Journal of Nematology 20(3):362–365. 1988.

Histology of the Interactions of *Paecilomyces lilacinus* with *Meloidogyne incognita* on Tomato[1]

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Abstract: Excised tomato roots were examined histologically for interactions of the fungus *Paecilomyces lilacinus* and *Meloidogyne incognita* race 1. Root galling and giant-cell formation were absent in tomato roots inoculated with nematode eggs infected with *P. lilacinus*. Few to no galls and no giant-cell formation were found in roots dipped in a spore suspension of *P. lilacinus* and inoculated with *M. incognita*. Numerous large galls and giant cells were present in roots inoculated only with *M. incognita*. *P. lilacinus* colonized the surface of epidermal cells as well as the internal cells of epidermis and cortex. The possibility of biological protection of plant surfaces with *P. lilacinus* against root-knot nematodes is discussed.

Key words: biological control, epiphyte, endophyte, *Meloidogyne incognita, Lycopersicon esculentum, Paecilomyces lilacinus,* rhizoplane, root-knot nematode.

The fungus *Paecilomyces lilacinus* (Thom) Samson has been reported as a potential biological control agent for root-knot nematodes and other plant-parasitic nematodes (1,6,13–15). *Paecilomyces lilacinus* is a common soil hyphomycete, closely related to *Penicillium* (17). It parasitizes eggs of *Meloidogyne* spp. and *Globodera pallida* (Stone) Behrens (5,14). This fungus also invades the females or cysts of a number of nematode species (6,8,13,14). It exhibits chitinase activity when grown on chitin-agar plates (8) and produces a peptidal antibiotic which has wide antimicrobial activity against fungi, yeast, and gram-positive bacteria (10,11).

Although the consistent association of *P. lilacinus* with eggs of *Meloidogyne* spp. and its ability to penetrate both eggs and females is documented (1,6,7,13,14), the mode of its parasitism is unknown. There are no reports on histological examinations of the effects of this fungus on *Meloidogyne* parasitizing tomato.

The objectives of this study were to 1) examine the histological changes of tomato roots inoculated with eggs of *Meloidogyne incognita* (Kofoid and White) Chitwood infested with *P. lilacinus* and 2) determine the effects of *P. lilacinus* on root-gall formation by *M. incognita* on excised tomato roots grown in vitro.

Materials and Methods

Seeds of tomato, *Lycopersicon esculentum* Mill. cv. Rutgers, were surface sterilized with 1% sodium hypochlorite for 7 minutes and rinsed three times with sterile distilled water. They were then placed in 1% water agar contained in autoclaved polypropylene sterilizing trays (15 × 28 × 12 cm) (Fisher Scientific Co., Pittsburgh, PA) at 25 C under continuous incandescent light conditions. Six days after seed germination, two 1-cm lengths of the primary root tip were excised and transferred to a petri dish containing the Skoog, Tsui, and White (STW) medium, cited by Koenning and Barker (16), adjusted to pH 5.2. The excised roots were kept in the dark at 25 C for 10 days.

Lateral roots that emerged were inoculated with nematode eggs and (or) fungus spore suspensions. Eggs of *M. incognita* race 1 ecological culture E-324 provided by the International *Meloidogyne* Project at Raleigh, North Carolina, were extracted from

Received for publication 22 September 1987.

[1] Paper No. 11262 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, NC 27695-7601. This research was supported in part by the Latin American Scholarship Program of American Universities (LASPAU) and by the International Potato Center (CIP) grant provided to Dr. J. N. Sasser at North Carolina State University, Department of Plant Pathology.

Use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Research Service of the product named nor criticism of similar ones not mentioned.

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The authors thank Drs. Robert Milholland, Edna Pableo, and Phillip Esbenshade, Mr. Marvin Williams, and Mrs. T. M. Nowaczyk for assistance and suggestions.

8-week-old galled tomato roots with 1% sodium hypochlorite (9) and were added to each petri dish in an aqueous suspension at the rate of about 200 eggs per dish.

The isolate of *P. lilacinus* obtained from Peru was reported to be effective in controlling nematodes (13). The fungus was grown on potato dextrose agar (PDA) medium at 25 C for 10 days. Nematode eggs and excised roots were separately treated with a spore suspension of *P. lilacinus* (2.1×10^7 spores/ml) for 30 minutes and 30 seconds, respectively. Sterile tap water was used for the controls. All root cultures were grown on STW medium incubated in the dark at 25 C.

Each treatment consisted of two 1-cm lengths of excised tomato roots per dish, replicated nine times. Treatments were *P. lilacinus* alone, *M. incognita* eggs alone, nematode eggs infested with the fungus, fungus plus nematode eggs, and noninoculated control. Root segments were fixed in formalin-propionic acid-propanol (FPP), dehydrated with an isopropyl alcohol series, infiltrated, and embedded in Paraplast-plus tissue embedding medium (Monoproject Scientific Division of Sherwood Medical, St. Louis, MO). Sections, 12-μm thick, were cut with a rotary microtome, mounted on clean slides with Haupt's adhesive and 3% formalin, and stained with the Triarch Quadruple Stain by using the Model 172 Histomatic Slide Stainer (Fisher Scientific Co.) (3). Histological examinations of the roots with the nematode, the fungus, or both were made 2 and 4 weeks after inoculation.

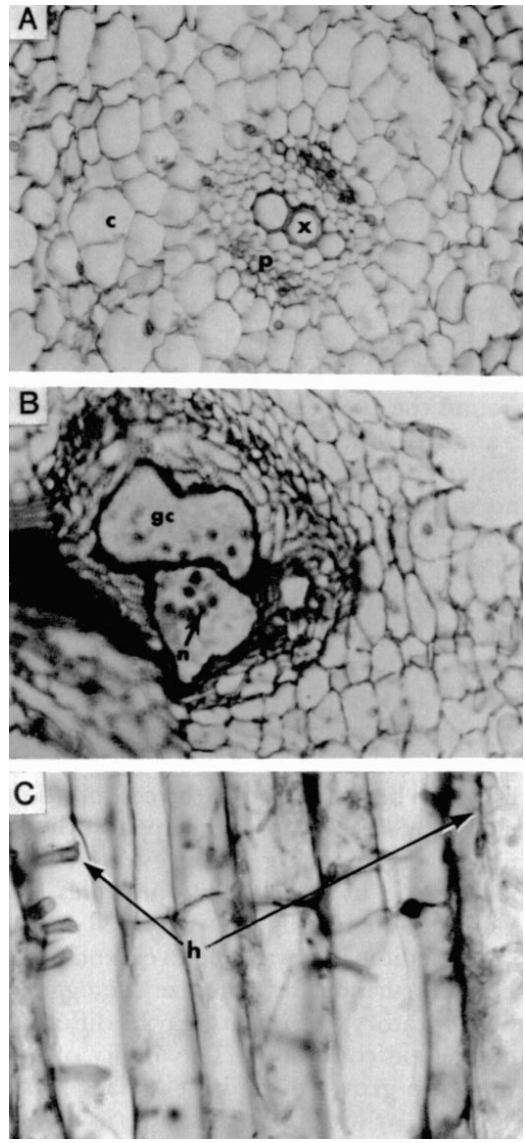

FIG. 1. Root sections showing the effects of *Paecilomyces lilacinus* on root galling by *Meloidogyne incognita* race 1 on tomato 2 weeks after inoculation. A) Healthy root of noninoculated control showing xylem (x), phloem (p), and cortical (c) tissue (×320). B) Giant cells (gc) and their nuclei (n) induced by *M. incognita* (×160). C) Hyphae of *P. lilacinus* growing on the surface of epidermal cells and intracellularly in the cortical cells (×320).

RESULTS AND DISCUSSION

Root galling and histology of tomato fixed 2 weeks after inoculation were different among the treatments. At that time, the roots inoculated with *M. incognita* eggs alone were larger and had more galls (30–32 galls per dish) than those dipped in the fungal spore suspension plus nematode inoculation (0–2 galls per dish). No root galling was observed in the control and fungus alone (Fig. 1A). There was a brown discoloration, however, at the root tips where *P. lilacinus* was growing in all treatments.

Root sections of tomato inoculated with nematode eggs alone developed well-formed giant cells (4) in the vascular cylinders of the roots (Fig. 1B). Most giant

cells were multinucleate with dense cytoplasm and thick cell walls. Tomato roots dipped in fungus spore suspension and then treated with nematode eggs had a few galls but no giant cells. Hyphae of the fungus were observed growing both as an epiphyte (around the epidermis of the root cells) and as an endophyte (inside the cells of epidermis and cortex) (Fig. 1C). Root sections in which the nematode eggs were infected with *P. lilacinus* had neither galls nor giant cells. The hyphae were concentrated around the root-tip region. Tomato roots examined 4 weeks after inoculation were similar to those examined 2 weeks after inoculation.

Simultaneous inoculation of tomato roots with *M. incognita* and *P. lilacinus* may provide biological protection of plant surfaces from invasion by the former. The low numbers of galls (0–2 root galls per dish) and the lack of giant cells on roots dipped in a spore suspension, as compared with those without the fungus (30–32 galls per dish), can be attributed to the colonization of eggs by *P. lilacinus* which apparently inhibited egg hatching and prevented root penetration by *M. incognita* juveniles (5). Although the fungus grew very well on the STW medium and was able to extend from the root surface and colonize the eggs in the laboratory, the results may be different under field conditions. The ability of the fungus to grow on the host surface as an epiphyte, or within the host cells as an endophyte, makes it a good candidate as an agent for biological protection of plant surfaces. This approach seems to offer the greatest potential use for commercialization for both field crops and high value ornamental and fruit crops (2). A limitation to this approach is that this fungus primarily colonizes eggs and possibly females of *M. incognita* but not juveniles which can penetrate and develop inside the root tissue (7,14).

The ability of *P. lilacinus* to infect females of *M. incognita* inside root tissue was not determined, since none were observed in roots dipped in spore suspension. The colonization of tomato root tissue by *P. lilacinus*, however, enables it to be in a good position to inhibit or parasitize this nematode. Indeed, the host plant provides the niche where antagonists inhibit or displace pathogens, which is called the "passive role" of the host in biological control (2). Plants infected with other fungi have similar influences on nematodes and suppress nematode reproduction in some associations. *Globodera rostochiensis* Woll. did not increase the susceptibility of tomato roots to invasion by the fungus causing brown root rot, but the fungus decreased the rate of hatch, invasion, and cyst development by the nematode (12). The fungus possibly produced a factor that inhibited the nematode (12). In this study, the relationship between protection of plant surfaces and the biocontrol of the nematode is explained mainly by the fungal colonization of eggs by the fungus. Future experiments will be required to determine if this endophyte can colonize the developing females and eggs deposited in the gelatinous matrix. There is also a need for histopathological examinations of additional crops and their interactions with *P. lilacinus*.

P. lilacinus colonizes *M. incognita* eggs, preventing them from hatching and leaving fewer juveniles to penetrate root tissue (5,14). Also, the finding that *P. lilacinus* colonizes the root tissue as an epiphyte and endophyte contributes to our understanding of the mechanism of biological control against root-knot nematodes when roots of tomato or possibly other susceptible crop plants are treated with this fungus prior to planting.

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