Torpedograss response to herbicide treatment in saturated and flooded conditions

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ABSTRACT

Torpedograss (*Panicum repens* L.) is invasive in wetlands, forming monocultures in flooded areas that are prone to seasonal hydrologic fluctuations. We conducted a mesocosm study to assess the effect of water depth on torpedograss control using foliar applications of aquatic herbicides. Torpedograss was grown in mesocosms at two water depths, 15 cm (saturated) and 45 cm (flooded), and treated with spot treatment concentrations of one of four herbicides: the commonly used commercial standards glyphosate and imazapyr, or the selective graminicides sethoxydim and fluazifop-*P*-butyl. Biomass harvests 45 and 90 days after treatment (DAT) were used to determine plant response to herbicide applications. Plants grown in flooded conditions had similar biomass to those in saturated conditions before herbicide application. However, flooding decreased the root-to-shoot ratio as well as the amount of shoot biomass above the waterline. There was a significant effect of both flooding and herbicide application 45 and 90 DAT. Flooding had a greater impact on the activity of graminicides than the commercial standards by 90 DAT, with sethoxydim providing poor control of aboveground biomass and both sethoxydim and fluazifop-*P*-butyl providing poor control of belowground biomass. These results indicate that graminicide performance may be decreased when applied to torpedograss in flooded conditions. Further research is needed to optimize graminicide treatments for torpedograss where seasonal fluctuations in water levels may influence management outcomes.

Key words: fluazifop-*P*-butyl, glyphosate, imazapyr, *Panicum repens* L., sethoxydim

INTRODUCTION

Torpedograss is a creeping, perennial grass that is native to tropical and subtropical regions of Eurasia and Africa (Hossain et al. 1999). It was introduced to the southeastern United States before 1876 and was intentionally spread throughout Florida in the early 1900s for use as cattle forage (Langeland et al. 1998, 2008). It escaped cultivation and invaded over 70% of Florida's public waters by 1992 (Schardt 1994). Torpedograss possesses an extensive rhizome system that can account for 70 to 90% of its total biomass, facilitating its spread and displacement of native species (Smith et al. 1993). In addition to its impact on native plants, torpedograss negatively alters fish habitat, dissolved oxygen concentrations, and organic material accumulation in the wetlands it invades (Hanlon and Langeland 2000, Stone 2011).

Torpedograss is difficult to manage due to its extensive rhizome system, and herbicides are the primary method of controlling this species. For many years, the nonselective, systemic herbicides glyphosate and imazapyr have been widely used for controlling torpedograss and other invasive aquatic grasses (Smith et al. 1993, Hanlon and Langeland 2000). Although these are effective in controlling torpedograss, their broad-spectrum nature often results in nontarget damage to native species, and there is a need for more selective products to facilitate ecosystem restoration. Recent research has identified two promising graminicides (2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one, or sethoxydim, and (R)-2-[4-[[5-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]propanoic acid, or fluazifop-*P*-butyl) for controlling torpedograss. In mesocosm studies, both have shown comparable levels of control to glyphosate and imazapyr while having minimal impact on broadleaved native species (Enloe and Netherland 2017). Sethoxydim was granted a 24(c) special local needs label for invasive grass management in aquatic habitats in Florida (Anonymous 2017). Fluazifop-*P*-butyl is currently being evaluated for aquatic use under an experimental use permit but is not yet registered.

Torpedograss is able to grow in both upland and wetland habitats, including areas that are prone to fluctuating water levels. When anchored to the sediment, stem fragments are able to initiate root and shoot production and elongate to above the water's surface at depths of up to 75 cm (Smith et al. 2004). Established plants are able to thrive in flooding depths up to 75 cm and are able to tolerate depths of at least 125 cm (Smith et al. 2004). Invasive plants such as torpedograss often display high levels of phenotypic plasticity (defined as the altered phenotypic expression of a genotype that occurs in response to environmental factors), which enables these species to grow in a variety of environmental conditions (Hulme 2008, Davidson et al. 2011). For torpedograss, flooding has been shown to result in changes in morphological features such as stem number

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and biomass allocation (Hossain et al. 2002, Smith et al. 2004).

Previous research has demonstrated that responses to change in environmental factors, such as drought, temperature, and atmospheric carbon dioxide concentrations, can alter plant susceptibility to herbicides (Smeda and Putnam 1990, Ziska and Teasdale 2000, Pereira 2010). Anecdotal evidence from land managers suggests that torpedograss is more difficult to control using foliar applications of herbicides when plants are growing under flooded conditions, and field research indicates that control using sethoxydim may be dependent on several factors including season of treatment and hydroperiod (Enloe et al. 2018). In some species, flooding can cause changes in leaf area, photosynthesis, and root-to-shoot ratios, factors that can affect herbicide efficacy (Chen et al. 2002, Rood et al. 2010, Varanasi et al. 2016). It is possible that flooding results in morphological changes in torpedograss that make it less susceptible to foliar herbicides.

Since chemical control is the most effective method of controlling torpedograss, it is important to understand the effects of flooding on herbicide efficacy for this species. Additionally, it is important to determine the relative effects of flooding on efficacy of the commonly used broad-spectrum herbicides glyphosate and imazapyr, as well as the graminicides sethoxydim and fluazifop-*P*-butyl. To investigate this, we conducted a mesocosm experiment to evaluate herbicide efficacy on torpedograss in saturated and flooded conditions.

MATERIALS AND METHODS

Plant material and flooding treatments

Experiments were conducted at the University of Florida's Center for Aquatic and Invasive Plants in Gainesville, FL. Rhizome fragments (10 cm in length) were planted in 3.8 L pots filled with commercial builder's sand[1] mixed with slow release fertilizer[2] and maintained in greenhouse conditions before initiation of experiments. Pots were placed in shallow tubs filled with well water (7.4 pH) to a depth of 15 cm. At 8 wk after planting, 120 healthy plants were selected based on uniformity and new shoot growth and moved into 100 L mesocosms inside the greenhouse. The mesocosms were filled with the same water source to either 15 cm (top of the pots) to simulate saturated conditions, or to 45 cm (30 cm above the pot soil surface) to simulate flooded conditions. For the flooded treatment, water was initially filled to 15 cm and then raised over a 2-wk period to the pot level. Plants were then allowed to grow at the final water level for an additional 7 d before baseline data collection. Baseline above- and belowground biomass data were then collected on five plants per flooding treatment before herbicide application. Shoots were separated into two sections for biomass measurements: the portion of stems emerging above the waterline, and the portion submerged. All biomass was dried at 65 C until a constant weight before measurement. Biomass measurements were also used to calculate root-to-shoot ratios.

Herbicide treatments

Plants were treated on 30 June 2017 and 11 July 2017 in the first and second experimental runs, respectively. Spot treatment concentrations for each herbicide were used in this study and included the following treatments: sethoxydim[3] (3% v/v, equivalent to 5.0 kg a.i. ha^{-1}), fluazifop-*P*-butyl[4] (2.25 % v/v, equivalent to 5.0 kg a.i. ha^{-1}), glyphosate[5] (3% v/v, equivalent to 18.15 kg a.i. ha^{-1}), or imazapyr[6] (1% v/v, equivalent to 2.24 kg a.i. ha^{-1}). All herbicide applications were made with a methylated seed oil adjuvant[7] at 1% v/v. Foliar applications were made using a forced air CO_2-powered sprayer at an equivalent of 935 L ha^{-1} diluent delivered through a single TeeJet® 80-0067 nozzle[8] at 40 psi.

The water in the mesocosms was completely exchanged 4 h after treatment and 3 days after treatment (DAT) to diminish the possibility of herbicide activity or plant uptake through the water column. Aboveground biomass was harvested 45 DAT to determine initial response to herbicide. In addition, aboveground and belowground biomass were collected 90 DAT to determine regrowth potential. All biomass samples were dried at 65 C until a constant weight before measurement. Biomass measurements were used to calculate percent control (PC) for biomass treatments: ($PC = 100 \times \frac{untreated\ plants - treated\ plants}{untreated\ plants}$). The corresponding untreated plant biomass for each depth was used to calculate PC for depth × herbicide combinations.

Statistical analysis

The experiment was conducted using a completely randomized design with five replicates per treatment and two experimental runs. There were 12 treatment combinations: four herbicides at each of the two depths, plus two untreated controls (pretreatment and final) at each of the two depths. All analyses were conducted using RStudio® (RStudio Team 2015). Baseline data were analyzed using one-way ANOVA; however, due to failed normality, a natural log transformation was required for root-to-shoot ratios. Two-way ANOVAs were used to determine the effects of water depth and herbicide treatment on torpedograss biomass 45 and 90 DAT. Analysis of posttreatment biomass required a square root transformation to meet the assumption of homogeneity of variance. PC was analyzed separately for each flooding depth using a one-way ANOVA to determine the effect of herbicide treatment, but square root transformations were again required to meet the assumption of homogeneity of variance. Means were separated for all analyses using Tukey's honestly significant difference test (Tukey's HSD) in the agricolae package in RStudio (de Mediburu 2017).

RESULTS AND DISCUSSION

Initial response to flooding

Plants appeared to be healthy in both water depths, and there were no differences in torpedograss above- or belowground biomass between the flooding treatments before herbicide application (Table 1). These results differ

Table 1. Pretreatment (mean ± SE) comparisons for aboveground biomass, belowground biomass, and above-water biomass (AWB) as well as the root-to-shoot ratio for torpedograss before treatment with glyphosate, imazapyr, sethoxydim, or fluazifop.

Water Depth (cm)	Aboveground Biomass (g)	Belowground Biomass (g)	AWB (g)	Root-to-Shoot Ratio
15	23.2 ± 2.6 (a)[1]	31.6 ± 3.1 (a)	23.2 ± 2.6 (a)	1.35 ± 0.08 (a)
45	22.9 ± 2.6 (a)	25.5 ± 3.1 (a)	11.0 ± 2.6 (b)	1.07 ± 0.09 (b)

[1]Means within a column followed by the same letter are not significantly different according to Tukey's honestly significant difference test at $P \leq 0.05$; $n = 5$.

from previous research on torpedograss response to flooding, which found that aboveground growth decreased with rising water levels (Smith et al. 2004). These differences may be due to the length of inundation time in each study. Smith et al. (2004) harvested plants 12 wk after flooding, whereas we harvested 3 wk after the flooding treatment was initiated. Smith et al. (2004) also found that flooding decreased biomass production above the waterline; this is consistent with our results, where plants growing in saturated conditions had more than double the shoot biomass above the waterline (23.2 ± 2.6 g) than those in flooded conditions (11.0 ± 2.6 g).

Although no significant differences in total above- or belowground biomass were detected, there was a significant difference in the root-to-shoot ratio between flooding treatments. Plants growing in saturated conditions had a significantly higher root-to-shoot ratio (1.35 ± 2.6) than those in flooded conditions (1.07 ± 0.09) (Table 1). These data reflect research by Hossain et al. (2002), which found greater biomass allocation to shoot rather than rhizome tissue in flooded conditions. This shoot biomass allocation is a common response in flood-tolerant species, thus allowing plants to maintain gas exchange processes, and has been found in other wetland grass species as well (Baruch 1994, Insausti et al. 2001, Miller and Zedler 2003).

Response to herbicide treatments

By 45 DAT, torpedograss had manifested clear symptomology from all herbicide treatments. For the graminicides, strong chlorosis and necrosis of torpedograss shoot tissue above the waterline was observed, including a necrotic band at the base of the leaf whorl. These symptoms were slow to manifest and were generally observed 14 to 21 d after treatment. Although not quantified, visual effects of the graminicides were more apparent on shoot tissues above rather than below the waterline. For glyphosate, visual symptoms of chlorosis and necrosis of the shoots above the waterline were very apparent 7 to 14 DAT. For imazapyr, visual symptoms of chlorosis and reddening of the leaves followed by necrosis were slow to manifest and were present by 14 to 28 d after treatment.

For aboveground biomass at 45 DAT, there was a significant interaction between water depth and herbicide treatment (Table 2). Overall, plants growing in flooded conditions had greater aboveground biomass than those in saturated conditions, indicating that flooding may have hindered the effect of herbicide treatments. Plants treated with glyphosate had the lowest biomass compared to those treated with other herbicides. Glyphosate and sethoxydim provided the greatest percent reduction compared to untreated controls (97.5 ± 1.4% and 91.6 ± 2.6%, respectively) 45 DAT for plants growing in saturated conditions (Figure 1). For plants in flooded conditions, glyphosate (84.0 ± 3.7%) provided the greatest percent reduction in biomass compared to the untreated controls, while fluazifop-*P*-butyl (62.1 ± 7.2%) provided the lowest. Imazapyr (66.9 ± 3.8%) and sethoxydim (68.9 ± 3.3%) were similar to both glyphosate and fluazifop-*P*-butyl. The average aboveground biomass for untreated plants in saturated and flooded conditions was 37.1 ± 3.8 g and 51.3 ± 1.1 g, respectively.

Table 2. *F* Ratios resulting from a two-way ANOVA of torpedograss biomass 45 and 90 days after treatment (DAT) with fluazifop-*P*-butyl (2.25% v/v, or 5.0 kg a.e. ha^{-1}), sethoxydim (3% v/v, or 5.0 kg a.i. ha^{-1}), glyphosate (3% v/v, 18.15 kg a.i. ha^{-1}), or imazapyr (1% v/v, or 2.24 kg a.i. ha^{-1}), with water depth (15 or 45 cm) and herbicide as factors.

	Depth[1]	Herbicide	Depth × Herbicide
Aboveground biomass (45 DAT)	**49.37*** **	**85.07*** **	**5.19*** **
Aboveground biomass (90 DAT)	**9.59** **	**64.66*** **	0.68
Belowground biomass (90 DAT)	**9.78** **	**16.98*** **	0.22

[1]Statistically significant are values in boldface: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

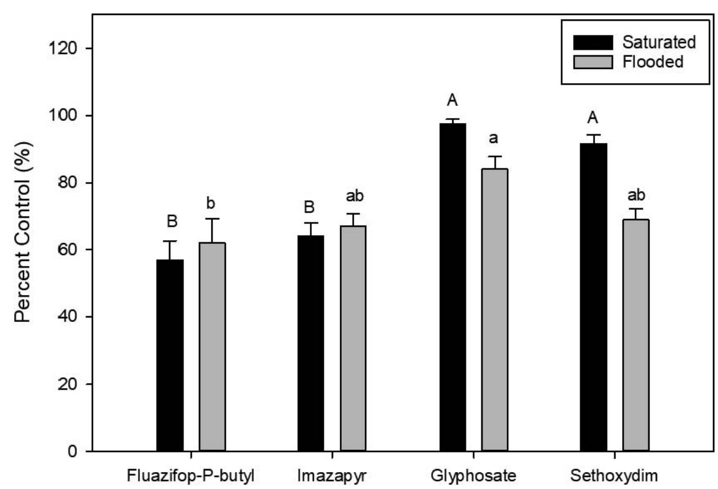

Figure 1. Percent control (PC) (± SE) of aboveground biomass 45 d after treatment with fluazifop-*P*-butyl (2.25% v/v, or 5.0 kg a.e. ha^{-1}), sethoxydim (3% v/v, or 5.0 kg a.i. ha^{-1}), glyphosate (3% v/v, 18.15 kg a.i. ha^{-1}), or imazapyr (1% v/v, or 2.24 kg a.i. ha^{-1}), for plants growing in saturated (15 cm of water) or flooded (45 cm of water) conditions. PC was calculated as $PC = 100 \times \frac{untreated\ plants - treated\ plants}{untreated\ plants}$. The average aboveground biomass (± SE) for untreated plants in saturated and flooded conditions was 37.1 ± 3.8 g and 51.3 ± 1.1 g, respectively. Means with the same letter are not significantly different according to Tukey's honestly significant difference (HSD) test at $P \leq 0.05$; $n = 5$. Each water depth was analyzed separately; uppercase letters indicate differences between plants growing in saturated conditions, and lowercase letters indicate differences between those in flooded conditions.

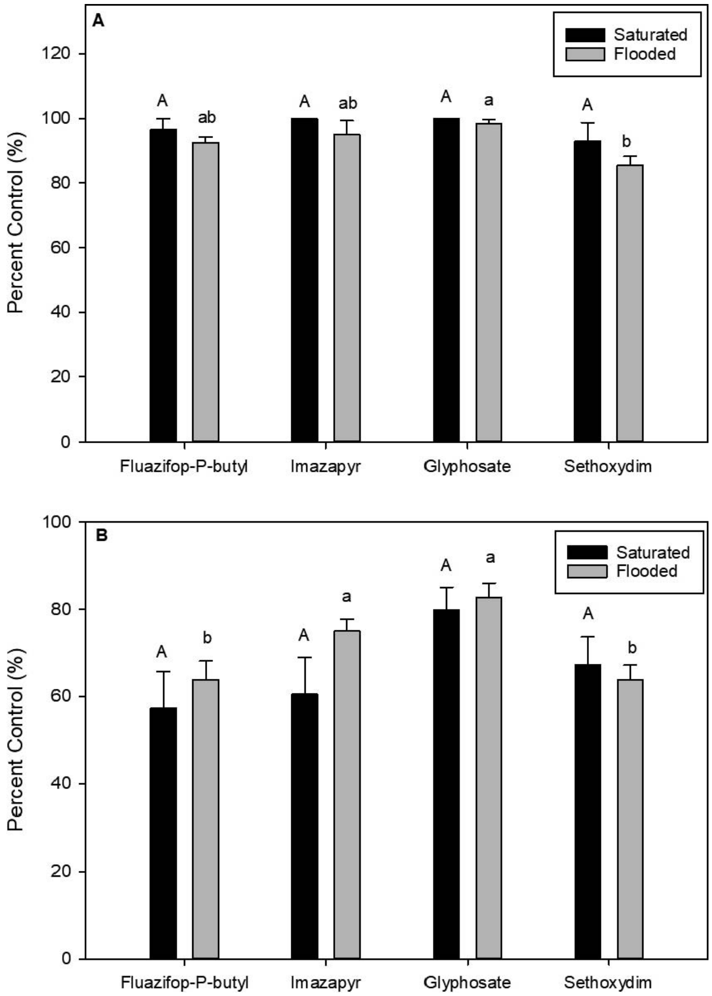

Figure 2. Percent control (PC) (± SE) of (A) aboveground biomass, and (B) belowground biomass, 90 d after treatment with fluazifop-*P*-butyl (2.25% v/v, or 5.0 kg a.e. ha^{-1}), sethoxydim (3% v/v, or 5.0 kg a.i. ha^{-1}), glyphosate (3% v/v, 18.15 kg a.i. ha^{-1}), or imazapyr (1% v/v, or 2.24 kg a.i. ha^{-1}), for plants growing in saturated (15 cm of water) or flooded (45 cm of water) conditions. PC is calculated as $PC = 100 \times \frac{untreated\ plants - treated\ plants}{untreated\ plants}$. The average aboveground biomass (± SE) for untreated plants in saturated and flooded conditions was 4.4 ± 0.8 g and 6.0 ± 1.1 g, respectively. The average belowground biomass (± SE) for untreated plants in saturated and flooded conditions was 101.8 ± 9.1 g and 59.2 ± 18.0 g, respectively. Means with the same letter are not significantly different according to Tukey's honestly significant difference (HSD) test at $P \leq 0.05$; $n = 5$. Each water depth was analyzed separately; uppercase letters indicate differences between plants growing in saturated conditions, and lowercase letters indicate differences between those in flooded conditions.

For both above- and belowground biomass 90 DAT, there were significant effects of both water depth and herbicide treatment, although there was no significant interaction between the two factors (Table 2). Aboveground biomass was significantly greater and belowground biomass significantly lower for plants growing in flooded conditions. This indicates greater regeneration capacity for plants in the deeper water treatment, because they allocated more resources to regrowth following herbicide application and shoot removal. Aboveground biomass was lowest in plants treated with glyphosate, imazapyr, and fluazifop-*P*-butyl, and belowground biomass was lowest in plants treated with glyphosate.

For plants growing in saturated conditions, there were no differences between herbicide treatments for both above- and belowground biomass with regards to PC (Figure 2). However, PC of aboveground biomass was greatest for plants growing in flooded conditions when treated with glyphosate (98.4 ± 1.2%) and lowest when treated with sethoxydim (85.5 ± 2.7%) (Figure 2A). Plants treated with imazapyr (95.2 ± 4.2%) and fluazifop-*P*-butyl (92.5 ± 1.8%) were controlled similarly to those that received foliar applications of glyphosate and sethoxydim. The average aboveground biomass for untreated plants in saturated and flooded conditions was 4.4 ± 0.8 g and 6.0 ± 1.1 g, respectively. For belowground biomass of flooded plants, glyphosate and imazapyr provided greater control (82.7 ± 3.3% and 75.0 ± 2.8%, respectively) than sethoxydim (63.7 ± 3.4) and fluazifop-*P*-butyl (63.9 ± 4.3%) (Figure 2B). The average belowground biomass for untreated plants in saturated and flooded conditions was 101.8 ± 9.1 g and 59.2 ± 18.0 g, respectively.

There was an overall effect of flooding on plant biomass throughout the study with plants in flooded conditions producing greater aboveground biomass 45 and 90 DAT than those in saturated conditions. This indicates that water level may impact herbicide efficacy for torpedograss, supporting anecdotal evidence from Florida land managers who have often noted limited control in flooded conditions. Flooding can induce several changes in plant growth and morphology such as growth rate, biomass allocation, or leaf area, which ultimately may have an impact on herbicide efficacy (Chen et al. 2002, Rood et al. 2010, Varanasi et al. 2016).

We found that the root-to-shoot ratios differed significantly between flooding treatments, with plants in flooded conditions having a lower root-to-shoot ratio than those in saturated conditions. It is possible that this had an effect on herbicide efficacy; however, in perennial rhizomatous species such as torpedograss, greater root-to-shoot ratios (rather than lower ones) typically reduce herbicide efficacy (Ziska et al. 2004). Plants growing in flooded conditions had significantly less shoot biomass above the waterline, and it is possible that flooding reduced herbicide uptake by limiting exposure of leaves to herbicide. Other factors that we did not measure, such as leaf area, may have also played a role. Further research is required to determine the causal mechanism for reduced herbicide efficacy in flooded conditions.

In saturated water, the graminicides sethoxydim and fluazifop-*P*-butyl provided comparable control of torpedograss to the commonly used herbicides glyphosate and imazapyr. This is consistent with previous research by Enloe and Netherland (2017), who demonstrated satisfactory control of torpedograss in mesocosm conditions using these two graminicides. However, although we found no significant difference between herbicide treatments in saturated conditions 90 DAT, the graminicides were less effective than glyphosate and imazapyr in flooded conditions. This effect is likely to be expressed much more extensively in field settings. Previous research suggests that multiple applications of sethoxydim and fluazifop may be necessary for adequate control, and due to their selective nature, multiple applications can be utilized with limited risk to native broadleaf species (Annen et al. 2005, Enloe and Netherland 2017, Enloe et al. 2018). Further research is required to determine if multiple applications may overcome the negative effect of flooding on efficacy for these two herbicides.

Many environmental factors can influence herbicide efficacy. In this study, water depth had a significant impact on torpedograss control and should be taken into consideration when planning operational management activities. The graminicides sethoxydim and fluazifop-*P*-butyl can provide desirable torpedograss control in saturated conditions and can significantly limit off-target damage to native and nontarget species, but may not be as effective when plants are growing in flooded conditions. Future research efforts should focus on optimizing graminicide use in flooded conditions to overcome this challenge by investigating the effects of multiple applications, various flooding depths, and the duration of inundation on efficacy. In addition, research is needed to determine if this effect occurs in other grass species that invade areas prone to flooding, such as West Indian marsh grass (*Hymenachne amplexicaulis*).

SOURCES OF MATERIALS

[1]Builder's sand, Argos USA, 924 S. Main St., Gainesville, FL 32601.

[2]SA-50 Controlled Release Fertilizer (14-14-14), Southern Agricultural Insecticides, Inc., P.O. Box 218, Palmetto, FL 34220.

[3]TIGR® herbicide, SePRO Corporation, 11550 N. Meridian St., Suite 600, Carmel, IN 46032.

[4]Fusilade® II, Syngenta Crop Protection, LLC, P.O. Box 18300, Greensboro, NC 27419.

[5]Rodeo®, Dow AgroSciences, 9330 Zionsville Rd, Indianapolis, IN, 46268.

[6]Habitat®, SePRO Corporation, 11550 N. Meridian St., Suite 600, Carmel, IN 46032.

[7]MSO Concentrate, Loveland Products, Inc., 14520 Co Rd 64, Greeley, CO 80631.

[8]TeeJet® nozzle, TeeJet Technologies Illinois, LLC, 1801 Business Park Drive, Springfield, IL 62703.

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