Control of Edaphic Populations of Woolly Apple Aphid using Entomopathogenic Nematodes and a Systemic Aphicide[1]

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ABSTRACT The effects of an entomopathogenic nematode, *Steinernema carpocapsae* (Weiser), and an experimental systemic aphicide, RH-7988, on edaphic populations of the woolly apple aphid, *Eriosoma lanigerum* (Hausmann), were investigated. Laboratory experiments showed that presence of the nematode in a culture of woolly apple aphids increased the mortality rate. Nematodes were found inside the body cavity of several aphids with entry possibly being through the anus via a droplet of honeydew. Field trials in an unsprayed six-year-old and four-year-old apple orchard tested the efficacy of broadcast spray and topdressing applications, respectively, of nematodes at a rate of 376,600 nematodes/m^2. The broadcast spray trees had fewer aphid colonies on roots than the untreated controls ($P = 0.10$), but the topdressing treatment had no effect. The systemic aphicide, RH-7988, was tested in a two-year-old apple orchard. Two rates of foliar and soil application were tested, with all treatments significantly reducing arboreal woolly apple aphid populations. Edaphic populations were also significantly reduced one month after treatment, but no difference was found four months after treatment. Both control methods show promise as potential management options for edaphic woolly apple aphid populations.

KEY WORDS Apple, *Eriosoma lanigerum, Steinernema carpocapsae,* biological control, aphicide.

The woolly apple aphid, *Eriosoma lanigerum* (Hausmann) (Homoptera: Aphididae), feeds on shoots, branches, wounds and roots of apple trees. Arboreal populations generally are controlled with standard orchard spray programs and biological control, especially the parasite *Aphelinus mali* (Haldeman) (Hymenoptera: Aphelinidae). Control of edaphic populations, feeding on roots, however, has not been adequately examined. Studies on the control of woolly aphids on roots have been conducted only on nursery stock (Lohrenz 1911, Greenslade 1936, Smith 1942, Stanley 1951, Attri and Sharma 1971) and potted trees (Klostermeyer and Williams 1980), and most of these studies used chemicals which are no longer registered. There are no currently recommended control methods for edaphic populations of woolly apple aphids in orchards.

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[2] This paper reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation for its use by USDA.
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Brown (1986) has shown that roots of most mature apple trees in eastern West Virginia were infested with woolly apple aphids, and Brown and Schmitt (1990) have shown that edaphic populations reduce apple tree growth in the orchard. This study was conducted to examine the possibility of controlling edaphic woolly apple aphids in an orchard environment. The insect-parasitic nematode, *Steinernema carpocapsae* (Weiser) (Rhabditida: Steinernematidae), and an experimental, selective aphicide with systemic activity, RH-7988 (Rohm and Haas Co., Philadelphia, PA), were used for these trials.

Materials and Methods

Nematodes-Laboratory Study. The experiment was begun in ten glass petri dishes, 9 cm inside diameter. Into each dish were added 40 ml of dry sterile sand and 12 ml deionized water. A 1.5 to 3.0 cm section of infested apple stem (M.7A clones) from a laboratory colony of woolly apple aphids was placed into a groove in the sand. The laboratory colony was established with aphids collected in Kearneysville, WV, on 31 January and 1 February, 1989. The ten dishes were divided into two groups so that each group contained approximately equal total lengths of infested stems. The number of aphids per dish ranged from 33 to 123 (mean of 83) as determined from counts at the end of the study. Into each of five dishes were added approximately 2700 active nematodes in 0.1 ml of deionized water. The water was placed at each end of the infested twig. The nematode density used was equivalent to the 376,600/m^2 (35,000/ft^2) recommended for field use (BioLogic, Willow Grove, PA). The remaining five dishes were treated with 0.1 ml of deionized water, without nematodes, as a control. All ten dishes were placed in the dark for the duration of the study except during examinations.

At daily intervals, each dish was examined and the number of dead (nonresponsive when touched with a metal probe) woolly apple aphids removed and counted. Ten dead aphids from each dish were examined for nematodes. Each was examined externally under a stereomicroscope for nematodes and then was crushed in deionized water to access internal parasitism. Each dish was also examined under a dissecting stereomicroscope for active, free-living nematodes. Water was added to the dish during examination if the sand appeared dry. At the end of 5 days, the study was concluded because of high mortality and the large number of aphids wandering on the sand in the control dishes. All living and dead aphids were counted at that time. Percent mortality was compared between treated and control dishes with a t-test for each day of the experiment.

Nematodes-Field Studies. Two methods of applying nematodes to orchard trees were tested. On 28 July, 1989 an aqueous suspension of nematodes was sprayed on the soil around the base of 16 six-year-old trees in a Hagerstown sandy loam soil. The soil surface was moist as a result of an unusually wet July, including a 1.25 cm rainfall on 25 July (Fig. 1). Treated trees were in a block of an experimental orchard in Kearneysville, WV; control trees were adjacent to the treated trees. Selection of treated and control trees was not randomized in order to minimize any migration of nematodes into the root zones of control trees. These trees were sprayed with permethrin (0.2 lbs AI/A, 0.22 kg AI/ha) on 21 April, 19 May, and 30 June, 1989 to build up woolly apple aphid populations (Penman and Chapman 1980). This was the only insecticide used on these trees.

Because the nematodes require an aqueous environment, a pretreatment of 11 liters of water were sprayed on the ground covering the area within the drip line of all 16 treatment trees and 18 control trees, applied at the rate of 15 liters/min with a handgun sprayer. The treatment trees received an additional 11 liters of water containing 485,500 nematodes per liter. The area covered was 14 m^2, giving the recommended rate of 376,600 nematodes/m^2 (35,000/ft^2). The soil at the base of the check trees was then sprayed with another 11 liters of water as a control. The sprays were concentrated at the base of the tree trunk and at root sprouts, but covered the entire area under the tree branches. Application was made between 1700 and 2000 hrs EDT. Another 3.8 cm of rain fell on 30 July. On 26 October all treatment and control trees were uprooted and the number of woolly apple aphid colonies on the roots were counted. Colonies were recorded in size classes of 1-5, 6-20, 21-50, or more than 50 aphids per colony. A chi-square test was used to compare treatment with the control.

The second method of nematode application was as a topdressing on 25 September. Approximately seven million nematodes in vermiculite (Scanmask, BioLogic, Willow Hill, PA) were mixed with deionized water and added to 4700 cm^3 (5 quarts) of peat moss. Five, four-year-old 'Delicious' trees were treated, and another five in the same row of trees were used as controls with a one tree buffer between treated and control trees to minimize the effect of nematode migration. The only insecticide applied to these trees was permethrin as described in the previous test. Water was applied at a rate of 15 liters per tree to wet the soil. Nematodes were spread on the soil surface beneath the branches of the treated trees in 940 cm^3 of peat moss at a rate of 376,600 nematodes/m^2 (35,000/ft^2). An equal amount of peat moss without nematodes was spread around the base of control trees. Another 15 liters of water/tree, on treated and control trees, were sprayed to facilitate nematode penetration into the soil. Soil moisture was high at the time of treatment because of 1.2 cm rainfall during the previous four days and 5 cm during the following four days (Fig. 1). Trees were uprooted on 26 October and data were collected and analyzed as in the broadcast spray study.

RH-7988. Seventy-two 'Delicious' trees were planted in May, 1989, in a Latin square design replicated four times in Kearneysville, WV. Each Latin square had three rows of six trees, with a plot consisting of two trees. In July, 1989, the root closest to the soil surface of half the trees, one tree from each two-tree plot, was exposed and reburied after placing a 2 to 5 cm section of apple stem infested with woolly apple aphids from a laboratory colony in contact with the root. Treatments were a control, and four rates of RH-7988 (two foliar rates, two soil drench rates). Two squares were treated with a soil drench and two with a foliar spray. The only other insecticide applied on these trees was permethrin, applied at the same rate and dates as in the nematode studies. RH-7988 was applied to the trees as a foliar spray at 0.07 and 0.14 kg ai/ha (1/16 and 1/8 lb ai/A; or 0.17 and 0.34 g ai/tree) and soil drench at 0.28 and 0.56 kg ai/ha (1/4 and 1/2 lb ai/A; or 0.68 and 1.35 g ai/tree) on 7 June 1990. The foliar spray used 1.25 liters (1/3 gal) of water per tree and the soil drench used 7.5 liters (2 gal) of water per tree. The number of woolly apple aphid colonies above the ground was counted every two weeks prior to treatment. Counts of arboreal colonies were made 4, 7, 14, 21, and 32 days after treatment. Half of the

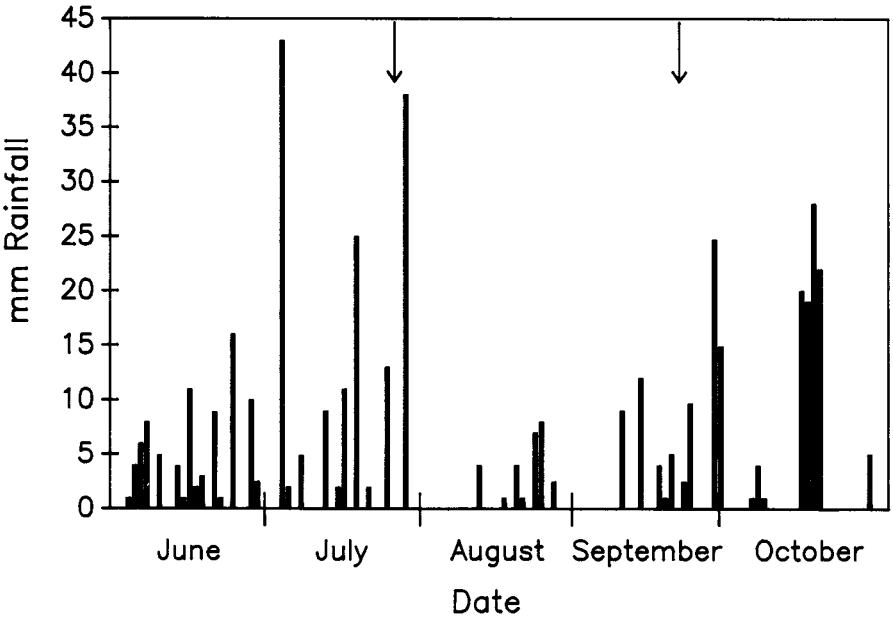

Fig. 1. Daily rainfall in Kearneysville, West Virginia, from June to October, 1989. Dates of nematode application are indicated with arrows: 28 July, broadcast spray applied; 25 September, topdressing applied.

trees (one Latin square from each application method) were uprooted on 5 July 1990, to evaluate root galling and to count active colonies on roots; the remaining trees were uprooted and evaluated on 12-14 October, 1990. Root galling is a result of prolonged feeding by woolly apple aphids, and therefore would not have been affected by a treatment one month earlier. Root galls are a permanent record of past infestation, serving as the only possible estimate of pre-treatment infestation levels, whereas the number of aphid colonies would reflect the effectiveness of a treatment. Arboreal woolly apple aphid control was evaluated with Latin square analysis of variance and Duncan's multiple range test. Edaphic woolly apple aphid control was analyzed with chi-square.

Results

Nematodes-Laboratory Study. Percent mortality in the treated and control petri dishes is presented in Fig. 2. There was significantly greater mortality in the inoculated dishes from 2 to 5 days after inoculation with nematodes. Four days after inoculation there was an average mortality of 77%, with 100% mortality in two of the five dishes. Control mortality averaged 24%, ranging from 3 to 57%.

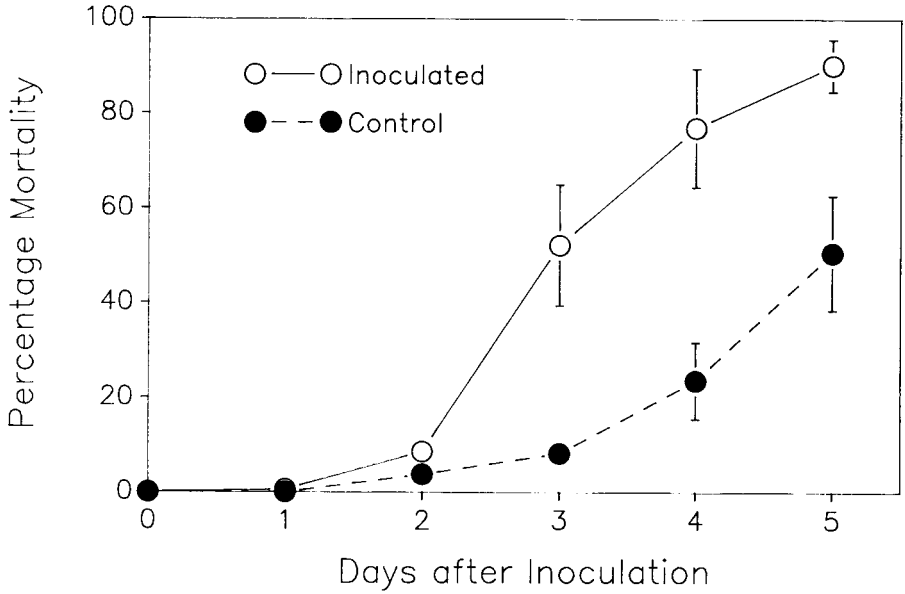

Fig. 2. Mortality of woolly apple aphid in petri dishes in the laboratory inoculated with the parasitic nematode, *Steinernema carpocapsae,* and uninoculated controls. Error bars represent standard error, sample size was five dishes per treatment; where error bars are absent the standard error is smaller than the size of the data point.

Live nematodes were observed in droplets of honeydew during examination of inoculated petri dishes. Woolly apple aphid honeydew occurs as spheres contained within the hydrophobic wax secreted by the aphids. Nematodes were frequently in these spheres of honeydew, whether attached to the aphid or detached. Live nematodes were observed in all the inoculated petri dishes throughout the study; none were observed in the control dishes. No nematodes were found externally on dead aphids. The body of the woolly apple aphid is hydrophobic because of wax secretions and therefore presents an inhospitable environment for nematodes. Several dead aphids from the inoculated dishes (about 5 to 10% of those examined) had live nematodes internally. Upon crushing in water, nematodes were readily seen swimming about the internal organs of the aphid.

Nematodes-Field Studies. The numbers of woolly apple aphid colonies on the roots of the trees in the two field studies are reported in Table 1. In the broadcast application, there were significantly fewer colonies in the treated trees ($X^2 = 7.30$, $P = 0.10$). There were no differences in the number of colonies in the topdressing treatment. All the trees had a similar intensity of root galling, indicating that all the trees have had about the same intensity of woolly apple aphid population pressure prior to this study.

Table 1. Numbers of woolly apple aphid colonies by size class on roots of apple trees treated with nematodes and on untreated trees.

Application method	Treatment	No. trees	Number of colonies by size class (aphids per colony) 1-5	6-20	21-50	>50	Total
Broadcast spray	Control	18	10	7	9	3	29
	Inoculated	14	3	5	3	2	13
					$X^2 = 7.30$, $p < 0.10$*		
Topdressing	Control	5	7	1	0	1	9
	Inoculated	5	9	1	4	1	14
					$X^2 = 4.25$, $p > 0.10$*		

* Chi square analyses tested for deviations from random distribution of aphid colonies between control and inoculated; expected frequency matrix was calculated with fixed column totals. Null hypothesis: the probability of an observed colony being on a control tree was equal to the probability of it being on an inoculated tree.

RH-7988. Results of the insecticide treatment on arboreal populations of woolly apple aphids is given in Table 2. There were no pre-treatment differences among plots. Both the foliar and soil drench applications reduced woolly apple aphid populations. The soil drench significantly reduced the number of aphid colonies for one week after treatment at both rates of the insecticide. In the foliar application, the number of aphid colonies was significantly reduced for two and three weeks at 0.07 and 0.14 kg ai/ha, respectively. After 9 July, the woolly apple aphid population was too low to reveal any differences.

Although the number of colonies on roots was small, only two of the nine colonies observed were found on treated trees one month after treatment (Table 3). Both the foliar and soil drench applications significantly reduced the number of root colonies ($X^2 = 4.77$, $P = 0.05$; $X^2 = 6.83$, $P = 0.01$; respectively). The analysis compared only control versus treated trees, regardless of the concentration of insecticide. In the second evaluation, in October, only five trees had colonies on the roots (three on control trees, two on trees receiving the low rate); too few to draw meaningful conclusions.

Discussion

Insect-parasitic nematodes have been used to control a wide range of soil inhabiting and wood boring insects (Poinar 1979, Kaya 1985). This study is the first to report mortality in an aphid caused by nematodes. Small host size prevents successful parasitism (i.e., reproduction in the host) of the woolly apple

Table 2. Arboreal woolly apple aphid colonies per tree before and after treatment with the systemic aphicide, RH-7988. Means followed by a different letter are significantly different within row and within method of application as determined by Duncan's multiple range test, P = 0.05.

	Soil Drench			Foliar Spray		
Date	Control	0.28*	0.56*	Control	0.07*	0.14*
8 May	1.54 a	0.00 a	0.18 a	0.00 a	0.67 a	0.17 a
16 May	0.54 a	0.42 a	0.45 a	1.33 a	0.67 a	0.58 a
22 May	3.09 a	3.83 a	2.54 a	3.67 a	3.00 a	4.50 a
29 May	2.00 a	1.00 a	1.36 a	1.58 a	1.50 a	1.25 a
5 June	10.36 a	9.92 a	9.45 a	13.33 a	14.83 a	13.67 a
7 June			Plots Treated			
11 June	6.18 a	1.50 b	0.45 c	4.67 a	0.58 b	0.08 c
14 June	5.09 a	2.50 b	1.45 c	3.75 a	1.00 b	0.50 b
21 June	3.45 a	1.42 a	1.36 a	2.50 a	0.67 b	0.67 b
28 June	2.54 a	0.92 a	1.00 a	1.17 a	0.50 a	0.08 b
9 July	0.83 a	0.33 a	0.00 a	0.17 a	0.50 a	0.17 a

* Application rate in kg ai/ha.

aphid by *S. carpocapsae*. However, nematodes were clearly responsible for aphid mortality and did enter the aphid's body cavity. A possible cause of mortality could be infection of the aphids by the nematodes' symbiotic bacteria (Poinar and Thomas 1966). Another cause might be physical damage caused by the nematodes. A single infective nematode could puncture the bodies of several aphids.

The field test results demonstrate a potential for biological control of edaphic populations of the woolly apple aphid. Despite the low number of trees and aphid colonies (averaging less than 3 colonies per tree), we were able to show a statistical decrease in the number of woolly apple aphid colonies on roots as a result of a broadcast spray of nematodes. Both the field and laboratory tests indicate that more research on the use of nematodes for biological control of woolly apple aphids is warranted.

The systemic aphicide, RH-7988, did control both edaphic and arboreal populations of *E. lanigerum*. Woolly apple aphid populations were at a peak when treated and declined rapidly even in the control plots, as is the general pattern in eastern West Virginia (MWB, unpublished data). Populations on treated trees declined significantly faster than on the control trees, however. As with the nematode study, despite low populations on roots and small number of trees sampled, we demonstrated that the aphicide was able to provide some degree of

Table 3. Number and percentage of edaphic woolly apple aphid colonies on RH-7988 treated and control apple trees evaluated on 5 July 1990 (treatment on 7 June 1990).

	Soil Drench			Foliar Spray		
	Control	0.28*	0.56	Control	0.07	0.14
Number of colonies	3	0	1	4	1	0
Percent trees infested	60	0	20	67	17	0
Statistic†	$X^2 = 6.83$, $p < 0.01$			$X^2 = 4.77$, $p < 0.05$		

* Application rate in kg ai/ha.

† Chi square analyses tested for independence among control and treated, with and without aphids, expected frequency matrix was calculated using fixed row and column totals. Null hypothesis was that treatment had no effect on the probability of a tree being infected.

control. The results of this study show that RH-7988 is systemic in activity against the woolly apple aphid and is translocated in the tree, both from the roots to the leaves and from the leaves to the roots. Laboratory studies have shown that RH-7988 does not affect nematodes (JJJ, unpublished data) or beneficial insects (Rohm and Haas Co. 1988) at the rates used, making this material compatible with biological control of woolly apple aphid and other pests in the orchard.

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References Cited

Attri, B. S. and P. L. Sharma. 1971. Granular systemic insecticides for the control of woolly aphid, *Eriosoma lanigerum* (Hausm.), on apple (*Malus pumila* Mill.). Indian J. Agric. Sci. 41:627-631.

Brown, M. W. 1986. Observations on woolly apple aphid, *Eriosoma lanigerum* (Hausmann) (Homoptera: Aphididae), root infestation in eastern West Virginia. Melsheimer Entomol. Ser. 36:5-8.

Brown, M. W. and J. J. Schmitt. 1990. Growth reduction in nonbearing apple trees by woolly apple aphids (Homoptera: Aphididae) on roots. J. Econ. Entomol. 83:1526-1530.

Greenslade, R. M. 1936. Horticultural aspects of woolly aphis control together with a survey of the literature. Imperial Bur. Fruit Production, Tech. Comm. No. 8. East Malling Res. Sta., Kent, England.

Kaya, H, K. 1985. Entomogenous nematodes for insect control in IPM systems, pp. 283-302. *In* M. A. Hoy and D. C. Herzog [eds.], Biological Control in Agricultural IPM Systems. Academic, Orlando, Fla.

Klostermeyer, L. E. and H. E. Williams. 1980. Apple, woolly apple aphid control on containerized nursery apple trees, 1980. Insecticide and Acaricide Tests 6:25.

Lohrenz, H. W. 1911. The woolly aphis, *Schizoneura lanigera*. J. Econ. Entomol. 4:162-171.

Penman, D. R. and R. B. Chapman. 1980. Woolly apple aphid outbreak following use of fenvalerate in apples in Canterbury, New Zealand. J. Econ. Entomol. 73:49-51.

Poinar, G. O., Jr. 1979. Nematodes for Biological Control of Insects. CRC Press, Boca Raton, Fla.

Poinar, G. O., Jr. and G. M. Thomas. 1966. Significance of *Achromobacter nematophilus* Poinar and Thomas (Achromobacteraceae: Eubacteriales) in the development of the nematode DD-136 (*Neoaplectana* sp. Steinernematidae). Parasitology 56:385-390.

Rohm and Haas Company. 1988. RH-7988 Selective Systemic Aphicide. Technical Bulletin-1988. Philadelphia, PA .

Smith, C. F. 1942. The use of cyanide in controlling the root form of the woolly apple aphid. J. Econ. Entomol. 35:908-910.

Stanley, W. W. 1951. Experiments to control the woolly apple aphis on nursery stock. J. Econ. Entomol. 44:1006-1007.