TOXICITY, PERSISTENCE AND ANTAGONISM OF AVERMECTIN B_1 AGAINST STORED-PRODUCT INSECTS

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Abstract

Avermectin B_1, a novel, natural product insecticide-acaricide-nematicide, was found to be extremely effective against 6 beetle and moth pests of stored products. At a dose of 320 parts per billion (ppb) in wheat, all adult rice weevils, lesser grain borers and sawtoothed grain beetles were killed after 3 wks, and all red flour beetle adults were sluggish. For the Angoumois grain moth and lesser grain borer, 98% suppression of progeny occurred at doses of 10 ppb and 20 ppb, respectively. For all other species except the Indianmeal moth, 96-100% suppression of progeny was achieved at doses of 160 ppb or less. The Indianmeal moth required 640 ppb for complete suppression of progeny. The half-life for decay of biological activity of avermectin B_1 on wheat at 12.5% moisture, 80°F and 60% rh, was 3-6 mo. Piperonyl butoxide, when present in ground wheat medium in 10,000-fold excess over avermectin B_1, significantly reduced the toxicity of the latter to larvae of the Indianmeal moth. Paradoxically, Indianmeal moth larvae with greatly elevated titers of mixed function oxidase (induced by pretreatment of the larvae with pentamethylbenzene) were slightly more tolerant of avermectin B_1 than noninduced larvae. The significance of these observations is discussed.

A need exists for more effective and broader spectrum insecticides than those currently available as protectants of grain and other stored products. This need is underscored by a combination of developing resistance in several pest species (Beeman *et al.* 1982, Haliscak and Beeman 1983) and a rather limited spectrum of activity associated with existing stored-grain insecticides. Malathion is currently the only residual insecticide registered for direct application to stored grain in this country.

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Avermectins are a family of novel and effective insecticide-acaricide-nematicides with an unprecedented mode of action (Putter et al. 1981, Mellin et al. 1982). They have shown a broad spectrum of activity against species representing the major orders of insects (e.g. Lofgren and Williams 1982). We therefore decided to test avermectin B_1 against a broad spectrum of stored-product insect pests. Since avermectin may undergo metabolic activation (Putter et al. 1981) we also tested the effects of an inducer and an inhibitor of mixed-function oxidase on avermectin toxicity to larvae of the Indianmeal moth.

Materials and Methods

Rearing and Bioassay. All insects were obtained from cultures that have been maintained for many years at the U.S. Grain Marketing Research Laboratory and have no prior history of exposure to insecticides. Newton wheat was purchased from a local source. Kernels were cleaned and tempered to 12.5 ± 0.3% as determined by a Motomco® moisture meter. Whole wheat, ground wheat or wheat-base medium were prepared and treated with acetone solutions of test substances as described previously (Beeman 1983). To assay the toxicity of freshly applied avermectin, 125 g lots of wheat or ground wheat medium were infested with 50 adult coleopterans or 50 lepidopteran eggs 24 h after treatment with avermectin. For beetles, acute adult mortality was assessed after 3 wks, at which time all original adults were discarded. Progeny were counted at periodic intervals thereafter until all F_1 adults had emerged. To assay the toxicity of aged residues of avermectin, the same lots of treated wheat were frozen at -15° C for 3 days to kill any insects remaining from the original infestation, held for 28 wks (from the date of the original treatment) at 80° F and 60% r.h., then reinfested and monitored as before.

Two protocols were used for synergism/antagonism tests with the Indianmeal moth. In the first experiment, diet was treated simultaneously with avermectin B_1 and piperonyl butoxide in 3 ml acetone per 100 g diet. After evaporation of solvent overnight, eggs were added. Adult emergence was scored after 1 month and at periodic intervals thereafter. In the second experiment larvae were preexposed to pentamethylbenzene for 14 days to induce mixed function oxidase, then were transferred to avermectin-treated diet, either in the absence or continued presence of pentamethylbenzene. Adult emergence was scored 16 days later and at periodic intervals thereafter.

Chemicals. A crystalline sample of avermectin B_1 (a 4:1 mixture of avermectins B_{1a} and B_{1b}) was obtained from Merck Sharp and Dohme Research Laboratories, Rahway, New Jersey. An EC formulation of Ro 10-3108/018 containing 50% ai (a 3:1 mixture of cis/trans 2-ethyl-3-[3-ethyl-5-(4-ethylphenoxy)-pentyl]-2-methyloxirane and cis/trans 2-ethyl-3-[5-(4-ethylphenoxy)-2,3-dimethylpentyl]-2-methyloxirane) was obtained from HLR Sciences, Inc., Nutley, New Jersey. Technical grade(89%) Piperonyl Butoxide (Butacide®) was a gift from the Fairfield American Corp., Medina, N.Y.

Mixed-Function Oxidase Assay. For enzyme assay, Indianmeal moth larvae were reared in coarse-ground whole grain (corn, wheat, oats or sorghum) containing 20 ppm of the juvenile hormone mimic Ro 10-3108/018 to enhance and stabilize mixed function oxidase (mfo) activity. Pentamethylbenzene (4000 ppm) was included in the meal to determine the inducibility of mfo in the moth larvae under various conditions. At day 18 of larval development, uniform-sized feeding larvae were weighed and the whole guts were removed and rinsed in cold 1.15% KCl.

The method of mfo assay was a slight modification of that reported by Krieger and Wilkinson (1969). The standard reaction mixture consisted of 10 whole guts in 1 ml of 5 mM Tris buffer pH 7.8 containing G-6-P (2.4×10^{-3}M), G-6-P dehydrogenase (1.6 units), NADP (5.1×10^{-5} M) and aldrin (20 µg), the latter added in 10 µl ethanol. Incubations were carried out for 30 min in 20 ml glass scintillation vials shaken in a water bath at 32°C. The reaction was stopped by addition of 2 ml acetone. A small amount of sodium sulfate was then added, and the tubes were extracted twice with 2 ml portions of hexane. The combined hexane extracts were diluted to a volume of 10 ml, and analyzed for dieldrin content by gas-liquid chromatography (GLC). A Varian 3700 GLC was used in conjunction with a ^{63}Ni electron-capture detector. A 20 x 1/8 inch stainless steel column was packed with 5% OV-101 on 100-120 mesh Chrom G-HP. Injector, column and detector were held at 240°C, 160°C and 310°C, respectively. Nitrogen carrier gas was run at 30 ml/min. Analyses were quantified by peak areas with reference to standard curves.

Results and Discussion

Toxicity and Residual Effectiveness. Data for the acute adult toxicity of fresh residues of avermectin B_1, as well as percent suppression of progeny, are given in Table 1. The wide dose range used prevented successful log-probit analysis in most cases. However, the extremely high toxicity of the avermectin to all species is evident. Acute mortality of 100% occurred after 3 wks exposure of adult rice weevils, lesser grain borers and sawtoothed grain beetles to a dose of 320 parts per billion (ppb). Adults of the red flour beetle were much more tolerant, since no mortality occurred at 320 ppb, and only 36% mortality occurred at 2.6 ppm. However, all red flour beetles exposed to doses as low as 160 ppb were noticeably sluggish. For all 4 beetle species, avermectin B_1 was more effective in progeny suppression than in acute kill of adults. This tendency was most evident in the red flour beetle and least evident in the rice weevil. For the latter, the avermectin was only marginally more effective in suppressing progeny than in killing adults. The Angoumois grain moth and the lesser grain borer were the two species most sensitive to avermectin, 98% suppression of progeny occurring at doses of 10 and 20 ppb, respectively. The Indianmeal moth and red flour beetle were the two least sensitive species, 640 and 320 ppb, respectively, being required for 100% suppression of progeny.

Table 1. Activity of avermectin B_1 (fresh residues) against moths and beetles and their F_1 progeny in wheat [a].

Species[b]	Stage	No. of adult progeny found in untreated sample	Acute toxicity (% mortality) to adults or % reduction[c] of F_1 adult progeny at doses (ppm) of								
			.01	.02	.04	.08	.16	.32	.64	1.3	2.6
AGM	-----	301	98	100	100	100	100	100	100	100	100
IMM	-----	45	0	4	(2)	0	22	60	100	100	100
RW	parents		0	0	29	76	100	100	100	100	100
	progeny	1238	39	33	52	92	99	100	100	100	100
LGB	parents		6	23	61	92	96	100	100	100	100
	progeny	346	53	98	100	100	100	100	100	100	100
STGB	parents		16	7	61	64	96	100	100	100	100
	progeny	13	(46)	38	0	100	100	100	100	100	100
RFB	parents		0	0	0	0	0	0	6	24	36
	progeny	625	5	8	22	44	96	100	100	100	100

[a] Adults (eggs in the case of the Indianmeal moth) were added to wheat or diet 24 hr after avermectin B_1. Each value represents a single determination (average of 2 in the case of untreated samples).

[b] Abbreviations are as follows: AGM = Angoumois grain moth, IMM = Indianmeal moth, RW = rice weevil, LGB = lesser grain borer, STGB = sawtoothed grain beetle and RFB = red flour beetle.

[c] Values in parentheses indicate percent increase.

Table 2 shows the biological activity of these same residues of avermectin B_1 after ageing and reinfesting the wheat. A loss of biological activity to all species except the Indianmeal moth and red flour beetle is apparent after 6 months. For most species, the biological activity of 6 month-old residues is on the order of 25-50% of that of fresh (day-old) residue, indicating a half-life of 3-6 months for decay of biological activity. The obligate internal feeders (Angoumois grain moth and rice weevil) were exceptional in that a plateau occurred at 97-99% mortality in the dose-response curve for progeny suppression in aged residues, i.e. a few progeny reached adulthood even at the highest doses tested, in spite of the fact that most progeny were suppressed at much lower doses. These survivors, developing inside the kernels, were apparently protected from avermectin residues which must not have penetrated extensively into the endosperm.

Modification of Avermectin Toxicity. It is uncertain whether activation or detoxification represents the initial stage of avermectin metabolism in animals. An undescribed microbial metabolite with nematicidal activity forms in avermectin-treated soil (Putter et al. 1981). Microsomes from mammalian liver metabolize avermectins B_{1a}, H_2B_{1a} and H_2B_{1b} to their C^{24}-methyl alcohol derivatives, and, in the case of H_2B_{1a} and H_2B_{1b}, to the corresponding monosaccharides (Miwa et al. 1982), but the toxicity of these hydroxylated metabolites has not been reported. Table 3 shows the effect of piperonyl butoxide (PB) on avermectin B_1 toxicity to Indianmeal moths exposed from the egg stage. These data clearly show that PB antagonizes the toxicity of avermectin B_1. However, we cannot conclude from these data alone that avermectin B_1 is activated by mixed-function oxidase (mfo). In the first place, mfo inhibitors can actually induce higher levels of mfo after prolonged exposure of the animal (Thongsinthusak and Krieger 1975). In the second place the dose of PB used was ~ 10,000-fold higher than the avermectin dose, and thus we must consider the possibility that PB may have exerted a protective action by reducing the intake or absorption of avermectin B_1 possibly via a change in the physical properties of the medium. In a separate experiment (data not shown), 2000 ppm of mineral oil was equal in effectiveness to the same dose of PB in protecting the Indianmeal moth from the toxic effects of a 0.1 ppm dose of avermectin B_1. However, 2000 ppm of sunflower oil had no protective effect.

To help resolve these uncertainties, we tested the effect of an inducer of mfo on avermectin toxicity to the Indianmeal moth. We chose pentamethylbenzene as the inducing agent, since this substance is known to be an effective mfo inducer in lepidopterans (Brattsten and Wilkinson 1973). First, we confirmed that pentamethylbenzene was an effective inducer of mfo in Indianmeal moth larvae. Pentamethylbenzene (4000 ppm) in wheat diet was shown to produce a 730% increase (on a per insect basis) in aldrin epoxidase activity in the gut tissues of 14-day-old larvae exposed from the egg stage (Table 4). Large increases in mfo activity were also measured in larvae exposed to pentamethylbenzene in ground corn, oats and sorghum (Table 4). A dose of 2000 ppm also produced a large increase in mfo activity (data not shown). We then compared avermectin toxicity to preinduced and

Table 2. Activity of avermectin B_1 (aged residues) against moths and beetles and their F_1 progeny in wheat [a].

Species[b]	Stage	No. of adult progeny found in untreated sample	Acute toxicity (% mortality) to adults or % reduction[c] of F_1 adult progeny at doses (ppm) of								
			.01	.02	.04	.08	.16	.32	.64	1.3	2.6
AGM	-----	270	94	96	96	98	98	99	97	98	99
IMM	-----	31	--	--	--	--	--	--	--	100	100
RW	parents		--	--	--	--	100	100	100	100	100
	progeny	1218	--	--	--	--	82	93	97	99	99
LGB	parents		--	0	17	25	82	96	94	100	100
	progeny	714	--	90	98	99	100	100	100	100	100
STGB	parents		32	52	27	52	92	100	100	100	100
	progeny	10	(50)	50	40	30	90	100	100	80	100
RFB	parents		--	--	--	--	7	0	0	37	78
	progeny	254	--	--	--	--	21	96	100	100	100

[a] Adults (eggs in the case of the Indianmeal moth) were added to wheat or diet 6 mo. after avermectin B_1. Each value represents a single determination (average of 2 in the case of untreated samples).

[b,c] See Table 1, footnotes b and c.

Table 3. Joint action of avermectin B_1 (AVM) and piperonyl butoxide (PB) in the Indianmeal moth[a].

Treatment	% Mortality[b]
control	2.4 ± 4.4
AVM .08 ppm	84.4 ± 3.8
AVM .08 ppm + PB 2000 ppm	29.0 ± 14.0
AVM .2 ppm	100 ± 0
AVM .2 ppm + PB 2000 ppm	53.0 ± 10.6
PB 2000 ppm	4.4 ± 4.4

[a] Fifty eggs were added to 100 g of medium in pint jars 48 hr after treatment of the medium. Adult emergence was measured after 27 days (49 days in the case of avermectin-treated samples).

[b] Based on the difference between the number of eggs added and the number of adults emerging. Values are means ± S.D.

Table 4. Aldrin epoxidase activity in whole guts of pentamethylbenzene-induced and noninduced larvae of the Indianmeal moth reared on 4 grains[a].

Diet	Activity of aldrin epoxidase (ng dieldrin /gut/30 min)	
	Induced	Noninduced
corn	57.5 ± 10.8	5.3 ± 3.8
oats	53.6 ± 12.7	6.6 ± 1.2
wheat	39.4 ± 9.5	5.4 ± 3.7
sorghum	39.9 ± 4.4	10.2 ± 2.3

[a] Values are means ± SD of 4 independent determinations. Detection limit ~1 ng/gut/30 min.

noninduced 14-day-old larvae under conditions which precluded the possibility of a physical or chemical interaction between the avermectin and pentamethylbenzene. This was accomplished by transferring 14-day-old preinduced or noninduced larvae to pentamethylbenzene-free, avermectin-treated medium.

Avermectin B_1 was found to be slightly less toxic to preinduced than noninduced larvae (Table 5). Furthermore, this slight antagonistic interaction occurred whether the inducer was present before, during, or both before and during exposure to the avermectin (Table 5). Contrary to the data in Table 4, this result suggests that mfo-catalyzed detoxification may make a small contribution to the overall metabolic fate of avermectin B_1 in the Indianmeal moth. Comparison of Tables 4 and 5 also shows that avermectin B_1 was much less toxic to 14-day-old larvae than to larvae exposed from the egg stage.

Avermectins are known to exert a slow-killing action in arthropods and nematodes. In our experiments, this was particularly evident in Indianmeal moth larvae. Many 14-day-old larvae exposed to 2 ppm of avermectin B_1 became stunted and acquired a characteristic pale green cast. Although these larvae failed to complete development, they lived for many months. Perhaps the primary GABAergic action of the avermectins is nonlethal, and the direct cause of death is starvation or some other secondary stress. Indianmeal moth larvae, being resistant to starvation and capable of larval diapause, may therefore survive for long periods despite disruption of GABA-mediated systems.

Acute toxicity may or may not accurately reflect level of insect control, since non-acutely toxic, behavior-modifying substances may suppress reproduction or prevent development (Beeman 1982), and conversely, acutely toxic chemicals, especially short-acting ones, may not suppress reproduction. The avermectins are clearly in the former category. Ostlind *et al.* (1979) reported that not all adult flour beetles (*T. confusum*) were killed, even after 2 wks exposure to 100 ppm of avermectin B_1. Using different criteria however, we found that adults of the closely related species *T. castaneum* were noticeably sluggish after several days exposure to doses almost 1000-fold lower, and that progeny development was totally suppressed at doses 300-fold lower than the dose used by Ostlind *et al.* Thus, using acute toxicity as the sole criterion, they concluded that malathion was at least 10-fold more potent than avermectin B_1 against *T. confusum*, whereas we conclude, using subtle and long term effects as criteria, that avermectin B_1 is considerably more potent and effective than malathion in *T. castaneum*. Species differences within the genus *Tribolium* are probably not involved.

At the present time, the high cost of avermectins and their considerable mammalian toxicity precludes their being considered for registration as stored-grain protectants, in spite of their superior performance. However, these substances represent a major innovation in both pesticide chemistry and mode of action, and cheaper routes of production as well as more selective analogs are likely to follow.

Table 5. Toxicity of avermectin B_1 (AVM) to pentamethylbenzene-induced and noninduced larvae of the Indianmeal moth reared on wheat[a].

Treatment	% Mortality[b]
control	0 ± 0
AVM 2 ppm noninduced	75.5 ± 10.8
AVM 2 ppm preinduced	57.5 ± 5.0
AVM 2 ppm postinduced	53.8 ± 6.3
AVM 2 ppm pre- and post induced	57.5 ± 5.0

[a] Eggs were transferred to wheat diet 24 hr after treatment of the diet with acetone (control, noninduced and postinduced) or with 2000 ppm of pentamethylbenzene (preinduced). After 14 days, induced and noninduced larvae were transferred to AVM-treated diet in the presence (postinduced) or absence (preinduced and noninduced) of pentamethylbenzene. Adult emergence was scored after 64 days post-egg (30 days for controls).

[b] Based on the difference between the number of 14-day-old larvae added and the number of adults emerging. Values are means ± S.D.

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