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Evaluation of Bioherbicidal Control of Tropical Signalgrass, Crabgrass, Smutgrass, and Torpedograss

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Tropical signalgrass (TSG) causes serious problems for sod production and turf maintenance in Florida. Other grasses such as large crabgrass (CG), smutgrass (SG), thin paspalum (TP), and torpedograss (TG) can be problematic as well. Several emulsion formulations composed of mycelium or mycelium-free culture filtrate (or both) of the fungal pathogen *Drechslera gigantea* (DG) and Sunspray 6E oil were tested with or without ammonium sulfate or pelargonic acid (*n*-nonanoic acid; a natural product registered as a biorational herbicide) in greenhouse and field trials. A 30% Sunspray 6E oil formulation containing DG mycelium (10 g), DG culture filtrate (70 ml), and 4.5 g of ammonium sulfate caused 88 to 100% injury on TSG, CG, SG, and TG in greenhouse trials. The injury resulted from disease as well as phytotoxicity of the culture filtrate, oil, and ammonium sulfate. An emulsion formulation composed of 30% Sunspray 6E oil and 70% DG culture filtrate amended with 2% (v/v) pelargonic acid killed SG 2 wk after application. DG formulations containing ammonium sulfate or pelargonic acid produced lower levels of injury when treated grasses were exposed to a 24-h dew period compared with those treated and not exposed to dew. Formulations containing DG mycelium, DG culture filtrate, and ammonium sulfate or pelargonic acid are effective and promising for control of weedy grasses. Further evaluations of these formulations under field conditions are justified.
Nomenclature: Ammonium sulfate; nonanoic acid (pelargonic acid); large crabgrass, *Digitaria sanguinalis* (L.) Scop.; smutgrass, *Sporobolus indicus* (L.) R. Br.; thin paspalum, *Paspalum setaceum* Michx.; torpedograss, *Panicum repens* L.; tropical signalgrass, *Urochloa subquadripara* (Trin.) R. D. Webster; *Drechslera gigantea* (Heald & Wolf) Ito.
Key words: Biological control, biorational herbicide, grass weeds, plant pathogens, *Drechslera gigantea*, ammonium sulfate, pelargonic acid, turf, sod.

La tolerancia de los zacates *Cynodon dactylon L.* 'Tifsport' y de 'tifdworf', *Zoysia japonica L.* 'Meyer', y *Paspalum virginatum Swartz* 'Salam' a los herbicidas oxadiazon (2240 g/ha) o quinclorac (840 hg/ha) aplicados 1 semana antes del espigamiento (WBS), en el espigamiento (AS), 2 semanas después del espigamiento (2 WAS) y 4 semanas después del espigamiento (4 WAS) fueron investigados en el campo. El control de malezas fue también evaluado. Para ambos herbicidas solamente el tiempo de aplicación AS dañó el césped en más del 22%, mientras que el daño para otros períodos de aplicación fue de un 9 a un 19%, 5 semanas después de la siembra. Cuando se evaluó el daño en el césped 8 semanas después de la siembra, siguiendo el tiempo de aplicación (AS) , éste permaneció en un 19% mientras que el daño pare cualquier otro intervalo de aplicación fue del 8% o menos. Los períodos de aplicación: 8 WAS y 1 WBS, AS, 2 WAS y 4 WAS alcanzaron 89, 79, 94 y 99% de cobertura respectivamente, cuando se promediaron con todas las otras especies de zacates, cultivares y herbicidas. Para las 13 WAS, todas las especies de cultivares alcanzaron al menos 90% de cobertura en la parcela.. Las aplicaciones anteriores al espigamiento de oxadiazan proporcionaron de un 98 a un 100% en el control de la *Eleusine indica L.* y del *Oldenlandia corymbosa L.* La aplicación de quinclorac al momento de la siembra proporcionó más del 70% de control de estas malezas. Los resultados indican que oxadiazon y quinclorac aplicados al momento de la siembra causan daños inaceptables en el césped. Si el *Eleusine indica L.* y el *Oldenlandia corymbosa L.* son problemáticos, el oxadiazon es una opción factible para el control de estas malezas pero no así el quinclorac.

Tropical signalgrass (TSG), a perennial grass species native to tropical Asia (Institute of Pacific Island Forestry 2002), is found in Florida, Maryland, Hawaii, and Puerto Rico (USDA 2002). It is one of the most serious weeds in St. Augustinegrass sod production in Florida, and its presence increases production costs and lowers turfgrass quality (Teuton et al. 2004). TSG has many common names, including signalgrass, small-flowered alexandergrass, corigrass, green summergrass, two-spiked panic, and two fingergrass (Speedy 2002).

TSG is difficult to control because of its tolerance to available chemical herbicides, lack of herbicide selectivity, or its ability to outgrow control treatments. Teuton et al. (2004) reported that none of the 20 POST herbicide treatments they tested provided acceptable control. Use of host-specific plant pathogens as bioherbicides may provide a practical non-chemical means of control for weedy grasses (Chandramohan et al. 2002d). It has been shown that three indigenous fungal pathogens could be used as bioherbicides to control several weedy grasses that affect agricultural crops and natural areas in Florida (Chandramohan and Charudattan 2001). These pathogens (named below) were used in a bioherbicide system

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consisting of a three-pathogen mixture. This system allows for custom blending of different pathogens to control different grass species. For instance, a triple-pathogen bioherbicide system containing *Drechslera gigantea* (DG), *Exserohilum longirostratum* (EL), and *E. rostratum* (ER) effectively controlled crowfootgrass [*Dactyloctenium aegyptium* (L.) Willd.], green foxtail [*Setaria viridis* (L.) Beauv.], guineagrass [*Urochloa maxima* (Jacq.) R. Webster], johnsongrass [*Sorghum halepense* (L.) Pers.], large crabgrass [*Digitaria sanguinalis* (L.) Scop.], napiergrass (*Pennisetum purpureum* Schumach.), natalgrass [*Melinis repens* (Willd.) Zizka], southern sandbur [*Cenchrus echinatus* L.], Texas panicum [*Panicum taxanum* Buckl.], torpedograss, vaseygrass (*Paspalum urvillei* Steud.), and yellow foxtail [*Setaria pumila* (Poir.) Roemer & J.A. Schultes] under greenhouse and controlled field conditions (Chandramohan and Charudattan 2001; Chandramohan et al. 2002a).

In field trials, these pathogens were confined to the area of application and did not spread (Chandramohan et al. 2002a). Furthermore, Chandramohan et al. (2002c) found that bermudagrass [*Cynodon dactylon* (L.) Pers.] turf sustained some injury from the pathogen mixture applied in an emulsion but recovered, whereas bahiagrass (*Paspalum notatum* Flueggé), creeping bentgrass (*Agrostis stolonifera* L.), centipedegrass [*Eremochloa ophiuroides* (Munro) Hack.], and seashore paspalum (*Paspalum vaginatum* Sw.) were immune. St. Augustinegrass and zoysiagrass were resistant (developed a few discrete leaf spots). The susceptible weedy grasses remained diseased after treatment with the emulsion–pathogen mixture, whereas the turf grasses continued to grow healthy if they were immune to the pathogen mixture or recovered from the initial resistance reaction and grew healthy. In resistant turf grasses such as St. Augustinegrass, this biocontrol system could be used in an over-the-top or spot-spray application to control TSG, whereas in bermudagrass, only spot treatment may be feasible because of the potential for injury.

In previous studies, DG alone or in combination with EL and ER was determined to be capable of suppressing several weedy grasses, including TSG (Chandramohan and Charudattan 2001; Chandramohan et al. 2002a,b,c,d; Teuton et al. 2003). On the basis of the results from our greenhouse trials (data not shown), we determined that DG alone was as effective as the combination of DG, EL, and ER. Considering the cost and technical difficulties of using all three pathogens compared with DG alone, we chose to use only DG in further studies.

DG culture filtrate contains phytotoxic metabolites that mainly consist of ophiobolin A and three other related minor metabolites (Evidente et al. 2006a,b) that can augment the effects of DG mycelium. It is also known that the Sunspray 6E[1] oil used in the emulsion formulation is compatible with DG and the bioherbicidal activity of the fungus is not affected when it is formulated in the oil emulsion (Chandramohan and Charudattan 2001; Chandramohan et al. 2002a). Higher amounts of oil in the emulsion increase the level of phytotoxic injury; hence, to augment the level of injury to the grasses, we tested two oil concentrations, 10 and 30% v/v. Our objectives were to: (1) assess the field efficacy of a bioherbicide system consisting of DG to manage TSG in Florida turf and sod, (2) determine the most effective combination of the bioherbicide mixture with two adjuvants, ammonium sulfate[2] [$(NH_4)_2SO_4$; a foliar desiccant that can be a source of nitrogen for fungal growth] and pelargonic acid[3] (*n*-nonanoic acid 96%; a fatty acid that occurs naturally as esters in the oil of pelargonium, and a registered biorational herbicide), and (3) develop an effective system to apply the bioherbicide for management of grassy weeds in turf and sod production in Florida.

Materials and Methods

Culturing DG. DG was grown for 3 to 5 d in tomato juice broth (200 ml of tomato juice and 800 ml of water per liter) on a shaker (100 rpm, 25 C). The mycelium and culture filtrate were separated and the latter was autoclaved at 121 C for 10 min before it was used in the bioherbicidal formulations.

Field Trials. *Field Trial I at Duda & Sons Sod Inc., Melbourne, FL.* In this trial, the efficacy of DG was tested against torpedograss (TG) in Bermudagrass 419 sod, TSG in St. Augustinegrass sod, and thin paspalum (TP) in St. Augustinegrass sod. For TSG and TP, 16 completely randomized individual patches of the respective weed infestation were used for each treatment. For TG, four completely randomized patches were used for each treatment. The patches were approximately 1 m^2 each and four treatments were used in this experiment: (a) mycelial suspension of DG (M) (10 g of fresh DG mycelium + 10 ml of Sunspray 6E oil emulsion + 80 ml of tap water), (b) autoclaved culture filtrate of DG (F) (90 ml of culture filtrate + 10 ml of Sunspray 6E), (c) control (C) (10 ml of Sunspray 6E oil emulsion + 90 ml of uninoculated culture broth, and (d) unsprayed control (U).

Three applications were made at 1-wk intervals and the experiment ran for 7 wk after the first spray. Treated patches were marked with flags and orange paint and the plants were rated for disease/injury severity weekly or biweekly for 7 wk after the initial treatment. The disease/injury was rated on a scale of 1 to 10 (1 = 1 to 10%; 2 = 11 to 20%; 3 = 21 to 30%; 4 = 31 to 40; 5 = 41 to 50%; 6 = 51 to 60%; 7 = 61 to 70%; 8 = 71 to 80%; 9 = 81 to 90%; 10 = 91 to 100% disease/injury).

Field Trial II at Duda & Sons Sod Inc., Fort Lonesome, FL. Results from field experiment I indicated a low level of efficacy of the DG formulation. Therefore, in experiment II, the formulation was amended with ammonium sulfate. The formulations were tested against TSG in St. Augustinegrass. Sixteen completely randomized individual patches of TSG infestation ($\sim$ 0.5 m^2) were used for each of the following nine treatments: (a) mycelial suspension (M) (10 g of fresh DG mycelium + 10 ml of Sunspray 6E oil emulsion + 80 ml of tap water), (b) mycelium and autoclaved culture filtrate (MF) (10 g of fresh DG mycelium + 10 ml of Sunspray 6E oil emulsion + 80 ml of autoclaved culture filtrate), (c) mycelium + ammonium sulfate (MA) (10 g of mycelium and 0.125 g of ammonium sulfate suspended and dissolved respectively in

80 ml of water and emulsified with 10 ml of Sunspray 6E oil), (d) mycelium + culture filtrate + ammonium sulfate (MFA) (10 g of fresh DG mycelium + 80 ml of autoclaved culture filtrate + 0.125 g of ammonium sulfate + 10 ml of Sunspray 6E oil emulsion), (e) culture filtrate only (F) (90 ml of culture filtrate + 10 ml of Sunspray 6E), (f) culture filtrate + ammonium sulfate (FA) (90 ml of culture filtrate + 0.125 g of ammonium sulfate + 10 ml of Sunspray 6E oil emulsion), (g) oil and uninoculated culture broth only—control (C) (10 ml of Sunspray 6E oil emulsion + 90 ml of uninoculated culture broth), (h) ammonium sulfate + uninoculated culture broth only (A) (10 ml of Sunspray 6E oil emulsion + 0.125 g of ammonium sulfate + 90 ml of uninoculated culture broth), and (i) unsprayed control (U). Three applications were made at 2-wk intervals and the experiment ran for 8 wk after the first spray. Treated patches were marked with flags and orange paint and rated biweekly for disease/injury severity using the scale described above.

Compatibility of Adjuvants with DG. Results from the field trials indicated an unsatisfactory level of efficacy of the DG bioherbicide formulations and to be practical, the efficacy needed to be improved by the addition of substances that can increase DG's pathogenicity or the formulations' phytotoxicity. To this end, a higher proportion of Sunspray 6E oil, DG culture filtrate, ammonium sulfate, and pelargonic acid were tested. Unlike the oil and the phytotoxins in the DG culture filtrate, the effects of ammonium sulfate and pelargonic acid on DG are unknown. Hence, we designed experiments to test their effects on DG's growth and sporulation in culture, spore germination, infectivity, and virulence on TSG. We also tested the effects of ammonium sulfate and pelargonic acid at different concentrations as adjuvants to improve the formulations' bioherbicidal efficacy possibly through a dual action of increased foliar injury and fungal nutrition. To determine whether DG was compatible with ammonium sulfate, the fungus was grown in vitro in the presence of this adjuvant. Effect of pelargonic acid on spore production, germination, and virulence was not tested because it is known to be inhibitory to fungi (Jalal and Read 1983).

Effect of Ammonium Sulfate and Pelargonic Acid on DG Growth and Sporulation. To evaluate the effects of amendments on the growth and sporulation of DG, the fungus was grown in Petri plates containing 20 ml of tomato juice[4] agar (TJA) (to test ammonium sulfate) or potato dextrose agar[5] (PDA) (for pelargonic acid). For both culture media, agar was added at a rate of 40 g/L to assure solidification of the medium containing higher concentrations of ammonium sulfate or pelargonic acid. Six-millimeter-diam discs from 2-wk-old DG cultures were placed in the center of the plates containing TJA or PDA. Six concentrations of ammonium sulfate (0, 1.5, 2.5, 3.5, 4.5, and 5.5%) or pelargonic acid (0, 0.5, 1, 1.5, 2, and 2.5%) were tested. For each concentration, there were 10 replicate plates for the mycelial growth study and 3 for the sporulation study. The cultures were incubated at 25 C in the dark and colony diameter (as average of two diagonal measurements per colony) was measured 2, 4, and 6 d after the cultures were started. The plates were scanned using a flatbed scanner[6] at 200 dots per inch and growth was measured at two points along the perimeter of the colony using the image size tool available with Adobe Photoshop CS2 Program.[7] Spore production was quantified with a hemacytometer using spore suspensions prepared from 4-wk-old cultures. Each replicate culture was quantified separately. The suspensions contained 0.02% Tween 80[8] to prevent clumping of spores.

Effect of Ammonium Sulfate on Spore Germination. DG spores produced on TJA without ammonium sulfate were suspended in solutions of ammonium sulfate at 0, 1.5, 2.5, 3.5, 4.5, and 5.5% w/v in water for 6 and 24 h and examined for germination. Spores produced on TJA containing ammonium sulfate at 0, 1.5, 2.5, 3.5, 4.5, and 5.5% w/v were harvested from the plates and suspended in solutions of the same ammonium sulfate concentrations for 6 and 24 h before examination. Three replicate spore samples were examined for each concentration and the number of germinated spores was counted from the first 100 spores on a slide viewed under a microscope. A spore was considered germinated when the germ tube was longer than the average width of the spores at their widest.

Effect of Ammonium Sulfate on Virulence of DG Spores. DG spores harvested from 4-wk-old cultures grown on TJA amended with different concentrations of ammonium sulfate (0, 1.5, 2.5, 3.5, 4.5, and 5.5% w/v) were washed and suspended in water at 1×10^6 spores/ml. Two 8-µl droplets of spore suspensions were placed with a 3-mm-diam capillary tube on 6-cm-long segments of TSG leaves kept in Petri plates lined with moistened sterile filter papers. Two droplets were placed per leaf segment and the leaf surface under the droplet was slightly wounded by gently pressing with the capillary tube to facilitate infection. Control treatment consisted of leaf segments treated with droplets of sterile water only and wounded as described above. Four replicate plates were used for each treatment (total of 16 spots per treatment). The diameter of disease lesions caused by conidia from each treatment was measured 4 d after application.

Greenhouse study. DG was grown for 3 to 5 d in tomato juice broth (200 ml of tomato juice and 800 ml of water per liter) on a rotary shaker (100 rpm, 25 C). The mycelium was separated from the culture broth by filtration and used in the formulations. The efficacy of the formulations was rated against TSG grown in a commercial potting mix[9] in plastic trays (52 by 25 by 6 cm) or large plastic pots (25 cm diameter by 22.5 cm height). Oil emulsion formulations of different compositions consisting of DG mycelium, DG culture filtrate, oil, ammonium sulfate, or pelargonic acid were tested. On the basis of the results of the preliminary experiments, a final selection of the most effective concentrations of the adjuvants was made (4.5% w/v for ammonium sulfate and 2% v/v for pelargonic acid) and tested twice in the greenhouse on TSG, CG, TG, and SG. The fungal formulation consisted of 10 g of fresh mycelium (blended to yield 2×10^7 mycelial fragments per ml in the final formulation) + 70 ml of culture filtrate + 30 ml of Sunspray 6E + ammonium sulfate (4.5% w/v in the final formulation) or pelargonic acid (at 2% v/v in the final formulation). DG mycelium was prepared as described. TSG, CG, TG, and SG

Table 1. Disease/injury rating on tropical signalgrass, torpedograss, and thin paspalum when rated 1 to 7 wk after treatment.[a]

Target weed	Treatment	Disease/injury rating after week:[b] 1	2	4	5	7
Tropical signalgrass	Culture filtrate (F)	1.1 ab[c]	2.3 b	3.1 a	2.9 a	3.3 a
	Mycelium (M)	1.3 a	3.4 a	3.1 a	1.6 b	0.7 b
	Noninoculated culture broth: Control (C)	0.8 b	1.5 c	1.5 b	0.8 bc	0.2 b
	Untreated (U)	NM[d]	0.1 d	0.00 c	0.3 c	0.0 b
Torpedograss	Culture filtrate (F)	1.0 ac	1.0 b	1.8 b	1.8 a	1.5 ab
	Mycelium (M)	0.8 a	4.3 a	3.8 a	2.3 a	2.5 a
	Noninoculated culture broth: Control (C)	0.0 b	0.3 bc	0.5 c	0.8 b	0.5 bc
	Untreated (U)	0.0 b	0.0 c	0.0 c	0.0 b	0.0 c
Thin paspalum	Culture filtrate (F)	1.1 bc	1.1 b	1.4 b	0.7 a	0.0 a
	Mycelium (M)	2.1 a	2.9 a	2.4 a	0.7 a	0.1 a
	Non inoculated culture broth: Control (C)	0.4 bc	0.3 c	0.4 c	0.2 b	0.0 a
	Untreated (U)	0.5 c	0.1 c	0.0 c	0.1 b	0.0 a

[a] Treated on July 10, 2007 in the field at Duda & Sons Sod Inc., Melbourne, FL.

[b] Disease/injury severity was evaluated on a scale of 1 to 10 (1 = 1–10%; 2 = 11–20%; 3 = 21–30%; 4 = 31–40; 5 = 41–50%; 6 = 51–60%; 7 = 61–70%; 8 = 71–80%; 9 = 81–90%; 10 = 91–100% disease/injury).

[c] Values within a column, for each weed, followed by the same letter(s) are not significantly different according to Duncan's multiple range test (P = 0.05).

[d] Abbreviation: NM, not measured.

grown on a commercial potting mix in plastic pots (25 cm diameter by 22.5 cm height) were treated, three pots per treatment. Applications were made with a hand-held, low-pressure sprayer as described. After application, all pots were kept on a greenhouse bench without exposure to dew. Treated plants were rated for disease/injury severity after 1, 2, 3, 4, 5, 6, 7, and 10 wk after inoculation (except for SG, which was rated after 1 and 2 wk only in the first trial) as percentage of foliar disease/injury as described above.

Statistical Analysis. All laboratory and greenhouse experiments were repeated at least once. The data were analyzed using the Statistical Analysis System software package (SAS Institute 2003). All multiple comparisons were first subjected to ANOVA and significant differences among treatment means were determined with Tukey's studentized range test or Duncan's new multiple range test as appropriate. All comparisons were performed at P = 0.05. The disease/injury severity data were subjected to repeated-measures ANOVA to determine if there are time or treatment–time interaction effects.

Results and Discussion

Field Trials. *Field Trial I at Duda and Sons Sod Inc., Melbourne, FL.* On TSG, by 7 wk after the spot treatments were applied, the fungus-free culture filtrate (treatment F) provided about 21 to 30% injury (Table 1). The fungus only (mycelium treatment M) produced about 21 to 30% injury as early as 2 wk after treatment, but the weed outgrew the injury by week 7, at which time the effect of this treatment was negligible and indistinguishable from the control treatments (C and U). The highest level of injury and hence the level of weed suppression obtained in this study by week 7, namely 21 to 30%, is insufficient for practical TSG management.

On TG, as early as 2 wk after treatment, the mycelium treatment (M) provided about 31 to 40% injury and by week

Table 2. Disease/injury rating on tropical signalgrass (TSG) when rated 2, 4, and 8 wk after treatment.[a]

Treatment	Disease/injury rating after week[b]: 2	4	8
Mycelium (M)	0.5 cd[c]	0.6 abc	2.6 a
Mycelium + filtrate (MF)	0.8 bc	0.6 abc	2.7 a
Mycelium + ammonium sulfate (MA)	1.1 b	0.9 ab	2.9 a
Mycelium + filtrate + ammonium sulfate (MFA)	1.8 a	1.2 a	3.1 a
Filtrate (F)	0.3 d	0.8 abc	3.3 a
Filtrate + ammonium sulfate (FA)	0.4 cd	0.4 bc	2.9 a
Oil + uninoculated culture broth: control (C)	0.0 d	0.2 c	2.5 a
Ammonium sulfate + uninoculated culture broth (A)	0.2 d	0.4 bc	2.7 a
Untreated (U)	NM[d]	NM	0.6 b

[a] Treated on August 21, 2007 in the field at Duda & Sons Sod Inc., Fort Lonesome, FL.

[b] Disease/injury severity was evaluated on a scale of 1 to 10 (1 = 1–10%; 2 = 11–20%; 3 = 21–30%; 4 = 31–40; 5 = 41–50%; 6 = 51–60%; 7 = 61–70%; 8 = 71–80%; 9 = 81–90%; 10 = 91–100% disease/injury).

[c] Values within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test (P = 0.05).

[d] Abbreviation: NM, not measured.

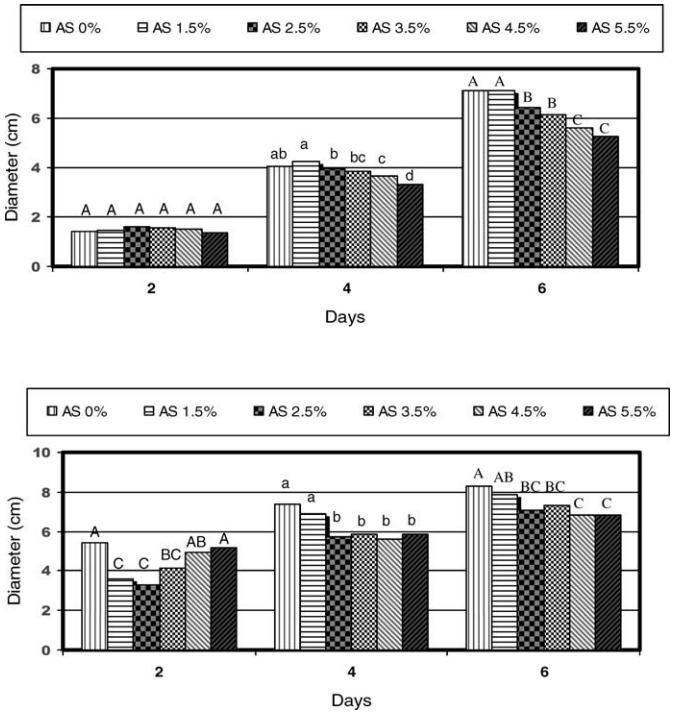

Figure 1. Effect of ammonium sulfate in the tomato juice agar (TJA) medium on the radial growth of *Drechslera gigantea* (DG) 2, 4, and 6 d after the cultures were started. Upper graph represents the first trial and the lower graph represents the second trial. AS, ammonium sulfate. Columns within each trial with same letter(s) are not significantly different according to Tukey's studentized range test (P = 0.05).

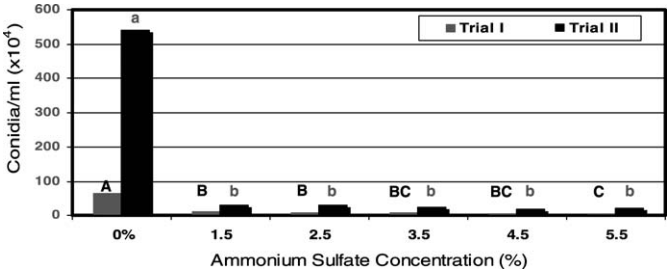

Figure 2. Effect of ammonium sulfate concentrations in the tomato juice agar (TJA) medium on the yield of *Drechslera gigantea* (DG) conidia from 4-wk-old cultures. Columns within each trial with same letter(s) are not significantly different according to Tukey's studentized range test (P = 0.05).

4, the injury level remained significantly higher compared with the other treatments (Table 1). By week 7, injury from treatment M (21 to 30%) was significantly higher than the two control treatments (C and U) but comparable with treatment F. The level of injury, 21 to 30% by week 7 with the best treatment (M), is unsatisfactory from a practical weed control objective.

On TP, treatment M provided 21 to 30% injury by week 2 and the injury level remained significantly higher compared with the other treatments (Table 1). However, by week 7, TP plants were totally recovered in all treatments (Table 1); therefore, TP is considered unmanageable by the DG formulations tested (Table 1).

Field Trial II at Duda and Sons Sod Inc., Fort Lonesome, FL. By 2 wk after treatment, MFA that consisted of DG mycelium, culture filtrate, and ammonium sulfate at 0.125% w/v (in the final formulation) inflicted the highest level of injury to about 11 to 20% of the foliar biomass of TSG (Table 2). By week 8, all treatments except the untreated (U) produced 21 to 30% injury, which is well below the acceptable level for TSG management (Table 2).

Compatibility Trials. *Effect of Ammonium Sulfate and Pelargonic Acid on DG Growth.* DG did not grow on PDA plates containing pelargonic acid, which is not surprising given the reported fungitoxicity of this compound (Jalal and Read 1983). Thus, there are no data to present on the effects of pelargonic acid on DG.

Figure 1 presents the radial growth of DG on the TJA medium containing different concentrations of ammonium sulfate up to 6 d after the cultures were started. With increasing concentration of ammonium sulfate above 1.5%, there was a reduction in DG growth, but even at the highest concentration (4.5%), the fungus was able to grow to an acceptable level.

Effect of Ammonium Sulfate on DG Sporulation. Adding ammonium sulfate to the medium at any of the tested

Table 3. Effect of ammonium sulfate concentrations on germination of *Drechslera gigantea* (DG) spores.

	Treatment		% Conidia germination after:			
			6 h		24 h	
Treatment code	Ammonium sulfate concentration (%) conidia produced on:	Ammonium sulfate concentration (%) conidia suspended in:	Trial 1	Trial 2	Trial 1	Trial 2
1	0	0.0	12.0 a[a]	4.0 a	20.7 a	4.7 a
2	0	1.5	10.0 ab	3.7 ab	19.3 a	1.7 bcd
3	0	2.5	10.3 ab	2.0 abcd	14.7 ab	1.7 bcd
4	0	3.5	6.7 ab	1.3 cd	14.3 ab	0.3 d
5	0	4.5	6.3 b	1.3 cd	19.3 a	1.3 cd
6	0	5.5	8.0 ab	1.3 cd	8.7 b	1.0 cd
7	1.5	1.5	0.0 c	0.7 cd	1.0 c	0.3 d
8	2.5	2.5	0.0 c	0.3 d	1.3 c	0.0 d
9	3.5	3.5	0.7 c	1.3 cd	0.7 c	0.7 cd
10	4.5	4.5	0.7 c	2.7 abc	1.7 c	3.3 ab
11	5.5	5.5	0.0 c	1.7 bcd	0.0 c	2.3 bc

[a] Values within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test (P = 0.05).

Table 4. Effect of *Drechslera gigantea* (DG) emulsion formulation containing ammonium sulfate or pelargonic acid on tropical signalgrass (TSG).

	% Disease/injury severity after week:															
	1		2		3		4		5		6		7		10	
Treatment	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Emulsion[a] + culture filtrate + fungus	53 c[b]	43 d	72 b	63 c	95 a	73 b	99 a	83 ab	99 a	92 a	99 a	90 a	99 a	88 a	98 a	75 a
Emulsion + culture filtrate + fungus + 4.5% ammonium sulfate	80 a	75 c	99 a	88 b	100 a	78 b	100 a	77 b	100 a	77 c	100 a	77 b	100 a	73 b	100 a	65 ab
Emulsion + culture filtrate + 4.5% ammonium sulfate (fungus-free)	37 d	33 e	37 c	37 d	43 c	33 c	53 c	25 c	67 b	25 d	67 b	20 c	67 b	17 c	57 b	10 c
Emulsion + culture filtrate + 2% pelargonic acid (fungus-free)	67 b	87 b	67 b	88 b	70 b	88 a	80 b	83 ab	87 ab	83 bc	87 ab	83 ab	87 ab	77 ab	77 ab	50 b
Emulsion + 2% pelargonic acid (fungus-free, culture filtrate-free)	85 a	98 a	95 a	99 a	99 a	96 a	99 a	90 a	100 a	90 ab	100 a	90 a	100 a	87 a	100 a	67 ab
Emulsion + culture filtrate (fungus-free, ammonium sulfate-free, pelargonic acid-free)	0 e	10 f	10 d	0 e	33 d	0 d	60 c	0 d	67 b	0 e	67 b	0 d	67 b	0 d	60 b	0 c
Emulsion only (culture filtrate-free, fungus-free, ammonium sulfate-free, pelargonic acid-free)	0 e	10 f	5 de	0 e	20 e	0 d	20 d	0 d	20 c	0 e	20 c	0 d	18 c	0 d	15 c	0 c
Untreated control	0 e	0 g	0 e	0 e	0 f	0 d	0 e	0 d	0 c	0 e	0 c	0 d	0 c	0 d	0 c	0 c

[a] Emulsion was composed of Sunspray 6E and tomato juice broth (control) or DG mycelium, DG culture filtrate, and ammonium sulfate or pelargonic acid at the specified concentrations. Formulation (400 ml) was used to spray plants in 3 replicates (pots) (133 ml per pot). No dew was provided.

[b] Values (rounded to nearest whole number) within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P = 0.05$).

concentrations led to a decrease in the number of conidia (asexual spores) of DG (Figure 2); the length of conidia decreased and the spore-wall thickness increased. Also, with increasing concentration of ammonium sulfate in the medium, conidia appeared unhealthy, with malformation and deterioration of the spore septa. Figure 2 presents the yield of DG conidia produced after 4 wk of growth on TJA medium containing different concentrations of ammonium sulfate. In general, DG spore production was strongly repressed by the presence of ammonium sulfate in the medium, whereas the mycelial growth was less affected and the level of growth remained adequate (Figure 1).

Effect of Ammonium Sulfate on Spore Germination. Germination of DG spores was negatively affected by ammonium sulfate. Spores produced on TJA medium without ammonium sulfate germinated when incubated in ammonium sulfate solutions but spores produced on TJA containing

Table 5. Effect of *Drechslera gigantea* (DG) emulsion formulation containing ammonium sulfate or pelargonic acid on crabgrass (CG).

	% Disease/injury severity after week:															
	1		2		3		4		5		6		7		10	
Treatment	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Emulsion[a] + culture filtrate + fungus	40 b[b]	27 b	87 c	53 b	95 b	87 b	90 b	91.7 b	92 b	92 b	93 a	96 b	91 b	96 b	80 b	83 b
Emulsion + culture filtrate + fungus + 4.5% ammonium sulfate	87 a	98 a	100 a	99 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a
Emulsion + culture filtrate + 4.5% ammonium sulfate (fungus-free)	40 b	NT[c]	95 b	NT	100 a	NT	100 a	NT	100 a	NT	100 a	NT	100 a	NT	100 a	NT
Emulsion + culture filtrate + 2% Pelargonic acid (fungus-free)	85 a	100 a	98 ab	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	98 a	100 a
Emulsion + 2% pelargonic acid (fungus-free, culture filtrate-free)	88 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a
Emulsion + culture filtrate (fungus-free, ammonium sulfate-free, pelargonic acid-free)	17 c	0 c	17 e	0 c	20 c	0 c	32 c	0.0 c	33 c	0 c	38 b	0 c	40 c	0 c	40 c	0 c
Emulsion only (culture filtrate-free, fungus-free, ammonium sulfate-free, pelargonic acid-free)	5 d	0 c	22 d	0 c	22 c	0 c	20 d	0.0 c	20 d	0 c	20 c	0 c	20 d	0 c	15 d	0 c
Untreated control	0 d	0 c	0 f	0 c	0 d	0 c	0 e	0.0 c	0 e	0 c	0 d	0 c	0 e	0 c	0 e	0 c

[a] Emulsion was composed of Sunspray 6E and tomato juice broth (control) or DG mycelium, DG culture filtrate, and ammonium sulfate or pelargonic acid at the specified concentrations. Formulation (150 ml) was used to spray plants in three replicate pots (50 ml per pot). No dew was provided.

[b] Values (rounded to nearest whole number) within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P = 0.05$).

[c] Abbreviation: NT, not treated.

Table 6. Effect of *Drechslera gigantea* (DG) formulation containing ammonium sulfate or pelargonic acid on torpedograss (TG).

Treatment	% Disease/injury severity after week: 1 Trial 1	1 Trial 2	2 Trial 1	2 Trial 2	3 Trial 1	3 Trial 2	4 Trial 1	4 Trial 2	5 Trial 1	5 Trial 2	6 Trial 1	6 Trial 2	7 Trial 1	7 Trial 2	10 Trial 1	10 Trial 2
Emulsion[a] + culture filtrate + fungus	23 ab[b]	23 bc	27 b	25 c	28 b	23 b	23 c	27 c	23 cd	20 c	33 c	10 c	47 b	5 c	90 a	2 b
Emulsion + culture filtrate + fungus + 4.5% ammonium sulfate	27 a	30 b	37 a	50 b	47 a	67 a	50 a	77 a	67 a	78 a	91 a	78 a	93 a	72 a	95 a	27 a
Emulsion + culture filtrate + 4.5% ammonium sulfate (fungus-free)	15 cd	13 cd	12 d	13 d	12 d	22 b	13 d	20 c	13 d	20 c	12 d	10 c	10 cd	5 c	10 cd	2 b
Emulsion + culture filtrate + 2% pelargonic acid (fungus-free)	17 bc	57 a	18 c	67 a	20 c	68 a	27 c	58 b	27 c	50 b	27 c	40 b	20 c	30 b	15 c	9 b
Emulsion + 2% pelargonic acid (fungus-free, culture filtrate-free)	23 ab	60 a	27 b	68 a	28 b	73 a	37 b	68 ab	43 b	60 b	47 b	53 b	50 b	43 b	43 b	7 b
Emulsion + culture filtrate (fungus-free, ammonium sulfate-free, pelargonic acid-free)	8 de	10 de	7 de	0 e	7 e	0 c	10 de	0 d	10 d	0 d	10 d	0 c	10 cd	0 c	8 cde	0 b
Emulsion only (culture filtrate-free, fungus-free, ammonium sulfate-free, pelargonic acid-free)	5 ef	10 de	5 ef	0 e	5 e	0 c	5 ef	0 d	5 de	0 d	5 de	0 c	3 d	0 c	3 de	0 b
Untreated control	0 f	0 e	0 f	0 e	0 f	0 c	0 f	0 d	0 e	0 d	0 e	0 c	0 d	0 c	0 e	0 b

[a] Emulsion was composed of Sunspray 6E and tomato juice broth (control) or DG mycelium, DG culture filtrate, and ammonium sulfate or pelargonic acid at the specified concentrations. Formulation (100 ml) was used to spray plants in three replicates (pots) (33 ml per pot). No dew was provided.

[b] Values (rounded to nearest whole number) within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P = 0.05$).

ammonium sulfate germinated very poorly or not at all when incubated in ammonium sulfate solutions (Table 3). The inhibitory effect on spore germination was more pronounced at higher than lower concentrations of ammonium sulfate.

Effect of Ammonium Sulfate on Virulence of DG Spores. DG spores produced on TJA medium containing different concentrations of ammonium sulfate failed to cause lesions or caused small, inconspicuous lesions and hence are considered avirulent. However, in the second trial, all conidia, whether produced on ammonium sulfate-free medium or medium containing any concentration of ammonium sulfate did not differ in their virulence (data not shown). Despite the negative effect of ammonium sulfate on mycelial growth of DG, the fungus was still able to grow to an acceptable level and since mycelium is the preferred form of DG inoculum, the adverse effect of ammonium sulfate on DG sporulation is not a concern.

Greenhouse Study. Fungal formulations containing 4.5% (w/v) ammonium sulfate or fungus-free, culture filtrate-free formulation containing 2% pelargonic acid were highly effective against TSG when rated 3 wk after treatment

Table 7. Effect of *Drechslera gigantea* (DG) formulation containing ammonium sulfate or pelargonic acid on smutgrass (SG).

Treatment	% Disease/injury severity after week: 1 Trial 1	1 Trial 2	2 Trial 1	2 Trial 2	3 Trial 2	4 Trial 2	5 Trial 2	6 Trial 2	7 Trial 2	10 Trial 2
Emulsion[a] + culture filtrate + fungus	33 c[b]	20 c	78 c	55 b	80 b	88 b	88 b	90 b	80 b	60 b
Emulsion + culture filtrate + fungus + 4.5% ammonium sulfate	63 b	50 b	88 b	95 a	100 a	100 a	100 a	100 a	100 a	100 a
Emulsion + culture filtrate + 4.5% ammonium sulfate (fungus-free)	10 d	17 c	20 d	87 a	98 a	98 a	100 a	100 a	100 a	100 a
Emulsion + culture filtrate + 2% pelargonic acid (fungus-free)	100 a	99 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a
Emulsion + 2% pelargonic acid (fungus-free, culture filtrate-free)	100 a	99 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a
Emulsion + culture filtrate (fungus-free, ammonium sulfate-free, pelargonic acid-free)	0 e	0 d	10 e	0 c	0 c	0 c	0 c	0 c	0 c	0 c
Emulsion only (culture filtrate-free, fungus-free, ammonium sulfate-free, pelargonic acid-free)	0 e	0 d	5 f	0 c	0 c	0 c	0 c	0 c	0 c	0 c
Untreated control	0 e	0 d	0 e	0 c	0 c	0 c	0 c	0 c	0 c	0 c

[a] The fungal formulation consisted of 10 g of fresh DG mycelium (w/v), 70 ml of DG culture filtrate (v/v), and 30 ml of Sunspray 6E (v/v). To this was added 4.5% ammonium sulfate or 2% pelargonic acid. Formulation (250 ml) was used to spray plants in three replicates (pots) (83 ml per pot). No dew was provided. First trial was terminated 2 wk after inoculation.

[b] Values (rounded to nearest whole number) within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P = 0.05$).

(> 99% injury in trial 1, or > 78% injury in trial 2) for disease/injury (Table 4), whereas large crabgrass (CG) were completely killed 3 wk after treatment by several treatments (emulsion containing culture filtrate and 4.5% ammonium sulfate either with or without the fungus, and the fungus-free emulsion containing 2% pelargonic acid either with or without the culture filtrate [Table 5]). Fungal formulation containing 4.5% ammonium sulfate was also effective (91% injury in trial 1, 78% injury in trial 2) against TG 6 wk after application, whereas pelargonic acid formulations were not effective against TG (Table 6). For smutgrass (SG), fungus-free emulsion amended with 2% pelargonic acid with or without the DG culture filtrate resulted in 100% control 1 wk (trial 1) or 3 wk (trial 2) after treatment, whereas fungal formulations containing 4.5% (w/v) ammonium sulfate resulted in 100% control after 3 wk (Table 7). With treatments that did not provide 100% control (Tables 4–7), the treated plants overcame the disease/injury, and by 7 to 10 wk were healthy again.

Results from this study indicate some possibilities for further development of a DG formulation composed of 10 g of DG mycelium, 70 ml of DG culture filtrate, 30 ml of Sunspray 6E oil, and 4.5 g of ammonium sulfate per 100-ml volume. Sunspray 6E oil containing 1 or 2% pelargonic acid (96% purity) is also highly effective against the grasses tested. From the results presented herein, it can be concluded that: (1) the bioherbicidal efficacy of an oil emulsion formulation composed of DG mycelium and Sunspray 6E oil was improved by adding fungus-free DG culture filtrate, ammonium sulfate, or pelargonic acid. (2) A practical level of control of TSG is possible with an emulsion formulation containing fungal mycelium, culture filtrate, and 4.5% ammonium sulfate or an emulsion formulation containing 2% pelargonic acid alone. (3) A fungus-free emulsion containing 2% pelargonic acid causes 100% SG and CG kill 1 and 2 wk after application in the greenhouse, respectively. (4) None of the formulations tested provided an acceptable level of TG control.

Further field studies are needed to evaluate these formulations with DG, particularly those containing ammonium sulfate and pelargonic acid. Additional studies should also be done to determine the feasibility of developing and possibly registering the most effective combinations as bioherbicides for spot treatment of grassy weeds in turf, sod, and pastures.

Sources of Materials

[1] Sunspray oil 6E, Sunoco, Inc., 1735 Market St. Ste. Ll, Philadelphia, PA 19103 (http://www.sunocoinc.com).

[2] Ammonium sulfate [$(NH_4)_2SO_4$], Fisher Scientific, 1 Reagent Ln., Fair Lawn, NJ 07410 (http://www.fishersci.com).

[3] Pelargonic acid (*n*-nonanoic acid 96%): Sigma-Aldrich, Inc., 3050 Spruce St., Saint Louis, MO 63103 (http://www.sigmaaldrich.com).

[4] Tomato juice, Publix Supermarkets, Inc., Lakeland, FL 33802 (http://www.publix.com).

[5] Potato dextrose agar, Becton, Dickinson and Company, Microbiology Systems, 39 Loveton Cir., Sparks Glencoe, MD 21152 (http://www.bd.com).

[6] Flatbed scanner, HP Scanjet 4570C; Hewlett-Packard Company, 3000 Hanover Street, Palo Alto, CA 94304-1185 (http://www.hp.com).

[7] Adobe Photoshop CS2 program, Adobe Systems Incorporated, 345 Park Avenue, San Jose, CA 95110-2704 (www.Adobe.com/PhotoshopCS4).

[8] Tween 80, Polyoxyethylene sorbitan mono-oleate; Fisher Scientific, 1 Reagent Ln., Fair Lawn, NJ 07410 (http://www.fishersci.com).

[9] Metro-Mix 300, Sungro Horticulture Canada Ltd., P.O. Box 189, Seba Beach, AB T0E 2B0, Canada (http://www.sungro.com).

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