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Use of Chitin for Controlling *Heterodera avenae* and *Tylenchulus semipenetrans*[1]

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Abstract: The nematicidal effect of chitin, relative to other pesticides, was evaluated against two plant-parasitic nematodes, *Heterodera avenae* and *Tylenchulus semipenetrans.* Wheat seedlings, grown in soils artificially or naturally infested with *H. avenae,* were treated with 0.4% (w/w) ClandoSan (CLA) prepared from crustacean chitin, aldicarb (Temik 15G), or ethylene dibromide (EDB 90EC). The CLA treatment significantly increased wheat straw, ear, and average grain dry weights of nematode-infected plants, compared with the other two treatments. In an experiment covering two consecutive seasons, all three treatments reduced the number of cysts in the soil by 60%. In a one-season experiment, CLA reduced the number of cysts by 51% and aldicarb or EDB reduced cyst number by about 40%. A reduction of 50–90% in *T. semipenetrans* population densities on roots of two citrus rootstocks was recorded following an application of 0.2% (w/w) CLA to the soil.

Key words: cereal cyst nematode, chitin, citrus, citrus nematode, control, *Heterodera avenae, Triticum aestivum, Tylenchulus semipenetrans,* wheat.

In previous studies (9–11), several aspects of the effect of chitin on nematode-infected plants have been investigated: the nematicidal activity and direct effect on host plants (11), mode of action (9), and the effect of root temperature on mineralization of chitin in soil and on microbial population buildup (10). Studies were limited mainly to the root-knot nematode *Meloidogyne javanica* (Treub.) Chitwood. The objective of the present work was to evaluate the nematicidal effect of chitin on two other sedentary plant-parasitic nematode species: the cereal cyst nematode, *Heterodera avenae* Wollenweber; and the citrus nematode, *Tylenchulus semipenetrans* Cobb.

Materials and Methods

Cereal cyst nematode: In experiment 1 (1985–86) wheat (*Triticum aestivum* L. cv. Lakhish) was sown in 750-cm^3 plastic pots containing 1.2 kg soil (6% clay, 6% silt, 88% sand; pH 8.3–8.5, organic matter 0.3%) which previously had grown wheat and was naturally infested with *H. avenae.* In order to determine the population density of *H. avenae,* soil samples were collected at random from the naturally infested field with a 5-cm-d tube to a depth of 15 cm. Each sample was mixed in a plastic bag, and cysts were extracted from a 200-g subsample to determine cyst numbers per 100 g soil and egg numbers per 20 cysts (1).

Before planting, half of the soil portion had been autoclaved twice for use as a control. ClandoSan (CLA), a crustacean chitin powder; ethylene dibromide (EDB 90EC), water diluted; or aldicarb (Temik 15G) were mixed in a concrete mixer with both infested and uninfested soils at a rate of 0.4% (w/w), 3 µl, and 2 mg per pot, respectively. Aldicarb and EDB application rates are equivalent to the recommended field application rates for nematode control. The pots were maintained from November to March in a screenhouse and were fertilized monthly with a 4-2-8 nutrient solution (Fertilizers and Chemicals Ltd., Haifa, Israel) at a rate of 5 ml/liter water. Shoot fresh and dry weights and ear fresh weights were determined 4 months after planting.

From April to October the pots with rootball intact were maintained, unirrigated, in the screenhouse. In November, after removing the roots from each pot, the soil rootball was treated as detailed in the previous paragraph and replaced in the orig-

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inal pots. Wheat was sown, and the experiment was repeated. Four months later the experiment was terminated and the following were recorded: 1) straw dry weight; 2) ear dry weight; 3) average and total grain dry weights; 4) number of cysts per 100 g soil (1); and 5) number of eggs and larvae per cyst, extracted from 20 cysts from each pot (1).

Experiment 2 (1986), a 1-year experiment similar to experiment 1, used fresh soil similar to the soil in experiment 1 naturally infested with *H. avenae* (population density had not changed significantly from experiment 1). Four months after planting the same five variables were recorded as in experiment 1.

In experiment 3 (1987), wheat was sown in naturally sandy loam soil (15% clay, 38% silt, 47% sand; pH 7.9–8.1, organic matter 0.8%) in 750-cm^3 plastic pots and grown under the conditions described for experiment 1. Before planting, half of the soil portion was inoculated with 26 *H. avenae* cysts (from the same field as experiment 1) per 100 g soil; the other half served as an uninoculated control. The infested and uninfested soils were treated with CLA at 0.4% (w/w) or ethylene dibromide (EDB 90EC) at 3 µl/pot. Four months after planting, the same five variables were recorded as in experiment 1.

In all experiments, plants were arranged in a randomized block design, with 10 replications of each treatment.

Citrus nematode: Seedlings of sour orange (*Citrus aurantium* L.), a rootstock susceptible to the citrus nematode, were planted in soil (12% clay, 36% silt, 52% sand; pH 7.9–8.1, organic matter 0.7%) in 750-cm^3 plastic pots. The soil was infested with 1,500–2,000 juveniles and preparasitic adults of *T. semipenetrans* per 100 g soil. They were maintained in a growth chamber at 25 ± 1 C. Before planting, half of the soil was autoclaved twice, and 0.2% (w/w) CLA was mixed in a concrete mixer with one-fourth of the infested and uninfested soils and then apportioned into pots. After planting, one-fourth of the pots were treated with aldicarb (Temik 15G) at 2 mg/pot and one-fourth with ethylene dibromide (EDB 90EC) at 3 µl/pot. These soil treatments were done in situ by surface application and watering.

Seedlings of rootstock 48/21 (*Poncirus trifoliata* × Poorman orange hybrid), an experimental citrus rootstock highly resistant to the citrus nematode (2), were grown in a peat-sand mixture (1:2) in 750-cm^3 plastic pots, irrigated weekly with 1.5 ml/liter of a 2-4-8 nutrient solution and maintained in a plant growth chamber at 25 ± 1 C. Before planting, half of the peat-sand mixture was mixed with 0.2% (w/w) CLA. Half of all pots, both CLA amended and not amended, were inoculated with ca. 18,000 juveniles and preadults of *T. semipenetrans* per pot.

Five months after inoculation, all plants were harvested and weighed. Juveniles and preadult nematode stages were extracted by incubating roots in modified Baermann funnels for 48 hours and were counted.

Pots were arranged in a randomized block design, with 10 replications of each treatment. The test was performed twice.

Results and Discussion

Cereal cyst nematode control: ClandoSan was as effective as EDB or aldicarb in increasing plant yield and controlling the nematode. Straw dry weights of *H. avenae*-infected wheat treated with CLA were higher by 20 (nonsignificant), 48, and 162% (significant) ($P < 0.05$) than those of nematode-infected, untreated plants in experiments 1, 2, and 3, respectively (Fig. 1A). Ear dry weights of nematode-infected plants increased significantly ($P < 0.05$) by 51, 38, and 286% in the CLA treatments and 21, 24, and 206% in the EDB treatments, compared with untreated infected plants in experiments 1, 2, and 3, respectively (Table 1). Aldicarb did not affect this parameter (Table 1). Comparing grain dry weights of CLA-treated and untreated infected plants revealed significant differences ($P < 0.05$) of 35, 62, and 290% in experiments 1, 2, and 3, respectively. In aldicarb-treated or EDB-treated plants, these differences were only 23, 32, and 190% (Table 1).

In all experiments, wheat grown in CLA-

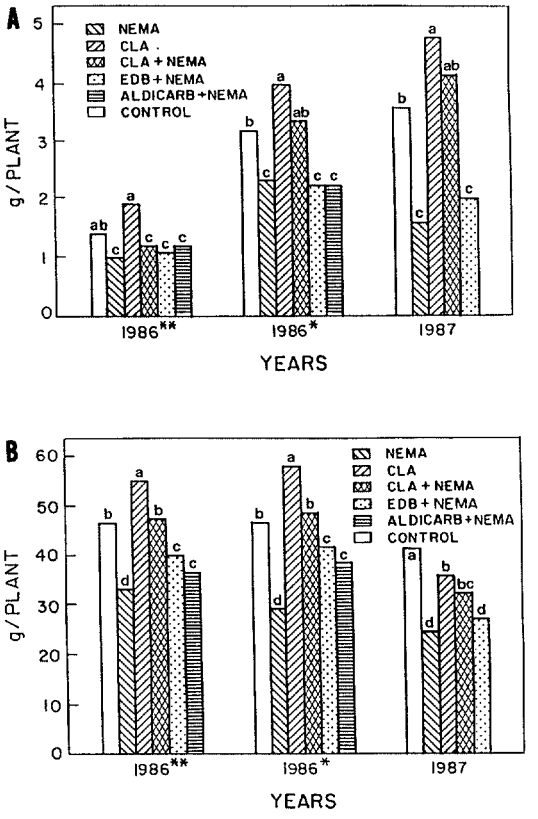

FIG. 1. Wheat yield from *Heterodera avenae*-infested soil. * = data from experiment 1, a two-season test; ** = data from experiment 2, a one-season test; 1987 = data from experiment 3, a one-season test. Within years, means followed by a common letter are not significantly different ($P = 0.05$) according to Duncan's multiple-range test. Twice autoclaved infested soil was used as a control. A) Straw dry weights of wheat from soil infested with *Heterodera avenae* and treated with aldicarb (2 mg/pot), ethylene dibromide (3 µl/pot), or 0.4% (w/w) ClandoSan. B) Total grain yield of wheat from soil infested with *Heterodera avenae* and treated with aldicarb (2 mg/pot), ethylene dibromide (3 µl/pot), or 0.4% (w/w) ClandoSan.

amended soil infested with *H. avenae* yielded about 1.5 times more grain than that grown in untreated infested soil (Fig. 1B). This yield increase on nematode-infested and sometimes in uninfested soil amended with CLA (Fig. 1, Tables 1, 2) is due in part to a fertilizer effect from ammonia released during the microbial decomposition of CLA (10). Neither aldicarb nor EDB affected yields of nematode-free plants.

As a nematicide, CLA was as effective as EDB or aldicarb against *H. avenae*. Aldicarb, CLA, or EDB added to the soil decreased the number of cysts per 100 g soil by 60% in experiment 1 and by 51% (CLA) and 40% (aldicarb and EDB) in experiment 2 (Table 2). In experiment 3 the decrease in *H. avenae* population level was insignificant (Table 2). In experiment 1, aldicarb, CLA, and EDB treatments resulted in 40–50% fewer eggs per cyst, whereas in experiments 2 and 3, only EDB produced such results (Table 2).

The reduction in phytonematode numbers following chitin amendment to the soil was, at least in part, due to the presence of fungi, bacteria, or actinomycetes with chitinolytic capacity (7,8). Nematode numbers increased as a result of the reduction in fungi known to be parasitic on *H. avenae* females (3,4). We may assume, therefore, that the outstanding efficacy of CLA in experiment 1, a two-season experiment, was probably due to a cumulative effect, resulting in a higher buildup of chitinolytic micro-organisms. Moreover, the biocontrol influence probably would be more ap-

TABLE 1. Ear and grain dry weights of wheat seedlings grown in soil infested with *Heterodera avenae* and treated with ClandoSan, aldicarb, or ethylene dibromide (EDB).

Treatment	Ear dry wt. (g/plant)			Grain dry wt. (g/ear)		
	Exp. 1 1985–86†	Exp. 2 1986	Exp. 3 1987	Exp. 1 1985–86†	Exp. 2 1986	Exp. 3 1987
Control‡	2.25 b	2.40 b	1.85 abc	1.87 cd	1.89 bc	1.39 a
Nematode	1.82 c	2.10 d	0.51 d	1.54 e	1.46 d	0.29 d
ClandoSan	2.55 ab	3.07 a	2.15 a	2.35 a	2.39 a	1.56 a
ClandoSan + nematode	2.75 a	2.89 b	1.97 ab	2.08 ab	2.36 ab	1.13 b
Aldicarb + nematode	1.96 bc	2.30 cd		1.89 cd	1.77 bc	
EDB + nematode	2.20 b	2.60 c	1.56 abc	1.89 cd	1.93 bc	0.84 c

Data are means of 10 replications. Within columns, means followed by a common letter are not significantly different ($P = 0.05$) according to Duncan's multiple-range test.

† Two-season test.

‡ Twice autoclaved, infested soil.

TABLE 2. *Heterodera avenae* cysts and eggs extracted from soil inoculated with *H. avenae* and treated with ClandoSan, aldicarb, or ethylene dibromide (EDB) and planted in wheat.

Treatment	Cysts/g soil			Eggs/cyst		
	Exp. 1 1985–86†	Exp. 2 1986	Exp. 3 1987	Exp. 1 1985–86†	Exp. 2 1986	Exp. 3 1987
Untreated	285 a	226 a	172 a	356 a	282 a	99 a
ClandoSan	112 b	111 b	149 a	211 bc	343 a	132 a
Aldicarb	111 b	126 b		219 bc	328 a	
EDB	117 b	148 b	141 a	162 c	191 b	44 b

Data are means of 10 replications. Within columns, means followed by a common letter are not significantly different ($P = 0.05$) according to Duncan's multiple-range test.
† Two-season test.

parent in soils with established populations of antagonistic organisms, as in the naturally infested soils in experiments 1 and 2, than in the artificially infested soil in experiment 3.

Citrus nematode control: Shoot fresh weights of citrus plants growing in soil with the various treatments did not differ; however, roots of the susceptible sour orange rootstock had significantly ($P = 0.05$) fewer nematodes when treated with CLA, aldicarb, or EDB. Decreases of as much as 90% were observed. From the roots of untreated infected plants, 1,220 ± 27 nematodes/g root were extracted, compared with only 90 ± 15 from CLA-treated roots. A nematicidal effect was evident in the resistant rootstock 48/21 as well; infected untreated roots yielded 58 ± 7 nematodes/g root, whereas CLA-treated roots yielded only 29 ± 5. Therefore, our results confirm those of Mankau and Das (5), who, under different experimental conditions, reduced the citrus nematode population by 83% with a chitin application.

Although ClandoSan's mode of action was not investigated in this study, its ability to control the two plant-parasitic nematode species may be assumed to be mediated by two related processes, as previously reported (6,9,10). CLA decomposition releases ammonia (10), which acts as a nematicide on the second-stage juveniles and stimulates an increase in chitinolytic microflora (6,8,10). Nematode eggs, egg sacs, and cysts are parasitized (8), resulting in an overall reduction in nematode numbers in following generations. Therefore, CLA apparently is a potential nematicide against *H. avenae* and *T. semipenetrans* in addition to *Meloidogyne* spp. (6,11).

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