with Herbicide 2 in the lupin crop.

Photo 4 (22 cm row spacing) and Photo 5 (44 cm row spacing) show the effect of row spacing on the growth of RR[®] canola. The row spacing of 44 cm reduced density of wheat from 136 to 106 plant m⁻² (a reduction of 22%) (Table 5). The emergence of the wheat crop treated with trifluralin was 9% lower than with pyroxasulfone in 2013 (Table 5). At the 44 cm row spacing, wheat grain yield was reduced by 29% compared to the 22 cm row spacing (Table 5). Nitrogen source and rate did not affect the density of wheat (Table 2). The density of canola was reduced from 38 plants m⁻² in 22 cm to 26 plants m⁻² in 44 cm row spacing (a reduction of 28%) (Table 5).

In addition, the N₃ (UAN) treatment reduced density (establishment) of canola by 15% compared to N₁ and 18% compared to N₂ but increased crop vigour of canola by 19% compared to N₁ and 12% compared to N₂ (Figure 3). The canola grain yield increased progressively with increases in N treatments, except at the 22 cm row spacing with the application of pyroxasulfone (alternative herbicide) (Table 6).

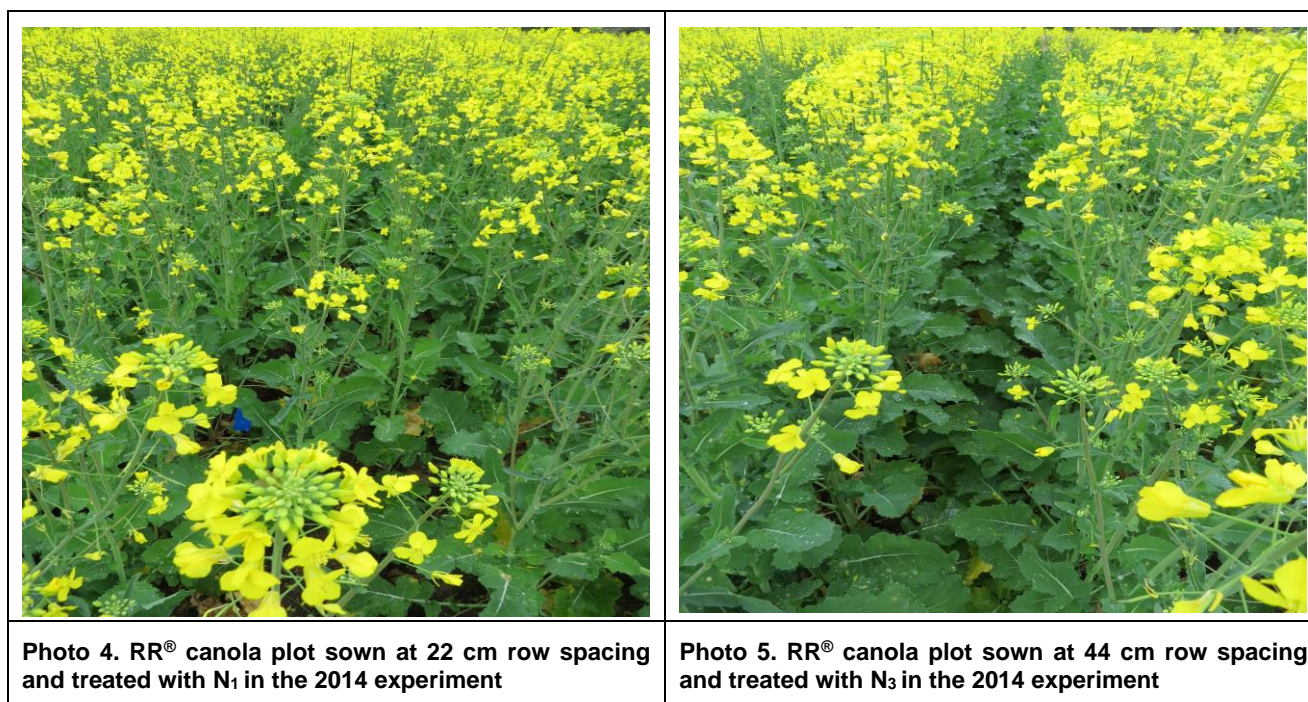


Photo 4. RR[®] canola plot sown at 22 cm row spacing and treated with N₁ in the 2014 experiment

Photo 5. RR[®] canola plot sown at 44 cm row spacing and treated with N₃ in the 2014 experiment

Table 4 The interaction of crop row spacing, herbicide type and applied nitrogen on the density of ryegrass plants in the 2013 wheat and the 2014 RR[®] canola at Cunderdin, WA¹.

Row spacing (cm)	Herbicides in wheat crop	Nitrogen (kg N ha ⁻¹)	Ryegrass in 2013 wheat crop (plants m ⁻²)	Ryegrass in 2014 RR [®] canola crop (plants m ⁻²)	
				3 WAE	12 WAE
22	Trifluralin (H1)	N ₁	54	489	7
		N ₂	51	380	7
		N ₃	59	540	6
	Pyroxasulfone (H2)	N ₁	44	508	6
		N ₂	42	486	6
		N ₃	76	296	6
44	Trifluralin (H1)	N ₁	62	650	8
		N ₂	67	510	9
		N ₃	105	540	10
	Pyroxasulfone (H2)	N ₁	35	448	5
		N ₂	47	432	4
		N ₃	51	488	5
P-value			0.05	ns	0.001
LSD.05			34.8	-	1.08

¹N₁ = 25 kg N ha⁻¹ as Urea, N₂ = 50 kg N ha⁻¹ as Urea; N₃ = 50 kg N ha⁻¹ as urea ammonium nitrate; WAE = week after emergence; RR = Roundup Ready.

Table 5 The effect of herbicides and row spacing on the crop emergence, and grain yield of crops from 2012 to 2014 in a lupin – wheat – RR[®] canola rotation at Cunderdin, WA¹.

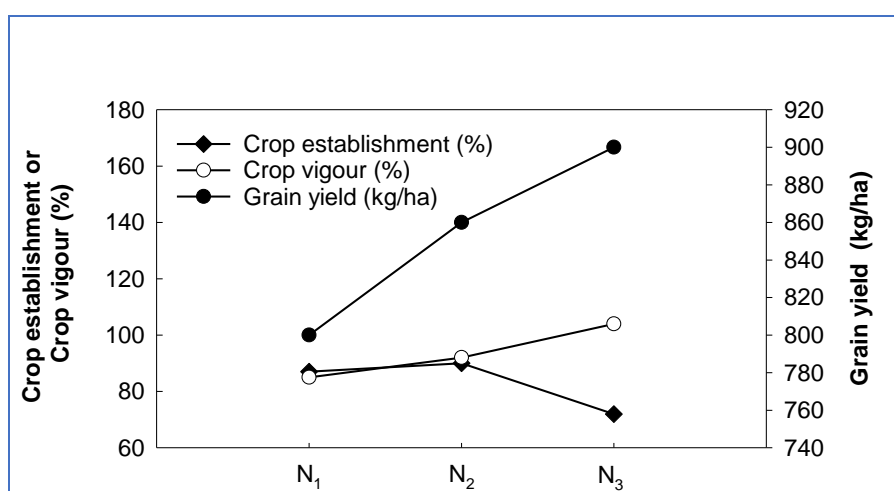
Herbicides/Row spacing	Crop density (plants m ⁻²) 5WAE			Crop grain yield (kg ha ⁻¹)	
	2012 lupin	2013 wheat	2014 canola	2012 lupin	2013 wheat
Herbicide 1	57	115	31	394	1490
Herbicide 2	55	127	33	604	1870
LSD.05	ns	10.4	ns	85.7	192
22 cm	62.7	136	38	536	1970
44 cm	49.1	106	26	402	1390
LSD.05	3.72	18.0	3.5	ns	332.0

¹Herbicide 1 = conventional herbicide: trifluralin for wheat and simazine for lupin; Herbicide 2 = alternative herbicides: pyroxasulfone for wheat and dimethenamid-p for lupin; WAE= weeks after emergence.

Table 6 The interaction of crop row spacing, herbicide type and applied nitrogen on canola grain yields in 2014, at Cunderdin, WA¹.

Row spacing (cm)	Herbicides in wheat crop	Nitrogen (kg N ha ⁻¹)	Grain yield (kg ha ⁻¹)
22	Trifluralin (H1)	N1	695
		N2	803
		N3	840
	Pyroxasulfone (H2)	N1	827
		N2	767
		N3	785
44	Trifluralin (H1)	N1	618
		N2	686
		N3	726
	Pyroxasulfone (H2)	N1	640
		N2	767
		N3	785
	P-value		0.016
	LSD.05		112.4

¹N₁ = 25 kg N ha⁻¹ as Urea, N₂ = 50 kg N ha⁻¹ as Urea; N₃ = 50 kg N ha⁻¹ as urea ammonium nitrate (Flexi N).

**Figure 3** The effect of N on crop establishment (%), crop vigour (%) and grain yield of RR[®] canola in the 2014 season at Cunderdin, WA. N₁ = 25 kg N ha⁻¹ (Urea), N₂ = 50 kg N ha⁻¹ (Urea); N₃ = 50 kg N ha⁻¹ (UAN). LSD (p=0.05) for crop establishment = 4.66%, crop vigour = 4.01%, and grain yield = 81.1 kg ha⁻¹.

Discussion

Relative Effectiveness of the Management Approaches on Ryegrass

RR[®] canola technology was effective in controlling the ACCase-resistant ryegrass population. At the end of this three-year crop rotation, the reduction in ryegrass was 99% compared to 1000 plants m⁻² in the untreated buffer zone 1 in 2012. Most of the decrease can be attributed to the two applications of glyphosate in the RR[®] canola in 2014 (Photo 5).



Photo 5. A close-up photo showing dead ryegrass plants after second application of glyphosate in the RR[®] canola in the 2014 experiment Cunderdin, WA

By comparison, the other management approaches used had only modest or minor effects on ryegrass control. The rotation of herbicides, together with the rotation of crop species, reduced the ACCase-resistant ryegrass from 1000 plants m⁻² to 586 (range 338 to 835) plants m⁻² in the lupin crop in 2012, from 525 plants m⁻² to 61 (range 44 to 79) plants m⁻² in the 2013 wheat crop, and from 910 plants m⁻² to only 7 (range 4 to 10) plants m⁻² in the RR[®] canola crop in 2014.

Our results agree with published literature. For example, Zhang et al. (2014) compared RR[®], Clearfield[®] (CL) and Triazine-Tolerant[®] (TT) canola and found that RR[®] canola produced the highest grain yield at both the low (Cunderdin) and high (Kojonup) rainfall areas of WA. In a five-year-rotation study, Stanton et al. (2010) found that glyphosate-tolerant (i.e. RR[®]) and TT canola achieved high levels of ryegrass control and attained higher yields than the conventional canola system.

Comparing the efficacy of weed control and yield advantages of herbicide tolerant crops with a standard herbicide treatment (sethoxydim plus ethametsulfuron) in a multi-site-year study in Canada, Harker et al. (2000) found that weed control in HT canola was highest with glyphosate, followed by imazethapyr/imazamox, and then glufosinate. In their study, the yield increases of glyphosate treatments over the standard treatment ranged from 13 to 39% but at some sites only. There is a general perception among some members of the public that the use of RR[®] canola could pose a risks to human health (when GM canola is consumed) and to the broader environment.

Row Spacing Effects on Crops and Ryegrass

Despite the effects of grain yields, there was little effect of narrow row spacing of lupin, wheat, or canola on the ryegrass density. In general, Fischer and Miles (1973) and Acciaresi and Chidichimo (2007) had earlier reported that seeding rate being constant, a reduction in the crop row spacing would increase the distance between plants within the row and is likely to result in an increased plant growth and grain yield due to lower intra-specific competition among the crop plants. An increase in grain yield at 22 cm row spacing was only found in wheat (42% higher in 22 cm than at 44 cm row spacing) in the present study. In wheat, the wider row spacing of 44 cm reduced the density of wheat by 22% which likely explained the decrease in grain yield.

In contrast, the decreased plant populations of lupin and canola did not affect the grain yields. Amjad and Anderson (2006) found a decline in wheat plant density with increased row spacing, even though the seed rate was constant. Unlike cereals, increased row spacings of canola do not usually result in grain yield reductions because canola plants are sufficiently plastic in producing similar biomass and grain yield in wide and narrow rows. This plasticity suggests that wide row spacing is an option for sowing canola (Harries et al., 2015).

Further, Patil and De (1978) reported that plants of *Brassica campestris* L. sown in wide rows utilized less water during the vegetative and flowering stages than the plants sown in close row spacing. In contrast, Kirkland (1993) and Weiner et al. (2001) have shown that very narrow row spacing (10 cm) or planting in a uniform grid can maximize the grain yield of cereal crops at higher seeding rates. Compared to wide row spacing, narrow row spacing is likely to facilitate crop plant with greater competitive ability than weeds (Minkey et al., 2000).

Herbicide Effects on Resistant Ryegrass in the Lupin and Wheat Crops

The present results suggest that there is scope for improved ryegrass control and crop grain yield by switching to alternative herbicides in lupin. Simazine was less effective than dimethenamid-p for ryegrass control in lupin. This may be due to the low rainfall (51% of long-term average) in May 2012, which resulted in the lupin crop being sown under dry conditions. Rainfall occurred about 16 days after sowing, and perhaps, simazine did not reach the root zone of ryegrass seedlings.

Previous studies had indicated that about 12.5 mm of rainfall in the USA (Peters, 2014) or 25 to 30 mm of rainfall in Australia (Nufarm, 2019) were needed after application on dry soil to disperse soil-applied herbicides, such as simazine into the soil, so that the herbicides can be absorbed by roots of weed seedlings. Gunasekara (2004) reported that the persistence of simazine is expected to be longer under dry conditions than wet conditions. Despite a half-life of 3-36 days of dimethenamid-p (APVMA, 2007), weed control in lupin by simazine was much lower than dimethenamid-p. The reason for lower efficacy of simazine in this study is unclear and needs further investigation.

Both herbicides applied in the wheat crop provided similar efficacy in controlling ryegrass in 2013. Although the vapour pressure of pyroxasulfone is lower than trifluralin, the similar efficacy of trifluralin was probably associated with the longer half-life of trifluralin than pyroxasulfone (Preston, 2017). However, the effects of herbicides applied in 2012 or 2013 were confined to those crops and had no significant influence on the density of ryegrass at 12 WAE in the canola crop of 2014.

The higher initial density of ryegrass with N₃ at 44 cm than N₁ or N₂ in the 2013 wheat crop, in the presence of soil-applied herbicide trifluralin (Table 4), suggests a possible stimulation of ryegrass emergence by the N₃ treatment.

These results demonstrate that greater herbicide incorporation by soils, thrown by the tines of the sowing machine, from the crop rows to the inter-row spaces, and increased competition from crop plants in narrow row spacing than wide row spacing, might have contributed to the greater reduction of ryegrass in this study at 22 cm than at 44 cm row spaces.

Although the effect of N fertilizers on the emergence of ryegrass was somewhat unclear in our study, Agenbag and De Villiers (1989) found that ammonium-containing fertilizers including UAN (N₃)

were quite effective in stimulating germination and emergence of wild oat (*Avena fatua*) in sandy and loamy soil. However, the reason for a reduction of ryegrass density in the wider row spacing under pyroxasulfone is unclear from our study and needs further investigation.

In a related study, Yamaji et al (2016) reported that the low water solubility and the low vapour pressure of pyroxasulfone, applied to a sandy loam soil in a field that was free of clods, led to limited horizontal diffusion of this molecule on the soil surface, and also posed a low risk of volatilization.

As such, light incorporation in the wider row spacing might have maintained the availability of more pyroxasulfone molecules in the wheat crop to be accessed by ryegrass roots in the present study. Pyroxasulfone has the potential to provide weed control for an extended duration with low risk of runoff or volatilization (Yamaji et al. 2016).

In our study, no residual effects of the herbicides applied in previous wheat and lupin crops and their row spacing were evident on the initial density of ryegrass in the 2014 RR[®] canola crop. However, the grain yield of RR[®] canola in 2014 was influenced by the interaction of row spacing and herbicides (applied in 2013 wheat crop) and N.

Nitrogen Effects on Crops and Ryegrass

The hypothesis that placement of N fertilizer in the seeding row would favour crop N uptake relative to weeds was not supported by the results of our study. Indeed, the highest N rate, supplied as UAN (N₃), had higher initial weed density than N₁ or N₂ in the 2013 wheat crop, indicating a possible stimulation of ryegrass emergence by N₃.

The application of N₃ did not influence the density of the crop or ryegrass nor grain yield of the wheat crop. In contrast to our finding, Nelson (2019) reported greater grain yield and protein content in a wheat crop from applications of N₃.

The lack of response of grain yield to N₃ in the present study may be related to the soil N supply. Alternatively, the effective depth of N₃ placement in a wheat crop might need further investigation. However, in terms of the aims of our study, there was no support for the notion that N fertilizer placement close to the row of wheat could increase its competitiveness with ryegrass.

In our study, application of N₃ increased crop vigour and grain yield of RR[®] canola even though the ryegrass density at 3 WAE was not influenced by N treatments. Hence, with the placement of N fertilizer

close to the RR[®] canola seeding row had no positive effect in suppressing weed competitiveness relative to the crop plants.

Herbicide Resistance and GM crops – Opportunities and Constraints

ACCase resistance in ryegrass was verified at the Cunderdin site, which is not a new occurrence within WA. Owen et al. (2014) reported that 96% of the ryegrass populations tested from WA were resistant to ACCase-inhibiting herbicide, diclofop-methyl, and the ALS-inhibiting herbicide, sulfometuron. Cross-resistance to these two modes of action (MOA) herbicides is also evident in 95% of the ryegrass populations.

Ryegrass has also evolved resistance to ACCase in other regions of southern Australia. Boutsalis et al. (2012) reported up to 60% of the southeast Australian ryegrass populations had resistance to the ACCase herbicides such as diclofop-methyl, tralkoxydim, and pinoxaden.

Owen et al. (2014) also noted that resistance to other herbicide modes of action (MOAs) was significantly lower than for ACCase-inhibiting herbicides, with only 27% of the populations containing plants with resistance to other herbicides including glyphosate. The Cunderdin population of ryegrass was quite susceptible to glyphosate. Hence, this study at Cunderdin could be considered representative of the weed control challenges with herbicide-resistant ryegrass across the grain growing regions of WA, and possibly, elsewhere in Australia.

Despite the existence of resistance to glyphosate in some weed species, GR crops represent more than 80% of the 120 million ha of transgenic crops grown annually world-wide. The economic advantages of the technology, as well as the simple and superior weed control by glyphosate, are the reasons for its wide-scale adoption (Duke and Powles, 2009).

In the 1990s, researchers developed the canola crop with resistance to herbicides (CropLife Canada, 2020). This technology enables a farmer to use a herbicide without damaging the crop to control weeds that otherwise might compete with the canola for water and nutrients. This means that farmers can practice no-till or conservation tillage, which may reduce soil erosion, improve soil quality, reduce greenhouse gas emissions, and cut water use (CropLife Canada, 2020).

On the positive side, RR[®] canola (with GM herbicide resistance traits) has allowed producers to

achieve superior weed control with the use of less total applied herbicide. Without the RR[®] canola technology, there will be ongoing selection pressure for weeds to develop resistance to the few other herbicide options available for use within canola crops. Despite the afore-mentioned, well-publicized advantages, the use of RR[®] technology for improved weed control and crop yields, needs to be considered in a broader context.

In our view, RR[®] canola (GM herbicide resistance traits) has allowed producers to achieve superior weed control with the use of less total applied herbicide. Without the RR[®] canola technology, there will be ongoing selection pressure for weeds to develop resistance to the few other herbicide options available for use in canola crops.

However, there are some genuine concerns among the communities about the RR technology that uses the glyphosate molecule. Hursh (2011) has rightly pointed out that glyphosate is such a widely used herbicide that the RR[®] canola varieties may trigger other weed control issues, particularly if canola volunteer plants are not controlled.

In a survey of soybean fields containing waterhemp (*Amaranthus rudis* J. D. Sauer) infestations across Missouri of the US, Rosenbaum and Bradley (2013) confirmed glyphosate resistance in 69% of the 144 populations of waterhemp. They noted that populations of glyphosate-resistant waterhemp were more likely to occur in fields where no other weed species were present at the end of the season. These were also the fields where continuous cropping of soybean was practised, which exclusively received glyphosate for several consecutive seasons, compared to fields with glyphosate-susceptible waterhemp. Evans et al. (2016) also reported that occurrence of glyphosate-resistant weed biotypes of *Amaranthus tuberculatus* (Moq.) J. D. Sauer was greatest in fields, which received the most frequent glyphosate applications at high annual rates with only a few herbicides from other MOAs on a yearly basis.

They also noted that where other herbicide MOAs were mixed with glyphosate at the time of application, the likelihood of GR *A. tuberculatus* was reduced. Based on the meta-analysis, Chow (2019) reported that people who are highly exposed to glyphosate have up to 41% increased risks of developing non-Hodgkin Lymphoma (NHL) while the Environmental Protection Agency of the USA (EPA) declared that glyphosate is not likely to be carcinogenic to humans.

In Australia, based on the risk assessment by the OGTR (2012), it was concluded that the risks posed by the commercial release of RR[®] canola to human health, safety and the environment are no greater than those posed by conventional (non-GM) canola. Broadly, we agree that the continuous use of RR[®] canola may also increase the number of documented cases of glyphosate-resistance in annual ryegrass and other weeds in Australia. Glyphosate-resistant biotypes of ryegrass will survive in RR[®] canola unless other interventions, such as (a) alternative knockdown herbicides are used prior to sowing, cultivation at or prior to sowing and/or (b) in-crop herbicides from other mode of action (MOA) are used. Such practices are part of the best management package recommendations for minimising the risk of increased selection for the glyphosate-resistant biotypes (Preston, 2017).

RR[®] canola growers in Australia are encouraged to undertake a paddock risk assessment and develop a resistance management plan before growing RR[®] canola (Pritchard, 2014).

Glyphosate should not be used in the year following RR[®] canola (Pritchard, 2014). Fortunately, ryegrass plants with glyphosate resistance have a 'fitness penalty' (i.e. crops can compete better with glyphosate-resistant ryegrass than with glyphosate-susceptible ryegrass). This means some IWM tactics, such as growing a competitive crop, are likely to work better with glyphosate-resistant ryegrass than glyphosate-susceptible ryegrass (Pritchard, 2014). However, if RR[®] canola is grown frequently, not only will it increase the risks of diseases, but also the risks of evolving more glyphosate-resistant weed biotypes (GRDC, 2018).

Canada is now the biggest single producer of canola. More than 20 million metric tonnes of canola were produced in 2018, about half of it in Saskatchewan (CropLife Canada, 2020). Beckie et al. (2006) examined some agronomic, economic, and environmental impacts of herbicide-resistant (HR) canola, soybean, corn, and wheat in Canada after 10 years of growing herbicide-resistant (i.e., glyphosate-resistant, GR or Genetically-modified, GM) cultivars. They found that the rapid adoption of herbicide-resistant canola and soybean brought a net economic benefit to farmers.

Herbicide-resistant (HR) crops often have improved weed management, produced greater yields or economic returns, and have similar or reduced environmental impacts, compared with their non-HR crop counterparts.

In Canada, there has been no measured changes in volunteer weed problems associated with HR crops. However, in zero-tillage systems when glyphosate is used alone to control canola volunteers, there have been issues with weed biotypes with evolved resistance.

Weed shifts, as a consequence of HR canola, have also been documented, but a reduction in weed species diversity was not noted although gene flow from glyphosate-resistant canola to wild populations of bird's rape (*Brassica rapa* L.) in eastern Canada occurred (Beckie et al., 2006).

The frequent use of HR crops in rotations and application of the same mode-of-action herbicide and/or multiple in-crop herbicide applications of the same mode of action over time can result in intense selection pressure for weed resistance. Therefore, diversifying the cropping systems and rotations are the key to sustainable agriculture. As such, the use of HR crops must adhere to this fundamental principles of farming and cropping systems diversity (Beckie et al., 2006).

Conclusions

The ryegrass population in our study was highly resistant to diclofop-methyl and clethodim but was highly susceptible to glyphosate. The initial soil seed bank of this herbicide-resistant ryegrass population was 6518 ± 291 plants m^{-2} . None of the management factors, except herbicides, significantly decreased ryegrass density. Indeed, the fertilizer treatment N₃ (UAN) increased the emergence of ryegrass (more in 44 cm than 22 cm rows). This aspect needs to be investigated by further studies.

In the lupin crop of our study, dimethenamid-p reduced the ryegrass density by 61%, while simazine reduced the ryegrass density by 21% compared with the untreated buffer zone. The herbicides applied in the 2013 wheat crop had no significant effects on the resistant ryegrass density. However, the most striking weed control (99%) was in RR[®] canola, attributed to the double application of glyphosate.

This high level of weed control should reduce the soil seed bank of resistant ryegrass, over time. Once the ryegrass seed bank has been reduced to a low level, it is important for sustainable grain production to implement IWM practices and maintain low seed bank levels of herbicide-resistant ryegrass to minimize further development of herbicide resistant populations. Such IWM practices should include a range of physical, chemical, biological and mechanical approaches of weed control to deplete

soil seed bank of weeds, kill resistant populations by effective and selective herbicides from different modes-of-action, and stop viable seed being set by resistant ryegrass. Additionally, IWM practices should also prevent viable weed seed being added to the soil seed bank, and the introduction of viable weed seed from external sources.

Finally, we emphasize that although the RR[®] canola technology appears to be a useful tool for effectively controlling resistant ryegrass and, perhaps, other weed species, this technology should be used carefully and judiciously. This requires strictly following the management guidelines of the OGTR (2018) to minimise the risks of further developments of glyphosate resistance in ryegrass and other weeds and potential health hazards.

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The authors also wish to state that this work was conducted some time back (2012-2014) but it has not been published in any other journal.

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