CHITIN AMENDMENTS FOR CONTROL OF *MELOIDOGYNE ARENARIA* IN INFESTED SOIL. II. EFFECTS ON MICROBIAL POPULATION.

G. Godoy, R. Rodríguez-Kábana, R.A. Shelby and G. Morgan-Jones
Department of Botany, Plant Pathology and Microbiology, Auburn University, Agricultural Experiment Station, Auburn, Alabama 36849, U.S.A.
Accepted:
30.III.1983
Aceptado:

ABSTRACT

Godoy, G., R. Rodríguez-Kábana, R.A. Shelby, and G. Morgan-Jones. 1983. Chitin amendments for control of *Meloidogyne arenaria* in infested soil. II. Effects on microbial population. Nematropica 13:63-74.

The effect of chitin on soil microflora and on nematodes was studied in microplots containing a sandy loan [pH = 6.0 and organic matter content $<$ 1% (w/w)] infested with *Meloidogyne arenaria* (Neal) Chitwood. Ground chitin was added to soil at rates of 0-4.0% (w/w). The chitin was allowed to decompose for 10 weeks, during which time soil samples were collected every 15 days. After 10 weeks, soil from each plot was transferred to the greenhouse and planted with 'Summer Crookneck' squash (*Cucurbita pepo* L.) to assess degree of root galling caused by the nematode. Chitin treatments at rates of 0.4% and above reduced root galling; however, at rates $\geq$ 0.8%, chitin amendments were phytotoxic to the plants. Chitin amendments at rates of 1% and above resulted in an increase in pH, conductivity, nitrate-nitrogen, ammoniacal-nitrogen and chitinase activity.

Fungal populations were stimulated by chitin amendments at rates of 1% and above. Elements of a mycoflora previously associated with parasitism of eggs of *Heterodera glycines* Ichinohe and *M. arenaria* were isolated from chitin-amended soils. The predominant fungus was *Malbranchea aurantiaca* Sigler & Carmichael which in tests *in vitro* has been shown to parasitise eggs of *M. arenaria*.
Additional key words: biological control, chitinase, nematode control, root-knot nematode

RESUMEN

Godoy, G., R. Rodríguez-Kábana, R.A. Shelby, y G. Morgan-Jones. 1983. Enmiendas con quitina para combatir *Meloidogyne arenaria* en un suelo infestado. II. El efecto en la población microbiológica. Nematrópica 13:63-74.

El efecto de enmiendas de quitina en la microflora y nematodos del suelo fué estudiado en un experimento con microparcelas. En el estudio se usó un suelo arenoso [pH = 6.0 y contenido de materia orgánica $<$ 1% (p/p)] naturalments infestado con *Meloidogyne arenaria* (Neal) Chitwood. La quitina, previamente triturada, fue añadida al suelo a concentraciones de 0-4% (p/p) y dejada descomponer por 10 semanas. Muestras de suelo fueron tomadas cada 15 dias durante este período, después del cual porciones de suelo de cada parcela fueron transferidas a pequeñas macetas

en el invernadero. En cada maceta se sembraron semillas de calabacín 'Summer Crookneck' (*Cucurbita pepo* L.) para determinar el grado de nodulación causado por el nematodo. Los tratamientos de quitina a concentraciones sobre 0.2% redujeron el número de agallas; sin embargo las concentraciones sobre 0.8% resultaron fitotóxicas para el calabacín.

Las enmiendas de quitina a concentraciones del 1% o más dieron lugar a incrementos en los valores de pH, conductividad, nitratos, ammoniaco y actividad quitinolítica. También, se observó una estimulación de la microflora a estas concentraciones de quitina. Especies de hongos, las cuales han sido previamente asociadas con parasitismo de huevecillos de *Heterodera glycines* Ichinohe y *M. arenaria* y degradación de quistes de *Heterodera* spp. fueron aisladas de suelos con quitina.

La especie fungosa más predominante en el estudio fué *Malbranchea aurantiaca* Sigler & Carmichael. En estudios *in vitro* este hongo demostró ser parásito de huevecillos de *M. arenaria*.

Palabras claves adicionales: control biológico, quitinasas, control de nematodos, nematodo de los nódulos.

INTRODUCTION

The effectiveness of soil organic amendments for the control of plant parasitic nematodes has been studied extensively (8,9,11,12). A review of the literature reveals that the most effective amendments are those with low carbon:nitrogen ratios. Among those materials chitin has been found to reduce populations of *Meloidogyne incognita* (Kofoid & White) Chitwood, *M. arenaria* (Neal) Chitwood, and *Pratylenchus penetrans* (Cobb) Chitwood & Oteifa (9,11,12).

Chitin, poly-β-(1$\rightarrow$4)-N-acetyl-D-glucosamine, is a polysaccharide widely distributed in nature and is a by-product of seafood processing. It is an important constituent of fungal cell walls, marine invertebrates, insects (17), and is a component of the middle layer of egg shells of tylenchoid nematodes (2).

The precise mode of action of chitin against plant parasitic nematodes has not yet been fully elucidated. During its degradation in soil chitin generates ammonia, which is nematicidal (21,25).

Application of chitin to soil stimulates microbial activity (1,13). Several investigators have suggested that the nematicidal activity of chitin is partially through stimulation of antagonistic bacteria and actinomycetes (3,10). The role of fungi in the process was not considered until recently when reports indicated that a specific mycoflora capable of parasitizing nematode eggs developed in soil in response to chitin amendments (11).

This paper reports the quantitative and qualitative effects of chitin amendments on the soil microflora, and the isolation and identification of those fungal components with potential for biological control of nematodes.

MATERIALS AND METHODS

Soil for the study was a sandy loam with a pH of 6.0 and organic matter content of less than 1% and was collected from a peanut field infested with *Meloidogyne arenaria* (Neal) Chitwood in Headland, Alabama. The soil was screened (1mm) and mixed 1:1 (v/v) with sand (this mixture will henceforth be referred to as soil). Microplots were prepared by burying 30-cm-long sections of 15 cm-diam. polyvinyl chloride (PVC) pipes 22 cm into the ground.

Unbleached crustacean chitin flakes (United States Biochemical, Cleveland, Ohio) were ground, sieved (1mm), and mixed with infested soil to provide chitin concentrations of 0, 0.2, 0.4, 0.8, 1.0, 2.0, and 4.0% (w/w). The soils were thoroughly mixed in a cement mixer to assure even chitin distribution. Amended soils were apportioned in 5-kg quantities into each microplot. Each chitin treatment was represented by eight microplots arranged in a randomized-complete-block design. The plots were kept moist (*ca.* 60% field capacity) and free from weeds during the experiment. Soil samples (200 g) were collected every 15 days over a period of 10 weeks for laboratory analysis. The samples consisted of two 2.5-cm-diam. soil cores obtained from each microplot to a depth of 20 cm. One hundred and fifty grams of each sample were air-dried on aluminum foil for 48 h at 27 C. The dried soil was stored in polyethylene bags in the dark at 4 C prior to determining pH, conductivity, nitrate-nitrogen, ammoniacal-nitrogen, and chitinase activity. The remaining soil was stored moist at 5 C and used within 24 h for determination of microbial populations.

The levels of infestation of *M. arenaria* were determined 10 weeks after initiation of the experiment using summer crookneck squash as a bioindicator. One kg of soil from each plot was transferred to 1.5-L PVC pots and each pot was planted with 5 squash seeds; the pots were arranged in a completely randomized design in a greenhouse.

Plants were allowed to grow for 5 weeks, when they were examined to determine: 1) the number of root galls, 2) galling index, 3) shoot height, and 4) fresh weight of shoots and roots. The galling index was based on a scale from 0-10 as described by Zecht (27), where 0 represented no galls and 10 severe galling.

The pH of a suspension of 10 g of air-dried soil in 10 ml of demineralized water shaken for 30 min was determined with a Corning® model 12 pH meter. For determinations of conductivity, this soil suspension was diluted with an additional 10 ml of demineralized water, and 10 ml of the diluted suspension was centrifuged (20 min at 4000 *g*). Conductivity of the supernatant was determined using an Industrial

Instruments® Conductivity Bridge, Model RC 16 B2, with a conductivity cell (k = 1.0).

Nitrate-nitrogen was determined from 10 g of soil and analyzed by the phenyldisulphonic acid method (6). Ammoniacal-nitrogen was determined using a Nessler procedure after extraction from soil with acidified NaCl solutions as described by Jackson (6). Chitinase activity was measured as described elsewhere (11).

Microbial populations of soil samples were determined following a modification of the dilution plate method of Rodríguez-Kábana (20). Ten grams of moist soil were placed in a 500-ml Erlenmeyer flask containing 400 ml of sterile water and stirred for 5 min. To determine the number of fungi, a sample from the moving suspension was withdrawn with a Pasteur pipette. One drop of the sample was delivered into a petri dish and covered with chitin-Rose-Bengal agar (pH = 5.5). The agar contained 0.2% (w/w) colloidal chitin and mineral salts (5), together with 2 ml/L of a 1% (w/v) aqueous solution of Rose Bengal and 50 ml/L of streptomycin sulfate to inhibit fungal and bacterial growth, respectively. The dish contents were thoroughly mixed, allowed to solidify, and placed in an incubator at 29 C. Numbers of bacteria and actinomycetes were determined by transferring 10 ml of the soil suspension to a 500 ml flask with 200 ml of water and following the procedure used for fungi, except that the culture medium was adjusted to pH 7.0 and did not contain Rose Bengal or streptomycin sulfate. There were 5 plates per sample for each of the microbial determinations. Microbial colonies on the plates were counted 48 h (bacteria and fungi) and 120 h (actinomycetes) after inoculation. The number of propagules per gram of soil was calculated on the basis of the amount of dry (105 C, 24 h) soil per drop of soil suspension. Fungal colonies on the plates were transferred to fresh 0.2% chitin-mineral salt agar and potato dextrose agar (PDA) plates for further identification and determination of their parasitism of *M. arenaria* eggs (5).

Data were analyzed following standard procedures for analysis of variance. Differences between means were evaluated for significance with a modified Duncan's Multiple Range Test (23). Differences referred to in this paper were significant at the 5% or lower level of probability.

RESULTS

Chitin amendments at rates greater than 1.0% prevented gall formation by *M. arenaria* on squash roots (Table 1). Generally, plants from soils containing 0.2 and 0.4% chitin had taller and heavier shoots than those from soils containing a higher percentage chitin or from un-

Table 1. Effect of chitin amendment of soil infested with *M. arenaria* on growth of squash plants.

Chitin %	Shoot height (cm)	Shoot fresh weight (g)	galls/g root	Galling[y] index
0	18.90 b[x]	3.21 b	9.95 b	2.97 a
0.2	21.50 a	3.87 a	17.03 a	3.37 a
0.4	20.03 ab	3.56 ab	4.28 c	2.31 b
0.8	18.81 b	2.23 c	4.28 c	2.48 b
1.0	17.57 b	2.20 c	1.39 d	1.01 c
2.0	14.70 c	1.71 cd	0.10 d	0.23 d
3.0	14.85 c	2.16 c	0.03 d	0.03 d
4.0	12.08 d	1.46 d	0.00 d	0.00 d

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

[y]Based on a scale from 0-10 where 0 represented no galls and 10 a severe degree of galling.

amended soils. Chitin rates of 0.8% or above were detrimental to plant growth.

Soil chitinase activity was analyzed at all samplings but the first; it increased throughout the 10-week period in soil amended with chitin at rates of 1.0% or higher (Fig. 1). Maximum activity was recorded 10 weeks after the addition of 2% chitin to soil. There were no significant differences in chitinase activity in soils treated with 0, 0.2, and 0.4% chitin. Results obtained for pH, conductivity, nitrate-nitrogen and ammoniacal nitrogen are presented in Fig. 2. Analysis of the soils for the variables could not be performed for the 2nd week sampling. Soils treated with chitin at rates of 1.0% and above had a higher pH than control soils at the 4th week sampling. Soils receiving chitin levels between 0.2 and 0.8% had lower pH values than control soils in all samples analyzed. A decrease in pH noted after 6 weeks in soils with more than 0.8% chitin continued to decrease with time. The conductivity of water extracts of soils with chitin at 0.2% or higher was greater than that of unamended soils at all sampling times.

A greater amount of ammoniacal-nitrogen was detected in soils with 1% or more chitin than in those with less chitin at the 4 and 6-week sampling time. Generally, amounts of ammoniacal-nitrogen declined 8

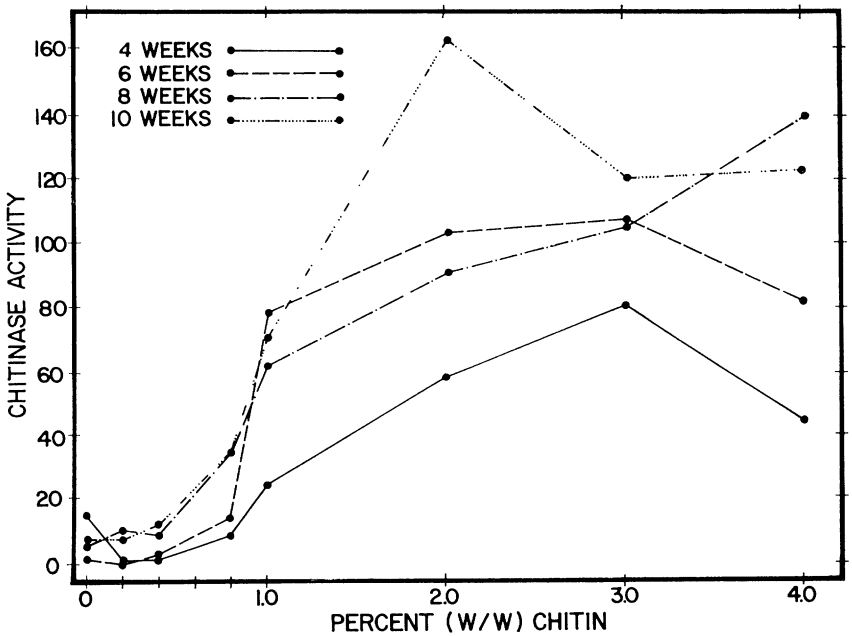

Fig. 1. Effect of chitin application to soil on chitinase activity (μg of N-acetylglucosamine/hr/g of soil).

to 10 weeks after amending the soil. The highest concentrations of ammoniacal-nitrogen were recorded for soils with 2.0% chitin.

Maximal nitrate-nitrogen levels were detected at the 6th week sampling in soils with 0.8% to 4.0% chitin.

Microbial populations were determined for soils at all but the 4th week sampling. Bacterial populations increased in chitin-treated soils at rates of 0.4-3.0% during the first 2 weeks of the experiment (Fig. 3A). Numbers of bacteria declined after the 2nd week, and remained lower than the initial counts throughout the remainder of the experiment (data not presented).

Actinomycetes were most numerous in soils amended with chitin at at 0.2% to 1.0% (Fig. 3B). The largest populations were present at the final sampling of soils containing chitin levels of 0.8-1.0%. Bacteria and actinomycete populations were generally lowest in soils with 3.0 and 4.0% chitin.

The soil mycoflora sampled after 2 weeks was greater in soils containing chitin at rates above 0.4% (Fig. 3C); this was followed by a slight decline at the 6th week. Fungal counts increased again after the 6th week and maximal counts were recorded for soils with 2.0% chitin.

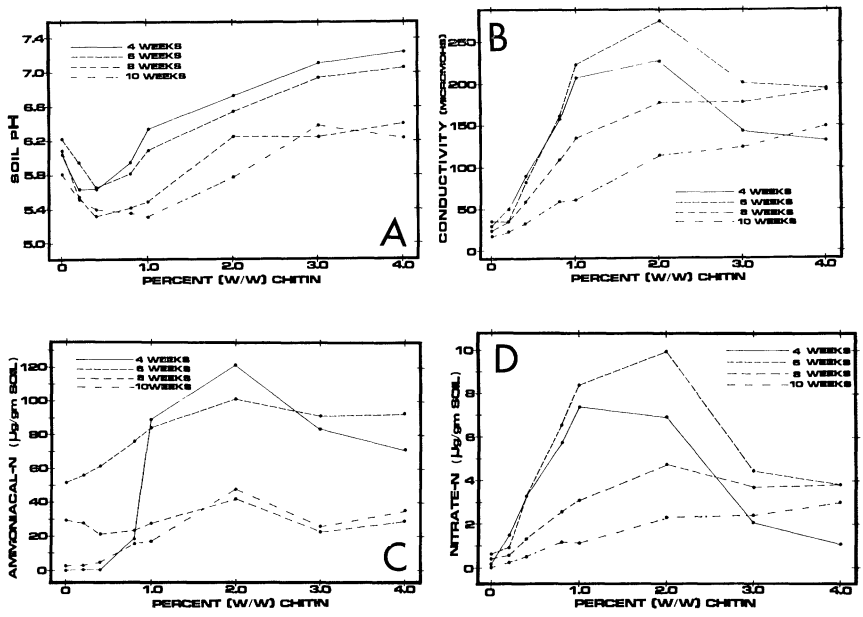

Fig. 2. Effect of chitin amendments on pH (A), conductivity of water extract (B), and on ammoniacal (C) and nitrate (D) nitrogen content of amended soils.

The fungi isolated from the chitin-amended soil are listed in Table 2. All, except *Cladosporium cladosporioides* (Fres.) de Vries, *Geotrichum candidum* Link ex Leman, and *Neocosmospora vasinfecta* E. F. Smith, showed chitinolytic activity.

Fungi isolated from unamended soil were: *Cunninghamella elegans* Lendner., *Rhizopus nigricans* Ehrenb. ex Corda, *Trichoderma harzianum* Rifai, and *Fusarium solani* (Mart.) Sacc.

DISCUSSION

Our results are in accordance with the findings of Mankau & Das (10), Miller et al. (12), and Mian et al. (11) which demonstrated that the addition of chitin to soil reduced nematode populations. However, the amounts of the polymer required for effective control differed for each study.

When chitin is added to soil, it is depolymerized through the action of chitinases to yield N-acetylglucosamine. Ammonia is liberated from this compound or from one of its subsequent derivatives (1). Ammonia is nematicidal to several species of ecto- and endoparasitic nematodes (21,25); however, its nematicidal activity in the field is short-lived (21).

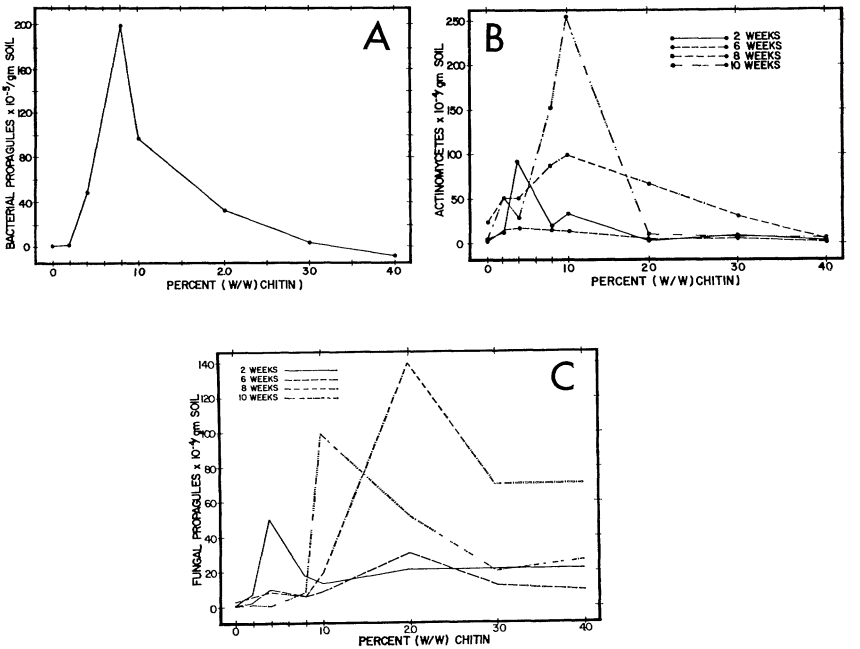

Fig. 3. Effect of chitin amendments on soil microflora determined using chitin-mineral salts agar. A. Bacteria; B. Actinomycetes; C. Fungi.

The action of chitin against nematodes has been attributed mainly to the release of ammonia (12), and to a lesser extent to stimulation of microorganisms resulting in an unfavorable environment for nematodes (10).

The addition of chitin to soil stimulates organisms that can degrade and utilize the polysaccharide through production of chitinases. Chitin decomposition products, such as ammonia and glucosamine, are utilized by microorganisms as carbon and nitrogen sources (1). Actinomycetes are considered the predominant microorganisms in chitin degradation and chitinase formation (26). Previously, actinomycetes have been thought to be antagonistic to *Meloidogyne* spp. and other nematodes (3,22).

Several investigators have reported significant reductions in fungal populations of chitin-amended soils, and have suggested that chitin amendments are detrimental to the soil mycoflora (3,13). Our results do not agree with these reports since fungal counts were high at the end of the experiment. Sampling time, and the techniques and media used could explain differences between results of the different investigations.

Release of ammoniacal nitrogen into the soil results in increased pH and the accumulation of nitrate-nitrogen through nitrogen which in

Table 2. Predominant fungi isolated from soils amended with chitin.

Fungal species	Chitinolytic activity
1. *Aspergillus fumigatus* Fres.	+
2. *Chaetomium indicum* Corda.	+
3. *Cladosporium cladosporioides* (Fres.) de Vries	−
4. *Cunninghamella elegans* Lendner.	+
5. *Fusarium equiseti* (Corda) Sacc. sensu Gordon	+
6. *Fusarium solani* (Mart.) Sacc.	+
7. *Geotrichum candidum* Link ex Leman	−
8. *Humicola fuscoatra* Traaen	+
9. *Malbranchea aurantiaca* Sigler & Carmichael	+
10. *Melanospora zamiae* Corda	+
11. *Neocosmospora vasinfecta* E.F. Smith	−
12. *Pseudeurotium ovale* Stolk	+
13. *Rhizoctonia*-like fungus. Unidentified	+
14. *Rhizopus nigricans* Ehrenb. ex Corda	+
15. *Thielavia basicola* Zopf	+
16. *Trichoderma harzianum* Rifai	+
17. *Verticillium chlamydosporium* Goddard	+

turn results in increased conductivity values of the soil extract. These biochemical processes involved in soil chitin degradation have been discussed in a previous study (11). Results from this study are in agreement with those interpretations. The presence of a large number of fungi with chitinolytic activity supports the view advanced earlier (11) that a particular mycoflora develops in soil in response to the addition of chitin. Most of the fungi isolated can produce a wide variety of exocellular enzymes in addition to chitinase (4), and at least half of them are known to be involved in cyst and egg wall degradation of *Heterodera* and *Meloidogyne* spp. (4,15,16,18).

In this study, *Verticillium chlamydosporium* Goddard frequently was isolated from chitin-amended soil. This fungus has been reported as an important egg parasite of the cyst nematode *Heterodera schachtii* Schmidt in Europe (24). It has also been repeatedly encountered in Alabama as an endogenous parasite of females and eggs of the root-knot nematode *M. arenaria* (14).

Humicola fuscoatra Traaen, *Pseudeurotium ovale* Stolk and *Thielavia basicola* Zopf were previously isolated from chitin-amended soils. These fungal species were pathogenic to eggs of *M. arenaria* and *H. glycines*

(11). Species of *Fusarium* are consistently found associated with cysts and eggs of *Heterodera* spp. in Europe and the United States (16,18). *F. solani* attacks young females and penetrates the eggs of *Heterodera glycines* (18). Another species of *Fusarium* frequently encountered in our study was *F. equiseti* (Corda) Sacc. This fungus has been isolated from cysts of *Globodera rostochiensis* Mulvey & Stone (4) and has also been implicated in cyst and egg wall degradation of *Heterodera avenae* (Wollenweber) Filipjev (7).

Cladosporium cladosporioides and *Neocosmospora vasinfecta* have been isolated from cysts of *H. glycines* where they occurred, apparently, in pure culture. When tested for parasitism on eggs, however, the results were negative (5). Furthermore, there is no evidence of their involvement in cyst wall degradation. *Aspergillus fumigatus* Fries was also among the fungal species encountered in chitin-amended soils. This species has been isolated previously from the surface of sclerotia of *Sclerotinia sclerotiorum* (Lib.) Fuck. buried in soil, and has been shown to produce exocellular enzymes, such as chitinase, and to degrade sclerotia (19). Other species of *Aspergillus* produce toxins with nematicidal activity in culture (9). *Aspergillus* spp. are among the most abundant fungal species capable of degrading chitin (26). *Chaetomium indicum* Corda, also isolated from chitin-amended soils in these studies, has been isolated from cysts of *H. glycines* (15). *C. indicum* produces a wide variety of exocellular enzymes which allow it to grow on substrates of widely differing composition (4). It has been suggested that this fungus may be involved in long-term degradation of cyst exocuticle in soil (15).

The fungal species most predominant in this study was *Malbranchea aurantiaca* Sigler & Carmichael. This species possesses chitinolytic activity and is capable of parasitizing eggs of *M. arenaria.* In conclusion, our results support earlier findings (11) indicating that elements of a selected mycoflora capable of parasitizing eggs of *M. arenaria* and *H. glycines,* and of degrading cysts of *Heterodera* and *Globodera* species, develop in soil in response to the addition to chitin. This selected mycoflora could be responsible for the recorded effectiveness of chitin amendments against nematode populations.

LITERATURE CITED

1. ALEXANDER, M. 1977. Introduction to soil microbiology, Second Edition. John Wiley and Sons. 467 pp.
2. BIRD, A.F., and M.A. McCLURE. 1976. The tylenchoid (nematoda) egg shell: Structure, composition and permeability. Parasitology 72:19-28.

3. BROWN, L.R., D.J. BLASINGAME, C.M. LADNER, C. TEICHERT, and S. BROWN. 1979. The use of chitinous seafood wastes for the control of plant parasitic nematodes. Report. Bureau of Marine Resources. Mississippi Department of Wildlife Conservation. 42 pp.
4. DOMSCH, K.H., and W. GAMS. 1980. Compendium of soil fungi. Vol I. Academic Press, New York, N.Y. 859 pp.
5. GODOY, G., R. RODRIGUEZ-KABANA, and G. MORGAN-JONES. 1982. Parasitism of eggs of *Heterodera glycines* and *Meloidogyne arenaria* by fungi isolated from cysts of *H. glycines*. Nematropica 12:111-119.
6. JACKSON, M.L. 1958. Soil chemical analysis. Prentice Hall, Englewood Cliffs, N.J. 498 pp.
7. JAIN, D.K. 1979. Occurrence of nematophagous fungi and their mode of action on the cereal cyst nematode (*Heterodera avenae*). C.A.B. 5(2):88-89 (Abstr.).
8. MANKAU, R. 1963. Effect of organic amendments on nematode populations. Phytopathology 53:881-882 (Abstr.).
9. MANKAU, R. 1969. Nematicidal activity of *Aspergillus niger* culture filtrates. Phytopathology 59:1170.
10. MANKAU, R., and S. DAS. 1969. The influence of chitin amendments on *Meloidogyne incognita*. J. Nematol. 1:15-16 (Abstr.).
11. MIAN, I.H., G. GODOY, R.A. SHELBY, R. RODRIGUEZ-KABANA, and G. MORGAN-JONES. 1982. Chitin amendments for control of *Meloidogyne arenaria* in infested soil. Nematropica 12: 71-84.
12. MILLER, P.M., D.C. SANDS, and S. RICH. 1973. Effect of industrial residues wood fiber wastes and chitin on plant parasitic nematodes and some soil-borne diseases. Plant Dis. Rep. 57:438-442.
13. MITCHELL, R., and M. ALEXANDER. 1962. Microbial processes associated with the use of chitin for biological control. Soil. Sci. Soc. Am. Proc. 26:556-558.
14. MORGAN-JONES, G., G. GODOY, and R. RODRIGUEZ-KABANA 1981. *Verticillium chlamydosporium,* fungal parasite of *Meloidogyne arenaria* females. Nematropica 11:115-119.
15. MORGAN-JONES, G., B. OWNLEY GINTIS, and R. RODRIGUEZ-KABANA. 1981. Fungal colonization of *Heterodera glycines* cysts in Arkansas, Florida, Mississippi, and Missouri soils. Nematropica 11: 155-164.
16. MORGAN-JONES, G., and R. RODRIGUEZ-KABANA. 1981 Fungi associated with cysts of *Heterodera glycines* in an Alabama soil. Nematropica 11:69-77.

17. MUZZARELLI, R.A. 1977. Chitin. Pergamon Press, New York, N.Y. 309 pp.
18. OWNLEY GINTIS, B., G. MORGAN-JONES, and R. RODRIGUEZ-KABANA. 1982. Mycoflora of young cysts of *Heterodera glycines* in North Carolina soils. Nematropica 12:295-303.
19. RAI, J.N., and S. DHAWAN. 1978. Lysis of sclerotia of *Sclerotinia sclerotiorum* and its possible relation with chitinase activity. Indian. Mycol. Plant Pathol. 8(1):103-107.
20. RODRIGUEZ-KABANA, R. 1967. An improved method for assessing soil fungus population density. Plant Soil 26:393-396.
21. RODRIGUEZ-KABANA, R., P.S. KING, and M.H. POPE. 1981.. Combinations of anhydrous ammonia and ethylene dibromide for control of nematodes parasitic of soybeans. Nematropica 11:27-41.
22. SAKA, V.W. 1978. Waste mycelium, sewage sludge and crab chitin as soil amendments to control plant parasitic nematodes *Meloidogyne incognita* and *Pratylenchus penetrans*. C.A.B. 39B:5 (Abstr.).
23. STEEL, R.G., and J.D. TORRIE. 1960. Principles and procedures of statistics. McGraw-Hill Book Co., New York, N.Y. 481 pp.
24. TRIBE, H.T. 1979. Extent of disease in populations of *Heterodera* with special reference to *H. schachtii*. Ann. Appl. Biol. 92:61-72.
25. VASALO, M.A. 1967. The nematicidal power of ammonia. Nematologica 13:155 (Abstr.).
26. VELDKAMP, H. 1955. Aerobic decomposition of chitin by microorganism. Meded. Landbouw., Wageningen 55:127-174.
27. ZECHT, M.T. 1971. A rating scheme for field evaluation of root-knot nematode infestation. Planzenschutz-Nacht. 24:141-144.