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Application of a Bacterial Pathogen, *Ralstonia solanacearum*, with a Wet-blade Mower for Biological Control of Tropical Soda Apple, *Solanum viarum*

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ABSTRACT

Tropical soda apple (*Solanum viarum* Dunal; TSA) is an invasive noxious weed in Florida and several southeastern U.S. states. To develop a bioherbicide agent that could be integrated with mowing, a recommended management practice for TSA, we screened several isolates of bacterial pathogens of Solanaceous plants and established that a *Ralstonia solanacearum* (=*Pseudomonas solanacearum*) (RS) isolate (10 Q, Race 1), originally from tomato, was capable of killing TSA without affecting tomato. RS is a xylem-invading, wilt-causing pathogen that when applied to cut main stems of TSA prevented regrowth and killed TSA plants under greenhouse conditions. To determine the effectiveness of this model bacterial bioherbicide agent under field conditions, a novel application method using a wet-blade mower was tested. Whereas wet-blade systems are used to deliver chemical herbicides and plant growth regulators to target weeds while mowing, none has been tested to deliver a biocontrol agent. We used the Burch Wet Blade^{TM} mower system (BWB) and conducted the study in a pasture with 18% TSA coverage. RS cells suspended in sterile tap water at 1.4 x 10^9 CFU units/ml were used as inoculum. Treatments included a BWB-applied control (culture medium without RS), RS applied at 23 L/ha with BWB, and RS applied at 23 L/ha with BWB plus RS over-sprayed at 560 L/ha with a backpack sprayer. Both RS treatments reduced TSA regrowth compared to the control (P = 0.0003). There was no difference between the wet-blade-applied RS treatment and the wet-blade + over-sprayed RS treatment. The wet-blade mower was an effective, practical means of application of the bacterial wilt pathogen to control TSA and it may have broader applicability to other types of wilt-causing pathogens.

Keywords: application technology, bacterial wilt, bioherbicide, Burch Wet-Blade, host range, Lifetest analysis, noxious weed
Abbreviations: BWB, Burch Wet-Blade^{TM}; **GPA**, gallons per acre; **IFAS**, Institute of Food and Agricultural Sciences; **RS**, *Ralstonia solanacearum*; **TSA**, tropical soda apple

INTRODUCTION

Tropical soda apple (*Solanum viarum* Dunal; TSA), a member of the Solanaceae family, is an exotic invasive plant from South America that is a designated noxious weed in the United States (Anonymous 2009). First observed in South Florida around 1987, it spread rapidly throughout the state covering an estimated half million hectares by 1995 (Mullahey *et al.* 1993, 1998). TSA infests open pastures and wooded areas in cattle ranches as well as natural areas (Langeland and Burks 2008). The plant is copiously covered with sharp spines that can range from 1 to 2 cm in length. A defense against herbivory, these spines discourage animals from consuming the leaves and stems but not the fruit. Because of the needle-like spines, dense infestations of TSA hinder movement of cattle, workers, and field management practices. A prolific producer of seeds with high rates of germination (Mullahey *et al.* 1993), TSA is seed-dispersed by cattle, water, and wildlife, and transported in contaminated manure, soil, hay, grass seeds, and sod (Mullahey *et al.* 1998). Consequently, TSA has great propensity to spread and is now established in several southeastern U.S. states (Cuda *et al*. 2002; Bryson and Byrd 2007). Helped by the mild winters in Florida, TSA grows year-round and perennates in some parts of the state, which further increases the chances of its spread.

Chemical herbicides, mowing, and biological control are used to manage TSA in Florida (Sellers *et al.* 2009). A combination of methods and materials are needed to control TSA because no single approach is satisfactory under all conditions. Accordingly, in an effort to find suitable biological control agents, the University of Florida-IFAS began a program in 1994 which resulted in the release and establishment of a chrysomelid beetle, *Gratiana boliviana,* as a classical biocontrol agent (Mullahey *et al.* 1994; Medal *et al.* 1995, 2006). Several fungal and bacterial pathogens were also collected in Brazil and Florida and screened against TSA. While none of the fungi proved promising as a biocontrol candidate, a few isolates of the bacterial pathogen *Ralstonia solanacearum* (RS) were highly virulent on TSA (Charudattan and DeValerio 1995).

RS is a soilborne bacterial pathogen of worldwide distribution. It consists of a diverse group of related strains representing more than one species (Prior and Fegan 2005). RS strains cause bacterial wilt that is characterized by chlorosis, necrosis, systemic wilting, and rapid plant mortality on a wide range of hosts. The host range includes tomato, potato, and banana as well as several important plants in the Solanaceae, Musaceae, Zingiberaceae, and other monocot and dicot families, depending on the race of the pathogen strain. In preliminary trials, some of the RS isolates we screened were highly virulent on TSA but not tomato or several other hosts, which suggested the possibility to find a TSA-restricted isolate to evaluate as a biocontrol agent.

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Research Note

As a vascular wilt pathogen that normally requires natural openings or wounds to enter plants, it is necessary to deliver RS into the plant in order to obtain satisfactory levels of weed control. Since mowing is one of the recommended practices for controlling TSA (Mislevy *et al*. 1999; Sellers *et al.* 2009), we tested the Burch Wet-Blade™ mower (BWB) system for field application of RS. The wet-blade mower system, of which there are a few different types, enables application of an herbicide while mowing. Designed for liquid pesticide applications, these systems have been used primarily with chemical herbicides and plant growth regulators for weed control, weed suppression, or to reduce the mowing frequency (Henson *et al.* 2003; Hixson *et al.* 2007). A feature of the wet-blade systems that was important to our work was the capability for instantaneous introduction of the bacterial suspension as the TSA stems are cut. This was expected to maximize bacterial entry into the cut stem due the suction created by xylem cavitation that pulls the liquid inward (Wahlers *et al.* 1997). Other advantages of wet-blade systems include the ability to apply low volumes (L/ha) and rates (active ingredient/ha) of herbicides, site-directed application, low or no drift, worker safety, low or no nontarget effects, single-step mowing and spraying, and cost savings. In studies done by Mullahey and Williams (2001) and Sellers and Mullahey (2008), the BWB system was used with different levels of success to control TSA, southern wax myrtle, and melaleuca. With TSA, 70% to 95% control could be achieved with several chemical herbicides (Mullahey and Williams (2001).

The objectives of this study were two-fold, namely to determine the feasibility of using RS as a bioherbicide agent for TSA and to test the feasibility of the BWB system as an effective, practical means of application of this pathogen in the field. In this paper, we present the results from different methods of inoculation of TSA with RS in a greenhouse and in the field, selection of a strain for field trials based on its host range and relative virulence to TSA and some Solanaceous crop plants, and the concept of controlling TSA with RS delivered using the BWB mower system.

MATERIALS AND METHODS

Greenhouse trials

Three greenhouse experiments were done to select the best inoculation method and a highly virulent RS isolate based on pathogenicity tests on TSA and other plants in the Solanaceae. The experiments had a completely randomized design with three to five replicates per treatment. The experiments were repeated at least once to verify the results. Test plants were grown to a height of 8 to 16 cm in potting mix, in 8- to 30-cm-diameter pots, before inoculation.

1. Determination of pathogenicity and virulence by foliar spray and stem injection

Seven RS isolates, 10, 10Q, W2, W4, H2, 446, and 506, all originally from tomato in Florida and belonging to Race 1 were used in this study. In the first method, the plants were sprayed with a suspension of bacterial cells plus 0.2% v/v Silwet L-77 (silicone-polyether copolymer, Loveland Industries, Inc., Greely, CO). The bacterial cells were grown on nutrient agar in Petri plates, rinsed off the plates with sterile water, and the Silwet was added for the foliar spray. The foliage was sprayed to drip. For stem injection, the cells were rinsed off the Petri plates with sterile 0.1 M phosphate buffer solution (PBS). In both cases, the cell concentration was adjusted to an optical density of 0.30 A at 600 nm which equaled approximately 5 x 10^8 cells per ml. Controls were sterile water plus 0.2% v/v Silwet L-77 solution only or 0.1 M PBS only for foliar spray and stem injection, respectively, without the bacterium. There were three plants (replicates) per treatment and the plants were observed for 4 weeks after inoculation. The plant host reaction was expressed as susceptible reaction characterized by bright yellow chlorosis of the leaves followed by rapid necrosis of leaves and shoot, wilting, and compete plant death or immune reaction characterized by the absence of disease symptoms.

2. Cut-and-swab inoculation

In the second greenhouse experiment, isolates 446 and 506, both from tomato, which were the most virulent of the isolates screened, were tested on large, mature TSA plants. The 187-day-old, 0.6-m-tall plants were inoculated by clipping the main stem 3 cm above the soil surface and swabbing the cut surface with 1-day-old bacterial cells suspended in sterile tap water. The inoculum was applied at two cell densities of 0.74 and 1.74 A at 600 nm, which equaled approximately 1×10^9 and 3×10^9 cells per ml, respectively. Control plants were inoculated by swabbing with sterile tap water. There were five replicates per treatment and the plants were observed for resprouts from the cut stems for 12 weeks after inoculation. The number resprouts per stem and the average height of resprouts were analyzed by analysis of variance and Duncan's mean separation.

3. Host-range study

In the third experiment, the host ranges of five RS isolates, W2, W4, 10, 10Q, and H2, were compared on 30 plant species in the Solanaceae, including five cultivars of tomato, two cultivars of pepper, two accessions of eggplant, and 27 other *Solanum* spp. Twenty to 30-cm-tall greenhouse-grown seedlings were screened by one of two methods: 1) injecting a suspension of fresh bacterial cells or 2) stabbing plant stems with a sterile wooden toothpick dipped in fresh cells. The bacterial cells for inoculation were prepared from 24 h cultures on Difco nutrient agar (Difco Laboratories, Detroit, MI). For stem injection, the cells were rinsed off the Petri plates with sterile water, centrifuged, and suspended in sterile 0.1 M PBS at approximately 1×10^8 cells per ml. For stem-stab inoculation, the cells were picked directly off the plates with the toothpick. Controls consisted of injecting with a phosphate buffer solution or stabbing them with a culture medium-coated wooded pick without the bacterium. There were three plants (replicates) per species/cultivar. In all three trials, plants were rated for symptoms of wilting and death, partial shoot kill, or no reaction using a visual rating system of resistant (R), slightly susceptible (SS), moderately susceptible (MS), or highly susceptible (HS). Thus, the scale ranged from a nonsymptomatic, apparently healthy plant (R) to a plant with dead shoots or entire plant death (HS).

Field trials

Two trials, each replicated at two locations or over two years (1998 and 1999), were done to test hand-inoculated and BWB-applied treatments. Isolate 10Q was used in field trials; it was one of two isolates highly virulent on TSA and capable of killing this weed.

1. Hand-inoculation trials

The hand-inoculated trials were done in Sumter and Levy Counties, Florida, using a completely random design with 30 and 20 plants (replicates) per treatment, respectively. One-day-old RS inoculum was produced in shake cultures in autoclaved nutrient broth (Difco) amended with TSA stem (20 g/L) and root (10 g/L). The bacterial cells were filtered through cheesecloth and centrifuged for 40 min at 23,500 rpm. Bacterial pellets were suspended in sterile distilled water to concentrations of 1.7×10^9 and 3.4×10^9 cells per ml (equal to optical densities of 1.04 and 1.96 A at 600 nm) for the Sumter and Levy sites, respectively. Control treatments were uninoculated media extracts without the bacterial cells. The Sumter site was located in open pasture under full sunlight; the Levy site was under partial shade in an oak hammock and consequently the plants at this site were spindly compared to those at the Sumter site. Plants at both sites ranged from 30 to 60 cm tall and, if not flowering and fruiting, were mature enough to do so. Seedlings were not included in the trials. Treatments at both sites were: injected inoculated, injected control, cut and swabbed inoculated, and cut and swabbed control. For the injected treatments, approximately 3-mm diameter hole was drilled at the base of the main stem with a cordless drill containing a 3-mm diameter drill

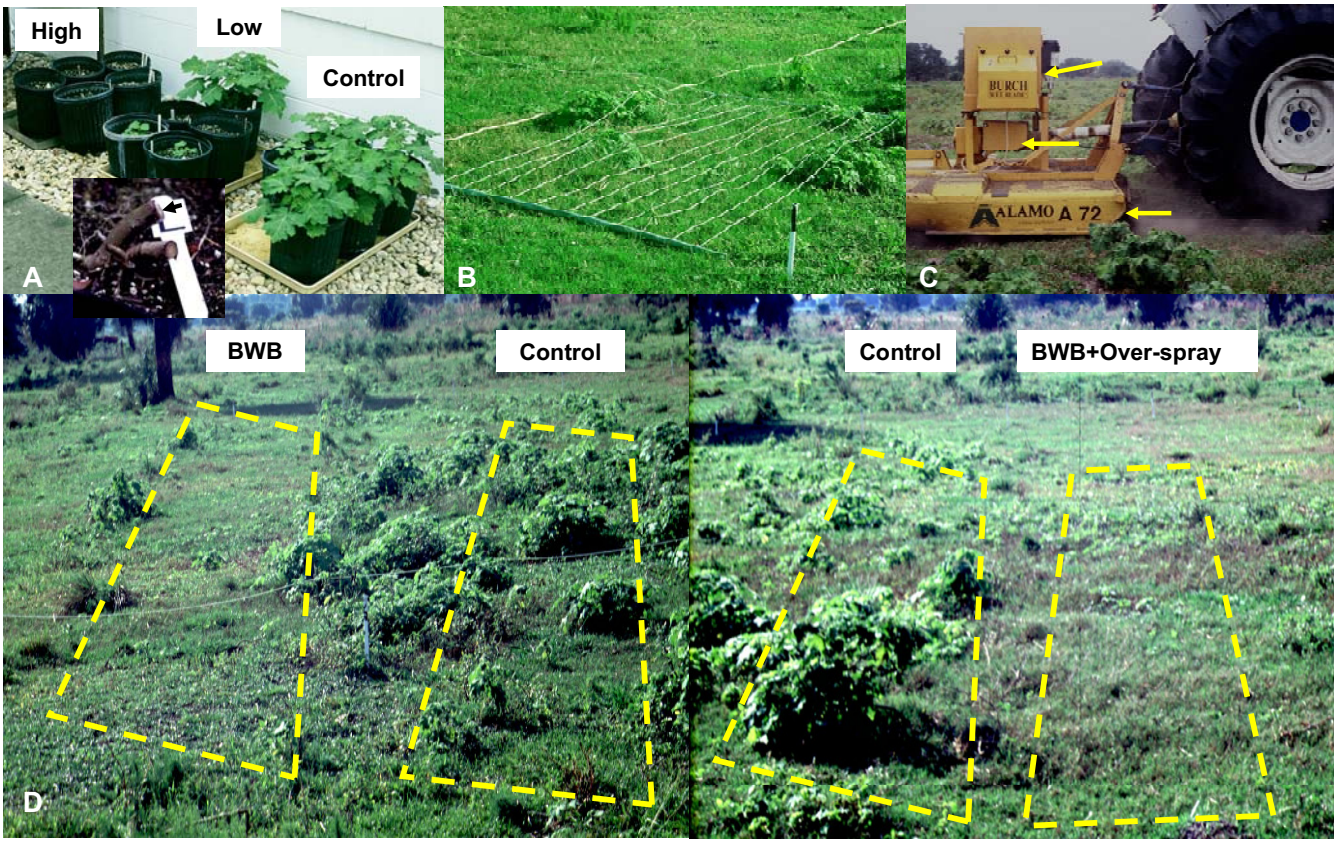

Fig. 1 Effect of *Ralstonia solanacearum* (RS) on regrowth and survival of tropical soda apple (TSA). Clockwise from top left: (**A**) TSA plants cut and swabbed with RS inoculum as described under Materials and Methods, showing strong regrowth of control plants, partial and sparse regrowth of plants treated with the low concentration of RS inoculum, and complete lack of growth and death of plants treated with the high inoculum concentration. The insert shows inoculated cut stems with bacterial ooze (arrow) that is typical on RS infection. (**B**) A grid used to measure the area of coverage by TSA regrowth. (**C**) The Burch Wet-blade mower system with arrows pointing to (from top to bottom) the liquid reservoir, the fluid delivery tube, and the mower blade casing, all mounted behind a tractor. (**D**) TSA plots, from left to right: mowed with BWB+RS, a control plot mowed with BWB without RS, another control plot, and RS applied with BWB plus in an over-spray. These images were taken 4 weeks after inoculation (**A** and **D**) or on the day of inoculation (**B** and **C**).

bit and the cavity filled with RS inoculum using a syringe. For the cut-and-swab treatment, the main stem in each plant was clipped with a pruning shear at 3 cm above the soil surface and the cut surface was swabbed with the bacterial suspension. The control plants were treated similarly but the cavities were filled with uninoculated culture medium. The plants were observed for 4 weeks after inoculation.

The treatment response was compared using the Lifetest procedure (SAS Version 9.2 Documentation). Used in pharmaceutical studies, the Lifetest procedure enables determination of whether the time required for a specific event to occur is dependent on the treatment. The Lifetest procedure was well suited to analyze the results from the Sumter and Levy counties trials because it is designed accommodate data sets where the subjects enter or leave the sampling at different times. For example, when screening patients' responses to medicines, both the time that the patients start to take a particular medicine and the time they respond to it can vary. In our study, the events such as wilting or sprouting, measured as percentage of plants that resprouted in control and inoculated treatments, were assessed over time as they occurred. Chi-square analysis was used to compare the treatment effects.

2. Burch Wet-Blade trials

Two experiments, one in each of two consecutive years (1998 and 1999), were established in Hendry County, Florida to test the effectiveness of the BWB mower as a delivery system for RS. The mower consisted of a commercial mowing deck retrofitted with the BWB delivery system and was loaned to one of the authors (JJM) by Burch Company, 1515 Mockingbird Lane, Suite 820, Charlotte, NC for testing in Florida. The mower system made a 2-m wide mowing swath and was calibrated to deliver 23 L/ha fluid (bacterial suspension or water). The pastures had an initial TSA coverage of about 18%. RS was produced the same way as for the Sumter and Levy counties trials except that the cultures were allowed to grow for up to 72 hrs before harvest. Also, in the second year, the amount of plant amendments to the culture medium were reduced to 1 g/L TSA stem and 0.5 g/L roots. The longer period of culturing was necessary to produce the large quantity of inoculum required. Bacterial cells were collected by centrifugation and resuspended in sterile distilled water to a cell concentration equal to an optical density of 0.80 A at 600 nm (1.4×10^9 cells per ml). The experiment had a completely random design with four replicates per treatment. Sampling units were 61.3 and 55.7 m^2 in years 1 and 2, respectively. Treatments included a BWB-applied control (culture medium without RS), RS applied at 23 L/ha with BWB, and RS applied at 23 L/ha with BWB plus 560 L/ha applied with a compressed-air backpack sprayer. The over-spray was applied with a 2-m wide spray boom by following the BWB mower on foot. A reason the over-spray treatment was included was to have an extra check on the wet-blade mower system.

The first year trial ran from October 9 to December 15, 1998, and the second year trial from December 15 to March 21, 1999. During the first year trial, the cumulative rainfall at the trial site was 198 mm, while in the second year it was much drier with only 26 mm cumulative rainfall. The average minimum-maximum daily temperatures were 22.2-32.2°C and 16.2-23.6°C for Years 1 and 2, respectively.

The TSA coverage in square meters was estimated by using a dot grid (**Fig. 1B**), which was laid over the sampling area in sections. Each dot on the grid represented 0.02 m^2 of coverage. This device enabled quantification of the size of area covered by the regrowing TSA. It was a more accurate estimator of the treatment effects than plant counts because it provided a uniformly scaled measurement of TSA regrowth relative to the initial coverage.

The treatments were analyzed using regression analysis by

comparing the change in coverage of TSA in square meters over time:

SqM (predicted) = intercept + slope (INTSQM)

where INTSQM is initial square meters covered by TSA.

The initial square meter was included in the analysis because there were differences between treatments at the time of mowing.

RESULTS AND DISCUSSION

Greenhouse trials

1. Determination of pathogenicity and virulence by foliar spray and stem injection

Of the seven isolates tested, four (10, 10Q, 446, and 506) were pathogenic and virulent to TSA when injected into the stem. On the stem-inoculated TSA plants, the disease developed rapidly, generally 4 days after injection with the virulent isolates 10, 10Q, 446, and 506. The leaves above the point of inoculation turned bright yellow and necrotic around the midrib followed by complete wilting of the plant. Leaf abscission was common. The plants were completely dead in about 10 days.

None of the RS isolates caused disease when applied in a foliar spray with Silwet L-77. This is likely due to the lack of necessary inoculum threshold to cause disease; foliar-applied inoculum would be less capable of delivering a critical level of inoculum into the plant tissues compared to stem injection that would deliver high levels of inoculum directly into the stem. Since RS is a vascular pathogen that normally requires a wound to enter the plant, the stem injection is clearly the more effective inoculation method.

2. Cut-and-swab inoculation

With isolates 446 and 506, the treatment differences were evident 2 weeks after inoculation when the control plants were beginning to resprout and appeared healthy while the inoculated plants failed to resprout (**Fig. 1A**). After 12 weeks, 100% of the plants treated with the high inoculum level were killed whereas only the shoot number and height were reduced in the low inoculum level treatment (**Table 1**). This is important because a reduction in shoot size could in turn reduce fruit and seed production, i.e., rate of spread (Bryson and Byrd 2007).

3. Host-range study

The results of the host-range study revealed differences in the pathogenicity and virulence of the isolates to different species and cultivars. While our aim was to find an isolate with restricted in host range to TSA, several hosts were moderately to highly susceptible to different isolates. Host reaction to isolates 10 and 10Q that were highly virulent to TSA varied: pepper and tomato cultivars were resistant to isolate 10 but not to 10Q. Eggplant accession 34 was slightly susceptible to both 10 and 10Q while accession 35 was highly susceptible to 10Q. Since there was no distinct host selectivity between these isolates, 10Q was chosen for further evaluations.

Field trials

1. Hand-inoculation trials

While hand-inoculation is not a practical method, the results from the Sumter and Levy counties trials showed that the bacterium was capable of controlling TSA under field conditions. At the Sumter site, 69% of the inoculated plants resprouted and grew normally after 4 weeks compared to 100% of the control plants that regrew and remained healthy ($Pr> \chi^2 = 0.0014$). At the Levy County site, there were 10 and 40% resprouted plants, respectively, after 3 weeks ($Pr> \chi^2 = 0.0004$) (**Table 2**). As previously stated, the plants at the Sumter site were more robust because they were growing in full sun, and the control plants resprouted strongly at this site. By contrast, plants at the Levy site were spindly because they were growing in the shade, and as a result, the injection inoculation procedure was more traumatic to these plants. This was further evidenced by the high incidence of wilting without disease symptoms among the control plants. Even so, the proportion of inoculated plants that resprouted was significantly lower than in the control plants. Another, characteristic observed on the injection-inoculated plants was the localization of the disease symptoms. This was observed more frequently on large plants in which wilting occurred only above the point of inoculation.

Table 1 Results of greenhouse inoculation of mature tropical soda apple plants 12 weeks after inoculation.

Treatment	Number sprouts per stem	Average height per sprout
No bacterial inoculum	A 2.6	A 24.9 cm
Low bacterial inoculum	B 0.8	B 11.4 cm
High bacterial inoculum	C 0.0	B 0.0 cm

Duncan's mean separation p=0.05.

Table 2 Results from injected treatments [a].

County	Inoculated % Resprouted	Control % Resprouted	$Pr> \chi^2$
Sumter	69	100	0.0014
Levy	10	40	0.0004

[a] Control plant did not exhibit bacterial wilt symptoms and resprouted normally.

Table 3 Results from cut and swabbed treatments.

County	Inoculated - % Resprouted	Control- % Resprouted [a]	$Pr > \chi^2$
Sumter	21	100	0.0001
Levy	10	70	0.0001

[a] Control plant did not exhibit bacterial wilt symptoms and resprouted normally.

Treatment responses for the Sumter and Levy County sites analyzed, each separately, by the Lifetest procedure indicated that once a plant wilted, it would often deteriorate badly therefore it was not possible to rate in subsequent weeks. At the Sumter site, in treatments where sprouting was assessed, there was a high incidence of insect defoliation of young succulent shoots by Colorado potato beetle (*Leptinotarsa decemlineata*) and tomato hornworm caterpillar (*Manduca quinquemaculata*). If a plant that sprouted was severely defoliated by insects, then it too was not possible to be evaluated later.

For the cut-and-swab treatments, a resprouted plant was one that, after clipping, regrew and coppiced. At the Sumter site, 21% of inoculated plants resprouted compared to 100% of the control plants after 4 weeks ($Pr> \chi^2 = 0.0001$). At the Levy County site, 10% of the inoculated plants and 70% of the control plants resprouted after 3 weeks ($Pr> \chi^2 = 0.0001$) (**Table 3**). The response at the Sumter County site was greater because the control plants resprouted strongly while those inoculated did not. By contrast, at the Levy site, where the plants were smaller under the shaded conditions, the control plants resprouted less and a much smaller proportion of the bacteria-inoculated plants resprouted.

2. Burch Wet-Blade trials

The bacterial inoculum was loaded into the spray tank of the BWB mower system (**Fig. 1C**) and the system calibrated to deliver precise amounts of inoculum. The inoculum was dispensed uniformly and precisely as calibrated over the treatment areas (**Fig. 1D**). Healthy control plots adjacent to inoculated plots showed no adverse effects, indicating no off-target effects. An application rate of 23 L/ha for the wet-blade inoculum delivery was chosen because it is a typical delivery rate applied by the BWB and the possibility of

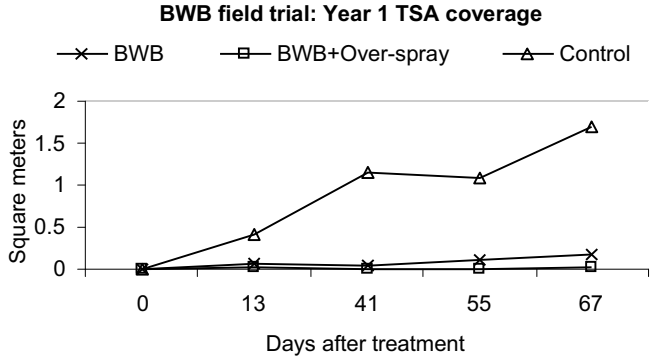

Fig. 2 Year 1 field trial of RS applied with BWB, illustrating the square meters of TSA regrowth in BWB without RS (Control), BWB with RS (BWB), and RS applied with BWB and in an over-spray (BWB+Over-spray), as described under Materials and Methods.

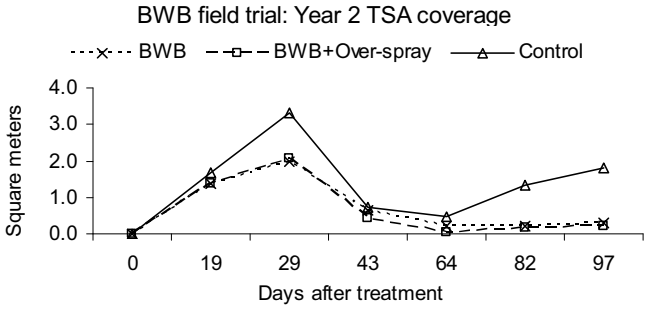

Fig. 3 Year 2 field trial of RS applied with BWB, illustrating the square meters of TSA regrowth in BWB without RS (Control), BWB with RS (BWB), and RS applied with BWB and in an over-spray (BWB+Over-spray), as described under Materials and Methods.

controlling TSA at such a low volume was a targeted goal. At this rate, it is believed that the inoculum was delivered at its lowest effective threshold. The amount of over-spray, 560 L/ha, was derived from the amount used for the cut-and-swab treatment in the Sumter and Levy counties trials. Accordingly, a rate that had proven success was chosen as an alternative treatment. For a comparative measure, this provided a pseudo common treatment between the experiments in north and south Florida.

Two weeks after inoculation in the first-year experiment, plants in the control plots resprouted while plants in the treated plots did not. After 67 days, TSA regrowth resulted in 5.9, 0.1, and 0.1% ground cover in the control, BWB, and BWB + over-spray treatments, respectively (**Fig. 2**). However, there was no difference between BWB-applied RS and the BWB/spray-applied RS.

In the second year, TSA in the control treatment regrew strongly until week 4 after treatment, but the coverage declined after week 4 and recovered again after 8 weeks (**Fig. 3**). TSA regrowth in the two RS treatments paralleled the control treatment but the plants did not regrow to the same extend as the control. By week 8 and beyond the RS treatments were significantly different from the control.

Both RS treatments reduced TSA regrowth by more than 90% of the predicted coverage in year 1 ($P = 0.0003$).

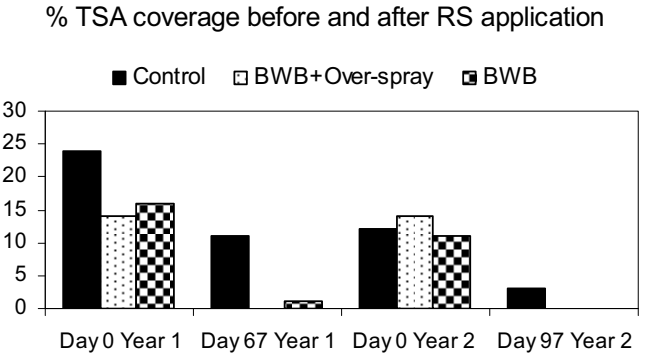

Fig. 4 Percent TSA coverage before (Day 0) and after (Day 67 or 97) application of RS in Years 1 and 2, respectively. The bacterial pathogen was applied with BWB or BWB and an over-spray, as described under Materials and Methods. The control consisted of BWB with water only (no RS).

The predictive equations from regression analyses of treatment responses for trials from Years 1 and 2 are presented in **Table 4**. The less dramatic results from the second year trial could be due to the drier conditions and cooler average temperatures that prevailed: As described under Materials and Methods, the cumulative average rainfall and maximum and minimum temperatures during the second year trial were much lower and less conducive for both TSA growth and infection by RS than during the first year trial.

Both the BWB and BWB + over-spray treatments reduced the cover of TSA to nil, or almost nil, compared to the control in both years of the field study (**Fig. 4**) and these treatment effects are shown in the paired-plot images in **Fig. 1D**.

The following conclusions are drawn from these results: 1) When applied as a cut-stem treatment, RS is capable of controlling TSA; 2) application of RS with a single mowing pass by the BWB system can provide 90% or better TSA control; and 3) the BWB mower system is an effective tool to inoculate TSA with RS.

Due to its broad host range and potential for prolonged survival in soil, the RS isolate used in our study will not be developed further as a bioherbicide. Another reason not to pursue RS was our discovery and development of *Tobacco mild green mosaic tobamovirus* as a bioherbicide for TSA (Charudattan and Hiebert 2007). Nevertheless, this study clearly demonstrated the novelty and feasibility of applying a wilt-causing pathogen with the Burch Wet-Blade mower system to control TSA.

Anderson and Gardner (1999) used an isolate of RS from ginger (*Zingiber officinale*) to control kahili ginger (*Hedychium gardnerianum*) in Hawaii. Kahili ginger, a native of the Himalayas and widely sold as an ornamental plant, is an invasive weed in the Hawaiian tropical forests. Application of a cell suspension of the ginger isolate to wounded roots or injecting it into shoots killed the inoculated khalili ginger within a few weeks after inoculation. Although the destructiveness of this strain to edible ginger was a concern, the authors suggested that the risk of contaminating ginger plantings is unlikely because of the remoteness of kahili ginger infestations in forests where the biocontrol operations would take place. The efficacy of this

Table 4 Predictive equations from regression analyses of treatment responses from Burch Wet-Blade trials from Years 1 and 2.

Treatment	Equation
Control	SqM (pred) = 1.69 + 0.364 (INTSQM) P = 0.0002*
Wet-blade + Over-spray	SqM (pred) = 0.11 - 0.00006 (INSQM) P = 0.9933*
Wet-blade	SqM (pred) = 0.76 - 0.0093 (INSQM) P = 0.8733*
Control	SqM (pred) = 2.06 - 0.041 (INSQM) P = 0.4413*
Wet-blade + Over-spray	SqM (pred) = 0.05 + 0.036 (INSQM) P = 0.8563*
Wet-blade	SqM (pred) = 0.06 + 0.061 (INSQM) P = 0.3088*

* Pr that slope is significantly different from zero.

bioherbicide agent was established in field trials but it is not known whether any further work has been carried out.

Silwet L-77 has been shown to facilitate the entry of bacterial cells into leaves via the stomata (Zidack *et al.* (1992). Application of bacteria in an aqueous solution of Silwet helps to negate the need to injure the plant before or during inoculation. However, in our study, foliar application of RS was not effective even under disease conducive-conditions in the greenhouse.

The wet-blade mower systems may be effective in delivering other vascular-invading and wilt-inducing pathogens such as *Xanthomonas campestris* pv. *poae* to control *Poa annua*, *Chondrostereum purpureum* against broadleaved woody weeds, and *Tobacco mild green mosaic tobamovirus* against TSA.

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