EVALUATION OF *DRECHSLERELLA DACTYLOIDES*, *DRECHSLERELLA BROCHOPAGA*, AND *PAECILOMYCES LILACINUS* FOR BIOCONTROL OF *ROTYLENCHULUS RENIFORMIS*

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ABSTRACT

Castillo, J. D., K. S. Lawrence, J. W. Kloepper, and E. van Santen. 2010. Evaluation of *Drechslerella dactyloides*, *Drechslerella brochopaga*, and *Paecilomyces lilacinus* for the biocontrol of *Rotylenchulus reniformis*. Nematropica 40:71-85.

Experiments were conducted to evaluate the biological control potential of *Drechslerella dactyloides*, *Drechslerella brochopaga*, and *Paecilomyces lilacinus* against the reniform nematode, *Rotylenchulus reniformis* under *in vitro*, and in greenhouse conditions. Pathogenicity tests *in vitro* with *Drechslerella dactyloides* and *D. brochopaga* revealed conidial germination at 14 hours and at 72 hours trapping-rings had formed and ensnared reniform nematodes. *Paecilomyces lilacinus* conidia germinated in 12 hours and parasitized nematode eggs within 24 hours after the initial exposure. Pathogenicity of the fungi was examined in the greenhouse in autoclaved and non-autoclaved soil. In autoclaved soil, all three fungi reduced ($P \leq 0.05$) the number of vermiform *R. reniformis* nematodes in soil 60 days after planting. *Paecilomyces lilacinus* also reduced ($P \leq 0.05$) numbers of eggs extracted from the cotton roots. In non-autoclaved soil, *D. dactyloides* reduced *R. reniformis* vermiform life stages, but none of the fungal isolates affected the number of *R. reniformis* eggs extracted from the roots. *Drechslerella dactyloides*, *D. brochopaga*, and *P. lilacinus* parasitize *R. reniformis in vitro* and in the greenhouse using autoclaved soil. However, the fungi did not reduce numbers of *R. reniformis* in non-autoclaved soil. These results illustrate these fungi are parasites of *R. reniformis*; however they need to have an advantage to compete with native soil microorganisms.

Key words: Biological control, *Drechslerella dactyloides*, *Drechslerella brochopaga*, *Paecilomyces lilacinus*, *Rotylenchulus reniformis*.

RESUMEN

Castillo, J. D., K. S. Lawrence, J. W. Kloepper, and E. van Santen. 2010. Evaluación de *Drechslerella dactyloides*, *Drechslerella brochopaga*, y *Paecilomyces lilacinus* para el biocontrol de *Rotylenchulus reniformis* bajo condiciones *in vitro*, e invernadero. Nematropica 40:71-85.

Se realizaron experimentos para evaluar el potencial de control biológico de *Drechslerella dactyloides*, *Drechslerella brochopaga* y *Paecilomyces lilacinus* contra el nematodo reniforme, *Rotylenchulus reniformis* bajo condiciones *in vitro* y de invernadero. Evaluaciones de patogenicidad *in vitro* de *Drechslerella dactyloides* y *D. brochopaga* revelan que la germinación de la conidia inicia después de 14 horas y 72 horas más tarde se formaron los anillos constrictores y los nematodos fueron atrapados. Las conidias de *Paecilomyces lilacinus* germinaron después de 12 horas de ser liberadas y los huevos de los nematodos fueron parasitados 24 horas después de ser expuestos a éste hongo. Evaluaciones de patogenicidad bajo condiciones de invernadero fueron realizadas en dos tipos de suelos (autoclavado y no autoclavado). En suelo autoclavado, los tres hongos redujeron ($P \leq 0.05$) el número de vermiformes de *R. reniformis* en suelo 60 días después de la siembra. *Paecilomyces lilacinus* redujo ($P \leq 0.05$) el número de juveniles y huevos en las raíces de algodón. En suelo no autoclavado, *D. dactyloides* redujo vermiformes de *R. reniformis* pero ninguno de los hongos evaluados redujo el número de huevos en las raíces. *Drechslerella dactyloides*, *D. brochopaga*, y *P. lilacinus* parasitan *R. reniformis in vitro* y en el invernadero utilizando suelo autoclavado. Sin embargo, estos hongos no redujeron el número de *R. reniformis* en suelo

no autoclavado. Los resultados demuestran que aunque éstos hongos son parásitos de *R. reniformis* necesitan tener una ventaja adicional para competir con microorganismos nativos del suelo.

Palabras clave: Control biológico, *Drechslerella dactyloides*, *Drechslerella brochopaga*, *Paecilomyces lilacinus*, *Rotylenchulus reniformis*.

INTRODUCTION

Rotylenchulus reniformis is a threat to cotton production in the United States, especially in the mid south where 7% of the total cotton production is lost due to this nematode (Blasingame *et al.,* 2009). Currently, there are no resistant cotton cultivars to *R. reniformis* (Weaver *et al.*, 2007; Usery *et al.,* 2005). Management options are limited to the use of crop rotation (Davis *et al.,* 2003; Gazaway *et al.,* 2000) and nematicides, such as aldicarb, metam sodium, oxamyl, 1,3 dichloropropene, avermectin, and thiodicarb (Koenning *et al.,* 2007; Starr *et al.*, 2007; Kinloch and Rich, 2001; Lawrence *et al.,* 1990).

Nematicides are proven to maintain cotton yields by reducing nematode damage in the roots (Koenning *et al.,* 2007; Kinloch and Rich, 2001; Lawrence *et al.,* 1990), but there is a concern of the toxicological and environmental effects of its application (Koenning *et al.,* 2004; Jatala, 1986) and efficacy is being lost due to degradation. Soil microflora accelerated aldicarb degradation, and even increasing the dose does not increase nematode control (McLean and Lawrence, 2003). A similar trend has been observed in seed treatments with abamectin, which increase cotton yields by reducing *R. reniformis* penetration and infection until taproots reach 5 cm length. Later nematode suppression decreased when the taproot length increase (Lawrence and Lawrence, 2007; Faske and Starr, 2007). Hence, there is a need to search for new control strategies such as biocontrol that can be incorporated into the integrated pest management system. Using more than one control strategy against *R. reniformis* will reduce its damage in cotton crops.

Biological control of nematodes in cotton is not practiced in the U.S. at this time (Robinson, 2007). There is often a misconception that nematodes are impossible to control using biocontrol (Sikora, 1992). During years research has been conducted on nematode biocontrol agents with promising results; however, field trials had been inconsistent and nematode reduction is lower compared to the control provided by a nematicide. To improve nematode biocontrol is necessary to understand factors such as the complexity of root-nematode-biocontrol agent interaction, improve formulation process of the biocontrol agent (longer shelf-life of the product), and use more than one biocontrol agent (with different mode of action) against a specific nematode to target its different life stages.

Biocontrol can play a future role in management of *R. reniformis* since numerous fungal and bacterial antagonists have been reported colonizing *R. reniformis*. The fungi *Pochonia chlamydosporia, Paecilomyces lilacinus* and an unidentified fungus named Arkansas Fungus (ARF) have been reported to parasitize eggs of *R. reniformis* (Wang *et al.,* 2005; Wang *et al.,* 2004; Walters and Barker, 1994), and *Arthrographis* sp., *Pseudorobillarda* sp., and *Fusarium equiseti* have been reported to reduce vermiform stages (McLean *et al.,* 2000). Strains of the bacterium *Pseudomonas fluorescens* were isolated from the rhizosphere of cotton plants in India and reduced the

population of *R. reniformis* 70.4% in soil and 44.8% in roots (Jayakumar *et al.,* 2003). Soils from Lower Rio Grande Valley of Texas have been reported to be suppressive to *R. reniformis* by up to 95%, but the organisms involved in this suppression have not been identified (Robinson *et al.,* 2008). Recently, isolates of *Pasteuria* spp. were isolated from *R. reniformis* from Alabama, Florida, and Mississippi cotton fields (Hewlett *et al.,* 2009).

In Alabama cotton crops, *Drechslerella dactyloides* (formerly *Arthrobotrys dactyloides*), *Drechslerella brochopaga* (formerly *Dactylaria brochopaga*), *Paecilomyces lilacinus*, and *Fusarium oxysporum* have been isolated from *R. reniformis* nematodes (Castillo *et al.,* 2008). *Drechslerella dactyloides* and *D. brochopaga* were previously reported reducing populations of *Meloidogyne graminicola* in rice (Singh *et al.,* 2007; Kumar and Singh, 2006), and were also found parasitizing juveniles of *R. reniformis* nematodes (Castillo *et al.*, 2008). These two fungi produce constricting rings that trap the vermiform stage of the nematode. *Paecilomyces lilacinus* reduces populations of eggs of *R. reniformis* in tomato plants by up to 36% at harvest season (Walters and Barker, 1994). *Paecilomyces lilacinus* penetrates the egg shell by production of penetrating hyphae and appressoria (Lopez-Llorca *et al.,* 2008).

The current study was designed to examine the biocontrol potential of *D. dactyloides*, *D. brochopaga*, and *P. lilacinus* isolated from *R. reniformis nematodes found across Alabama.* Our hypothesis is that these fungi are pathogens that will reduce *R. reniformis* populations.

MATERIALS AND METHODS

Three strains of *Drechslerella dactyloides* (strains named with two initials from the place they were isolated: *BW-D. dactyloides, GH-D. dactyloides, HN-D. dactyloides*), *Drechslerella brochopaga*, and *Paecilomyces lilacinus* were isolated from *R. reniformis* nematodes found across cotton crops in Alabama (Castillo *et al.,* 2008).

In vitro observations

Inoculum preparation

Conidia were collected from seven-day old pure cultures of *Drechslerella dactyloides*, *D. brachopaga*, and *Paecilomyces lilacinus* on corn meal agar (CMA) (Sigma Chemical Co.). Plates were flooded with 10 ml of sterile water, and conidia were dislodged aseptically with a spatula and transferred to a sterile beaker. Conidial solutions were enumerated, quantified using a hemacytometer, and standardized to 6250 conidia/ml each for *D. dactyloides, D. brochopaga*, and 7500 conidia/ml of *P. lilacinus.*

Rotylenchulus reniformis life stages were extracted from stock cotton plants cv. ST 5599 BGRR planted in 500 cm^3 pots containing a sandy loam soil (sand, silt, clay of 67.5-20-12.5; 1.4% OM) in the Plant Science Research Center of the Alabama Agricultural Experiment Station on the campus of Auburn University. Vermiform nematode stages were extracted from the soil by gravity screening followed by sucrose centrifugation-flotation, and eggs were washed from cotton root systems by shaking the roots in 6.0% NaOCl for 4 minutes at 120 rpm (Hussey and Baker, 1973). Extracted vermiform life stages and eggs were disinfected by immersion in streptomycin sulfate (200 mg/L) (Sigma Chemical Co.) for 30 seconds, followed by a second 30 second wash in vancomycin (10 mg/L) (Sigma Chemical Co.), and a final rinse in sterilized distilled water.

In the *in vitro* colonization test treatments were: 1) *D. dactyloides* + 20 *R. reniformis* vermiforms, 2) *D. brochopaga* + 20 *R. reniformis* vermiforms, 3) *P. lilacinus* + 20 *R. reniformis* eggs, 4) 20 vermiforms *R. renifor-*

mis, and 5) 20 eggs of *R. reniformis*. Each treatment contains four replicates. Treatments 4 and 5 that contain eggs or vermiforms were used as controls to verify that there was no fungal or bacterial contamination in the cultures. Conidia were pipetted onto glass slides with 3.5 cm circular depression containing 1% CMA adjusted to 6.6 pH. To observe colonization of vermiforms trapped by the rings produced by *D. dactyloides* and *D. brochopaga,* 20 juveniles of *R. reniformis* were placed by hand on the 3.5 cm diameter of depression slides. To record the infection by the nematode egg-parasite *P. lilacinus* twenty eggs of *R. reniformis* were added to the slides. All the cultures were incubated at 22°C without light. Observations of conidia germination parasitism of the nematode eggs and vermiform life stages by the fungi were recorded every 6 hours. A linear correlation of the percentage of nematodes infected vs. time were analyzed on SAS Software SAS 9.1.3 (SAS Institute Inc.) using the REG procedure for the linear regression of each of the fungi, and the GLM procedure was used to compare the linear regressions of *D. dactyloides* and *D. brochopaga*.

SEM

The vermiform life stages of *R. reniformis* nematodes trapped by rings of *D. dactyloides* and *D. brachopaga*, eggs parasitized by conidia of *P. lilacinus*, and females were observed using SEM. Vermiform life stages and eggs parasitized by fungi were removed from the culture and placed on 12mm diameter aluminum stubs. Fixation of the tissues included vapor exposure to a 2% aqueous solution of osmium tetroxide (OsO_4 2%) in the dark for 2 hours. Samples were air dried for 2 more hours, and afterward the stubs were transferred to the sputter coater (EMS 550x) for gold layering. SEM observations were made using a Carl Zeiss EVO 50 microscope.

Greenhouse trials

Three cotton plants cv. ST 5599 BGRR plants were planted in 500 cm^3 pots containing the sandy loam soil and 1% v/v of fungal inoculum was added to each pot. The fungal inoculum was mixed into the soil to evenly distribute the fungus. Additionally, 3000 *R. reniformis* vermiform life stages and eggs in 3 ml of water were added to each pot by pipeting at planting.

The fungal isolates were cultured for 7 days on water agar (WA) and then transferred to a flask carrier culture. In a 250 ml conical flask, 150 cm^3 of the carrier (oat seed, wheat seed, or 6% v/v cornmeal sand mix) were added and moistened with 100 ml of tap water. Imbibed seed were autoclaved twice at 121°C and 103.4 kPa for 30 minutes on two consecutive days. Two 5 mm diameter fungal disks from the periphery of 7 day-old WA cultures were aseptically transferred to each flask. Fungal cultures were increased in a growth chamber at 27°C during 30 days and shaken daily to distribute the fungi evenly.

The greenhouse colonization test treatments was designed as a factorial arrangement of a RCBD with the main factor being the six fungal isolates, the second factor was the fungal carrier and the third factor was the autoclaved or natural soil. The six fungal isolates were increased on three different fungal carriers (oat seed, wheat seed, or cornmeal/sand) and placed in two types of soil (autoclaved or natural non-autoclaved) in the presence or absence of the nematode. In addition, one control contained the fungal carrier without any fungi and the second or absolute control added no fungal carrier at all. Each treatment combination was replicated five times and the entire experiment was repeated twice, for a total of 960 experimental units. Plants were allowed to grow in the greenhouse for 60 days and then harvested.

Parameters measured included plant height; fresh and dry shoot weight and fresh and dry root weight were recorded. *Rotylenchulus reniformis* vermiform life stages were extracted from the soil and eggs were extracted from the cotton roots as previously described. Vermiform life stages and eggs were counted using an inverted TS100 Nikon microscope. Data were imported, linearized, and tabulated in SAS 9.1 (SAS Institute Inc.) using PROC GLIMMIX to check the residuals, and means were compared by Dunnett's test at ($P \leq 0.05$) level of significance.

RESULTS

In vitro observations and SEM

In the presence of *R. reniformis*, *Drechslerella dactyloides* conidia began germinating 8-12 hours after culturing on the CMA slides at 27°C (Fig. 1A). Hyphal strands spread across the plate within 14 hours (Fig. 1B), and nematode trapping rings were formed by 32 hours (Fig. 1C, D). When the ring formation was completed, 5% of the nematodes were trapped at 72 hours (Fig. 1E), 16% at 78 hours, 24% at 84 hours, 35% at 90 hours, and 59% after 96 hours. Nematode entrapment followed a linear pattern over time. The linear regression model obtained for *D. dactyloides* is % of nematodes captured = -103.6 + 1.6 * time (hours) with an r^2 = 0.84. Based on the regression, conidia are able to germinate, form rings, and start trapping nematodes within 64 hours, and can kill the nematodes within 127 hours (Fig. 2). *Drechslerella brochopaga* growth was similar to that of *D. dactyloides* but differences in timing were observed. The conidia started to germinate at 10 hours after culturing, which was consistently two hours slower than *D. dactyloides*. Hyphae extended across all the plates at 14 hours, which was similar to the hyphal extension observed with *D. dactyloides*. Ring formation was initiated at 24 hours with complete ring formation by 32 hours after culturing. First nematodes were trapped after 66 hours, which was 6 hours before any nematodes were trapped by *D. dactyloides*. At 72 hours 5% of the nematodes were trapped, at 78 hours 15%, at 84 hours 30%, at 90 hours 47%, and after 96 hours 69% (Fig. 2). The linear regression model obtained for *D. brochopaga:* is % of nematodes captured = -125.5 + 1.9 * time (hours) with an r^2 = 0.81. *Drechelerella brochopaga* should require 118 hours to kill the nematodes (Fig. 2). *Drechslerella dactyloides* and *D. brochopaga* are ring-trapping fungi, and the amount of time required from germination of the conidia to rings trapping *R. reniformis* differed by 9 hours but this time difference was not significant ($P < 0.0994$).

Paecilomyces lilacinus is an egg pathogen and the infection process required less time than the nematode-trapping ring fungi. Germination of conidia was observed at 12 hours after culturing (Fig. 3A). The first eggs were surrounded by hyphae at 18 hours and were parasitized by 24 hours (Fig. 3B-C). *Paecilomyces lilacinus*-colonized eggs were observed with conidiophores with conidia rupturing through the egg shell after 72 hours (Fig. 3D). This fungus began parasitizing eggs at 18 hours (2% of the total eggs) and increased mortality in a linear fashion killing 82% of the eggs present after 42 hours. Linear regression of % of eggs parasitized = -28.1 + 2.4 * time (hours) with an r^2 = 0.89; then eggs could be parasitized after 53 hours (Fig. 4).

SEM observations illustrate the differences between the conidia of *D. dactyloides* and *D. brochopaga* (Fig. 5A-B). Nematode trapping rings and the assimilative hyphae were observed inside the body of the parasitized *R. reniformis* nematodes (Fig. 5C-D). *Paecilomyces lilacinus* surrounded the nematode eggs with hyphae and lemon shaped conidia (Fig. 6A-B).

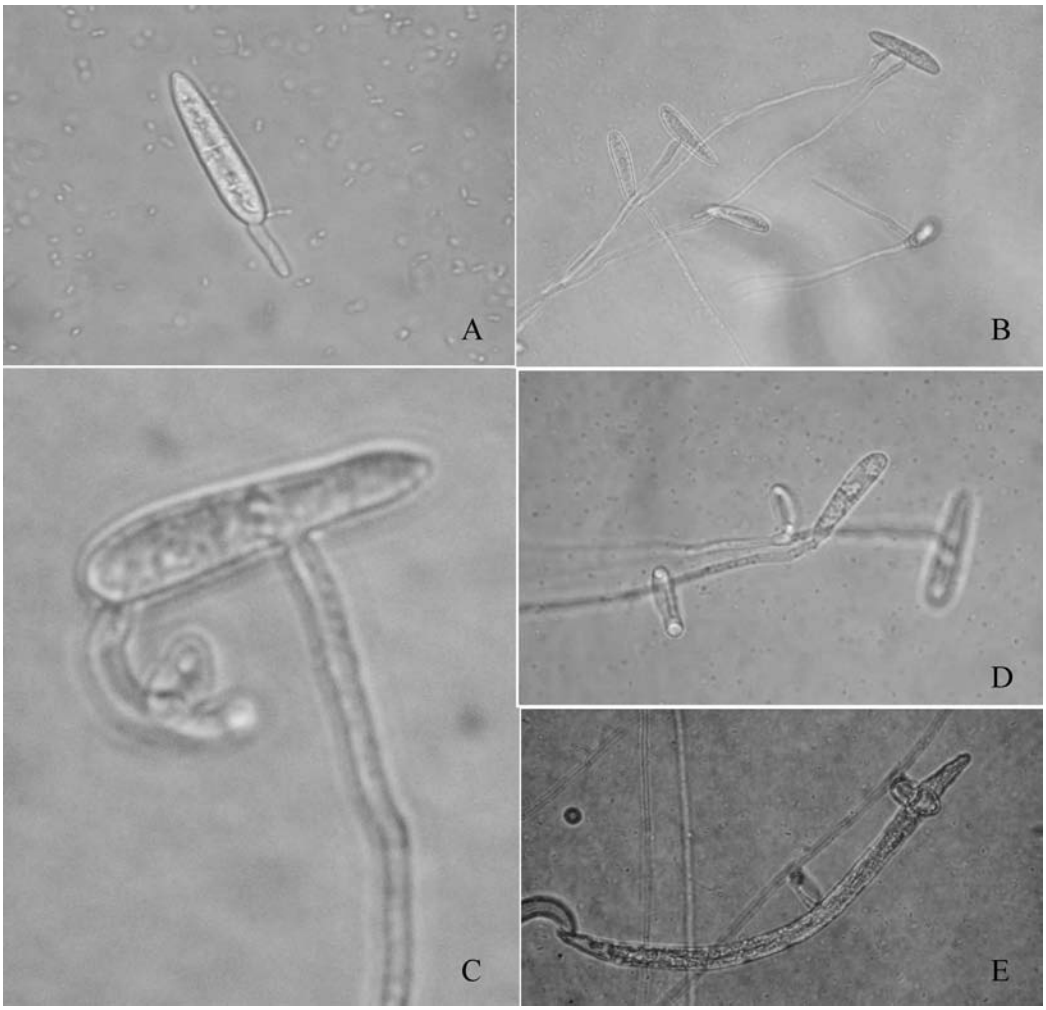

Fig. 1. *Drechslerella dactyloides* and *Drechslerella brochopaga* trapping process. (A) Initiation of conidia germination 8-12 hours (400x), (B) hyphae growth at 14 hours (400x), (C) initial of ring formation at 16 hours (400x), (D) Complete formation of rings at 24-36 hours (400x), (E) Nematode trapped by a ring at 72 hours (400x).

Greenhouse trials

Phytopathogenicity to the cotton plants was not observed for any of the fungal isolates. Plant shoot mass was not affected by the fungal isolates, soil types (autoclaved or non-autoclaved), presence or absence of *R. reniformis*, or the fungal carrier. No interactions were observed between the fungal isolates, carrier, and presence or absence of the nematode ($P < 0.998$), and no significant differences were found among the carriers within the soil treatments ($P < 0.074$). However, in autoclaved soil shoot mass was greater than in non-autoclaved soil ($P < 0.001$). In treatments with corn meal carrier, plant shoot mass was lower than plants with oat ($P < 0.015$) and wheat

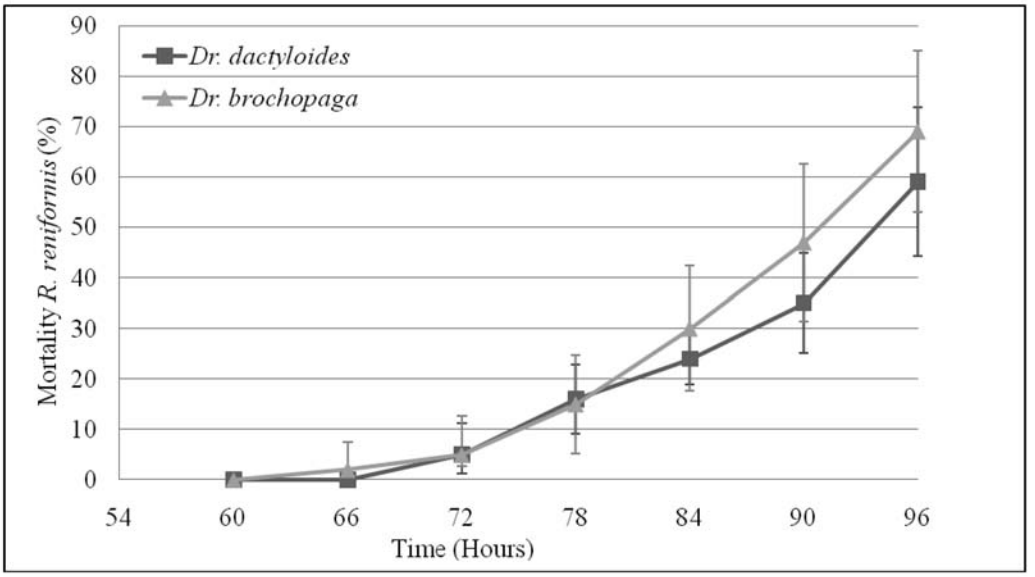

Fig. 2. Percentage of capture of *Rotylenchulus reniformis* vermiforms in time, by *Drechslerella dactyloides* and *D. brochopaga* under *in vitro* conditions. Linear regression model for *D. dactyloides*: Percent of nematodes captured = -103.6 + 1.6 * Time (hours) $r^2 = 0.84$. Linear regression model for *D. brochopaga*: Percent of nematodes captured = -125.5 + 1.9 * Time (hours) $r^2 = 0.81$.

carriers ($P < 0.039$). Also, in treatments without nematodes the shoot mass was higher ($P < 0.019$) (Fig. 7-A).

Root mass was affected by soil treatments, fungal inoculums carrier, and the presence or absence of the nematode ($P < 0.006$). Cotton root mass was 60% higher in natural soils than in autoclaved soils ($P < 0.001$). Plants infected with the nematode had 41% less root mass than the plants without the nematode ($P < 0.001$). Cornmeal carrier in non-autoclaved soil increased root mass than plants inoculated with oat ($P < 0.001$) and wheat carrier ($P < 0.001$). In the presence of the nematode, root mass of the plants exposed to the oat carrier was higher than the wheat and cornmeal carriers in the autoclaved or natural soils ($P < 0.001$) (Fig. 7-B). Plant height was not affected by the soil combinations ($P < 0.975$), however, the interaction between soil, fungal carrier, and presence of nematodes was significant ($P < 0.0126$). Plants grown in natural soil were taller than the ones from autoclaved soil and also taller when nematodes were not present ($P < 0.001$) (Fig. 7-C).

Treatments in the non-autoclaved soil present lower nematode populations than autoclaved soil. The total number of *R. reniformis* nematodes in the soil was lower in all six fungal isolate treatments compared to the two controls ($P < 0.001$) in the autoclaved soil. In non-autoclaved soil, the fungal isolates BW-*D. dactyloides* ($P < 0.003$) reduced 64% populations of *R. reniformis* compared to the two controls (Table 1). The total number of *R. reniformis* eggs extracted from the roots was 48% lower averaged over all six fungal isolates treatments compared to the absolute control in the autoclaved soil. However, *P. lilacinus* ($P < 0.007$) reduced *R. reniformis* populations 46% and 60% when compared to the two

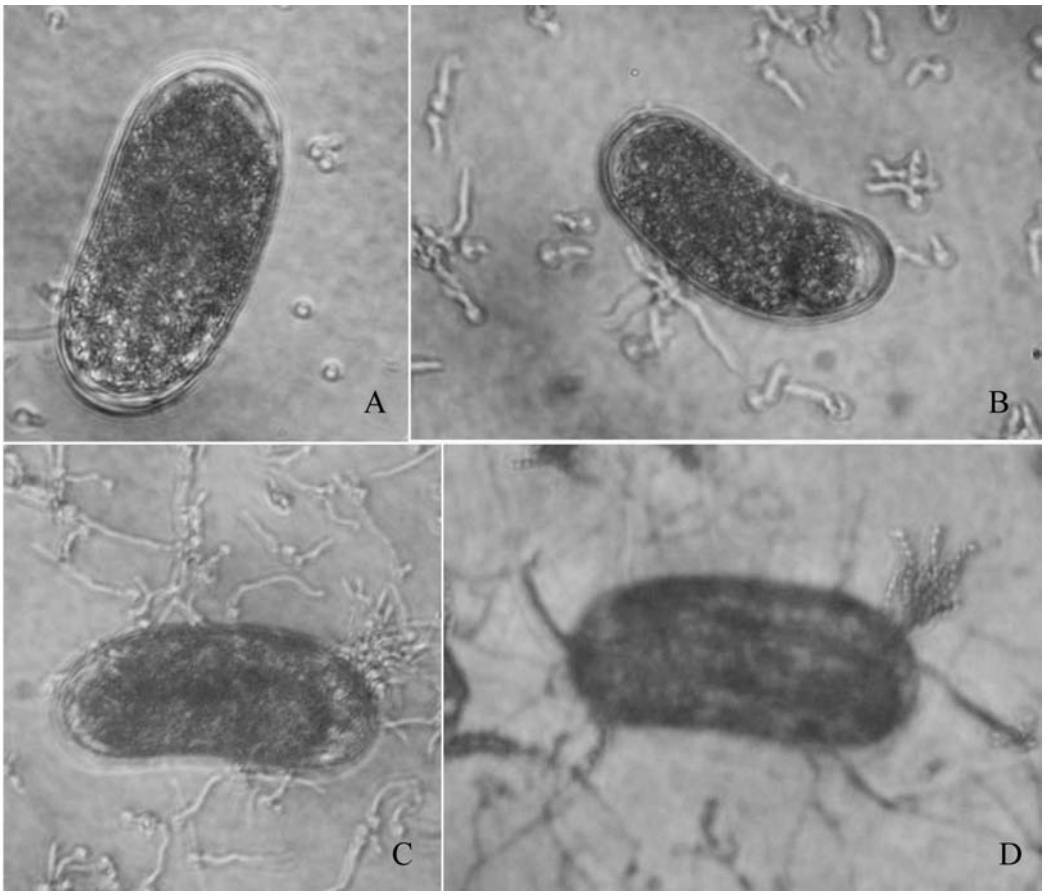

Fig. 3. *Paecilomyces lilacinus* attacking reniform egg. (A) Reniform nematode egg and non germinated conidia 12 hours (400 x), (B) Germinated conidia surrounding reniform egg at 18 hours (400 x), (C) Egg parasitized by *P. lilacinus* 24-40 hours (400 x) (D) Sporulation of *P. lilacinus* within the egg 72 hours (400 x).

controls. There was no reduction of nematode population in natural soil (Table 2). The numbers of eggs and vermiform life stages of *R. reniformis* per gram of root was significantly reduced over all fungal isolates by 62% compared to the no carrier control ($P < 0.001$), but no difference was observed compared to the carrier control ($P < 0.06$), and no nematode reduction was observed in natural soil (Table 3).

DISCUSSION

Drechslerella dactyloides (isolates: GH, HN), *D. brochopaga*, and *Paecilomyces lilacinus* reduced populations of *R. reniformis* in autoclaved soil but not in non-autoclaved soil in the greenhouse. The lack of efficacy in non-autoclaved soils suggests that when fungi are applied at planting they compete with native soil microflora and must over-

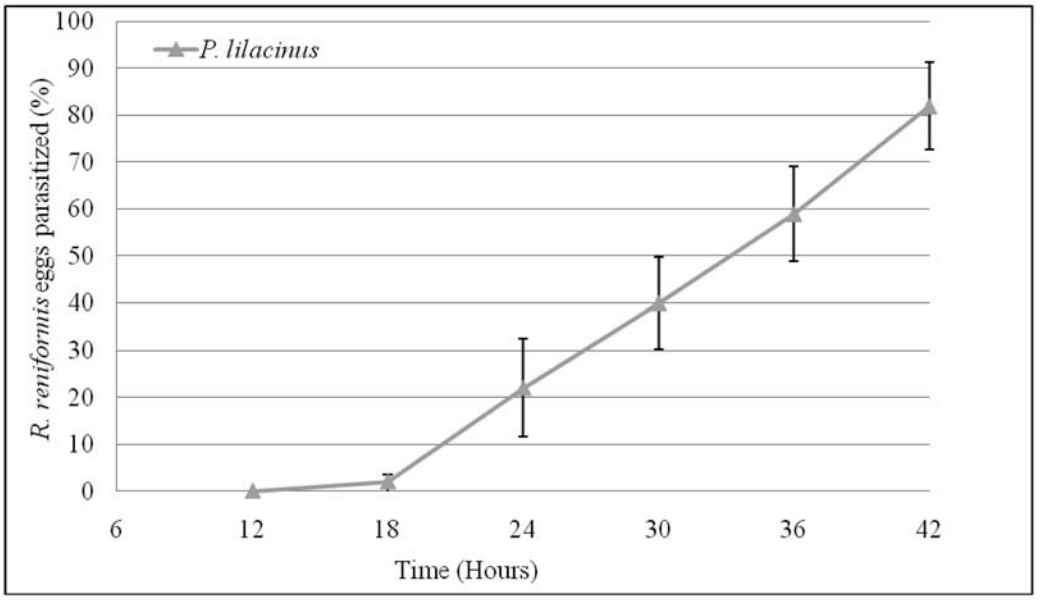

Fig. 4. Percentage of *Rotylenchulus reniformis* eggs parasitized by *Paecilomyces lilacinus* under *in vitro* conditions. Linear regression model: Percentage of eggs parasitized = -28.1 + 2.4 * Time (hours) $r^2 = 0.89$.

come fungistasis in order to establish in the rhizosphere and reduce nematode populations (Kerry, 2000; Persson and Jansson, 1999). Only the isolate, BW-*D. dactyloides* showed this ability and controlled *R. reniformis* in sandy loam non-autoclaved soil in the greenhouse trials.

To have successful control with the tested fungi, it is necessary to understand the influence of the soil factors (moisture, pH, temperature) in the plant-fungi-nematode interaction. Additionally, formulation of the fungal carrier needs to be improved to allow the fungi to compete in the natural soil environment and colonize cotton rhizosphere. The formulations used in this study, seeds of wheat and oat, and cornmeal mixed with sand, are probably used by many microorganisms as a food source. Fungi evaluated reduced *R. reniformis* population in autoclaved soil when there was no competition with other microorganisms. In contrast, in non-autoclaved soil where constant competition among microorganisms is occurring the nematode reduction was not significant. Furthermore, during inoculum preparation we frequently observed that *D. dactyloides* and *D. brochopaga* had a slower growth rate and that the cultures can easily became contaminated by different fungi with a faster growth rate (data not shown). Hence, different fungal carriers have to be evaluated with the objective of enhancing the survival and antagonism of the nematode-parasite fungi. Support for the premise that changing the carrier can improve the efficacy of the fungal biocontrol agent comes from Stirling *et al.,* 1998, where using *D. dactyloides* in granules formulations with kaolin and vermiculite as carriers and arabic gum as a binder reduced numbers of *Meloidogyne javanica* by over 90% and reduced galls on tomato roots by 57-98%. Cabanillas *et al.,* 1989 also obtained higher fungus-infected egg masses using pellets

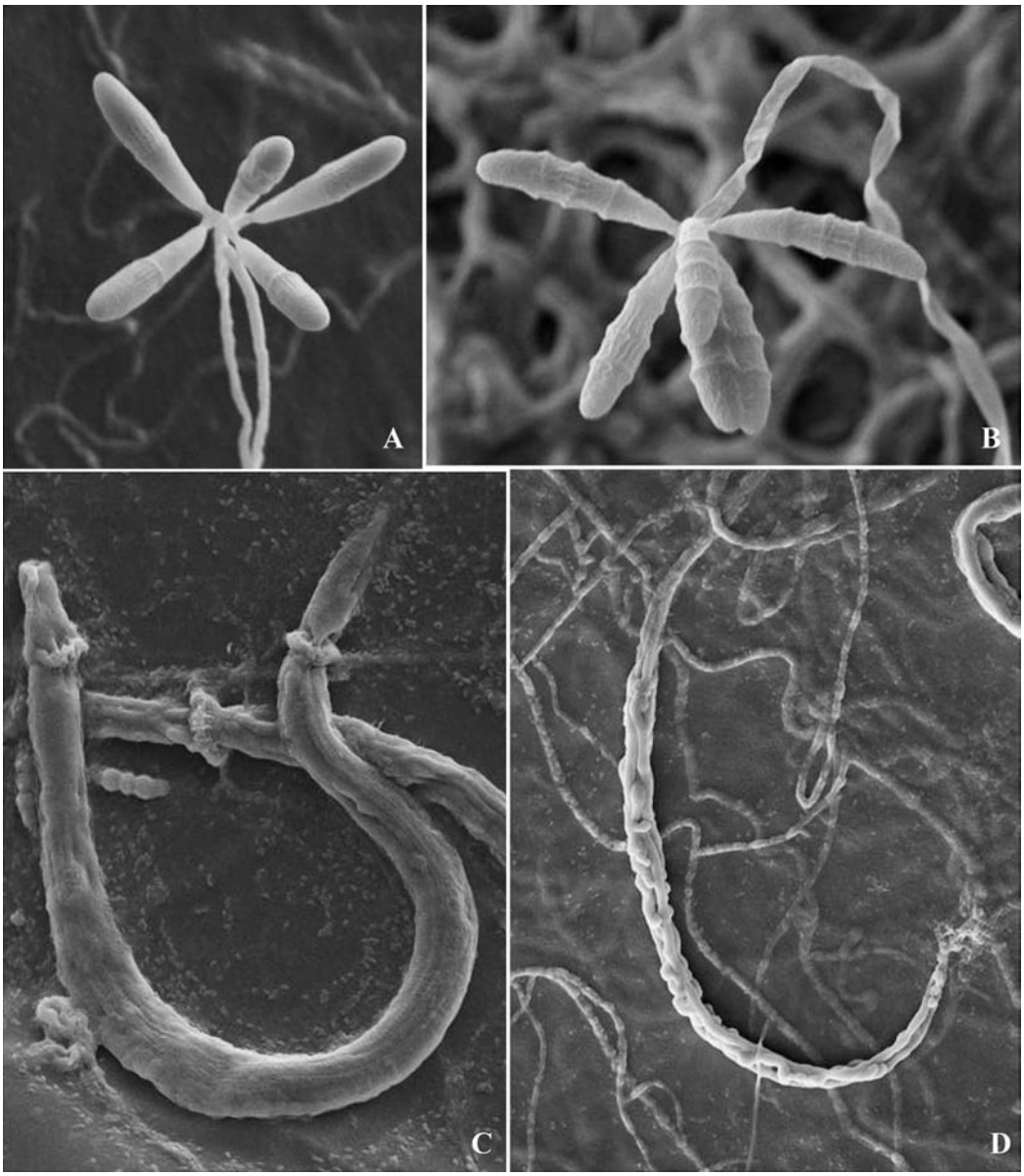

Fig. 5. Conidia of *D. dactyloides* and *D. brochopaga*. (A) *D. dactyloides* (2300x), (B) *D. brochopaga* (3000x), (C) *Rotylenchulus reniformis* vermiform trapped by constricting rings (2500x), (D) Assimilative hyphae inside *R. reniformis* vermiform (1000x).

(32%) than when used wheat (16%) as a fungal carrier.

Under *in vitro* conditions, conidia of *D. dactyloides* and *D. brochopaga* require at least 72 hours (3 days) to germinate, produce trapping-rings, and start trapping *R. reniformis* vermiforms. Kumar and Singh (2006) reported that in this same 3-day time

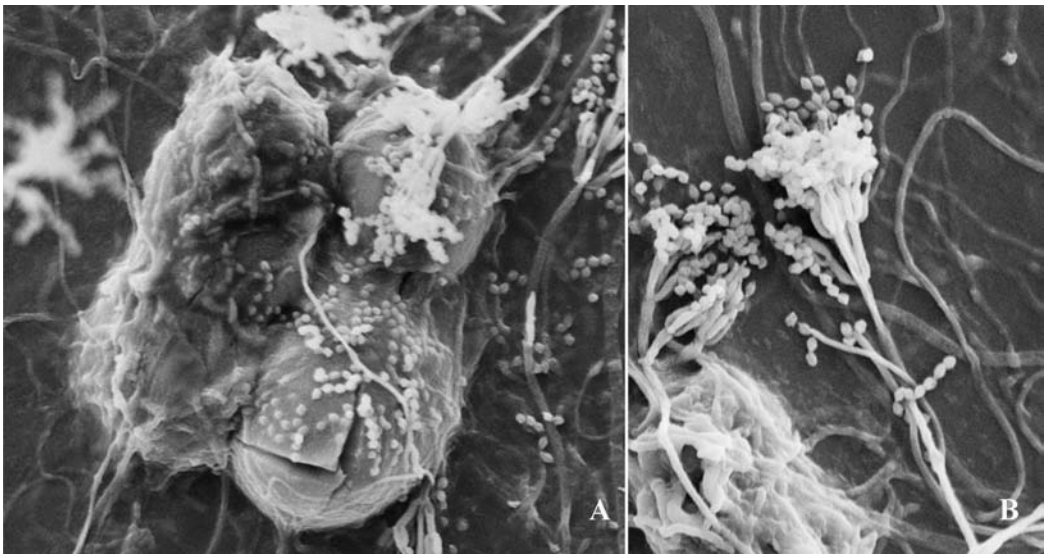

Fig. 6. (A) *Rotylenchulus reniformis* egg parasitized by *P. lilacinus* (1400x), (B) Conidiophore with lemon shaped conidia of *P. lilacinus* (2250x).

period, a strain of *D. dactyloides* captured 95% of the total population of *Meloidogyne incognita.* This increased efficacy compared to our results likely results from the fact that Kumar and Singh (2006) released the nematodes in 8-day-old fungal cultures of *D. dactyloides,* while in our study the conidia and *R. reniformis* vermiforms were released at the same time.

Trapping times of *D. brochopaga* reported by Singh *et al.* (2007) where the fungus trapped 60% of *Meloidogyne graminicola* in 96 hours (4 days) coincide with our study. Also, ring trapping formation time is also similar to the studies mentioned above. The conidia of *D. dactyloides* and *D. brochopaga* have a lag phase when they are adapting to changing environments and are beginning to form trapping rings to ensnare *R. reniformis* vermiforms. Times of application of these fungi have to be evaluated. *In vitro* infection times of these fungi suggests that they require time to grow mycelia and form trapping rings before they start trapping nematodes. It is possible that releasing these fungi before planting can improve the biocontrol in natural soils.

Paecilomyces lilacinus starts parasitizing eggs after 24 hours under *in vitro* conditions. This fungus has a fastest growth rate, invading eggs within 48 hours. *Rotylenchulus reniformis* second stage juveniles develop and hatch in 6-7 days (Sivakumar and Seshadri, 1971), which make them sensitive to parasitism by *P. lilacinus* before colonizing the roots.

Under greenhouse conditions *P. lilacinus* reduced the number of eggs of *R. reniformis* in autoclaved soil when the fungus was applied at planting, which coincides with Reddy and Khan (1988) where *R. reniformis* was controlled by this fungus after 60 days. Also, Cabanillas and Barker (1989) reported increased tomato yields and 36% reduction in tomato root galling by applying *P. lilacinus* 10 days after transplanting

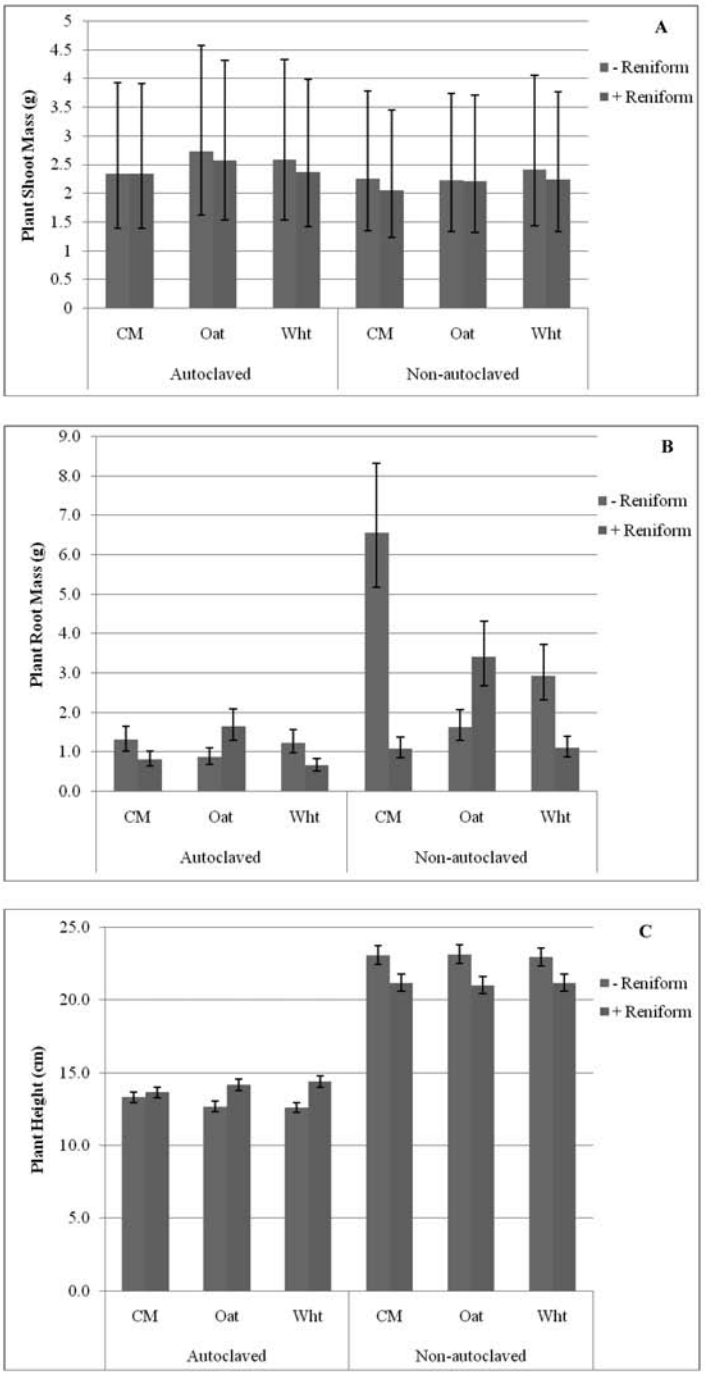

Fig. 7. Effect on cotton shoot mass (A), root mass (B), and plant height (C), using corn meal, oat, and wheat as fungal carriers with autoclaved and non-autoclaved soils ($P < 0.05$)

Table 1. *Rotylencuhlus reniformis* numbers per 500 $cm^3$of soil as influenced by *Drechslerella dactyloides*, *D. brochopaga*, and *Paecilomyces lilacinus* in autoclaved and non-autoclaved soil ($P < 0.05$).

	Autoclaved			Non-autoclaved		
		Dunnett P vs.			*R. reniformis*/ 500 cm^3	
Fungi	*R. reniformis*/ 500 cm^3	No carrier	Carrier	*R. reniformis*/ 500 cm^3	No carrier	Carrier
No carrier	4851			2138		
Carrier	5539			2107		
BW-*D. dactyloides*	1726	0.0001	0.0001	1138	0.0039	0.0051
GH-*D. dactyloides*	2495	0.0022	0.0002	1492	0.2001	0.2350
HN-*D. dactyloides*	2209	0.0002	0.0001	1590	0.3823	0.4357
HN-*D. brochopaga*	2471	0.0018	0.0001	1890	0.9695	0.9845
GH-*P. lilacinus*	2036	0.0001	0.0001	1373	0.0721	0.0876

Table 2. Number of *Rotylenchulus reniformis* eggs and juveniles extracted from cotton roots as influenced by *Drechslerella dactyloides*, *Dr. brochopaga*, and *Paecilomyces lilacinus* in autoclaved and non-autoclaved soil ($P < 0.05$).

	Autoclaved			Non-autoclaved		
		Dunnett P vs.			Dunnett P vs.	
Fungi	Eggs and juveniles/ Root	No Carrier	Carrier	Eggs and juveniles/ Root	No Carrier	Carrier
No carrier	5028			1659		
Carrier	3797			1772		
BW-*D. dactyloides*	2498	0.002	0.131	1388	0.874	0.637
GH-*D. dactyloides*	2779	0.013	0.384	1436	0.950	0.766
HN-*D. dactyloides*	2683	0.007	0.278	1881	0.975	1.000
HN-*D. brochopaga*	2534	0.003	0.153	1192	0.327	0.168
GH-*P. lilacinus*	2019	0.001	0.007	1411	0.916	0.701

and at transplanting under microplot conditions.

In the current study there was no *R. reniformis* reduction of eggs in non-autoclaved soil, which suggest that in future studies the fungus has to be applied before planting and placed close to where the seed will germinate to guarantee the root colonization by the fungus. *Paecilomyces lilacinus* has been shown to grow around and in the epidermis of the roots (Cabanillas *et al.*, 1988).

We expect that optimizing management of *R. reniformis* will require the selection of additional different antagonists that attack the nematode during its different life stages. For example, select different species of fungi that attack nematode-egg stage and combine them with strains of fungi that attack juveniles and females. These biocontrol agents combined with other practices such as crop rotation, and seed treatments can improve *R. reniformis* management in cotton crops.

Table 3. *Rotylenchulus reniformis* eggs and juveniles per gram of cotton root as affected by *Drechslerella dactyloides*, *Dr. brochopaga*, and *Paecilomyces lilacinus* in autoclaved and natural soils ($P < 0.05$).

	Autoclaved			Non-autoclaved		
Fungi	Eggs and juveniles/ gr of root	Dunnett P vs. No Carrier	Dunnett P vs. Carrier	Eggs and juveniles/ gr of root	Dunnett P vs. No Carrier	Dunnett P vs. Carrier
No carrier	474.9			117.3		
Carrier	304.2			123.4		
BW-*Dr. dactyloides*	178.4	0.0001	0.0526	101.6	0.9672	0.8698
GH-*Dr. dactyloides*	222.8	0.0023	0.4697	91.9	0.7097	0.5258
HN-*Dr. dactyloides*	213.7	0.0012	0.3400	139.3	0.9224	0.9867
HN-*Dr. brochopaga*	182.4	0.0001	0.0685	84.0	0.3956	0.2571
GH-*P. lilacinus*	179.8	0.0001	0.0576	95.3	0.8316	0.6585

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