TOXICITY OF SELECTED TREMORGENIC MYCOTOXINS AND RELATED COMPOUNDS TO *SPODOPTERA FRUGIPERDA* AND *HELIOTHIS ZEA*

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A series of tremorgenic mycotoxins and related compounds were tested for oral toxicity to the fall armyworm (*Spodoptera frugiperda*) and corn earworm (*Heliothis zea*) by incorporation of materials into artificial diets and examining mortality and weights after 7 days. Significant mortality to both insect species was caused with dihydroxyaflavinine and roseotoxin B, while significant mortality to *H. zea* was also caused by penitrem A at 25 ppm. After 7 days, weights of larvae treated with 25 ppm penitrem A, roseotoxin B, and verruculogen were less than 50% of controls for both insect species. Weights of *H. zea* larvae treated with 25 ppb of penitrem A were less than 50% those of control larvae. Relative toxicities of the tremorgens and related compounds to insects compared to vertebrates are discussed.

Mycotoxins and other fungal metabolites are thought to serve as chemical defense systems for the fungi that produce them, and may also be of use in protecting the resource from consumption by other organisms[1,2]. Insects are one group of organisms which may be the target of these fungal chemical defenses. For example, mycotoxins such as aflatoxin B_1 and trichothecenes are toxic to many insects[3].

The tremorgens are one group of mycotoxins that has received no attention as far as toxicity to insects is concerned. These tremor-producing compounds are primarily indole alkaloid-based, but others are cyclic peptides[4]. Many of the indole alkaloid-based tremorgens are structurally analogous to other fungal secondary indole alkaloids that have very limited toxicity to mammals[4]. Based on the similarity between the structure of tremorgenic mycotoxins and other substances previously shown to be insecticidal, it appears that the tremorgenic mycotoxins could be toxic to insects as well. For example, cyclic peptides such as the piericidins and destruxins, which are microbially produced, are toxic to insects[5]. Higher plant-derived indole alkaloid-based compounds are also insecticidal[6].

The tremorgenic mycotoxins are produced by some fungi[4] that infect field crops such as corn, oats, barley, and other grasses (*e.g.* see host range descriptions by refs 7 and 8). These crops may also be hosts of the corn earworm, *Heliothis zea* or the fall armyworm, *Spodoptera frugiperda*[9]. Thus, this research examines the relative toxicity of representative tremorgens and their analogs to these two insects.

†† The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

Materials and Methods

Insects

Larvae of *S. frugiperda* and *H. zea* were reared on pinto bean-based diet at 27±1°C, 40±10% relative humidity, and 14 : 10, light : dark photoperiod. Neonate larvae were used for all assays.

Chemicals

Cytochalasin H, dihydroxyaflavinine, paspaline, paxilline, penitrem A, roseotoxin B and verruculogen were isolated, purified, and identified according to published procedures (cytochalasin H[10], dihydroxyaflavinine[11], paspaline[12], paxilline[13], penitrem A[14], roseotoxin B[15], verruculogen[16]). Mephenesin was obtained from Sigma Chemical Co. All other chemicals were of reagent grade.

Bioassays

Due to the limited amounts of chemicals available, toxicity was determined from a 25 ppm wet weight (100 ppm dry weight) starting point. Any chemicals showing greater than 50% weight reduction or 10% mortality were tested at doses of decreasing magnitudes.

Chemicals were incorporated into 5 ml aliquots of unsolidified pinto bean diet[17] by adding the chemicals in 125 μl of acetone to unsolidified diet and mixing vigorously with a vortex mixer[18]. Diets were sectioned into 20 pieces, and each piece was placed in a separate well of a 24-well tissue culture plate. A single neonate larva was placed in each well, and the plates were sealed with parafilm to prevent desiccation of the diet[18]. Plates were held under the same conditions the larvae were reared under, and inspected at 2, 4, and 7 days for mortality. Surviving insects were weighed when 7 days old. Forty insects, total, were used for each compound. A solvent blank was used as the control. Controls were used as standards and did not differ significantly in mortality or weight during the course of the different assays.

Results

Of the compounds tested, roseotoxin B caused the greatest mortality at 25 ppm (Table 1) in both insect species. Greater than 10% mortality was also obtained with dihydroxyaflavinine for *S. frugiperda*, and penitrem A and dihydroxyaflavinine for *H. zea*. Although it caused little mortality, verruculogen did cause large reductions in rates of development as indicated by 7-day weights, in both insect species. Roseotoxin B and penitrem A also caused significant toxicity to both insect species at lower doses. Penitrem A reduced weights of *H. zea* by greater than 50% even at 25 ppb (larvae weighed 13.2±1.4 mg).

Since penitrem A has been reported to interfere with nervous system activity in mice, which could be reversed by the glycine agonist mephenesin[19], the ability of mephenesin to alter toxicity of penitrem A in both insect species was tested. In this case, the toxicity (mortality) of penitrem A was enhanced by mephenesin in both species of insects (*S. frugiperda* predicted 8%, found 20%; *H. zea* predicted 38.8%, found 88.9%; predictions based on sums of control, mephenesin, and penitrem A mortalities).

Discussion

A tremorgen isolated from ryegrass infected with endophytic fungi is toxic to a stem weevil[20]. The results of the present study demonstrate that a number of tremorgenic mycotoxins and their relatives can be toxic to insects. Dihydroxyaflavinine occurs at levels higher than 100 ppm (dry weight) in the resting stage structures produced by *Aspergillus flavus* (sclerotia), which are not fed upon by the fungivorous nitidulid *Carpophilus hemipterus*[21]. The levels naturally present are sufficient to be toxic to the insects tested in the present study. However, the naturally occurring levels of other tremorgens or their relatives have not been reported. Of the compounds tested in the present study, penitrem A was as toxic as permethrin (81% mortality at 2.5 ppm) to *H. zea* larvae tested in the same

Table 1. Toxicity of tremorgenic mycotoxins and related compounds to *Spodoptera frugiperda* and *Heliothis zea*.

Compound	*S. frugiperda*		*H. zea*	
	Mortality (%)	7-Day weight (mg)	Mortality (%)	7-Day weight (mg)
25 ppm				
Control	0.0	38.4±2.8	0.0	33.2±1.9
Penitrem A	5.4	9.0±3.1*	28.2*	1.1±0.4*
Roseotoxin B	100.0*	—	38.7*	0.1±0.0*
Verruculogen	5.0	7.7±0.8*	0.0	6.7±0.7*
Dihydroxyaflavinine	20.0*	24.7±3.1*	20.0*	22.6±2.3*
Cytochalasin H	8.1	16.3±2.1*	2.6	28.9±2.1*
Paspaline	0.0	30.6±2.3*	0.0	24.5±1.1*
Paxilline	10.0*	27.3±2.2*	2.7	21.3±1.6*
2.5 ppm				
Control	0.0	38.4±2.8	0.0	33.2±1.9
Penitrem A	5.0	26.1±2.1*	15.0*	1.5±0.3*
Roseotoxin B	2.5	11.9±1.2*	2.5	4.9±0.3*
Verruculogen	0.0	30.8±2.2*	7.5	25.7±1.5*
Dihydroxyaflavinine	0.0	32.6±3.4*	2.8	34.1±1.6
0.25 ppm				
Control	0.0	38.4±2.8	0.0	33.2±1.9
Penitrem A	15.0*	30.5±3.3*	20.5*	6.0±0.6*
Roseotoxin B	4.9	29.0±2.1*	0.0	37.4±2.4

Some losses of insects occurred, and mortality values have been adjusted accordingly. Weights are based on survivors of mortality studies and are means±standard errors. Mortality values in columns followed by an * for the same dose are not significantly different from controls at $P<0.05$ by chi square analysis[26]. Weights followed by an * in the same columns for the same dose are not significantly different from controls at $P<0.05$ by linear contrast analysis of variance[26].

manner. Roseotoxin B was as toxic as malathion to both insects (5% mortality and 27% weight reduction to *H. zea* and 30% mortality and 45% weight reduction to *S. frugiperda* at 2.5 ppm for malathion). Verruculogen, dihydroxyaflavinine, and cytochalasin H were as toxic as nicotine to both insects (2.5% mortality, 28% weight reduction in *H. zea*; 0% mortality and 41.4% weight reduction in *S. frugiperda* at 25 ppm for nicotine). Thus, these fungal metabolites are comparable in toxicity to commercially available insecticides.

The tremorgenic mycotoxins and their analogs are of varying toxicity to vertebrates[4] (Table 2). Generally, their relative toxicity to vertebrates ranges penitrem A>roseotoxin B, cytochalasin H>verruculogen>paxilline>paspaline, dihydroxyaflavinine (Table 2). In the present study, in considering mortality and then reduction in development (weights), the relative toxicity of these compounds to the insects tested, generally ranged roseotoxin B>dihydroxyaflavinine>penitrem A>verruculogen>cytochalasin H>paxilline>paspaline for *S. frugiperda*, and roseotoxin B>penitrem A>dihydroxyaflavinine>verruculogen>paxilline>paspaline>cytochalasin H for *H. zea*. This information demonstrates differences in relative toxicity of these mycotoxins to vertebrates and insects, especially in regard to the toxicity of dihydroxyaflavinine (greater to insects) and cytochalasin H (greater to vertebrates).

The mode of action of penitrem A in vertebrates is assumed to be nervous system interference by antagonizing the production of glycine (an inhibitory neurotransmitter), which results in tremorgenic effects[19]. This activity can be suppressed by mephenesin in mice, which increases production of glycine and also substitutes for it in the nervous system[19]. No tremorgenic symptoms were noted in the insects tested. The toxicity of penitrem A was enhanced by mephenesin in both insect species tested, which is opposite of the effect seen with mice. The activity of mephenesin may be due to enhancing whatever effect that the penitrem A is having, regardless of whether the site of action is the

Table 2. Toxicity of tremorgenic mycotoxins and analogs to vertebrates[4].

Compound	Toxicity (LD_{50}, mg/kg)			
	Mouse		Chick	
	Oral	ip	Oral	ip
Penitrem A		1.05		
Roseotoxin B		166[a]	12.5	
Cytochalasin H			12.5	
Verruculogen	126.7	2.4	265.5	15.2
Paxilline	>227[b]		>100[b]	
Paspaline		>500[c]		
Dihydroxyaflavinine[11]			>300[c]	

[a] LD_{100}.
[b] No mortality, but tremors occur.
[c] No effect (including tremors).

nervous system or not. Although there is some evidence that glycine may be a neurotransmitter in the insect nervous system[22,23], its role is not conclusive. However, paxilline and verruculogen inhibit GABA (γ-amino butyric acid) receptors in rat brain and *Torpedo* electric organ[24]. Since insects also possess GABA receptors, this mode of action is also possible, although the insect GABA receptor differs from that of mammals[25].

Overall, there appears to be some selective factor which governs the toxicity of these metabolites to insects that differs from that of vertebrates. The low toxicity of dihydroxyaflavinine to vertebrates, coupled with its high toxicity to insects suggests that these compounds represent chemical leads for a new class of insecticides. The large number of different tremorgens and their analogs which are produced by fungi provides a fertile area for discovering new insecticides.

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