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BACILLUS THURINGIENSIS PREPARATIONS AS A MEAN FOR THE CONTROL OF LEPIDOPTEROUS AVOCADO PESTS IN ISRAEL

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ABSTRACT

An Integrated Pest Management (IPM) program was developed for control of the insect pests in avocado plantations of Israel. This program included *inter alia* control of lepidopterous pests by means of *Bacillus thuringiensis (B.t.)* preparations. Three important and two less important lepidop- terous pests damage avocados in Israel. The giant looper, *Boarmia selenaria* (Schiffermlllller) (Lepidoplera: Geometridae), the honcydew moth, *Cryptoblabes gnidiella* (Milliere) (Lepidoptera: Pyralidae), and the carnation leaf roller, *Cacoecimorpha pronubana* (HUbner) (Lepidoptera: Tortricidae), are the most important pests in some regions, where the Egyptian cotton bollworm, *Spodoptera litloralis* (Boisduval) (Lepidoptera: Nocluidae), and the tomato looper, *Chrysodeixis chalcites* (Esper) (Lepidoptera: Nocluidae), are of lesser importance. The honcydew moth is usually attracted to the honcydew of the mealybug, *Pseudococcus longispinus* (Targioni-Tozzelti) (Hemip- tera: Pseudococcidae), which is successfully controlled by two *parasitoids,Arhopoideus peregrinus* (Compere) and *Anagyrus fusciventris* (Gerault) (Hymenoptera: Encyrtidae). The *B. thuringiensis* preparations have no adverse effect on those wasps. Besides the use of *B. thuringiensis* preparations, an egg parasitoid, *Trichogramma platneri* Nagarkalti (Hymenoptera, Trichogrammatidae), was introduced as an additional biological control agent against the giant looper and the honeydew moth. For this beneficial wasp the *B. thuringiensis* preparations are harmless. Efforts arc being made to find virulent strains of *B. thuringiensis* for control of the lepidopterous avocado pests.

INTRODUCTION

The avocado, *Persea americana* Miller (Ranales: Lauraceae), which originated from Mexico, Central America and the Andean region, has been grown on a commercial scale for (he last century in climatic zones similar to those zones in which citrus is grown. According to the FAO 1984 report (Table 1, from Wolstenholme, 1987) Mexico is the largest producer, accounting for 28.8% of the world total, followed by the U.S.A. (13.8%), Dominican Republic, Brazil, Peru, Indonesia, Haiti, Israel and Venezuela. From a total of 1568 x 10^3 tons of yearly production (1983), the developing countries produced 1277 x 10^3 and developed countries only 290x 10^3 tons. For world trade avocado is produced mainly in Israel, South Africa, the USA, Spain and to a smaller extent, Martinique and Kenya. Thus, most of the crop produced in developing counlries is for local consumption. Because of its high nutritional value the avocado is an important, although relatively expensive, food source for these countries. The biggest importers are France and England. In Israel avocado is an important export crop and efforts are being made to supply high quality fruit without any damage or residuals. In order to control the pests which damage avocado, an Integrated Pest Management (IPM) program was developed (Swirski et al., 1988; Wysoki et al., 1981) and is in use. The most important pests — such as the long-tailed mealybug, *Pseudococcus longispinus* (Targioni-Tozzelti) (Hemiptera:

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TABLE 1
World avocado production according to FAO report, 1984 (Wolstenholme, 1987)

Region	10^3 tons	% of total
North and Central America	982.0	62.6
South America	323.0	20.6
Asia	140.5	9.0
Africa	116.5	7.4
Oceania	3.0	0.2
Europe	2.0	0.1
Total	1568.0	100.0
Developed countries	290.0	18.5
Developing countries	1277.0	81.4

Pseudococcidae) and the japanese bayberry whitefly, *Parabemisia myricae* (Kuwana) (Homoptera: Aleyrodidae) — are controlled by means of predators and parasitoid wasps (Swirski et al., 1980; 1986a; 1987; Wysoki, 1977; Wysoki and Cohen et al., 1983). Parasitoids have been introduced (Wysoki 1985, 1987) against a newly introduced pyriform scale, *Protopulvinaria pyriformis* (Cockerell) (Homoptera: Coccidae). This IPM program also included control of lepidopterous avocado pests by means of *Bacillus thuringiensis* preparation. *B. thuringiensis* preparations do not interfere with the biological equilibrium in avocado orchards and are harmless to the natural enemies of the avocado pests.

Three important and two less important lepidopterous pests damage avocados in Israel. The giant looper, *Boarmia selenaria* (Schiffermüller), (Lep.: Geometridae), the honeydew moth, *Cryptoblabes gnidiella* (Millière) (Lep.: Pyralidae), and the carnation leaf roller, *Cacoecimorpha pronubana* (Hübner) (Lep.: Tortricidae), are the most important pests in some regions, while the Egyptian cotton bollworm, *Spodoptera littoralis* (Boisduval) (Lep.: Noctuidae), and the tomato looper, *Chrysodeixis chalcites* (Esper) (Lep.: Noctuidae) are of less importance. The honeydew moth is usually attracted to the honeydew of *P. longispinus*, which is successfully controlled by two parasitoid wasps, *Arhopoideus peregrinus* (Compere) and *Anagyrus fusciventris* (Gerault) (Hymenoptera: Ercyrtidae). The *B. thuringiensis* preparations have no adverse effect on these wasps. Besides the use of *B. thuringiensis* preparations, an egg parasitoid, *Trichogramma platneri* Nagarkatti (Hymenoptera: Trichogrammatidae), was introduced as an additional biological control agent against the giant looper and the honeydew moth. *B. thuringiensis* preparations are harmless to this beneficial wasp. Efforts are being made to identify more virulent strains of *B. thuringiensis* for control of lepidopteran avocado pests. Many strains and serovars of *B. thuringiensis* were tested for their activity against lepidopteran larvae (e.g., *kurstaki, entomocidus, thuringiensis, aizawai, ostriniae* and *kenyae*).

Giant looper, *Boarmia (Ascotis) selenaria* (Schiffermüller)

The giant looper, an important geometrid pest of avocado plantations in Israel, is also a serious pest of many other agricultural crops, such as coffee in Kenya and Tanzania; tea in Georgia (USSR), Formosa and India; citrus in South Africa and Sicily; peanuts in Madagascar; alfalfa in Hungary; mulberry in Japan; teak trees in Burma; and apples and pecans in Israel (Izhar et al., 1979; Wysoki, 1982). In Israel it causes extensive damage to avocado by eating foliage and gnawing fruits in the regions where cotton is planted (Wysoki, 1982; Wysoki and Izhar, 1980a, 1986). Local natural enemies control populations of *B. selenaria* effectively (Wysoki and Izhar, 1980b), but in those regions where cotton is widely planted the biological balance is upset by the drift of insecticides from aerial sprays on cotton fields. In orchards commercial products of *B. thuringiensis* serovar *kurstaki* are used to control the pest (Fig. 1). Since only young caterpillars (up to 1.5 cm in length) are sensitive to the bacterium (Cohen et al., 1983; Izhar et al., 1979; Wysoki et al., 1981; Wysoki

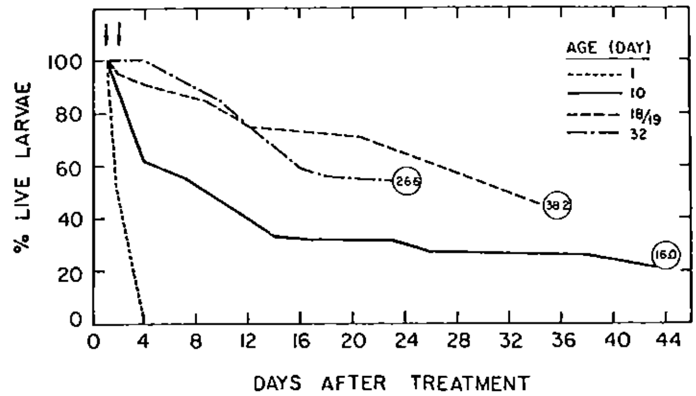

Fig. 1. Percentage of live larvae surviving at different periods after *Boarmia selenaria* larvae of various ages were fed avocado leaves dipped in a 0.5% aqueous dilution of a *Bacillus thuringiensis* preparation for 2 days. The percentage of emerging moths is indicated by circles. Arrows indicate days on which treated leaves were fed (Izhar et al., 1979).

and Izhar, 1980a), a monitoring system, involving virgin female baited traps (attracting males), was developed. It is recommended to use *B. thuringiensis* 2 weeks after the peak of male trappings (preoviposition and incubation periods) and at the time of field observations of the appearance of young caterpillars. This method of control has been used successfully since 1977 in many avocado groves (Wysoki and Izhar, 1986). Although it is very effective, additional steps for improvement have been undertaken, including replacement of virgin female baited traps by traps baited with synthetic pheromones, importation of egg parasitoids such as *T. platneri* (Wysoki and Renneh, 1985) (successfully introduced into California for control of lepidopterous pests), and exploration for more virulent strains of *B. thuringiensis* to control not only the early stages, but also the later stages of larval development.

Much effort was expended to bioassay different strains and varieties of *B. thuringiensis*. Table 2 lists the serovars that have been tested against the giant looper. Sensitivity to the various serovars is not uniform, although there is an indication that *B. selenaria* is susceptible to serovars 3a, 3b (*kurstaki* and 1 (*thuringiensis*), and less susceptible to serovar 6 (*entomocidus*), 7 (*aizawai*), 4a, 4c (*kenyae*), and 8a, 8c (*ostriniae*). In laboratory and field experiments, the commercial preparation Toarow CT, containing dead spores of *B. thuringiensis* var. *kurstaki*, has similar activity against the giant looper as the products which contain live spores (Wysoki et al., 1988). An experimental unregistered formulation (CB-105) containing two delta-endotoxins was tested only in the laboratory and its performance was practically the same as that of such commercial preparations as Dipel W.P. (Hadar et al., 1987). The phagostimulants Coax^{R} and Gustol^{R} were tested to see if improvement of the efficacy of *B. thuringiensis* against the giant looper could be obtained. In laboratory and field trials, as well as in semicommercial-scale applications, the minimum concentration of *B.t.* required for satisfactory larval mortality could be lowered by the addition of Coax^{R} to the spray formulation; however, with Gustol^{R}, no such beneficial effect could be observed (Hadar et al., 1986).

Honeydew moth, *Cryptoblabes gnidiella* (Millière)

The honeydew moth, *C. gnidiella*, is a pest of many agricultural crops. It damages figs, peaches, apples, pears, citrus, grapes, corn, sorghum, anona, cotton, avocado and other crops (for pertinent references, see Wysoki et al., 1975). It is usually attracted to the honeydew of aphids or mealybugs, and to the sticky exudate of damaged fruit, but is found in agricultural crops not harboring these

TABLE 2
List of isolates of *Bacillus thuringiensis* tested against *Boarmia selenaria*

Serovar	Isolate	References
7, *aizawai*	IH-A	Cohen et al., 1983
	HA3	Cohen et al.
	T63L4	Cohen et al.
	AO18	Cohen et al.
	AO7	Cohen et al.
	HD-249	unpublished data
	7–10	Wysoki & Jarvinen, 1986
	7–17	Wysoki & Scheepens, 1986
	7–21	Wysoki & Scheepens, 1986
	7–23	Wysoki & Scheepens, 1986
	7–29	Wysoki & Scheepens, 1986
	7–30	Wysoki & Scheepens, 1986
	ABG 6104	Navon et al., 1983
	ABG 6105	Navon et al., 1983
	serotype 17	Wysoki & Scheepens, 1988
6, *entomocidus*	Bt 24	Cohen et al., 1983; Wysoki & Jarvinen, 1986
4a,,4c, *kenyae*	Ken 2	Wysoki & Jarvinen, 1986
	Ken 6	Wysoki & Jarvinen, 1986
	400–1	Wysoki & Scheepens, 1988
	400–2	Wysoki & Scheepens, 1988
	400–3	Wysoki & Scheepens, 1988
	400–4	Wysoki & Scheepens, 1988
	400–5	Wysoki & Scheepens, 1988
	400–6	Wysoki & Scheepens, 1988
	400–7	Wysoki & Scheepens, 1988
	400–8	Wysoki & Scheepens, 1988
	400–9	Wysoki & Scheepens, 1988
	400–10	Wysoki & Scheepens, 1988
	400–11	Wysoki & Scheepens, 1988
	400–12	Wysoki & Scheepens, 1988
	400–14	Wysoki & Scheepens, 1988
3a, 3b, *kurstaki*	HD-1-S-1980	Navon et al., 1983; Wysoki & Jarvinen, 1986
	HD-1	Cohen et al., 1983
	30–2	Wysoki & Scheepens, 1988
	30–11	Wysoki & Scheepens, 1988
	30–12	Wysoki & Scheepens, 1988
	30–13	Wysoki & Scheepens, 1988
	30–15	Wysoki & Scheepens, 1988
	HD-251	Wysoki & De Haan, 1988
	HD-263	Wysoki & Scheepens, 1988, unpublished data
8a, 8c, *ostriniae*	BL8a8c	Wysoki & Scheepens, 1988
1, *thuringiensis*	Bt3A	Cohen et al., 1983
not identified	B40	Cohen et al., 1983
	B49	Cohen et al., 1983

insects. In avocado plantations in Israel, *C. gnidiella* was found to be usually attracted to the honeydew of *P. longispinus* in orchards adjacent to cotton fields. The drift of the insecticides form the adjacent cotton fields killed the natural enemies of the mealybugs and caused heavy outbreaks of the long-tailed mealybug, which attracted *C. gnidiella*. The caterpillars of the honeydew moth at first feed on the honeydew, but subsequently they gnaw on the fruits.

A monitoring system for *C. gnidiella* is available and a sex attractant of this pest was developed and is on the market (Bjostad et al., 1981). Because of the high susceptibility of all stages of the caterpillars to *B. thuringiensis* preparations, it is not essential to carry out monitoring in the field to detect young caterpillars, but early control of this pest avoids heavy damage to the fruit. Usually death of the caterpillars occurs within 24 h of initial spray treatment (Fig. 2). Recently, use of commercial *B.t.* preparations at concentrations of 0.5 or 1.0% has been necessary because of the size and density of the trees, and the presence of hidden fruit, covered by dense foliage.

Since the caterpillars are active in avocado orchards mainly during July to November, the period when *B. thuringiensis* is not applied against *B. selenaria*, extra treatments against *C. gnidiella* are sometimes needed.

A commercial preparation of *B.t.* containing live spores (Dipel WP; Thuricide HP) and a commercial preparation of delta-endotoxin containing killed spores of *B.t.* serovar *kurstaki* (Toarow CT) was tested. In laboratory experiments on avocado fruit and in artificial medium, the 6–8 day-old larvae were killed between 24 h and 4 days after treatment, depending on their age and the concentration of the preparation used (Wysoki et al., 1988). Research on the control of this pest is directed at gaining an understanding of the phenology, population dynamics, and early detection of the pest, and/or at discovery and development of new and more virulent varieties of *B.t.* for use at lower concentrations.

Carnation leaf roller, *Cacoecimorpha pronubana* (Hübner)

The carnation leaf roller, a tortricid native to southern Europe, Sicily, Corsica, Italy and Spain

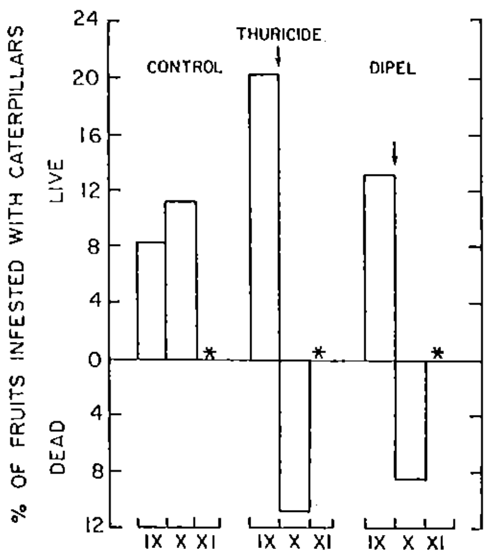

Fig. 2. Effect of treatment with Dipel 0.5% and Thuricide 0.5%, in combination with Colfix 0.05%, as a sticker, on the percentage of fruits infested with live or dead caterpillars of *Cryptoblabes gnidiella*. Asterisks indicate no live caterpillars; arrows indicate dates of spraying (Wysoki et al., 1975).

(Anon., 1975; Carter, 1984; Millière, 1859), is a polyphagous insect and has been found on more than 140 plant species, including agricultural crops such as pears, peaches, plums, apples, cabbage, tomatoes, cherries, roses (Izhar and Ben Yehuda, 1987; Fisher, 1924; von Winning, 1939), and on avocado in Israel (Wysoki and Izhar, 1976).

Research on this pest is aimed at its control without disturbing the ecological equilibrium. Susceptibility of *C. pronubana* to *B. thuringiensis* has been evaluated by laboratory experiments using semi-artificial medium for rearing of the larvae. Bioassays were performed using 6 day old (mostly second instar) larvae and Dipel WP.

Select quantities of *B. thuringiensis* powder were suspended in 0.011% Tween-80 solution to facilitate obtention of a homogeneous suspension. A semi-artificial medium, a modification of the diet developed by Shorey and Hale (1965), was prepared in a 1-liter Waring^R Blendor at maximum speed. One ml of a *B. thuringiensis*-Tween 80 suspension per 100 ml of diet (control contained 1 ml of distilled water with Tween 80 solution per 100 ml of diet) was mixed with the medium to obtain a homogenous mixture. Doses of 25, 100, 150, and 100 μg/ml diet were tested, with is equivalent to respectively 400, 1600, 2400 and 3200 IU *B. thuringiensis*/ml diet.

Treated and untreated diets were allowed to solidify and dry for 2 h, after which they were cut into small cubes. The bioassay was carried out in transparent plastic cups (25 mm diam, 13 mm depth) and two larvae were placed in each cup, along with 0.5 cm^3 of diet. In order to absorb excess moisture, pieces of filter paper were placed in the bottom of each cup; to admit fresh air, the lids of the cups were perforated. Cups were stored at 25 ± 2°C, with a 16L:8D photo period and relative humidity (RH) of 75 ± 5%. Mortality was recorded every day, for 8–10 days. Larvae were weighed before the test, and at the end of the bioassay, weight of the surviving larvae was determined.

Five-day and 7-day dosage-mortality regressions were computed using the log-probit transformation (Finney, 1971). After correcting for control mortality by Abbott's formula (1925), data were subjected to probit analysis. The 5-day dosage mortality regression was not significant, and therefore the LC_{50} estimates were not meaningful. The 7-day dosage regression was significant and resulted in an LC_{50} estimate of 151.0 μDg Dipel WP/ml diet (= 2416 IU) [fiducial limits: 116.1–218.5 μg Dipel/ml diet]. The equation of this regression is: $Y = 1.783X + 1.115$ (Fig. 3).

Tortricidae are known to be relatively insensitive to *B. thuringiensis* preparations. *C. pronubana*

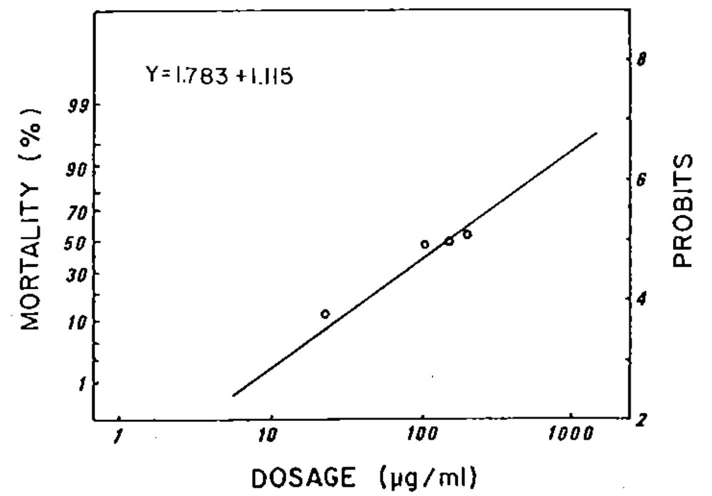

Fig. 3. Seven-day dose-mortality regression of bioassay for larvicidal activity of *Bacillus thuringiensis* on larvae of *Cacoecimorpha pronubana* (A.H. de Meyer and M. Wysoki, unpublished data).

is reported to be controlled satisfactorily only with a dose at least three times higher than the 0.3% *B. thuringiensis* preparation normally used (Burges and Jarrett, 1978).

Considering an LC_{50} of 151.0 μg Dipel/ml for *C. pronubana*, it is questionable if field sprays of 0.5% Dipel would be effective against *C. pronubana* larvae. On the other hand, at low dosage application (25 μg/ml), larvae of *C. pronubana* cease to feed and development is halted. Treated larvae gained almost no weight with some visible shrinkage, whereas non-treated larvae grew steadily (Table 3). The retarded development of the treated larvae may result ultimately in death before the adult stage is reached. Similar symptoms have been reported in *Choristoneura fumifera* (Yamvrias and Angus, 1970), *B. selenaria* (Cohen et al., 1983), and several other species of Lepidoptera (Ignoffo and Gregory, 1972) when treated with *B. thuringiensis*.

In order to obtain the information required for good control management of *C. pronubana*, attention should be focused on the susceptibility of the larval stages to *B. thuringiensis*, the effect of *B. thuringiensis* ingestion by larvae on pupal and adult stages, and the susceptibility of larvae to other *B. thuringiensis* serovars and isolates.

Although *C. pronubana* is considered to be not very susceptible to *B.t.* preparations (Burges and Jarrett, 1978), preliminary results show that with 1% concentrations of *B.t.* commercial products, good results were obtained in the avocado plantation at Bet haEmeq (Y. Izhar, personal communication).

TABLE 3
Mortality after 5 and 7 days of *Cacoecimorpha pronubana* larvae provided with a *Bacillus thuringiensis*-infected diet (According to A.H. de Meyer and M. Wysoki, unpublished data)

Dose (μg/ml)		Weight (mg)		% Mortality*		
		Initial	After 8 days	After 5 days	After 7 days	n
–	control	–	4.248	18	18	50
25	Dipel	–	0.603	26(9)	30(14.6)	50
–	control	–	–	6	6	50
25	Dipel	–	–	4	4	50
–	control	0.532	1.089	5.6	5.6	36
100	Dipel	0.532	0.569	30.6(26.5)	50.0(47.0)	36
–	control	0.579	6.220	2.6	2.6	40
150	Dipel	0.704	0.442	17.5(15.3)	50.0(48.7)	40
–	control	0.578	6.220	2.6	2.6	40
200	Dipel	0.640	0.446	25.0(23.0)	55.0(53.8)	40

*In parentheses, Abbott's corrected mortality.

Egyptian cotton worm, *Spodoptera littoralis* (Boisduval)

The Egyptian cotton worm, a polyphagous noctuid, is a serious pest of many agricultural crops, especially of vegetables and cotton. The population of this pest does not build up in avocado plantations. Caterpillars, newly emerged from eggs layed on avocado leaves, die after a short time. The only damage is caused by large caterpillars migrating from adjacent defoliated cotton fields, and the caterpillars usually damage leaves and fruit near the ground. Sometimes young avocado seedlings are defoliated by *S. littoralis* caterpillars. In these instances commercial preparations based on *B. thuringiensis* v. *kurstaki* have no effect as the pest is not sensitive to the strain used (Sneh et al., 1981). Newly bioassayed strains of *aizawai* kill young stages of this pest (Navon et al., 1983). With migrating large caterpillars, safety belts dusted with benzene hexachloride and placed on the ground around the orchards, protect the orchards from invasion.

Tomato worm, *Chrysodeixis chalcites* (Esper)

The tomato worm, a noctuid polyphageous pest, attacks many agricultural crops in Israel, including solanaceous vegetables and cotton (Rivnay, 1962; Yathom and Rivnay, 1968) and occasionally avocado leaves, but has not been found to feed on avocado fruits. It is not considered a serious pest in commercial avocado orchards, and therefore no precautions or control measures for orchards are taken against *C. chalcites*. In some nurseries *C. chalcites* caterpillars attack young seedlings; commercial preparations of *B. thuringiensis* var. *kurstaki* provide satisfactory control of the pest here (Y. Izhar, personal communications).

Effect of *Bacillus thuringiensis* preparations on natural enemies of avocado pests

Because avocado pests have a large number of natural enemies (Swirski et al., 1986b; Wysoki and Izhar, 1980b; Wysoki et al., 1981), some of the natural enemies have been examined for susceptibility to *B. thuringiensis* preparations. The honeydew moth, *C. gnidiella*, is attracted to honeydew of the long tailed mealybug, which is controlled by two encyrtid wasps, *Archopoides (Hungariella) peregrinus* (Compere) and *Anagyrus fusciventris* (Girault). In several laboratory experiments no adverse affect of a commercial preparation of *B.t.* (Dipel[R]) on these wasps was observed. Not only was the preparation harmless, but also probably due to additional food ingredients in the product, it delayed mortality of the wasp (Fig. 4). The parasitoid wasps were observed in avocado orchards 7 days after initial treatment with preparations of *B.t.* (Wysoki et al., 1975).

To improve the biological control of the giant looper, an egg parasitoid wasp, *T. platneri*, was introduced into Israel in 1983 (Wysoki and Renneh, 1985; Wysoki et al., 1987). This parasitoid is already established in avocado plantations in California, where it was introduced to control two important lepidopterous pests, *Amorbia cuneana* Walsingham (Tortricidae) and *Sabulodes aegrotata* (Guenee) (Geometridae) (Oatman et al., 1983). Under laboratory conditions, this egg parasitoid attacks both *B. selenaria* and *C. gnidiella*. Because of the commercial use of *B.t.* preparations against *A. cuneana* and *S. aegrotata*, a bioassay was conducted to determine possible detrimental effects on these wasps by two *B.t.* commercial preparations (Bactospeine and Dipel) at very high concentrations. Statistical analyses done by ANOVA (t-test $P < 0.05$) showed no significant differences between treated and untreated wasps (Fig. 5) (S. Wellinga, personal communication). Other species of *Trichogramma* are also known not to be affected by *B.t.* such as *T. cacoeciae* Marchal (Franz et al., 1980; Hassan, 1983; Hassan et al., 1983) and *Trichogramma pretiosum* Riley (Oatman et al., 1983). Other serovars of *B.t.* have no adverse effect on several *Trichogramma* species: serovar *thuringiensis* on *T. cacoeciae* (Hassan and Krieg, 1975), serovar

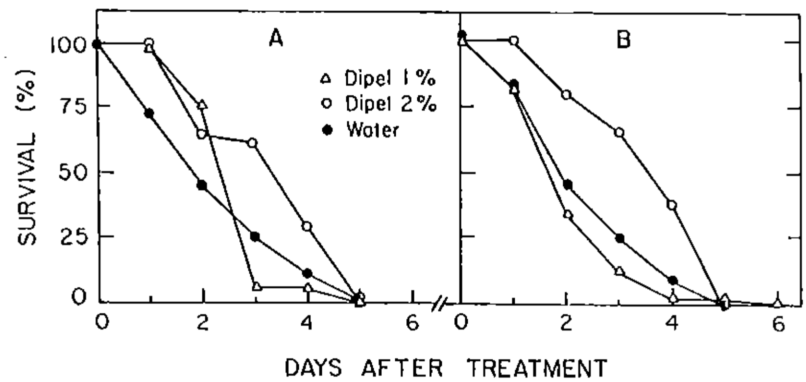

Fig. 4. Effect of treatment with 1% and 2% Dipel on the survival rate of (A) *Arhopoideus peregrinus* and (B) *Anagyrus fusciventris* (Wysoki et al., 1975).

galleriae and serovar *israelensis* on *T. evanescens* Westwood (Salama and Zaki, 1985), and serovar *israelensis* on *T. cacoeciae* (Krieg et al., 1980). Another unidentified strain of *B.t.* was found harmless to *T. nubiliale* Ertle and Davis (Tipping and Burbitis, 1983). According to Bull and Coleman (1985), microbial pesticides such as *B.t.* are fully compatible with releases of *Trichogramma* species, and therefore the possible introduction of additional *Trichogramma* species should not hinder continued use of *B.t.* for insect control.

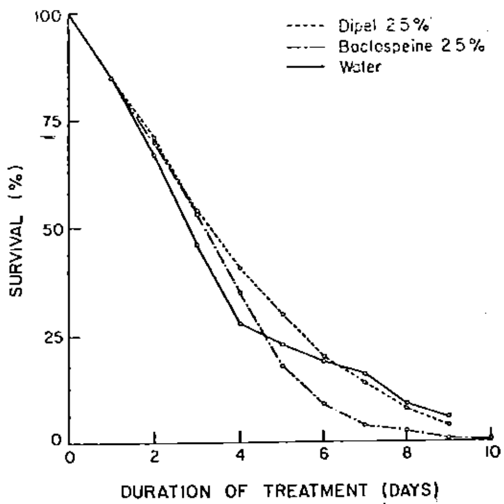

Fig. 5. Effect of treatment with 2.5% Dipel and 2.5% Bactospeine on the survival rate of *Trichogramma platneri* in a 75% honey/water solution (S. Wellinga and M. Wysoki, unpublished data).

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