Influence of Chitin Soil Amendments and Flutolanil Application on Black Root Rot of Strawberry[1]

J. A. LaMONDIA and W. H. ELMER[2]

Department of Plant Pathology and Ecology
The Connecticut Agricultural Experiment Station
Valley Laboratory, P.O. Box 248
Windsor, CT 06095
and
P.O. Box 1106
New Haven, CT 06504

Abstract. Chitin (ground crab shell) or cottonseed meal at rates of 0.1% or 1.0% (w/w) to soil reduced recovery of lesion nematodes (*Pratylenchus penetrans*) from strawberry roots in growth chamber experiments after 6 and 9 weeks. Two weeks after treatment, the higher rates of both amendments produced concentrations of ammonium in soil lethal to nematodes, and phytotoxic to strawberries. Field experiments using both amendments at N rates of 71 and 213 kg/ha did not significantly reduce nematode populations one year later despite a strong downward trend between chitin application rate and nematode reproduction. Populations of *Trichoderma* spp. antagonistic to the soilborne strawberry pathogen, *Rhizoctonia fragariae,* were increased 3 weeks later by high rates of chitin, but not by meal. Nine months following chitin treatment, recovery of chitinolytic bacteria and actinomycetes was increased, but populations of *Trichoderma* spp. were not affected. Severity of black root rot associated with *R. fragariae* and *P. penetrans* increased with chitin application, possibly due to the increased release of ammonium. At the higher rate of chitin application, however, an inverse correlation occurred between populations of *Trichoderma* spp. and black root rot ratings after 9 months, but disease suppression in these plots was minimal. In other experiments, 6.7 t/ha of a commercial nematicide formulation (CNF) containing 25% chitin, was tested alone and in combination with flutolanil, a systemic fungicide with activity against certain species of *Rhizoctonia,* for suppression of strawberry black root rot. The CNF increased strawberry leaf area, but had no effect on recovery of lesion nematode or berry yield. Flutolanil had no effect on black root rot or berry yield alone or in combination with CNF. Concentrations of up to 10 ug/ml in *in vitro* tests failed to restrict radial growth rates of *Rhizoctonia fragariae.*

[1] Accepted for publication 13 March 1992.

[2] We thank Dr. L. Krusberg, University of Maryland, College Park, MD, for the crab shell chitin, Nourse Farms, South Deerfield, MA, for the tissue cultured 'Honeoye' strawberry crowns, NorAm Chemical Co., Wilmington, DE, for the flutolanil 50 WP, T. Jones, Shelton, CT, for use of his strawberry fields, and E. O'Dowd and J. Morrison for technical assistance.

Mention of a trademark or propietary product does not constitute a guarantee or warranty of the product by the Connecticut Agricultural Experiment Station, or imply approval to the exclusion of other products that may also be suitable.

Introduction

Black root rot of strawberries in Connecticut is a disease complex involving the lesion nematode, *Pratylenchus penetrans* (Cobb) Filip & Schur.-Stek., and isolates in anastomosis groups AGA, AGG, and AGI of the soilborne binucleate fungus, *Rhizoctonia fragariae* Hussain & McKeen (11,15). Black root rot can be reduced by soil fumigation, but the effects of fumigation are often inconsistent (29). Fumigation may destroy natural antagonists of the pathogens and allow reintroduction and proliferation of the pathogens on planting stock (6). Because strawberry plantings are perennial, and because black root rot is a debilitating disease that becomes progressively worse over several years, control strategies are needed that can be safely applied to established plantings.

Chitin (poly-B-(1-4)-N-acetyl-D-glucosamine) is a polysaccharide that is a component of fungal cell walls, insects (17), and tylenchoid nematode eggs (3). Chitin or chitin-based soil amendments have been shown to reduce soil populations of nematodes (8,16,19,23,25) including *P. penetrans* (15), and soilborne fungi, such as *R. solani* Kuhn (6,16,21,22). Two processes would reduce soil densities of the pathogens. Chitin decomposition results in the release of ammonium, which is toxic to nematodes (26), and also stimulates chitinolytic bacteria, actinomycetes, and fungi, such as *Trichoderma* spp., which increase competition with, or parasitism of, the pathogens (16,19,23,24,25,26).

Flutolanil (a,a,a-trifluoro-3-isopropoxy-o-toluanilide) (Nor-Am Chemical Co, Wilmington, DE) is a new systemic

fungicide with activity against certain strains of *Rhizoctonia* spp. (2). Its efficacy in suppressing *R. fragariae* and black root rot of strawberry has not been addressed. The objectives of this research were to determine the effects of chitin or chitin-based soil amendments and flutolanil applications on *P. penetrans, R. fragariae,* strawberry growth, yield, development of black root rot and soil densities of chitinolytic microflora.

Materials and Methods

Growth Chamber Experiment

Field soil (Merrimac fine sandy loam: 71.8% sand, 23.0% silt, 5.2% clay, 2.2% organic matter, pH 6.2) was fumigated with methyl bromide, and then amended 6 months later with ground crab shell chitin (4.8% N; 3.1% P, 0.3% K) to 0.1% or 1.0% (w/w). Cottonseed meal (6.0% N, 2.5% P, 1.5% K) was amended into the same soil at 8 or 80 g per 10 kg soil which was the equivalent amount of organic nitrogen added as with chitin amendments. Cottonseed meal was included as a control due to its similar N-content and mineralization process. Chitin-treated soil received 0.18 g or 1.8 g K_2P0_4 per 10 kg soil to balance potassium levels in meal and chitin-amended soils. Each amended soil was thoroughly mixed in a concrete mixer for 20 min prior to placing 450 cm^3 of each treated soil into 11.3-cm-diam. plastic pots. Twenty four tissue-cultured 'Honeoye' strawberry crowns (Nourse Farms, South Deerfield, MA) were planted into these pots (1 plant per pot). An equal number of crowns were also planted into soils amended with very low amounts of chitin or cottonseed meal (0.001% N) to serve as controls.

P. penetrans inoculum was prepared by blending monoxenic carrot cultures of *P. penetrans* for 15 sec, decanting and sieving to remove carrot pieces, pelleting the nematodes by centrifuging for 5 min at 2,000 RPM, and suspending the pellet in water (11). Non-infested carrot disks treated in a similar manner served as controls.

R. fragariae isolates AG-A (R-190), AG-G (R-95), and AG-I (R-92) were grown for 2 weeks at 18-24 C on fescue (*Festuca arundinacca* Schreb) seeds which had been autoclaved twice for 1 hr on consecutive days (11). Sterile seeds served as an uninoculated control.

One week after planting, the plants in twelve randomly chosen pots were inoculated with both pathogens by making three equidistant 1 cm-diam. holes with a pencil halfway between the pot edge and crown. Holes were 2-3 cm deep. Six fescue seeds colonized by *R. fragariae* (two seeds per AG isolate; equivalent to 3 g of infested seed per 4 kg soil) were inserted into each hole. A suspension of 5,000 adults, juveniles and eggs of *P. penetrans* was then evenly added to the three holes with a pipette and then holes were covered with a small amount of moist soil. The 12 other pots in each treatment received sterile fescue seeds and distilled water only. Pots were randomly arranged in a growth chamber at 20 C for a 12 hr photoperiod under cool white fluorescent lights. Four of these replicates were sampled for ammonium weekly by taking three 1-cm-diam. cores per pot. The soil was analyzed for ammonium using the procedures of Lunt et al. (14), so that mineralization of the amendments to ammonium could be monitored.

Four replicate pots of each treatment were destructively sampled after 6 and 9 weeks for total leaf area, root and shoot weight, root length, black root rot ratings, lesion nematodes, and *Trichoderma* spp. in the soil. Leaf area (LA) per petiole was estimated by measuring the length (L) and width (W) of the center leaflet using (L x W) in the equation LA = 3.02 + 1.77 L x W (R^2 = 0.98). Roots were gently washed free of soil, pressed between absorbent paper towels, weighed, and placed on a 35 x 40 cm piece of plastic divided into 1 cm^2 grids, and photographed. Slides of photographed roots were projected and the lengths of healthy and rotted roots were estimated using the line-intersect method (11,28). Main and lateral roots were distinguished from fine feeder roots. The percentage of rotted roots was determined by dividing by the total root length and multiplying x 100. Nematodes were extracted by shaking 3 g subsamples of root tissue that were chopped into 0.5 cm pieces in 50 ml water in 125 ml Erlenmeyer flasks for 10 days. Populations of *Trichoderma* spp. were estimated by agitating 2 g soil samples in 200 ml with a magnetic stirrer on a stir plate for 15 min. Serial dilutions of 0.1 ml were distributed with a pipette onto selective media (4), spread over the surface with a sterile glass rod, and incubated at ambient temperatures (2-3 plates per dilution). Populations were expressed as colony-forming-units/g soil (oven dry weight equivalent).

Field Experiments

Two rates of chitin or cottonseed meal (equivalent to 71 and 213 kg/ha of N) were applied on 1 September 1989 to a commercial strawberry field in Shelton, CT, following bed renovation. The soil (Agawam fine sandy loam) was naturally infested with the strawberry nematode pathogens, *P. penetrans, Meloidogyne hapla* Cobb and the soilborne fungal pathogen, *R. fragariae* (strains AG-A, AG-G, and AG-I). Chitin or cottonseed meal was applied to forty 0.5 m^2 double-row bed plots of 4-yr-old 'Raritan' strawberries at rates of 165 or 495 g cottonseed meal and 206 or 618 g chitin per plot (10 plots per treatment). Treatments were applied to the center of the rows, mixed in by hand, and these areas were allowed to be colonized by runners. Nematode populations in roots and soil were determined immediately prior to treatment and in the following spring (May 1990), and fall (October 1990) by removing ten cores 2.5 cm diam. by 15 cm deep and two plants from each plot. Nematodes were extracted from soil by sugar centrifugation and from roots by incubation in water on a shaker for 10 days. One and three weeks after treatment, soils were similarly sampled for populations of *Trichoderma* spp. and total fungi. Total fungal populations were estimated in the same manner as *Trichoderma* spp. except that serial dilutions were spread onto acidified potato-dextrose-agar. Chitinolytic organisms were enumerated on chitin agar (12) in the same manner as

described for fungi. Not all organisms were recorded at each sampling time.

In another study, 1-yr-old nursery grown crowns of susceptible 'Honeoye' plants (Nourse Farms, South Deerfield, MA) were planted into a Cheshire sandy loam soil at the Lockwood Farm in Hamden, Connecticut in 1990. Soils were naturally infested with *P. penetrans* and were artificially infested with *R. fragariae* (AG-A) by rototilling 10 g of colonized fescue seed into 32 plots (2.4 m^2). Rows were 3.1 m long spaced 0.9 m apart and contained 10 crowns spaced 15 cm apart. One day prior to planting, 16 randomly selected plots received a commercial nematicide formulation containing 25% chitin (CNF) (Igene Biotechnology, Columbia, MD), at 6.72 t/ha (containing 1.68 t/ha chitin, 160 kg/ha of N) which was rototilled in to a depth of 10-15 cm. One week after planting, flutolanil was applied to eight CNF-treated plots and eight nontreated plots at 3.3 kg/ha in a backpack sprayer in 3,900 l/ha. The experiment was designed as a complete randomized block factorial with four blocks (two replicates per block). Flutolanil was applied again in August 1990, and all treatments were applied again the following spring. Four to six weeks later, leaf area was estimated as described above. Flowers and all but one runner per plant were removed during the season. This designated runner was allowed to root next to the mother plant and then dug in October 1990. The fresh shoot weight of the runner, numbers of *P. penetrans* in the roots, and black root rot ratings (BRR) based on a scale of 1-5 where 1 = no discoloration or root rot, 2 = 1-10% BRR, 3 = 11-25% BRR, 4 = 26-50% BRR, 5 = 51-100 % BRR were recorded. Berry yield was recorded every 5 days for four pickings in June 1991. Factorial analyses were used to examine interactions.

In Vitro *Flutolanil Assay*

Flutolanil was dissolved in acetone and added in 1 ml amounts to flasks containing 50 ml of cooled (48-50°C) liquid potato-dextrose-agar (Difco Co., Detroit, MI) at rates to yield 0, 0.01, 0.05, 0.10, 0.50, 1.0, 2.5, 5 and 10 ug a.i./ml. Five plates (10 cm-diam.) were poured from each amended flask. One day later, plates were seeded in the center with a 4 mm agar plug removed from the outer margins of a colony of *R. fragariae* AG-A (R-190). Radial expansion of the colony was measured in the widest and narrowest diameters after 2, 4 and 7 days at 25°C. The geometric mean growth was computed and used to calculate growth rate on each amended agar.

Results

Growth Chamber Experiment

Treatment effects were not different between the 6 and 9 week samplings, so only data taken after 9 weeks are presented. Addition of chitin or cottonseed meal to soil increased shoot weight and leaf area of 'Honeoye' strawberries (Table 1). Root weights, however, were increased only at the lower rates of chitin and cottonseed meal. The lengths of the main plus lateral roots and the feeder roots were similar for all treatments, with the exception of the high rate of cottonseed meal, which significantly reduced root length.

The percentage of rotted and blackened roots increased with application rate of chitin and cottonseed meal; control roots were whiter and healthier in appearance than roots in amended soils (Table 2). The high rate of cottonseed meal had phytotoxic effects which caused root death prior to the 6

Table 1. The effect of chitin or cottonseed meal on strawberry growth at 20°C in a growth chamber.

Treatment, N(kg/ha)	*Leaf area* (cm^2)	Shoot wt (g)	Root wt (g)	*Root length (cm)* main/lat	feeder
control-chitin, 1.2	101	4.5	4.8	91.3	275
control-meal, 1.2	120	3.9	5.4	102.9	259
0.1% chitin (w/w), 120	158	8.8	7.1	99.5	291
1.0% chitin (w/w), 1200	174	7.8	4.6	93.6	228
0.08% meal (w/w), 120	167	7.7	7.3	100.9	281
0.8% meal (w/w), 1200	132	6.9	4.6	70.1	182
LSD (P = 0.05)	33	1.2	0.8	16.8	47
CONTRASTS					
1. Chitin vs. meal	0.08	NS	0.05	NS	NS
2. Controls vs. amendments	<0.001	0.01	<0.001	NS	NS
3. High vs. low amendments	NS	<0.001	NS	NS	0.01
4. High chitin vs.high meal	NS	NS	NS	NS	NS
5. Low chitin vs. medium and high chitin	0.04	0.05	<0.001	NS	NS

Table 2. The effect of chitin or cottonseed meal in growth chamber tests (20·C) on strawberry root infection by the lesion nematode (*Pratylenchus penetrans*), black root rot incidence, and soil populations of *Trichoderma* spp.

Treatment	*Pratylenchus* per 3 g root	*Percent rotted roots* main & lateral	feeder	*Trichoderma* CFU X 10^4/g
control-chitin	47.6	32.6	21.0	0.98
control-meal	51.0	29.5	21.0	3.03
0.1% chitin (w/w)	39.8	44.1	36.6	2.30
1.0% chitin (w/w)	5.8	60.6	57.6	2.49
0.08% meal (w/w)	31.5	43.3	42.4	2.99
0.8% meal (w/w)	2.0	81.7	71.1	0.17
LSD (P=0.05)	12.9	11.6	14.4	0.62
CONTRASTS (P)				
1. Chitin vs. meal	NS	NS	NS	NS
2. Control vs. amendment	<0.001	<0.001	<0.001	NS
3. High vs. low amendments	<0.001	<0.001	0.01	<0.001
4. High chitin vs. high meal	NS	0.05	NS	<0.001
5. Low chitin vs. med. & high chitin	0.05	0.05	0.05	<0.001

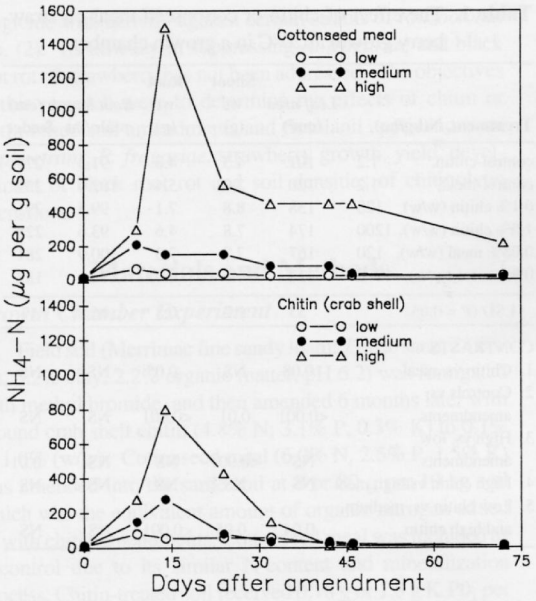

Figure 1. Ammonium concentration detected over time in soil amended with low (0.001%, w/w), medium (0.01%, w/w) and high (1.0%, w/w) of cottonseed meal or ground crab shell chitin. The equivalent rates of nitrogen added are 0.7, 70, and 700 µg/g soil, respectively.

week sampling period (data not shown). Some of these damaged plants recovered and initiated new growth after 9 weeks. Numbers of lesion nematodes recovered from roots, however, were significantly reduced by the addition of high rates of chitin or cottonseed meal.

Over the course of these experiments, most of the ammonium released during mineralization of chitin or meal was greatest approximately 2 weeks after amendment (Figure 1). At this time, high cottonseed meal-amended soil produced almost twice the amount of ammonium as did high chitin-amended soil and continued to produce more than the control soil after 75 days. In all other treatments, ammonium levels declined to pre-amended concentrations after 45 days.

Field Experiments

The addition of chitin or cottonseed meal to soil in a renovated strawberry bed that was naturally infested with the black root rot organisms did not significantly decrease nematode numbers, but there was a trend towards lower numbers of *P. penetrans* in plots amended with chitin (Table 3). Neither chitin nor cottonseed meal affected *M. hapla* populations. Nine months after treatment (May 1990), plants had more black root rot when treated with chitin than plants treated with cottonseed meal, but there were no differences after 14 months (October 1990) between treatments (data not shown). The higher application rate of chitin increased black root rot in a manner similar to that in growth chamber experiments even though these applications in the field were much less than in growth chamber studies.

Table 3. The influence of chitin or cottonseed meal on nematode infection and black root rot of field grown strawberries.

Treatment kg/ha[b]	*P. penetrans* Pf/Pi[c]	*M. hapla* Pf/Pi	Percent root rot[a] May/90
cottonseed meal 1183	14	5	20
cottonseed meal 3550	25	4	13
chitin 1478	4	28	33
chitin 4433	1	5	41
Kruskal-Wallis (P)[d]	0.11	NS	0.02

[a] Statisitical analyses performed using arcsine of the square root transformation.
[b] Equivalent to 71 and 213 kg/ha of nitrogen.
[c] Ratios of the final population (October '90)/initial population (September '89).
[d] Kruskal/Wallis Test at (P = 0.05) is a nonparametric rank test.

The application of chitin or cottonseed meal to field soil did not affect total populations of soil fungi or *Trichoderma* spp. until 3 weeks after treatment, when total fungi increased in soil treated with the higher chitin rate (Table 4). Much of this increase was due to elevated levels of *Trichoderma* spp. After 9 months, chitin-amended soil had significantly greater densities of chitinolytic organisms, but not after 14 months.

There was no interaction between the effects of applying CNF and flutolanil on growth, yield, disease suppression, or nematode recovery from the roots, so only main effects will be discussed (Table 5). The application of CNF to field-grown strawberries at time of transplanting in 1990 resulted in a significantly higher mother plant leaf area and runner leaf weight, but not berry yield the following year. CNF did not reduce densities of nematodes recovered from the roots after 4 months. Black root rot ratings were significantly more severe after 4 months in plots treated with CNF. Flutolanil applications had no effect on black root rot ratings, plant growth or berry yield.

In Vitro *Flutolanil Assay*

Isolate (R-190) of *R. fragariae* (AG-A) had an averaged radial expansion rate of 0.92 cm/day (± 0.021), and was not inhibited on flutolanil-amended agar containing 10 ug/ml (data not shown).

Discussion

Chitin amendments at 0.2 to 4.0% (w/w) have previously been shown to control plant parasitic nematodes (8,15,23,25), and reduce the saprophytic and pathogenic activity of *Rhizoctonia* (15,20,21). In our growth chamber experiment, chitin at 1.0% (w/w) greatly reduced recovery of lesion nematodes from strawberry roots. Equivalent amounts

Table 4. The influence of chitin or cottonseed meal on soil populations of total soil fungi, species of *Trichoderma,* and chitinolytic bacteria and actinomycetes in strawberry field soil.

		CFU[a] X 10^4/g soil (air dry equivalent)								
		Week 1		Week 3		May '91		October '91		
Treatment,	kg/ha[b]	TF[c]	Tric[c]	TF	Tric	Tric	Chit[c]	TF	Tric	Chit
cottonseed meal,	1183	5.3	3.7	2.8	1.3	0.4	45.2	2.6	1.0	40.8
cottonseed meal,	3550	4.7	1.8	2.3	1.5	0.4	28.9	2.0	0.8	37.4
chitin,	1478	5.1	1.4	2.0	1.1	0.2	61.4	2.3	1.1	31.2
chitin,	4433	5.1	2.7	3.1	2.1	0.2	106.6	2.1	0.5	32.8
Treatment										
Kruskal/Wallis Test[d]		NS	NS	0.01	NS	NS	0.03	NS	NS	NS
ANOVA		NS	NS	0.01	NS	NS	0.02	NS	NS	NS

[a] All colony-forming unit (CFU) data were analyzed following a log (x + 1) transformation.
[b] Equivalent to 71 and 213 kg/ha of nitrogen.
[c] Total fungi (TF), *Trichoderma* spp. (Tric), and chitinolytic organisms (Chit, primarily bacteria and actinomycetes) were determined by placing soil dilutions onto acidified potato detrose agar, *Trichoderma*-selective media (4),or chitin agar (12).
[d] Kruskal/Wallis Test at (P = 0.05) is a nonparametric rank test.

of nitrogen applied as cottonseed meal were equally as effective. These results suggest that release of ammonium during decomposition of these amendments may be responsible for the nematicidal effects. Nitrogen added as urea is completely transformed into ammonium in about 5 days (26) and most of the ammonium produced by the decomposition of organic fertilizers, such as cottonseed meal, occurred within 2 weeks (27). Levels of ammonium have been reported to be nematicidal at concentrations between 125 to 245 µg/g soil (7,18). This is consistent with the nematode control we observed in soils with elevated ammonium. Although chitin acted as a fertilizer and increased plant growth, the higher concentrations (1%, w/w) were often phytotoxic.

Because of difficulties associated with quantifying *Rhizoctonia* spp. on roots and in soil, we evaluated root length and percent root rot as indicators of infection by *R. fragariae* and pathogenesis. The phytotoxic effects of high ammonium levels observed in the growth chamber experiment may have resulted in increased *R. fragariae* infection and disease. It is well known that ammoniacal forms of nitrogen are conducive to many *Rhizoctonia* diseases (10). For example, black root rot of sugar beets caused by *R. solani* was more severe in plants fertilized with ammonium than with nitrates (1). A number of other cortical and root diseases caused by *Rhizoctonia* spp. increase in severity with increasing levels of ammonium in soil (10). Our findings and the research of others have demonstrated that high levels of ammonium may be phytotoxic (8,23,24) and increase disease (10,23). Therefore, the amount of ammonium that may be safely applied as chitin in a given year may be insufficient to limit nematodes and fungi via ammonium activity. For these reasons, we were particularly interested in determining whether or not chitin influenced the soil microbial populations and enhanced biological control of pathogenic nematodes and fungi (8,23,24).

The biocontrol activity of *Trichoderma* spp. against *Rhizoctonia* spp. has been demonstrated (18), and suppression of *Rhizoctonia* has been correlated with increased soil populations of *Trichoderma* spp. (5,6,13). Soil populations of *Trichoderma* are reported to increase and be sustained following chitin amendments (9,22). In Israel, root rot of strawberry caused by *R. solani* was reduced by the application of *T. harzianum* Rifai, but only when such application was continual (6). We hypothesized that if chitin amendments could increase and support high populations of naturally occurring *Trichoderma* spp. in soils, control of black root rot could be sustained. However, in growth chamber and field experiments we were unable to significantly increase or sustain naturally occurring populations of *Trichoderma* spp. In the growth chamber, high rates of meal actually reduced *Trichoderma* levels, possibly due to high ammonium production. Application of chitin after renovation to an established strawberry field had a brief effect on total soil fungi and stimulated the number of chitinolytic organisms (primarily bacteria and actinomycetes) up to 9 months after application. Chitin, but not cottonseed meal, resulted in a trend toward lower *Pratylenchus* populations after one year. These data seem consistent with the dual mode of action, ammonium generation followed by increased levels of chitinolytic organisms, suggested by other researchers (8,24), but in our studies chitin application consistently increased black root rot ratings instead of suppressing the disease.

CNF, a 25% chitin-containing nematicide amended with urea and other fertilizers, resulted in an initial growth stimulation in first year strawberries, possibly due to the slow release of nitrogen, but did not reduce root infection by *P. penetrans.* Like chitin in the previous experiments, CNF increased black root rot ratings, possibly due to increased ammonium levels in soil.

Table 5. The effect of 25% chitin-nematicide formulation (CNF) and flutolanil on strawberry yield, growth and black root rot.

Treatment	1991 Yield (kg/ha)	Leaf area dm²	Sampled Runners in 1990 BRR[b] rating	*P. penetrans* per 3 g root	Leaf fresh weight (g)
Control[c]	14347	0.6	2.5	63.3	16.0
Flutolanil[d]	15420	0.6	2.7	91.8	16.7
CNF[e]	17102	0.8	3.0	102.0	22.9
Flutolanil + CNF	15027	0.8	3.3	97.2	22.1
LSD	NS	0.17	0.6	NS	5.22
ANOVA (P)					
Flutolanil	NS	NS	0.03	NS	NS
CNF	NS	<0.001	<0.001	NS	<0.001
Flutolanil X CNF	NS	NS	NS	NS	NS

[a] Leaf area measured 1 mo after treatment.
[b] Black root rot; rated on scale of 1-5 where 1 = no discoloration or root rot (BRR), 2 = 1-10% of the roots had BRR, 3 = 11-25% of the roots had BRR, 4 = 26-50% of the roots had BRR, 5 = 51-100 % of the roots had BRR.
[c] Control = equivalent amount N applied as 10:10:10.
[d] 3.6 kg/ha applied banded over the row.
[e] 3.3 metric tons/ha.

Flutolanil, a new systemic fungicide with specificity against basidiomycetous fungi (2), had no effect against black root rot of strawberries in our field evaluation. The predominant isolate of *R. fragariae* (AG-A) present in these plots was found to be insensitive to up to 10 ppm flutolanil *in vitro*. It is unknown if the observed insensitivity to flutolanil is specific to this isolate or anastomosis groups (AGA) of *R. fragariae*. Flutolanil is toxic to several anastomosis groups of *R. solani* and will suppress diseases associated with these isolates (2); however, flutolanil has been inconsistent in suppressing root rot of winter wheat caused by AG-8 of *R. solani* and *R. oryzae* Ryker & Gooch (20). Additional research with flutolanil as a potential control of *R. fragariae* and black root rot of strawberries should evaluate other isolates and AG groups *in vitro* and in the field. We conclude that the inclusion of chitin soil amendments or flutalonil applications in strawberry culture may not be effective in suppressing black root rot or increasing yield, and that chitin may indirectly increase disease severity by releasing ammonium.

Literature Cited

1. Afanasiev, M. M., and W. E. Carlson. 1942. The relation of phosphorus and nitrogen ratio to the amount of seedling diseases of sugar beets. **Ann. Amer. Soc. Sugar Beet Tech.**, pp. 1-5.
2. Araki, F. 1985. Moncut (flutolanil), a new systemic fungicide. **Jpn. Pestic. Inf.** 47:23-25.
3. Bird, A. F., and M. A. McClure. 1976. The tylenchoid (nematoda) egg shell: structure, composition and permeability. **Parasitology** 72:19-28.
4. Davet, P. 1979. Technique pour l'analyse des population de *Trichoderma* et de *Gliocladium virens* dans le sol. **Ann. Phytopathol.** 11:529-533.
5. Elad, Y., I. Chet, and Y. Henis. 1982. Degradation of plant pathogenic fungi by *Trichoderma harzianum*. **Can. J. Microbiol.** 28:719-725.
6. Elad, Y, I. Chet, and Y. Henis. 1981. Biological control of *Rhizoctonia solani* in strawberry fields by *Trichoderma harzianum*. **Plant Soil** 60:245-254.
7. Galper, S., E. Cohn, Y. Spiegel, and I. Chet. 1990. Nematicidal effect of collagen-amended soil and the influence of protease and collagenase. **Rev. Nematol.** 13:67-71.
8. Godoy, G., R. Rodriquez-Kabana, R. A. Shelby, and G. Morgan-Jones. 1983. Chitin amendments for control of *Meloidogyne arenaria* in infested soil. II. Effects on microbial populations. **Nematropica.** 13:63-74.
9. Harman, G. E., I. Chet, and R. Baker. 1981. Factors affecting *Trichoderma hamatum* applied to seeds as a biocontrol agent. **Phytopathology** 71:569-572.
10. Huber, D.M. and R.D. Watson. 1974. Nitrogen form and plant disease. **Annu. Rev. Phytopathol.** 12:139-165.
11. LaMondia, J. A., and S. B. Martin 1989. The influence of *Pratylenchus penetrans* and temperature on black root rot of strawberry by binucleate *Rhizoctonia* spp. **Plant Dis.** 73:107-110.
12. Lingappa, Y., and J. L. Lockwood. 1962. Chitin media for selective isolation and culture of actinomycetes. **Phytopathology** 52:317-323.
13. Liu, S. and R. Baker. 1980. Mechanism of biological control in soils suppressive to *Rhizoctonia solani*. **Phytopathology** 70:404-412.
14. Lunt, H. A., H. G. M. Jacobson, and C. L. W. Swanson. 1950. **The Morgan soil testing system.** Connecticut Agr. Exp. Sta. Bull. 541. 60 pp.
15. Martin, S. B, 1989. Identification, isolation frequency, and pathogenicity of anastomosis groups of binucleate *Rhizoctonia* spp. from strawberry roots. **Phytopathology** 78:379-384
16. Miller, P. M., D. C. Sands, and S. Rich. 1973. Effect of industrial mycelial residues, wood fiber wastes, and chitin on plant parasitic nematodes and some soilborne diseases. **Plant Dis. Rpt.** 57:438-442.
17. Muzzarelli, R. A. 1977. Chitin. Pergamon Press, New York, N.Y. 309 pp.
18. Papavivas, G. C. 1985. *Trichoderma* and *Gliocladium:* Biology, ecology and potential for biocontrol. **Annu. Rev. Phytopathol.** 23:23-54.
19. Rodriquez-Kabana, R., D. Boube, and R.W. Young. 1989. Chitinous materials from blue crab for control of root-knot nematode. 1. Effect of urea and enzymatic studies. **Nematropica** 19:53-74.
20. Smiley, R. W., W. Uddin, S. Ott, and K. E. L. Rhinhart. 1990. Influence of flutolanil and tolclofos-methyl on root and culm diseases of winter wheat. **Plant Dis.** 74:788-791.

21. Sneh, B. and Y. Henis. 1972. Production of anti-fungal substances active against *Rhizoctonia solani* in chitin-amended soil. **Phytopathology** 62:595-600.
22. Sneh, B., J. Katan, and Y. Henis. 1971. Mode of inhibition of *Rhizoctonia solani* in chitin-amended soil. **Phytopathology** 61:1113-1117.
23. Spiegel, Y., E. Cohn, and I. Chet. 1986. Use of chitin for controlling plant-parasitic nematodes. 1. Direct effects on nematode reproduction and plant performance. **Plant Soil** 95:87-95.
24. Spiegel, Y., I. Chet, and E. Cohn. 1987. Use of chitin for controlling plant-parasitic nematodes. II. Mode of action. **Plant Soil** 98:337-345.
25. Spiegel Y., E. Cohn, and I. Chet. 1989. Use of chitin for controlling *Heterodera avenae* and *Tylenchulus semipenetrans*. **J. Nematol.** 21:419-422.
26. Spiegel, Y., I. Chet, E. Cohn, S. Galper, and E. Sharon. 1988. Use of chitin for controlling plant-parasitic nematodes. III. Influence of temperature on nematicidal effect, mineralization and microbial population buildup. **Plant Soil** 109:251-256.
27. Swanback, T. R. 1952. **The nitrogen fertilization of Connecticut tobacco.** Connecticut Agr. Exp. Sta. Bull. 559. 23 pp.
28. Tennant, D. 1975. A test of a modified line intersect method of estimating root length. **J. Ecol.** 63:995-1001.
29. Yuen, G. Y., M. N. Schroth, A. R. Weinhold, and J. G. Hancock. 1991. Effect of soil fumigation with methyl bromide and chloropicrin on root health and yield of strawberry. **Plant Dis.** 75:416-420.