SUSCEPTIBILITY OF THE WOOLLY APPLE APHID PARASITOID, *APHELINUS MALI* (*HYM.: APHELINIDAE*), TO COMMON PESTICIDES USED IN APPLE ORCHARDS IN ISRAEL

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The effects of one acaricide (cyhexatin), two fungicides (penconazole and sulfur), and six insecticides (azinphos-methyl, chlorpyrifos, imidacloprid, pirimicarb, triazamate and vamidothion) on the adult stage of the parasitoid *Aphelinus mali* (Haldeman), a parasite of the woolly apple aphid *Eriosoma lanigerum* (Hausmann), were investigated under laboratory conditions. Chlorpyrifos (an organophosphorus insecticide-OP) was found to be highly toxic to the adult wasps. Vamidothion (OP) was more toxic to the parasitoid than azinphos-methyl (OP). On the other hand, both chlorpyrifos and azinphos-methyl were found to be harmless to the immature stages of the parasitoid in a test conducted under semi-field conditions. Of the other insecticides, imidacloprid was more toxic to the adult parasitoid than pirimicarb and triazamate. Neither cyhexatin nor penconazole had a considerable toxic effect upon the parasitoid. In contrast, sulfur was found to be moderately toxic to the parasitoid under laboratory conditions, as well as in a field survey in which we followed populations of *E. lanigerum* and *A. mali* parasitization in sulfur-treated plots, and in a plot treated with *Ampelomyces quisqualis* Ces., a fungus antagonist to powdery mildew. The implications of these results to IPM programs of apple orchards in Israel are discussed.

KEY-WORDS: *Aphelinus mali, Eriosoma lanigerum,* pesticides, IPM.

The woolly apple aphid, *Eriosoma lanigerum* (Hausmann), is a pest of apple orchards infesting both the stems and roots of host trees (Baker, 1915). It has been shown that *E. lanigerum* can reduce the rate of growth of young trees (Brown & Schmitt, 1990), and can also weaken mature trees by both feeding on roots and by inducing root and shoot galls which damage the xylem and interrupt water transportation (Klimstra & Rock, 1985). Heavy infestations of *E. lanigerum* can cause yield reduction, orchard decline and even death of trees (Klimstra & Rock, 1985; Brown et al., 1994). Outbreaks of *E. lanigerum* have often occurred as a result of pesticide applications which have decimated biological control agents (McLeod, 1954; Penam & Chapman, 1980).

The most important natural enemy of *E. lanigerum* is the parasitoid *Aphelinus mali* (Haldeman). *A. mali* has been introduced from its native home (North America) throughout

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the world in order to control this pest, and has been reported as a successful biological control agent in 40 countries (DeBach, 1964). The parasitoid was first introduced into Israel in 1935 by Bodenheimer, who concluded that *A. mali* was rather ineffective in controlling *E. lanigerum* under the climatic conditions of this country (Bodenheimer, 1947).

The present study is based on two events observed in commercial apple orchards in Israel: 1. Severe outbreaks of *E. lanigerum* occurring after vamidothion applications (the only recommended insecticide for this pest in Israel); and 2. Effective biological control of *E. lanigerum* by *A. mali* observed in organic orchards (*i.e.,* orchards free of any pesticide treatment at all or where only natural substances, such as sulfur, are allowed as pesticides). The present study is also based on previous reports of suspected resistance in *A. mali* populations to DDT (Johansen, 1957), to diflubenzuron (Ravensberg, 1981) and to azinphos-methyl (Croft, 1982), which supports the possibility of finding pesticide-resistant strains of *A. mali* in Israel orchards.

The present study was aimed at evaluating the susceptibility of *A. mali* to common pesticides used in apple orchards in Israel. The study includes three aspects: (a) laboratory bioassays to determine the susceptibility of adult *A. mali* to these pesticides; (b) observations of *A. mali* populations in an organic apple orchard sprayed with sulfur and with a parasitic fungus against powdery mildew disease; and c) a semi-field trial to investigate the effect of organophosphorus (OP) insecticides, with widespread use in Israeli orchards, on the immature stages of the parasitoid.

MATERIALS AND METHODS

Laboratory Bioassays

Apple twigs infested with *E. lanigerum* which had been parasitized by *A. mali* were collected during August and September from an organic orchard at Keshet, in the Golan Heights region. The twigs were placed in a rearing cage (60 × 40 × 60 cm) covered with organdy cloth, were soaked on a wet foam, and were replaced every 10-14 days with new infested twigs. The cages for parasitoid rearing were maintained in a controlled chamber under conditions of 25 ± 1°C, 55 ± 5% RH and 14:10 L:D.

Disks of apple leaves (ca 40 mm in diameter) from the organic orchards were dipped for 10 sec. in aqueous dilutions of the test compounds. Deionized water was used as a control. Common pesticides for controlling apple pests were used in the bioassays (table 1). A treated apple leaf was placed on acrylic foam in a plastic vial (45 mm in diameter and 100 mm height), containing some water to provide humidity. Another piece of foam was used to close the vial. Twenty-five *A. mali* wasps, aged 1-5 days, were aspirated from the rearing cages and placed in each vial on the treated leaf disk, and mortality was determined after 24 h. Each treatment was carried out with 5 replicates of between 15 to 25 *A. mali* adults per replicate. Treatments were repeated on three-five different days. We tested between three-six concentrations of the investigated compounds. Control mortality was corrected according to Abbott's (1925) formula before analysis. POLO-PC (LeOra Software, 1987) was used to estimate, probit regression parameters.

Observations of *A. mali* Populations in an Organic Apple Orchard

Sampling of the parasitoid population was conducted in the Keshet organic apple orchard (var. included Jonathan, Golden Delicious, Star King and Granny-Smith) during 1987 and 1991. The sample plot (ca 1.5 ha) was commercially treated with sulfur against powdery

TABLE 1

Pesticides tested on A. mali *wasps in laboratory bioassays*

Active ingredient	Brand name	Manufacturer	Chemical group
Acaricides			
cyhexatin	Lintex 25 WP*	Agrolinz, USA	triphenyltin
Fungicides			
penconazole	Ophir 10 EC**	Ciba-Geigy, Switzerland	triazole
sulfur	Gofrativ 90 WP	Makhteshim, Israel	
Insecticides			
azinphos-methyl	Cotnion 20 WP	Makhteshim, Israel	organophosphorus
chlorpyrifos	Dorsan 48 EC	Makhteshim, Israel	organophosphorus
imidacloprid	Confidor 35 WP	Bayer, Germany	nitromethylene
pirimicarb	Pirimor 50 WP	Zeneca, UK	carbamate
triazamate	Aphistar 14 EC	Rohm & Hass, USA	triazole
vamidothion	Kilval 40 EC	Rhône Poulenc, France	organophosphorus

* WP-wettable powder.
** EC-emulsifiable concentrate.

mildew disease. In 1991, a substitute for sulfur, based on the parasitic fungus *Ampelomyces quisqualis* Ces. (AQ), was tested in an additional plot and compared with the sulfur- treated plot.

In each plot, four-six (25-30 cm long) twigs were randomly removed from eight trees infested with *E. lanigerum.* The twigs were examined, and the aphids were sorted into two groups: live and parasitized (mummies with emergence holes) by *A. mali.* Analysis of parasitization was restricted to the periods of infestation. The time at which wax filaments (produced by aphid colonies) invading higher portions of the cortex reached a height of 1.5 m above ground, in at least 50% of the trees, was considered the establishment of infestation; the time at which the wax, and therefore the aphid colonies, disappeared from the tree cortex was defined as the end of infestation. Differences in percent parasitization between treatments were tested with Wilcoxon's Signed-Ranks Test (Sokal & Rohlf, 1981).

SUSCEPTIBILITY OF IMMATURE *A. MALI* TO ORGANOPHOSPHORUS INSECTICIDES UNDER SEMI-FIELD CONDITIONS

Potted trees (apple rootstock sensitive to the aphid, *Malus domestica*) were used for this experiment. Trees were grown in a controlled room (20°C) with artificial illumination (16:8 L:D), and were irrigated daily. When the trees reached a height of 1 m, they were transferred to an adjacent room, under the same temperature and illumination regime, and were then infested with parasitoid-free *E. lanigerum.* After the aphids became established (ca 1 month), about 100 *A. mali* adults were released on each tree. *E. lanigerum*-infested twigs in which parasitization exceeded 50% were marked and selected for the experiment.

Azinphos-methyl (emulsifiable concentrate-EC 25%) and chlorpyrifos (EC 48%), at a concentration of 0.2%, were applied until runoff to six trees each on September 3, 1995.

Another six trees were left as a control. Mummies with emergence holes were removed from the twigs of treated and control trees before the application, leaving only mummies carrying immature parasitoids. Subsequently, mummies showing emergence holes were recorded 7, 15 and 30 days after the treatment. Differences between treatments on day 30 after spraying were tested with an analysis of frequencies (Sokal & Rohlf, 1981).

RESULTS

Laboratory Bioassays

One hundred percent mortality of adult *A. mali* was obtained with chlorpyrifos at all the tested concentrations (1, 2, 5 and 10 ppm; recommended field rate: 720 ppm). Mortality curves that represent the response of *A. mali* wasps to two other OP insecticides, azinphos-methyl and vamidothion, are shown in fig. 1 and table 2. Azinphos-methyl had a moderate effect upon the adult wasp: The calculated LC_{90} for this compound was one-third of the recommended field rate (rfr) concentration. In contrast, vamidothion was highly toxic to *A. mali* adult wasps: LC_{90} for this compound was calculated at approximately 12 ppm, which is 1/66 of the rfr concentration. Table 2 also shows the toxicity of imidacloprid, pirimicarb and triazamate to *A. mali* adults. Based on LC_{50}, LC_{90} and rfr, imidacloprid was found highly toxic to the wasp. In contrast, pirimicarb and triazamate had a lesser effect upon the wasp.

Regarding the effect of the tested fungicides (penconazole and sulfur) and the acaricide (cyhexatin) upon *A. mali* adults, the laboratory results showed that these compounds had a small or moderate effect upon the wasp (table 2). While penconazole did produce some degree of mortality in the adult wasp, mortality never exceeded the 20% level (the highest

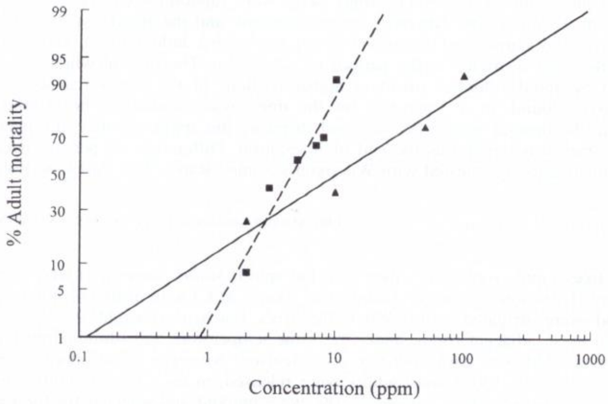

Fig. 1. Log concentration-response curves (on a probit scale) of the effect of azinphos-methyl (▲) and vamidothion (■) on *A. mali* parasitoids.

concentration tested of this compound was 400 ppm). Similarly, cyhexatin had a small effect upon the wasp: even at high concentrations (*i.e.*, 600 ppm) the LC_{90} was never reached with this compound. On the other hand, sulfur had a small to moderate effect upon the wasp; the LC_{90} was not reached in the laboratory, but the LC_{50} (567 ppm) was slightly greater than half of the rfr concentration (900 ppm) (table 2).

TABLE 2

Comparative toxicity of various pesticides tested on A. mali *wasps in the laboratory.*

Compound	n	Slope (± SEM)	LC_{50} (ppm) (95% CL)	LC_{90} (ppm) (95% CL)	RFR[1] (ppm)
Insecticides					
azinphos-methyl	437	1.33 (0.24)	13.5 (*)	124 (*)	400
vamidothion	562	2.97 (0.35)	4.49 (2.26-6.54)	12.13 (7.89-62.58)	800
imidacloprid	386	1.77 (0.24)	0.16 (0.11-0.22)	0.88 (0.69-1.22)	350
pirimicarb	356	1.09 (0.10)	171.8 (43.7-809.5)	**	500
triazamate	532	2.21 (0.33)	84.2 (63.2-107.8)	319.2 (227.3-556.3)	210
Fungicides and Acaricide					
penconazole	502	***	***	***	25
sulfur	502	3.76 (0.41)	567 (*)	**	900
cyhexatin	431	3.53 (1.26)	253 (*)	**	375

[1] RFR-Recommended Field Rate.
* Polo-PC did not calculate the 95% confidence limits.
** Even at high concentrations, 90% mortality was never reached.
*** Even at high concentrations mortality never went above 20%.

FIELD AND SEMI-FIELD OBSERVATIONS

The activity of *A. mali* in sulfur and in *Ampelomyces quisqualis*-treated plots is shown in table 3. Low levels of parasitization (less than 30%) were detected in sulfur-treated plots during the entire period of the study. In contrast, parasitization in the *A. quisqualis*-treated plot reached levels of up to 70% by the end of the infestation period. No differences in the level of parasitization were found between the sulfur treated plots ($T_s = 4$, $P > 0.05$). In contrast, the level of parasitization in both sulfur treated plots significantly differed from that in the *A. quisqualis* plot ($T_s < 1$, $P < 0.05$).

Table 4 shows the effect of azinphos-methyl and chlorpyrifos on the developing stages of the parasitoid under semi-field conditions. The percent of mummies with emergence holes of the parasitoid on the three sampling dates was very similar in the treated and control trees. A small statistical difference in the frequency of emergence holes between sprayed

Of the other insecticides tested in this study, imidacloprid was the most toxic to the parasitoid, followed by triazamate and pirimicarb. Although *A. mali* was highly susceptible to imidacloprid in the laboratory, the field effect of this compound upon the wasp may be less severe. In the laboratory, *A. mali* was directly exposed to the pesticide. In the field, in contrast, imidacloprid is applied to the root system, thus reducing the parasitoid's direct contact with the insecticide. There is still a need to investigate the actual field effect of imidacloprid on the parasitoid, however, as there are some indications that the compound may be toxic to other beneficial insects even when it is applied as a drench (Pfluger & Schmuck, 1991/2).

Pirimicarb was found to have a very moderate effect upon the adult parasitoids (*e.g.*, even at high concentrations of 200 ppm, mortality did not reach levels higher than 70%). This low susceptibility of the parasitoid to pirimicarb in the laboratory seems to also hold true for the field, although we did not study the effect of this compound under field conditions. Growers in Israel, using this product against *E. lanigerum*, have reported high levels of *A. mali* parasitization in their orchards (Cohen, 1994). Regarding triazamate, there are no current studies, nor reports, on its effect upon the parasitoid in the field.

A. mali was just slightly sensitive to the acaricide, cyhexatin, and to the fungicide, penconazole (table 2). On the other hand, it was highly sensitive to sulfur, both in the laboratory (table 2) and under field conditions (table 3). The detrimental effect of sulfur on natural enemies is well known (Rosen, 1967; Harpaz & Rosen, 1971). Thus, it was not surprising to find that sulfur had a considerable effect upon *A. mali*. However, the fact that mortality in the laboratory, even at high concentrations of the pesticide, was not 100%, and that some activity of the parasitoid was found in heavily sulfur-sprayed orchards, suggests that populations of *A. mali* may have developed some degree of tolerance to this pesticide. Tolerance of parasitoids to sulfur has been reported in the past in heavily sprayed agro-ecosystems (Doutt & Smith, 1971; Wetzel & Dickler, 1994). Since sulfur continues to be an effective and widely-used fungicide, the presence of potentially heterogeneous populations might be important for the establishment of integrated pest management (IPM) programs in apple orchards in Israel.

While the laboratory toxicological tests cannot be directly related to field results, they could provide some indication as to which pesticides might be good candidates for field trials. As an example, while both vamidothion and triazamate are effective insecticides against *E. lanigerum* (unpublished results; Gloria, 1982), the former was found to be more toxic to the parasitoid at lower concentrations than the latter (table 2). Further, vamidothion was found by Gloria (1982) to be highly detrimental to the early developing stages of *A. mali*. In addition, we also found that, unlike triazamate, vamidothion had a long persistence effect upon field populations of *E. lanigerum* (Cohen, 1994). This lasting effect, which delayed the resurgence of woolly aphid populations in the treated orchards, indirectly affected the parasitoid populations, which disappeared from the orchard. As a result, a second application of vamidothion was needed. In contrast, the shorter-term effect of triazamate resulted in an initial reduction of the *E. lanigerum* population and preservation of the parasitoid population in the field, which was then able to control the recovering pest population (Cohen, 1994). Thus, in view of the laboratory effect of triazamate upon the parasitoid, and its short-term effect upon *E. lanigerum*, this insecticide may be recommended as a management alternative in an IPM program in apple orchards. Similarly, the mild laboratory effect of azinphos-methyl and penconazole upon the parasitoid is important for an IPM program, as these pesticides form the basis of current plant protection strategies in apple orchards. In addition, the mild effect, or lack of effect, of azinphos-methyl upon the developing stages of the parasitoid (table 4) strengthens the possibility of including this pesticide in apple orchard IPM, at least as far as its effect upon *A. mali* is concerned.

While we still lack a lot of information regarding the effect of pesticides used in apple orchards upon other beneficial arthropods, the present study provides an initial framework for the development of sound control strategies in Israeli orchards. The results of these toxicological studies on beneficial insects, as well as studies on the use of other strategies of pest control (*e.g.*, mating disruption, mass-rearing and release of natural enemies) which are currently being undertaken, are expected to provide the basic framework for an IPM program in Israel's orchards.

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RÉSUMÉ

Sensibilité du parasitoïde, *Aphelinus mali* (Hym. : Aphelinidae) aux pesticides couramment utilisés en verger de pommiers en Israel

Les effets d'un acaricide (cyhéxatin), de deux fongicides (penconazole et soufre), et de six insecticides (azynphos-méthyl, chlorpyrifos, imidaclopride, pyrimicarbe, triazamate et vamidothion) ont été testés en conditions de laboratoire sur les adultes d'*Aphelinus mali* (Haldeman), parasitoïde du puceron du pommier *Eriosoma lanigerum* (Hausman). Le chlorpyrifos (organophosphoré) s'est révélé hautement toxique pour les adultes ; le vamidothion était plus toxique que l'azinphos-methyl. Par ailleurs, le chlorpyrifos et l'azinphos-méthyl étaient sans danger pour les stades immatures du parasitoïde, comme l'a démontré un test effectué en conditions semi-naturelles. Parmi tous les autres insecticides, l'imidaclopride était plus toxique pour les parasitoïdes adultes que le pyrimicarbe et le triazamate. Ni le cyhéxatin ni le penconazole n'ont eu un effet important sur *A. mali*. A l'inverse, le soufre s'est révélé modérément toxique pour le parasitoïde dans les conditions de laboratoire, ainsi que dans une étude au champ au cours de laquelle a été suivi le parasitisme des populations d' *E. lanigerum* et d'*A. mali* dans des parcelles traitées au soufre, et dans une parcelle traitée avec *Ampelomyces quisqualis* Ces., un champignon antagoniste du mildiou. Les implications de ces résultats dans les programmes de lutte intégrée en vergers de pommier en Israël sont discutées.

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