LABORATORY BIOASSAYS OF THE ACUTE AND ANTIFEEDANT EFFECTS OF AVERMECTIN B1 AND A RELATED ANALOGUE ON *SPODOPTERA EXIGUA* (HÜBNER)[1,2]

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Abstract: In diet-incorporation bioassays, an analogue of avermectin (L656,748) was ca. 1,500 times more active against neonate *Spodoptera exigua* (Hübner) than avermectin B1 (Abamectin). LC_{50} values in ng active ingredient/ml diet (with 95% fiducial limits) were 0.480 (0.447 - 0.515) for the analogue and 755.4 (616.4 - 897.2) for avermectin B1. In leaf disk bioassays where both third instar larvae and leaf disks were treated, the avermectin analogue proved to be as toxic as a methomyl standard with 24 h post-application. Avermectin B1 was significantly less active. In residue tests using leaf disks, both avermectin analogue and methomyl caused significant levels of mortality for third instar larvae for at least 14 d post-application, by which time avermectin B1 was no longer effective. Although residues of avermectin B1 produced significant antifeedant effects immediately following application, this effect was not evident by 7 d post-treatment. Only residues of the avermectin analogue produced significant reductions in food consumption per larvae after 14 d. Thus, the analogue of avermectin has considerably more potential for use against *S. exigua* than avermectin B1.

Key Words: *Spodoptera exigua,* beet armyworm, avermectin, Abamectin, food consumption.

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Avermectin B1 (Abamectin), a macrocyclic lactone produced by the mycelia of *Streptomyces avermitilis,* has substantial biological activity against a variety of economically important arthropods (Grafton-Cardwell and Hoy 1983; Burts 1985; Robertson et al. 1985; Grout and Morse 1986). The primary mode of action appears to be as a mimic or disrupter of the neurotransmitter gamma aminobutyric acid (Fritz et al. 1979; Kass et al. 1980; Mellin et al. 1983), but the visual system also is impaired in some insects (Agee 1985). Studies documenting both the selectivity of avermectin B1 (Hoy and Cave 1985) and sublethal effects on pest species (Robertson et al. 1985; Wright 1984; Beach and Todd 1985), suggest that this compound might be useful in integrated pest management (IPM) programs.

Avermectin B1 has potential for incorporation into IPM programs in celery and tomatoes for control of the leafminer, *Liriomyza trifolii* Burgess, as this chemical does not damage the parasite-based biological control system (Trumble 1985). Since the complex of Eulophid and Pteromalid species can provide up to 90% control of the leafminer in celery (Trumble and Nakakihara 1983), and the problems associated with development of insecticide resistance by *L. trifolii* are escalating (Keil et al. 1985), maintenance of parasite activity is becoming increasingly important. Unfortunately, avermectin B1 does not provide economically effective suppression of the beet armyworm (BAW), *Spodoptera exigua* (Hübner) (Zehnder and Trumble 1985), which is also a key pest in celery and tomato.

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Therefore, the current practice of applying broad spectrum pesticides such as methomyl for BAW control, which kills the leafminer parasites without suppressing leafminer populations (Oatman and Kennedy 1976; Trumble and Toscano 1983), effectively eliminates the selective advantage of avermectin B1.

Although several approaches have potential for solving this problem, we examined the possibility that an analogue of avermectin B1 (L656,748) might have a broader range of biological activity which would include BAW. We report here several laboratory bioassays designed to compare the impact of avermectin analogue, avermectin B1, and methomyl on BAW survival and food consumption.

MATERIALS AND METHODS

Diet-Incorporation Bioassay

All BAW used in the following trials were from a laboratory colony established in 1982 from larvae collected in an untreated research plot in Orange Co., CA, and maintained on a modified lima bean diet (Patana 1969) at 27 ± 1°C, LD:16-8. The colony has been supplemented with several hundred wild insects to preserve the gene pool. The age of neonate BAW was "standardized" by selecting individuals which eclosed during a 4 h hour period. Thirty larvae (< 4 h old) were tested per dose, with seven doses per treatment plus a control. The entire assay was replicated four times. Doses (ng AI/ml diet) tested were: avermectin B1 (18 g AI/liter EC, MK 936) = 100, 200, 400, 800, 1200, 1800, 2400; avermectin analogue (6.0 g AI/liter EC, L656,748) = 0.1, 0.2, 0.3, 0.45, 0.6, 0.8, 1.10, and 2.0. Suspensions and controls were made by adding materials to an aqueous 0.1% Tween 80 solution to produce 50 ml.

Each dose was added to 1300 ml of artificial diet (Patana 1969) after the diet had cooled to 45.3°C in an ice bath. The mixture was blended for two minutes and then ca. 15 ml were poured into 30 ml plastic cups. The diet was allowed to dry for 1 - 4 h before one neonate BAW was placed in each cup. Cups were covered with opaque plastic lids and placed in an environmental chamber set at 27 ± 1°C and LD: 16-8. Larval mortality was assessed at day 7 in all treatments. Data initially were analyzed using the Proc Probit Procedure (SAS Inst. Inc. 1985) after correction for control mortality with Abbotts (1925) formula, and then judged for suitability for the probit model using the criteria of Vandekar and Dulmage (1982). Control mortality did not exceed 10%.

Leaf-Disk Bioassay and Food Consumption Trials

Larvae used in these tests were from the laboratory colony described previously. Ages were standardized by selecting test populations from eggs oviposited during a 12-h period, rearing them on a lima bean diet at 27 ± 1°C, and exposing them to pesticide application and/or residues within 12 h following molt to the third instar.

Spray applications for contact + residue and residue-only trials were made using a carbon dioxide pressurized backpack sprayer operating at a pressure of 2.81 kg per sq. cm and calibrated to deliver 935 liter/ha. A water control was included in each test. Rates in kg/ha for each experimental compound are listed on the tabular material. All tests consisted of four replicates of 20 larvae per treatment (five larvae per diet container), and four treatments: methomyl, avermectin analogue, avermectin B1, and a control. Thus, the total number of larvae used per

test was 320. A few larvae (never more than 5% per treatment, normally less than 2%) were lost due to cannabalism. Since the cause of death could not be determined precisely (i.e., whether cannabalism occurred after death or death occurred due to cannabalism), these insects were excluded from the analyses.

Larvae were classified as dead when no heartbeat could be detected visually, or as moribund when no response occurred to prodding, but the heart was still beating. Examination intervals varied from 4 h to 24 h, and have been listed with the tabular material for each test. All data were analyzed using the GLM procedure of SAS (SAS Inst. 1985) following an arcsine square root transformation, and means were compared with Duncan's New Multiple Range Test (DNMRT, Duncan 1955).

In contact + residue tests, larvae were placed on leaf disks in screen cages (3 mm high × 2.2 cm diameter), and the cages were randomly assigned to treatments. The insects, leaf disks and screen cages were treated, thus exposing larvae to direct contact plus residue even if the larvae left the disks and moved onto the screen. Since leaf disks (2.1 cm diameter) in the cages were changed at 24 h intervals, celery plants were treated at the initiation of the study, held in the laboratory at 22 - 25°C, and replacement leaf disks were cut from these plants (variety HK 5270-R). The leaf disks rested on moist cotton which prevented the plant material from drying too rapidly, and were covered with 25 mesh screening to inhibit movement off of the leaf disk. The wide mesh screen also allowed free air movement across the disks, preventing larval "fumigation" by vapor action from treated surfaces. Following treatment, cages were held at 27 ± 1°C in a photoperiod of LD: 16-8.

In residue-only tests, larvae were placed randomly in cages with leaf disks taken from celery (variety HK 5270-R) which had been treated as described previously. Where larvae were exposed to residue immediately following pesticide application, the leaf disks were allowed to dry for approximately 1 h prior to introduction of the larvae. Following pesticide applications, test plants were placed outdoors beneath an overhang, where they were protected from the nearly 3.8 cm of rain that fell during the first week and a half on the test. Additional leaf disks were collected as needed from these plants at 1 - 3 wk posttreatment. The plants received ca. 4.5 - 5 h of direct radiation (outdoor sunlight) each day. Thus, the degradation rates of the pesticides were less than could be expected if the plants had been exposed to actual field conditions, but the variable of rainfall was eliminated. Outdoor temperatures during the study ranged from 4.4 - 15.6°C during the first week, 4.4 - 18.3°C during the second week, and 1.6 - 21.1°C through the third week. As in the previous tests, larvae on treated disks were held at 27°C and a photoperiod of LD: 16-8.

Leaf disks from each replicate in the residue studies were changed at 24 h intervals for the 72 h following each test. Samples were frozen immediately. Recordings of leaf consumption initially were made with a Li-Cor Leaf Area Meter (Li-Cor Inc., Lincoln, NE) to determine the area of the leaf which was completely eaten through by the larvae. Since BAW larvae also may feed on just the leaf surface (i.e., epidermis and palisade mesophyll), and such damage will not be recorded by the leaf area meter, the additional surface area eaten was cut from the disks with a scalpel and the leaf consumption then was reassessed with the leaf area meter. All data were corrected for ca. 9.1% shrinkage of the leaf disks which occurred during storage (as measured by undamaged, frozen disks, n = 20).

RESULTS AND DISCUSSION

Diet-Incorporation Bioassay

In diet bioassays, avermectin analogue was ca. 1,500 times more toxic to BAW than avermectin B1 (Fig. 1). The dosage required to cause 90% mortality was 1,972 ng AI/ml diet (95% fiducial limits = 1,563 - 2,857) for avermectin B1 versus only 1.067 (0.956 - 1.226) for the avermectin analogue. In spite of the wide variation in toxicity, the parallel slopes generated for the probit lines suggest that response of the test population was similar for both compounds.

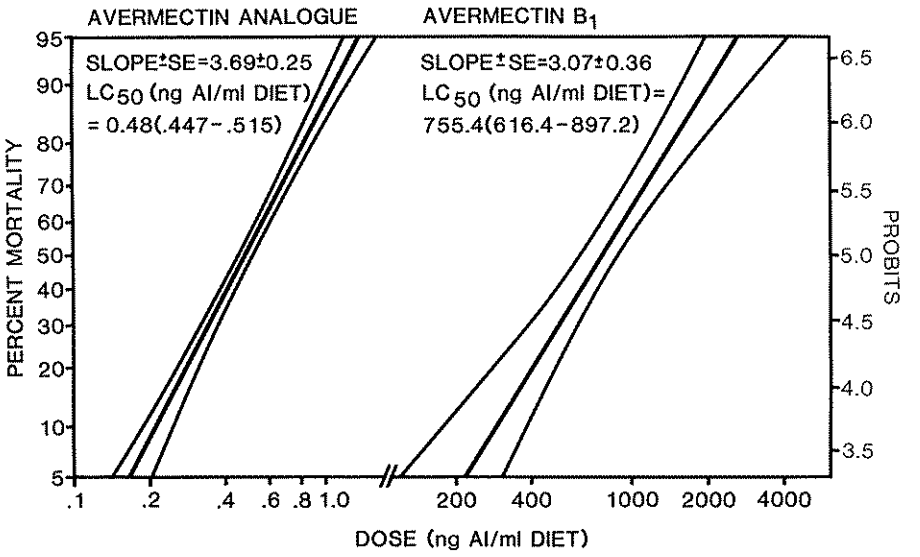

Fig. 1. Comparative toxicities of diet-incorporated avermectin B1 (abamectin) and a chemically derived analogue of avermectin to first instar *Spodoptera exigua* larvae. Curved lines denote the 95% fiducial limits.

Leaf-Disk Bioassay and Food Consumption Trials

In contact + residue tests, the avermectin analogue proved to be as toxic as the methomyl standard by 24 h posttreatment (Tables 1 and 2). Avermectin B1 acted substantially slower, but by 72 h all larvae were moribund, regardless of whether the application rate was 0.0112 (Table 1) or 0.0224 kg/ha (Table 2). Larvae in the avermectin B1 treatments from both tests were maintained for an additional 48 h, at which time all were dead. Although this suggests that avermectin B1 should be an effective control compound for BAW, field trials have demonstrated that this chemical is not efficacious (Zehnder and Trumble 1985).

Incomplete coverage and failure to directly contact larvae are possible causes for the lack of effective BAW suppression by avermectin B1 in field situations. Late instar BAW exhibit a negatively phototactic behavior, and hide either at the center of the celery plant or underground during the daylight hours (Griswold and Trumble 1985). Thus, the largest larvae are protected in refugia during the normal

Table 1. Cumulative percent mortality of third-instar *Spodoptera exigua* over 72 h resulting from contact + residual activity of selected insecticides.

Treatment and kg AI/ha	Cumulative % mortality posttreatment*			
	4 h	24 h	48 h	72 h
methomyl 1.008	100.00 a	100.00 a	100.00 a	100.00 a
avermectin 0.0112 analogue	64.25 b	97.37 a†	98.75 a†	100.00 a
avermectin B1 0.0112	1.25 c	10.90 b	30.64 b	62.85 b†
control (water only)	0.00 c	0.00 c	0.00 c	0.00 c

* Means of four replicates of 20 larvae per treatment; means in columns followed by the same letter are not significantly different at the $P \leq 0.05$ level, DNMRT (Arcsine transformation, untransformed means presented).

† Remaining larvae moribund; larvae exposed to avermectin B1 died within 48 h following the termination of the 72 h test.

Table 2. Cumulative percent mortality of third-instar *Spodoptera exigua* over 72 h resulting from contact + residual activity of selected insecticides.

Treatment and kg AI/ha	Cumulative % mortality posttreatment*				
	3 h	18 h	24 h	48 h	72 h
methomyl 1.008	100.00 a	100.00 a	100.00 a	100.00 a	100.00 a
avermectin 0.0112 analogue	81.25 b	86.25 b	100.00 a	100.00 a	100.00 a
avermectin B1 0.0224	10.00 c	33.75 c	48.75 b	63.75 b	73.75 b
control (water only)	0.00 c	0.00 c	1.25 c	6.25 c	11.25 c

* Means of four replicates of 20 larvae per treatment; means in columns followed by the same letter are not significantly different at the $P \leq 0.05$ level, DNMRT (Arcsine transformation, untransformed means presented).

pesticide application period. While contact with avermectin B1 residues immediately following application resulted in good control, a significant loss in effectiveness was evident at 7 d (Table 3). By 14 d the impact of remaining residues on mortality was undetectable with this assay.

The avermectin analogue demonstrated relatively longer residual effects (Table 3). Even after residues aged for 14 d, exposure resulted in many larvae being moribund at 48 h, and 30% larval death after 72 h. This compound, therefore, has considerably more potential for controlling BAW in the field than avermectin B1. Paralysis of the larvae exposed to sublethal residues of avermectin analogue may have additional value in IPM programs for celery if this chemical, like avermectin B1, has little impact on beneficial Hymenoptera (Trumble 1985). Thus this chemical may have potential not only as a pesticide, but as a means of extending the "window" of suitability of *S. exigua* as a host for parasites and/or predators. Such an extension would be achieved without risk to the grower, as the larvae do not feed following the onset of morbidity.

The trends in leaf area eaten per larvae were nearly identical whether measurements were made of areas where leaves were eaten completely through or overall surface area eaten was assessed. In all treatments, larvae fed on just the surface of the leaf (i.e., epidermis and palisade mesophyll tissues) in approximately

Table 3. Cumulative percent mortality of third instar *Spodoptera exigua* exposed to residual activity of selected insecticides following application.

Days post application	Treatment	Cumulative % mortality posttreatment* 4 h	24 h	48 h	72 h
0	methomyl	100.00 a	100.00 a	100.00 a	100.00 a
	avermectin analogue	30.00 b	100.00 a	100.00 a	100.00 a
	avermectin B1	12.50 c	43.50 b	50.66 b	75.10 b
	control (water only)	2.50 c	2.50 c	2.50 c	2.50 c
7	methomyl	38.75 a	70.02 a	89.67 a	96.25 a
	avermectin analogue	0.00 b	29.00 a	33.97 a	57.69 a
	avermectin B1	1.25 b	6.92 c	10.62 c	27.42 c
	control (water only)	1.25 c	1.25 c	2.50 d	2.50 d
14	methomyl	0.00 a	16.60 a	40.70 a	55.92 a
	avermectin analogue	0.00 a	0.00 b	11.59 b	30.56 b
	avermectin B1	0.00 a	0.00 b	0.00 c	3.95 c
	control (water only)	0.00 a	0.00 b	0.00 c	0.00 c

* See previous table for application rates; means of four replicates of 20 larvae per treatment per date; means in columns within days posttreatment followed by the same letter are not significantly different at the $P \leq 0.05$ level, DNMRT (Arcsine transformation).

the same proportion. Therefore, there were no clear behavioral differences in leaf area consumption which could be ascribed to any given treatment.

The leaf area consumed by larvae treated with pesticides was variable. Exposure to residues of either the avermectin analogue or methomyl was adequate to cause 100% mortality immediately following application, and significantly reduce feeding 7 d post application (Table 4). Only the avermectin analogue

Table 4. Impact of chemical residue on leaf area consumption by *Spodoptera exigua.**

Days posttreatment	Treatment	Area of leaf "holed" (sq. cm)†	Total surface area eaten (sq. cm)
0	control	0.098 a	0.138 a
	avermectin B1	0.045 ab	0.049 ab
	avermectin analogue	0.000 b	0.000 b
	methomyl	0.000 b	0.000 b
7	control	0.128 a	0.196 a
	avermectin B1	0.168 a	0.295 a
	avermectin analogue	0.005 b	0.021 b
	methomyl	0.027 b	0.032 b
14	control	0.083 b	0.123 c
	avermectin B1	0.145 a	0.262 a
	avermectin analogue	0.257 c	0.041 d
	methomyl	0.147 a	0.183 b

* Values represent average leaf area eaten per larvae in 24 h. Means in columns within days posttreatment are not significantly different if followed by the same letter ($P \leq 0.05$, DNMRT), see Table 2 for application rates.

† "Holed" = eaten completely through the leaf.

significantly reduced feeding at 14 d posttreatment. Avermectin B1 residues reduced leaf area consumption at 0 d posttreatment, which was consistent with earlier reports showing a feeding reduction by the alfalfa weevil on alfalfa immediately (48 h) following application (Pienkowski and Mehring 1983). However, BAW feeding in our trials was equivalent to or greater than that of control insects for 7 and 14 d old residues. Thus, the short antifeedant effect on BAW, coupled with incomplete coverage in field situations and a possible stimulation of feeding by 7 + d-old residues, may help explain the lack of economically useful suppression of BAW provided by avermectin B1 in celery.

An increase in leaf area consumption was noted for larvae feeding on methomyl-treated leaves 14 d after application. Pienkowski and Mehring (1983) noted a similar increase in food consumption for alfalfa weevil larvae on foliage treated with carbofuran.

Although the avermectin analogue has demonstrably more activity against BAW than avermectin B1, additional field-oriented research will be necessary to document the impact on: 1) BAW populations and damage, 2) *Liriomyza trifolii* survival and fitness, and 3) parasitoid survival, success, and fitness. With this information, the potential for inclusion of the avermectin analogue in current integrated pest management and resistance management programs can be assessed.

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