TECHNICAL PAPER

Design and testing of field application tools for a bioherbicide with a plant virus as active ingredient

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Abstract

SolviNix LC is a novel commercial bioherbicide containing a plant virus as the active ingredient (ai). It is registered in the USA for the control of tropical soda apple (*Solanum viarum,* TSA), an invasive weed of pastures and woodlands. As no prior example or experience exists for the application of a plant virus as an herbicide, we devised and tested tools and methods for field application.

Our objectives were to design a practical, economical, and effective tool and method that could be easily melded into weed management practices. This should be accomplished by delivering a minimal effective amount of the ai (in dosage and rate). As TMGMV, like all plant viruses, requires physical damage to the plant by abrasion or wounds to enter the tissues, we designed, assembled, and tested four tools and a few modifications thereof that simultaneously abraded and applied the bioherbicide to the leaves. We also tested two commercially available herbicide wipers and modifications to them to treat individual plants. Of the tools tested, high-pressure sprayers, either a backpack sprayer or an all-terrain vehicle (ATV) mounted sprayer, delivering the herbicide at $\geq$ 0.55 MPa ($\geq$80 psi), provided the desired level of weed kill (85% or higher). Here we describe the different application tools, the test results, and the rationale for the application tool/method presently included in the label, a backpack sprayer. Given the novelty of the application systems we tried, this report could be instructive to others facing a similar challenge.

Keywords: *Tobacco mild green mosaic tobamovirus,* TMGMV, plant virus, tropical soda apple, *Solanum viarum*, SolviNix, bioherbicide.

Introduction

Solanum viarum Dunal (tropical soda apple, TSA) is a serious weed in pastures and surrounding woodlands in the USA and several other countries. It is a species native to Argentina, Brazil, Paraguay, and Uruguay and is a designated Noxious Weed in the USA (e-CFR, Part 360 Noxious Weed Regulations, 2020). Following its introduction into Florida in 1984, TSA spread rapidly to several south-eastern U.S. states in the 1990s (CABI, 2020). Following several years of coordinated, aggressive management by these states, TSA is now confined to Florida where it is a recurring problem in cattle pastures and surrounding woodlands.

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TSA has been introduced into 19 countries of which 10 have reported it to be invasive, including Australia (reported from New South Wales [NSW WeedWise, 2018] and Queensland [Csurhes, 2012]), India, Myanmar, Nepal, Vietnam, and others (CABI, 2020). In New South Wales, TSA is under a Biosecurity (Tropical Soda Apple) Control Order (Christie, 2017). The biology, distribution, economic and environmental impacts, and management of TSA have been well studied and published (e.g. Cuda et al., 2000; Mullahey et al., 1993, 1994a, 1994b; Salaudeen et al., 2013).

In our search for a biological control agent for TSA, we discovered an unusual but previously known phenomenon of virus-elicited systemic necrosis that consistently killed this plant. The virus is a naturally occurring (non-engineered, unmodified) Tobacco mild green mosaic virus Strain U2 (TMGMV U2). In December 2014, the U.S. Environmental Protection Agency (EPA) granted an unrestricted registration for our product SolviNix LC as a bioherbicide for TSA and TMGMV U2 as an herbicide active ingredient (ai). It is the world's first example of an herbicide ai that is a plant virus. To assemble the registration data package, we researched the biology of the host-virus interaction, sequenced an isolate of the registered strain, screened 435 plant species to delineate the host range of the virus, and developed a process to mass-produce the virus for commercial use.

With no prior example or experience to guide us in the application of a plant virus as a commercial herbicide, we devised and tested several tools and methods for field application. The method had to be practical, effective, and easily melded into weed management practices. As TMGMV, like all plant viruses, requires physical damage to the plant by abrasion or wounds to enter the plant tissues, the challenge was to design a tool that *simultaneously abraded and applied the bioherbicide to the leaves*. This should be accomplished by delivering a minimal effective amount of the ai (in dosage and rate) to render the herbicide economical and environmentally acceptable. Here we describe the different application tools, the test results, and the rationale for the application tool/method included in the label.

Tobacco mild green mosaic virus (TMGMV) (scientific name: *Tobacco mild green mosaic tobamovirus*, is a species of *Tobamovirus.* It was previously called Tobacco mosaic virus U2 (TMV U2) and is worldwide in distribution wherever tree tobacco (*Nicotiana glauca* Graham), an occasional ornamental and more commonly a naturalized weed, and cultivated tobacco, *N. tabacum* L., are grown.

As the name implies, TMGMV causes a mild mosaic symptom in tobacco compared to the closely related TMV. Also, compared TMV, TMGMV attacks fewer hosts (a narrower host range) (Description of Plant Viruses [DPV], 2020).

Unlike animal viruses that infect their hosts through receptors on host cells, for TMGMV to be effective as a bioherbicide, the virus particles (the ai in the bioherbicide) have to be introduced into living TSA cells through microscopic or macroscopic injuries to the cell walls. The virus can then infect the cells, replicate, and move from cell to cell, triggering the systemic lethal necrosis, a form of severe hypersensitive response to the virus from TSA (Charudattan and Hiebert, 2007).

TMGMV and other tobamoviruses are called "mechanically transmitted" viruses. They have no known natural vectors such as arthropods or nematodes that transmit the virus and must be physically inoculated into the plant cells to cause disease. On a small scale in the laboratory and greenhouse, rubbing individual leaves with a piece of sterile cheesecloth dipped in a virus extract is used, which is impractical to use on a field scale. Hence, it was necessary to design and test a new equipment or modify conventional herbicide applicators.

Our priorities were to devise an equipment/tool that is effectively inoculates the plants, was easy to operate, and could be purchased from us or self-assembled by the users. Moreover, the application should ensure a consistently high level of control (weed kill) while delivering only a small amount/volume of the virus. The method must injure the foliage mildly without tearing off the inoculated leaves or breaking the branches, which would preclude virus replication and triggering of the lethal host reaction. The challenge was enormous due to the lack of prior art to such a method of herbicide application.

Background

From the start, field-wide application of the bioherbicide was not contemplated as TSA occurs in patches rather than in large monocultures. It was equally important to avoid non-target application, i.e., leaving virus residues in pasture areas devoid of TSA. The cost of the bioherbicide to the user was another important consideration. Therefore, we attempted to design an application tool to treat scattered TSA patches and areas inaccessible to larger equipment, such as in wooded areas. Based on our prior work (Charudattan and Hiebert, 2007), we decided that two methods of application would be necessary: 1) spot application to treat plants in sites inaccessible to spray vehicles and 2) an ATV (all-terrain vehicle) mounted sprayer to treat scattered, patchy infestations in open fields. Also, from the earlier work it was established that more than 85% TSA control could be obtained using 10 µg of ai per ml applied at the rate of about 5 ml per plant in spot treatments.

Materials and Methods

Unless stated otherwise, the following application tools/systems were conceptualized and developed jointly by the authors and custom designed and assembled by Dr. Wayne Currey from commercially available components. A liquid concentrate of a purified preparation of TMGMV U2, the registered bioherbicide formulation of SolviNix LC, was used (SolviNix Labels, **www.bioprodex.com,** 2014).

The tools and methods were tested and validated under an Experimental Use Permit (EUP) issued to BioProdex, Inc. by the EPA. The trials were done in approximately 18.45 hectares (45.6 acres) at six sites in six Florida counties (see details in **Tables 1 and 2**).

As a standard practice, the SolviNix LC was mixed with a volume of water required to provide the chosen ai/ml and the approximate volume for the area or number of plants to be treated. No adjuvant, surfactant, or abrasive such as carborundum power was used. Indeed, none of these additives should be used (SolviNix Labels, 2014) as they will either render SolviNix to be noninfective (adjuvants/surfactants) or could be unsafe to applicators (carborundum). The effects of virus inoculation on disease development and symptoms sequence are presented under Results in **Figure 11** (see later).

The timing of application is critical to assure efficacy: SolviNix should be applied from spring to fall in subtropics and from late spring to late summer in temperate zones. So, these trials were done between late March and early September. Each of the following systems (except S-4 M-1, S-6 and its two modifications, **Table 1**) was tested at least twice at two or more sites to confirm consistency of results. As this was a qualitative comparison intended to select tools and methods that consistently provided 85% or higher levels of TSA kill, no statistical analysis was deemed necessary.

System 1 (S-1): This system consisted of a MeterJet™ spray gun (Spraying Systems Co., Glendale Heights, IL, USA, **https://www.spray.com** having a TeeJet 0001 nozzle (TeeJet Technologies, Glendale Heights, IL, USA, **https://www.teejet.com**) set to deliver 2 mL of SolviNix per discharge at 0.55 MPa (80 psi) force at the point of discharge (**Figure 1**). The sprayer was connected to a high-pressure backpack cylinder containing CO_2 as the propellant. The spray gun was attached to the cylinder with a high-pressure hose fitted with a Series 2 HKGL Eaton Hansen quick-disconnect coupling (Eaton, Jacksonville, Florida, **https://www.eaton.com**).

Figure 1. The S-1 sprayer gun for spot- spraying with a pre-set 2-mL volume of SolviNix per discharge at 0.55 MPa (80 psi).

S-2: Like S-1, S-2 was operated with a backpack high-pressure CO_2 cylinder but unlike S-1, it had a meter-long) wand with a TeeJet nozzle having a D-1 or D-2 orifice plate in a Quick Cap and an extra gasket (**Figure 2**).

Also, unlike S-1, S-2 was not designed to discharge a pre-set volume of SolviNix; the discharge duration was used to regulate the volume sprayed. The SolviNix was sprayed at 0.41 to 0.55 MPa (60 to 80 psi) forcing it to penetrate the leaves.

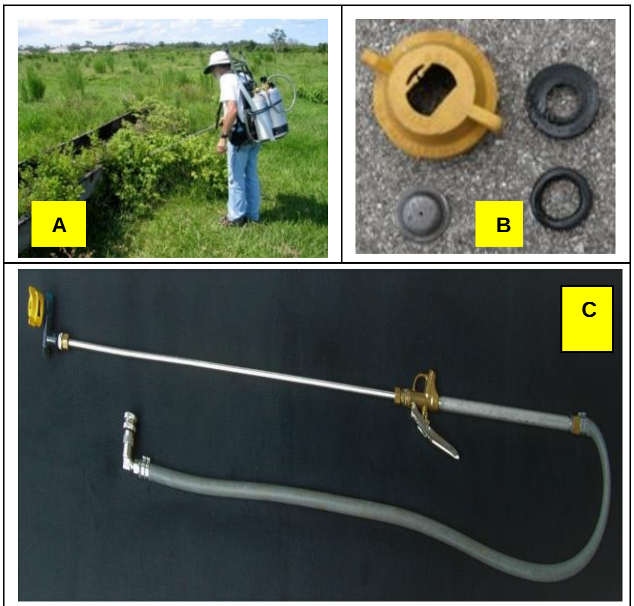

Figure 2. (**A**) S-2 backpack sprayer with an herbicide tank and a pressurized CO_2 cylinder.(**B**) Parts of the nozzle assembly consisting of (clockwise from top left) Quick Cap, a standard washer, an extra washer, and a TeeJet D-2 orifice plate. (**C**) A fully assembled trigger-controlled wand with the nozzle assembly and a Series 2 HKGL Eaton Hansen quick-disconnect coupling to connect the hose to the tank.

S-3: This system was similar to S-2 but was operated with a 12-volt battery-powered electric pump mounted on an all-terrain vehicle (ATV) (**Figure 3**). It could also be operated off the battery of a pickup truck. It was designed to generate a spray stream at 0.48 to 1.38 MPa (70 to 200 psi). Unlike S-2, S-3 was attached to a long pressure hose to reach sites inaccessible to the ATV or a truck.

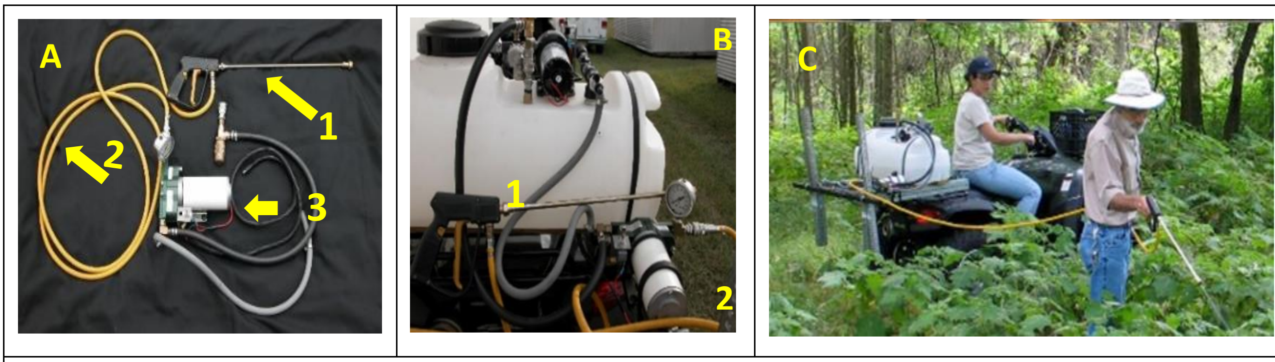

Figure 3. (**A**) The high-pressure, trigger-controlled spray wand of S-3 (arrow 1) fitted with the TeeJet nozzle assembly (**Figure 3 B, C**), a long hose (arrow 2), and a high-pressure electric pump (arrow 3). (**B**) A fully assembled S-3 with an ATV-mounted tank, the spray wand (arrow 1), and the high-pressure electric pump with a pressure gauge (arrow 2). (**C**) The mobility and reach afforded by S-3 is shown.

S-4: The S-4 was designed with a 3.05-meter-wide (10-foot) spray boom providing a 3.7-meter (12-foot) swath coverage. The system was mounted on an ATV as shown in **Figure 4**. The spray boom was designed to work with a row of plastic doormats (available in local hardware stores), 46 cm width by 56 cm long, hung from a metal rod mounted about 8 cm behind the boom.

Figure 4. (**A**) The S-4 with a spray boom and abrasive mats fitted on one side of the ATV with the tufted, abrasive side of the mats facing forward and hung from a bar about 8 cm (3 inches) behind and below the spray boom. (**B**) The row of mats (shown back side facing the camera) were flexible as they passed over the TSA canopy. (**C**) Frontal (**C1**) and rear views (**C2**) of S-4 making passes over TSA plants.

The mats were intended to abrade the leaves as they passed over the TSA foliage (**Figure 4 B**). The spray boom was fitted with seven TeeJet Airmix 110-03 flat fan nozzles and the system was fed with SolviNix from a spray tank by a CO_2 high-pressure cylinder, both housed in a fiberglass crate mounted on the ATV (arrow, **Figure 4 A**).S-4 was first tested twice in a replicated study at the University of Florida field research station (site details in **Table 2**).

The test site was prepared by tilling and treatment with a preplant chemical herbicide approved for use for this site. The plots were 3.05 m^2 (10 ft^2) and each plot was transplanted with 24 TSA seedlings in four rows each with six seedlings. The transplants were allowed to grow for approximately 12 weeks to reach maturity to the pre-flowering stage at inoculation.

S-4 was tested by applying six treatments of 0 (water control) and, 3.12, 6.25, 12.5, 25, or 50 µg/ml of SolviNix with five replicates plots per concentration. The study was concluded 44 days (first trial) or 34 days (second trial) after the treatments were applied at which time the final counts of living plants per plot were taken and percent TSA kill was calculated The results are presented in **Table 2**.

Figures 5 illustrates the effectiveness of the S-4 system and that of SolviNix as an herbicide. The system was tested in a replicated field trial and further modified to improve efficacy.

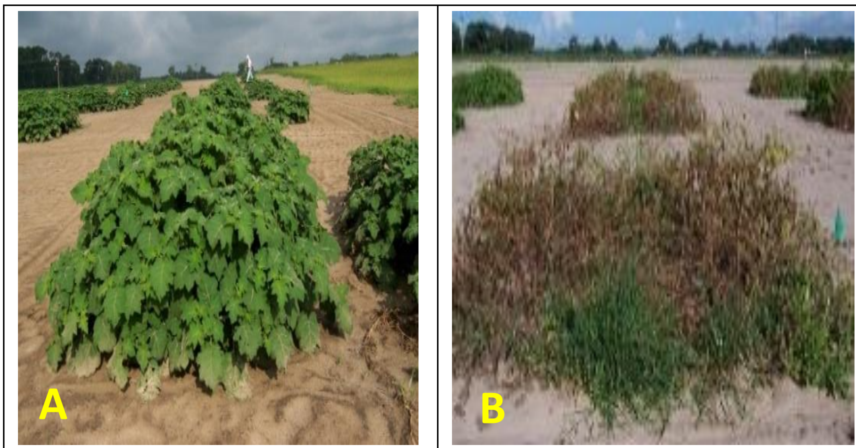

Figure 5. Replicated plots of TSA plants treated with SolviNix using the S-4 system (**Figure 5**). (**A**) The TSA plants had been treated two days before this picture was taken. (**B**) The plots to the left of the centre row show untreated (green, alive) TSA; the row of plots in the middle and the two plots to the right were treated with SolviNix (brown, dead). Grass weeds (green) are seen in the foreground plot (21 days after treatment).

The system was modified three ways to improve leaf abrasion. Modification 1 (M-1) consisted of using 11 cm (4.5 inch) wide strips of the plastic mats (compared to the 46 cm wide mats in S-4) (**Figure 6 A, B, C**).

It was conjectured that the narrower strips unlike the broader mats would pass through the TSA canopy and abrade the leaves from different angles unlike the wider mats that tended to simply ride over the canopy and miss wiping a large proportion of the lower leaves.

Figure 6. In M-1 modification of S-4, the mats were vertically cut into 11-cm (4.5-inch) strips, front (**A**) and back (**B**) views. The strips were attached and hung from a metal bar on an ATV as in S-4. (**C**) Application of SolviNix with M-1 in a TSA-infested mixed pasture-pecan grove.

In the second modification M-2, the 11-cm wide plastic mats were replaced with a row of 0.76-meter (2.5-foot) lengths of 5.1-cm (2-inch) width double-loop chain (**Figure 7A**). The chain lengths were hung 2.5 cm (1 inch) apart, from a 3.05-meter metal bar about 8 cm (3 inches) behind the spray boom.

It was thought that the metal links would help abrade the leaves as the chain lengths passed through TSA canopy, amidst the branches. In M-3, the spray boom of S-4 was fitted with a section of chicken-mesh fencing weighted down with a galvanized metal bar (**Figure 7B, 7C**), assisting the chicken-mesh to abrade the leaves as it passed over TSA canopy.

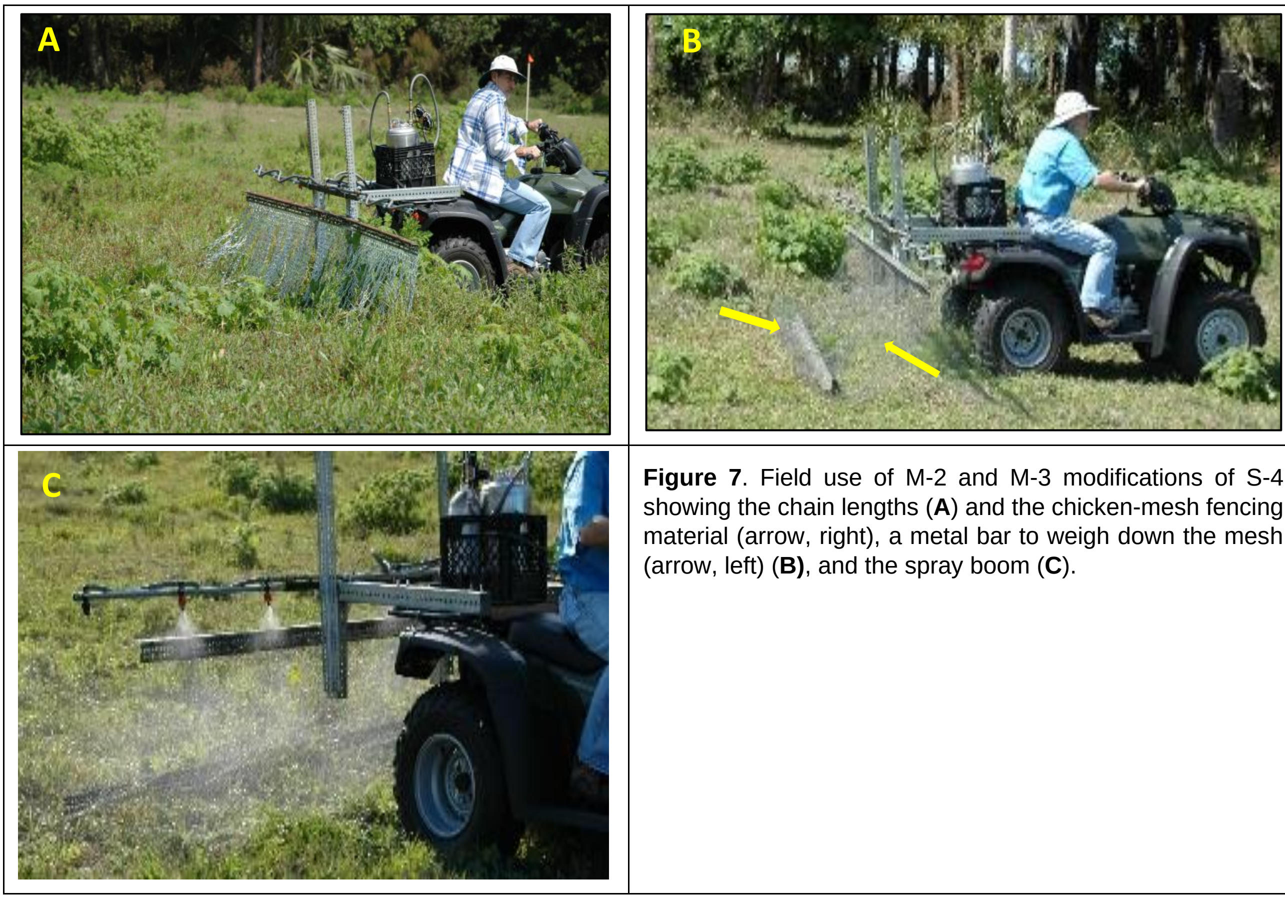

Figure 7. Field use of M-2 and M-3 modifications of S-4 showing the chain lengths (**A**) and the chicken-mesh fencing material (arrow, right), a metal bar to weigh down the mesh (arrow, left) (**B)**, and the spray boom (**C**).

S-5: A commercial wiper system developed for chemical herbicide applications called the Alley Cat Herbicide Wiper (Alley Cat Farm Equipment, Boynton Beach, Florida (**www.weedwipe.com**) was tested.

The wiper system consisted of a row of water-permeable synthetic fibre mats backed with plastic mats with both fitted with tubes delivering measured volumes of herbicide (**Figures 8 and 9**).

The flow rate was controlled by a pressure regulator attached to an ATV-mounted 56.8 litter (15-gallon tank). As the wiper passed over the TSA foliage, the mats abraded the leaves and deposited a liquid film of SolviNix on the leaves (**Figure 9**).

S-5 was tested at delivery rates of 28 L/ha to 131 L/ha (3 to 14 GPA), and further improvements were tried to increase abrasiveness. This was done by (1) placing a stretch of chicken-mesh fencing in front of the wiper pads (modification M-1a) (**Figure 8A)** or (2) behind the pads (modification M-2a; image not shown), or (3) by adding a section of galvanized reinforcement mesh to the chicken-mesh fencing in front of the wiper pads (M-3a) (**Figure 8B**). These additions were made without interfering with the wiper's designed fluid delivery system.

Figure 8. (**A**) Application system S-5, Alley Cat Herbicide Wiper. Water permeable material (blue) that allows flow-through while remaining moist with *SolviNix* LC and rubber backing material. (**B**) Attached with fluid-delivery tubes. (**C**). The S-5 wiper system in use in the field.

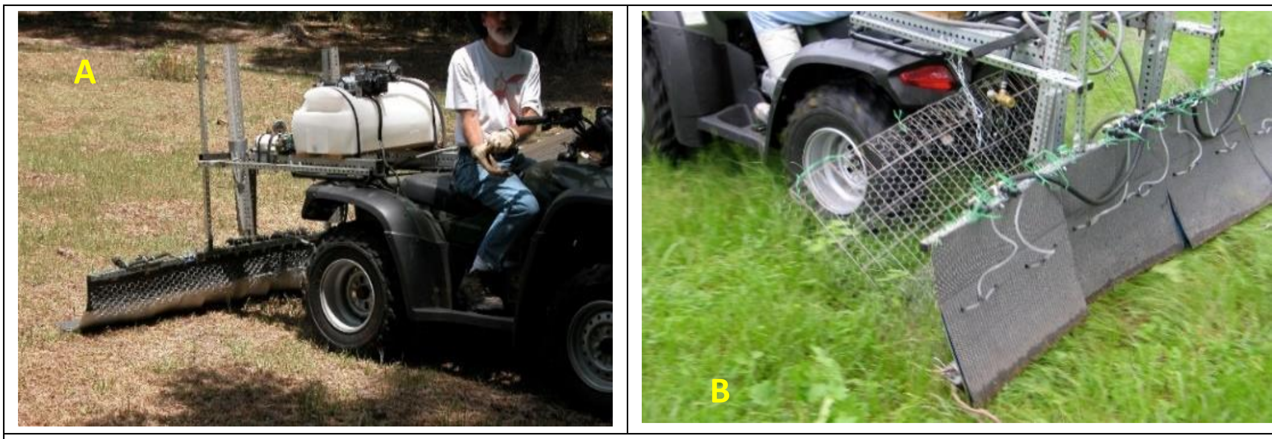

Figure 9. Modification M-1a to the Alley Cat herbicide wiper (S-5) with chicken-mesh fencing placed before the pad (**A**) and M-3a with galvanized reinforcement mesh and chicken-mesh before the pad (**B**), both modifications shown fully assembled and ready to be used.

S-6: The S-6 was the Microwipe (**Figure 10**) herbicide wiper made by the Micron Group (Micron Group, Bromyard Industrial Estate, Herefordshire, U.K., https://www.Microngroup.com/microwipe).

We tested it as a hand-held "clean-up" tool for scattered TSA plants. It is made of plastic tubes in the shape of a "T", with the see-through long end doubling as the handle and herbicide reservoir and the wick looped from the opaque top of the "T" (**Figure 10A, 10 B**). The wick, when fully wet, enabled the herbicide to be wiped on the foliage. To add abrasiveness to the tool, we wrapped chicken-mesh fencing or drywall sanding and plastering screen around the top of the "T", behind the wick (**Figure 10C**).

Results

Once infected by TMGMV, the TSA plant invariably dies following a typical disease development and symptoms expression sequence; the different results reported here were due to the variable efficacy of the tools and methods in delivering the virus into the leaves. The virus-host reaction in these field trials mirrored the repeated observations from greenhouse trials (Charudattan and Hiebert, 2007), but at a slightly slower speed (**Figure 11**).

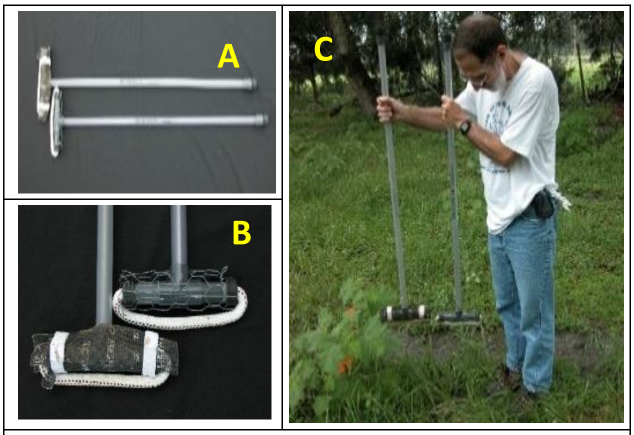

Figure 10. (**A**) S-6 Microwipe applicators with chicken-mesh fencing (**B right**), or drywall sanding screen wrapped behind the wick to improve abrasion (**B, left**). The reservoir (the long handle) is filled with an appropriate dilution of SolviNix LC and after the wick is fully moist, TSA plants are rubbed and scoured as shown in **C**.

The appearance of necrotic local lesions, an expression of host resistance reaction, is the first symptom seen around 14 days (usually on the eighth day in the greenhouse) following infection (**Figure 11 B**). Since the lesions are expressed only in infected leaves, they are not easy to find in the field. Easier and more widely seen is systemic chlorosis of the plant and epinasty and wilting in apical shoots (**Figure 1 C**), the second step in the symptom sequence.

This is followed by the onset of wilting of the entire plant (**Figure 1 D),** progressive death and drying of leaves and branches (**Figure 1 E**), and complete death with the dried plant left standing until trampled by cattle or brought down by natural forces (**Figure 1 F**). Mature green fruits present when the plant is treated may mature and turn yellow carrying viable seeds. Alternatively, if the fruits are immature, they may rot and shrivel up in time (**Figure 11 G)**.

The relative efficacies of the application systems along with site and application details are provided in Table 1. Although S-1 precisely delivered the pre-set volume, it was difficult to aim and consequently gave poor results (<10% TSA kill, results not shown). It was difficult to hit the leaves with brief spurts of fluid as the stream tended to miss the leaves by passing through open spaces between leaves. Therefore, S-1 was not considered suitable and it was not tested further.

S-2 and S-3 systems were highly effective and were considered suitable SolviNix application tools. The average level of efficacy of SolviNix applied with high-pressure spot sprays (S-2 and S-3) averaged 89% (range 58-100% in 12 trials). Both systems performed equally well.

The boom sprayer system S-4 without modifications gave an average of 75% TSA kill in two trials averaged across SolviNix treatments (control not included). The TSA kill ranged from 13% to 96% in the two trials without the expected correspondence between ai/ml concentration and percent kill (Table 2). Hence, to improve efficacy and consistency of S-4, modifications were tried. Of the tree modifications, M-1 gave 50% kill in a single trial and it was not tested further. M-2 and M-3 yielded, respectively, 54% (43-65%, five trials) and 60% (53-66%, four trials) TSA kill.

Likewise, the efficacy of the Alley Cat wiper system, S-5, without modification, was also only fair (61%, 50-73%, three trials). The modifications M-1a (45%; 40 and 50%, two trials), M-2a (45%; 40 and 50%, two trials), and M-3a (40%, one trial) were also not efficacious (Table 1).

The S-6, Microwipe, without modifications was not effective (no data taken). With modifications, it provided 75% TSA kill (with chicken mesh or drywall sanding screen). The addition of the chicken mesh and/or the reinforcement mesh to S-4 or S-5 modifications introduced a major drawback: the meshes severely tore the TSA leaves or broke off the branches, removing them from being the virus replication sites.

Discussion

We screened three Solanaceae-adapted tobamoviruses for infectivity and possible usefulness as biological control agents for TSA: Tomato mosaic virus (ToMV), Tobacco mosaic virus U1 (TMV U1), and TMGMV U2 as these were available to us to study. As previously reported (Charudattan and Hiebert, 2007), TMGMV U2 killed all inoculated TSA plants in repeated greenhouse trials whereas the other two viruses elicited only nonlethal systemic mosaic or systemic mosaic and mottling in this host.

Thus, by pure chance, we discovered that TMGMV U2 elicited systemic necrosis and killed TSA, an invasive weed of interest to us. This host-virus interaction underlines the novelty of SolviNix; there is no similar example among herbicides.

However, we are not the first to consider TMGMV U2 for biological control of a weed; in 1986, Professor J. W. Randles, The University of Adelaide, Waite Agricultural Research Institute, proposed that TMGMV U2 (referred to in the paper by its older designation as TMV U2) could be used as a biological control agent for *Echium plantagineum* (Paterson's curse, purple viper's-bugloss; Boraginaceae) in Australia (Randles, 1986).

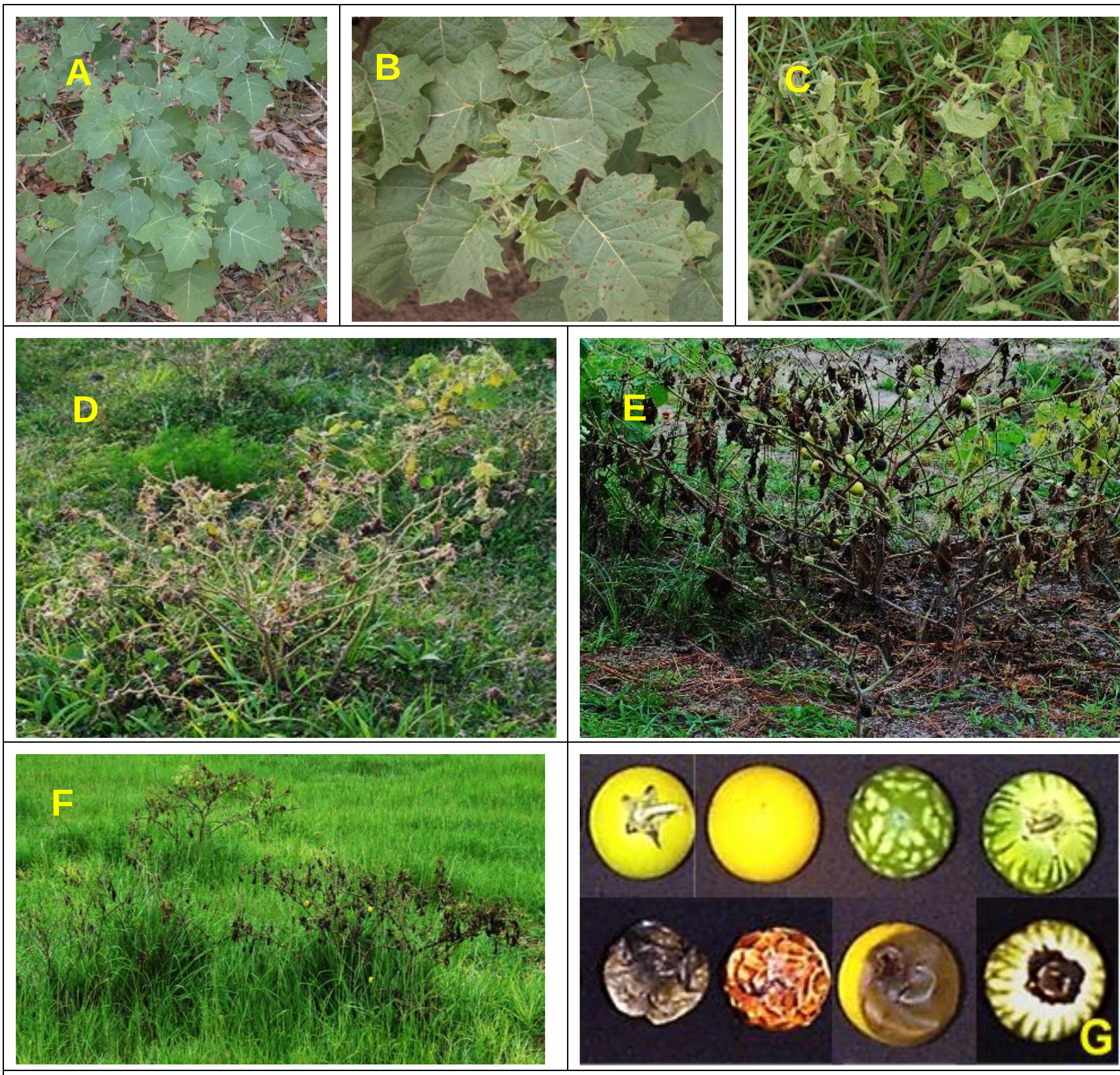

Figure 11. Sequence of disease and symptoms development in TMGMV U2-infected TSA plants in the field. A healthy, uninfected plant (**A**); foliar necrotic local lesions (**B**); chlorosis of the plant with epinasty in shoot apices and onset of wilting (**C**); wilting of the entire plant (**D)**; advanced wilting and drying of the plant with partially green branches (**E**); fully wilted, dried plants (**F**); and the effects on fruit: top row, right to left: unaffected mature green fruit and mature, ripe, yellow fruit; fruit viewed from adaxial (stalk) and abaxial sides; bottom row, right to left: mature green fruit with rotting around the stalk, rotting of mature yellow fruit, and two stages of dead, dried fruit (**G**).

In this plant, TMGMV U2 elicited yellowing and systemic mosaic and reduced leaf production and increased leaf senescence in pot trials. It reduced seed production in inoculated plants in the field. However, unlike in the TMGMV U2-TSA interaction, the virus did not kill *E. plantagineum*.

It is the invariable causation of 100% mortality of infected TSA, leaving no living, systemically infected plants in the field, was the prime consideration on which the SolviNix registration decision was based. For, it was reasoned that with no surviving, systemically infected TSA plants left to serve as virus reservoir, there is no danger of virus spread to other susceptible species. Therefore, the use of TMGMV U2 as a bioherbicide was acceptable.

TMGMV is readily transmitted mechanically in the laboratory by abrading the leaf with virus solution. Therefore, we expected all tools/methods to perform relatively well. Yet, none of the tools/methods we developed to abrade the leaves while delivering the virus solution was as effective in the field as spraying with a high-pressure sprayer. It was unexpected, particularly since this experience was contrary to the belief that TMGMV, like TMV, is highly contagious.

In fact, in field trials, we observed many cases of healthy TSA plants growing in contact with TMGMV-infected plants that failed to become infected. Also, there was no evidence of virus spread within the field following SolviNix applications.

The results from these EUP trials were used to develop label directions for application of SolviNix LC. As spot spraying is generally the prevailing TSA control method used in Florida and a high level of TSA kill of 85% or higher was consistently obtained by high-pressure (0.55 MPa, 80 psi) spraying of TSA canopy with a backpack or ATV-based sprayers, only these methods were considered for listing in the label.

However, initially, only the high-pressure backpack sprayer, which can be more easily cleaned and is cheaper to buy and use than an ATV-mounted system, was approved for listing on the SolviNix label (see Labels under Products, **www.BioProdex.com**). We are testing an ATV-mounted boom sprayer like the S-4 capable of spraying at pressures higher than in the trials reported here for possible future addition to the label.

Conclusions

SolviNix LC reliably provides $\geq$85% TSA kill when spot-sprayed with a high-pressure (> 0.55 MPa) backpack sprayer, which is listed in the label. SolviNix LC performs effectively and satisfactorily in commercial usage.

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Table 1. Efficacy of SolviNix and application systems in field trials.

Application type; System used	Area treated (ha)	Applied Dose (µg a.i/mL)	Rate (L/ha)	Number of days after treatment	% TSA kill
Site designation, County, site description: C Cowart, Flagler county, cattle, and blueberry farm: GPS coordinates: N29°28.619; W081°23.405. Total area treated at this site: 0.81 ha, 2 ac.					
Spot spraying; S-3	2	10	N/Ap	37	98
Site designation, County, site description: Dinner Island Wildlife Management Area, Hendry county, Cypress dome: GPS coordinates: N 26°28.55; W 081°10.289. Total area treated at this site: 2.02 ha, 5 ac.					
Spot spraying; S-2	4.76	10	N/Ap	34	99
Spot spraying; S-2	0.24	10	N/Ap	34	100
Site designation, County, site description: D Crawford, Hendry county, wetland pasture in a homestead: GPS coordinates: N26°32.349; W081°23.730. Total area treated: 4.05 ha, 10 ac.					
Spot spraying; S-3	2.0	10	N/Ap	53	90
Large-area application; S-4: M-2	2.0	10	22.5	53	65
Large-area application; S-4: M-2	2.0	5.7	35	53	58
Large-area application; S-4: M-3	2.0	10	22.5	53	59
Large-area application; S-4: M-3	2.0	5.7	35	53	66
Site designation, County, site description: M Dunn, Alachua county. GPS coordinates: N 29°38.564; W 082º06.676. Total area treated: 0.03 ha (0.07 ac).					
Spot spraying; S-3	N/Av	10	N/Ap	46	90
Spot spraying; S-3	N/Av	10	N/Ap	21	90
Large-area application; S-4: M-1	1.2	50	20	26	50
Large-area application; S-4: M-2	0.96	10	20	46	54
Large-area application; S-5	0.28	10	2.14	46	60
Large-area application; S-5	0.5	10	5.4	46	73
Large-area application; S-5: M-1a	0.63	10	10.8	24	50
Large-area application; S-5: M-1a	0.63	50	10.4	22	40
Large-area application; S-5: M-2a	0.63	10	14.4	21	50
Large-area application; S-5: M-2a	0.63	50	10.4	22	40
Large-area application; S-5: M-3a	0.63	50	10.4	22	40
Microwipe; S-6	N/Av	50	N/Ap	N/Ap	N/T
Microwipe; S-6, Chicken-mesh	N/Av	50	N/Ap	22	75
Microwipe; S-6, Drywall sanding screen	N/Av	50	N/Ap	22	75
Site designation, County, site description: R Crawford, Collier county, open pasture. GPS coordinates: N26°24.081 W081°26.343. Total area treated: 6.07 ha (15 ac).					
Spot spray; S-2	2.87	10	N/Ap	36	96
Spot spray; S-3	2.87	10	N/Ap	36	96
Large-area application; S-4: M-2	0.88	10	22.5	35	52
Large-area application; S-4: M-2	0.88	5.7	35	35	43
Large-area application; S-4: M-3	0.88	10	22.5	35	61
Large-area application; S-4: M-3	0.88	5.7	35	35	53
Large-area application; S-5; site disturbed	5.73	10	3.5	48	N/T
Site designation, County, site description: E Tucker, Elkton, St Johns county, cull pile/cattle feed. GPS coordinates: N29°46.527; W081°25.540. Total area treated: 3.08 ha (7.6 ac).					
Spot spraying; S-2	1.26	10	N/Ap	24	80
Spot spraying; S-2	1.26	10	N/Ap	21	85
Spot spray; S-3; nozzle with D-2 orifice plate	1.26	10	N/Ap	36	85
Spot spray; S-3; with an 80-03 flat fan nozzle	1.26	10	N/Ap	36	58
Large-area application; S-5	1.26	20	5	49	50
N/Av = Not available; N/Ap = Not applicable, ~5 mL per plant; N/T = data not taken; test abandoned.					

Table 2. Efficacy of S-4 in a replicated field trial applying six SolviNix concentrations

Treatments:	SolviNix a.i. µg/mL						Average of all treatments by trial:
	0 (control)	3.1	6.3	12.5	25	50	
	Percent TSA Kill						
Trial 1:	0	92	71	92	96	96	89
Trial 2:	0	13	38	86	79	83	60
	Average of all treatments from two trials:						75
Site designation, County, site description: Plant Science Research and Education Unit, University of Florida, Marion county. GPS coordinates: N 29°24.624; W 082º10.201. Total area treated: 0.03 ha, 0.07 ac.							