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Effects of Selected Herbicides on the Efficacy of Tobacco Mild Green Mosaic Virus to Control Tropical Soda Apple (*Solanum viarum*)

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Experiments were initiated to determine if the tropical soda apple (TSA) biological control agent, *Tobacco mild green mosaic tobamovirus* (TMGMV), could be mixed with synthetic herbicides to provide effective broad-spectrum weed control. When TMGMV was mixed with 2,4-D ester or amine, metsulfuron, or hexazinone, TSA control ranged between 80 and 100%. On average, TMGMV increased TSA control by 81% as compared to these herbicides applied alone. Treatment applications were made by rubbing only three leaves, not as a broadcast application. Although this is not the optimum method for herbicide application, it does indicate the level of control the herbicide alone potentially provided relative to the herbicide/TMGMV mixture. Results indicate that the majority of TSA control was due to virus and that the herbicides mixed with TMCMV did not interfere with the virus's ability to infect TSA. Additions of organosilicone adjuvants or low rates of crop oil or nonionic adjuvants to TMGMV solutions resulted in greater infection of TSA. The finding that TMGMV remains infective when mixed with herbicides will allow greater flexibility for landowners attempting to control TSA and other troublesome weeds.

Nomenclature: 2,4-D amine; 2,4-D ester; dicamba; hexazinone; metsulfuron; tropical soda apple; *Solanum viarum* Dunal SOLVI.

Key words: Biocontrol, herbicide-virus interaction, pasture, TMGMV.

Tropical soda apple (TSA) is a perennial weed that currently infests over 400,000 ha in Florida (Mullahey 1996), but also extends as far north as Tennessee and Pennsylvania (Mullahey et al. 1998). This weed infests pastures, roadsides, citrus groves, and natural areas of Florida (including local, state, and Federal preserves) where it outcompetes native plant species (Langeland and Burks 1998). The invasiveness of this weed has led it to be listed on both the Florida and Federal Noxious weed lists (Anonymous 2006a,b).

Many experiments have been conducted to determine a cost-effective and efficacious means of controlling TSA. Previous research concluded that triclopyr, picloram, glyphosate, and aminopyralid were the most effective herbicide options (Call et al. 2000; Ferrell et al. 2006; Mislevy et al. 1999; Mullahey et al. 1993). However, picloram and glyphosate are not readily used because the former is not registered for use in Florida and the latter is nonselective and highly injurious to desirable forage grasses. This has led to great reliance on triclopyr in the past for TSA control, whereas aminopyralid is now the most common herbicide used. Regardless if triclopyr or aminopyralid is used, both have potential drawbacks that can limit their use. Triclopyr often provides inconsistent control if plants are mature or fruiting at application (Mislevy et al. 1997) and lacks soil residual activity (Vencill 2002). Therefore, mowing is often required prior to the triclopyr application to ensure that all plants are actively growing in order to maximize control (Ferrell and Mullahey 2005). However, even if this is accomplished and complete control of emerged plants is achieved, rapid reinfestation of TSA from seed is common. Alternatively, aminopyralid is highly effective on TSA of all sizes and maturities (Ferrell et al. 2006). However, vegetable crops, including tomato, pepper, and melons, are commonly planted in Florida as part of a forage grass renovation program. With the significant level of residual control achieved with aminopyralid on TSA, the replant interval for other solanaceous crops (tomato, pepper, eggplant, etc.) is currently unknown. Therefore, in rotational pastures an alternative means of controlling TSA is needed.

A search for suitable pathogens to be used as a bioherbicide was conducted. It was determined that *Tobacco mild green mosaic tobamovirus* (TMGMV) induced a systemic, lethal, hypersensitive response in TSA (Charudattan et al. 2004). TMGMV, a member of the *Tobamovirus* genus of plant viruses, is a mechanically transmitted RNA virus that only infects plants (Brunt et al. 2007). Other closely related viruses were tested, such as *Tobacco mosaic virus* U1 and *Tomato mosaic tobamovirus*, but only mild leaf mosaic/mottling was observed and TSA death did not occur (Charudattan et al. 2004). This indicated that TMGMV was the best option of the strains tested for use as a bioherbicide for TSA control.

TMGMV is highly effective on TSA and has been shown to control 100% of plants that have become infected by the virus (Charudattan et al. 2004). TMGMV is not absorbed into healthy plant leaves, but requires entry through a wound (Charudattan et al. 2004). It has been established that TSA plants could be infected by broadcast applications at 420 kPa or low-pressure applications with slight wounding of the plant (Charudattan et al. 2004). In addition to being highly effective and easily applied, it has been shown that TMGMV could be used safely as a bioherbicide despite its infectivity to several solanaceous species besides TSA. Considering that applications of TMGMV only kills TSA among the weed species present at the site of application, additional herbicides might have to be used to increase the weed control spectrum of this bioherbicide. Additionally, many herbicides are formulated with organic solvents and other additives; it is unknown if tank mixes with commercially formulated herbicide products will denature and deactivate the virus.

The objectives of this study were to determine if TMGMV can retain it efficacy against TSA when combined with pasture herbicides and spray adjuvants. The herbicides and adjuvants used here are all commonly used for weed and brush control in Florida.

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Materials and Methods

TSA plants were propagated from seed in a shade-house enclosure. Potting soil[1] was placed in seedling trays and TSA seeds that were collected throughout Florida were sown at a depth of 3 to 4 mm. At the three- to four-leaf stage, TSA seedlings were transferred to 4 L pots at a density of one plant per pot. Plant age at time of experiment initiation ranged from 60 to 90 d. However, all plants were of uniform age within an experimental run. Temperature and daylength were ambient conditions occurring from June through November in Gainesville, FL. During the growing period, average temperature ranged from 27 to 20 C, and relative humidity of 70 to 80% was common.

TSA inoculation. Herbicides were selected that are commonly used for weed control on pastures and rangelands, but possess marginal activity on TSA (Call et al. 2000). Those with marginal activity were chosen so that TSA death would most likely be due to TMGMV and not confounded by herbicide effects. The herbicides used were 2,4-D amine[2] and ester[3] (1 kg ha^{-1}); 2,4-D + dicamba[4] (0.72 + 0.25 kg ha^{-1}); dicamba[5] (1 kg ha^{-1}); metsulfuron[6] (0.02 kg ha^{-1}); and hexazinone[7] (0.75 kg ha^{-1}). Florida producers commonly use these herbicides at the above rates with applications made at 280 L ha^{-1}. In this experiment, each herbicide was added to 20 ml of water and final solution concentration was equal to an application made at 280 L ha^{-1}.

The TMGMV isolate used for experimentation was maintained in infected, frozen, tobacco tissue in the plant virus collection at the Plant Pathology Department, University of Florida, Gainesville, FL. A virus extract from the stored tobacco was combined with 20 mM sodium phosphate buffer (pH 7.2). An aliquot of the virus-buffer solution, sufficient to obtain a virus concentration of 50 mg ml^{-1}, was added to the herbicide solutions within 1 h of inoculation. Also, 20 mg of carborundum (600-grit) was added to this solution to serve as an abrasive to aid virus entry. Three leaves on each TSA plant were inoculated by gently rubbing the leaf on the adaxial surface with a piece of cheesecloth soaked in herbicide/virus solution. Three leaves on each plant were treated in this manner. After inoculation, the plants were watered daily and fertilized with a hose-attached spreader weekly for 60 d. Visual estimates of percent control were obtained (0 = no control and 100 = complete control) prior to the plant harvest for testing using antigen-trapped indirect enzyme-linked immunosorbant assay (ELISA). The experiment contained 14 individual treatments: six herbicides with and without virus as well as a positive and negative virus control (negative control consisted of buffer solution and carborundum only). Each treatment was replicated five times and the experiment was repeated three times.

The use of a spray adjuvant commonly is recommended in Florida to enhance herbicide performance. The most commonly used adjuvants in pasture and rangeland are nonionic surfactants and crop oil concentrates, with minor uses of the organosilicones. The adjuvant experiment was conducted in a similar fashion to that described above. Nonionic surfactant[8] (0.125 and 0.5% v/v), crop oil concentrate[9] (0.5 and 1.5% v/v), and organosilicone[10] (0.025 and 0.25% v/v) application rates were based on the minimum and maximum application concentrations as suggested by the manufacturer. Because adjuvants are commonly used in combination with herbicides, determining if they interfere with virus infection is of great importance. This experiment had three replications per treatment and was conducted twice. A negative virus control was also included.

Tropical soda apple leaf tissue was tested for TMGMV using ELISA. The procedure was a modification of that used by Siegmann et al. (1998), Elliott et al. (1996), and Yeh and Gonsalves (1984). Absorbance readings at a wavelength of 405 nm were taken at 15-min intervals using a microplate reader.[11] Each sample was replicated in three wells and the average of the 1-h incubation values was used for evaluation. In most cases, A_{405} values equal to or greater than two times the average of the control plants (noninoculated) were considered positive (Sutula et al. 1986). In some cases, values of 100 points but not two times greater than the controls were considered positive.

The herbicide experiment consisted of a completely randomized design with 5 replications, and was repeated three times. The surfactant experiment was also completely randomized, but had three replications and was repeated twice. An analysis of variance test was performed and a significant treatment by run interaction was observed. Therefore, data were reported separately for each experimental run. For these data, a one-tailed *t*-test with pair-wise comparisons was used to determine if treatments provided significantly greater control than the untreated.

Results and Discussion

We observed that 2,4-D ester and amine, metsulfuron, and hexazinone provided between 80 and 100% TSA control when applied in combination with TMGMV (Table 1). Control of TSA with these herbicides in the absence of TMGMV ranged between 0 and 29%. The presence of TMGMV with these herbicides, on average, increased TSA control by 81% over those treated with herbicide alone. We can conclude from these data that TMGMV was the primary agent causing TSA injury.

Control achieved with dicamba + TMGMV ranged between 70 and 89% and between 36 and 77% with dicamba alone. Likewise, 2,4-D + dicamba resulted in 84 to 98% TSA control with the virus and 21 to 58% without. The increased activity of these herbicides on TSA was expected because dicamba has been previously shown to provide a significant level of TSA control (Akanda et al. 1997). Although the dicamba concentration in this experiment was lower than the 2.2 kg ha^{-1} as used by Akanda et al. (1997), the rate of 1 kg ha^{-1} as used here is more common and still provided significant activity.

Using ELISA analysis, TMGMV was generally not detected in plants that were not inoculated, except in the case of treatments with dicamba and hexazinone where three of 15 and one of 15 plants were infected, respectively. This occurrence was likely due to untreated plants accidentally brushing against inoculated plants. The cumulative detection ratio as determined by ELISA for the positive control (None +) was 10 of 15 (Table 2), whereas the ratios of 2,4-D amine and 2,4-D + dicamba were both 11 of 15. The detection ratios ranged from four of 15 for 2,4-D ester to six of 15 for dicamba and hexazinone and seven of 15 for metsulfuron.

The lower-than-expected detection ratio of the positive controls, as determined by ELISA, might be due to the nature

Table 1. Visual estimates of tropical soda apple control 60 d after herbicide treatment with or without the biocontrol agent *tobacco mild green mosaic virus*. Means differing by a sum greater than the LSD value are considered statistically different at 0.05 level of significance.

			Control		
Herbicide	Virus	Rate	June 21, 2006	September 9, 2006	November 15, 2006
	kg ha^{-1}		%		
Untreated	+	—	96	90	96
	−		0	0	10
2,4-D amine	+	1.0	95	100	95
	−		16	8	26
2,4-D ester	+	1.0	89	92	80
	−		19	5	—
2,4-D + dicamba	+	0.72 + 0.25	84	98	90
	−		21	40	58
Dicamba	+	1.0	89	70	88
	−		36	57	77
Metsulfuron	+	0.02	92	94	92
	−		15	0	—
Hexazinone	+	0.75	95	93	96
	−		12	0	29
LSD 0.05			12	16	15

of the lethal hypersensitive reaction and the sampling method. Systemic infection of TMGMV-inoculated plants is determined by the presence of virus in new leaves that developed after inoculation. Although the virus can often be detected in the inoculated leaves, this does not indicate a systemic infection because it might never spread beyond that leaf and move throughout the plant. Also, one to three leaves can develop before the virus reaches the apical meristems. Frequently in TSA plants that develop a lethal hypersensitive reaction, the virus never reaches the newest leaves before most of the leaves abscise and the plant collapses (personal observation). If these new leaves are sampled, virus might not be detectable using ELISA in spite of the fact that the rest of the plant is dead. This phenomenon is often observed in young, rapidly growing plants (unpublished data).

Stress is another factor that can affect plant response to a herbicide or virus. Virus infection rates are reduced when TSA plants are stressed by drought or flooding. Likewise, experimental plants that are stressed, such as being large and pot-bound, occasionally become infected but fail to die or die very slowly (unpublished data).

The pH of four of the six herbicide solutions ranged from 5 to 7, well within the range for virus viability. The pH of both the dicamba and hexazinone solutions was between 7 and 8. The activity of TMV, a close relative of TMGMV, is reduced and then eliminated as pH exceeds pH 8.0 (Hull 2002). This likely is the case for TMGMV and might also account for the lower ELISA absorbance values and visual ratings for these herbicides.

Perhaps the most significant unknown is viral replication and movement in a plant that is responding physiologically to a herbicide. The development of tiny new distorted leaves following the epinasty and abscission symptoms typical of TMGMV disease development suggests that viral progression might have been altered. This resulted in plants that lost most or all of the leaves after inoculation but did not die.

These phenomena, as well as other environmental factors, might account for the variations between the studies and the lower than expected control by some of the virus–herbicide combinations. However, we can conclude that TMGMV remains infective and can be mixed with all of the herbicides to achieve at least 80% control and that greatest control can

Table 2. ELISA analysis of infection ratio of tropical soda apple control 60 d after herbicide treatment with or without the biocontrol agent tobacco mild green mosaic virus.

			Detection rate		
Herbicide	Virus	Rate	June 21, 2006	September 9, 2006	November 15, 2006
	kg ha^{-1}				
None	+	—	4/5	2/5	4/5
	−		0/5	0/5	0/5
2,4-D amine	+	1.0	3/5	4/5	4/5
	−		0/5	0/5	0/5
2,4-D ester	+	1.0	0/5	3/5	1/5
	−		0/5	0/5	0/5
2,4-D + dicamba	+	0.72 + 0.25	2/5	5/5	4/5
	−		0/5	0/5	0/5
Dicamba	+	1.0	3/5	0/5	3/5
	−		1/5	0/5	2/5
Metsulfuron	+	0.02	2/5	2/5	3/5
	−		0/5	0/5	0/5
Hexazinone	+	0.75	2/5	2/5	2/5
	−		0/5	0/5	1/5

Table 3. Impact of adjuvant on the ratio of TMGMV infection of tropical soda apple.

		Rate of detection	
Adjuvant	Concentration (%)	September 9, 2006	November 15, 2006
Crop oil	0.5	3/3	2/3
Crop oil	1.5	1/3	1/3
Nonionic	0.125	2/3	2/3
Nonionic	0.5	0/3	1/3
Organosilicone	0.025	2/3	3/3
Organosilicone	0.25	2/3	2/3
Blank	—	0/3	0/3
Virus only	—	3/3	2/3

be achieved with 2,4-D amine; 2,4-D + dicamba; and hexazinone.

Adjuvants. A representative from each of the previously mentioned adjuvant classes was selected and mixed with the virus solution at the minimum and maximum concentration as suggested by the manufacturer. It was observed that high detection ratios (five of six) occurred when crop oil and organosilicone were used at their lowest concentrations (Table 3). Additionally, the highest concentration of the organosilicone and the lowest concentration of the nonionic surfactant also had little impact on the virus because detection ratios were four of six. However, the highest concentrations of the crop oil and nonionic surfactant adversely affected virus performance and decreased the detection ratio to two of six and one of six, respectively.

The reason for reduced virus activity when mixed with nonionic surfactant and crop oil adjuvants is likely due to the detergent properties of these products. Nonionic adjuvants have both hydrophilic and lipophilic portions on the molecule, which allow increased herbicide solubility in water (Hazen 2000). However, this hydrophilic/lipophilic balance also allows the adjuvant to interact with other molecules. It has been reported that sodium dodecyl sulfate (SDS), which also acts as a detergent, can effectively denature the protein coat of tobacco mosaic virus (Rafikova et al. 2004), a close relative to TMGMV. Therefore, it is likely that the active ingredients in the nonionic adjuvant, when used at higher rates, denatured the virus and led to reduced incidence of infection. The same trend was observed with the crop oil adjuvant. Considering that crop oil adjuvants possess a blend of emulsifiable oils and fatty acid esters (similar to that of nonionic adjuvants), it is likely that higher rates resulted in a sufficient concentration of the detergent to interfere with the virus.

From these data, we conclude that TMGMV can be mixed with all the herbicides tested in this experiment without significant reduction in virus activity. Additionally, organosilicone adjuvants and low rates of crop oils and nonionic adjuvants can be included with TMGMV/herbicide mixtures. Previous research has shown that broadcast applications performed at 420 kPa or low-pressure applications with slight wounding of the plant are sufficient to induce infection (Charudattan et al. 2004). Currently, additional research is ongoing to determine if different apparatus can be attached in front of a low-pressure application to adequately induce wounding and subsequent infection. Therefore, if adequate application techniques can be developed, this bioherbicide can be mixed with herbicides for broad-spectrum postemergence control of TSA and other commonly occurring weeds. This combination could be particularly helpful in Florida and its neighboring states if TSA control is necessary in areas where aminopyralid can not be used or if herbicide drift onto nearby sensitive crops is a concern.

Sources of Materials

[1] Metro-mix 300, Scotts–Sierra Horticultural Products Co., Marysville, OH 43040.

[2] Weedar 64, Nufarm Inc., Burr Ridge, IL 60527.

[3] 2,4-D LV 4, Nufarm Inc., Burr Ridge, IL 60527.

[4] Banvel + 2,4-D, Micro Flo Co., LLC, Memphis, TN 38117.

[5] Banvel, Micro Flo Co., LLC, Memphis, TN 38117.

[6] Cimarron 60DF, E. I. duPont de Nemours and Co., Wilmington, DE 19898.

[7] Velpar 2L, E. I. duPont de Nemours and Co., Wilmington, DE 19898.

[8] Induce, 90% blend. Helena Chemical Co., Collierville, TN 38017.

[9] Agri-Dex, 99% blend. Helena Chemical Co., Collierville, TN 38017.

[10] Silwet L-77, 99% blend, Helena Chemical Co., Collierville, TN 38017.

[11] EL800 Universal Microplate Reader, Bio-Tek Instruments, Inc., 100 Tigan St., Winooski, VT 05404.

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