Efficacy of *Bacillus thuringiensis* preparations containing dead and live spores against two avocado pests: the giant looper, *Boarmia selenaria* (Lep.: Geometridae) and the honeydew moth, *Cryptoblabes gnidiella* (Lep.: Phycitidae)

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Abstract. The activity of Toarow CT, a commercial preparation containing dead spores of *Bacillus thuringiensis* var. *kurstaki*, was compared with that of two other commercial preparations containing live spores, against the giant looper, *Boarmia* (*Ascotis*) *selenaria* (Schiffermüller), and the honeydew moth, *Cryptoblabes gnidiella* (Millière). In laboratory experiments Toarow CT was compared with Dipel W.P. and in field trials with Thuricide HP. In laboratory trials 60–80% of *B. selenaria* larvae aged 8 and 15 days were killed by a product concentration of 0·5% and 80–90% by a concentration of 1%, respectively, for Toarow CT and Dipel WP; 100% mortality of 15-day-old giant looper was reached only on the ninth day after initial treatment. In field trials, after 1 week some larvae remained in Toarow CT-treated plots, but after 2 weeks no live larvae were found after Toarow CT and Thuricide HP treatments. Because of the great sensitivity of *C. gnidiella* to *B. thuringiensis* preparations, the 6- to 8-day-old larvae were killed on avocado fruit and on artificial medium, between 24 h and 4 days after treatment, depending on their age and the concentration of preparations used.

Keywords: *Bacillus thuringiensis*; biological pest control; avocado; *Boarmia selenaria*; *Cryptoblabes gnidiella*

Introduction

Boarmia (*Ascotis*) *selenaria* is a serious pest of various crops in the world: avocado in Israel, coffee in Kenya and Tanzania, tea in Taiwan, India and Georgia, USSR, and citrus in Sicily and South Africa; it has also been recorded on peanuts, alfalfa, apples and pecans (for pertinent references *see* Wysoki, 1982). Control of this pest is based on the use of preparations containing *Bacillus thuringiensis* (Izhar, Wysoki and Gur, 1979). As only young larvae, up to 1·5 cm in length, are sensitive to the bacterium, a monitoring system based on traps baited with virgin females is used. On the basis of the fluctuation in numbers of males trapped (about 2 weeks after the peak trapping) and monitoring of the appearance of young larvae in the orchards, spraying of orchards with *B. thuringiensis* preparations from the air or ground is recommended (Wysoki and Izhar, 1986). An additional significant pest in avocado orchards is the honeydew moth, *Cryptoblabes gnidiella*, and its control also is based on the use of commercial preparations of *B. thuringiensis* (Wysoki *et al.*, 1975). The sensitivity of this pest to these preparations is high and usually results in the death of the larvae after 24 hours. *C. gnidiella* is polyphagous, attacking also citrus, apricots, peaches, plums, grapes, pomegranates, cotton, loquats, corn, sorghum and other crops (Bodenheimer, 1930; Avidov and Gothilf, 1960; Swailen and Ismail, 1972; Srivastava and Singh, 1975). The purpose of the investigations described were to determine if Toarow CT, which contains the *B. thuringiensis* δ-endotoxin with killed spores, will have the same influence on *B. selenaria* and *C. gnidiella* as the commercial preparations containing live spores, which are today used to control the pests in avocado orchards.

Materials and methods

The preparations tested were wettable powders containing *Bacillus thuringiensis* var. *kurstaki*, commercially available as the following formulations: Toarow CT, with dead spores of the bacterium, con-

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taining 7·0% of crystal protein (70 B.t.×unit/mg as potency) manufactured by Toagosei Chemical Industry Co., Japan (represented in Israel by Isaac Federman Ltd); Dipel W.P. containing 3·2% active ingredient (B.t.), 16000IU/mg and at least 25 billion viable spores per gram, manufactured by Abbott Lab., USA (represented by Chemical and Technical Equipment Ltd); and Thuricide HP containing 3·2% active ingredient (B.t.), 16000IU/mg and at least 30 billion viable spores per gram, manufactured by Sandos Wagner, USA (represented by Pazchim Ltd). Colfix (comprising 40% polyvinyl resins and 60% adjuvants) manufactured by Sipcam, Italy (represented by Rimi Ltd), was added to the water suspensions as a sticker at the rate of 0·05%.

Laboratory breeding of *B. selenaria* larvae took place at 27±1°C and 45% relative humidity. The larvae were reared in plastic cages, 15×35×29cm, covered by glass, with two openings (each 25×7·5cm), covered with cloth to ensure adequate ventilation. Sawdust and absorbent paper were placed on the bottom of each cage to absorb moisture and as a pupation site for the larvae. A plastic grid was placed on plastic containers and used as a tray for the food medium. The artificial medium was prepared according to Shorey and Hale (1965), slightly modified by replacing the antibiotics with vitamin solutions (Navon, Wysoki and Keren, 1983). The pupae were kept in plastic boxes with net-covered openings until emergence of the adults, when they were transferred to plastic mating cages, 15×35×29cm, covered with glass. A net was hung between the glass cover and the bottom, providing a surface for the mating adults and also enabling the eggs to drop through the net to the bottom of the cage. In the centre of the cage, on the net, a piece of cotton wool soaked in water was placed to provide drinking water for the adults. The eggs were collected from the bottom of the mating cages and kept in cloth-covered plastic containers until hatching.

The products for testing were added to the artificial diet or leaves were dipped in different concentrations of the products dissolved in water. For the laboratory test, larvae of different ages (*see* Results) were used. For laboratory experiments on leaves, 8-day-old larvae (third instar, 10–15mm long) were used. The experiments were carried out in plastic containers, 6cm in diameter and 4cm high, on the bottom of which absorbent paper (Whatman 40) was placed to absorb moisture, and on the top of which a perforated plastic cover was placed for ventilation. Five larvae were inserted into each container in 20 replications per treatment, for a total of 100 per treatment. Young avocado leaves were collected from the orchards and dipped in various substances at concentrations of 0·5% and 1%, to which was added 0·5% Colfix. The dipped leaves were inserted into the plastic containers as food for the caterpillars (larvae); control leaves were dipped in Colfix 0·05% only. The larval mortality was checked daily for 6 days and in certain cases even longer.

Field trials were carried out at 'En haMifraz, Western Galilee, on avocado trees cv. Haas, in which the efficacy of Toarow CT was compared with that of Thuricide HP. In these trials larval mortality and the damage caused to the avocado tips were investigated. The effects of the sprays were checked after 1 or 2 weeks.

The efficacy of Toarow CT against *C. gnidiella* was also studied. The honeydew moth larvae were reared on the same artificial food as were *B. selenaria* larvae. Six-day-old larvae were used in experiments on a food medium, and 8-day-old larvae on fruit. In the experiments on fruit, two plastic rings (22mm wide, 35mm deep and 10mm high) were glued on to avocado (cv. Haas) fruit, the upper part of the ring being covered with a piece of silk material. The larvae were inserted into the rings (five to a ring) 3 days before the experiment, for acclimatization. After spraying, the mortality rate was recorded.

Results

In a series of experiments on leaves, the efficacy of Toarow CT and of Dipel WP against 8-day-old caterpillars of *B. selenaria* was checked. The efficacy of Toarow CT at 0·5% and 1% was similar to and even somewhat better than that of Dipel WP (*Table 1, Figure 1a*). The number of larvae surviving after 7 days was eight and four, after treatment with Toarow CT at 0·5% and 1%, respectively, compared with 16 and eight larvae after treatment with Dipel WP at the same concentrations. In addition, the weight of the surviving larvae was extremely low: 14·0mg with 0·5% Toarow CT and 6·5mg with 1% Toarow CT, as compared with 185·8mg in the control group. The weight of the larvae surviving treatment with Dipel WP was similar to that of larvae surviving Toarow CT treatment.

In an additional experiment, with 15-day-old larvae, the two preparations gave rise to similar mortality rates at the same concentrations (*Figure 1b*) and the weight of the surviving larvae was low in comparison with the

TABLE 1. The mean weight of surviving *Boarmia selenaria* larvae 7 days after the initial treatment, when the caterpillars were 8 or 15 days old

	8 days old at beginning of experiment		15 days old at beginning of experiment	
Treatment	No. of surviving larvae	Weight (mg) $\bar{x}\pm$SE	No. of surviving larvae	Weight (mg) $\bar{x}\pm$SE
Control (Colfix 0·05%)	45	185·8±99·5	37	216±176
Toarow CT 0·5%+ Colfix 0·05%	8	14·0± 5·9	6	69± 40
Toarow CT 1%+ Colfix 0·05%	4	6·5± 3·0	1	40±—
Dipel WP 0·5%+ Colfix 0·05%	16	13·7±13·5	9	56± 37
Dipel WP 1%+ Colfix 0·05%	8	9·9± 7·3	2	32± 4

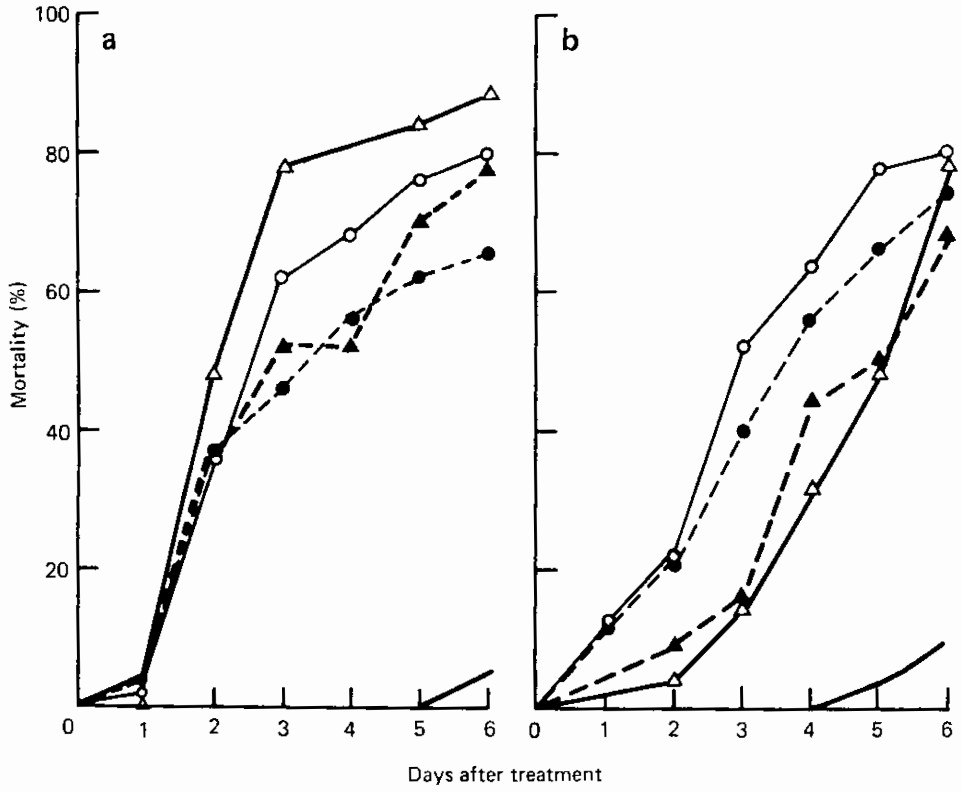

FIGURE 1. Mortality rate of *Boarmia selenaria* larvae on treated avocado leaves. (a) Larvae 8 days old; (b) larvae 15 days old. ———▲——— Colfix 0·05% + Toarow 0·5%; ———△——— Colfix 0·05% + Toarow 1·5%; ———●——— Colfix 0·05% + Dipel 0·5%; ———○——— Colfix 0·05% + Dipel 1·5%; ——— control (Colfix 0·05% alone).

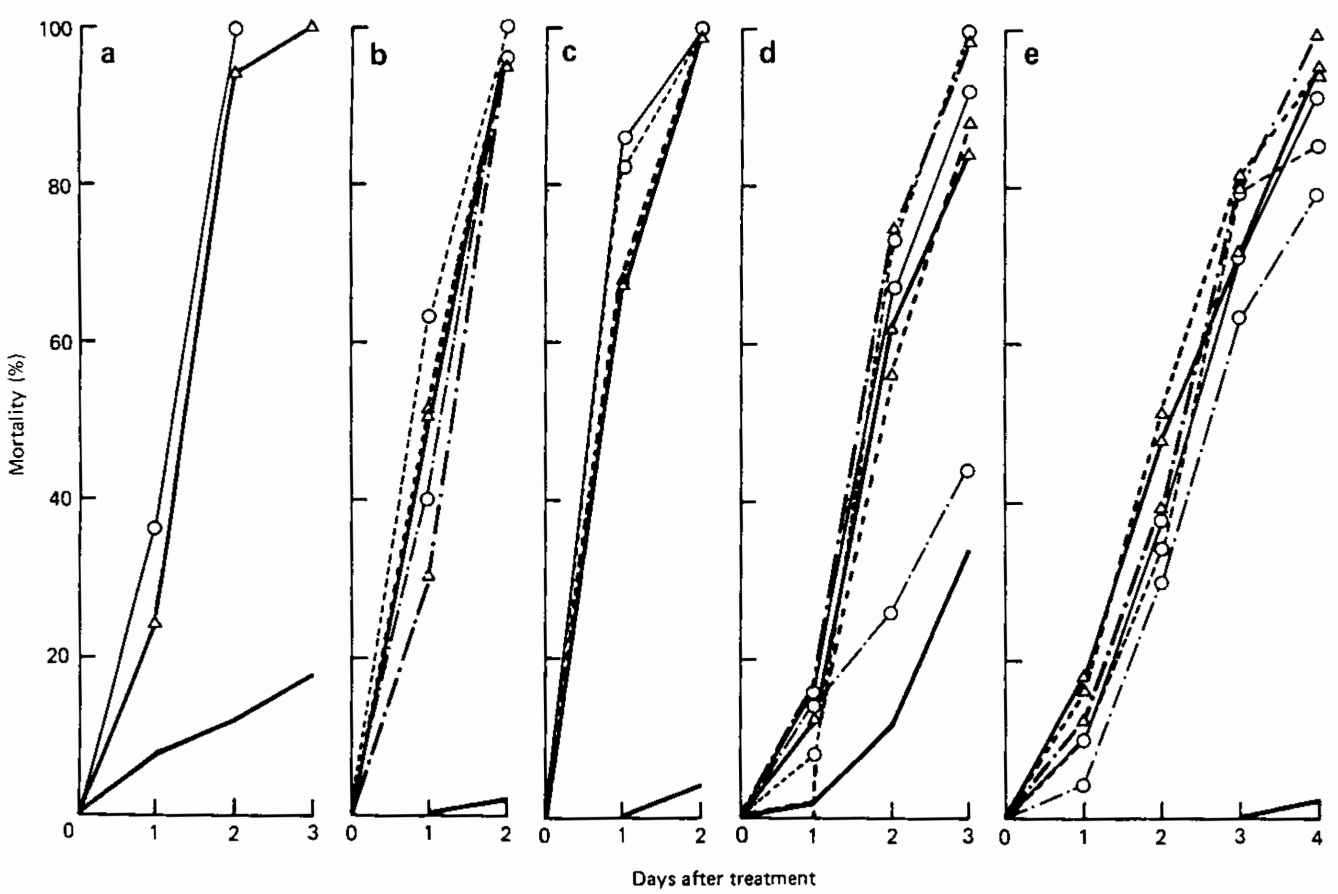

FIGURE 2. Mortality rate of *Boarmia selenaria* larvae on treated artificial medium. Larvae aged (a) 1 day; (b) 2 days; (c) 6 days; (d) 10 days; (e) 12 days. ———△——— 0·0125% Toarow; ———○——— 0·0125% Dipel; ———△——— 0·00625% Toarow; ———○——— 0·00625% Dipel; —·—△—·— 0·003125% Toarow; —·—○—·— 0·003125% Dipel; ——— control.

control (216 mg): 40 mg with 1% Toarow CT and 32 mg with 1% Dipel WP (*Table 1*).

Because of the sensitivity of young and neonate larvae, additional experiments were carried out on the effect of these preparations on *B. selenaria* which were grown on artificial food (Shorey and Hale, 1965). The antibiotic was removed from the food medium and replaced by a vitamin mixture (Navon *et al.*, 1983). The experiments were carried out on caterpillars (1, 2, 6, 10 and 12 days old) and used a very low concentration of the products—between 0·1% and 0·003125% (*Figure 2*). The mortality rate of the 1- and 2-day-old larvae with either Toarow or Dipel was very high, reaching 100% after 2 days. Even 6-day-old caterpillars were very sensitive and died after 2 days. Larvae that were 10–12 days old died after 3 days, but with lower concentrations of the two preparations tested the mortality rate did not reach 100%.

The efficacy of Toarow CT was compared with that of Thuricide HP against *B. selenaria* larvae in cv. Haas avocado orchards (4 ha for each treatment) at 'En haMifraz. Before each spraying the caterpillars on the trees were counted. Spraying was carried out on 26 July, followed 1 and 2 weeks later by counts of larvae and damaged tree tops. In each treatment 5 kg preparation per hectare was used plus 0·05% Colfix. The number of *B. selenaria* was reduced after 1 week, and after 2 weeks no live caterpillars were found in the treated area; only one larva was found in the area treated with Thuricide HP (*Table 2*).

In laboratory experiments performed to determine the effect of Toarow CT on honeydew moth caterpillars, the results seemed to be no different from those with Dipel WP. Because of the great sensitivity of the pest to *B. thuringiensis* here as well, good results were obtained very rapidly (*Figures 3* and *4*).

Discussion

Bacterial preparations containing *Bacillus thuringiensis* var. *kurstaki* are used commercially in avocado orchards against two important lepidopterous pests—the giant looper, *Boarmia selenaria* and the honeydew moth, *Cryptoblabes gnidiella.* The activity of the bacterium against the giant looper is limited to caterpillars up to 1·5 cm in length (Izhar *et al.*, 1979). Control of the pest was therefore carried out according to a timetable determined by sex traps, utilizing virgin females (Wysoki and Izhar, 1986). Two weeks after the peak of trapping, the avocado orchards are checked for infestation, and spraying with *B. thuringiensis* is recommended according to the results. Because of these limitations it was important to find additional, more virulent strains or varieties of the bacterium which would control larger caterpillars as well. In the past a number of *B. thuringiensis* strains have been examined (Cohen, Wysoki and Sneh, 1983; Wysoki and Jarvinen, 1986) but their efficacy was unsatisfactory.

Toarow CT, which is characterized by inactive spores, showed an effect against the two tested species similar to that of preparations with live spores, namely Dipel WP and Thuricide HP. The honeydew moth is very sensitive to preparations containing *B. thurin-*

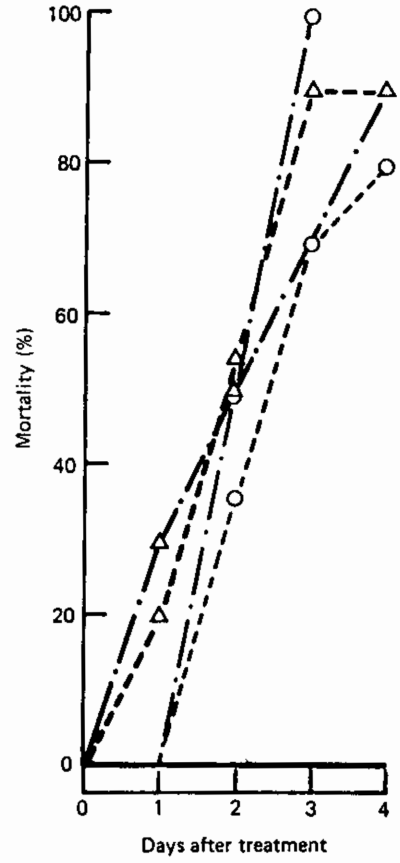

FIGURE 3. Mortality rate of 6-day-old *Cryptoblabes gnidiella* larvae on treated artificial medium. —·—Δ—·— Colfix 0·05% + Toarow 0·25%; – – –Δ– – – Colfix 0·05% + Toarow 0·10%; —·—○—·— Colfix 0·005% + Dipel 0·25%; – – –○– – – Colfix 0·05% + Dipel 0·10%; ——— control (Colfix 0·05% alone).

TABLE 2. Field trials with Toarow CT (5 kg/ha) and Thuricide HP (5 kg/ha) against *Boarmia selenaria* larvae at 'En haMifraz

Preparation	Date (1984)	Number of trees	Total number of tree tops counted	Total number of damaged tree tops	Number of *B. selenaria* larvae: Small (>1·5 cm)	Medium (1·5–2·5 cm)	Large (<2·5 cm)
Before spraying	26 February	30	900	Not counted	331	85	41
Toarow CT	1 July	25	500	221	17	0	8
Thuricide HP		50	1000	391	4	2	3
Toarow CT	8 July	50	1000	236	0	0	0
Thuricide HP		50	1000	34	0	0	1

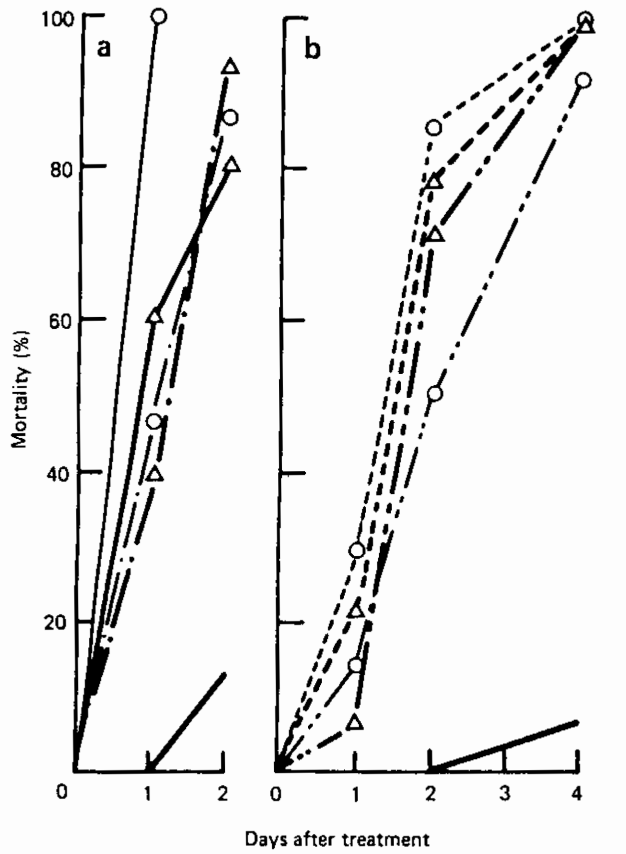

FIGURE 4. Mortality rate of 8-day-old *Cryptoblabes gnidiella* larvae on treated avocado fruits. (a) commercially recommended concentrations (0·5%, 1·0%); (b) low concentrations (0·00625% and 0·003125%). Toarow ——Δ—— 0·1%; ---Δ--- 0·05%; ——Δ—— 0·00625%; —·—Δ—·— 0·003125%; Dipel ——○—— 0·1%; ---○--- 0·05%; ——○—— 0·00625%; —·—○—·— 0·003125%; —— control.

giensis with viable spores (Wysoki *et al.*, 1975), and Toarow CT (with dead spores) demonstrated this sensitivity as well. Two other products of the Toagosei Chemicals Industry Co., Toarow and Toarow B.t., were tested against *B.* (*Ascotis*) *selenaria*, which is a pest of tea in Japan, and were found to be effective (Takaki, 1975). On the other hand, the Toarow CT preparation is not effective against another lepidopterous pest, the European corn borer, *Ostrinia nubilalis* Hübner (Navon and Melamed-Madjar, 1986), because both toxin crystals and active spores are necessary for control of this pest (Mohd-Salleh and Lewis, 1982).

Although *B. thuringiensis* preparations are considered to be safe for use, several infections caused by *B. thuringiensis* have been reported: corneal ulcer in man (Samples and Buettner, 1983), bovine mastitis (Gordon, 1977), and experimental diarrhoea in monkeys (Bennett, 1986). Bennett (1986) therefore suggested that bacterial insecticides based on *B. thuringiensis* should be screened for the production of diarrhoea enterotoxin, a water-soluble protein toxin produced by the vegetative cells in the growth phase. Bacterial insecticides with dead spores can preclude such developments. Moreover, Toarow CT product has been registered in Japan (since 1981), where the use of commercial preparations containing viable spores has not been approved, because they are considered to induce an infectious disease in silkworms.

Notes and acknowledgements

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