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Effects of Culture Age on Conidia Production, Viability and Pathogenicity of *Dactylaria higginsii*

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ABSTRACT

Effects of culture age on conidia production, viability and pathogenicity of *Dactylaria higginsii*, a potential bioherbicide for controlling *Cyperus rotundus* (purple nutsedge) were investigated both in the laboratory and the greenhouse. This fungus was capable of producing conidia after five days of culturing on potato dextrose agar (PDA), with the highest conidia production obtained from the 15 day-old culture compared to other culture ages. Conidia harvested from 15 days of culturing on PDA, produced higher viability and were more pathogenic to purple nutsedge compared to conidia harvested before or after 15 days of culturing. The infection rate of conidia harvested from the 15 day-old cultures was faster (r_L0.40 logit/day) compared to the infection rate of conidia harvested from other culture ages.

Keywords: Mycoherbicide, purple nutsedge, *Dactylaria higginsii*, harvesting age

ABSTRAK

Kesan usia kultur kulat ke atas penghasilan konidia, kemandirian dan kepatogenan *Dactylaria higginsii*, kulat yang berpotensi sebagai bioherbisid untuk mengawal *Cyperus rotundus* (rumput halia hitam) telah dikaji pada keadaaan makmal dan rumah hijau. *Dactylaria higginsii* berupaya menghasilkan konidia selepas lima hari setelah dikulturkan di atas agar dekstosa ubi kentang (PDA), dengan penghasilan konidia tertinggi pada hari yang ke 15, dengan kebolehidupan tertinggi dan lebih patogen kepada *C. rotundus* berbanding konidia pada usia sebelum hari yang ke 15. Kadar jangkitan konidia yang berusia 15 hari juga lebih cepat (r_L0.40 logit/hari) berbanding kadar jangkitan konidia pada hari-hari yang lain.

Kata kunci: Mikoherbisid, rumput halia hitam, *Dactylaria higginsii*, umur penuaian

INTRODUCTION

Dactylaria higginsii, the pathogen of purple nutsedge, has good potential as a mycoherbicide to control *Cyperus rotundus* (Kadir and Charudattan, 2000). It causes a foliar disease, which is capable of reducing

growth components of purple nutsedge (Kadir and Charudattan, 2000; Kadir *et al.,* 1999; Kadir *et al.,* 2000a; Kadir *et al.,* 2000b) under certain conditions. It is highly host-specific to *Cyperus* spp. To develop this pathogen as a mycoherbicide, it is essential to understand the conditions under which a high level of disease incidence can be achieved. In addition to understanding the conditions under which this patho-system works, the physical condition of the conidia is of paramount importance because it greatly affects the number, stability, durability, and virulence of fungal propagules. Most mycoherbicide research and development has concentrated on finding methods of mass conidia production and preservation of fungal conidia of many potential mycoherbicides, but only a few achieved commercial successes (Amsellem *et al.,* 1999). One major constraint has been the problem of producing fungal conidia that consistently produce high level of disease, with longer viability (shelf-life). Viability and virulence of the fungal conidia may be related to the age of the harvested conidia. For example, Russo (1997) showed that there was a significant regression trend between inoculum age and seedling mortality. The ability of the conidia to incite disease decreases with conidia age and the maximum seedlings mortality was achieved from conidia harvested from the 14 day-old fungal culture. It is hypothesized that there are, nonetheless, significant differences in the viability and pathogenicity (virulence) among conidia age of *D. higginsii* that can greatly influence the measurement of infectivity level. Currently, there is no information available on the optimum *D. higginsii* conidia to be harvested that are both viable and infective to the host. The work presented here will be the first detailed study in this regard. The objective of this study was to determine the conidia harvesting age that is most virulent to the host.

MATERIALS AND METHODS

An isolate of *Dactylaria higginsii* that was isolated from purple nutsedge in Gainesville, Florida was used in this experiment. Tray inoculation was carried out in two trials. In the first trial, all trays were inoculated at the same time and the conidia were harvested every five days after inoculation until day 25, and in the second trial, the trays were inoculated every five days until day 25, and the conidia were harvested at the same time at the end of the trial period.

In both methods, a fiberglass tray with the dimension of 34 cm by 42 cm by 3 cm deep, containing 250 mL PDA amended with 37 mg/L of streptomycin sulfate and 25 mg/L of chloramphenicol was used. Exactly 15 mL of the conidia suspension, containing 2.5 x 10^5 conidia/mL was sprayed with the aerosol spray onto PDA. The tray was covered with a sterile Reynold's oven bag (35.5 cm x 50.8 cm) and incubated at 28 ^oC under 12 h photoperiod, with lighting provided by fluorescent lamps (150 $\mu e/s/m^2$). Conidia were harvested by flooding the surface of the agar with 150 mL of sterilized water and scrapping the agar surface with a rubber spatula. The resulting conidia suspension was passed through a layer of cheesecloth, and the final conidia count was determined with a hemacytometer.

Conidia Viability Test

Conidia suspension was spread onto 15% water agar plates. The water agar plates, with 10 replications per culture were placed randomly in an incubator at 28 ± 1 ^oC in the dark for 18 h. The percentage of germinated conidia was assessed 18 h after the plate inoculation by counting 50 conidia per plate. Germination was defined as a conidium having a germ tube equal to the length of at least half the width of the conidium (Wyss *et al.,* 2001)

Pathogenicity Testing (Virulence of Conidia)

Young, actively growing purple nutsedge plants (at the four to six leaf stage) were inoculated by spraying conidia suspension containing 2.5 × 10^6 conidia/mL. The plants were inoculated at the rate of 3 mL conidial suspension per plant by using an aerosol sprayer. The conidial suspension contained either purified corn oil (2% v/v), Metamucil (0.5% w/v), or a mixture of purified corn oil and Metamucil as a carrier. The control plants were inoculated with water plus 2% purified corn oil.

Disease Assessment

Disease severity and disease incidence was assessed on plants that were spray inoculated with conidia suspension. Disease severity was assessed by estimating the proportion of leaves with lesions and necrotic or dying leaves per plant. Disease incidence was assessed by estimating the proportion of diseased plants over the total number of plants inoculated for each treatment. Disease severity was used to estimate the virulence of the conidia. Disease intensity (both disease severity and incidence) was assessed by using a modified Horsfall-Barratt disease rating scale (Kadir and Charudattan, 2000) 15 days after inoculation.

Statistical Analysis

All experiments were conducted twice by using a completely randomized design with four or ten replicates as described. All percentages were arcsine-transformed prior to analysis. Data from similar experiments were pooled if a test of homogeneity justified such pooling. The treatment means showing significant effects were separated by Fisher's Protected Least Significant Different Test at the 5% level of significant. All statistical analyses were performed with the Statistical Analysis System (SAS Institute, Cary, NC).

RESULTS

Dactylaria higginsii started to sporulate 5 days after inoculation of PDA surface with conidial suspension. The conidia production was low initially (7.5×10^7 conidia per 250 mL PDA, Figure 1), but started to increase exponentially thereafter until 15 days after inoculation. Conidia were abundantly produced 10, 15, and 20 days after inoculation. Conidia production was not significantly different among harvesting dates, however, was significantly reduced when conidia harvest was done 25 days after tray inoculation (Table 1).

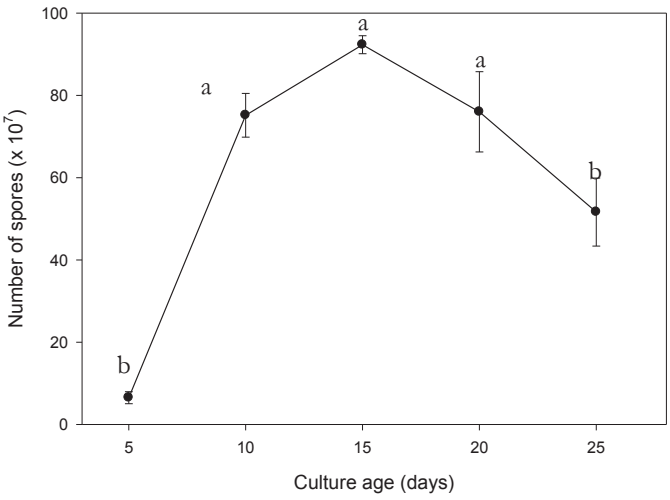

Fig. 1. Relationship between culture age with conidia production of *D. higginsii*. Differences between culture ages were significant (as indicated by different lower case letter) according to Fisher's Protected LSD test at $P < 0.05$. Each data point is the means of two trials, each with ten replicates, respectively. Vertical bars represent standard error of the mean

Table 1. Analysis of variance (ANOVA) of the effect of culture age on conidia production, conidia germination and conidia pathogenicity (virulence).

Variables	df	Mean[b]	R^2	C.V.
Number of conidia	4	60.73×10^7 **[c]	0.75	30.92
Conidia length	4	26.50 *m*m ns [d]	0.60	1.04
Conidia diameter	4	6.26 *m*m ns	0.71	5.20
Germination	4	94.47% **	0.76	3.07
Disease incidence	4	55.02% **	0.95	12.02
Disease severity[a]	4	43.18 **	0.86	25.00

Note:
[a] assessed based on the disease diagrams, and is assessed 14 days after plant inoculation
[b]mean = mean from two experiments each replicated four times
[c]**significance at $P < 0.001$
[d]ns = not significant

Conidia germination was affected by harvest dates (Table 1: $P < 0.001$, $R^2 = 0.75$). Conidia germination from 5 day-old cultures was significantly low, which indicated that the conidia were immature and that conidia maturation was required. This finding corroborated with the finding reported by Isacc (1992), who reported that in some fungi, conidia must attain maturity prior to germination. Higher conidia germination was obtained from 15 day-old conidia, but was not significantly higher when compared to 10 and 20 day-old, respectively (Figure 2a). These conidia were dark colored and the cells contained numerous granules. These granules may contain large amounts of storage materials such as glycogen and trehalose, a similar finding has been reported on *Pyricularia oryzae*, a pathogen of rice (Abe, 1936). Conidia germination was significantly reduced when the conidia were harvested 25 days after inoculating the tray. Low conidia germination in the 25 day-old culture may be attributed to the higher number of presumably empty conidia as indicated by the absence of granulation in the cells and also the appearance of colorless cell content. This could be due to the aging process of the conidia. Aged conidia may become nutrient depleted, especially trehalose, which quickly lead to a decrease in physiological activity. Trehalose is the most abundant sugar in spores, and it seems to be involved in germination (Undeen, 1990). The aged conidia may contain a high level of toxic or waste materials, which eventually resulted in autolysis or self-digestion.

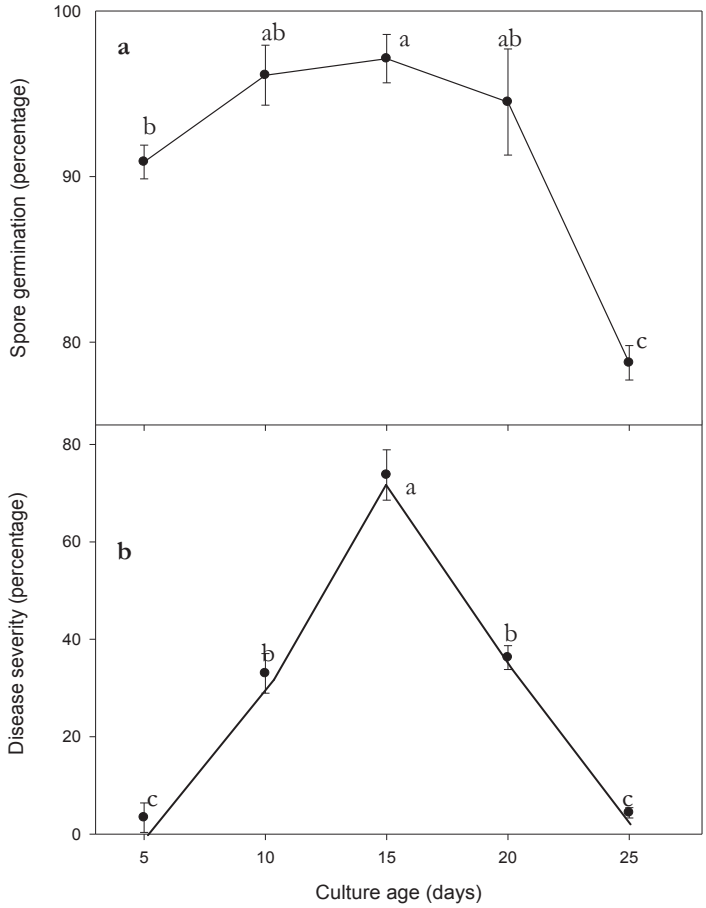

Fig. 2. Effect of culture age on (a) conidia germination of *D. higginsii* conidia on 1.5 % water agar; (b) disease severity on purple nutsedge. Disease severity, based on Horsfall-Barratt rating scale modified by Kadir and Charudattan (2000), was assessed 14 days after inoculation. Differences between harvest dates were significant (as indicated by different lower case letter) according to Fisher's Protected LSD test at $P < 0.05$. Each data point is the means of two trials, each with ten and four replicates, respectively. Vertical bars represent standard error of the mean

Disease intensity (as described by disease incidence and severity) was significantly affected by the conidia harvest date (Table 1, Figure 2b). Purple nutsedge plants inoculated with conidia obtained from the 5 day-old culture developed a low disease level, which could be attributed to immature conidia (explained earlier) and most of the symptoms that developed on these plants were confined to the tip and margins of the leaf laminas. The lesions turned brown rapidly and did not spread. Disease intensity increased exponentially when the plants were inoculated with 10 day-old conidia and reached the maximum level of disease severity with 15 day-old conidia. About 85% of the plants inoculated with the 15 day-old conidia suspended in corn oil were killed 14 days after inoculation. Russo (1997) reported similar findings in which he found that the seedling mortality correlated with the age of the conidia. He reported that the 14 day old *Colletotrichum orbiculare* conidia caused 100% melon seedlings mortality. Disease intensity was lower when the plants were inoculated with 20 day-old conidia and significantly decreased thereafter. The F-test for culture age effect was

significant at $P < 0.001$, indicating that differences exist among culture age with regards to disease severity. Using a Protected Fischer's LSD test with pre-assigned $P = 0.05$, the disease severity caused by each culture age was different from that of every other (Figure 2b) and this observation was further supported by a different rate of disease progress. The disease progress rate was higher (r_L= 0.42 logit/day) for the conidia harvested from the 15 day-old culture (Table 2, Figure 3) as compared to every other progress rate. None of the control plants developed disease.

It is of paramount importance that conidia be harvested at the right time as conidia harvesting date greatly influenced conidia viability and conidia infectivity. Disease severity is positively correlated with conidia maturity, however, it is not directly correlated with percentage of conidia germination (Figure 4).

Table 2. Regression analysis of the conidia age on disease development on purple nutsedge two weeks after inoculation with *Dactylaria higginsii* at the rate of 2.5×10^6 conidia/mL

Conidia age	R^2 (%)	MSE	Intercept	Rate parameter (Slope)[y]
5 day-old	93	5.79	-5.51	0.23a
10 day-old	88	12.52	-4.39	0.33b
15 day-old	97	19.11	-3.30	0.42c
20 day-old	79	9.80	-3.34	0.30b
25 day-old	75	9.45	3.92	0.29ab

Note: [y]slope refers to infection rate, the values were calculated from the transformed disease severity values using Logistic model (Berger, 1981). Numbers within the same column followed by different letters are significantly different ($P = 0.05$) according to Protected Fisher's LSD test.

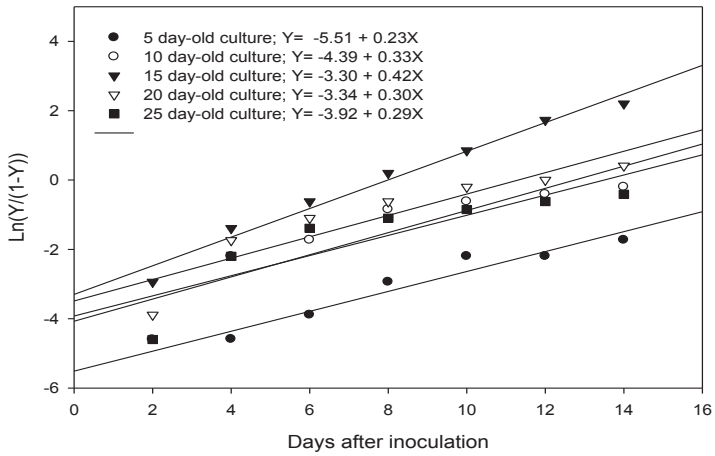

Fig. 3. Effect of conidia age on disease progress on purple nutsedge plants inoculated with*Dactylaria higginsii*. Disease severity values were transformed using the Logistic model: ln (Y/(1-Y)).

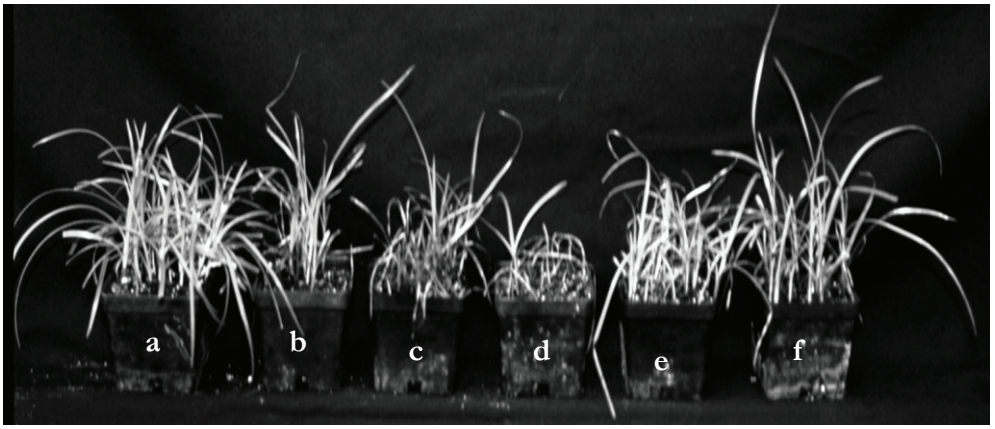

Fig. 4. Effect of conidia age on disease development on purple nutsedge plants two weeks after inoculation with *Dactylaria higginsii*: uninoculated control (a), 5 day old conidia (b); 10 day old conidia (c); 15 day old conidia (d); 20 day old conidia (e); and 25 day old conidia (f).

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