CHITIN AND *PAECILOMYCES LILACINUS* FOR CONTROL OF *MELOIDOGYNE ARENARIA*

A. K. Culbreath, R. Rodriguez-Kabana, and G. Morgan-Jones
Department of Plant Pathology, Auburn University, Alabama Agricultural Experiment Station, Auburn, Alabama 36849, U.S.A.

ABSTRACT

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   Soil infested with *Meloidogyne arenaria* was amended with chitin at rates of 0, 0.1, 0.2, 0.4, 0.8, and 1.0% (w/w). Rice colonized with *Paecilomyces lilacinus* was also added to the soil at 1, 2, and 5 g/kg soil to have all possible combinations of rates for the 2 materials. The treated soils were kept moist for 2 wk and were then planted with squash (*Cucurbita pepo*) seed. After 6 weeks, the squash was removed and the soils were replanted with 4-wk-old tomato (*Lycopersicon esculentum*) seedlings. The tomatoes grew for an additional 6 wk before the experiment was terminated. The number of galls/g of root (GR) in squash was not affected by the *P. lilacinus* treatments; however, GR values increased for soils treated with 0.1-0.8% chitin. *P. lilacinus* and chitin amendments reduced GR values and the number of juveniles/g tomato roots. Results indicate that combinations of chitin and *P. lilacinus* amendments are effective for control of *M. arenaria*.
   Additional key words: biological control, mucopolysaccharides, organic amendments, soil enzymes, root-knot nematodes.

RESUMEN

Culbreath, A. K., R. Rodriguez-Kabana, y G. Morgan-Jones. 1986. La quitina y el *Paecilomyces lilacinus* para combatir *Meloidogyne arenaria*. Nematropica 16:153-166.

   Se estudio el efecto de la quitina sobre *Meloidogyne arenaria* anadiendose el polimero a niveles de 0, 0.1, 0.2, 0.4, 0.8, y 1.0% (w/w) a un suelo infestado con el nematodo. Tambien, se anadio al suelo arroz colonizado por *Paecilomyces lilacinus* a razon de 1, 2, y 5 g/kg de suelo de manera a tener representadas en el estudio todas las combinaciones posibles entre todos los niveles de los dos tipos de enmiendas. Los suelos con los tratamientos se mantuvieron humedecidos por 2 semanas seguido lo cual se sembraron con semillas de calabacin (*Cucurbita pepo*). Las plantas resultantes se las dejo desarrollar por 6 semanas cuando fueron removidas y los suelos fueron replantados con plantulas de tomate (*Lycopersicon esculentum*) de 4 semanas. Se dejaron crecer los tomates por 6 semanas cuando se examinaron y se dio por terminado el experimento. El numero de agallas/g de raiz (AR) de calabacin no fue afectado por los tratmientos con *P. lilacinus* aunque se observo un aumento en el numero AR en suelos tratados con quitina a niveles de 0.1-0.8%. Los tratamientos tanto con quitina como con *P. lilacinus* disminuyeron los valores AR y el numero de larvas/g de raices de tomate. Los resultados señalan que las enmiendas con quitina y *P. lilacinus* son efectivas para combatir *M. arenaria*.
   Palabras claves adicionales: combate biologico, enmiendas organicas, manejo de plagas, enzimas del suelo, nematodo nodulador.

INTRODUCTION

Additions of chitin to soil can result in control of plant-parasitic nematodes (2,5,10,11,12,19,23). The action of the polymer on nematodes is based on the release of ammonia (a compound toxic to nematodes) through the activities of deaminating enzymes and chitinases present in soil (2,11,18,19). Also, chitin treatments selectively promote the development of a specialized soil microflora capable of degrading the polymer (3,6,7,15). Certain species of this microflora are capable of destroying eggs of *Heterodera glycines* Ichinohe and of *Meloidogyne* spp. It is possible that the susceptibility of the eggs to attacks by these micro-organisms may be based, at least in part, on the presence of chitin in the egg shell (1) and in the gelatinous matrix of egg masses of *Meloidogyne* spp. (24). Indeed, there is a degree of correlation between ability to attack nematode eggs and chitinolytic activity in fungal species (5,7). The fungus *Paecilomyces lilacinus* is an egg parasite of species of *Meloidogyne, Globodera,* and *Heterodera* (8,13,14,16). There is also evidence that some isolates of this fungal species are chitinolytic and that its virulence against nematode eggs varies considerably between individual biotypes (20). Isolates of *P. lilacinus* when added to soil infested with *M. arenaria* (Neal) Chitwood can suppress populations of the nematode, but not to a practical level (20). Since *P. lilacinus* is chitinolytic, its activity in soil should be stimulated by chitin amendments. This study was conducted to determine the value of combination treatments of *P. lilacinus* and chitin amendments to soil for control of *M. arenaria*.

MATERIALS AND METHODS

Soil for the experiments was obtained from a peanut (*Arachis hypogaea* L.) field infested with the root-knot nematode, *M. arenaria*. The field had been in peanut culture for the preceeding 8 years and was planted to hairy vetch (*Vicia villosa* Roth) each winter. The soil was a sandy loam, pH = 5.8 and organic matter content < 1.0% (w/w). The soil was screened (1 mm) to remove large particles and debris, and mixed 1:1 (v:v) with fine builder's sand (avg. particle size ≤ 0.5 mm). The soil-sand mixture is referred to as soil in the remainder of this paper. The soil was moistened (60% field capacity) and apportioned in 1-kg amounts into 3-L capacity polyethylene bags until mixed with the appropriate amendment.

The culture of *Paecilomyces lilacinus* was a Peruvian isolate provided by Dr. Parvis Jatala (Centro Internacional de la Papa, Lima, Peru) and originally isolated in his laboratory from diseased *M. incognita* eggmasses. The fungus was maintained on potato dextrose agar (PDA). Long grain rice (*Oryza sativa* L.) was boiled in water for 2 min., strained, and

apportioned into 500-ml Erlenmeyer flasks to give 150 ml of the rice in each flask. The flasks were then closed with styrofoam stoppers and sterilized by autoclaving for 30 min. After cooling to room temperature each flask was inoculated with 10 ml of a spore suspension of *P. lilacinus.* The inoculated flasks were incubated in the dark at 28 C for one week, when all the rice grains were completely covered by heavily sporulating colonies of the fungus. The colonized rice was spread on aluminum foil on a table top and dried at room temperature (25 ± 2C). The dried inoculum was transferred to polyethylene bags and kept at 4 C in the dark until used.

Unbleached crustacean chitin (U.S. Biochemical Corp., Cleveland, Ohio 44122) were ground (≤ 0.5 mm) and the resulting powder was mixed into soil at rates of 0, 0.1, 0.2, 0.4, 0.8, and 1.0% (w/w). *P. lilacinus*-colonized rice grain was added (whole rice grain) at 0, 1.0, 2.0, and 5.0 g/kg soil so that all possible combinations of chitin and *P. lilacinus* rates were represented. Previous studies had demonstrated that the amount of organic matter added to the soil by these rates of the *P. lilacinus* inoculum had no effect on *M. arenaria* (unpublished data). The amended soils were transferred to one-liter-capacity cylindrical 10-cm-diam PVC pots. The pots were placed in a greenhouse where the soil was kept moist to allow for decomposition of the amendments. Each chitin-*P. lilacinus* combination treatment included 8 replications (pots) arranged in a completely randomized design. After 2 weeks each pot was planted with 5 'Summer Crookneck' squash (*Cucurbita pepo* L.) seeds. After 6 weeks, squash plants were carefully removed from the soil. The root system of each plant was examined to determine the number of galls caused by *M. arenaria* and root and shoot weight were recorded. The total number of plants and the height of shoots were also determined. The pots were replanted with 4-week-old 'Rutgers' tomato (*Lycopersicon esculentum* Mill.) seedlings. After six weeks each tomato plant was removed and examined as described for the squash plants. In addition, soil samples were collected from each pot to determine nematode and microbial population densities. Nematodes were collected from the soil with the "salad bowl" incubation technique (21). *M. arenaria* juveniles were recovered from entire root systems with the same technique (72-hr incubation). Numbers of chitinolytic fungi, bacteria and actinomycetes in the soils were assessed using chitin media as described by Godoy *et al.* (5). All data were analyzed following standard procedures for analysis of variance (25). Fisher's least significant differences were also calculated according to standard procedures (25), as were regression analyses and curve fitting (9). All differences referred to in the text were significant at the 5% or lower level of probability.

<div align="center">RESULTS</div>

First Crop: Squash. The number of galls caused by *M. arenaria* per g of fresh root (GR) increased in response to the addition of chitin to the soil in the range of 0-0.4% (Fig. 1A). GR values for plants from soil with the two highest chitin levels were not different from those for control plants. This general pattern of response to chitin amendments was true for all levels of *P. lilacinus.* Factorial analysis of the data revealed no significant interaction between chitin and *P. lilacinus* amendments on GR. Although the effect of *P. lilacinus* on GR was negligible, the pattern of response to chitin amendments was as described above (Fig. 1B). The relation between GR values and the percent of chitin added to the soil (X) was best described (R^2 = 0.965) by a typical normal function:

$$GR = (13.307[0.797]^X)/(X^{0.0277})$$

The interaction between the effects of chitin and *P. lilacinus* amendments on fresh shoot weights was significant, so that no statements could be made on the main effects of the two types of amendments on the variable. Shoot weights were lowest for plants grown in soil amended with chitin at rates of 0.4% or higher and highest in those from soils with no chitin or those treated with either 0.1 or 0.2% of the polymer (Table 1).

No significant interaction occurred between the effects of *P. lilacinus* and chitin treatments on fresh root weight (Table 1). *P. lilacinus* amendments had no significant effect on root weights when considered independently of the effects of chitin; however, chitin amendments resulted in reductions in root weights. The relation between fresh root weight in grams (Rw) and the percent chitin added to soil (X) was described (R^2 = 0.905) by the function:

$$Rw = (0.1546[0.7585]^X)/(X^{0.1662})$$

Second Crop: Tomato. Chitin and *P. lilacinus* treatments had significant effects on GR values in tomato roots (Fig. 2A). The interaction between the effects of chitin and *P. lilacinus* on GR was not significant. Chitin amendments at all levels above 0.1% resulted in significant reductions in GR values (Fig. 2B). Independent of the effects of chitin, a pattern of decline in GR in response to increasing rates of *P. lilacinus* was observed. The relation between the percent chitin added to the soil (X) and GR values was described (R^2 = 0.946) by
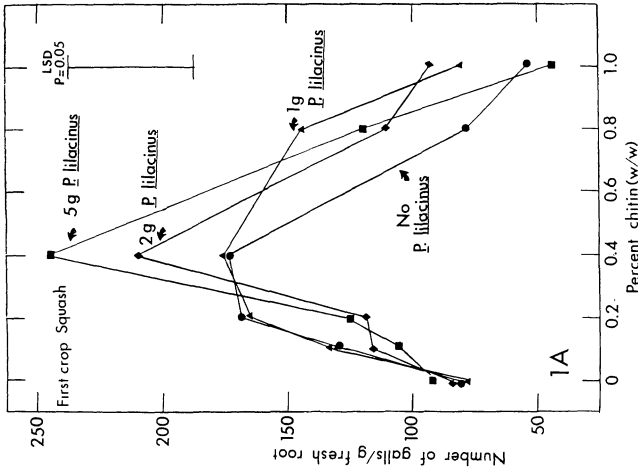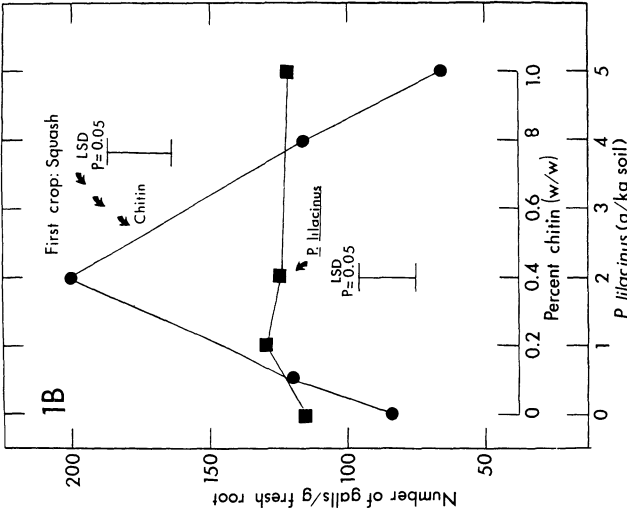
$$GR = 60.77[0.1275^X] X^{0.0298}$$

Fig. 1. Effects of chitin and *Paecilomyces lilacinus* soil amendments on the number of galls caused by *Meloidogyne arenaria* in 'Summer Crookneck' squash (*Cucurbita pepo*) growing in soil infested by the nematode. A. Results of combination treatments; B. Effects of each type of amendment considered independently of the others on numbers of galls.

Table 1. Effect of amending soil with chitin and rice colonized with *Paecilomyces lilacinus* on the growth of 'Summer Crookneck' squash in soil infested with *Meloidogyne arenaria*.

Percent (w/w) chitin added	P. lilacinus (g/kg soil)									
	0.0		1.0			2.0		5.0		
	Shoot weight (g)	Root weight (g)	Shoot height (cm)	Shoot weight (g)	Root weight (g)	Shoot weight (g)	Root weight (g)	Shoot height (cm)	Shoot weight (g)	Root weight (g)
0.0	2.111	0.354	14.28	2.345	0.335	1.881	0.317	14.67	1.821	0.272
0.1	1.652	0.255	14.75	1.567	0.255	1.731	0.241	13.38	1.349	0.215
0.2	1.085	0.192	13.33	0.871	0.167	1.415	0.238	14.22	1.212	0.196
0.4	0.764	0.166	12.51	0.601	0.120	0.729	0.160	11.81	0.804	0.160
0.8	0.690	0.104	9.90	0.627	0.119	0.657	0.106	9.98	0.560	0.095
1.0	0.762	0.209	11.67	0.850	0.105	0.716	0.094	12.37	0.981	0.138
LSD (P=0.05)	0.303	0.074	1.80	0.303	0.074	0.303	0.074	1.80	0.303	0.074

The equivalent relation between GR and the amount of *P. lilacinus* inoculum in g per kg soil (Xp) was linear ($R^2 = 0.815$) and expressed as:

$$GR = 23.56\text{-}4.211Xp$$

The addition of chitin to the soil had a pronounced effect on root population densities of juveniles of *M. arenaria* (Fig. 2C). In soil without *P. lilacinus,* chitin additions at all rates above 0.1% resulted in significant reductions in juvenile population densities. Also, when chitin was combined with 1.0 g of *P. lilacinus* inoculum, the 0.1% level of chitin was also effective in reducing juvenile populations. *P. lilacinus* amendments to soil without chitin also reduced juvenile populations when the inoculum was added at rates of 2.0 and 5.0 g/kg soil. Factorial analysis of the data on populations of juveniles in the roots revealed a significant interaction between the effects of chitin and those of the *P. lilacinus* inoculum.

Numbers of *M. arenaria* juveniles in soil were reduced (≤ 2 juveniles/ 100 cm^3 soil) in all soils that received chitin, *P. lilacinus*, or combinations of the two amendments (data not presented). Differences between these treatments were not significant; however, all soils treated with the amendments had lower numbers of juveniles than the untreated soil.

The interaction between the *P. lilacinus* amendments and chitin treatments on fresh tomato shoot weights was significant. *P. lilacinus* applied to soil without chitin had no effect on shoot weights at rates below 5.0 g/kg soil (Table 2); however, the 5.0-g rate resulted in increased shoot weights. Shoot weights in the experiment were highest for plants in soils amended with chitin at 0.2% + *P. lilacinus* at 5.0 g/kg soil. The use of chitin alone without *P. lilacinus* had no effect on shoot weights; however, several combination treatments of chitin at 0.2 or 0.4% and *P. lilacinus* at 2.0 or 5.0 g/kg soil resulted in increased shoot weights.

Factorial analysis of tomato fresh root weights revealed a significant interaction between the effects of chitin and of *P. lilacinus* amendments. Although additions of chitin with or without the 1.0-g rate of *P. lilacinus* had no effect on root weights (Table 2), this was not the case when chitin was combined with either the 2.0 g or the 5.0 g rates of *P. lilacinus.* Several combinations of chitin at 0.1 and 0.2% and *P. lilacinus* at 2.0 or 5.0 g/kg soil resulted in heavier roots than those of plants from untreated soil. Treatments with chitin at either 0.8% or 1.0% + *P. lilacinus* at the 2.0 or 5.0 g rates resulted in reduced root weights when compared with root weights from untreated soil. Applications of *P. lilacinus* without chitin did not affect root weights.

The interactions between chitin and *P. lilacinus* treatments on numbers of actinomycetes (Fig. 3A), bacteria (Fig. 3B), or fungal propagules
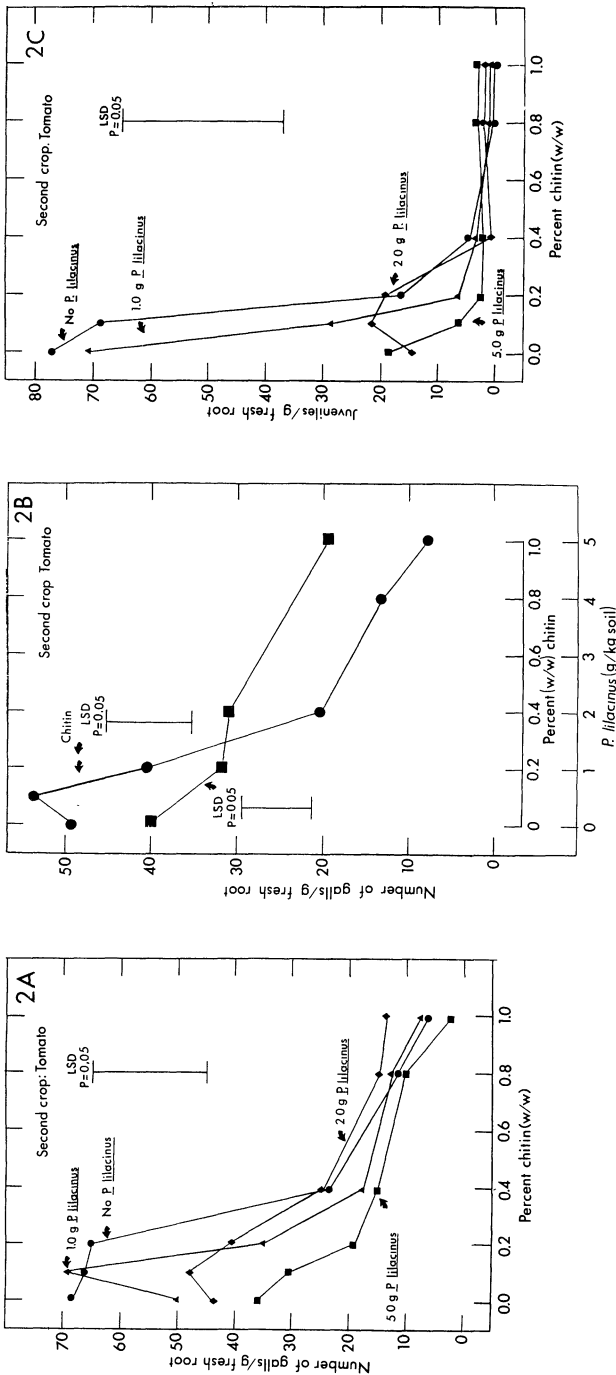
Fig. 2. Effects of chitin and *Paecilomyces lilacinus* soil amendments on development of *M. arenaria* in 'Rutgers' tomatoes (*Lycopersicon esculentum*) planted in the soils following a crop of 'Summer Crookneck' squash. A. Effects of combination treatments on numbers of root galls caused by the nematode; B. Effects of each type of amendment considered independently of the other on numbers of galls; C. Relation between treatment levels and numbers of *M. arenaria* juveniles in the roots.

Table 2. Effect of amending soil with chitin and rice colonized with *Paecilomyces lilacinus* on the growth of 'Rutgers' tomato in soil infested with *Meloidogyne arenaria*.

Percent (w/w) chitin added	P. lilacinus (g/kg soil)											
	0.0		1.0			2.0		5.0				
	Shoot weight (g)	Root weight (g)	Shoot height (cm)	Shoot weight (g)	Root weight (g)	Shoot weight (g)	Root weight (g)	Shoot height (cm)	Shoot weight (g)	Root weight (g)		
0.0	3.327	1.595	16.75	2.456	1.127	3.747	1.425	19.15	5.549	2.085		
0.1	2.639	1.652	17.68	3.482	1.716	5.004	2.330	19.04	6.811	2.551		
0.2	2.724	1.613	15.77	3.406	1.636	5.028	2.396	20.15	7.684	3.100		
0.4	3.779	1.875	17.03	3.445	1.507	5.091	2.142	17.47	5.071	2.097		
0.8	4.683	2.010	14.97	3.060	1.027	3.137	1.117	13.96	1.778	0.694		
1.0	2.591	1.170	16.61	2.712	0.937	2.833	1.050	15.21	2.870	1.171		
LSD (P=0.05)	1.698	0.713	2.71	1.698	0.713	1.698	0.713	2.71	1.698	0.713		

(Fig. 3C) were significant. Applications of *P. lilacinus* without chitin had no effect on populations of chitinolytic microorganisms. The addition of chitin at rates of 0.2% or higher to soil resulted in increased numbers of actinomycetes and bacteria. Actinomycete populations were highest for all treatments with *P. lilacinus* with chitin rates of 0.1 or 0.8%. Soils without *P. lilacinus* amendments had the highest numbers of actinomycetes when chitin was added at the 0.2 or 0.4% rates.

Numbers of fungi in soil with no *P. lilacinus* amendment increased in response to chitin rates up to 0.4%; however, number of fungal colonies in soil with the two highest chitin rates were not different from the number for untreated soil. No conspicuous pattern of response to the *P. lilacinus* or chitin amendments was observed with fungal populations in soils treated with combinations of the two amendments. All soils treated with the *P. lilacinus* amendment had higher numbers of colonies of this fungus than the other soils in the study.

DISCUSSION

Previous studies have shown (2,5,11,19) that chitin amendments are effective against *M. arenaria* in acidic soils at rates of 0.5% (w/w) or higher. It was the objective of this study to determine not only if the effectiveness of *P. lilacinus* amendments against *M. arenaria* could be enhanced by chitin, but also if the fungal amendment could increase that of ᴄhitin against the nematode. Results of this study indicate that time may be an important factor in determining the activity of amendments against nematodes. Data from the first crop (squash) differ significantly from those obtained from the second crop (tomato). *P. lilacinus* and chitin amendments were ineffective in reducing galling caused by *M. arenaria* in squash; indeed some of the chitin amendments actually resulted in increased numbers of galls in the roots. Populations of *M. arenaria* in the soil at initiation of the experiment were known to consist of juveniles. The activity of *P. lilacinus* against *M. arenaria* is based on its ability to destroy eggs of the nematode (7,20) but it is, as far as we know, ineffective against juveniles. Also, the effectiveness of chitin against *M. arenaria* juveniles depends on the quick release of NH_3 in sufficient quantity to reach nematicidal levels. NH_3 in acid soils is nematicidal at very high concentrations (4,22); these concentrations could not result from the lower amounts (< 0.8%) of chitin used in the study. Therefore, the NH_3 released after chitin amendment probably had no effect on juvenile populations. The juveniles survived, penetrated squash roots, and caused galling of the roots. It was only after the tomato crop that the chitin and, to a lesser degree, the *P. lilacinus* amendments, reduced galling by *M. arenaria* and populations of the nematode in roots and soil. Results thus suggest that consideration of
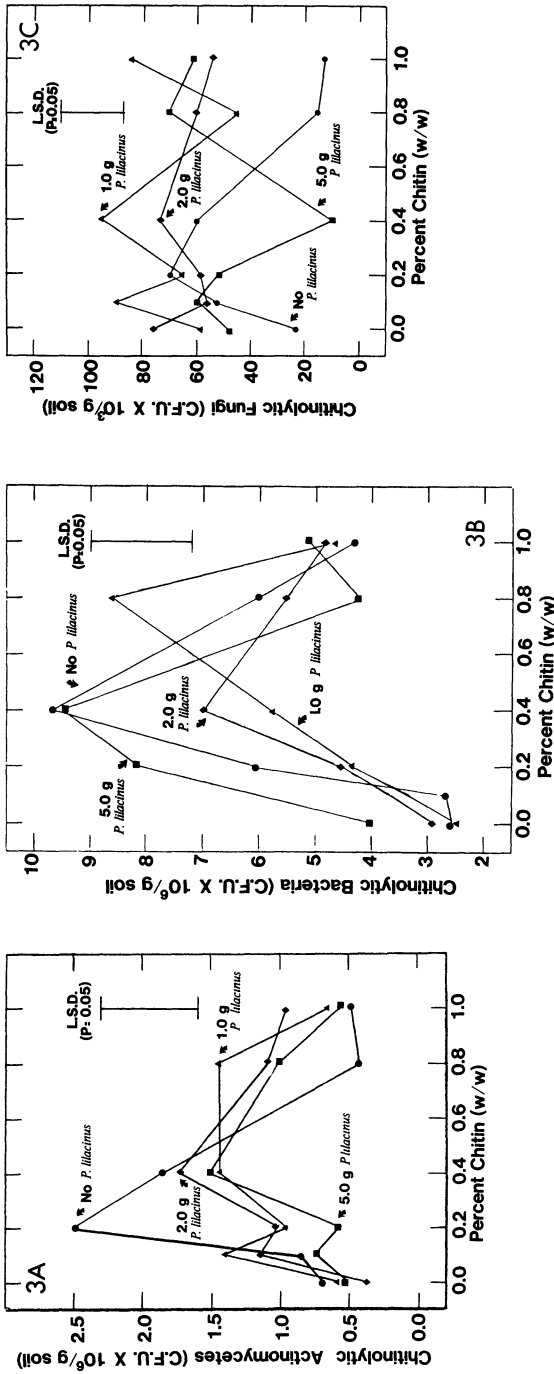
Fig. 3. Populations of chitinolytic microorganisms in soil amended with chitin and *Paecilomyces lilacinus* following cropping with squash and tomatoes. A. Actinomycete colony forming units (C.F.U.); B. Bacteria; C. Fungi.

the time factor may be important in the interpretation of the effects of these types of amendments. The lag-period observed for suppressive activity against nematodes indicates that, to be effective, these amendments must be present in soil for sufficient time to induce a buildup of a selective antagonistic chitinolytic microflora. Microbial population counts in the present, and in previous studies (5,11,18), have indicated that a chitinolytic microflora is enhanced by additions of chitin to soil. This stimulation was still evident in the present study even after the second crop (tomato). Fungal counts also indicate that *P. lilacinus* can establish itself in soil and become the predominant chitinolytic fungal species present. Nevertheless, we do not interpret the decline in *M. arenaria* populations after tomato as due solely to the activities of *P. lilacinus*. It is more likely that a microflora consisting of a number of microorganisms antagonistic to the nematode developed as a result of the applications of the amendments. This is supported by the general pattern of enhancement in numbers of actinomycetes, bacteria and, for some chitin levels, of fungal propagules in soil. Thus, it appears that no single microbial species was totally responsible for control of *M. arenaria*. Our interpretation is, however, tentative since the biological mechanisms involved in the observed results remain to be fully elucidated.

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