Direct and indirect toxicity of triclopyr and a nonionic surfactant on the salvinia weevil

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ABSTRACT

Biological control of giant salvinia (Salvinia molesta Mitchell) with the salvinia weevil (Cyrtobagous salviniae Calder & Sands) is considered the cornerstone of giant salvinia control programs in the southern portion of the invaded U.S. range. However, secondary invasion by Cuban bulrush [Oxycaryum cubense (Poepp. & Kunth) Lye] onto floating mats of giant salvinia might disrupt the dispersal and impact of the weevil. Additionally, it is uncertain how herbicides used for Cuban bulrush management may impact the weevil via direct or indirect effects. To improve the biological control of giant salvinia and manage Cuban bulrush, it is important to determine the impact (i.e., toxicity) of the aquatic herbicide triclopyr on both the weevil and giant salvinia. Therefore, laboratory and mesocosm studies were conducted to determine the direct and indirect toxicity of triclopyr (1.68 and 3.36 kg acid equivalent [a.e.] ha^{-1}) alone and with a nonionic surfactant (0.25% v v⁻¹) on adult salvinia weevils and determine the impact of triclopyr on giant salvinia biomass. In laboratory bioassays, the direct application of triclopyr (3.36 kg a.e. ha^{-1}) alone and in combination with a nonionic surfactant resulted in 32 and 37% weevil mortality, respectively. The higher rate treatments were different than the reverse osmosis (RO) water treatment but not the nontreated reference 7 days after treatment (DAT). However, no other differences in insect mortality were detected among treatments. Additionally, no differences were detected among treatments when triclopyr was applied to giant salvinia to measure indirect weevil mortality and plant biomass 7 DAT. These results suggest that triclopyr has limited impacts on the salvinia weevil and could be used to control Cuban bulrush in salvinia weevil-rearing sites.

Key words: biological control, chemical control, Cyrtobagous salviniae, integrated pest management, Oxycaryum cubense, Salvinia molesta.

INTRODUCTION

Giant salvinia is considered one of the worst aquatic weeds in southeastern United States (Harley and Mitchell 1981,

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McFarland et al. 2004). This free-floating aquatic fern is native to southern Brazil. In the late 1990s, it was introduced along the Texas-Louisiana border in the Toledo Bend Reservoir and has since invaded at least 13 other states (Owens et al. 2004, Coetzee and Hill 2020). Giant salvinia infestations are managed via chemical, physical, mechanical, and biological control methods. Although chemical control methods can provide rapid results, they can become costly for land managers because of the size of infested sites (Mudge et al. 2013, 2016; Coetzee and Hill 2020). In 2001 the salvinia weevil was released in Louisiana as a biological control agent for giant salvinia (Tipping et al. 2008). Its effectiveness in controlling giant salvinia is attributed to the feeding habits of both adult and larval life stages (Sands et al. 1983, Tipping et al. 2008, Wahl et al. 2020).

Adults feed on the meristematic tissue of new and older fronds, causing scars resembling bullet holes on the emergent fronds (Sands and Schotz 1984, Sands et al. 1986). Although the adults may be found on top of the salvinia mat, the majority of their time is spent between feeding, reproducing, or resting within buds, between fronds, along the rhizomes, within the roots (underwater), or in deeper plant layers within the mat (Sands et al. 1983, Julien et al. 1987, Tipping et al. 2008, Wahl et al. 2020). The adult weevil behavior and location within the mat is a key factor when investigating whether biological control can be combined with chemical control. Previous research investigating integrated control of giant salvinia evaluated the direct and indirect effects of aquatic herbicides (Mudge et al. 2013, Wahl et al. 2018, Moran et al. 2023). Based on these laboratory studies, direct exposure refers to a topical application of the herbicide onto the adult weevil to simulate the insect coming into direct contact with a droplet of spray solution while located on the surface of the plant. The indirect effects were measured by allowing natural dispersal of the weevils throughout the plant mat before applying herbicide to the foliage of the plant canopy, resulting in a more realistic type of exposure.

Giant salvinia mats serve as a floating substrate that can be colonized by native and invasive emergent plants. Cuban bulrush is a free-floating, epiphytic perennial sedge that is native to South America and the West Indies (McLaurin et al. 2019, Clarke et al. 2023). Cuban bulrush has been found within mats of giant salvinia, and it has quickly spread across the southeastern United States, especially in Louisiana, Mississippi, and Texas, where both species thrive concurrently (USFWS 2018, McLaurin et al. 2019, Nachtrieb et al. 2019). Once established, it forms large floating islands, or tussocks, by weaving its runners between the established roots or rhizomes of other aquatic vegetation such as giant

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salvinia and water hyacinth [*Eichhornia crassipes* (Mart.) Solms] (Mallison et al. 2001, Bryson et al. 2008). Large tussocks of Cuban bulrush and giant salvinia have significant detrimental effects on aquatic ecosystems such as blocking sunlight and decreasing dissolved oxygen levels in the water, as well as impeding navigation and limiting access to waterways, disrupting drainage, and altering water quality (Mallison et al. 2001, Turnage 2018). The formation of thick mats leads to the blockage of sunlight penetration and decreased dissolved oxygen levels.

Natural resource managers use foliar and submersed herbicide treatments to control Cuban bulrush, with triclopyr being a commonly used herbicide (Turnage 2022). Triclopyr's mode of action targets auxin pathways that causes uncontrolled growth and eventual plant death and is highly effective against terrestrial and aquatic weeds, including Cuban bulrush (Watson and Madsen 2014, Turnage 2020). Currently, chemical management remains the primary control method since no Cuban bulrush biological control agents are available and the use of harvesters, shredders, or other mechanical control can be costly and time consuming (University of Florida 2023). Since Cuban bulrush is frequently found in mats of giant salvinia in field (Bryson and Carter 2008, Nachtrieb et al. 2019) and weevil-rearing pond (Pham 2023) settings, it is important to screen herbicides that are efficacious against Cuban bulrush and provide selectivity to giant salvinia and the weevil so that critical weevil-rearing sites for biological control programs are protected. Therefore, the direct and indirect toxicity of triclopyr and a surfactant were investigated to determine the impact of the auxin herbicide on adult salvinia weevil mortality. In addition, assessing whether exposure of giant salvinia to triclopyr will result in damage to the plant and a reduction in biomass under short-term exposures was also investigated. These findings may improve salvinia weevilrearing efforts and provide vital information about the interactions between chemical and biological control methods for combating an invasive aquatic plant.

METHODS AND MATERIALS

Plant and insect colonies

Tertiary growth stage giant salvinia was reared at the LSU AgCenter Aquaculture Research Facility in Baton Rouge, LA, in 1,325-L outdoor tanks that contained pond water (pH 8.5 to 9.0) amended with peat moss (14 g) to lower and maintain a pH of 5.0 to 6.0. Nutrients (10 mg L^{-1} N^{1}) were also added periodically to maintain healthy plant growth throughout the course of the trial. Using Berlese funnels, adult salvinia weevils were extracted from giant salvinia obtained from an outdoor weevil-rearing pond at the LSU AgCenter Reproductive Biology Center in St. Gabriel, LA (Wahl et al. 2018). Weevil-infested plant material was dried for 72 h in the Berlese funnels, which allowed the adults to crawl down into collection bags² containing 1 g of fresh giant salvinia and nutrient-amended water (pH 5.0 to 6.0; Wahl et al. 2016). Harvested salvinia weevils were acclimated in a growth chamber³ for 7 days at 24 C (\pm 1) and a 14h:10-h (light:dark) photoperiod in 0.5-L plastic containers

(7.6 cm by 8.6 cm by 11.7 cm) with fresh giant salvinia and nutrient-amended water (pH 5.0 to 6.0).

Direct impacts of triclopyr with surfactant on adult salvinia weevils

Laboratory bioassays were conducted and repeated one month apart at the LSU Department of Entomology in Baton Rouge, LA, in August and September 2022. These bioassays were designed to simulate the direct exposure of the salvinia weevils to the herbicide triclopyr and a nonionic surfactant (Mudge et al. 2013, Wahl et al. 2018, Moran et al. 2023). Herbicide stock solutions were prepared by diluting triclopyr⁴ and the surfactant⁵ into separate containers with reverse osmosis (RO) water that was the equivalent to a diluent of 935 L ha⁻¹ or the amount of water used commercially in a spray tank when treating giant salvinia in field settings. Treatments included low and high rates of triclopyr (1.68 and 3.36 kg a.e. ha^{-1}), a surfactant at 0.25% $v v^{-1}$, and combinations of the low and high rates of triclopyr with surfactant (Table 1). Nontreated and RO control groups were included to evaluate any adult mortality that occurred without chemical exposure (Mudge et al. 2013, Wahl et al. 2018).

Prior to herbicide exposure, 15 acclimated adult salvinia weevils were removed from the plant material and placed into Petri dishes using soft forceps. Each Petri dish was randomly assigned a treatment (n = 7), and each treatment was replicated three times (n = 3; 45 salvinia weevils per treatment). From the stock solutions, 2 µl of RO water, herbicide, surfactant, or a combination of the two was pipetted directly onto each weevil in the Petri dishes (Mudge et al. 2013, Wahl et al. 2018). The solution was allowed to dry for 10 min, and the adults were then placed onto 10 g (±0.5) of fresh giant salvinia that were inside plastic containers with 200 ml of nutrient-amended water. The containers were covered with a fine mesh (approximately 0.1 cm) and returned to the growth chamber set at the conditions described above.

At 7 days after treatment (DAT), all adult weevils were removed from the plant material and placed into Petri dishes using soft forceps. After 10 min of acclimation, each adult was individually examined for mortality. The acclimation period was utilized since salvinia weevils can become rigid and immobile when disturbed (Mudge et al. 2013,

TABLE 1. TRICLOPYR AND SURFACTANT TREATMENTS APPLIED DIRECTLY AND INDIRECTLY TO ADULT SALVINIA WEEVILS IN LABORATORY AND MESOCOSM SETTINGS.

Treatment ¹	Rate ²
Nontreated Reverse osmosis water ³ Low triclopyr High triclopyr Low triclopyr + nonionic surfactant High triclopyr + nonionic surfactant Nonionic surfactant	

¹Two microliters of treatment solution applied to each weevil.

²The herbicides and surfactants were mixed in reverse osmosis water at an equivalent of 935 L ha⁻¹ diluent to provide a solution similar to a commercial herbicide application to control giant salvinia.

³Reverse osmosis water treatment applied only in direct exposure trial.

Wahl et al. 2018). Mortality was recorded if no movement was observed after 10 min, indicating no signs of life, or if they were found deceased at the bottom of the container. Conversely, if the salvinia weevils exhibited movement, such as antennae movement, they were recorded as alive.

Indirect impacts of triclopyr with surfactant on adult salvinia weevils

An outdoor mesocosm experiment was conducted and repeated at the LSU AgCenter Aquaculture Research Facility in September 2022 to evaluate the indirect effects of a foliar triclopyr application on adult salvinia weevils residing within the giant salvinia mat. These experiments were repeated with a 2-wk interval. Mesocosm experiments were housed in 76-L plastic containers (49.5 cm diam by 58.4 cm tall) and filled with pond water (pH 8.5 to 9.0). Approximately 14 g of peat moss was added to each mesocosm to maintain pH between 6.0 and 7.0. Fertilizer¹ (10 mg L⁻¹ N) was added to the water column, and equal amounts of giant salvinia were placed into each mesocosm container to cover approximately 70% of the water surface.

Weevil-infested giant salvinia was collected from an outdoor weevil-rearing pond at the LSU AgCenter Reproductive Biology Center in St. Gabriel, LA, and weevils were live extracted using the Berlese funnel extraction method (Wahl et al. 2018). Upon extraction, 20 adult salvinia weevils were counted and placed in 0.5-L containers with 10 g (\pm 0.5) of fresh giant salvinia with 200 ml of water that contained the same fertilizer as describe above (n = 5; 100 salvinia weevils per treatment). The adults were immediately released into their randomly assigned mesocosms. To ensure the weevils acclimated to the mesocosm environment and established themselves within the plant material prior to herbicide application, all mesocosms were covered with fine mesh (approximately 0.1 cm) to prevent weevil migration and allowed to acclimate for 7 days.

The indirect exposure experiments included a nontreated reference and five chemical treatments: low and high rates of triclopyr (1.68 and 3.36 kg a.e. ha^{-1}), a surfactant at 0.25% v v⁻¹, and combinations of the herbicide (low and high rates) and surfactant (Table 1). Treatments were randomly assigned to each mesocosm and replicated five times. Herbicide treatments were applied to the foliage using a forced-air CO₂-powered sprayer at an equivalent of 935 L ha^{-1} of diluent delivered through a single brass flat fan 80-0067 nozzle⁶ at 138 kPa (Mudge et al. 2013, Wahl et al. 2018).

At 7 DAT, weevil-free and weevil-infested giant salvinia were destructively harvested by collecting the plant material in each mesocosm and placed into labeled plastic bags. The plant material was transferred to nets where excess water from each bag was drained for 5 min, and the wet weights of the plants were measured and recorded⁷. Plant material was placed into Berlese funnels for 72 h to extract salvinia weevils and dry the giant salvinia (Harms et al. 2009, Wahl et al. 2016). Surviving salvinia weevils were collected and counted from collection bags containing 100% EtOH that were attached to the end of the Berlese funnels. Weevil mortality was assigned to any missing weevils that had not moved out of the plant material during the extraction process. Following the 72-h weevil extraction period, the plants were placed on a scale to obtain dry weight for each treatment.

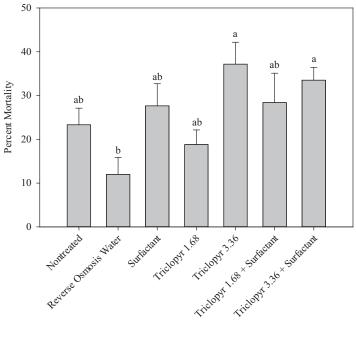
Statistical analysis

In the direct toxicity study, there were no differences in adult salvinia weevil mortality between bioassay trials, so the data were pooled (P = 0.07). In the indirect toxicity study, no differences were seen between the trials for adult salvinia weevil mortality (P = 0.7), so the two mesocosm trials were also pooled. The data from both studies met the assumptions of normality and homogeneity of variance, so one-way analysis of variance (ANOVA) tests were performed for both experimental studies. Significant differences among groups were determined at a significance level of $\alpha = 0.05$ and further analyzed using a post-hoc Tukey's test. All statistical tests were performed using JMP[®] Pro 16.2.0⁸.

RESULTS AND DISCUSSION

Direct impacts of triclopyr with a surfactant on adult salvinia weevils

Adult salvinia weevil mortality differed among the direct exposure treatments in the laboratory experiment (P = 0.008; Figure 1). The mortality from the high rate of triclopyr alone (3.36 kg a.e. ha⁻¹) and high rate in combination



Treatments

Figure 1. Percent mortality (\pm SE) of adult salvinia weevils 7 days after direct exposure to triclopyr applications and a nonionic surfactant (0.25% v v⁻¹) in a laboratory setting (P = 0.008). Numbers following triclopyr on x-axis represent herbicide rates in kg a.e. ha⁻¹. Treatments with different letters are statistically significant according to the post-hoc Tukey's test at $\alpha < 0.05$ (n = 6).

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with the nonionic surfactant $(0.25\% \text{ v v}^{-1})$ treatments was approximately threefold higher for adult salvinia weevils compared to the RO water control treatment (Figure 1). However, no differences were found in weevil mortality when comparing the nontreated reference to any herbicide or surfactant treatments. These data indicate that a foliar application of triclopyr can be applied where weevilinfested giant salvinia coexists with Cuban bulrush and there will be limited impacts to the biological control program. Notably, the direct mortality was higher compared to other trials (Mudge et al. 2013, Wahl et al. 2018, Prinsloo et al. 2022), potentially because of the study being conducted later in the growing season (August and September) with older weevils. Accurate aging of the weevils was not feasible, as they were randomly selected from weevil-rearing ponds maintained under field conditions.

Previous studies have demonstrated varying levels of triclopyr toxicity among invertebrate species. Although triclopyr has been shown to be highly toxic to certain aquatic invertebrates, such as netspinning Caddisfly spp. (Hydropsyche) and brushlegged mayfly spp. (Isonychia) (Kreutzweiser et al. 1994, Tu et al. 2001), its impact on terrestrial invertebrates has exhibited greater variability. For instance, studies evaluating triclopyr toxicity to the broom seed beetle [Bruchidius villosus F. (Chrysomelidae)] and the oil palm pollinating weevil [Elaeidobius kamerunicus Faust (Coleoptera: Curculionidae)] observed 0% and 63% mortality, respectively (Affeld et al. 2004, Setyawan et al. 2020). Lindgren et al. (1998) determined triclopyr amine had no impact on oviposition of the classical biological control agent black-margined Loosestrife Leaf Beetle (Galerucella calmariensis), which is used to manage purple loosestrife (Lythrum salicaria) This variability prompted the concern that triclopyr would significantly impact salvinia weevil mortality; however, direct exposures to salvinia weevils in a laboratory setting did not support this hypothesis. The highest triclopyr rate applied directly to salvinia weevils resulted in a mortality rate of 37%, which is notably lower than the 63% mortality observed when the oil palm pollinating weevil was exposed to a lower field rate of triclopyr (Setyawan et al. 2020). This discrepancy suggests that salvinia weevils may possess a higher tolerance to triclopyr compared to other terrestrial invertebrates. Further studies are necessary to investigate the long-term impacts of triclopyr exposure on salvinia weevil adults, such as fecundity and feeding behavior.

Indirect impacts of triclopyr with a surfactant on adult salvinia weevils

Although weevil mortality was observed across all treatments in the outdoor indirect exposure trial (22 to 40%), no differences were found among treatments (P = 0.17; Figure 2). These results suggest that triclopyr rates had minimal effect on adult salvinia weevil mortality after indirect exposure when applied to the foliage of giant salvinia. These results support the use of foliar applications of triclopyr by natural resource managers to target Cuban bulrush in areas where salvinia weevils have previously been released.

Future studies should investigate the behavior of adult salvinia weevils following foliar applications of triclopyr to

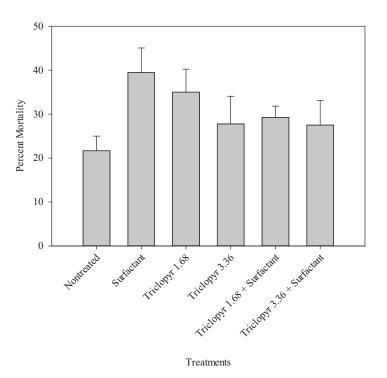
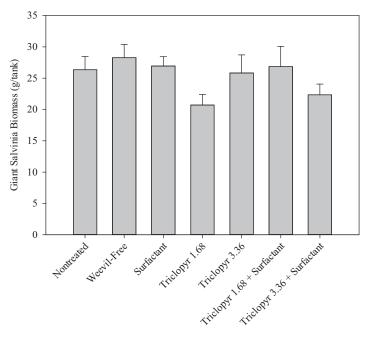


Figure 2. Percent mortality (\pm SE) of adult salvinia weevils 7 days after indirect exposure to foliar applications of triclopyr and a nonionic surfactant (0.25% v v⁻¹) targeting giant salvinia in a mesocosm setting (P = 0.17; n = 10). Numbers following triclopyr on x-axis represent herbicide rates in kg a.e. ha⁻¹.

plants in mesocosm or field settings, as adult salvinia weevils can easily disperse to healthier plants via flight (Room et al. 1981, Micinski et al. 2016). Recording flight behavior following an herbicide application would determine if impacts other than mortality may hinder the weevil's ability to colonize and disperse within the mat of giant salvinia and/or to healthy/nontreated plant material. Moreover, previous studies observed adult salvinia weevils underneath the fronds, within the submerged fronds, or on the submerged rootlike structure (Forno et al. 1983, Grodowitz et al. 2014), indicating that they would be sheltered by the plant mat from the herbicide application. However, insects feeding on tissue treated with triclopyr could present unknown issues since herbicide half-life in plants can vary from 3 to 24 days after application (Ganapathy 1997, USEPA 1998, Shaner 2014, Tomlin 2015). Carruthers et al. (2023) examined the impacts of two formulations of triclopyr, imazapyr, glyphosate, and a methylated seed oil adjuvant on adult South American thrips (Pseudophilothrips ichini), through both direct application and feeding studies of treated plants. Pseudophilothrips ichini survival was significantly reduced by direct application of triclopyr, and insect survival was reduced by 50% after insects fed on triclopyr-treated plants. In addition, the Brazilian peppertree (Schinus terebinthifolia) biocontrol agent demonstrated aversive behaviors such as departure from triclopyr-treated plants following foliar and basal bark applications. Similar postherbicide application research is warranted to determine if triclopyr poses indirect or delayed toxicity to salvinia weevils feeding on herbicide treated plants.

In recent years, the aquatic herbicides 2,4-D, flumioxazin, metsulfuron-methyl, penoxsulam, and a few adjuvants (e.g., nonionic, methylated seed oil, and blended products have been tested for toxicity against salvinia weevils (Mudge et al. 2013, Wahl et al. 2018, Prinsloo et al. 2022, Moran et al. 2023). Wahl et al. (2018) determined that a direct exposure of the other auxinic aquatic herbicide 2,4-D as being less toxic (10% mortality) to the weevils. Foliar applications of 2,4-D also had minimal activity (i.e., selectivity) on giant salvinia biomass (Wahl et al. 2018), which is important since it is also efficacious against Cuban bulrush at similar rates (Turnage 2018, 2020) and it may also be useful for selective control of Cuban bulrush where weevil rearing is needed for integrated pest management (IPM) strategies.

During the indirect exposure studies, visible injury (e.g., chlorosis) of giant salvinia was observed within 1 wk after foliar triclopyr application. However, no differences were seen in giant salvinia biomass among treatment groups, which was expected since this trial was concluded 7 DAT (P = 0.1786; Figure 3). The average giant salvinia biomass for the control and weevil-free treatments was 26.3 g (± 2.1 , SE) and 28.2 g (± 2.12 , SE), respectively. The average giant salvinia biomass across all herbicide and surfactant treatments was 24.5 g (± 2.2 , SE; p = 0.23; Figure 3). Triclopyr, a herbicide that mimics auxin, disrupts plant growth by acidifying and loosening cell walls, thus leading to the destruction of vascular tissue. This mechanism provides an explanation for the observed plant injury 7 DAT (Tu et al. 2001). Although triclopyr is used to control Cuban bulrush, its effectiveness on giant salvinia is not well understood (McFarland et al. 2004). Triclopyr is generally used to control



Treatments

Figure 3. Impact of triclopyr and a nonionic surfactant $(0.25\% \text{ v v}^{-1})$ on giant salvinia dry weight (\pm SE) 7 days after herbicide was applied to the foliage of plants in a mesocosm setting (P = 0.23; n = 10). Numbers following triclopyr on x-axis represent herbicide rates in kg a.e. ha⁻¹.

broadleaf and woody species (Shaner 2014). However, Glueckert et al. (2023) evaluated three formulations of triclopyr against Old World Climbing Fern (*Lygodium micro-phyllum*) and found all to provide control of the invasive species.

One of the primary objectives of this research was to determine short-term impacts of triclopyr on weevil mortality either through direct or indirect applications, which is the reason this research was concluded 7 DAT. Therefore, future studies should monitor plant response (e.g., biomass reduction, percent injury, and recovery) following triclopyr and surfactant applications for an extended period to determine long-term impacts on giant salvinia (Mudge et al. 2013, 2016). By extending the trial, impacts on the nutritional value of plants, impacts to palatability, and biomass reductions that force the insects to migrate to nontreated/ healthy material can be monitored.

In conclusion, an IPM approach is especially needed where Cuban bulrush and giant salvinia coexist and both weeds need to be eliminated or when giant salvinia is considered a nontarget species and must remain unharmed to support the salvinia weevil. Direct and indirect applications of triclopyr had minimal short-term impacts on the mortality of the salvinia weevil; therefore, triclopyr can be used for controlling Cuban bulrush when it coexists with giant salvinia and the biological control agent.

SOURCES OF MATERIALS

¹Miracle-Gro All Purpose Plant Food (24–8–16, N–P–K), Scotts Miracle-Gro Company, 14111 Scottslawn Rd, Marysville, OH 43041.

²Whirl-Pak[®], Nasco[™], 1000 Abernathy Rd NE, Atlanta, GA 30328.

 $^3\mathrm{Model}$ I-36VL, Percival Scientific Inc., 505 Research Drive, Perry, IA 50220.

⁴Triclopyr[®] 3, Alligare, 1565 5th Avenue, Opelika, AL 36801.

⁵Surface[®] Nonionic Surfactant, Alligare, 1565 5th Avenue, Opelika, AL 36801.

⁶TeeJet, TeeJet Technologies, P.O. Box 7900, Wheaton, IL 60187.

⁷A-Series Precision Balance, VWR[®], Suite 200 100 Matsonford Road, Radnor, PA 19087.

 $^{8}\mathrm{JMP}^{\ast}$ Pro 16.2.0, SAS Institute Inc., 100 SAS Campus Dr, Cary, NC 27513.

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