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Effect of *Dactylaria higginsii* on Purple Nutsedge (*Cyperus rotundus*) Interference with Pepper (*Capsicum annuum* L)

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ABSTRAK

Satu kajian rumah hijau telah dijalankan untuk menilai kesan patogen berkulat, Dactylaria higginsii *pada nutsedge ungu yang dicampur dengan lada hitam 'Capistrano'* (Capsicum annuum). *Tanaman nutsedge ungu yang tumbuh daripada umbi pada peringkat awalnya ditanam dengan kepadatan 40, 80, 160 dan 320 tanaman m bersama-sama lada hitam di dalam pot komersial sederhana bergaris pusat 35-cm, dengan keadaan pengairan dan pembajaan yang tidak terhad. Tiga hingga empat-peringkat-daun nutsedge ungu dan empat-peringkat-daun tanaman lada hitam telah disiram dengan* D. hingginsii *dalam 0.5% Metamucil, suatu pembawa: rawatannya cuma pembawa sahaja, 10^4 conidia ml^{-1} + pembawa, atau 10^6 conidia ml^{-1} + pembawa. Secara signifikan, nutsedge ungu pada kesemua umbi yang padat mengurangkan hasil lada hitam tanpa kehadiran* D. hingginsii. *Peratus hasil lada hitam menyusut lebih tinggi dalam rawatan bersama dengan 10^4 conidia ml^{-1}. Walau bagaimanapun, peratus kesusutan hasil lada hitam adalah sangat kecil jika dirawat dengan* D. higginsii *pada 10^6 conidia ml^{-1} berbanding kawalan tanpa tumbuhan berumput. Secara signifikan, kadar perkembangan penyakit dalam rawatan bersama 10^6 conidia ml^{-1} ($r^G = 0.113$ 0.123) lebih cepat berbanding yang dirawat bersama 10^4 conidia ml^{-1} ($r^G = 0.049$ 0.050). Pada 10^6 conidia ml^{-1},* D. higginsii *mengurangkan pencampuran nutsedge, memberi kawalan yang lebih tinggi pada nutsedge, dan meningkatkan hasil lada hitam berbanding kawalan berumput.*

ABSTRACT

Greenhouse studies were conducted to evaluate the effect of the fungal pathogen, Dactylaria higginsii, *on purple nutsedge interference with 'Capistrano' pepper* (Capsicum annuum). *Purple nutsedge plants established from tubers were planted at initial densities of 40, 80,160, and 320 plants m^2 with pepper in 35-cm diam pots with a commercial potting medium, under nonlimiting fertilization and irrigation conditions. Three to four-leaf-stage purple nutsedge and four-leaf-stage pepper plants were inoculated by spraying* D. higginsii *in 0.5% Metamucil, a carrier; the treatments were carrier only, 10^4 conidia ml^1 + carrier, or 10^6 conidia conidia ml^1 + carrier. Purple nutsedge at all tuber densities significantly reduced pepper yield in the absence of* D. higginsii. *Percentage yield loss of pepper was greater in treatment with 10^4 conidia ml^1. However, percentage yield loss of pepper was negligible in treatments with* D. higginsii *at 10^6 conidia ml^1 when compared to the non-weedy control. The disease progress rate was significantly faster in treatments with 10^6 conidia ml^1 ($r_G = 0.113 - 0.123$) compared to 10^4 conidia ml^1 ($r_G = 0.049 - 0.050$). At 10^6 conidia ml^1,* D. higginsii *reduced nutsedge interference, provided greater nutsedge control, and increase pepper yield compared to weedy checks.*

INTRODUCTION

Purple nutsedge is rated as one of the world's worst weed and has been reported in more than 70 countries. It competes and interferes with crops particularly early in the growing season and heavy infestation of purple nutsedge can cause high yield loss in vegetable crops. Although (chemical) herbicides inhibit the growth of the weed, adverse environmental factors and plant-growth stages at the time of application act against the effect of the herbicide (Gricher *et al.* 1992). Several other nonchemical methods have been used, but none have provided acceptable control. Long-term, sustained control of purple nutsedge has been difficult to achieve.

Research has recently commenced into the use of a bioherbicide to reduce interference by purple nutsedge in cropping systems. *Dactylaria higginsii*, a fungal pathogen of purple nutsedge has been reported to be capable of controlling this weed (Kadir and Charudattan 1996, Kadir *et al.* 1997a; 1997b). However, its potential to reduce nutsedge interference in cropping system has not been studied. Therefore, the objectives of this research are: 1) to determine the effective inoculum concentration needed to reduce interference from the purple nutsedge and 2) to determine the effect of *D. higginsii* on the interference of purple nutsedge on pepper.

MATERIALS AND METHODS

Experimental Method

The experimental method used in this study was the additive series approach. In this method, the density of one species (usually, called the indicator crop) is held constant and the density of the other species (the weed) is varied. Since the latter is added into the first of this bipartite series, this approach is called the additive series. This system uses the response of the first species in fixed density as an indicator of the relative aggressiveness or competitive ability of the second species to the first. This system is applicable in cropping systems with encroaching weeds and in intercropping systems (Cousens 1990; Nickel *et al.* 1990).

Purple Nutsedge Interference

The experiment was carried out in a greenhouse in spring 1996 and repeated in autumn 1996, using transplants pepper cv 'Capistrano' as the indicator crop. A mixture of pepper and purple nutsedge were grown in 30 cm (diam) × 10 cm (height) pots filled with 0.07 m^3 of commercial potting medium (Metro Mix 220, Scott-Sierra Horticultural Product Co., Maryville, OH.) consisting of horticultural vermiculite, Canadian spaghnum peat, and horticultural perlite. Each pot contained one transplant of pepper and one of the following purple nutsedge densities: 0, 40, 80, 160, and 320 tubers per m^2. Plants in pots were watered by drip irrigation three times daily to stimulate soil moisture in the field. Soil fertility was maintained by adding water-soluble Peters Professional All Purpose Plant Food (20:20:20 + Trace Elements, Spectrum Group, Div. of United Industries Corp., St Louis, MO) at the recommended rate of 3.785 liters of solution (9.5 g/3.785 liters water) for 0.09 $meter^2$ bed, every two weeks.

Fungal Inoculation

Inoculum used in this experiment was produced in trays on a thin layer of PDA (Kadir 1997). Three inoculum concentrations were used: 0 [0.5% Metamucil (w/v), used as a humectant; as a control]; 10^4 conidia/ml with 0.5% Metamucil (w/v); and 10^6 conidia/ml with 0.5% Metamucil (w/v). One hour before the plants were inoculated, they were misted for 5 min to wet the leaf surfaces. The 4-leaf old pepper plants and 3-4-leaf old purple nutsedge were inoculated by spraying the conidial suspension with an aerosol sprayer until the excess fluid dripped off the foliage. Starting 6h. after inoculation, the greenhouse misters were turned on for 5 min at every 6h. interval for the first 24h. to maintain leaf wetness. This was to ensure that *D. higginsii*, which requires a dew-duration period of at least 12h. for disease development, would be able to infect purple nutsedge under greenhouse conditions.

Data Collection

Disease severity was assessed every five days using the Horsefall Barratt scale (Horsefall and Barratt 1945), modified by Kadir (Kadir 1997). The values of the total portion of disease were transformed by using the Gompertz model transformation (Berger, 1981) of the form:

$$\text{Gompit } y = -\ln(-\ln(y))$$

to linearize the disease progress curve. The area under the disease curve (AUDPC) was calculated from this linearized curve using the equation (Campbell and Madden, 1990):

$$\text{AUDPC} = \sum_{i}^{n+1} \left(\frac{y_i + y_{i+1}}{2} \right) \left(t_{i+1} - t_i \right).$$

Pepper was harvested at 50 and 65 days after transplantation and the yield was recorded as fruit weight (in gram) per plant. Pepper fruits were harvested twice, since the yields during the first harvest did not show any expected trend. The data from the first and second harvest were

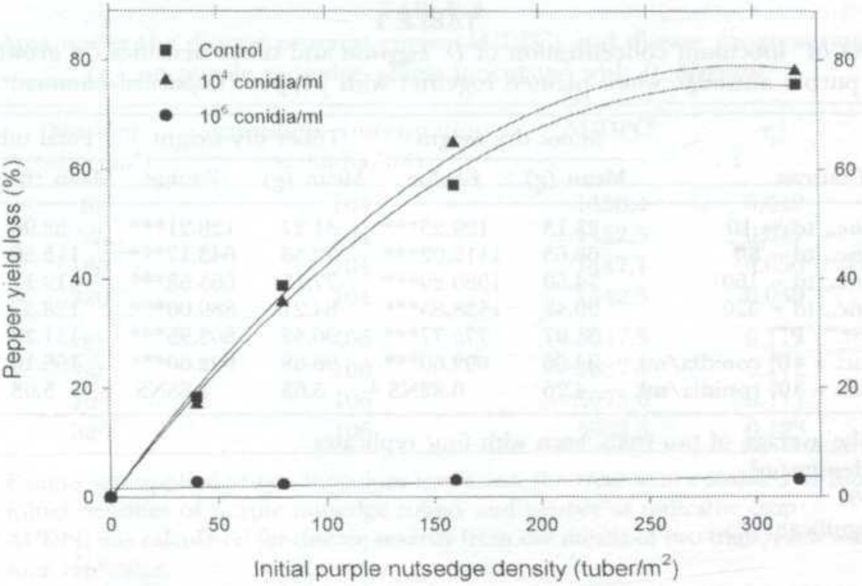

Fig. 1. Effect of inoculation of purple nutsedge with D. higginsii *on the percentage yield loss of* C. annuum. *Each data point represents the mean value from two trials each with four replicates. Control = noninoculated control; 10^4 conidia ml^{-1} = inoculated with 10^4 conidia ml^{-1} at the rate of 90 ml m^{-2}; and 10^6 conidia ml^{-1} = inoculated with 10^6 conidia ml^{-1} at 90 ml m^{-2}.*

pooled and recorded as the total yield per plant. Final tuber numbers of purple nutsedge were recorded at the final harvest time (65 days after transplantation), from each pot. The tubers and the bulbs were separated after washing the soil from the roots and rhizomes. Both were recorded as tubers. The shoot plus tuber biomass was determined at harvest time by weighing the shoots and tubers after they were dried at 75°C for 5 days. These parameters represent weed-growth components.

Statistical Analysis

The study was a factorial experiment with two factors (tuber densities as the main factor and inoculum concentration as the sub-factor). The experiment had a randomized complete block design with four replications. Mean values of four replications were used for statistical analysis. Orthogonal contrasts of the log inoculum concentration and tuber densities, and of the slopes of the linear regression models, was performed to determine the individual effect of tuber density and inoculum concentration and their interactions on weed-growth components and crop yield. Linear regression of AUDPC against yield was done to determine their relationship.

RESULTS

Homogeneity of variance among treatments were noted in the levels of control of the weed-growth components and disease severity of Dactylaria leaf blight on inoculated purple nutsedge from both trials. The data on weed growth components and disease severity, the latter expressed as the AUDPC, were therefore combined and averaged over both trial dates.

Effect of D. higginsii *on weed-growth components and pepper yield*

The initial planting density of tubers had a significant effect on shoot and tuber dry weight of purple nutsedge in noninoculated control and in treatments where plants were inoculated with 10^4 conidia/ml (Table 1). The final shoot and tuber dry weight of purple nutsedge increased with increasing purple nutsedge tuber density. Exception was the treatment in which the purple nutsedge plants were inoculated with *D. higginsii* at 10^6 conidia/ml. The final shoot and root dry weight were significantly reduced in these treatments regardless of initial planting densities of tuber compared to the non-inoculated weedy control and treatments where purple nutsedge plants were inoculated with 10^4 conidia/ml.

TABLE 1

Effect of inoculum concentration of *D. higginsii* and tuber densities on growth of purple nutsedge when planted together with pepper (*Capsicum. annuum*)[a].

Contrast	Shoot dry weight		Tuber dry weight		Final tuber number	
	Mean (g)	F-value	Mean (g)	F-value	Mean (no.)	F-value
Quad log conc., td [b]= 40	23.13	129.25***[c]	31.27	128.21***	52.96	4.38***
Quad log conc., td = 80	66.63	1115.02***	72.38	643.17***	115.50	288.95***
Quad log conc., td = 160	74.60	1080.20***	77.15	665.58***	119.17	308.47***
Quad log conc., td = 320	90.42	1538.85***	84.21	889.00***	128.37	432.06***
Quad td, conc. = 0	91.97	775.77***	96.42	803.95***	151.28	386.25***
Quad td, conc. = 10^4 conidia/ml.	94.66	692.60***	96.68	692.60***	155.10	297.62***
Quad td, conc. = 10^6 conidia/ml.	4.26	0.82NS [d]	5.63	1.38NS	5.63	0.18NS

[a] Values are the average of two trials, each with four replicates

[b] td = tuber density/m^2

[c] ***$P < 0.0001$

[d] NS = Not significant

The slope comparison for relationship of the weed-growth components of purple nutsedge with initial planting densities of tubers is shown in Table 2. The slopes of the non-inoculated control and treatments where purple nutsedge plants were inoculated with 10^4 conidia/ml were comparably similar, but were significantly lower in treatment where purple nutsedge plants were inoculated with 10^6 conidia/ml.

The percentage of yield loss of pepper was significantly high even at 40 tubers/m^2 (19.07% for the control and 15.42% for 10^4 conidia/ml. The application of 10^4 conidia/ml of *D. higginsii* did not have any significant effect in reducing the yield loss of pepper. The percentage yield loss of pepper was significantly reduced irrespective of tuber densities, when purple nutsedge were inoculated with 10^6 conidia/ml. This could be explained by the reduction in weed growth components (explained earlier).

Effect of D. higginsii *on AUDPC*

Purple nutsedge plants inoculated with 10^4 conidia/ml developed low levels of disease compared to plants inoculated with 10^6 conidia/ml. Almost all of the plants in the 10^6 conidia/ml treatments died. Secondary spread of *D. higginsii* from the previously diseased leaves caused subsequent infection on the regrowth, thus very little or no regrowth were observed.

The disease severity of the inoculated plants was expressed as the AUDPC (Table 3). The

TABLE 2

Slope values and comparisons of slopes from linear regression of growth components of purple nutsedge and the initial tuber densities of purple nutsedge in pepper recorded 65 days after inoculation with *D. higginsii*

Treatments	Slope values		
	Shoot dry weight (g)	Tuber dry weight(g)	Final tuber numbers
Control	1.02	1.20	2.71
10^4 conidia/ml	1.11	1.14	2.52
10^6 conidia/ml	0.05	0.06	0.05
		Contrasts of slope values	
Control vs 10^4	NS [a]	NS	NS
Control vs 10^6	***[b]	***	***
10^4 vs 10^6	***	***	***

[a] NS = Not significant.

[b] *** = $P< 0.001$.

TABLE 3
Area under the disease progress curve (AUDPC) and disease progress rate (r_G) on purple nutsedge plants inoculated with *D. higginsii*.[a]

Densities (tuber/m²)	Inoculum concentration (conidia/ml)	AUDPC[b]	r_G^c
40	104	1456.4	0.049
80	104	1452.5	0.047
160	104	1447.1	0.050
320	104	1442.3	0.049
40	106	5647.5	0.113
80	106	5887.5	0.112
160	106	5975.0	0.117
320	106	5962.5	0.123

[a] Fungus was applied at two inoculum levels and the treatment consisted of four initial densities of purple nutsedge tubers and pepper as indicator crop.

[b] AUDPC was calculated for disease severity from the means of two trials, each with four replicates.

[c] Disease progress rate was calculated by using the Gompertz model (Berger, 1981).

AUPDC values of treatment where purple nutsedge plants were inoculated with 10^4 conidia/ml were lower compared to AUDPC values of treatment where purple nutsedge plants were inoculated with 10^6 conidia/ml. The disease progress rates (r_G) of the treatment where purple nutsedge plants were inoculated with 10^4 conidia/ml ($r_G = 0.047$ - 0.050) was slower compared to the apparent infection rates ($r_G = 0.112$ - 0.123, Table 3) of the experiment where purple nutsedge plants were inoculated with 10^6 conidia/ml.

TABLE 4
Slope values and comparisons of slopes from linear regression of percentages of yield loss of *C. annuum* on initial tuber densities of purple nutsedge recorded 65 days after inoculation with *D. higginsii*.

Treatment	Slope values
Control	0.75
10^4 conidia/ml	0.74
10^6 conidia/ml	0.06
	Contrasts of slope values
Control vs 10^4	NS[a]
Control vs 10^6	***[b]
10^4 vs 10^6	***

[a] NS = not significant

[b] *** = $P < 0.001$

DISCUSSION

D. higginsii did not infect pepper. This was expected as this fungus had been previously determined to be host specific to *Cyperus* spp. (Kadir and Charudattan 1999). Infection was observed on purple nutsedge in the control, due to cross-contamination but the level of infection was below 5% severity. This low level would not account for any significant effect on the yield of pepper or the final weed-growth components of purple nutsedge. The final weed-growth components of purple nutsedge were influenced by the initial planting density of tubers, however, these components were significantly reduced in treatments where nutsedge plants were inoculated with 10^6 conidia/ml of *D. higginsii*. The nutsedge plants in these treatments were severely diseased. Tubers from the diseased plant resprouted, but the growth was suppressed. This finding is contradictory to the report by Marambe (1996) who found that purple nutsedge, even when completely defoliated, tends to increase shoot and tuber numbers. However, his study was done in the absence of a crop, unlike our study which was carried out in the presence of pepper plant. The shading provided by the vigorously growing pepper plant probably helped to maintain humid conditions and promote disease development with severe secondary infection.