

Effect of Selected Pesticides on Conidial Germination and Mycelial Growth of *Dactylaria higginsii*, a Potential Bioherbicide for Purple Nutsedge (*Cyperus rotundus*)¹

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Abstract: The suitability of a bioherbicide as a component of an integrated weed management program not only relies on its field efficacy, but also on its compatibility with other pest control measures that may be employed during the cropping season. The effects of selected pesticides applied according to label rates on *Dactylaria higginsii*, a biological control agent for purple nutsedge, were determined using mycelial growth on pesticide-amended potato dextrose agar (PDA) and conidial germination as indicators of pesticide sensitivity. Among the pesticides tested, the herbicides oxyfluorfen and sethoxydim and the fungicides fosetyl-Al and thiophanate methyl inhibited *D. higginsii* mycelial growth and reduced or completely inhibited conidial germination; the herbicide diuron, the fungicides metalaxyl and copper hydroxide, and the insecticide cyromazine reduced mycelial growth but did not reduce conidial germination. The miticide dicofol reduced mycelial growth and completely inhibited conidial germination while the herbicide imazapyr had no adverse effect on either the mycelial growth or conidial germination of *D. higginsii*.

Nomenclature: Copper hydroxide; cyromazine; dicofol; diuron; fosetyl-Al, Aluminum tris (ethyl phosphonate); imazapyr; metalaxyl; oxyfluorfen; sethoxydim; thiophanate methyl; purple nutsedge, *Cyperus rotundus* L. #³ CYPRO, *Dactylaria higginsii* (Luttrell) M. B. Ellis.

Additional index words: Biological control, bioherbicide, pesticide sensitivity, conidial germination.

Abbreviations: CRD, complete randomized design; RPA, rice polish agar.

INTRODUCTION

Purple nutsedge is one of the most severe weed problems in vegetable production systems worldwide. Purple nutsedge infestation causes significant yield reduction in vegetable crops including tomato (*Lycopersicon esculentum* Mill.) (53% reduction), garlic (*Allium sativum* L.) (89%), okra (*Abelmoschus esculentus* [L.] Moench) (62%), cabbage (*Brassica oleracea* var. *capitata*) (35%), bell pepper (*Capsicum annuum* L.) (73%), carrot (*Daucus carota* L.) (39 to 50%), cucumber (*Cucumis sativus* L.) (43%), and radish (*Raphanus sativus* L.) (70%) (Keeley 1987; Morales-Payan et al. 1998; Santos et al. 1996; William and Warren 1975).

Because long-term suppression of purple nutsedge is

difficult to achieve with existing chemical and nonchemical methods, other means of reducing its impact on crops, such as the use of biological control agents, have been investigated. Kadir and Charudattan (2000) reported that under greenhouse conditions, the application of the hyphomyceteous fungus *Dactylaria higginsii* (Luttrell) M. B. Ellis (Mitosporic fungi) on purple nutsedge resulted in significant reduction in shoot number (72%), shoot dry weight (73%), and tuber dry weight (67%). In field studies, repeated applications of *D. higginsii* at the rate of 10⁶ conidia per milliliter provided >90% purple nutsedge control (Kadir et al. 2000). The application of *D. higginsii* on purple nutsedge in tomato crop reduced interference and resulted in increased tomato yield (Kadir et al. 1999).

Plant pathogens that are developed as bioherbicides are evaluated primarily for their biocontrol efficacy and potential for commercial application. Additionally, studies are needed to determine compatibility with other pesticides because tank mixing would be advantageous to growers. The application of a mixture of a bioherbicide and a herbicide can increase the damage to the target weed (Bannon et al. 1988; Cardina and Littrell 1986; Quimby 1985; Scheepens 1987; Smith 1991; Wymore and Watson 1988) or broaden the range of weed species

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³ Letters following this symbol are a WSSA-approved computer code from *Composite List of Weeds*, Revised 1989. Available only on computer disk from WSSA, 810 East 10th Street, Lawrence, KS 66044-8897.

that can be controlled by the biocontrol agent (Khodayari and Smith 1988). According to Altman et al. (1990) and Charudattan (1993), the addition of herbicides may increase the level of weed control by stressing the weed and rendering it more vulnerable to the colonization by the biological agent. However, some pesticide-bioherbicide interactions can result in reduced levels of weed control due to inhibition of the biological control agent (Charudattan 1985; Rayachhetry and Elliott 1997; Ridings 1986).

Hence, it is important to determine any antagonistic, synergistic, or additive effects when combining synthetic pesticides with the bioherbicide. Accordingly, this study was conducted to determine the sensitivity of *D. higginsii* to various pesticides.

MATERIALS AND METHODS

Source of Isolate. The *D. higginsii* isolate used in this study was originally isolated from diseased purple nutsedge plants collected in Gainesville, FL. Mycelial plugs and conidia for the experiments were obtained from 4-wk-old *D. higginsii* cultures grown on rice-polish agar (RPA; 20 g rice polish [flour removed from brown rice], 16 g of agar in 1 L of water).

Pesticides Used. The pesticides used in this study were the fungicides fosetyl-Al,⁴ copper hydroxide,⁵ metalaxyl,⁶ and thiophanate methyl⁷; the herbicides diuron,⁸ glyphosate,⁹ imazapyr,¹⁰ oxyfluorfen,¹¹ and sethoxydim¹²; the miticide dicofol¹³; and the insecticide cyromazine.¹⁴ The bases for selection were (1) recommended for use in tomato, (2) use in areas adjacent to tomato fields (ditch banks, rights-of-way), or (3) to compare the effect of different chemical classes/active ingredients to *D. higginsii*.

⁴ Aliette WDG, Bayer CropScience, 2 T. W. Alexander Drive, Research Triangle Park, NC 27709.

⁵ Kocide DF, Griffin LLC, P.O. Box 1847, Valdosta, GA 31603.

⁶ Ridomil Gold EC, Syngenta Crop Protection, 410 Swing Road, Greensboro, NC 27409.

⁷ Topsin M, Cerexagri, Inc., Suite 402, King of Prussia, PA 19406.

⁸ Karmex DF, Griffin LLC, P.O. Box 1847, Valdosta, GA 31603.

⁹ Roundup Ultra, Monsanto Company, 800 North Lindbergh Boulevard, St. Louis, MO 63167.

¹⁰ Arsenal, BASF Corporation, 3000 Continental Drive North, Mount Olive, NJ 07828.

¹¹ Goal 2XL, Dow AgroSciences, LLC, 9330 Zionsville Road, Indianapolis, IN 46268.

¹² Poast, BASF Corporation, 3000 Continental Drive North, Mount Olive, NJ 07828.

¹³ Kelthane MF, Dow AgroSciences, LLC, 9330 Zionsville Road, Indianapolis, IN 46268.

¹⁴ Trigard, Syngenta Crop Protection, 410 Swing Road, Greensboro, NC 27409.

Effect of Pesticides on Mycelial Growth. To determine the effect of the pesticides on mycelial growth, 60 × 15 mm petri dishes containing 10 ml of PDA per plate were prepared, with and without pesticides (control). The pesticides tested and their rates are listed in Table 1. Plates were inoculated with a 6-mm mycelial plug of *D. higginsii* and incubated under 12 h of ambient light per day at room temperature. Colony diameter was computed by subtracting the initial diameter (6 mm) from the final diameter measurements, which were taken after 4 wk of incubation. The experiment was performed twice in a completely randomized design (CRD) with five replications per treatment. The effect of herbicides and fungicides/insecticide/miticide on *D. higginsii* growth was tested in separate experiments.

Effect of Pesticides on Conidial Germination. To determine the effect of the pesticides on conidial germination, conidia of *D. higginsii* harvested from 4-wk-old cultures on RPA were mixed with sterile water amended with the various pesticides. Pesticide concentrations used in these assays were used in accordance with the recommended rates (Table 1). The conidia were incubated with the pesticides for 1 h at room temperature. After incubation, a 200- μ l droplet of each conidia and pesticide mixture was evenly spread on water agar (14 g of microbiological agar in 1 L of water) plus 2% sucrose in 100 × 15 mm Petri plates. The inoculated plates were incubated for 24 h at room temperature under 12 h ambient light, after which the conidial germination was determined. Percentage conidial germination was determined by counting the number of germinated and non-germinated conidia out of 30 conidia on each plate. The experiment was performed twice in a CRD with four replications per treatment.

Data Analysis. Data from similar trials were pooled when the variances were homogenous. All data were analyzed using the Proc general linear model procedure of SAS¹⁵ with $P = 0.05$. Conidial germination data were transformed using the arcsine square-root transformation before analysis was performed. The means were compared using Duncan's multiple range test.

RESULTS AND DISCUSSION

Effect of Pesticides on Mycelial Growth. The herbicides sethoxydim and oxyfluorfen completely inhibited the growth of *D. higginsii* grown on pesticide-amended PDA, whereas significant growth reductions were ob-

¹⁵ SAS 9.1, SAS Institute Inc., SAS Campus Drive, Cary, NC 27513.

Table 1. Pesticide active ingredient, pesticide class, chemical class, formulation, mode of action, and rates tested on *Dactylaria higginsii*.

Pesticide active ingredient	Pesticide class	Chemical class	Formulation	Mode of action	Label rate ^a	Rate per liter of medium ^b
Fosetyl-Al	Fungicide	Organophosphate	Wettable powder	Systemic; stimulates plant defenses	5.6 kg ha ⁻¹	44.94 ml
Copper hydroxide	Fungicide/ Bactericide	Inorganic-copper	Dry flowable	Enzyme function disruptor	2.125 kg ha ⁻¹	11.24 g
Metalaxyl	Fungicide	Xylolalanine	Emulsifiable concentrate	RNA synthesis inhibitor	1.17 L ha ⁻¹	3.57 ml
Thiophanate methyl	Fungicide	Benzimidazole	Wettable powder	DNA synthesis inhibitor	0.575 kg ha ⁻¹	0.599 g
Imazapyr	Herbicide	Imidazolinone	Aqueous solution	ALS/AHAS inhibitor	3.5 L ha ⁻¹	21.42 ml
Oxyfluorfen	Herbicide	Diphenylether	Emulsifiable concentrate	Cell membrane disrupter	2.34 L ha ⁻¹	12.5 ml
Diuron	Herbicide	Substituted urea	Dry flowable	Photosynthesis inhibitor	0.45 kg ha ⁻¹	23.97 g
Sethoxydim	Herbicide	Cyclohexenone derivative	Liquid emulsifiable concentrate	ACC-ase ^c inhibitor; lipid biosynthesis inhibitor	14.84 ml/L	14.84 ml
Glyphosate	Herbicide	Amino acid derivative	Soluble concentrate	Amino acid synthesis inhibitor	2% solution	20.8 ml
Dicofol	Miticide	Organochlorine	Emulsifiable concentrate	Contact poison, electron transport inhibitor	7.041 ml/L	7.041 ml
Cyromazine	Insecticide	Triazine	Wettable powder	Insect growth regulator; molting and pupation inhibitor	1.99 g/L	1.99 g

^a Rates were computed on the basis of recommended label rates for ground application to control weeds, insects, mites, or diseases in tomato or pepper. Imazapyr rates were based on label recommendation for ground application on weeds in railroad, rights-of-way, fence rows, pumping installations, and similar areas.

^b Medium refers to either potato dextrose agar or water.

^c Abbreviation: ACC-ase, Acetyl-CoA carboxylase.

served with diuron and glyphosate (Figure 1). Among all the herbicides tested, only imazapyr had no adverse effect on mycelial growth. The fungicides fosetyl-Al and thiophanate methyl completely inhibited mycelial growth, whereas metalaxyl, copper hydroxide, the miticide dicofol, and the insecticide cyromazine reduced my-

celial growth compared with that of the nontreated control (Figure 2).

Reduction of *D. higginsii* colony growth on PDA amended with diuron, a photosynthesis inhibitor in plants, was probably due to the effect of toxic metabolites that were released during its breakdown during the

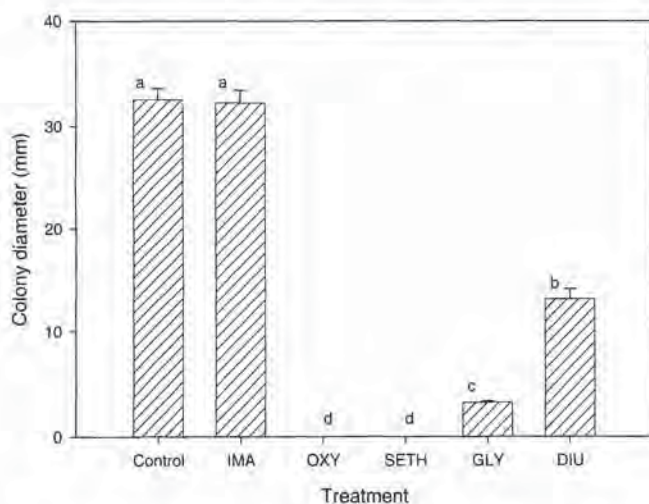


Figure 1. Effect of herbicides on the mycelial growth of *D. higginsii*. Control indicates water; IMA, imazapyr; OXY, oxyfluorfen; SETH, sethoxydim; GLY, glyphosate; DIU, diuron. Bars represent 10 means from 2 similar trials. Vertical lines indicate standard errors of mean. Bars with the same letter are not significantly different at $P = 0.05$ as determined by Duncan's multiple range test.

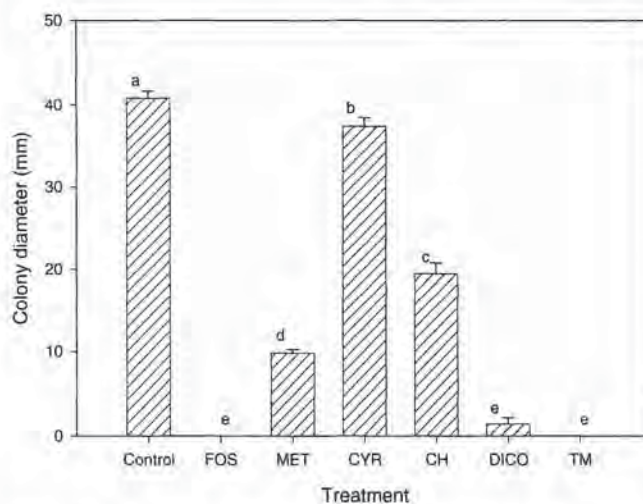


Figure 2. Effect of fungicides and an insecticide/miticide on the mycelial growth of *D. higginsii*. Control indicates water; FOS, fosetyl-Al; META, metalaxyl; CYR, cyromazine; CH, copper hydroxide; DICO, dicofol; TM, thiophanate methyl. Bars represent 10 means from 2 trials. Vertical lines indicate standard errors of mean. Bars with the same letter are not significantly different at $P = 0.05$ as determined by Duncan's multiple range test.

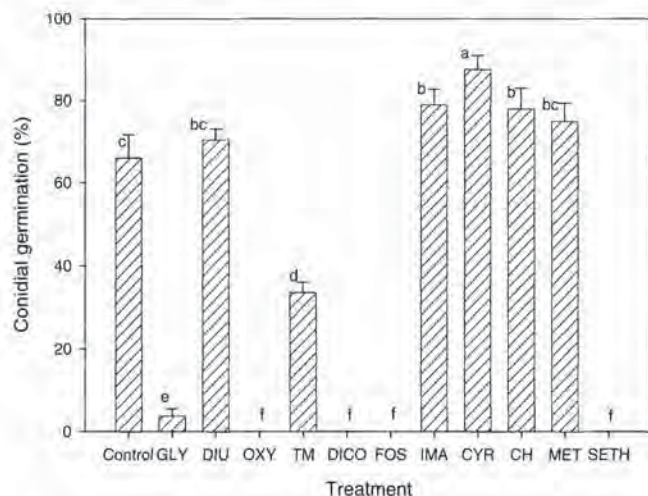


Figure 3. Effect of various pesticides on the germination of *D. higginsii* conidia. Control indicates water; GLY, glyphosate; DIU, diuron; OXY, oxyfluorfen; TM, thiophanate methyl; DICO, dicofol; FOS, fosetyl-Al; IMA, imazapyr; CYR, cyromazine; CH, copper hydroxide; MET, metalaxyl; SETH, sethoxydim. Bars represent eight means from two trials. Vertical lines indicate standard errors of mean. Bars with the same letter are not significantly different at $P = 0.05$ as determined by Duncan's multiple range test.

course of the experiment. According to data presented by Tixier et al. (2000), microorganisms are able to break down diuron, resulting in the release of monodemethylated and didemethylated metabolites, which are more toxic than diuron itself. A possible explanation for the nontoxic effects of imazapyr is its tendency to be photodegraded; because imazapyr has a half-life of 2 d in aqueous solution (Mallipudi et al. 1991), it is possible that exposure of the plates that contained imazapyr to light (12 h/d) degraded it before it could have an effect on the colony growth of *D. higginsii*. On the other hand, the negative effect of cyromazine may be due to its ability to inhibit chitin synthesis, a process that occurs in insects as well as in fungi. Nikkomycin Z, a fungal chitin synthase competitive inhibitor, was able to block chitin synthesis in sheep blowfly (*Lucilia cuprina* [Wiedemann]) (Diptera: Calliphoridae) (Tellam et al. 2000).

Effect of Pesticides on Conidial Germination. Among the herbicides tested, sethoxydim, glyphosate, and oxyfluorfen either completely suppressed or significantly reduced germination of *D. higginsii* conidia. Diuron and imazapyr did not reduce germination; conidia exposed to these herbicides had equal or higher germination than conidia exposed to water only (control) (Figure 3). Among the fungicides, only fosetyl-Al and thiophanate methyl inhibited or reduced conidial germination. Exposure to two other fungicides, metalaxyl and copper hydroxide, did not have an adverse effect on the conidia germination (Figure 3). Exposure to the miticide dicofol

resulted in no germination, whereas exposure to the insecticide cyromazine resulted in germination that was higher than the control.

Mycelial growth of *D. higginsii* was reduced or completely suppressed by 10 of the 11 pesticides tested, whereas conidial germination was reduced or completely inhibited by exposure to only 6 of the 11 pesticides. Pesticides that reduced both mycelial growth and conidial germination were the fungicides fosetyl-Al and thiophanate methyl; the herbicides oxyfluorfen, sethoxydim, and glyphosate; and the miticide dicofol. Pesticides that reduced mycelial growth but did not reduce conidial germination were the herbicide diuron, the fungicides copper hydroxide and metalaxyl, and the insecticide cyromazine. The differential reaction of *D. higginsii* mycelia and conidia to the same pesticide is probably due in part to the length of exposure; mycelia were exposed to the pesticide for 4 wk, whereas the conidia were exposed for 1 h. Differential sensitivity of mycelia and conidia to the same compound has been reported for another hyphomyceteous fungus, *Metarrhizium anisopliae* (Metschnikoff) Sorokin strain ESC-1, a biocontrol agent for the German cockroach, *Blattella germanica* (L.). Pachamuthu et al. (1999) observed that *M. anisopliae* mycelium was more sensitive to insecticides than the conidia. This phenomenon is believed to be due, to some extent, to the dissimilar mechanisms involved in mycelial development and conidial germination. Germination of conidia may be less affected by exogenous materials because the process primarily involves the utilization of reserved nutrients and a minimal amount of carbon from the environment or substrate, whereas mycelial growth requires the utilization of exogenous carbon and nitrogen (Gottlieb 1976; Smith and Grula 1981; St. Leger et al. 1989). In the course of its growth, mycelia may take up other materials that are present in the substrate, including pesticides.

Here we compare the reaction of *D. higginsii* to the reactions of other fungal species (belonging to the same fungal class as *D. higginsii* or otherwise) to pesticides of the same class or of a similar mode of action. The only pesticide that did not reduce or suppress both mycelial growth and conidial germination was imazapyr, an imidazolinone herbicide that inhibits acetolactate synthase in plants. The imidazolinones imazapyr and imazethapyr did not reduce conidial germination of *Phomopsis amaranthicola* Roskopf, Charudattan, Shabana & Benny, a coelomycete with bioherbicidal potential against *Amaranthus* spp. (Wyss et al. 2004). Similarly, Rayachhetry and Elliot (1997) reported that imazethapyr

did not reduce the inoculum (hyphal) viability of *Botryosphaeria ribis* Gross. & Duggar, an ascomycete with bioherbicidal effects to the Australian mealeuca, *Meleuca quinquenervia* (Cav.) S. T. Blake.

Negative effects of exposure to glyphosate have been reported for other fungi as well; it reduced the aeciospore germination of the basidiomycete *Puccinia lagenophorae* Cooke, a biocontrol agent for *Senecio vulgaris* L. (Wyss and Muller-Scharer 2001) and was fungitoxic to another basidiomycete, *Chondrostereum purpureum* (Pers.:Fr.) Pouzar, a bioherbicide for forest weeds (Prasad 1994). However, the toxicity of glyphosate may be due to the formulation additive and not to glyphosate itself. A glyphosate formulation that did not contain any adjuvant¹⁶ did not reduce germination of the coelomycete *Aposphaeria amaranthi* (Ell. & Barth) (syn. *Microsphaeropsis amaranthi*), a potential bioherbicide for waterhemp (*Amaranthus rudis* Sauer) as much as the other glyphosate formulations that contain tallowamine surfactants (Smith and Hallett,¹⁷ unpublished data).

Sethoxydim, which completely inhibited mycelial growth and germination of *D. higginsii*, has been found to have an adverse effect on the conidial germination of the coelomycete *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. In Penz. f. sp. *malvae*, a bioherbicide for round-leaved mallow (*Malva pusilla* Sm.) (Grant et al. 1990). The herbicides sethoxydim and diuron and the fungicide fosetyl-Al have all been reported to be incompatible with *P. amaranthicola* (Wyss et al. 2004). Negative effects from exposure to thiophanate methyl have been reported on *C. gloeosporioides* f. sp. *malvae* (Grant et al. 1990) and *Beauveria bassiana* (Balsamo) Vuillemin, a hyphomycete (Todorova et al. 1998). Copper hydroxide reportedly killed conidia of *P. amaranthicola* (Wyss et al. 2004). Prasad (1994) also reported that metalaxyl did not reduce the mycelial growth of *C. purpureum*. Cyromazine, which reduced mycelial growth but not the conidial germination of *D. higginsii*, had a dissimilar effect on *P. amaranthicola*, conidia treated with cyromazine had reduced germination (Wyss et al. 2004). Fungi differ in their sensitivity to pesticides of the same chemical class or same mode of action. Therefore, a generalization cannot be made as to what types of fungi are more or less sensitive to a certain pesticide.

Long-term exposure of *D. higginsii* mycelia and short-term exposure of conidia to the same pesticides can pro-

duce different results. Because conidial germination was not reduced by short-term exposure to some pesticides, it is possible that the efficacy of *D. higginsii* will not be reduced by tank mixes if conidia are used. Additional studies are needed to investigate the differences between mycelial inhibition and conidial germination enhancement by the same compounds. The feasibility of tank mixing *D. higginsii* conidia with the compatible herbicides to improve the level of weed suppression also need to be verified by field studies.

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