

## Systemic Effects of a Neem Insecticide on *Liriomyza huidobrensis* Larvae

Phyllis G. Weintraub and A.R. Horowitz<sup>1</sup>

In an effort to expand the spectrum of larvicides effective against the pea leafminer, *Liriomyza huidobrensis* (Blanchard), we studied the effects of a neem-based insecticide (Neemix-45) on the development of the leafminer under laboratory conditions. Bean plants were treated with a soil drench of 1, 5, 10 or 25 ppm azadirachtin or by dipping leaves in 1 or 15 ppm azadirachtin at various times before or during the development of the leafminer. Treating the plants with the neem insecticide before exposure to egg-laying adults had a greater effect on inhibiting the development of pupae and adult eclosion than treatment at the 1st-instar larval stage. The systemic effects from a soil drench had a greater adverse effect on pupation and adult eclosion than leaf dipping. Drenching plants with 1 ppm azadirachtin 24 h before exposure to adults had a greater effect (0% adult eclosion) than leaf dipping at the same time period and concentration (15.6% adult eclosion). Similar results were obtained when drenching plants infested with 1st-instar larvae with 1 ppm azadirachtin (11.7% eclosion) vs dipping leaves at the same time period and concentration (44.7% eclosion).

KEY WORDS: *Liriomyza huidobrensis*; neem-based insecticide; Neemix-45; systemic effects by soil drenching; leaf dipping.

### INTRODUCTION

The pea leafminer, *Liriomyza huidobrensis* (Blanchard), is a serious pest of flower and vegetable crops in greenhouses and open fields. It appeared in Israel in 1992 and has been refractory to insecticides (14). Both the larvae and adults cause damage; the larvae primarily mine the spongy mesophyll, where chloroplasts are located (as opposed to *Liriomyza trifolii*, which mines the non-chloroplast-containing palisade mesophyll) (10), and the adult females puncture both the upper and lower leaf surfaces to feed and lay eggs. This results in reduction of both plant vigor and yield, and in cosmetic damage to leaves and stems.

There are currently no effective adulticides, and few effective larvicides (primarily abamectin and cyromazine) (12) against this pest; moreover, exclusive use of these larvicides could quickly generate resistance. Botanical insecticides derived from the seed of the neem tree, *Azadirachta indica* (Meliaceae), have shown promise due to their physiological (insect growth regulating) and antifeedant effects on a diversity of phytophagous insects (see reviews 1,7).

Although a recent report (5) has shown detrimental effects of neem insecticides to aphid predator and parasitoid populations in the laboratory, the same effects were not

---

Contribution from the Agricultural Research Organization. No. 2056-E, 1997 series. Received February 24, 1997; received in final form May 28, 1997.

<sup>1</sup>Dept. of Entomology, ARO, Gilat Experiment Station, M.P. Negev 85280, Israel [Fax: +972-7-9926485; e-mail: vprami@volcani.agri.gov.il].

observed under field conditions. These authors suggest that predators and parasitoids were not affected because of ultraviolet degradation of azadirachtin (the most important active ingredient of neem insecticides), avoidance of treated plant parts, and rapid recolonization. Further, in field tests, no translocation of azadirachtin-contaminated nectar and pollen to larval honey bees was detected, and the LD<sub>50</sub> of azadirachtin on larval honey bees was higher than in most other insect species (8). Neem insecticides are attractive for use in integrated pest management programs because of their low contact toxicity and need to be directly ingested by insects to be effective (11).

Neem formulations have been tested for their effects on feeding and oviposition in *L. trifolii* and *L. sativae*, but not *L. huidobrensis*, and results are equivocal, ranging from no deterrent effects (6,13) to a reduction in oviposition (2). It was found that *L. trifolii* larvae are adversely affected (reduction in pupation) by neem formulations when applied as a soil drench (6,9,13). Parkman and Pienkowski (9) also demonstrated adverse effects on the fecundity and longevity of adult *L. trifolii* when treated with azadirachtin, from a neem seed extract, in the larval stage. Initial trials utilized neem insecticides that differed widely in extraction procedures, formulation and azadirachtin content (7). Isman *et al.* (3) have shown that the azadirachtin content greatly affects insecticidal activity, and argue for standardization. Herein, we report the systemic effects of a commercially available neem insecticide, of known azadirachtin content, applied either as a soil drench or a leaf dip, on pupation and eclosion in *L. huidobrensis* under laboratory conditions.

## MATERIALS AND METHODS

### *Plants*

Bulgarian variety beans (*Phaseolus vulgaris*) were seeded in 10-cm-diam pots (4–6 per pot) with holes in the bottom, with soil consisting of equal parts peat moss, vermiculite and sand. Plants were grown at 24±2°C, with a photoperiod of 14:10 h (L:D), until two true leaves were fully expanded, unless otherwise stated.

### *Insecticide application*

Neemix-45 (4.5% azadirachtin, produced by W.R. Grace & Co. - Conn., Columbia, MD, USA) was used in all bioassays and applied to the soil or leaves of bean plants. Although this product contains additional triterpenes, application rates are expressed in terms of ppm of azadirachtin, the most important active constituent. Dilutions of Neemix-45 were prepared immediately before use in 0.01% aqueous solutions of the surfactant Agral 90 (Zeneca Agrochemicals, UK). Plants were not watered for 2 days before the soil was drenched (with 75 ml) to runoff with dilutions of 1, 5, 10 or 25 ppm azadirachtin, or 0.01% Agral 90 for control groups. In leaf-dip trials soil was first watered to runoff, then covered with thick layers of paper toweling to prevent neem dilutions from dripping off the leaves and onto the soil and thereby causing any systemic effect. Leaves were dipped for 10 sec in dilutions of either 1 or 15 ppm azadirachtin, or in the 0.01% Agral 90 solutions for control groups. To avoid possible adverse effects from the larvae being crowded on the leaf and stressed because of inadequate food, only leaves with 4 to 12 eggs or larvae were used.

### *Bioassay*

In all trials, plants were placed for 24 h in a cage with a small number of adults from the stock *L. huidobrensis* colony, after which eggs were counted. For the trial in which plants were treated 8 days before exposure to adult leafminers, the soil of 2-day-old seeded beans was drenched with azadirachtin dilutions and then watered as per standard practices thereafter; when two true leaves were fully expanded, plants were exposed to adult leafminers. In trials involving treatment of larvae, the number of 1st-instar larvae was counted before treatment by using a stereo-microscope with substage lighting. In all trials, plants were checked daily until the first 3rd-instar larvae were observed. Plants were then cut at soil level and stems were pushed through the parafilm covering of Erlenmeyer flasks filled with water. Parafilm thus prevented prepupae/pupae from falling inside the water-filled flasks. Prepupae were allowed to pupate on the leaves or where they had fallen, but were removed once daily after they were fully sclerotized, and were observed daily for eclosion. Pupation is expressed as percentage of the number of eggs, and eclosion as percentage of the number of pupae.

Soil drench trials consisted of four insecticide application regimes: (i) 8 days or (ii) 24 h before plants were exposed to leafminer adults; (iii) immediately after removing adults from the plants; or (iv) when 1st-instar larvae were observed. Leaf dip trials consisted of three insecticide application regimes: (i) dipping leaves 24 h before plants were exposed to leafminer adults; (ii) immediately after removing adults from the plants; or (iii) when 1st-instar larvae were observed.

### *Data analysis*

Each trial was repeated at least three times (for a minimum of 12 plants per concentration and treatment regime of azadirachtin). All data were transformed by arcsine, analyzed by completely randomized one-way analysis of variance (ANOVA), and means were separated by Tukey-Kramer multiple range testing.

## RESULTS AND DISCUSSION

### *Soil drench trials*

In all soil drench trials, pupation occurred over a 4–5 day period starting 7 days after the eggs had been laid. Adults eclosed over a 3–4 day period starting 8–9 days after the onset of pupation. The effect of concentrations of azadirachtin on the total percent pupation, as a function of eggs laid, and adult eclosion is listed in Table 1. There was no significant difference in the percent pupation, as a function of eggs laid, between each control group. There was no significant difference in the percent of adult eclosion from any control group.

When drenching occurred 8 days before exposure to adults, pupation occurred 8–10 days after egg-laying. As shown in Table 1, 1 ppm azadirachtin was not significantly different from control plants with regard to percent pupation, whereas 5 and 10 ppm azadirachtin had significantly ( $P < 0.05$ ) fewer pupae. No adults emerged from any of the pupae from neem insecticide-drenched soil. Since percent pupation was 0.4% with 10 ppm, plants were not treated with 25 ppm azadirachtin.

When the soil of plants was drenched with neem insecticide 24 h before exposure to adult leafminers, pupal emergence occurred 7–10 days post-egg-laying. There were no significant differences in the percent pupation among any of the drenched plants and control plants (Table 1). However, no adults emerged from any of the pupae from azadirachtin-drenched plants. In contrast, Meisner *et al.* (6), working with bean plants in sandy loam soil, obtained almost 75% reduction in *L. trifolii* pupation from a soil drench with a crude water extract of neem seed kernels applied 24 h before egg laying.

When the soil of plants was drenched with neem insecticide immediately after exposure to adult leafminers (*i.e.*, immediately after egg laying), pupal emergence was completed by 9 days post-egg-laying. There were no significant differences in the percent pupation between control and concentrations up to 10 ppm (Table 1) and results from treatment with 10 or 25 ppm were not significantly different. Of plants treated with 1 ppm immediately after egg laying, significantly fewer adults eclosed as compared with control plants ( $P < 0.05$ ); no adults emerged from pupae from plants treated with 5, 10 or 25 ppm azadirachtin. Larew *et al.* (4) obtained similar results with *L. trifolii* in chrysanthemum for pupation and eclosion. Parkman and Pienkowski (9), also working with *L. trifolii* in potted chrysanthemums, obtained substantially different results. There are a number of variables that could account for these differences, but extraction procedures/formulations are probably a primary source of variation.

When the soil of plants infested with 1st-instar larvae was drenched with neem insecticide, there were predictably higher rates of pupation (Table 1) and adult emergence than with treatment at earlier stages of development. Pupation occurred 7–10 days post-egg-laying, and adult emergence 7–12 days post-pupation. There were no significant differences ( $P < 0.05$ ) in the percent pupation between azadirachtin treatment groups up to 10 ppm and controls; 25 ppm azadirachtin was not significantly different from 10 ppm azadirachtin, but was different from the rest of the treatment groups. Adult emergence occurred from pupae treated with 1 and 5 ppm, but was significantly less ( $P < 0.01$ ) than in controls.

Meisner *et al.* (6) showed that a soil drench with a neem seed kernel extract of potted bean plants 1 day before exposure to adult *L. trifolii* had less of an effect on pupation than a soil drench 5 days before exposure to adult leafminers. Similarly, in our trials, plants in soil drenched with 5 and 10 ppm azadirachtin 8 days before exposure to adults had significantly less pupation than plants treated 24 h before, or immediately after, exposure to adult leafminers. Plants treated 8 days before exposure were barely breaking the soil surface and yet the increase in plant mass did not have a dilution effect of the azadirachtin. A similar result was observed by Larew *et al.* (4). They applied a single drench of azadirachtin to potted chrysanthemums; 21 days later the leaf surface had increased by 53%, and the effect on pupation was greater than in plants challenged 3 days after the azadirachtin drench.

#### *Leaf dip trials*

In all leaf dip trials, pupation occurred 7–10 days after eggs had been laid. Adults eclosed 7–11 days after the onset of pupation. The effect of concentrations of azadirachtin on the total percent pupation, as a function of eggs laid, and adult eclosion is listed in Table 2. There was no significant difference in the percent pupation or adult eclosion between each control group.

When leaves were dipped in azadirachtin 24 h before exposure to adult leafminers,

TABLE 1. Effect of Neemix-45, applied as a soil drench, on percent pupation and adult eclosion of *Liriomyza huidobrensis*

Conc. (ppm)	8 days before exposure to adults			24 h before exposure to adults			Immediately after egg laying			At 1st instar		
	No. of eggs	Pupation (%)	Eclosion (%)	No. of eggs	Pupation (%)	Eclosion (%)	No. of eggs	Pupation (%)	Eclosion (%)	No. of eggs	Pupation (%)	Eclosion (%)
0	141	85.9 aAB	51.2 A	203	72.4 aB	63.8 A	271	64.0 aAB	68.1 aA	235	94.9 aA	54.8 aA
1	156	64.5 aAB	0	149	64.1 aAB	0	351	50.4 abB	4.9 b	144	90.3 aA	11.7 b
5	80	10.9 bB	0	207	65.9 aA	0	189	59.7 aA	0	164	92.7 aA	5.2 b
10	104	0.4 bC	0	167	40.6 aB	0	237	51.4 abB	0	186	90.9 abA	0
25							148	19.6 bcB	0	150	70.7 bA	0

Within columns, numbers followed by different lower case letters are significantly different at  $P < 0.05$ . Within rows, percent pupation and percent eclosion, respectively, for each treatment, were evaluated and numbers followed by different upper case letters are significantly different at  $P < 0.05$ . Pupation is expressed as percentage of the number of eggs, and eclosion as percentage of the number of pupae.

TABLE 2. Effect of Neemix-45, applied as a leaf dip, on percent pupation and adult eclosion of *Liriomyza huidobrensis*

Conc. (ppm)	24 h before exposure to adults			Immediately after egg laying			At 1st instar		
	No. of eggs	Pupation (%)	Eclosion (%)	No. of eggs	Pupation (%)	Eclosion (%)	No. of eggs	Pupation (%)	Eclosion (%)
0	177	83.1aA	66.3aA	163	61.3aA	66.4aA	181	87.3aA	59.0aA
1	193	66.8aAB	15.6bB	282	36.9bB	10.6bB	162	91.4aA	44.7aA
15	244	64.6aAB	0c	321	29.0bB	0.8bA	124	79.8aA	1.8bA

Within columns, numbers followed by different lower case letters are significantly different at  $P < 0.05$ . Within rows, percent pupation and percent eclosion, respectively, for each treatment, were evaluated and numbers followed by different upper case letters are significantly different at  $P < 0.05$ . Pupation is expressed as percentage of the number of eggs, and eclosion as percentage of the number of pupae.

there was no significant difference between total percent pupation ( $P < 0.01$ ) (Table 2). However, there were significant differences in adult eclosion: 1 ppm azadirachtin caused ~75% reduction in pupation as compared with controls, and no adults emerged from the 15 ppm treatment groups.

Dipping leaves in azadirachtin immediately after removal from adult leafminers (at egg laying) caused differences ( $P < 0.05$ ) in the percent pupation and adult eclosion between controls and treatment, but not between treatments (Table 2). Pupation occurred from 7–11 days post-egg-laying, and adult eclosion 9–11 days after pupation. Female leafminers lay eggs on the leaf surface; eggs are often exposed and not fully covered by the leaf. Because the control pupation in this treatment regime is less than that of other control groups (but not significantly so), we believe that the surfactant may have adversely affected these freshly laid and exposed eggs. Exposure of the eggs to the surfactant may have caused greater efficacy of the two azadirachtin treatments.

When plants containing 1st-instar larvae were dipped in concentrations of 1 or 15 ppm, there were no significant differences in the total percentage pupation between azadirachtin treatments and control (Table 2). There were no significant differences in adult eclosion between control and 1 ppm; however, there were significant differences in adult eclosion between both control or 1 ppm and 15 ppm azadirachtin. Results with other *Liriomyza* spp. under similar treatment of larvae are equivocal: Webb *et al.* (13) obtained 100% mortality of *L. sativae* larvae and Dimetry *et al.* (2) obtained up to ~55% mortality. There are a number of variables that could account for these differences, but extraction procedures/formulations are, again, probably a primary source of variation.

Suppression of pupation and adult eclosion was less severe when soil drenching occurred later in the life cycle of the leafminer than earlier. However, dipping egg- or larvae-infested leaves in neem insecticide with a surfactant had less effect on pupation and adult eclosion than the systemic effects of a soil drench. Since azadirachtin is more efficacious as a systemic insecticide, and because it is very susceptible to photodegradation, we suggest further testing of it under field situations with drip irrigation systems.

## ACKNOWLEDGMENT

We thank Uri Rosenberg, Agron Agrochemical Development & Marketing, Ltd., Rehovot, Israel, for providing a sample of Neemix-45

## REFERENCES

1. Ascher, K.R.S. (1993) Nonconventional insecticidal effects of pesticides available from the neem tree, *Azadirachta indica*. *Arch. Insect Biochem. Physiol.* 22:433-449.
2. Dimetry, N.Z., Barakat, A.A., Abdalla, E.F., El-Metwally, H.E. and El-Salam, A.M.E.A. (1995) Evaluation of two neem seed kernel extracts against *Liriomyza trifolii* (Burg) (Dip. Agromyzidae). *Anz. Schädlingskd. Pflanzenschutz Umweltschutz* 68:39-41.
3. Isman, M.B., Koul, O., Luczynski, A. and Kaminski, J. (1990) Insecticidal and antifeedant bioactivities of neem oils and their relationship to azadirachtin content. *J. Agric. Food Chem.* 38:1406-1411.
4. Larew, H.G., Knodel-Montz, J.J., Webb, R.E. and Warthen, J.D. (1985) *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) control on chrysanthemum by neem seed extract applied to soil. *J. Econ. Entomol.* 78:80-84.
5. Lowery, D.T. and Isman, M.B. (1995) Toxicity of neem to natural enemies of aphids. *Phytoparasitica* 23:297-306.
6. Meisner, J., Yathom, S., Tal, S. and Ascher, K.R.S. (1985) The effect of various extracts of neem seed kernel on *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae). *J. Plant Dis. Prot.* 93:146-152.
7. Mordue (Luntz), A.J. and Blackwell, A. (1993) Azadirachtin: an update. *J. Insect Physiol.* 39:903-924.
8. Naumann, K. and Isman, M.B. (1996) Toxicity of a neem (*Azadirachta indica* A. Juss.) insecticide to larval honey bees. *Am. Bee J.* 36:518-520.
9. Parkman, P. and Pienkowski, R.L. (1990) Sublethal effects of neem seed extract on adults of *Liriomyza trifolii* (Diptera: Agromyzidae). *J. Econ. Entomol.* 83:1246-1249.
10. Parrella, M.P. and Bethke, J.A. (1984) Biological studies of *Liriomyza huidobrensis* (Diptera: Agromyzidae) on chrysanthemum, aster, and pea. *J. Econ. Entomol.* 77:342-345.
11. Schmutterer, H. (1988) Potential of azadirachtin-containing pesticides for integrated pest control in developing and industrialized countries. *J. Insect Physiol.* 34:713-719.
12. van der Staay, M. (1992) Chemical control of the larvae of the leafminer *Liriomyza huidobrensis* (Blanchard) in lettuce. *Meded. Fac. Landbouwwet. Rijksuniv. Gent* 57:473-478.
13. Webb, R.E., Hinebaugh, M.A., Lindquist, R.K. and Jacobson, M. (1983) Evaluation of aqueous solution of neem seed extract against *Liriomyza sativae* and *L. trifolii* (Diptera: Agromyzidae). *J. Econ. Entomol.* 76:357-362.
14. Weintraub, P.G. and Horowitz, A.R. (1995) The newest leafminer pest in Israel, *Liriomyza huidobrensis*. *Phytoparasitica* 23:177-184.