CHITIN AMENDMENTS FOR CONTROL OF *MELOIDOGYNE ARENARIA* IN INFESTED SOIL

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ABSTRACT

Mian, I. H., G. Godoy, R.A. Shelby, R. Rodríguez-Kábana, and G. Morgan-Jones. 1982. Chitin amendments for control of *Meloidogyne arenaria* in infested soil. Nematropica 12: 71-84.

The effect of amending soil with chitin on *Meloidogyne arenaria* (Neal) Chitwood was studied in a greenhouse experiment using silt loam [pH 6.5, org. matter < 1% (w/w)] from an infested peanut (*Arachis hypogaea* L.) field. Chitin was added to soil at rates of 0.5-4.0% (w/w). The chitin was allowed to decompose for 3 weeks, then the soil was planted with summer crookneck squash (*Cucurbita pepo* L.). After 6 weeks of growth, squash plants were examined to determine the level of root galling and the soil was analyzed for soil enzymic activity and microbial populations. No galls were found on plants from soil with 1% or more of chitin. Soils with 0.5 to 2.0% chitin produced plants which were significantly taller and with heavier shoots than those from untreated soil or from soil with 4.0% chitin. Chitin treatments of 2.0% or higher significantly reduced germination of seed, and chitin at rates above 1.0% resulted in a significant reduction in root weight. Chitin amendments resulted in increased aryl phosphatase, chitinase, and urease activities of soils. A specific microflora capable of growing on chitin mineral salts agar was isolated from chitin-treated soil. Fungal species isolated from the treated soils were: *Fusarium solani* (Mart.) Sacc., *F. udum* Butler, *Thielavia basicola* Zopf, *Humicola fuscoatra* Traaen, and *Pseudeurotium ovale* Stolk. *In vitro* tests demonstrated that all fungal species parasitized eggs of *M. arenaria*, and all but *T. basicola* also parasitized eggs of *Heterodera glycines* Ichinohe.

Additional key words: mucopolysaccharides, biological control, nematode egg pathology, soil enzymes, nematode control, soil fungi, root knot nematodes, soybean cyst nematode.

RESUMEN

Mian, I.H., G. Godoy, R.A. Shelby, R. Rodríguez-Kábana, y G. Morgan-Jones. 1982. Enmiendas con quitina para combatir *Meloidogyne arenaria* en un suelo infestado. Nematrópica 12: 71-84.

En un experimento de invernadero se estudió el efecto de enmiendas

con quitina para combatir *Meloidogyne arenaria* (Neal) Chitwood en un limo arenoso [pH = 6.0, mat. org. 1% (w/w)] proveniente de un campo de maní (*Arachis hypogaea* L.) infestado. La quitina se añadió al suelo en concentraciones de 0.5-4.0% (w/w) y se dejó descomponer por tres semanas seguido lo cual se sembró el suelo con semilas de calabacín "summer crookneck" (*Cucurbita pepo* L.). Después de seis semanas de crecimiento se examinaron las plantas para determinar el grado de nodulación causado por el nematodo. También, se analizó el suelo para determinar los niveles de actividades enzimáticas y de poblaciones de microorganismos. Las enmiendas de 1% o más eliminaron el agallamiento de las raíces. También, los tratamientos de 0.5% y 2.0% resultaron en plantas más altas y con tallos más pesados que las de suelos sin tratamientos o que las de los que recibieron el polímero en concentración de 4%. Las aplicaciones de 2% o más redujeron el grado de germinación de las semillas y las enmiendas en concentraciones superiores al 1% resultaron en disminuciones en el peso de las raíces. La adición de quitina al suelo causó aumentos en las actividades de aril-fosfatasa, quitinasa y ureasa del suelo. El suelo tratado con quitina desarrolló una microflora especifica con capacidad de crecimiento en un agar con quitina y sales minerales. Las especies fungosas aisladas de suelos con quitina fueron: *Fusarium solani* (Mart.) Sacc., *F. udum* Butler, *Thielavia basicola* Zopf, *Humicola fuscoatra* Traaen, y *Pseudeurotium ovale* Stolk. Pruebas *in vitro* demostraron que todas las especies fungosas eran parásitas de huevos de *M. arenaria* y que todas menos una (*T. basicola*) también eran parásitas de huevos de *Heterodera glycines* Ichinohe.

Palabras claves adicionales: mucopolisacáridos, combate biológico, patología de huevos de nematodos, enzimas del suelo, combate de nematodos, hongos del suelo, nematodos noduladores, nematodos enquistadores.

INTRODUCTION

Chitin, a polymer of N-acetyl-glucosamine, is common in nature. It is a constituent of cell walls or structural tissues of insects, crustaceans, and many species of fungi and other organisms (12). Chitin is found as a component of the middle layer of egg shells of nematodes (2,3). The addition of chitin or chitinous materials to soil for control of plant parasitic nematodes has been studied previously. Miller *et al.* (11) found that chitin and chitinous mycelial amendments suppressed populations of plant parasitic nematodes. Mankau and Das (10) used chitin amendments to control *Meloidogyne incognita* (Kofoid & White) Chitwood in soil; they proposed that chitin, when added to soil at rates of 1000 to 10,000 ppm, stimulated microbial activity creating conditions adverse to *M. incognita* including production of microbial toxins. The precise mode of action of chitin against nematodes, however, has never been ascertained. This study was conducted to determine the effectiveness of chitin amendments to soil against *M. arenaria* (Neal) Chitwood, and to determine the effects of the amendments on certain soil enzymes and the soil microflora.

MATERIAL AND METHODS

The effect of chitin on *M. arenaria* was studied in a greenhouse experiment using nematode infested soil from a peanut (*Arachis hypogaea* L.) field near Headland, AL. The soil was a silt loam with a pH of 6.5 and an organic matter content of $< 1\%$. The soil was screened (1 mm mesh) to remove large particles and crop debris, moistened to achieve 60% field capacity (F.C.), and apportioned in 1 kg amounts into 4-L capacity polyethylene bags. Crustacean chitin flakes (United States Biochemical Corporation, Cleveland, Ohio) were ground to pass a two-mm mesh sieve and mixed with the soil to give chitin concentrations of 0, 0.5, 1.0, 1.5, 2.0, and 4.0% (w/w). The amended soil was transferred to 1.5 L plastic pots and maintained at 60% F.C. in a greenhouse to permit decomposition of chitin. Each chitin concentration was represented by eight replications (pots) arranged in a randomized complete block design. After 3 weeks, the soils were planted with five, summer crookneck squash seeds (*Cucurbita pepo* L.) and the resulting plants were allowed to grow for six weeks after which time the number of *M. arenaria* galls on the roots was determined. Other variables recorded were fresh weights of roots and shoots and the shoot height. The general condition of the root systems was evaluated using a subjective scale ranging in value from one to five. A value of one was assigned to roots showing restricted development with widespread necrotic tissue and no root hairs or fine roots; a value of five corresponded to roots in excellent condition with abundant root hairs and rootlets and no necrotic tissue. The degree of root galling was also evaluated using a scale ranging from 0-10 as described by Zecht (18) in which a value of ten represented the most severe galling reaction, and 0 represented root systems free of galls.

Soil from each pot was collected after removal of squash plants. Twenty gms of the soil was stored moist at 5° C and the rest was spread on aluminum foil to dry at 25° C. The dried soil was kept at 4° C in the dark until analyzed and the moist soil was used to determine microbial populations.

Soil pH was determined electrometrically in a suspension of 10g of air-dried soil in 10 ml of demineralized water after shaking for 30 min. Following determination of pH the suspension received an additional 10 ml of water and, after thorough mixing, 10 ml of the suspension was centrifuged for 20 min at 4000 g. The conductivity of the supernatant was determined using an Industrial Instruments conductivity bridge with a conductivity cell (K = 1.0). The nitrate-nitrogen content of the supernatant was determined by the phenyldisulphonic acid method (9).

The general biological activity of the soil was assessed by determining the levels of three enzymatic activities: aryl phospatase, chitinase and urease. Aryl phosphatase activity was assessed using a modification of Hofman's method (8). A suspension of 10 g of air-dried soil in 10 ml of an aqueous solution of phenyl disodium phosphate (6.75 gm/1) in a 60 ml glass bottle was incubated for 3 h at 37° C. After incubation, 40 ml of water was added to the suspension and mixed. Ten milliliters of the diluted suspension was centrifuged for 20 min at 4000 g. The amount of phenol in 1 ml of the clear

supernatant was then determined (8). Controls included samples in which water was used instead of the substrate solution, and others in which twice-autoclaved soil (no enzymatic activity) was used. Enzymatic activity was expressed as the number of micrograms of phenol released per hour per gm of soil.

Chitinase activity was measured in 10 gm of air-dried soil in a 60 ml glass bottle. The soil was treated with 1.5 ml of toluene for 15 min, followed by the addition of 10 ml of 1% (w/v) colloidal chitin prepared by solubilizing in concentrated HCl as described elsewhere (7). The mixture was shaken and incubated at 37° C for 18 h. After incubation 10 ml of water was added to each bottle and 10 ml of the resulting suspension was centrifuged (20 min, 4000 g). One milliliter of the clear supernatant was used for determination of the amount of N-acetyl-glucosamine released following the method of Aminoff (1). Chitinase activity was expressed as the number of μg of N-acetyl-glucosamine released per hour per gm of soil.

Urease activity was determined according to the method of Rodriguez-Kábana and King (14). Ten milliliters of 10% (w/v) aqueous solution of urea was added to 5 gm of air-dried soil in a 60-ml glass bottle and incubated for 5 h at 37° C. After incubation, 20 ml of a 10% (w/v) aqueous solution of NaCl acidified with 0.1 N HCl (pH 2.5) and 20 ml of water were added. The suspension was thoroughly mixed and allowed to stand for 5 min for particle sedimentation. One milliliter of the suspension was placed in the middle well of an Obrink microdiffusion dish containing 4 ml of a 40% (w/v) solution of K_2CO_3 in the outer well, 1 ml of the same solution in the middle well and 2 ml of 0.1 N HCl in the center well. The dish was sealed immediately and ammonia was allowed to distill into the center well overnight. The amount of ammonia in 1 ml of solution from the center well was determined by Nesslerization (9). Urease activity was expressed as micrograms of ammoniacal nitrogen released per hour per gram of soil.

The number of fungi in the soil samples was determined following a modification of the method of Rodriguez-Kábana (13). Ten grams of moist soil was placed in a 500-ml Erlermeyer flask containing 400 ml of sterile water and magnetically stirred for 5 min. A sample from the moving suspension was withdrawn with a pasteur pipette. One drop of the sample was delivered into a petri dish and enough chitin-Rose Bengal agar (pH = 5.5) was added to give a 2-mm-thick layer. The chitin-rose bengal medium contained 0.2% colloidal chitin suspension and mineral salts (7), together with 2 ml of a 1% (w/v) Rose Bengal aqueous solution and 22 μg/ml of streptomycin sulfate to inhibit fungal growth and avoid bacterial growth, respectively. The dish contents were thoroughly mixed, allowed to solidify, and placed in an incubator at 29° C. The number of bacteria and actinomycetes was determined by transferring 10 ml of the soil suspension to a 500 ml flask with 200 ml of water and following the procedure as used for the fungi. The culture medium for bacteria was the same as that used for fungi except that the medium was at pH = 7.0 and it did not contain Rose Bengal or streptomycin sulfate. There were

five plates per soil sample for fungal and bacterial determinations. Microbial colonies on the plates were counted 48 hr (bacteria) and 120 hr (actinomycetes) after innoculation. The number of propagules per gram of soil was calculated on the basis of the previously determined amount of dry (105 C, 24 hr) soil per drop of soil suspension.

Fungal colonies isolated from the chitin-Rose Bengal plates were transferred to fresh, 0.2% colloidal chitin-mineral salts agar, and potato-dextrose agar (PDA) for identification (PDA medium) and to determine their pathogenicity (chitin medium) against eggs of *M. arenaria* and *Heterodera glycines* Ichinohe (race three). Mycelial growth, chitinolytic activity of the fungal species, and tests for pathogenicity were determined on chitin medium as described elsewhere (7).

Data were analyzed following standard procedures for analysis of variance. Differences between means were evaluated for significance according to a modified Duncan's multiple range test (17). Unless otherwise stated, reported differences were significant at the 5% or lower level of probability.

RESULTS

Chitin at rates of $< 2.0\%$ did not affect germination of squash seeds or seeding development but the addition of chitin at rates of two and four percent caused a reduction in the number of seeds that germinated and developed into seedlings (Table 1). Chitin amendments at all rates but the highest resulted in taller plants than those from untreated soil. Plants from soil treated with the highest chitin rate were shorter than all other plants in the experiment. Plants with the heaviest shoots were those from soil treated with the 1.0 and 1.5% rates and the lightest plants were those from untreated soil and soil that received the highest rate of chitin. The addition of chitin at rates higher than 1.0% caused reductions in the weight of roots. All chitin amendments at rates of 1.0% or higher essentially prevented gall formation giving a root-knot index value of 0; however, the two highest rates caused deterioration in the general appearance of the roots.

Data for pH, conductivity and nitrate nitrogen are presented in Table 2. Highest pH values were in soils that received the two highest chitin rates and lowest in those with 1% or less chitin. All soils receiving the amendments contained more nitrate nitrogen than control soils; greatest amounts of nitrate were observed in soils with 2.0 and 4.0% chitin. Conductivity of the water extracts of soils amended with 1.5% or higher rates of chitin was greater than that of extract of soils with no chitin or with those that received the lowest amount of the polymer. The extract with the highest conductivity value was that from soil with 4.0% chitin.

Aryl phosphatase activity was greatest in soil amended with 1.5% chitin and lowest in soil with no chitin (Fig. 1). Activity in amended soils was greater

Table 1. Effect of chitin amendments to soil infested with *Meloidogyne arenaria* on growth of squash plants growing in the soil.[V]

Percent (w/w) Chitin	Germination[X] Plants/Pot	Shoot Height (cms)	Fresh Shoot Weight (gms)	Fresh Root Weight (gms)	Root[Y] Condition Index)	No. Galls per Gm of Root	Galling[Z] Index
0.0	4.75 A[V]	15.43 B	1.81 C	0.52 A	2.90 A	61.12 A	3.98 A
0.5	5.00 A	21.04 A	3.91 B	0.46 AB	2.90 A	52.08 A	3.40 A
1.0	5.00 A	21.47 A	4.42 A	0.44 AB	2.58 AB	1.49 B	0.38 B
1.5	4.62 A	22.24 A	4.99 A	0.38 BC	2.65 AB	0.00 B	0.00 B
2.0	3.62 B	20.35 A	3.71 B	0.32 CD	2.44 B	0.00 B	0.00 B
4.0	2.25 C	12.10 C	1.75 C	0.27 D	1.49 C	0.00 B	0.00 B

[V]Figures within the same column followed by a common letter were not significantly different (P = 0.05); all figures represent averages of eight replications.

[X]Total number of seeds per pot = 5.00.

[Y]Index based on a scale ranging from one to five where one represented roots with restricted development and with necrotic tissue and no root hairs or rootlets; a value of five was assigned to roots with excellent growth.

[Z]Galling index based on a scale ranging from 0-10 where 10 represented the most severe galling reaction and zero root systems with no galls (18).

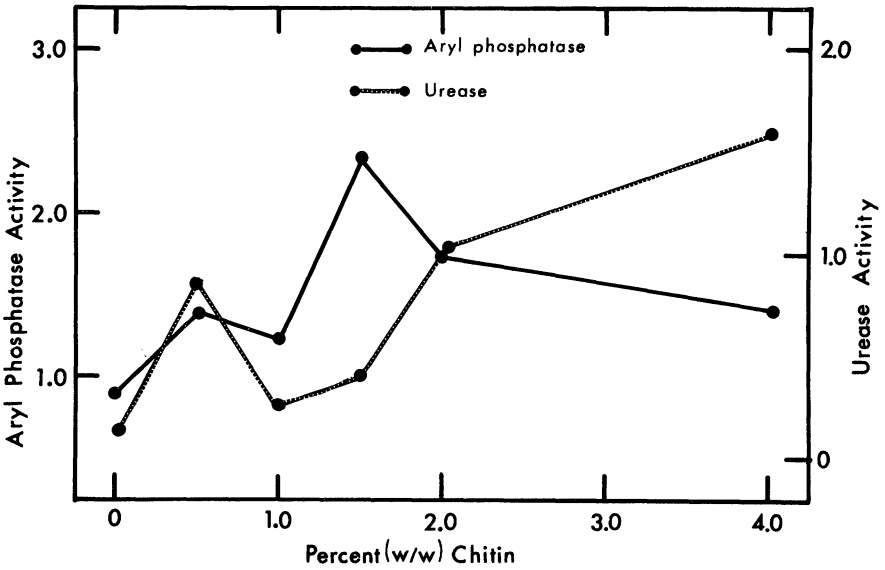

Fig. 1. Relation between aryl phosphatase and urease activities of soils and the amount of chitin added to soil.

Table 2. Effect of chitin amendments on pH, conductivity of water extract and nitrate nitrogen content of chitin amended soils infested with *Meloidogyne arenaria* following growth of squash plants.[x]

Percent (w/w) Chitin	Soil pH	Conductivity (μmohs)	Nitrate-N μg/gm soil)
0.0	6.52 BC	17.13 D	0.00 C
0.5	6.43 C	25.53 D	1.20 B
1.0	6.43 C	34.40 CD	1.19 B
1.5	6.61 B	47.87 BC	1.71 B
2.0	7.11 A	65.35 B	4.15 A
4.0	7.15 A	104.50 A	3.57 A

[x]Figures within the same column followed by a common letter were not statistically different (P = 0.05).

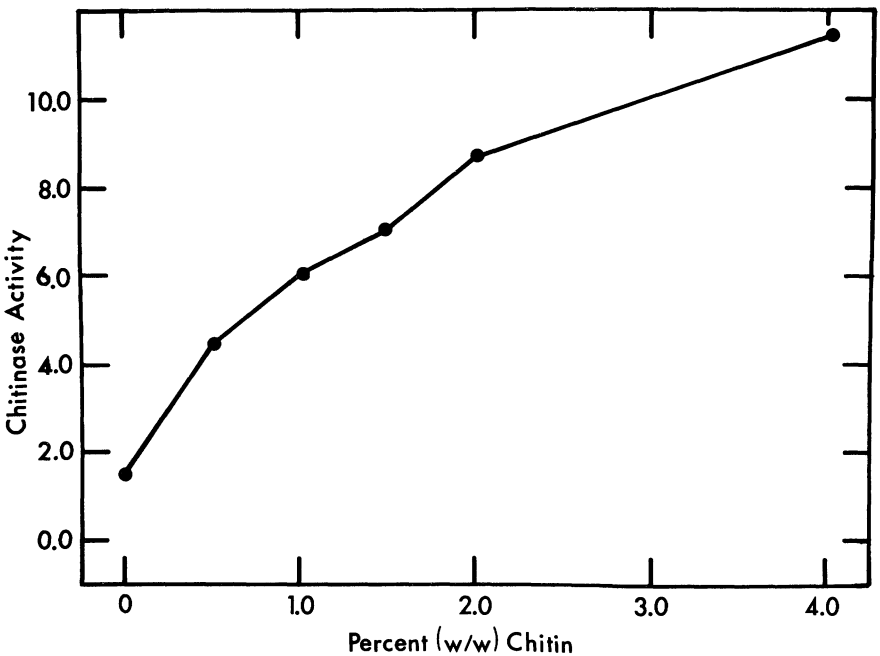

Fig. 2. Effect of chitin amendments on soil chitinase activity.

than in untreated soil. Differences in activity between soils with 0.5 and 1.0% chitin or between those that received the two highest rates of the polymer were not significant.

Chitinase activity increased directly in response to the amount of chitin added to soil (Fig. 2). The relation between this activity and the amount of chitin added to soil could be described by the equation $Y_c = 2.31X + 3.06$, where Y_c represented chitinase activity and X the percent chitin in the soil.

No differences in urease activity were detected between soils that received chitin at rates of 2.0% or less (Fig. 1); however, soil with 4.0% chitin had a higher urease activity than any other soil except the one with 2.0% chitin. The relation between urease activity and amount of chitin added to soil was linear and could be described by $Yu = 0.4422X - 0.0608$, where Yu = urease activity and X represent amount of chitin added. Changes in chitinase activity of soils were correlated ($r = 0.85$) with changes in urease activity; however, changes in urease activity were not correlated with those for aryl phosphatase.

Greatest numbers of bacteria and fungi were recorded from soils with 4.0% chitin (Table 3); numbers of these organisms in the other soils were not significantly different. Soils with the lowest numbers of actinomycetes were those with the 4.0% amendment and the highest numbers were in unamended

Table 3. Microbial population in chitin-amended soil infested with *Meloidogyne arenaria* determined nine weeks after addition of the polymer and following growth of squash plants.

	Thousands of propagules per gram of soil[y]		
Percent (w/w)	Actinomycetes	Bacteria	Fungi
0.0	59.2 A[x]	28.9 B	0.9 B
0.5	43.2 B	123.3 B	1.3 B
1.0	34.3 B	55.4 B	0.8 B
1.5	30.1 B	13.8 B	1.6 B
2.0	72.1 A	111.3 B	1.4 B
4.0	0.9 C	1105.6 A	3.5 A

[x]Figures within the same column followed by a common letter were not significantly different.
[y]Numbers of bacteria and actinomycetes were determined using a chitin-mineral salts medium; the medium used for fungi was identical except in that it was supplemented with Rose Bengal and streptomycin sulfate. Bacterial and fungal colonies were counted 48 hrs after inoculation and actinomycete colonies after 120 hrs.

soils and those with the 2.0% treatment.

No attempts were made to identify species of actinomycetes or bacteria in the plates. The fungi on the chitin-Rose Bengal plates were almost exclusively of the following species: an atypical form of *Fusarium solani* (Mart.) Sacc., *Fusarium udum* Butler, *Thielavia basicola* Zopf, *Humicola fuscoatra* Traaen, and *Pseudeurotium ovale* Stalk.

Fusarium solani, H. fuscoatra, P. ovale and *T. basicola* growing on chitin medium showed clearing of the medium (chitinolytic activity) within 48 hrs after inoculation; production of aleuroconidia, cleistothecia, and sporodochial sporulation was abundant together with mycelial development (Table 4); cultures of *F. udum* did not show clearing of the medium and sporulation was not abundant.

Cultures of *H. fuscoatra, F. solani* and *T. basicola* were more pathogenic to *M. arenaria* than to *H. glycines* eggs (Table 4); *P. ovale* and *F. udum* parasitized few eggs of either of the two nematode species.

DISCUSSION

Our results corroborate earlier findings on the effect of chitin on nematodes. Miller *et al.* found that chitin soil amendments to soil reduced populations of *Tylenchorhynchus dubius* (Butschli) Filipjev and *Pratylenchus penetrans* (de Man) Filipjev (11). Mankau and Das (10) had previously

Table 4. Chitinolytic activity, and degree of parasitism of eggs of *Heterodera glycines* and *Meloidogyne arenaria* by fungi isolated from soils amended with chitin.

Fungal Species	Chitinolytic[x] Activity	Radial[y] Mycelial Growth (cm)	Percent Eggs Parasitized *H. glycines*	*M. arenaria*
Fusarium solani (atypical)	+	8.0	10	28
Humicola fuscoatra	+	5.5	15	35
Pseudeurotium ovale	+	4.5	8	11
Thielavia basicola	+	4.5	0	20
Fusarium udum	-	6.0	4	6

[x]Denote presence (+) or absence (-) of a clearing zone around or beneath colonies growing on chitin-mineral salts agar.

[y]Determined on chitin-mineral salts agar seven days after inoculation.

demonstrated the effectiveness of the amendments for control of *M. incognita*. The minimally effective levels against the two nematodes in the study by Miller *et al.*, or for *M. incognita* in the experiments of Mankau and Das, were considerably lower than the levels required for suppression of galling by *M. arenaria* in our study.

The action of chitin against nematodes may be interpreted as resulting from the release of ammoniacal nitrogen into the soil through decomposition of the polymer by soil microorganisms. Ammoniacal nitrogen is toxic to some nematode species at rates of 500 kg/ha or higher (6,15). In this study, only chitin levels of 1% or more contained ammoniacal nitrogen in that concentration range. The release of ammoniacal nitrogen from chitin in the soil may be mediated through the action of deaminating enzymes. In this respect, the observed soil urease activity increase in response to chitin additions would be expected. However, we cannot but speculate on the exact steps involved in the deamination of chitin or its monomer, N-acetyl glucose amine, since chitin or the monomer have not been reported as substrates for soil urease (4).

Release of ammoniacal nitrogen into the soil results in increased soil pH and the accumulation of nitrate nitrogen through nitrification which in turn results in increased conductivity values of the soil extract. Our results would agree with this sequence of biochemical changes. The release of ammoniacal nitrogen into the soil may reach levels damaging to plants as was observed for the two highest chitin rates in the experiment; this was possibly due to accumulation of nitrites through the process of nitrification.

The addition of chitin to the soil may be expected to stimulate activity of soil microflora through promotion of species capable of degrading the polymer. This explains the observed increases in chitinase activity of chitin-treated soils through production of chitinase (s) by microorganisms. Microbial counts, however, were not correlated with chitinase activity. This lack of correlation might be anticipated because microbial counts were made 9 weeks after addition of chitin to the soil. Hence, the counts represent a single point in the succession of population changes following chitin addition. Therefore, the counts do not provide a good quantitative description of the processes involved in the decomposition of chitin. In contrast, chitinase activity represents the result of activities of enzymes produced by microorganisms active at the time of sampling together with others which have accumulated in the soil from earlier microbial activity.

Our results indicate that a particular mycoflora develops in response to addition of chitin to soil. All but one of the fungal species isolated from chitin-treated soil exhibited chitinolytic activity and all the species parasitized eggs of *M. arenaria* to some degree. The chitinolytic species could also parasitize eggs of *H. glycines*. These results suggest a relation between chitinolytic activity and the ability to parasitize the eggs. Nematode egg shells contain a layer composed of a chitin-protein complex (2,3), which may be a suitable substrate for chitinase(s). We believe, however, on the basis of other studies (7), that the mechanism of parasitism of eggs can involve enzymes

other than chitinase(s). Indeed, other studies (7) have shown that fungi isolated from cysts of *H. glycines* and having no apparent chitinolytic properties can be pathogenic to eggs of *M. arenaria* and *H. glycines.*

Some fungal species isolated from chitin-treated soil clearly represent elements of a specialized mycoflora not normally encountered in an active state in agricultural soils. Thus, *H. fuscoatra*, although reportedly isolated from various soils cannot be considered a common soil species (5); it has been isolated from soils in association with *Tuber magnatum* Pico, a truffle, and feathers of free living birds (5). *P. ovale* was originally isolated from cysts of *Globodera rostochiensis* and later, infrequently, from wheat field soils (5). In other studies in our laboratory, we have regularly isolated species of *Thielavia* from cysts of *H. glycines* from soybean field soils in a number of southeastern States of the U.S.A. Species of the genus *Fusarium* are, however, ubiquitous in soil.

Aryl phosphatase activity in soils results from production of phosphatases by a variety of microorganisms and plant roots (16). Although some chitinolytic organisms, and those producing urease(s) may be expected to produce phosphatases others may not. Similarly, microorganisms that do not produce chitinase(s) or urease(s) may produce phosphatases. Consequently, the data on aryl phosphatase represent a more general measure of biological activity than results obtained for chitinase and urease activities. Changes in aryl phosphatase activity in our study showed a pattern of increase in response to chitin rates over the range of 0-1.5% with sharp declines at rates higher than 1.5%. This pattern of response is similar to that observed for changes in fresh root weight of squash plants. This observation together with the lack of correlation found between aryl phosphatase activity and chitinase or urease activity suggests that chitin at high rates in soil may not only be phytotoxic but also detrimental to the general soil microflora.

We made no attempt to test the ability of actinomycetes or bacteria isolated from treated soils to parasitize nematode eggs. It is possible that many of these species are antagonistic to *M. arenaria* as suggested by Mankau and Das (10) for *M. incognita.*

Our study revealed that chitin amendments of 1% are required to suppress *M. arenaria* in soil. This rate is equivalent to approximately ten MT/ha on a broadcast basis. While this amount of chitin may be excessive for routine applications to row crops, it may be suitable for use in high value vegetable or ornamental crops. It is possible that the rate required for control of other nematode species may be lower than that needed to control *M. arenaria* (10, 11). The availability of waste chitinous materials in coastal areas of the United States and other countries suggests that use of these materials as soil amendments may provide an economical means to dispose of such waste, supply nitrogen to the soil and control nematodes.

CONCLUSIONS

Our results indicate that chitin amendments can be used to control *M.*

arenaria in infested soil but that the polymer can be phytotoxic at concentrations above 1%. Results also showed that elements of specific mycoflora capable of parasitizing eggs of *M. arenaria* and *H. glycines* develop in soil in response to chitin amendments.

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