Biological Control 54 (2010) 159–165

Contents lists available at ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon

Production and application of the bioherbicide agent *Dactylaria higginsii* on organic solid substrates

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ARTICLE INFO

Article history:
Received 18 February 2010
Accepted 4 May 2010
Available online 10 May 2010

Keywords:
Spore production
Organic substrates
Solid substrates
Dactylaria higginsii
Bioherbicides
Nutsedge

ABSTRACT

Purple nutsedge and yellow nutsedge are serious weeds in Florida and in many parts of the world. *Dactylaria higginsii* is a promising bioherbicide candidate for these weeds. This fungus does not sporulate in liquid culture. Thus, for mass production of *D. higginsii*, 19 solid substrates were tested, including dried, cut culms or foliage of 14 different plants prepared as hays, sugarcane bagasse, a commercial peat-based microbe carrier (BioAPT), and two recycled papers (kraft paper and newspaper). These substrates were tested alone or amended either with 0.01% indole butyric acid (IBA), which enhances growth and/or sporulation of *D. higginsii*, potato dextrose broth (PDB) for nutrient amendment, or PDB + IBA. Yield of *D. higginsii* conidia produced on these substrates was measured 4 and 12 weeks after inoculation. Conidia were tested for virulence on purple and yellow nutsedges in the greenhouse. Hays without amendments or amended with PDB yielded more conidia which were also more virulent than those produced on hays amended with IBA. Conidial yields were highest when the fungus was grown on purple nutsedge hay without amendments for 4 weeks but conidia produced on this medium had low virulence. Conidia produced on sorghum × sudangrass and cogongrass hays were slightly larger and thicker walled than those produced on other substrates. Conidia produced on sorghum × sudangrass were the most virulent on nutsedge seedlings. Purple nutsedge was more susceptible to *D. higginsii* than yellow nutsedge. Cogongrass and sorghum × sudangrass hay substrates offer a suitable and easy medium for large-scale production of *D. higginsii*.

1. Introduction

Purple nutsedge (*Cyperus rotundus* L.) and yellow nutsedge (*Cyperus esculentus* L.) are the most troublesome weeds in crops in many parts of the world (Holm et al., 1991). High yield losses of 70% in radish, 81% in beans (Santos et al., 1996; William, 1973), 43% in cucumber and rice (Okafor and De Datta, 1974, 1976; William and Warren, 1975), 50% in carrot, and 54% in lettuce (Morales-Payan et al., 1996; William and Warren, 1975) have been reported due to purple nutsedge interference. Yield reductions due to purple nutsedge are reported to be as high as 45% and 58% in cotton and soybean, respectively, in Philippines and India (Guantes and Mercado 1975; Kondap et al., 1982). Over 40% yield loss was incurred with 12 yellow nutsedge plants/m^2 in direct-seeded or transplanted watermelon (Buker et al., 2003). Nutsedges propagate primarily by tubers that can sprout repeatedly, often producing more than one culm at a time (Bendixen, 1973; Stoller et al., 1972; Thullen and Keeley, 1979). Most tubers sprout in the spring, but some can remain dormant for more than 3 years (Stoller and Sweet, 1987), with some yellow nutsedge tubers remaining dormant for up to 10 years (Neal, 1995). Consequently, nutsedge control strategies must include a long-term plan for preventing new tuber formation. Although both purple and yellow nutsedges produce viable seeds, they are not a significant factor because of poor seedling vigor (Lapham and Drennan, 1990; Stoller and Sweet, 1987). Yellow nutsedge is more cold- and drought-tolerant than purple nutsedge and infests a wider geographic area (Bendixen and Nandihalli, 1987; Day and Russell, 1955; Holm et al., 1991; Stoller, 1973).

In Florida, where this study was conducted, conventional production systems for horticultural crops rely on plastic mulching and methyl bromide soil fumigation for nutsedge suppression. When the use of methyl bromide as a soil fumigant is phased out, the losses due to nutsedge competition are expected to

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doi:10.1016/j.biocontrol.2010.05.002

increase in fumigation-dependent crops (e.g., Gilreath and Santos, 2004). Yields in organic production systems will continue to suffer due to the lack of effective weed controls. A promising approach for nutsedge management is biological control with the fungus *Dactylaria higginsii* (Kadir and Charudattan, 2000).

One of the constraints facing *D. higginsii* as a bioherbicide agent is the difficulty in large-scale production of its spores (conidia) for use as inoculum. Liquid fermentation systems are preferred for mass production of bioherbicide agents (Churchill, 1982; Stowell, 1991), but *D. higginsii* does not sporulate in liquid culture (Shabana et al., unpublished). Since fungi are adapted to grow and sporulate on solid substrates, agricultural products have been used for mass production of several fungal biocontrol agents (Connick et al., 1990; Zhang and Watson, 1997; Shabana and Hallett, 2006). Wyss et al. (2001) used several complex media containing biological materials as well as chemically defined agar media for mass production of this fungus. They found that conidium production was highly variable; fewer were produced on complex agar media than on chemically defined media. In an attempt to improve conidial yields of *D. higginsii*, Morales-Payan et al. (2004) tested the effects of several plant growth regulators and biostimulants and found that yield was improved by 0.01% indole butyric acid, 0.03% acetylthioproline, and 0.25% of commercial mixture of zeatine + indole acetic acid + gibberellic acid + micronutrients. They reported that when agar media were amended with 0.01% IBA, the conidial yield was increased by five times without affecting virulence.

The specific objectives of this research were to develop a low-cost solid substrate for mass production of high quality inoculum of *D. higginsii*. Our long-term goal is to develop a *D. higginsii*-infested hay for integrated management of nutsedges. Our premise is that a hay-based application of *D. higginsii* will prolong and promote weed-suppression from the combined activities of the biocontrol agent and the physical interference effects of the mulch.

2. Materials and methods

2.1. Fungal isolate used

The *D. higginsii* isolate used in this study was isolated in 1994 from naturally infected purple nutsedge leaves collected in Gainesville, Florida and reported as a biocontrol agent for purple nutsedge, yellow nutsedge, and other sedges (Kadir and Charudattan, 2000). The isolate has been preserved in soil tubes maintained at 10 °C, and a culture has been deposited as ATCC #74379 in the American Type Culture Collection.

2.2. Production of D. higginsii conidia on different plant-based solid substrates

The feasibility of production of *D. higginsii* inoculum, as conidia, was tested on 15 solid, plant-based substrates in the form of dried, cut culms or foliage of plants. In addition, four other substrates were tested, including an industrial byproduct (sugarcane bagasse), recycled paper (kraft and newspapers), and a commercial peat-based microbe carrier (BioAPT) (Table 1). The plant-based substrates were cereal rye (*Secale cereale* L.), pearl millet (*Pennisetum glaucum* [L.] R. Br.), sorghum × sudangrass (*Sorghum bicolor* (L.) Moench nothosubsp. *drummondii* (Steud.) de Wet ex Davidse), corn (*Zea mays* L.), cowpea (*Vigna unguiculata* [L.] Walp.), sunnhemp (*Crotalaria juncea* L.), Pensacola bahiagrass (*Paspalum notatum* Flüggé), cogongrass (*Imperata cylindrica* [L.] P. Beauv.), purple nutsedge (*Cyperus rotundus* L.), yellow nutsedge (*C. esculentus* L.), timothy hay (*Phleum pretense* L.), waterhyacinth (*Eichhornia crassipes* [Mart.] Solms), pine (*Pinus* sp.), cedar (*Cedrus* sp.), and sugarcane (*Saccharum officinarum* L.). All were used as dried cut leaves and stalks except for corn, waterhyacinth, pine, cedar, and sugarcane, which were used as husks, whole plants, needles, stem shavings, and bagasse, respectively. These were tested alone (group 1) or amended with 0.01% (w/v) indole butyric acid (IBA) at a rate of 4:1 (v:w; IBA solution:substrate dry weight) (group 2), potato dextrose broth (PDB; Difco, Becton, Dickinson and Company, Sparks, MD 21152) (group 3), or with PDB + 0.01% IBA (group 4). IBA is a plant growth regulator and biostimulant that was found in a previous study to promote conidium production in *D. higginsii* (Morales-Payan et al., 2004).

Table 1
Solid substrates evaluated for mass production of *Dactylaria higginsii* conidia.

	Substrate[a]	Scientific name	Plant parts used
1	Rye	*Secale cereale* L.	Leaves + culms
2	Millet	*Pennisetum glaucum* (L.) R. Br.	Leaves + culms
3	Sorghum x sudangrass	*Sorghum bicolor* (L.) Moench nothosubsp. *drummondii* (Steud.) de Wet ex Davidse	Leaves + culms
4	Corn	*Zea mays* L.	Husks
5	Corn	*Zea mays* L.	Leaves + culms
6	Cowpea	*Vigna unguiculata* (L.) Walp.	Leaves + culms
7	Sunnhemp	*Crotalaria juncea* L.	Leaves + culms
8	Bahiagrass	*Paspalum notatum* Flüggé	Leaves + culms
9	Cogongrass	*Imperata cylindrica* (L.) P. Beauv.	Leaves + culms
10	Purple nutsedge	*Cyperus rotundus* L.	Leaves + culms
11	Yellow nutsedge	*C. esculentus* L.	Leaves + culms
12	Timothy hay	*Phleum pratense* L.	Leaves + culms
13	Waterhyacinth	*Eichhornia crassipes* (Mart.) Solms	Leaves + stems + roots
14	Pine	*Pinus* L. sp.	Needles
15	Cedar	*Cedrus* sp.	Wood shavings
16	Sugarcane	*Saccharum officinarum* L.	Bagasse
17	Kraft paper	NA	NA
18	Newspaper	NA	NA
19	BioAPT	NA	NA

[a] The plant materials used in this study were collected from local farms/waterways around Gainesville, FL. Sugarcane bagasse was obtained from a manufacture (1500 Gator Boulevard, Belle Glade, Florida 33430). The recycled papers (kraft and newspapers) were obtained from local resources on the University of Florida campus at Gainesville, and a commercial granular peat-based microbe carrier (BioAPT) samples were obtained from American Peat Technology, LLC, Aitkin, MN <www.AmericanPeatTech.com>.

One-quarter liter flasks, each containing 3 g of plant material, 3 g washed coarse sand (1.4–2.0 mm diam.) and 12 ml deionized (DI) water, with and without the amendments, were autoclaved at 121 °C for 60 min. and after cooling, inoculated with 3 ml of *D. higginsii* mycelial suspension (Fig. 1). In the treatments amended with PDB or IBA solutions, those were added in equal volume as a substitute for the DI water. Sand was used to enhance aeration and increase the substrate surface for fungal growth and sporulation by preventing clumping of plant material. IBA solution in DI water (0.02% w/v) was prepared by dissolving the IBA in a small amount of ethanol, which was then mixed with water. Six milliliters of this solution were added to the substrates in each flask containing 6 ml DI water after autoclaving and cooling to bring the concentration of the IBA solution to 0.01%. The sugarcane bagasse, corn leaves and stalks, kraft paper, newspaper, and BioAPT (granular peat-based microbe carrier; American Peat Technology, LLC, Aitkin, MN <www.AmericanPeatTech.com>) substrates were tested without amendment following the same procedure as described above. BioAPT was tested alone or mixed with cogongrass, purple nutsedge, or sorghum × sudangrass hays at 1:1 (w/w). The inoculated flasks were incubated at 25 ± 2 °C on a laboratory bench.

Conidia were harvested by adding 50 ml of sterile DI water containing Tween® 20 at 0.01% (v/v) to each flask, shaking by hand for 1 min and filtrating through 2-layers of sterile cheesecloth into

Fig. 1. Cogongrass hay non-inoculated (left) vs. inoculated (right). Hay on the right is covered with dark *D. higginsii* mycelium and conidia, 4 weeks after inoculation.

50-ml sterile centrifuge tubes. The volume in each tube was then adjusted to 50 ml using sterile DI water containing Tween® 20 (0.01% v/v). Three replicate flasks were used per treatment. Conidial yield from these substrates was measured 4 and 12 weeks after inoculation with the aid of a haemacytometer. Three readings were recorded for each replicate, the counts averaged and the number of conidia per gram of substrate was calculated. Those that were harvested after 4 weeks were harvested a second time, 4 weeks after the first harvest. The experiments were performed twice with three replicates for each treatment.

2.3. Conidium production on substrates in combination with and without BioAPT

The commercial microbe carrier BioAPT was examined for its effects on conidium yield and virulence of *D. higginsii* produced in this carrier. BioAPT was combined with substrates that had been determined to promote conidiation and/or enhance virulence of *D. higginsii* conidia. The following five combinations were used: BioAPT + sorghum × sudangrass; BioAPT + cogongrass; BioAPT + purple nutsedge; purple nutsedge + cogongrass; and purple nutsedge + sorghum × sudangrass. Plant substrate-only controls, consisting of 3 g chopped plant material, 3 g sand (1.4–2.0 mm diam.) and 12 ml DI water in a 250-ml Erlenmeyer flask, were included for comparison. BioAPT + plant substrate combination treatments consisted of 3 g BioAPT and 3 g of sorghum × sudangrass, cogongrass, or purple nutsedge hays, and 12 ml DI water in a 250-ml Erlenmeyer flask. The treatments of purple nutsedge hay combined with cogongrass or sorghum × sudangrass hay consisted of 1.5 g of each hay and 12 ml DI water in a 250-ml Erlenmeyer flask. Flasks containing the plant substrates were autoclaved for 1 h and then inoculated with 3 ml of *D. higginsii* mycelial suspension. Four flasks (replicates) were prepared for each individual or combination treatment. All inoculated substrates in flasks were incubated at room temperature (25 ± 3 °C) and ambient light for 6 weeks.

Conidia produced in each flask were harvested by adding 50 ml sterile DI water containing 0.01% (v/v) Tween® 80 to the flask and shaking the flasks by hand for 1 min. The resulting suspension was poured through 2-layers of sterile cheesecloth into a 50-ml sterile centrifuge tube. The conidial suspension was adjusted to 50 ml by adding sterile DI water containing 0.01% (v/v) Tween® 80. Conidia were counted with the aid of a haemacytometer using three readings for each flask and four flasks per treatment (replicates).

2.4. Virulence of conidia produced on different solid substrates against purple nutsedge and yellow nutsedge

The purple and yellow nutsedge plants used in this experiment were grown from tubers purchased from Azlin Seed Service, Leland, Mississippi. Tubers of uniform size, ranging from 0.6–0.8 g fresh weight for purple nutsedge tubers and 0.4–0.6 g for yellow nutsedge tubers were used. Tubers were pre-sprouted by submerging in water in shallow trays (10-cm deep) for one week to allow uniform growth. The water was then drained and the trays were covered with plastic wrap to maintain moisture. Tubers were rinsed every other day until they sprouted. When enough tubers had sprouted, the culms were trimmed to 1 cm in length and four sprouted tubers were seeded per pot (10-cm-diam) containing commercial potting medium (Metro-Mix 300; Scott–Sierra Horticultural Products Co., Marysville, OH).

Since not all of the solid substrates supported conidium production, only conidia produced in sufficient numbers on a solid substrate were evaluated for their virulence on purple and yellow nutsedges. On this basis, conidia produced on sorghum × sudangrass, bahiagrass, cogongrass, purple nutsedge, and yellow nutsedge without amendments (group 1) or amended with PDB + IBA (group 4) were tested. Virulence was assessed 4 and 8 weeks after inoculation of the substrate (for group 1) or 8 weeks after inoculation (for group 4). Among all substrates, only conidia produced on cogongrass amended with IBA (group 2) and those produced on sorghum × sudangrass amended with PDB (group 3) were tested 4 weeks after inoculation.

For inoculation, nutsedge seedlings (4–6-leaf) were sprayed with a suspension containing 1×10^6 conidia per ml until run off and then placed in a dew chamber for 24 h at 27 °C in the dark. Four pots (replicates), each containing four nutsedge seedlings, were used per treatment. Disease incidence (DI; as a percentage of leaves showing any degree of disease) and disease severity (DS; proportion of plant tissue area diseased) were measured 3 weeks after inoculation. At the end of the experiment (3 weeks after inoculation), purple nutsedge and yellow nutsedge culms were cut at the soil surface, oven-dried at 60 °C for 3–6 days, and the dry weight was recorded.

2.5. Statistical analysis

Experiments were arranged in a completely randomized design with 3–4 replications and were repeated once. Data from both trials were pooled since a test of homogeneity of variance by the Bartlett test (Gomez and Gomez, 1984) justified such pooling. 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 (at $P = 0.05$) were determined with Duncan's new multiple range test. DI, DS, and nutsedge culm dry weight data were subjected to repeated measures ANOVA to determine if there are time and/or treatment-time interaction effects.

3. Results

3.1. Production of conidia on different solid substrates

In general, hay substrates without amendments or those amended with PDB generated the production of more conidia than

Table 2
Effect of solid substrates and amendments on production of *D. higginsii* conidia harvested twice[a].

Substrate	No amendments		Amended with indole butyric acid (IBA)		Amended with potato dextrose broth (PDB) + IBA		Amended wit PDB
	First harvest Spore/g (×1000)	Second harvest Spore/g (×1000)	First harvest Spore/g (×1000)	Second harvest Spore/g (×1000)	First harvest Spore/g (×1000)	Second harvest Spore/g (×1000)	First harvest Spore/g (×1000)
Rye	22 d[b]	40,138 a	0 c	0 a	24,053 cde	9628 bc	39 c
Millet	2000 d	5858 b	5 c	0 a	3167 e	0 c	13,333 ab
Sorghum	30,444 bc	39,025 a	867 c	21,461 a	34,277 bcde	3833 c	18,333 a
Corn husks	0 d	0 b	0 c	0 a	11,670 de	4222 c	74 c
Cowpea	5722 d	350 b	0 c	0 a	20,167 cde	0 c	18,059 a
Sunnhemp	0 d	850 b	6889 abc	0 a	3833 e	0 c	5650 bc
Bahiagrass	38,111 bc	27,595 ab	1904 bc	27,332 a	47,777 abcd	9889 bc	109 c
Cogongrass	6981 d	14,522 ab	7389 ab	29,272 a	64,000 ab	18,333 bc	56 c
Purple nutsedge	171,889 a	39,833 a	0 c	0 a	79,057 a	26,111 bc	33 c
Yellow nutsedge	52,000 b	39,405 a	0 c	0 a	17,003 cde	41,295 ab	0 c
Timothy hay	0 d	0 b	0 c	0 a	51,110 abc	73,889 a	0 c
Waterhyacinth	9667 cd	0 b	12,778 a	555 a	13,723 de	1239 c	12,725 ab
Pine straw	18 d	0 b	0 c	0 a	0 e	0 c	0 c
Cedar	0 d	0 b	0 c	0 a	0 e	0 c	0 c
Corn stubble	34 d	NC[c]	NC	NC	NC	NC	NC
Sugarcane bagasse	0 d	NC	NC	NC	NC	NC	NC
Kraft paper	756 d	NC	NC	NC	NC	NC	NC
Newspaper	0 d	NC	NC	NC	NC	NC	NC
BioAPT	0 d	NC	NC	NC	NC	NC	NC

[a] First harvest was 4 weeks after inoculation and the second was 4 weeks after the first harvest.
[b] Values within a column followed by the same letter(s) are not significantly different according to Duncan multiple range test ($P = 0.05$).
[c] NC = Not considered because no or sparse spores were obtained in the first harvest and no characteristic dark color of *Dactylaria* spores was found on the substrate.

hays amended with IBA (Table 2). Conidia could be harvested twice from some but not all of the solid substrates tested (Table 2).

Of the 19 substrates tested (Table 1), only five generated moderate to high levels of conidium production: purple nutsedge, cogongrass, yellow nutsedge, bahiagrass, and sorghum × sudangrass (Table 2). Purple and yellow nutsedges generated higher numbers of conidia when used without amendments than with any type of amendments. Cogongrass, bahiagrass, and sorghum × sudangrass generated the highest numbers of conidia when they were amended with IBA and PDB (Table 2). Overall, hays amended with IBA or PDB were the least supportive of conidium production (Table 2) while conidia yield was highest (1.7×10^8 conidia/g of substrate) when the fungus was grown on purple nutsedge hay without any amendments for 4 weeks (Table 2).

Conidia yield was higher from the first harvest (4 weeks after inoculation) than the second harvest (4 weeks after first harvest) when *D. higginsii* was grown on purple nutsedge, yellow nutsedge, and bahiagrass. However, the opposite was the case for sorghum × sudangrass and cogongrass (Table 2). Purple nutsedge substrate generated the highest conidia yield in the first harvest; there were no significant differences among the substrates in terms of conidia yield in the second harvest (Table 2).

Among the treatments that were harvested once 12 weeks after inoculation (Table 3), bahiagrass hay with no amendments generated the highest conidia yield (9.3×10^7 conidia/g) followed by purple nutsedge and sorghum × sudangrass hay substrates without amendments (6.7×10^7 and 6.6×10^7 conidia/g, respectively), but the differences were not significant (Table 3). The substrates without amendments yielded comparable numbers of conidia 12 weeks after inoculation (Table 3).

3.2. Effect of incubation period on conidium production on unamended hay substrates

The incubation period (4 or 12 weeks) did not affect conidial yield (ANOVA; $P = 0.6509$) while the substrate type had a significant effect on conidium production ($P = 0.018$). Among the substrates, purple nutsedge hay provided the highest conidial yield when harvested after a 4-week incubation period, followed by the other four substrates, with no significant differences among them (Fig. 2). When conidia were harvested 12 weeks after inoculation, there were no significant differences among the hay substrates in terms of conidial yield (Fig. 2). The incubation period (4 and 12 weeks) had no significant effect on the conidial yields obtained on all hay substrates except the purple nutsedge; on which the yield obtained after 4 weeks was higher than that obtained after 12 weeks ($P = 0.016$) (Fig. 2).

Table 3
Effect of solid substrates and amendments on production of *D. higginsii* conidia harvested once, 12 weeks after inoculation.

Substrate	No amendments Spore/g (×1000)	Amended with indole butyric acid (IBA) Spore/g (×1000)	Amended with potato dextrose broth (PDB) + IBA Spore/g (×1000)
Sorghum	65,944 aAB[a]	NC[b]	17,611 aB
Bahiagrass	93,611 aA	NC	10,072 abB
Cogongrass	8778 aB	185 B	9906 abB
Purple nutsedge	66,667 aAB	NC	14,911 abB
Yellow nutsedge	6667 aB	NC	6633 bB

[a] Values followed by the same lowercase letter (within a column) or uppercase letter(s) (among all treatments in all groups) are not significantly different according to Duncan multiple range test ($P = 0.05$).
[b] NC = Not considered because no or sparse spores were obtained in the first harvest and no characteristic dark color of *Dactylaria* spores was found on the substrate.

3.3. Effect of substrate combinations on conidium production

Combining the substrates with purple nutsedge hay or with the microbial carrier BioAPT did not improve the yield of *D. higginsii* conidia (Table 4). The commercial microbial carrier BioAPT by itself did not support sporulation (Table 4).

3.4. Effect of different solid substrates on virulence of conidia against purple nutsedge

In general, conidia produced on hay substrates without amendments were significantly more virulent on purple nutsedge, as measured from DI and DS, than those produced on hay amended

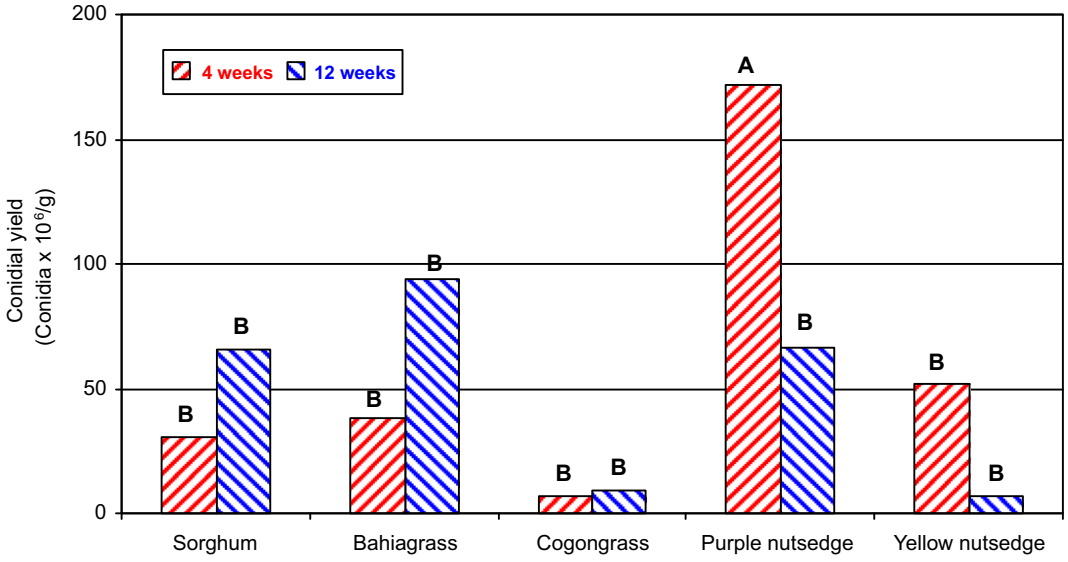

Fig. 2. Effect of incubation period on yield of *D. higginsii* conidia (count of conidia per gram of dry substrate) produced on hays without amendments. Bars with the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).

Table 4
Production of conidia of *D. higginsii* grown for 6 weeks on combinations of selected solid substrates with or without BioAPT.

Substrate/combination	Spore count per ml (×1000)	Spore count per g (×1000)
Sorghum × sudangrass	5915 a [a]	98,542 a
Cogongrass	1059 b	17,611 b
Purple nutsedge	470 b	7833 b
Sorghum × sudangrass + purple nutsedge	3 b	50 b
Cogongrass + purple nutsedge	70 b	1167 b
BioAPT	0 b	0 b
Sorghum × sudangrass + BioAPT	600 b	4979 b
Cogongrass + BioAPT	81 b	4750 b
Purple nutsedge + BioAPT	470 b	667 b

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

with IBA, PDB, or PDB + IBA ($P = 0.05$) (Table 5). Conidia produced on sorghum × sudangrass hay with no amendments were the most virulent on purple nutsedge (83% and 78% for DI and DS, respectively) followed by conidia produced on cogongrass hay without amendments (81% and 50% for DI and DS, respectively) ($P = 0.05$) (Table 5). Conidia produced on purple nutsedge and bahiagrass hays were the least virulent against purple nutsedge plants ($P = 0.05$). Typical symptoms start as tiny brown to dark brown spots that develop into eye-shaped lesions with gray centers and then coalesce together causing leaf blights and leaf withering (Fig. 3). There were no differences among the treatments with conidia derived from amended or non-amended substrates with regard to the number of living plants and dry weight of nutsedge culms at the end of the experiment (3 weeks after application) ($P = 0.05$) (Table 5).

3.5. Effect of different solid substrates on virulence of conidia against yellow nutsedge

None of the conidial inoculum produced on any type of substrate, with or without amendments, produced notable disease on yellow nutsedge. The level of DS was quite low (Table 6). Conidium produced on cogongrass hay without amendments yielded 16% and 5% DI and DS, while those produced on sorghum × sudangrass hay without amendments yielded 4.7% and 1% DI and DS. Conidia produced on purple nutsedge and bahiagrass hays were the least virulent against yellow nutsedge plants (Table 6).

4. Discussion

Solid substrates composed of agricultural byproducts have been used for production of inoculum of several bioherbicide agents (Morin et al., 1989; Connick et al., 1990; Zhang and Watson,

Table 5
Disease on purple nutsedge caused by *D. higginsii* conidia harvested from different solid substrates with and without amendments, 3 weeks after inoculation[a].

Substrate	No amendments		Amended with indole butyric acid (IBA)		Amended with potato dextrose broth (PDB) + IBA		Amended with PDB	
	DS[b] (%)	Shoot dry weight	DS[b] (%)	Shoot dry weight	DS[b] (%)	Shoot dry weight	DS[b] (%)	Shoot dry weight
Sorghum	78 aA[c]	1.13 abA	NA[d]	NA	1.0 bC	1.27 abA	12.5 aB	1.00 bA
Bahiagrass	16 dA	1.40aA	NA	NA	0.8 bB	1.23 abcA	NA	NA
Cogongrass	50 bA	1.16 abA	20.0 aB	1.04 aA	12.5 aB	0.83 bcA	NA	NA
Purple nutsedge	24 cA	0.86 bA	NA	NA	1.0 bB	0.75 cA	NA	NA
Yellow nutsedge	0.3 eA	0.69 bA	NA	NA	0.5 bA	0.85 bcA	NA	NA
Control	0 e	1.40 aA	0 bA	1.40 aA	0 bA	1.40 aA	0 bA	1.40 aA

[a] Conidia were harvested after a 4-weeks incubation on the substrates.
[b] DS = disease severity (percentage of symptomatic tissue).
[c] Values for each parameter followed by the same lowercase letter(s) (within a column) or uppercase letter (within a row) are not significantly different according to Duncan multiple range test ($P = 0.05$).
[d] NA = not applicable because of sparse spore yield that was insufficient for inoculation.

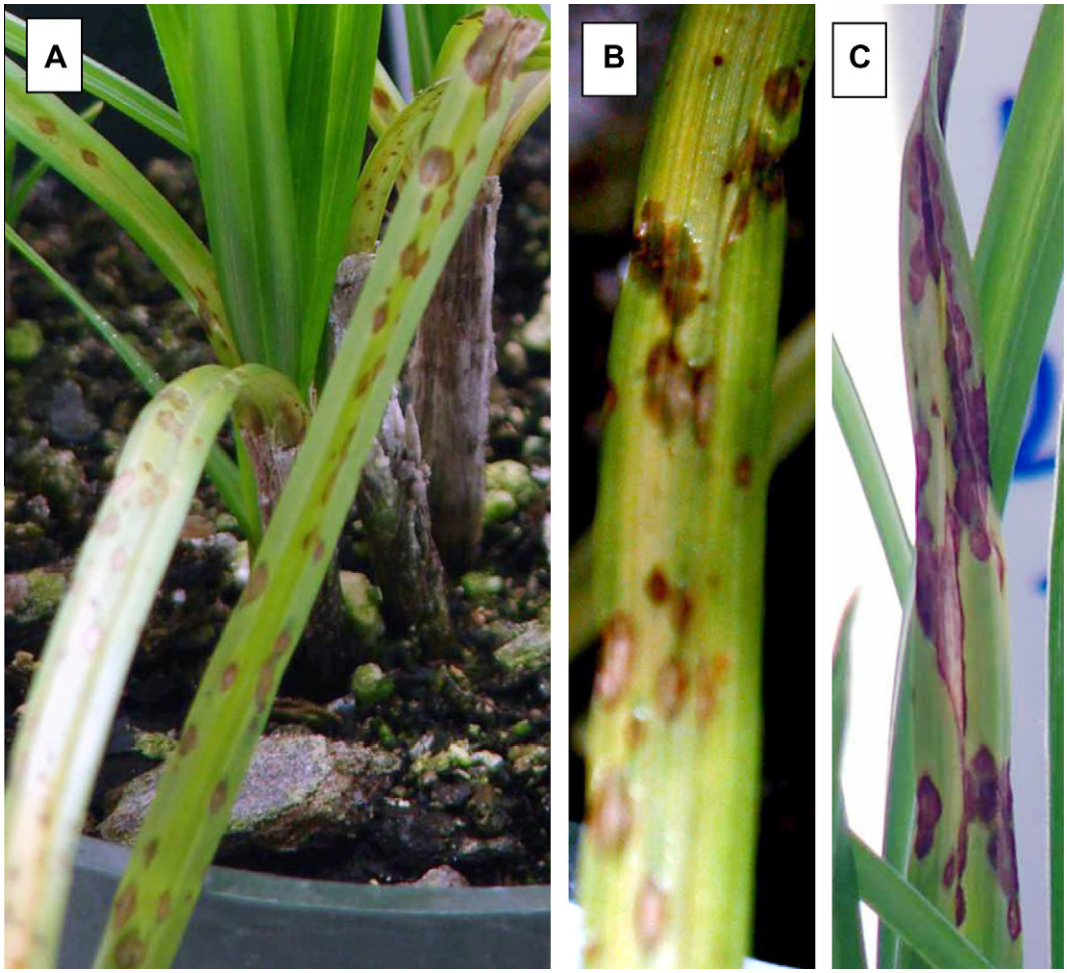

Fig. 3. Disease symptoms on purple nutsedge caused by *Dactylaria higginsii* conidia produced on sorghum × sudangrass hay substrate. Typical symptoms start as tiny brown to dark brown spots (a), that develop into eye-shaped lesions with gray centers (b) and then coalesce causing leaf blight (c).

Table 6
Disease on yellow nutsedge caused by *D. higginsii* conidia harvested from different solid substrates with and without amendments[a], 3 weeks after inoculation[a].

Substrate	No amendments		Amended with indole butyric acid (IBA)		Amended with potato dextrose broth (PDB) + IBA		Amended with PDB	
	DS[b] (%)	Shoot dry weight	DS[b] (%)	Shoot dry weight	DS[b] (%)	Shoot dry weight	DS[b] (%)	Shoot dry weight
Sorghum	1.0 bA[c]	0.94 bA[d]	NA[d]	NA	0.8 abA	1.20 aA	1.0 aA	1.41 aA
Bahiagrass	0.0 cA	0.89 bB	NA	NA	0.0 bA	1.43 aA	NA	NA
Cogongrass	5.0 aA	0.98 bB	1.0 aB	1.19 aB	2.0 aB	1.62 aA	NA	NA
Purple nutsedge	0.0 cA	1.22 bA	NA	NA	0.3 bA	1.22 aA	NA	NA
Yellow nutsedge	0.0 cA	1.28 abA	NA	NA	0.0 bA	1.31 aA	NA	NA
Control	0.0 cA	1.63 aA	0.0 bA	1.63 aA	0.0 bA	1.63 aA	0.0 bA	1.63 aA

[a] Conidia were harvested after a 4-weeks incubation on the substrates.
[b] DS = disease severity (percentage of symptomatic tissue).
[c] Values for each parameter followed by the same lowercase letter(s) (within a column) or uppercase letter(s) (within a row) are not significantly different according to Duncan multiple range test ($P = 0.05$).
[d] NA = not applicable because of sparse spore yield that was insufficient for inoculation.

1997), and, as demonstrated here, are also feasible for *D. higginsii*. Babu et al. (2004) reported that solid substrates with relatively low protein content, such as seeds of sorghum, millet, maize, rice, and maize stalks promoted sporulation of *Alternaria alternata*, with rice, wheat, sorghum, and maize yielding most conidia. Compared to the seeds used by Babu et al. (2004), the solid substrates tested in the present study are low in protein content. We tested hays that are readily available or that could be produced or collected at minimal cost. In general, yield of conidia of *D. higginsii* was highest when the fungus was grown on purple nutsedge followed by bahiagrass, sorghum × sudangrass, yellow nutsedge, and cogongrass hays. The effect of hays on conidium production is likely due to the large surface area available for sporulation, surface structural features that enable retention of fungal propagules, lack of particle aggregation, and appropriate nutrient and moisture contents, all of which may contribute to restricting mycelial growth in favor of conidium production.

A high level of virulence is an important feature of a successful biocontrol agent, and optimization of mass production of an agent strives for maximum yield of propagules with high 'inoculum potential' (Zhang and Watson, 1997; Charudattan, 2010). Conidia produced on sorghum × sudangrass and cogongrass were

significantly more virulent than those produced on any other medium, which may be related to the observation that conidia produced on those substrates were slightly larger in size and had a thicker cell wall (data not shown).

Despite that conidia produced on sorghum × sudangrass hay with no amendments were the most virulent on purple nutsedge with regard to disease development, this treatment was not different from the other treatments with regard to the number of living plants and dry weight of nutsedge culms at the end of the experiment. This may be due to infection by *D. higginsii* forcing nutsedge plants to produce new growth to compensate for the photosynthetic tissue lost due to foliar disease.

The finding that purple nutsedge was more susceptible to *D. higginsii* than yellow nutsedge is in line with the findings of Kadir and Charudattan (2000). While several sedges have been reported to be susceptible to *D. higginsii*, purple nutsedge appears to be the primary and most susceptible host for this pathogen.

Solid-state production of *D. higginsii* on hay substrates is a feasible system for large-scale production of conidia. On some hay substrates, two harvests of conidia can be obtained. There is no need to amend the hays with IBA or PDB since unamended hays yielded higher numbers of conidia that were also more virulent than those produced on amended hays. Moreover, the hay-based production of *D. higginsii* does not require specialized and expensive equipment or facilities. The inoculum can be produced in gas-permeable plastic bags containing a suitable moistened hay substrate and incubated under ambient conditions. Application of *D. higginsii* in organic mulch for management of nutsedge may be applicable to organic and conventional crop production. Further studies are underway.

Acknowledgments

Funding for this research was provided by a USDA-CSREES TSTAR Award #2006-34135-17493. We thank the Senior Vice-President for Agriculture and Natural Resources, University of Florida/IFAS for providing salary support for Yasser Shabana. We also thank the UF/IFAS Center for Aquatic and Invasive Plants for support and to Mark Elliott, Sahar Gomaa, and Gabriella Maia for their technical help.

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