EFFECTS OF CHITIN AMENDMENTS TO SOIL ON *HETERODERA GLYCINES*, MICROBIAL POPULATIONS, AND COLONIZATION OF CYSTS BY FUNGI.

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ABSTRACT

Rodríguez-Kábana, R., G. Morgan-Jones, y B. Ownley Gintis. 1984. Effects of chitin amendments to soil on *Heterodera glycines*, microbial populations, and colonization of cysts by fungi. Nematropica 14:10-25.

The addition of crustacean chitin to soil at rates of 0.5-4.0% (w/w) resulted in control of *Heterodera glycines* Ichinohe in the roots of 'Ransom' soybean (*Glycine max* Merr). Total numbers of chitinolytic fungi and actinomycetes in the soil 8 weeks after amendment increased non-linearly in relation to the amount of chitin added in the range of 0-3.0%. Several fungal species isolated from chitin-treated soil are known parasites of nematode eggs in the genera *Globodera, Heterodera,* and *Meloidogyne*. The number of cysts of *H. glycines* colonized by fungi and the number of fungal species in the cysts decreased with increasing levels of chitin in the soil; however, the frequency of occurrence of fungi belonging to the genera *Neocosmospora, Paecilomyces, Pythium,* and *Phytophthora* in the cysts was not affected by the amendments. *Additional key words: biological control, nonchemical control, ecology, population dynamics, microbial antagonism, waste management.*

RESUMEN

Rodríguez-Kábana, R., G. Morgan-Jones, y B. Ownley Gintis. 1984. Efectos de enmiendas al suelo con quintina sobre *Heterodera glycines*, la microflora y sobre la colonización de quistes por hongos. Nematrópica 14:10-25.

La incorporación de quitina de crustáceos al suelo a niveles de 0.5-4.0% (w/w) redujo las poblaciones de *Heterodera glycines* Ichinohe tanto en el suelo como en las raíces de soya (*Glycine max* Merr.) 'Ransom'. Los números totales de hongos y actinomicetos quintinolíticos en el suelo 8 semanas después de la incorporacíon del polisácarido en el suelo aumentaron de manera no-linear en proporción a la cantidad de quitina en el suelo entre 0 y 3.0%. Muchas de las especies fungosas de los suelos con quitina eran parásitos reconocidos de huevos de nematodos en los géneros *Globodera, Heterodera* o *Meloidogyne*. El número de quistes de *H. glycines* colonizados por hongos asi como el número de especies fungosas en los quistes disminuyeron en proporción al nivel de quitina en el suelo aunque las frecuencias de hongos en los géneros *Paecilomyces, Pythium, Phytophthora* y *Neocosmospora* que se encontraron en los quistes no fueron afectadas por el nivel de quitina en el suelo.

Palabras clavas adicionales: combate biológico, ecologia, dinámica poblacional, antagonismos microbiales, manejo de plagas.

INTRODUCTION

Chitin amendments to soil can result in effective control of root-knot nematodes (*Meloidogyne* spp.) (4,7,8). Decomposition of the polysaccharide in soil is mediated principally through the activities of fungi and actinomycetes (2,11,14) and to a lesser extent by bacteria (14). Many chitinolytic fungal species found in soil are capable of destroying eggs of root-knot and soybean cyst (*Heterodera glycines* Ichinohe) nematodes *in vitro* (3,5,8). Their action on the eggs is thought to involve enzymatic hydrolysis of the chitin in the chitin-protein layer of the egg shell, thereby affecting permeability (1,5). Once permeability is altered, egg contents, including developing larvae, become predisposed to possible physiological disorder brought about by entry of diffusible toxins. Although considerable information is available on the effectiveness of chitin amendments to soil for control of root-knot nematodes, information on the efficacy of the amendments for control of soybean cyst nematodes and other phytoparasitic nematodes is not available. Chitin amendments to soil may affect the specific composition of the mycoflora in cysts of *Heterodera* and *Globodera* species. This paper presents results of a study conducted to determine the effect of chitin amendments to soil on *H. glycines*. Mathematical analysis of the effect of the amendments on soil microbial populations is discussed.

MATERIALS AND METHODS

A sandy loam soil obtained from a soybean [*Glycine max* (L.) Merr.] field near Roba, Alabama was used in this study. The field had been under soybean monoculture for the previous 4 years and was infested with race 3 of *H. glycines* Ichinohe, *Helicotylenchus dihystera* (Cobb) Sher, *Meloidogyne incognita* (Kofoid & White) Chitwood, and *Pratylenchus brachyurus* (Godfrey) Goodey, had a pH = 6.0 and less than 1.0% org. matter. The soil was screened (1 mm) to remove large particles and then mixed 50:50 (v:v) with fine sand (<0.5 mm). A moist mixture of this soil with 60% field capacity was placed into polyethylene bags in 1 kg quantities. The mixture will be referred to as soil in the remainder of this paper. Finely ground (<0.1 mm) unbleached crustacean chitin (United States Biochemical Corporation, Cleveland, Ohio, U.S.A.) was added to the soil to have 0, 0.5, 1.0, 2.0, 3.0 or 4.0% (w/w) in the soil. There were 8 bags for each level of chitin. After thorough mixing, the contents of each bag were transferred to individual 10-cm diameter

cylindrical 1.5 L capacity plastic pots. The pots were then arranged in a completely randomized design in a greenhouse and the soil moisture maintained to allow decomposition of the chitin. After 4 weeks approximately 300 cm^3 soil was removed from each pot to extract cysts and to assess nematode populations (9,10,16). Each pot was then planted with 5 seeds of soybean cv. Ransom. The plants were allowed to develop for 8 weeks prior to their removal for examination of root systems and determination of the number of *H. glycines*. The roots were then weighed and incubated in water for 72 hr to extract the nematodes (16). Shoots of soybean plants were weighed and measured to determine their length. Soil from each pot was processed for assessment of chitonolytic fungal populations, bacteria, actinomycetes (8,14), and nematodes.

Mature brown cysts were recovered from soil at each sampling by a flotation technique described elsewhere (12,13). Ten cysts were randomly picked from each sample and placed on chitin agar with streptomycin sulfate (8,14). Following incubation for 5-15 days at 25C the resultant fungal colonies were identified and frequency of occurrence of fungal species in each sample was calculated.

Data were analyzed following standard procedures for analysis of variance and correlation analysis (17). Unless otherwise specified, differences referred to in the text were significant at 5% or lower level of probability.

RESULTS

Microbial populations. Numbers of chitinolytic fungi increased in response to the amount of chitin added to soil in the range of 0-2% (Fig. 1A); amendment of the soil with more than 2% chitin resulted in either no further increase in fungal propagules (3%) or a decline in numbers (4%) compared with the number present in soils with 2% chitin. Chitin-agar plates inoculated with unamended soil contained fungal colonies of species of *Rhizopus, Aspergillus,* and *Myrothecium* in addition to *Fusarium oxysporum* Schlecht. Colonies of *Aspergillus* spp., *F. oxysporum, Cylindrocarpon tonkinense* Bugnicourt, *Humicola fuscoatra* Traaen, *Malbranchea aurantiaca* Sigler & Carmichael, *Myrothecium verrucaria* (Alb. & Schw.) Ditm., *Paecilomyces lilacinus* (Thom) Samson, *P. variotii* Bainier, and *Pseudeurotium ovale* Stolk were identified in plates inoculated with chitin-amended soil.

Numbers of actinomycetes increased sharply with chitin levels in the range of 0-3.0% (Fig. 1B), but in soils that received 4% of the polymer their number was not significantly different from that in unamended soil. The number of bacteria in soils with 3 or 4% chitin was not different from that of control soil (Fig. 1C). However, bacterial populations were higher in soils with 0.5, 1.0, and 2.0% chitin than in soil with no chitin.

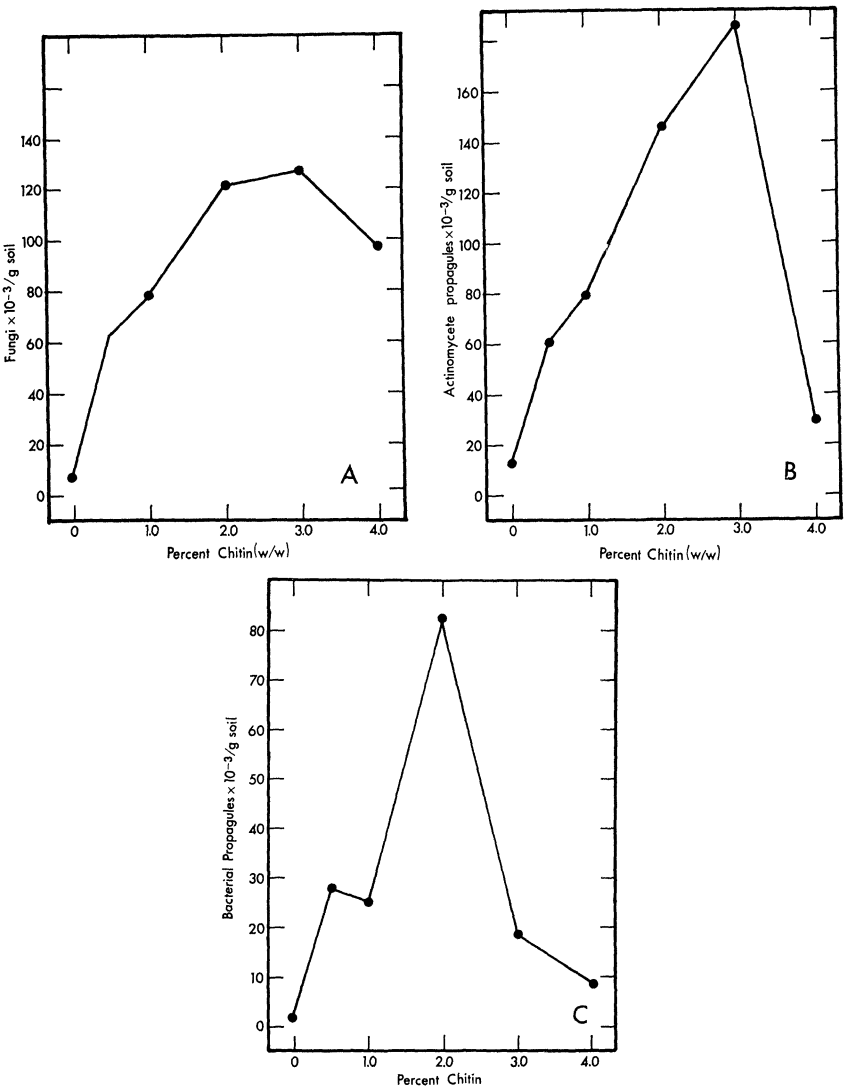

Fig. 1. Effect of chitin amendments to soil on populations of fungi (A), actinomycetes (B), and bacteria (C) determined using chitin agar media 8 weeks after addition of the polymer.

Nematode populations. The numbers of all plant-parasitic nematodes in the soil at the 4-week sampling were too low to permit comparisons on the effect of chitin treatments (Table 1). The number of non-phytoparasitic nematodes in soils treated with 1.0% or higher levels of chitin was larger than those in unamended soil.

Table 1. Effect of chitin amendments on nematode populations in soil 4 weeks after treatment.

Percent (w/w) chitin added	Nematodes per 100 cm^3 soil			
	H. glycines larvae	*H. dihystera*	*T. claytoni*	Non-phyto-parasitic
0.0	6.0	2.1	1.9	117
0.5	5.5	1.4	0.0	165
1.0	1.8	0.6	0.0	216
2.0	2.6	1.0	0.0	184
3.0	0.0	0.0	0.0	178
4.0	0.0	0.7	0.0	202
LSD (P=0.05)	N.S.[a]	N.S.	N.S.	55

[a]N.S. = not significant

Chitin amendments reduced populations of plant parasitic nematodes in soil and roots at the 8th week sampling (Tables 2,3). Populations of all the parasitic species, except *H. dihystera* at 0.5%, were reduced by all chitin amendments. In contrast, numbers of nonphytoparasitic nematodes were higher in soils with 0.5 and 1.0% chitin than in unamended soil and no chitin treatment reduced numbers of these nematodes below the number found in control soils.

Growth of soybeans. All chitin treatments resulted in marked reductions in fresh root weights (Table 4). However, the effect of the treatments on fresh shoot weights or shoot heights was not as pronounced. The 3% chitin treatment reduced shoot weight while 2 and 3% amendment resulted in shorter plants.

Fungi in the cysts. Fungal species isolated from cysts in samples taken after the 4th week are listed in Table 5. Species of *Fusarium* and *Paecilomyces* appeared consistently at all levels of chitin. *Chaetomium cochliodes* and *N. vasinfecta* also occurred in cysts from almost all soils. Other fungal species followed no apparent pattern and *Geotrichum candidum* and *Verticillium lecanii* were only isolated in cysts from unamended soil. Chitin amendments reduced the average number of fungal species isolated/plate from cysts (Table 6). Similarly, the number of cysts free of fungi was higher in chitin-treated soils than in control soils. The frequency of occurrence of *Fusarium* spp. in the cysts generally declined with increasing concentration of chitin. However, chitin amendments had no effect on the frequency of occurrence of *Paecilomyces* spp.

Table 2. Effect of chitin amendments on nematode populations in soil 8 weeks after treatment.

Percent (w/w) chitin added	Nematodes in 100 cm^3 soil				
	Larvae of *H. glycines*	Larvae of *M. incognita*	*H. dihystera*	*T. claytoni*	Non-phytoparasitic
0.0	25.5	2.5	11.0	15.1	157
0.5	5.2	0.6	6.1	4.7	273
1.0	0.5	0.0	3.2	0.6	249
2.0	0.0	0.0	0.4	0.0	153
3.0	0.0	0.0	0.0	0.0	119
4.0	0.0	0.0	0.0	0.0	103
LSD (P = 0.05)	10.7	1.8	6.0	8.7	86

Table 3. Effect of chitin amendments on the number of cysts and mature females of *H. glycines* and on other nematodes in the roots of 'Ransom' soybeans planted on the amended soil.

Percent (w/w) chitin added	Number per gm fresh root				
	H. glycines larvae	*M. incognita* larvae	*H. dihystera*	*P. brachyurus*	*H. glycines* (cysts + mature females)
0.0	19.5	82.4	25.9	10.6	9.6
0.5	2.5	0.7	38.7	1.0	0.6
1.0	0.0	0.0	6.5	0.4	0.1
2.0	0.0	0.0	0.5	0.0	0.0
3.0	0.0	0.0	0.0	0.0	0.0
4.0	0.0	0.0	0.0	0.0	0.0
LSD (P = 0.05)	8.8	59.7	13.3	3.2	2.4

Table 4. Effect of chitin amendments on development of soybeans cv. Ransom in soil amended with the polysaccharide.

Percent (w/w) chitin added	Fresh root weight (gm)	Fresh shoot weight (gm)	Shoot height (cm)
0.0	1.93	2.10	14.6
0.5	1.05	2.01	15.0
1.0	0.57	1.74	14.0
2.0	0.35	1.70	13.1
3.0	0.14	1.20	12.0
4.0	0.25	1.95	13.7
LSD (P = 0.05)	0.36	0.51	1.5

Fungal species isolated from cysts in the 8-week samples are listed in Table 7. The complex of species in these cysts was analogous in composition to that of the 4-week samples. Again, species in the genera *Fusarium* and *Neocosmospora* were present in all soils. *Chaetomium cochliodes* and species of *Paecilomyces, Pythium,* and *Verticillium* occurred in most soils. The average number of species isolated per plate declined with increasing concentration of chitin and the frequency of cysts free of fungi was higher in chitin-treated soil than in unamended soil (Table 8). The frequency of occurrence of *Fusarium* spp. in cysts was lower in those from soils with chitin than in unamended soil but, as for the 4-week sampling, chitin had no effect on the frequency of occurrence of *Paecilomyces* spp. Species of *Pythium* and *Verticillium* were isolated more frequently at the 8-week sampling than in the 4-week samples. However, *F. solani, G. candidum, C. tonkinense, P. americana, P. cinnamoni,* and *T. terricola* occurred in the first sampling but not in the 8-week one. Conversely, *C. lunata, G. catenulatum, H. fuscoatra,* species of *Mortierella* and *Mucor, S. chartarum,* and *T. harzianum* were isolated only in the 8-week samples.

DISCUSSION

Results of this study indicate that chitin amendments to soil can control the soybean cyst nematode and other endo- and ectoparasitic nematodes. The results confirm previous studies in which amending soil with the polysaccharide was shown to control root-knot nematodes (4,7,8). Additions of 1% or more chitin to soil are necessary to obtain

Table 5. Effect of chitin amendments on percent frequency of occurrence of fungal species isolated from cysts of *Heterodera glycines* in soil samples taken 4 weeks after addition of the polymer to soil.

Fungal species	Percent (w/w) chitin added to soil					
	0	0.5	1.0	2.0	3.0	4.0
Chaetomium cochliodes Palliser	5.0	2.5	0.0	2.5	10.0	1.2
Cylindrocarpon tonkinense Bugn.	1.2	1.2	0.0	0.0	0.0	0.0
Exophiala pisciphila McGinnis & Ajello	2.5	0.0	1.2	0.0	2.5	0.0
Fusarium oxysporum Schlecht	16.2	16.3	20.0	6.2	2.5	0.0
Fusarium solani (Mart.) Sacc.	21.2	5.0	3.7	2.5	0.0	1.2
Geotrichum candidum Link	1.2	0.0	0.0	0.0	0.0	0.0
Gliocladium roseum Bain	1.2	0.0	0.0	0.0	1.2	0.0
Neocosmospora vasinfecta E. F. Smith	15.0	7.5	2.5	3.7	0.0	3.7
Paecilomyces lilacinus (Thom) Samson	1.2	2.5	2.5	3.7	6.2	5.0
Paecilomyces variotii Bain	3.7	5.0	2.5	2.5	1.2	0.0
Penicillium decumbens Thom	2.5	7.5	0.0	0.0	0.0	0.0
Phoma americana Morgan-Jones & White	0.0	1.2	0.0	0.0	0.0	1.2
Phoma terrestris Mont.	16.2	6.2	8.7	0.0	0.0	1.2
Phytophthora cinnamoni Rands	0.0	7.5	13.7	0.0	0.0	0.0
Pythium sp.	0.0	2.5	0.0	0.0	1.2	7.5
Scytalidium fulvum Morgan-Jones & Gintis	0.0	1.2	2.5	0.0	0.0	0.0
Thielavia terricola (Gilman & Abbott) Emmons	0.0	0.0	1.2	0.0	0.0	0.0
Verticillium chlamydosporium Goddard	3.8	0.0	1.2	2.5	0.0	1.2
Verticillium lecanii (Zimm) Viegas	1.2	0.0	0.0	0.0	0.0	0.0
Unidentified yeast	0.0	5.0	6.2	0.0	0.0	0.0

Table 6. Effect of chitin amendments on the average number of fungal species and the frequency of occurrence of the most common genera isolated from brown cysts extracted from amended soil 4 weeks after application of the polymer.

Percent (w/w) chitin added	Average number of species/plate	Frequency of occurrence (%)					
		Fusarium	*Pythium* + *Phytophthora*	*Neocosmospora*	*Phoma*	*Paecilomyces*	Cysts with no fungi
0.0	5.4	38.7	0.0	7.5	13.7	7.5	17.5
0.5	4.4	22.5	7.5	7.5	7.5	7.5	37.5
1.0	3.5	23.7	13.7	2.5	8.7	5.0	37.5
2.0	4.0	8.7	0.0	3.7	0.0	6.2	71.2
3.0	2.1	2.5	1.2	0.0	0.0	7.5	73.7
4.0	2.2	1.2	7.5	3.7	2.5	5.0	60.0
LSD (P = 0.05)	1.3	14.3	N.S.[x]	N.S.	7.6	N.S.	20.0

[x]N.S. = not significant.

Table 7. Effect of chitin amendments on percent frequency of occurrence of fungal species isolated from cysts of *Heterodera glycines* in soil samples taken 8 weeks after addition of the polymer to soil.

Fungal species	Percent (w/w) chitin added to soil					
	0	0.5	1.0	2.0	3.0	4.0
Chaetomium cochliodes	0.0	3.7	1.2	0.0	7.5	1.2
Curvularia lunata (Wakker) Boedijn	0.0	1.2	0.0	0.0	0.0	0.0
Exophiala pisciphila	3.7	2.5	5.0	0.0	0.0	0.0
Fusarium oxysporum	25.0	15.0	6.2	7.5	5.0	3.7
Gliocladium catenulatum Gilman and Abbott	1.2	0.0	0.0	0.0	0.0	0.0
Gliocladium roseum	0.0	0.0	0.0	0.0	0.0	1.2
Mortierella sp.	0.0	0.0	0.0	0.0	0.0	3.7
Mucor sp.	0.0	0.0	0.0	3.7	0.0	2.5
Humicola fuscoatra	3.7	0.0	0.0	2.5	1.2	0.0
Neocosmospora vasinfecta	13.7	1.2	2.5	3.7	1.2	1.2
Paecilomyces lilacinus	2.5	0.0	1.2	0.0	1.2	0.0
Paecilomyces variotii	1.2	0.0	2.5	1.2	1.2	0.0
Penicillium sp.	1.2	0.0	0.0	0.0	0.0	0.0
Phoma terrestris	0.0	1.2	3.7	1.2	0.0	0.0
Pythium sp.	5.0	0.0	5.0	2.5	2.5	5.0
Scytalidium fulvum	0.0	0.0	1.2	0.0	0.0	0.0
Stachybotrys chartarum (Ebrenb.) Hughes	1.2	0.0	0.0	0.0	0.0	0.0
Tricoderma harzianum	0.0	0.0	2.5	1.2	0.0	0.0
Verticillium chlamydosporium	3.7	1.2	1.2	0.0	0.0	1.2
Verticillium lecanii	0.0	0.0	1.2	0.0	1.2	0.0

Table 8. Effect of chitin amendments on the average number of fungal species and the frequency of occurrence of the most common genera isolated from brown cysts extracted from amended soil 8 weeks after application of the polymer.

Percent (w/w) chitin added	Average number of species/plate	Frequency of occurrence (%)			
		Fusarium	*Paecilomyces*	*Pythium* + *Phytophthora*	Cysts with no fungi
0.0	4.0	25.0	3.7	5.0	38.7
0.5	1.6	15.0	0.0	0.0	73.7
1.0	2.9	6.2	3.7	6.2	66.2
2.0	2.0	7.5	1.2	2.5	76.2
3.0	1.7	5.0	2.5	2.5	78.7
4.0	1.4	3.7	0.0	5.0	81.2
LSD (P = 0.05)	1.2	12.5	N.S.[a]	N.S.	17.3

[a]N.S. = not significant.

consistent results. However, the present study confirmed previous findings (4,8) in that these levels of chitin are phytotoxic. Toxicity to plants by chitin amendments is probably the result of accumulation of nitrites and nitrates (8,14) due to the narrow C:N ratio of the amendments. It may be possible to broaden the C:N ratio of the amendments by including a source of easily metabolizable carbon together with the chitin and thus avoiding accumulations of nitrates and nitrites. This approach has been followed successfully with other materials with narrow C:N ratio (15). The problem of the phytotoxicity due to chitin amendments is one that will require further research. Our results nevertheless confirm that there is a mycoflora capable of decomposing chitin and tolerating high levels of metabolites derived and accumulated from decomposition of the polymer in soil (4,8,14).

There is a positive relation between changes in the number of chitinolytic fungi and the amount of chitin added to soil. Our results indicate that the rate dF/dx at which the number of chitinolytic fungi (F) changes in relation to the level of chitin added to soil (x) is dependent on a constant K; K represents the maximal number of fungal propagules than can develop, at least theoretically, in a soil in response to unlimited amounts of chitin, or expressed mathematically:

$$\frac{dF}{dx} = k\,(K-F) \qquad (I)$$

where k is a positive constant. The number of fungi at any level of chitin can then be obtained from (I), since

$$\left(\frac{1}{K-F}\right)\frac{dF}{dx} = k \qquad (II)$$

and assuming that K-F>0, then

$$\int \frac{1}{K-F}\,dF = kx + c \qquad (III)$$

where c is an arbitrary constant. The left side of (III) can be integrated to yield:

$$\int \frac{1}{K-F}\,dF = -\ln\,(K-F) + c' \qquad (IV)$$

where c′ is a constant. Combining (IV) and (III) we obtain:

$$-\ln\,(K-F) + c' = kx + c$$

and

$$F = K - e^{-kx + C} \qquad (V)$$

where C = c′–c = a positive constant. Our data (Fig. 1A) for chitin concentrations in the range of 0-3.0% fit (V) well ($r^2 = 0.95$) resulting in

$$F = 140 - e^{4.8447 - 0.8030x} \quad \text{(VI)}$$

The model implies that theoretically a limit of 140 fungal propagules per gm soil would be approached in the soil of the study when the amount of chitin added to soil was not limiting. In reality, our results indicated that chitin concentrations higher than 3.0% resulted in smaller fungal populations than predicted from (VI). We interpret this as a result of toxicity from accumulated metabolites from chitin decomposition in the soil.

The data for actinomycetes conformed also ($r^2 = 0.98$) to the model expressed in (V) so that the number of actinomycetes (Ac) was related to chitin levels in the range of 0-3.0% by the equation:

$$Ac = 300 - e^{5.6639 - 0.3203x} \quad \text{(VII)}$$

indicating that a limiting number (300) of actinomycete colonies/gm soil would be approached in the soil when chitin levels were not limiting and provided no toxic or deleterious effects developed in the soil to impede population development. As for the fungi, however, the 4.0% level of chitin was inhibitory to actinomycetes.

The data for bacteria did not fit the model and suggested that chitinolytic bacteria do not tolerate chitin levels in the soil higher than 2.0%. Apparently there is a similar pattern of response to chitin amendments for fungi and actinomycetes. Our results indicate that these two groups of organisms are mostly responsible for degradation of the polymer in soil, especially at high (71%) chitin concentrations. Previous studies have implicated actinomycetes (11,14) and fungi (4,14) as responsible for the degradation of chitin in soil. Also, we have shown a direct relation between increased soil chitinase activity in response to chitin amendments to soil and the number of chitinolytic fungi in soil (14); soil chitinase activity was not related to bacterial numbers in the amended soil.

The composition of the mycoflora of chitin-treated soils in this study was analogous to that of previous studies with a different soil (4,8). This indicates the presence of an ubiquitous specialized mycoflora associated with the decomposition of chitin in soils. Furthermore, several of the fungal species (e.g. *P. lilacinus, P. variotii, P. ovale*) have been shown to be capable of destroying eggs, juveniles or adults of several nematode species (2,3,6). This suggests that the reduction in plant-parasitic nematode numbers observed in response to chitin amendments may be at least partially caused by the presence and activities of these fungal species in the treated soil. The effect of these fungi on nonphytoparasitic nematodes is not known; however, our data indicate that it may be different from that for plant parasites. Nonphytoparasitic nematodes observed

in this study consisted of several unidentified species of saprophagous and microbivorous nematodes. These nematodes apparently could feed on fungi, bacteria, and other debris derived from chitin added to soil and increase in numbers in response to the amendment at least for some time.

Data obtained on fungal colonization of brown cysts from soil clearly indicate a progressive decrease in the number of species colonizing the cysts in response to increasing amounts of chitin in the soil. Significantly, however, the frequency of occurrence of some fungi in the cysts (*Paecilomyces, Neocosmospora, Pythium,* and *Phytophthora*) was not affected by changes in chitin levels. Thus there is a trend towards change in species composition of the mycoflora in the cysts in response to chitin levels favoring those fungal species which can apparently tolerate the metabolites and conditions derived from the decomposition of chitin. The significance of this trend vis-a-vis biological control of nematodes is immediately apparent in view of the ability of isolates of species of *Paecilomyces* to destroy eggs of cyst and root-knot nematodes (3,6). The trend also explains the increase in the frequency of cysts with no fungi in response to chitin levels; a reduction in the number of fungal species capable of developing (or just surviving) in chitin-treated soil necessarily means that the frequency of colonized cysts must decline. Significantly however, cysts from chitin-treated soil with no fungi in them also contained no viable eggs, indicating that conditions and metabolites produced through the decomposition of chitin resulted in death of the eggs.

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