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Effect of carrier volume and adjuvant with foliar applications of triclopyr on Brazilian peppertree

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ABSTRACT

Brazilian peppertree (*Schinus terebinthifolia* Raddi) is a troublesome invasive shrub that grows across many wetland environments in Florida. It is notorious for prolific epicormic sprouting from the root collar and lateral roots following management efforts, and this greatly frustrates management efforts. Although high and low volume foliar treatments with triclopyr are common, there is a lack of data examining how application volume might influence triclopyr efficacy. Furthermore, assessment of different adjuvant types in relation to triclopyr efficacy and carrier volume are also lacking. To address this, greenhouse experiments were conducted from 2019 to 2020 at the University of Florida Center for Aquatic and Invasive Plants in Gainesville, Florida. Triclopyr was applied to Brazilian peppertree at 1.12 kg ae ha^{-1} in conjunction with either a protein-based adjuvant at three rates (0.6, 1.2, and 2.3 L ha^{-1}) or a standard methylated seed oil (MSO) at 1.2 L ha^{-1}. Both were tested at low (187 L ha^{-1}) and high (935 L ha^{-1}) carrier volumes. We found that the protein-based adjuvant's performance was independent of rate and there were very few differences in triclopyr efficacy when applied with either adjuvant. No treatment effects were observed on percent defoliation at 75 or 180 d after treatment (DAT). Regardless of adjuvant type, plants treated at 187 L ha^{-1} exhibited 2.7 fewer epicormic shoots per plant and ~11% lower live cambium height at 180 DAT compared to those treated at 935 L ha^{-1}. After plants were excised at 180 DAT and allowed to regrow for 60 d, plants treated at the lower carrier volume regrew 1.6 fewer epicormic shoots per plant, which equated to ~25% less biomass per plant compared to those treated at the high carrier volume. Spray card data indicated adjuvant type did not influence above- or below-canopy spray coverage. However, the high application volume resulted in greater spray coverage both above and below the canopy than the low application volume. These data suggest that reducing carrier volume for triclopyr applications to peppertree can result in greater efficacy and this work supports the need for future research and development of low carrier volume application techniques for use in foliar triclopyr operations targeting Brazilian peppertree.

Key words: Brazilian peppertree, foliar application technique, methylated seed oil, protein-based adjuvant, *Schinus terebinthifolia* Raddi, triclopyr.

INTRODUCTION

Brazilian peppertree (*Schinus terebinthifolia* Raddi) is an invasive shrub that is widespread throughout peninsular Florida, south Texas, and Hawaii. As a member of the Anacardiaceae, it invades canal banks and, growing up to the water's edge, tolerates seasonal inundation in freshwater wetlands, and also tolerates considerable brackish conditions in coastal mangrove habitat. Due to its complex tangled canopy structure, dense brace root systems, toxic sap, and prolific epicormic shooting, Brazilian peppertree management is difficult for ground crews (Langeland et al. 2008). Mechanical and physical control methods for Brazilian peppertree have included cutting and mulching machines; however, these methods are labor intensive and do not provide long-term control due to prolific shooting from epicormic buds on the root collar and lateral roots (Cuda et al. 2006). A biological control agent [*Pseudophilothrips ichini* (Hood) Insecta: Thysanoptera: Phlaeothripidae] has recently been released that reduces plant biomass (Manrique et al. 2014). However, the long-term effectiveness of this insect to provide control is still uncertain. Chemical control methods have primarily relied on triclopyr, imazapyr, and glyphosate (Doren and Jones 1997, Gioeli and Langeland 1997) applied by a variety of techniques, including basal bark, cut stump, injection, and aerial- and ground-based foliar treatments (Bell 2019).

For invasive plant control, foliar herbicide applications can vary in carrier volumes, depending upon the approach used. For example, aerial treatments can be applied at 94 to 187 L ha^{-1}, whereas vehicle-mounted handgun treatments are often applied at very high volumes of 935 L ha^{-1}. Low application volumes are applied at higher herbicide concentrations but generally do not result in complete coverage, especially of the subcanopy. For high application volumes (935 L ha^{-1} or higher), the goal is to deliver a low-concentration herbicide solution in a manner that provides 100% coverage to all foliage (Kline and Duquesnel 1996). The difficulty in optimizing herbicide dose to the target in the most efficient manner is often exacerbated by prolific resprouters such as Brazilian peppertree (Woodall 1982, Doren 1997).

To our knowledge, the effect of carrier volume on Brazilian peppertree's response to triclopyr has never been directly investigated. However, evidence in the literature suggests that optimal herbicide carrier volume is specific to each herbicide and species combination (Knoche 1994).

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Some auxin herbicides have been shown to be more effective when applied at lower carrier volumes despite reductions in spray coverage due to a steepened herbicide concentration gradient at the leaf surface (Knoche 1994). For example, auxin type herbicides such as picloram and 2,4,5-T uptake via droplets on leaves of woody species increased when the concentration of the herbicide outside the leaf was higher (Davis et al. 1968). Likewise, 2,4-D exhibited greater control of common lambsquarters (*Chenopodium album* L.), white mustard (*Sinapis alba* L.), and wild mustard (*Sinapis arvensis* L.) as carrier volume decreased (Scoresby and Nalewaja 1984; Knoche 1994). However, reducing carrier volume can sometimes lead to reduced efficacy. For example, reducing triclopyr carrier volume from 281 to 47 L ha^{-1} resulted in marginal Virginia creeper [*Parthenocissus quinquefolia* (L.) Planch.] control due to poor lower canopy penetration (Tworkoski et al. 1988). Also, 2,4,5-T applied at lower carrier volumes significantly reduced phytotoxicity on upland cotton (*Gossypium hirsutum* L.) and mesquite [*Prosopis juliflora* (Sw.) DC.] (Behrens 1957). Several studies have also reported no effect of carrier volume on auxin herbicide efficacy (McKinlay et al. 1972, Merritt and Taylor 1977). Consequently, it is unknown how reducing carrier volume of foliar triclopyr applications in Brazilian peppertree would affect efficacy.

Although utility adjuvants serve a multitude of purposes related to herbicide delivery, historically, most activator spray adjuvants have fallen into basic categories such as stickers or spreaders (Penner 2000). However, some more recent adjuvants include protein blends that are difficult to categorize. One example is the protein-based adjuvant, AMP↑™ (hereafter referred to as AMP), which has been primarily registered as an adjuvant for submersed herbicide treatments. According to the product label, it consists of a yeast protein extract (55.0%), alcohol ethoxylate (22.5%), sodium laurel ether sulfate (7.5%), and inert ingredients (15%). The original patent suggests the protein polypeptide component enhances the activity of several herbicides, possibly through enhanced absorption (Ratajczyk et al. 2018). AMP is labeled for use in submersed herbicide applications up to 3.79 L product 1,233 m^{-3}. A mesocosm study indicated its addition to 2,4-D reduced median lethal dose (LC_{50}) values from 0.77 mg ae L^{-1} to 0.34 mg ae L^{-1} for Eurasian watermilfoil (*Myriophyllum spicatum* L.) (Ratajczyk et al. 2018). Additionally, preliminary data indicated it was comparable to a nonionic surfactant when mixed with triclopyr for foliar treatment of the invasive plants, including Old World climbing fern [*Lygodium microphyllum* (Cav.) R. Br.] and shoebutton ardisia (*Ardisia elliptica* Thunb.) (Elroy Timmer, pers. comm.). However, there are no directions on the product label regarding use for foliar herbicide applications, and therefore the adjuvant potential of AMP under terrestrial settings is largely unknown.

Given these issues, our objectives in this experiment were to evaluate effects of: 1) the potential of a novel protein-based adjuvant (AMP) compared to a commonly used adjuvant and 2) evaluate the role of carrier volume for foliar triclopyr applications to Brazilian peppertree.

MATERIALS AND METHODS

A greenhouse experiment was conducted twice in the summer of 2019 at the University of Florida's Center for Aquatic and Invasive Plants (29°43′17.55″N, 82°25′2.28″W). Brazilian peppertree plants were established from seed collected from a roadside infestation in Largo, FL (27°52′58.20″N, 82°48′42.21″W) approximately 3.9 km from the coast. Seedlings were transplanted into 1-L pots filled with potting mix[1] and maintained for 12 mo. These were then transplanted into 11.3 L-pots and grown for an additional 1 yr until saplings were approximately 0.9 m in height and had a root collar diameter of 2.5 to 3 cm. Throughout this period, plants were watered and fertilized with a slow-release fertilizer[2] as needed.

The experiment was established as a completely randomized design with four replications per treatment and an augmented factorial arrangement of treatments. Factors included a protein-based adjuvant[3] at three rates (0.6, 1.2, and 2.3 L product ha^{-1}), a methylated seed oil (MSO)[4] at two rates (0 and 1.2 L product ha^{-1}), and two carrier volumes (187 or 935 L ha^{-1}). All treatments except the nontreated control (NTC) contained the acid formulation of triclopyr[5] at 1.12 kg ae ha^{-1} and spray indicator dye[6] at 0.25% v v^{-1} for colorimetric coverage analysis. In this study we chose a low rate of triclopyr so that the effect of application volume and adjuvant system could be observed without being overcome by herbicide rate.

Treatments for the two experimental runs were applied on September 5 and September 12, 2019. Treatments were applied using a CO_2-pressurized backpack sprayer equipped with TeeJet 11002 DG nozzles[7] calibrated to deliver 187 or 935 L ha^{-1} at 276 kPa. Walking speed was the only application parameter adjusted using a metronome to change carrier volume to ensure droplet size spectrum was not profoundly impacted by nozzle size, type, or operating pressure among treatments (Butts et al. 2018). All treatments were applied outside of the greenhouse in an open environment and plants were not returned to the greenhouse until foliage was dry to eliminate off-target particle drift or herbicide vaporization.

For each treatment, three spray cards[8] per experimental unit (EU), 5 by 8 cm in size, were placed 91 cm above the ground on ring stands unobstructed by plant canopies to quantify spray coverage representing the "crown" canopy coverage. Likewise, four spray cards per EU were placed equidistant from the base on the soil surface directly below peppertree foliage to quantify spray interception by peppertree canopies. Following treatment applications, spray cards were left to dry for approximately 15 min. Cards were then scanned, converted to 8-bit format, threshold adjusted, converted to a binary format, and analyzed for percent coverage using ImageJ software (National Institute of Health; Schneider et al. 2012). In addition, the frequency of spray puddling was evaluated on each spray card as a binary variable (present or absent) by observing deeper blue coloring on card margins. However, the presence of spray puddling did not impact spray coverage quantification software.

Posttreatment assessment of Brazilian peppertree response included visual evaluations of plant defoliation, stem cracking, presence of epicormic shoots, live cambium height, and epicormic shoot biomass. Visual evaluations were recorded at 75 and 180 d after treatment (DAT), and all other variables were collected at 180 DAT. Visual evaluations were conducted using a scale of 0 to 100%, where 0% indicated no defoliation and 100% indicated no leaves on the plant. Presence of stem cracking (binary variable: present or absent) was characterized by cracks or breaks in the outer and inner bark with visible damage to the cambium around the entire stem above epicormic shoots (where present). This is classic auxin-type herbicide symptomology that readily manifests in young woody plants. Presence of epicormic shoots were defined as new shoots at least 1 cm in length emerging from the lower 30 cm of the primary stems of each experimental unit. We rarely observed new shoots emerging from lateral roots, but the pot size might have precluded extensive lateral root growth. Live cambium height was determined by scraping the bark of each experimental unit with a razor blade, starting at the terminal bud moving downward toward the potting soil surface. The distance from the soil surface to the maximum height of live cambium was then recorded. Epicormic shoots were then counted, harvested, dried in a forced-air oven at 65 C for 72 h, and weighed. Following this, all plants were cut with pruning shears at 10 cm above the soil surface. These plants were then maintained for an additional 60 d postharvest, at which time epicormic shoot number and biomass were collected again at 240 DAT.

Statistical analysis

Data were subjected to mixed-model ANOVA to test for main effects and interactions using R software (version 3.6.1) in the "stats" and "lme4" packages (Bates et al. 2015, R Core Team 2019). Percent data were arcsine-square root transformed to improve normality and homogeneity of variance. However, back-transformed means are presented for clarity. Adjuvant type and carrier volume were included as fixed effects, whereas adjuvant rate, experimental run, and replicate (nested in experimental run) were considered random effects (Blouin et al. 2011). The model was reduced to include the protein-based adjuvant rate as a random effect after failure to identify a significant rate effect in initial models ($P > 0.18$). Where significant effects (α=0.05) were detected, means were separated using Fisher's protected LSD test ($\alpha = 0.05$) in the "emmeans" package in R (Lenth 2020). Furthermore, a separate *t*-test ($\alpha = 0.05$) was conducted to determine significant differences between the NTC and treatments.

RESULTS AND DISCUSSION

Adjuvant type and carrier volume did not influence peppertree defoliation at 75 or 180 DAT (data not shown). At 75 DAT, defoliation was consistent across both adjuvant types and carrier volumes with a narrow range from 50 to 56%. At 180 DAT, defoliation was again consistent across adjuvant types and carrier volumes, with a range from 86 to 96%, excluding the NTC. These data suggest a comparable defoliation response by Brazilian peppertree to the two

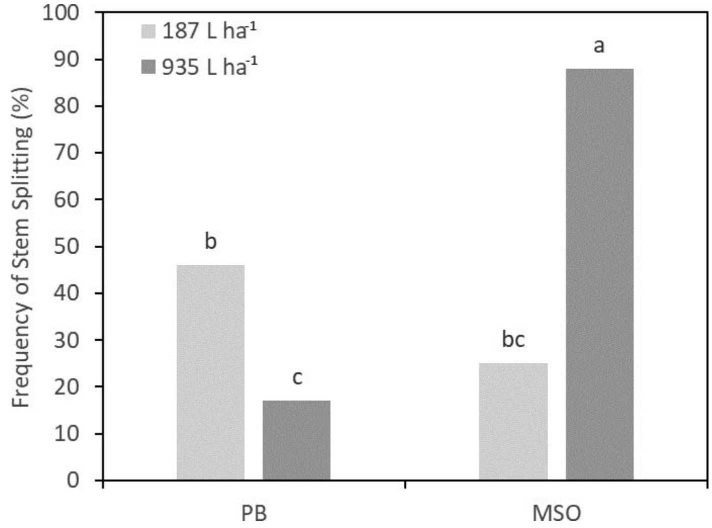

Figure 1. Frequency of Brazilian peppertree stem cracking 180 d after treatment in response to the interaction between adjuvant type (protein-based [PB] or methylated seed oil [MSO]) and carrier volume. No stem cracking was observed on nontreated plants. Treatment bars sharing the same letters are not different according to Fisher's protected LSD test ($\alpha = 0.05$).

adjuvants and carrier volumes tested. This is noteworthy as the protein-based adjuvant was as effective as the methylated seed oil commercial standard. Additionally, triclopyr, when applied at the rate used in this study, resulted in a very slow defoliation of Brazilian peppertree. This would seem ideal from an auxin type herbicide translocation standpoint, provided metabolism of the herbicide was not meaningful.

At 180 DAT, an interaction between adjuvant type and carrier volume was detected in Brazilian peppertree stem cracking frequency (Figure 1). The interaction was driven by a differential response between each adjuvant at the high carrier volume. Stem cracking with the protein-adjuvant applied at the low carrier volume was approximately 30% greater than the comparative high-volume treatment. The opposite occurred for MSO, which had 60% greater frequency of stem cracking in the high carrier volume compared to the low carrier volume. A higher frequency of stem cracking might suggest greater triclopyr absorption through thin bark that was facilitated by MSO, but not the protein-based adjuvant. Increased uptake of aminocyclopyrachlor with the addition of MSO has been documented in silk tree (*Albizia julibrissin* Durazz.) (Koepke-Hill et al. 2012).

Epicormic shoot presence was affected only by carrier volume at 180 DAT (Table 1). When pooled across adjuvant type, epicormic shoots were observed 28% more frequently on plants treated at 935 L ha^{-1} (72%) than 187 L ha^{-1} (44%). A similar pattern was also detected for epicormic shoot number, where the high carrier volume treatments resulted in 2.7 more epicormic shoots per plant than the low-volume treatments (Table 1). Epicormic shoot formation as a survival mechanism following stress events, such as herbicide treatment, is commonly observed in Brazilian peppertree (Force 1997). However, foliar-applied herbicide

TABLE 1. FREQUENCY AND NUMBER OF BRAZILIAN PEPPERTREE EPICORMIC SHOOTS PER PLANT AND LIVE CAMBIUM HEIGHT 180 D AFTER TREATMENT AS AFFECTED BY ADJUVANT TYPE AND CARRIER VOLUME IN TREATMENTS CONTAINING TRICLOPYR AT 1.12 KG AE HA^{-1}.

Main Effect	Epicormic Shoots Frequency (%)	Epicormic Shoots No. Plant^{-1}	Cambium Height % of Total Height
Adjuvant[1]			
AMP↑™	64 A[2]	5.5 A*	40 A*
MSO	50 A	2.7 A*	36 A*
Carrier volume (L ha^{-1})			
187	44 b	3.4 b*	34 b*
935	72 a	6.1 a*	45 a*

[1]AMP↑™ = protein-based adjuvant; MSO = methylated seed oil.
[2]Means within columns and main effects followed by the same letter are not different according to Fisher's protected LSD test (α = 0.05). Uppercase and lower-case letters are used to distinguish mean separations within main effects.
*An asterisk indicates a significant difference from the nontreated control at (α = 0.05). The nontreated control means were 0 shoots per plant, 100% cambium height, and 137 cm total height.

treatments, especially at lower rates as was tested in the current experiment, more frequently result in epicormic shoot formation compared to other application methods such as cut-stump or basal bark (Enloe et al. 2015). Consequently, these data suggest that reducing carrier volume in foliar treatments might have promoted greater initial herbicide uptake, resulting in greater inhibition of epicormic shoot formation.

Similar to epicormic shoot data, live cambium height did not differ between adjuvant types and ranged from 36 to 40% of the NTC height (Table 1). However, carrier volume did influence live cambium height which was 11% lower in the low carrier volume treatments (34%) than the high carrier volume treatments (45%). This indicated greater proportional stem necrosis and top down kill in the low carrier volume treatments compared to the high carrier volume treatments.

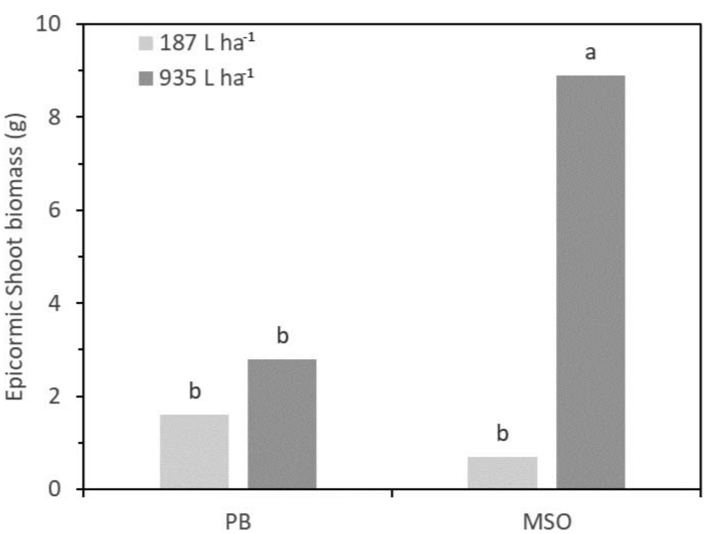

Figure 2. Brazilian peppertree shoot biomass 180 d after treatment in response to the interaction between adjuvant type (protein-based [PB] or methylated seed oil [MSO]) and carrier volume. Shoot biomass of nontreated plants was 0 g. Treatment bars sharing the same letters are not different according to Fisher's protected LSD test (α = 0.05).

TABLE 2. NEW BRAZILIAN PEPPERTREE GROWTH AT 240 D AFTER TREATMENT (DAT), WHICH WAS 60 D AFTER 180 DAT HARVEST, AS AFFECTED BY ADJUVANT TYPE AND CARRIER VOLUME IN TREATMENTS CONTAINING TRICLOPYR AT 1.12 KG AE HA^{-1}.

Main Effect	Epicormic Shoots, No. Plant^{-1}	Shoot Biomass, g Plant^{-1}
Adjuvant[1]		
AMP↑™	5.3 A*[2]	0.82 A*
MSO	2.9 B*	0.56 B*
Carrier volume (L ha^{-1})		
187	4.3 b*	0.66 b*
935	5.9 a*	0.89 a*

[1]AMP↑™ = protein-based adjuvant; MSO = methylated seed oil.
[2]Means within columns followed by the same letter are not different according to Fisher's protected LSD test (α = 0.05). Uppercase and lower-case letters are used to distinguish mean separations among main effects.
*An asterisk signifies a significant difference from the nontreated control at (α = 0.05). Mean epicormic shoots and shoot biomass in the nontreated control were 11.3 shoots and 2.89 g, respectively.

For epicormic shoot biomass at 180 DAT, there was a significant interaction between adjuvant type and carrier volume (Figure 2). The interaction was driven by significantly higher shoot biomass in the high carrier volume MSO treatment compared to all other treatments. However, treatments with the protein-based adjuvant did not exhibit differences in shoot biomass among carrier volumes. The differential biomass response among carrier volumes in MSO treatments, but not the protein-based adjuvant treatments, could be an artifact of the pooled protein-based adjuvant rates, whereas MSO was only tested at a single rate. Conversely, these biomass data might indicate that the protein-based adjuvant might buffer herbicide uptake across carrier volumes compared to MSO. However, further testing is needed to confirm these hypotheses.

At 240 DAT, which was 60 d following the initial epicormic shoot harvest and stem cutting, total new epicormic shoots and shoot biomass were affected by adjuvant type and carrier volume independently (Table 2). Treatments containing MSO resulted in 2.4 fewer new epicormic shoots per plant compared to protein-based adjuvant treatments. Treatments applied at 187 L ha^{-1} resulted in 1.6 fewer shoots per plant compared to 935 L ha^{-1} treatments. New shoot biomass data mirrored shoot number data (Table 2).

Spray coverage, as derived from spray card collectors, was only affected by carrier volume (Table 3). This suggests that neither adjuvant type differed in their impact on physical solution properties that would alter spray coverage. As expected, greater carrier volume resulted in greater spray coverage both at the crown and below the peppertree canopy. However, the difference in spray coverage between above and below canopy was 27% in the 187 L ha^{-1} treatments compared to 11% in the 935 L ha^{-1} treatments. Consequently, despite both carrier volumes resulting in some spray deposition below canopy, the total spray retention from the low volume applications was greater. This is also supported by the higher frequency of spray puddling on cards from the 935 L ha^{-1} treatments, where 14% of cards below canopy showed puddling. This was significantly higher than puddling frequency on spray cards from the 187 L ha^{-1} treatments, which was only 2%. Similar to the current study, Katovich et al. (1996) reported triclopyr spray retention on purple loosestrife (*Lythrum*

Table 3. Spray coverage and spray card puddling frequency above and below Brazilian peppertree canopy as affected by carrier volume and adjuvant type in treatments containing triclopyr at 1.12 kg ae ha^{-1} under greenhouse conditions.

	Spray Coverage, %		Frequency of Puddling, %	
Main Effect	Above Canopy	Below Canopy	Above Canopy	Below Canopy
Adjuvant[1]				
AMP↑™	93 A[2]	69 A	27 A	7 A
MSO	91 A	83 A	29 A	6 A
Carrier volume (L ha^{-1})				
187	89 b	62 b	8 b	2 b
935	96 a	85 a	51 a	14 a

[1]AMP↑™ = protein-based adjuvant; MSO = methylated seed oil.
[2]Means within columns followed by the same letter are not different according to Fisher's protected LSD test ($\alpha = 0.05$). Uppercase and lower-case letters are used to distinguish mean separations between main effects.

salicaria L.) increased as carrier volume decreased from 935 to 94 L ha^{-1} which in turn resulted in a greater amount of triclopyr retained per plant. However, reducing carrier volume too low could lead to reduced efficacy due to poor canopy penetration such as what has been reported in Virginia creeper (Tworkoski et al. 1988). Consequently, finding an optimal carrier volume is specific to each application scenario because practitioners should strive to maximize plant coverage yet reduce spray deposition below the target canopy that is wasted or could potentially affect nontarget species.

These studies provide valuable insights into several key issues regarding foliar treatment of Brazilian peppertree. First, our data indicated that a novel protein-based adjuvant generally resulted in comparable efficacy to a commonly used methylated seed oil-based product. This is noteworthy because there is little published information on novel adjuvant types such as this. However, in no case did we see enhanced activity of the protein-based adjuvant over the commercial standard at the single rate of triclopyr used. Future research with novel protein-based surfactants should incorporate additional active ingredients at multiple rates to better understand the ratios required to potentially enhance activity as the patent suggests (Ratajczyk et al. 2018).

Second, although short-term efficacy at 75 DAT was similar among carrier volumes, our study indicated a difference between them in longer-term (180 and 240 DAT) plant response data. In almost all cases, the high carrier volume resulted in greater frequency, number, and biomass of epicormic shoots and higher live cambium height than the low carrier volume. This presents a difficult issue for land managers, who frequently use high-volume foliar applications for Brazilian peppertree control. In general, low volume applications are more common in aerial treatments, which are limited to large area treatments. Additional research is needed to improve low volume ground-based foliar application techniques. This would be beneficial in terms of both improved treatment efficacy from improved spray retention on the canopy and less off-target issues from leaf runoff of the herbicide from high volume applications.

SOURCES OF MATERIALS

[1]Sungrow® MM510 potting mix, Sun Gro Horticulture, 770 Silver St, Agawam, MA 01001.

[2]Osmocote Complete Slow release fertilizer, The Scotts Company LLC, 14111 Scottslawn Rd, Marysville, OH 43040.

[3]Protein-based adjuvant, AMP↑™ Activator, Applied Biochemists, 1400 Bluegrass Lakes Pkwy, Alpharetta, GA 30004.

[4]Premium Methylated Spray Oil, Helena Chemical Company, 225 Schilling Blvd #300, Collierville, TN 38017.

[5]Tryclopyr, Trycera, Helena Chemical Co, 225 Schilling Blvd #300, Collierville, TN 38017.

[6]Spray indicator dye, Turf Mark® Blue, Becker Underwood Inc., 801 Dayton Ave, Ames, IA 50010.

[7]TeeJet 1102 DG nozzles, TeeJet® Technologies, Spraying Systems Co., 200 North Ave, Glendale Heights, IL 60139.

[8]Spray cards, Kromecote Photo Paper, CTI Paper USA, 1535 Corporate Center Dr #400, Sun Prairie, WI 53590.

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