D. Gotlieb *et al.* (2003) *Phytoparasitica* 31(3):217-225

Dry Mycelium of *Penicillium chrysogenum* Protects Cucumber and Tomato Plants against the Root-knot Nematode *Meloidogyne javanica*

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Incorporation into soil of dry mycelium of *Penicillium chrysogenum*, a waste product of the pharmacological industry, enhanced plant growth and reduced root galling caused by the root-knot nematode *Meloidogyne javanica* in cucumber and tomato plants. Incorporation into sandy loam soil in pots of dry mycelium at a concentration of 0.25% (w/w) resulted in complete protection of cucumber plants from the nematode. The number of juveniles recovered from soils containing dry mycelium was greatly reduced even at a concentration of 0.1% (w/w). In microplot studies conducted at two sites in two seasons, with three or four doses, dry mycelium caused a dose-dependent reduction in root galling index (GI) and promotion of plant growth of cucumber and tomato plants. In *in vitro* studies, the water extract of dry mycelium immobilized nematode juveniles and reduced the egg hatching rate, but these effects were partly reversible after a rinse in water. Soil-drenching of cucumber and tomato seedlings with water extract of dry mycelium did not reduce GI or number of root-invading juveniles. The results show that dry mycelium promotes plant growth and protects plants against nematode infection. Protection, however, does not operate *via* induced resistance.
KEY WORDS: *Meloidogyne javanica*; *Penicillium chrysogenum*; root-knot nematode; soil amendment.

INTRODUCTION

Plant-parasitic nematodes have been controlled mainly by chemical nematicides, but several effective nematicides and fumigants have been withdrawn from the market because of their deleterious effects on human health and the environment. In particular, the reduction in use and phasing out of methyl bromide in recent years have made nematode control more difficult (18). Cultural practices such as use of resistant varieties, crop rotations and soil amendments are often integrated into nematode control strategies. Organic soil amendments, especially those with low C/N ratios, have been reported to have nematicidal effects (25). Crab shell-based products and chitin, which is found in the exoskeletons of insects and crustaceans, and in cell walls of certain fungi as polymers, have been studied extensively for their effects on nematode and fungal pathogen control (2,26,27). These amendments in soil are subjected to microbial decomposition, and several volatiles with nematicidal activity, especially ammonia, are released (20,26).

Received Nov. 6, 2002; received in final form Jan. 1, 2003; http://www.phytoparasitica.org posting April 6, 2003.
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Besides the direct nematicidal effect of these volatiles in earlier decomposition processes, the build-up of antagonistic microorganisms is also thought to be involved in nematode control by organic amendments (12,26). However, this approach is usually limited due to the large quantity of amendments required for control and irreproducible efficiencies, which probably result from soil conditions (29). Enhancement of natural plant-defense mechanisms is another control strategy. Several compounds are known to elicit plant defense responses, which induce local and/or systemic resistance and protect plants from a wide range of pathogens. Salicylic acid, the most investigated compound, induces resistance in plants against several pathogens (28). β-aminobutyric acid, a natural non-protein amino acid, has been reported to induce resistance to a wide range of plant pathogens, including nematodes (7). Microorganisms and compounds of microbial origin have also been found to induce defense responses and/or resistance in plants toward pathogens (5,14,23,30). For example, oligomers of chitosan, which may be released by plant chitosanase from invading fungal pathogens, have been used to induce resistance against fungi (3,4). Dry mycelium (DM) of *Penicillium chrysogenum* from the antibiotics industry has been reported to induce resistance against *Verticillium dahliae* and *Fusarium oxysporum* f.sp. *vasinfectum* in cotton and against *F. oxysporum* f.sp. *melonis* in melon (8-10). Increases in free L-proline in melon, and peroxidase activities in melon and cotton, have been induced after treatments with DM (8,10).

As compared with fungal or bacterial pathogens, induced resistance against plant-parasitic nematodes has not been studied thoroughly. β-aminobutyric acid (BABA) has been shown to induce resistance against certain root-knot and cyst nematodes (7,21,22). Other compounds, such as salicylic acid and lipopolysaccharides, and microorganisms including avirulent nematodes and rhizobacteria, have been reported to increase resistance against nematodes (13,16,19,24). In the present study, the direct and indirect effects of DM of *P. chrysogenum* on the root-knot nematode *Meloidogyne javanica* were investigated in tomato and cucumber plants.

MATERIALS AND METHODS

Dry mycelium and extract DM of *P. chrysogenum* was provided by Biochemie Ltd. (Kundl, Austria). This nonviable fungal biomass was dried for 4 h at 110°C by the manufacturer and contains no residues of penicillin. DM contains ~90% organic matter, 7% N, 1% P and 2% K. Water extract of DM (DME) was prepared as follows: 100 g of DM was mixed with tap water to make 1000 ml suspension. The slurry was stirred overnight at 4°C, and filtered through Whatman No. 1 filter paper. The filtrate was centrifuged at 5000 RCF (relative centrifugal force) for 10 min and filtered through a 0.2-μm filter. This water extract (10% DME) had a pH of 3.5, which was modified to pH 7.0 using 1.0 N NaOH solution before filtration through a 0.2-μm filter.

Nematode Eggs of *M. javanica* were extracted from nematode-infected tomato (*Lycopersicon esculentum* cv. 'Hazera-144') roots with sodium hypochlorite solution (15). Second-stage juveniles (J2) of the nematode were collected daily from eggs, spread on a 30-μm sieve, and stored at 15°C. Juveniles aged for less than 3 days were used in the *in vitro* and pot experiments. J2 and eggs for *in vitro* experiments were washed with sterile water several times.