Toxicity and Pharmacodynamics of Avermectin in the Tobacco Budworm, Corn Earworm, and Fall Armyworm (Noctuidae: Lepidoptera)

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Short-term mortality effects as well as delayed disruption of metamorphosis were observed in third- and last-stage larvae of the tobacco budworm (TBW), *Heliothis virescens* (F.), corn earworm (CEW), *Heliothis zea* (Boddie), and fall armyworm (FAW), *Spodoptera frugiperda* J. E. Smith, after topical or oral treatments with avermectin (AVM). These effects were most severe in TBW, moderate in CEW, and least severe in FAW. Studies of the fate of topically applied [³H]AVM-B_1a indicated that similar but very low-level concentrations (1.1–3.5% of dose) were absorbed by all three species. Orally administered [³H]AVM-B_1a was readily absorbed from the midgut, metabolized, and excreted along with its metabolites by last-stage larvae of the three test species; the rates of these physiological events were slowest in TBW and about the same for CEW and FAW. At 4 and 8 h after oral administration of [³H]AVM-B_1a to last-stage larvae, significantly more radioactive material (ca. 2×) was recovered from the heads of TBW, while that in CEW and FAW was about the same. The magnitude of observed differences in the posttreatment fate of AVM does not appear sufficient to explain the substantial differences in susceptibility to the compound among the test species.

The avermectins comprise a group of closely related macrocyclic lactones (Figure 1) that are produced during fermentation reactions of the actinomycete *Steptomyces avermitilis* (Burg et al., 1979). These compounds were originally detected in research on new drugs for the treatment of gastrointestinal parasites of domestic animals (Egerton et al., 1979); they have since shown considerable promise for use in controlling various agricultural arthropod pests (Putter et al., 1981). Recent reports indicate the avermectins have remarkable systemic activity against certain ectoparasites of livestock (Benz, 1985; Drummond, 1985). These chemicals are also highly effective when administered in baits for control of fire ants, *Solenopsis spp.* (Williams, 1985), and have shown good potential for use against several arthropod pests of field and horticultural crops (Putter et al., 1981; Wolfenbarger et al., 1985; Wright et al., 1985). In a recent study with *Heliothis spp.*, Wright et al. (1985) observed short-term mortality effects after applications of avermectin B_1 as well as delayed effects on pupation and adult emergence.

In preliminary studies, we found substantial differences in susceptibility to avermectin among the noctuid species *Heliothis virescens* (F.), *Heliothis zea* (Boddie), and *Spodoptera frugiperda* J. E. Smith. This paper reports results of comparative studies of the toxicity and pharmacodynamics (Goodman and Gilman, 1965) of avermectin after application to different development stages of these three insect species.

MATERIALS AND METHODS

Insects. Colonies of the tobacco budworm (TBW), *H. virescens* (F.), the corn earworm (CEW), *H. zea* (Boddie), and the fall armyworm (FAW), *S. frugiperda* J. E. Smith, were reared in the laboratory on a semidefined artificial diet (Shaver and Raulston, 1971). The three strains used were obtained from standard colonies maintained in USDA laboratories at Stoneville, MS (TBW, CEW), or Brownsville, TX (FAW), and have been reared through many generations in the laboratory without exposure to insecticides. These strains are considered to be generally susceptible to insecticides.
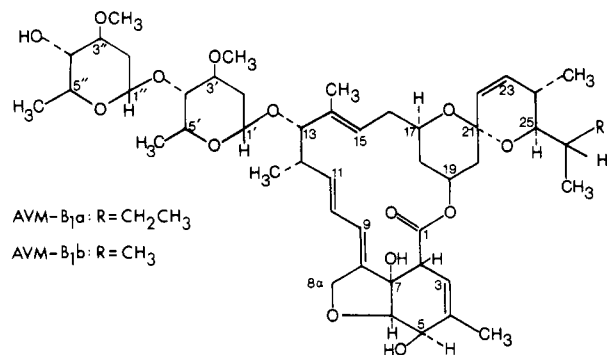
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Chemicals. Samples of avermectin B_1a (AVM-B_1a) radiolabeled with tritium at the 5-position (sp act. 1.74 mCi/mg) and unlabeled avermectin B_1 (AVM-B_1, MK-936; a mixture of 80% avermectin B_1a and 20% avermectin B_1b) were provided by Merck & Co., Inc., Rahway, NJ. These materials were >95% pure and were used as received. All other chemicals and solvents were obtained from various commercial sources.

Toxicity. Third- and last-stage larvae of the three insect species were used for evaluations of the acute and delayed effects of AVM-B_1. Third-stage larvae (25–30 mg) were lightly anesthetized with carbon dioxide and then treated on the dorsal abdominal surface with topical applications (1 μL/each) of acetone solutions containing graded doses of AVM-B_1. After treatment, larvae were confined individually in small cups of diet and observed daily on the four succeeding days to determine acute mortality effects and then periodically through 28 days posttreatment for possible delayed effects on pupal and adult development. Last-stage larvae (average weights: TBW, 370 mg; CEW and FAW, 460 mg) were anesthetized with carbon dioxide and orally treated with solutions (2 μL/insect) of AVM-B_1 in Me_2SO. These doses were administered with a calibrated, micrometer-driven syringe fitted with a blunt, 27-gauge hypodermic needle (15 mm long). For this treatment, the needle is inserted gently into the buccal cavity and eased through the alimentary canal until the tip reaches the midgut, where the dose is delivered. Treated last-stage larvae were also confined individually and observed daily on the four succeeding days for acute mortality effects and periodically thereafter through 14 days posttreatment for delayed effects on development.

Each test was replicated three or more times with 20 larvae/dose per replicate, using at least four concentrations of AVM. Data were analyzed by using a computerized probit analysis program based on the procedure of Finney (1971).

Absorption. Third-stage larvae of each species were treated topically as described with acetone solutions of [³H]AVM-B_1a (0.01 μg/larva) and then held individually without food at 27 °C in 20-mL glass scintillation vials. At the specified times posttreatment, duplicate samples of five larvae each were rinsed three times with 10-mL volumes of acetone. Rinsed larvae were then homogenized with acetone using a motor-driven Teflon pestle and glass

AVM-B₁a: R=CH₂CH₃

AVM-B₁b: R=CH₃

Figure 1. Structure of avermectin.

tube tissue grinder. Homogenates were centrifuged, and the precipitated solids were reextracted with acetone. Radioactivity in combined external rinses or in internal extracts was determined by liquid scintillation (LSC) counting as described below. Radioactivity in extracted tissues was analyzed by oxygen combustion (vide infra), and the results were combined with extraction data to give a single value for absorbed radioactive material.

Preliminary studies indicated that radioactivity in fecal pellets, which accumulated during the 8-h experimental period, was below the level of detection. Radioactive material that presumably was deposited in holding containers as a result of ruboff was measured directly by LSC following addition of counting fluid to the vials. Absorption tests were replicated at least four times.

Distribution. Actively feeding last-stage larvae were selected on the basis of both chronological age and apparent uniformity in physiological development for use in studies of the posttreatment distribution of radioactive material after oral administration of a solution of [³H]-AVM-B₁a in Me₂SO (0.1 μg in 2 μL of Me₂SO/insect). Treated larvae were held individually at 27 °C in glass vials until they were subjected to analysis.

At the specified times posttreatment, treated larvae were killed by freezing and then immediately thawed and dissected. A larva was pinned on top of a small piece (2 × 3 cm) of aluminum foil, which in turn rested on a similar sized piece of filter paper in a paraffin-filled dissecting dish. Dissections were performed with the aid of a binocular microscope. The cuticle was slit with microsurgical scissors along the entire length of the dorsal abdominal surface, taking care not to rupture any part of the digestive tract. (At this point, larvae were subjected to further selection for uniformity in physiological development—any larva whose midgut was not full of food that was in an apparently active state of digestion was rejected.) The larval cuticle was then pinned open to facilitate further dissection, and the midgut was removed, taking care to avoid loss of contents.

The excised midgut and remaining carcass were extracted individually with the Teflon pestle and glass tube tissue grinder. Tissues were homogenized first with ca. 5 mL of distilled water, then methanol (ca. 20 mL) was added, and the mixture was homogenized again. The homogenate was chilled and centrifuged, and precipitated solids were reextracted twice with 10-mL volumes of acetone. Excreta in holding containers were softened with a small volume of water, rinsed into the homogenization tube with methanol, and extracted as described for tissues. Different extracts were combined, radioassayed, and held in a freezer for further analysis. Any hemolymph that accumulated on the aluminum foil was recovered with a methanol rinse and combined with the corresponding carcass sample. Hemolymph that seeped through pin holes

in the foil and was absorbed by the underlying filter paper was counted directly by LSC, and any radioactivity so determined was combined with the data for carcass analyses. Extracted solids were air-dried and then analyzed for residual radioactivity with the oxygen combustion procedures described below; these data were combined with those reported for extractions.

Data were subjected to a computerized analysis of variance (ANOVA) using a general linear models program, and means were compared at the $p = 0.05$ level by Duncan's (1951) multiple-range test.

Metabolism. Studies of the metabolic fate of [³H]-AVM-B₁a in the three test species were done with last-stage larvae treated orally (0.1 μg in 2 μL of Me₂SO/insect) as described. Treated larvae were handled and extracted as described in the above study of distribution, except that there were no dissections; instead, intact live larvae were homogenized and processed to generate samples of total unexcreted radioactivity. Extracts of treated larvae and their excreta were radioassayed and then evaporated under vacuum and gentle heat (ca. 30 °C). The residue was dissolved immediately in a small volume of methanol and applied to TLC plates (Silplate F-254, 20 × 20 cm, 0.25-mm gel thickness, with fluorescent indicator; Brinkmann Instruments Inc., Westbury, NY). These plates were developed twice in one dimension in a solvent mixture of 10:3:1 (v/v) ethyl acetate, benzene, and 2-propanol (Bull et al., 1984). Radioactive zones were located by autofluorography and then scraped from plates and quantitated by LSC. For the autofluorography, plates were sprayed with a 10% solution of PPO (2,5-diphenyloxazole) in toluene until thoroughly moist; they were then air-dried and exposed at −30 °C to X-ray film (Kodak X-OMAT AR, Eastman Kodak Co., Rochester, NY) for a suitable period of exposure. In each test, three larvae were extracted individually at each of three to four posttreatment sample times, and each test was replicated at least four times. The data obtained were subjected to ANOVA as described.

Accumulation in Heads. In vivo experiments were conducted to determine whether there were any differences among the three test species in the posttreatment accumulation of radioactive materials in the heads of last-stage larvae. Larvae were treated as described with oral doses of Me₂SO solution of [³H]AVM-B₁a (1 μg in 2 μL/insect) and held at 27 °C for analysis at the specified times posttreatment. Treated larvae were killed by freezing and then immediately decapitated. Excised heads were placed for a brief period on filter paper to allow thawing and absorption of the major part of the hemolymph. Individual heads were then homogenized manually with a small volume of ethanol in an all-glass tissue grinder. Each homogenate was transferred to a 20-mL glass scintillation vial and allowed to air-dry. Then, 0.5 mL of tissue solubilizer (Soluene-100; Packard Instrument Co., Inc., Downers Grove, IL) was added to each vial and allowed to stand for 6 h. LSC cocktail was added, and solubilized samples were radioassayed repeatedly over time until any chemoluminescence that was present had abated and the sample counts were consistent over at least a 24-h period. Five individual larvae of each species were analyzed in each test, and tests were replicated four times at 4-h posttreatment and three times at 8 h. Data were subjected to ANOVA as described.

Analytical Procedures. All radioassays were done with conventional LSC procedures. Except for those generated by combustion analyses, samples derived from tests with [³H]AVM-B₁a were counted in a cocktail made with a standard fluor (Permablend II; Packard Instrument

Table I. Acute and Delayed Effects of AVM-B$_1$ on Third and Last Instar Noctuid Larvae[a]

species/stage treated	treatment	dose, µg/larva					
		acute effects			delayed effects		
		LD$_{50}$	FL	slope	LD$_{50}$	FL	slope
TBW							
3rd instar	topical	0.032	0.023 0.039	0.753	<0.01		
last instar	oral	0.501	0.403 0.617	0.841	0.081	0.00 0.191	−0.691
CEW							
3rd instar	topical	0.206	0.168 0.246	0.794	0.108	0.072 0.280	−0.670
last instar	oral	15.039	11.935 29.124	1.849	1.405	2.324 6.353	−0.609
FAW							
3rd instar	topical	>10.0			62.300	1.620 5.130	−0.365
last instar	oral	>10.0			28.500	6.150 7.990	−0.623

[a]Acute effects are larval mortality at 96-h posttreatment; delayed effects are reduced emergence of adults compared to untreated controls.

Co., Inc.) dissolved in toluene. Corrections were made as needed for instrument efficiency, quenching, or radioisotope decay.

Combustion analyses were done with a biological oxidizer (R. J. Harvey Instrument Corp., Hillsdale, NJ) operated at oven temperatures of 900 °C in the combustion area and 680 °C in the catalyst zone. Combustion gases were passed through a chilled glass trap to condense tritiated water. This radioactive material was recovered by rinsing the trap with several small volumes of LSC cocktail suitable for aqueous samples (the same fluor in a 2:1 (v/v) mixture of toluene and ethylene glycol monomethyl ether); the final volume counted was 20 mL. Periodic analyses of known standards indicated recovery was 95% or better.

RESULTS

Toxicity. Treated larvae responded in different ways to treatment with AVM-B$_1$. Among third-stage larvae that received topical applications, acute mortality effects usually occurred within the first 96-h posttreatment. Most of the larvae that survived that period were able to feed and undergo apparently normal development until the time of population. At that time, some developed into larval/pupal intermediate forms that soon died while others entered an apparently normal pupal stage but then died. Among the fifth-stage larvae that received oral doses of AVM-B$_1$, acute mortality effects also occurred during the first 96 h. Death in these tests was defined as no response by the larva to contact with a pointed probe. Many fifth-stage larvae, especially TBW and CEW, became prostrate shortly after treatment. These larvae evinced a definite reaction to probing, retained normal body color, and eventually tried to pupate. However, most of these prostrated larvae formed larval/pupal intermediates that soon died, or they remained in the larval stage well past the normal time of pupation; generally very few of these larvae formed pupae that developed into adults.

The data shown in Table I represent the concentrations of AVM-B$_1$ required to kill 50% of the treated larvae within 96-h posttreatment or to cause a 50% reduction in adult emergence compared to untreated controls. These data for adult emergence thus reflect the total lethal effects arising from both acute mortality and delayed mortality resulting from induced morphological and/or physiological aberrations. As indicated in Table I, there were substantial differences among species in their acute and delayed responses either to topical applications of AVM-B$_1$ during the third larval instar or to oral applications during the last larval instar. The order of susceptibility to both

Table II. Absorption of [^{3}H]AVM-B$_1$a following Topical Application to Third-Stage Larvae (Dose: 0.01 µg/Larva in 1 µL of Acetone)

species/h posttreat	distribn of radioact, % of dose (±SD)[a]			
	unabsorbed	absorbed	container	total
TBW				
0	100.0 ± 0.2	0.0 ± 0.1	0.0 ± 0.0	100.0
1	90.5 ± 6.4	1.3 ± 0.8	2.9 ± 3.2	94.7
2	93.6 ± 5.8	1.5 ± 1.0	0.9 ± 1.3	96.0
4	96.3 ± 3.9	2.4 ± 0.7	1.3 ± 1.4	100.0
8	92.5 ± 2.0	1.1 ± 0.1	0.8 ± 0.8	94.4
CEW				
0	100.0 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	100.0
1	90.3 ± 1.3	1.4 ± 2.0	1.5 ± 2.1	93.2
2	84.9 ± 7.8	2.2 ± 1.6	0.6 ± 0.5	87.7
4	79.7 ± 5.2	2.6 ± 2.4	1.1 ± 1.2	83.4
8	82.1 ± 3.3	3.5 ± 1.1	0.5 ± 0.4	86.1
FAW				
0	100.0 ± 0.8	0.0 ± 0.0	0.0 ± 0.0	100.0
1	84.4 ± 9.0	1.3 ± 0.9	5.8 ± 5.3	91.5
2	85.5 ± 5.6	1.8 ± 0.9	3.9 ± 5.5	91.2
4	85.0 ± 3.9	1.9 ± 0.7	5.2 ± 5.3	92.1
8	85.6 ± 3.4	2.0 ± 0.4	1.5 ± 2.3	89.1

[a]Data represent means of four replicates/time per species using five larvae/replicate.

methods of treatment was TBW ≫ CEW ≫ FAW. There were no apparent acute effects on either larval stage of FAW at the highest topical or oral dose tested (10 µg/insect), but we observed some reduction in adult emergence compared to untreated controls. AVM-B$_1$ was highly toxic to the tobacco budworm, especially after topical applications to the third-stage larvae; even at the lowest dose evaluated (0.01 µg/insect), eventual adult emergence was less than 10%.

Absorption. Studies of the absorption of topical doses (0.01 µg/insect) of [^{3}H]AVM-B$_1$a by third-stage larvae demonstrated there was very little internal accumulation of radioactive material in any of the three test species during the 8-h experimental period (Table II). Recoveries of radioactive material ranged from only 1.1 to 3.5% of the the dose in extracts of larvae that had been previously rinsed with solvent to remove unabsorbed [^{3}H]AVM-B$_1$a. Also, there was very little accumulation of radioactive material in containers used to hold treated larvae (0.5–5.2%). That which was detected in containers is attributed to contact loss of [^{3}H]AVM-B$_1$a because analyses of fecal pellets revealed only trace levels of tritium. These tests were not extended for a longer experimental time because beyond 8-h posttreatment most of the larvae began to molt.

Table III. Distribution of Radioactivity at Different Times Posttreatment of Last-Stage Larvae with [³H]AVM-B₁a (Oral Dose: 0.1 μg in 2 μL of Me₂SO)

species	x̄ reps	midgut	carcass[b]	excreta
		1 h		
TBW	24	82.0 ± 8.5 a	6.6 ± 6.9 a	1.5 ± 2.2 a
CEW	22	75.2 ± 11.0 b	8.4 ± 5.6 a	2.8 ± 4.1 a
FAW	30	74.5 ± 9.6 b	5.8 ± 5.1 a	7.4 ± 6.8 b
		2 h		
TBW	22	71.2 ± 9.9 a	10.1 ± 7.6 ab	3.7 ± 3.9 b
CEW	34	56.5 ± 14.3 b	11.7 ± 6.4 a	8.8 ± 7.9 ab
FAW	34	64.1 ± 8.1 c	7.3 ± 6.1 b	6.6 ± 5.5 a
		4 h		
TBW	8	38.1 ± 8.4 a	11.6 ± 4.6 a	41.9 ± 9.9 b
CEW	7	19.8 ± 9.5 b	9.3 ± 4.4 a	54.6 ± 10.7 a
FAW	7	42.0 ± 6.5 a	14.1 ± 5.7 a	27.9 ± 5.4 c

[a] Numbers in columns followed by the same letter are not significantly different ($p = 0.05$) according to Duncan's multiple-range test. Data represent total radioactivity recovered through extraction and combustion of extracted samples. [b] Carcass samples comprised all of the body that remained following midgut removal plus the hemolymph.

Distribution.
Results of studies of the posttreatment distribution of radioactive material after oral administration of [³H]AVM-B₁a (0.1 μg/insect) to last-stage larvae of the three test species are shown in Table III. In general, the diminution of administered radioactive material in the midgut was slowest in the TBW, significantly so during the first 2-h posttreatment, and most rapid in the CEW. Concentrations of radioactive material in carcass/hemolymph samples were relatively constant among species at the three posttreatment sample times. That levels of radioactive material in carcass/hemolymph samples did not accumulate to very high levels in any of these larvae indicates these insects were quite efficient in eliminating [³H]AVM-B₁a and its degradation products following absorption through the gut wall.

In these tests, many of the TBW larvae were partially immobilized within 1-h posttreatment. That is, they were unable to maintain a normal position but still were quite responsive to any type of disturbance, and their excretory processes did not appear to be unusually impaired, at least during the time required for these tests.

Metabolism.
The results of TLC analyses of different extracts of last-stage larvae treated with oral doses of [³H]AVM-B₁a (0.1 μg/insect) are shown in Table IV. Because of the complexity of the structure of AVM, very few of the many transformation products observed in previous studies with rat and steer liver microsomal preparations (Miwa et al., 1982), or with soil (Bull et al., 1984), have been identified. Since the concentrations of metabolites detected in these tests were quite small, no attempt was made to isolate or identify these products. Instead, the major goal was to show the relative rates at which the parent compound was degraded in the test species. Thus, the data have been simplified by combining observed radioactive TLC zones into three groups: one representing AVM-B₁a, another comprising the material remaining at the origin, and a third that includes all other extracted radioactive products.

In general, the analyses of internal extracts indicated that significantly greater concentrations of unmetabolized [³H]AVM-B₁a were recovered from TBW than from CEW or FAW larvae at 1-, 2-, and 4-h posttreatment. There were no significant differences between CEW and FAW in the recovery of the parent compound in internal extracts at the sample times. [³H]AVM-B₁a was also detected in

Table IV. TLC Distribution of Radioactive Extractable Products and Bound Residues in Fifth-Stage Larvae at Different Times Posttreatment with [³H]AVM-B₁a (Oral Dose: 0.1 μg in 2 μL of Me₂SO/Larva)

species	origin	misc metab	AVM-B₁a	bound resid[b]
		1-h internal		
TBW	8.4 ± 3.0 b	14.7 ± 1.0 a	75.6 ± 3.3 a	0.5 ± 9.3 a
CEW	18.0 ± 4.6 a	7.6 ± 1.6 b	60.0 ± 6.5 b	1.2 ± 0.6 a
FAW	7.9 ± 1.5 b	13.7 ± 2.7 a	66.1 ± 0.6 b	0.4 ± 0.2 a
		2-h internal		
TBW	10.4 ± 5.9 b	12.9 ± 1.9 a	66.5 ± 2.3 a	0.4 ± 0.3 b
CEW	23.8 ± 6.3 a	5.5 ± 1.4 b	41.4 ± 9.8 b	1.6 ± 9.3 a
FAW	19.2 ± 1.2 ab	11.8 ± 1.6 a	46.4 ± 4.9 b	0.5 ± 0.4 b
		4-h internal		
TBW	4.1 ± 1.6 b	8.2 ± 1.5 a	36.0 ± 1.6 a	0.5 ± 0.4 b
CEW	10.0 ± 5.0 b	2.4 ± 2.1 b	13.0 ± 8.0 b	2.6 ± 0.1 a
FAW	21.9 ± 2.0 a	8.0 ± 2.3 a	19.8 ± 8.5 b	0.7 ± 0.5 b
		4-h excreta		
TBW	5.1 ± 0.5 b	6.9 ± 3.1 a	30.1 ± 13.6 ab	
CEW	17.7 ± 9.5 a	5.2 ± 0.7 a	36.8 ± 7.0 a	
FAW	6.2 ± 6.2 ab	5.1 ± 1.4 a	19.5 ± 4.6 b	
		8-h excreta		
TBW	12.9 ± 6.7 a	14.2 ± 3.1 a	56.2 ± 6.5 a	0.5 ± 0.3 b
CEW	20.7 ± 9.0 a	8.2 ± 2.6 b	44.8 ± 10.1 a	2.9 ± 0.0 a
FAW	14.6 ± 5.0 a	14.3 ± 3.1 a	48.1 ± 4.1 a	0.7 ± 0.5 a

[a] Number in columns followed by the same letter are not significantly different ($p = 0.05$) according to Duncan's multiple-range test. Three or more replicates were processed for each time and species. [b] Data for bound residues represent results of combustion analyses of combined extracted internal and excreta samples.

extracts of excreta of treated larvae, but there were no clear trends in the relative distribution of the compound or its metabolites among species. Except in the 4-h internal samples, concentrations of a polar radioactive material retained at the origins of TLC plates were generally higher and combined amounts of miscellaneous metabolites were generally lower in CEW larvae than in TBW and FAW.

Bound residues of radioactive material in extracted tissues and excreta were generally inconsequential, but they were a little higher in CEW samples.

Accumulation in Heads.
Studies of the accumulation of radioactive material in the heads of last-stage larvae treated with oral doses (1 μg in 2 μL of Me₂SO/insect) of [³H]AVM-B₁a demonstrated that significantly greater concentrations were detected in TBW than in CEW and FAW (Table V). These differences were observed at both 4- and 8-h posttreatment. Amounts of radioactivity in the heads of CEW and FAW were statistically alike. The amounts of radioactive material measured at the two sample times were not significantly different in any species.

DISCUSSION

Precise reasons for the observed substantial differences in the susceptibility of TBW, CEW, and FAW larvae to AVM remain unexplained. The evidence indicates that, under the conditions of the tests, there were no apparent differences in the rates at which AVM penetrated the cuticle of third-stage larvae. Since only very small concentrations of topically applied AVM were absorbed by any species, the strong toxicity of the compound to TBW and CEW larvae of that stage is impressive. Although studies of changes in the posttreatment internal distribution and excretion of AVM after oral administration to last-stage larvae demonstrated that the compound and/or its metabolites were retained a little longer in the digestive

Table V. In Vivo Accumulation of Radioactivity in Heads of Fifth-Stage Larvae Treated with [³H]AVM-B₁a (Oral Dose: 1 μg in 2 μL of Me₂SO/Larva)

| species | dPM (±SD) at indic h posttreat | |
	4	8
TBW	4147 ± 1559 a	3980 ± 1422 a
CEW	2188 ± 1383 b	2118 ± 940 b
FAW	1966 ± 1150 b	1508 ± 840 b

[a] Numbers followed by the same letters are not significantly different ($p = 0.05$) according to Duncan's multiple-range test. Data represent means of 20 and 15 observations at 4 and 8 h, respectively.

tract and excreted more slowly in TBW than in CEW and FAW, the differences seem insufficient to explain the markedly greater susceptibility of TBW in both acute and delayed responses to such treatments. Orally administered AVM was metabolized significantly more slowly by last-stage TBW larvae than by CEW and FAW, but again these differences do not appear adequate to explain the greater susceptibility of TBW. Moreover, there were no significant differences between CEW and FAW in the metabolism of AVM, but there are substantial differences between these two species in their susceptibility to AVM.

If, as is suggested by these studies, differential rates of absorption and metabolism are not primary factors influencing tolerance to AVM among these noctuid test species, another possible explanation is differential sensitivity at the target site. Reports by Pong et al. (1980) and Pong and Wang (1982) indicated that, in preparations of brain tissue from mammals, AVM stimulates presynaptic release and postsynaptic binding of γ-aminobutyric acid (GABA), an important neurotransmitter in the central nervous system of mammals and in the peripheral nervous system of invertebrates. A recent report by Tanaka et al. (1984) suggests that GABA-related systems serve an important neurotransmitter function in the central nervous systems of insects. In a review of research on AVM-B₁a/GABA interactions, Wang and Pong (1982) concluded that AVM-B₁a intensified the action of GABA in certain arthropod and nematode species. In the present study, significantly greater levels of AVM-B₁a were recovered from the heads of TBW than from either CEW or FAW and presumably much of this was associated with the brain. However, there were no significant differences between the latter two species in levels of AVM-B₁a that accumulated in larval heads.

In previous studies of the mode of action and mechanisms of insect resistance to cyclo dienes, Kadous et al. (1983) reported there was less in vivo and in vitro binding of α-dihydropicrotoxinin to picrotoxinin receptors in the brains of insects from cyclo diene resistant strains of the German cockroach than in brains of insects from a susceptible strain. They also found that the cyclo diene resistant strain was also cross-resistant to picrotoxinin. Since the toxicity of picrotoxinin is related to its ability to counteract the normal function of GABA in the nervous system, Kadous et al. (1983) concluded that the mechanism of cyclo diene resistance in these cockroaches might be related to alternation of the neural receptor for picrotoxinin. In studies of the mode of action of DDT in houseflies (Chang and Plapp, 1983) and DDT and permethrin in the tobacco budworm and its predators, Chrysopa carnea Stephens (Chang and Plapp, 1983), it was found that differences in susceptibility to the insecticides were closely related to differences in receptor binding in neural tissues. It is possible that the differential toxicity of AVM to the insects used in this study is related, at least

in part, to its affinity for and interaction with GABA receptors.

Although differences in the metabolism of AVM and in its accumulation in brain tissue of treated larvae were demonstrated by this study, the magnitude of these differences does not seem sufficient to explain the dramatic differences in susceptibility to the compound among the three noctuid species investigated. Clearly, there is a need for comparative studies of the neurophysiology of these insects, especially of receptors involved in the mode of action of AVM. It is important to learn more about the receptor–toxicant interactions that eventually result in the acute toxic effects of AVM on these species, as well as in delayed effects on development. Knowledge gained from such studies would surely benefit efforts to develop AVM and its analogues for broader use in pest control.

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