THE EFFECTS OF A CHITIN-UREA SOIL AMENDMENT ON GLOBODERA TABACUM TABACUM POPULATION CHANGES UNDER SHADE TOBACCO

TOBACCO SCIENCE

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The preplant application of cottonseed meal-based fertilizer (213 kg N/ha) or chitin-urea amendments (213 or 426 kg N/ha) to soils infested with *Globodera tabacum tabacum* did not affect nematode soil densities or reproduction in shade tobacco over two years of application. Populations of chitinolytic organisms were higher in soil with chitin-urea amendments, but they were not correlated with nematode control or with nonviable *G. t. tabacum* juveniles and eggs. The application of chitin-urea amendments adversely affected leaf quality characteristics in 1989. There were no differences in the fresh weight leaf yields between meal-based fertilizer and chitin-urea amendments.

Additional key words: *Nicotiana tabacum*, tobacco cyst nematode.

INTRODUCTION

The tobacco cyst nematode, *Globodera tabacum tabacum* (Lownsbery & Lownsbery 1954) Behrens 1975 suppresses the growth and yield of Connecticut shade-grown cigar wrapper tobacco (5,8). Currently, *G. t. tabacum* is controlled by preplant soil fumigation with 1,3-dichloropropene (22) or by use of systemic nematicides such as oxamyl (5). Other nematicides have been shown to reduce *G. t. tabacum* densities and increase yields, but these tactics result in reduced leaf quality (22).

Chitin (poly-B-(1-4)-N-acetyl-D-glucosamine) is a polysaccharide component of fungal cell walls, insects (11), and tylenchoid nematode eggs (1). Chitin or chitin-based soil amendments reduce populations of plant parasitic nematodes (6,9,14,17,18), including cyst nematodes such as *Heterodera glycines* Ichinohe (15), *H. avenae* Wollenweber (17), and *H. schachtii* Schmidt (20). A dual mode of action has been proposed for the activity of chitin against nematodes. First, chitin decomposition results in the release of ammonia, which is toxic to nematodes (20). Second, the increase and stimulation of populations of chitinolytic bacteria, actinomycetes, and fungi may increase the biological control activity of amended soils (9,14, 17,18,19,20).

A commercially available chitin-urea amendment (Clandosan 618: 25% chitin amended with organic buffers, urea, and minerals) reduces nematode densities (14). The objectives of this research were: (1) to determine the effects of up to 4.4 metric ton/ha of Clandosan 618 applied in two consecutive years on population densities of *G. t. tabacum* in the soil; and (2) to determine the effects of this chitin-urea amendment on yields of shade tobacco and quality of cigar wrapper tobacco.

MATERIALS AND METHODS

Experiments were conducted in 1989 and 1990 at the Connecticut Agricultural Experiment Station Valley Laboratory in Windsor, Conn. Experimental plots (4.6 X 9.2 m) were established in a cloth-covered shade tent (12) in soil (Entic Haplorthod; 71.8% sand, 23.0% silt, 5.2% clay, pH 6.2, 4.0% organic matter) naturally infested with *G. t. tabacum*. Two weeks before plantings on 18 May 1989, 2.2 or 4.4 ton/ha of Clandosan 618 (10.4-2.3-1.3 N-P-K) or a cottonseed meal-base tobacco fertilizer (5.9-2.8-6.1 N-P-K) at an N rate equivalent to 2.2 ton/ha of Clandosan 618 were broadcast over the plots and incorporated by tilling to 15 cm deep. A preplant application of diazinon (2.2 kg [a.i.]/ha) and metalaxyl (1.1 kg [a.i.]/ha) was incorporated into all plots 24 hours before transplantation (12) according to standard commercial practice. Two-month-old seedlings of the shade tobacco cultivar '0-40' were transplanted on 1 June 1989 to four rows per plot. Plots were one meter apart with 35 cm between plants within rows. All plants were treated identically with respect to sidedress fertilization, cultivation, hand suckering, tying, and harvesting. Tobacco was sidedressed with 28 kg N/ha as cottonseed meal based fertilizer 2, 3, and 4 weeks after transplantation. Foliar insects were controlled by acephate (1 kg [a.i.]/ha) applied to all plots as needed. There were six replicate plots of each treatment.

G. t. tabacum densities in soil were determined by removing and bulking 50 cores (2.5-cm-diam to 15 cm deep) per plot before adding the cottonseed-based fertilizer or the chitin-urea amendment. After harvest and tilling with a disk, soils were sampled again. All soils were air dried and mixed well, and then *G. t. tabacum* cysts were extracted from 250 cm^3 of soil with a modified Fenwick can (5). Cysts were crushed in water and two aliquots of nematodes in suspension were counted to determine the number of free second-stage juveniles (J2) and J2 remaining inside eggs per cm^3 of soil. Viable and nonviable juveniles were visually differentiated and counted separately. Initial *G. t. tabacum* densities ranged 19-86 juveniles per cm^3 of soil. Initial densities averaged 50.1, 45.2, and 44.0 juveniles per cm^3 of soil for the plots that received 0.0, 2.2, and 4.4 ton/ha of the chitin-urea amendment, respectively. Population changes over a season were expressed as the ratio of final (Pf) to initial (Pi) juveniles per cm^3 of soil.

On 22 May 1990, conventional fertilizer or chitin-based nematicide treatments were applied to the same plots as in 1989. Tobacco seedlings (cultivar 0-40) were transplanted on 5 June 1990, and plots were subject to the same conditions and treatments as above.

In both 1989 and 1990, three leaves per plant were harvested on each of six weekly intervals starting on 31 July. The third harvest, typically the best quality, was cured and analyzed for quality characteristics by Culbro, Inc. in a blind test.

G. t. tabacum populations were sampled before treatment and after the final harvest as before. In 1990, populations of chitinolytic organisms were determined by dilution plating three 1-g aliquots of moist soil onto chitin agar (6,7). Serial dilutions of soil suspensions in sterile distilled water were distributed onto the media, spread over the surface with a glass rod, and incubated at ambient temperatures for 10 days. Populations of organisms that formed halos in the media due to chitin degradation were expressed as colony-forming units per gram of soil (oven dry wt equivalent).

Data were analyzed by analysis of variance (ANOVA), and means were separated by the protected least significant difference procedure (16). The ratios of final to initial nematode densities were transformed to arcsines before ANOVA to stabilize variance. Quality rating data from 1989

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Table 1. Effects of chitin-urea amendments or a conventional fertilizer treatment on changes in viable or nonviable *Globodera tabacum tabacum* population densities over two years.

Treatment	Application rate	Viable Pf/Pi[a]			Nonviable Pf/Pi[a]		
		1989	1990	Total	1989	1990	Total
	-- kg N/ha --						
Cottonseed meal fertilizer	213	3.8	2.1	7.7	2.7	1.3	4.0
Chitin-urea[b]	213	3.5	2.3	7.4	3.0	1.6	4.6
Chitin-urea[b]	426	2.6	2.1	5.3	2.2	1.4	3.1
ANOVA		NS	NS	NS	NS	NS	NS

[a]Pf/Pi (ratio of final to initial populations): total = fall 1990/spring 1989 nematode densities.
[b]Clandosan 618.

Table 3. The influence of two years of chitin-urea amendments or conventional fertilizer application on chitinolytic organisms in soil.

Treatment	Application rate	Chitinolytic organisms
	-- kg N/ha --	-- CFU X 10^5 --
Cottonseed meal fertilizer	213	2.4
Chitin-urea[b]	213	8.6
Chitin-urea[b]	426	12.6
LSD (P=0.05)		5.4

[a]Total chitinolytic organisms (bacteria, actinomycetes, and fungi) per g soil (oven dry weight equivalent).
[b]Clandosan 618.

and 1990 were analyzed by the nonparametric Kruskal-Wallis test (2).

RESULTS

The preplant application of the chitin-urea amendment to soils naturally infested with *G. t. tabacum* did not result in significant differences in nematode Pf/Pi ratios between conventional fertilization and chitin-urea treatments in either 1989 or 1990, or after two years of application (**Table 1**). Numbers of nonviable juveniles and eggs recovered from cysts were not different in 1989, 1990, or after two years of chitin-urea application when compared to conventional applications of cottonseed meal-based fertilizer.

There were no differences in fresh leaf weights between treatments that received the meal-based fertilizer or the chitin-urea amendment (**Table 2**). However, leaf quality characteristics differed among treatments in 1989. Leaf color and body were adversely affected by preplant chitin-urea application compared to conventional fertilizer applications. Leaf size, vein quality (thin, inconspicuous veins for wrapper leaves), burn (rate and color), and taste were not affected. In 1990, there were no differences in leaf quality among treatments; however, leaf quality was poor for all treatments.

After two successive years of treatments to field plots, the number of chitinolytic organisms, primarily bacteria and actinomycetes, was greater in plots receiving the chitin-urea amendment (**Table 3**). There were no significant differences in numbers of chitinolytic organisms between the two rates of chitin-urea application.

DISCUSSION

A chitin-urea soil amendment was not effective in reducing *G. t. tabacum* soil populations or in reducing nematode reproduction in shade tobacco over two years of application in Connecticut. Numbers of chitinolytic organisms in soil were higher after two years of application of the chitin-urea amendment. However, the increase in chitinolytic organisms was not correlated with an increase in nonviable, or potentially parasitized, *G. t. tabacum* juveniles and eggs.

The application of chitin-urea amendments significantly decreased body and color quality characteristics of cured leaves in 1989. Leaf quality was generally poor for all treatments in 1990, probably due to high (80-215 J2/cm^3 of soil) *G. t. tabacum* populations.

Cottonseed meals are the main sources of nitrogen in shade tobacco fertilizer, and they were used as an equivalent nitrogen control in these experiments. The breakdown of cottonseed meal proteins to ammonia usually reaches a maximum within two weeks. The production of nitrites from ammonia by *Nitrosomonas* and conversion of nitrite to nitrate by *Nitrobacter* occurs quickly in the light, well-drained soils where shade tobacco is produced (21). Urea and urea compounds are converted to ammonia and nitrates more quickly than cottonseed meal-based fertilizers (3,6). While urea may be substituted for meal fertilizers in shade tobacco production, rapid decomposition can lead to problems in timing nitrate availability with tobacco growth.

Miller et al. (10) showed that 242 kg N/ha applied as cottonseed meal may temporarily suppress tobacco root

Table 2. Effects of chitin-urea amendments or a conventional fertilizer treatment on leaf yield and quality of shade tobacco.

Treatment	Application rate	Yield[a]	Quality Characteristics[b]					
			Body	Color	Size	Veins	Burn	Taste
	-- kg N/ha --	-- kg/plot --						
			1989					
Cottonseed meal fertilizer	213	30.7	1.6	1.6	1.2	1.9	2.5	2.4
Chitin-urea[c]	213	32.0	2.4	2.0	1.6	2.0	1.8	2.0
Chitin-urea[c]	426	30.5	2.9	2.9	2.1	2.0	2.4	2.1
Kruskal-Wallis		-	*	*	-	-	-	-
			1990					
Cottonseed meal fertilizer	213	-	3.0	3.5	2.8	3.2	1.8	2.0
Chitin-urea[c]	213	-	3.0	3.3	3.0	3.3	2.0	2.2
Chitin-urea[c]	426	-	3.3	3.8	3.3	3.7	2.2	2.2

[a]Fresh weight yield, 18 leaves per plant harvested 3 leaves per occasion over 6 occasions.
[b]Quality ratings done by Culbro, Inc., in a blind test; ratings: 1 = excellent to 5 = poor. Rating data analyzed by the nonparametric Kruskal-Wallis Test.
[c]Clandosan 618.
*Significant at P=0.05. There were no significant differences in 1990 quality characteristics.

infection by *G. t. tabacum*. This effect may have been due to transient ammonia levels suppressive to nematodes. Root infection resumed as ammonia levels decreased (10).

The nematicidal effect of chitin-urea observed in other systems is thought to be due partially to the production of ammonia resulting from the decomposition of urea (13,19,20). Anhydrous ammonia reduces soil populations of a number of nematodes at rates in excess of 150 kg N/ha, and urea suppresses nematodes when applied at rates above 300 kg N/ha (13). Most reports of successful nematode control with chitin-urea amendments resulted from the incorporation of 0.3-4.0% amendments (w/w) in pots or small plots (4,6,15,17,20). These levels are higher than those applied in our experiments.

Studies of nematode control by means of inorganic N fertilizers indicated that effective levels far exceeded those required for crop fertilization, and they were more phytotoxic (13). The rates of cottonseed meal and chitin-urea applied in our experiments were not phytotoxic to tobacco, and this may explain the lack of nematicidal activity. Rates of chitin-urea from 1,093 to 1,868 kg/ha were phytotoxic to Brussels sprouts, but they did not control *Heterodera schachtii* populations (23).

Perhaps, because of the quick conversion of ammonia to nitrate by microbes in tobacco soils (21), and because of apparent losses of significant N fertilizers by leaching of nitrate (3,12) or evaporation of ammonia (21), greater applications of chitin-urea amendments than tested in our experiments may be required for control of *G. t. tabacum*. However, nitrate contamination of ground water is a significant hazard associated with shade tobacco production in Connecticut (12). Nitrate management is an important environmental concern and this would seem to exclude excessive rates of chitin-urea amendments, regardless of efficacy against *G. t. tabacum*.

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