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EFFECTS OF INDOLE ALKALOIDS FROM GRAMINEAE ON APHIDS

LUIS J. CORCUERA

Departmento de Biología, Facultad de Ciencias Básicas y Farmacéuticas, Universidad de Chile, Casilla 653, Santiago, Chile

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Key Word Index—Gramineae; *Schizaphis graminum*; *Rhopalosiphum maidis*; greenbug; indole alkaloids; gramine; tryptamine.

Abstract—The indole bases N_{ω}-methyltryptamine, 5-methoxy-*N*,*N*-dimethyltryptamine, 3-*N*,*N*-dimethylaminomethylindole (gramine) and 5-methoxytryptamine decreased survival of nymphs of *Rhopalosiphum maidis* with LD_{50} of 3.8, 3.5, 2.9 and 2.3 mM, respectively, after 48 hr of feeding with a synthetic diet. Gramine also decreased survival of *Schizaphis graminum* with an LD_{50} of 0.7 mM after 48 hr of feeding. The four compounds tested showed feeding deterrent activity on the aphids at concentrations as low as 0.5 mM. In addition, gramine showed toxic effects on *S. graminum*. Since the deleterious effects of gramine and related compounds in synthetic diets are observed at concentrations similar to those found in plants, it is possible that these molecules may have a role in protecting the plant against aphids.

INTRODUCTION

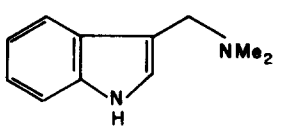
Indole alkaloids are responsible for toxicity of *Phalaris* pastures to sheep and cattle [1–3]. Several of these alkaloids are present in various species of Gramineae, Leguminosae and other plant families [4]. Simple indole bases such as 3-*N*,*N*-dimethylaminomethylindole (gramine, **1**), 5-methoxy-*N*,*N*-dimethyltryptamine (MOM_2T, **2**), 5-methoxytryptamine (MOT, **3**) and N_{ω}-methyltryptamine (MT, **4**) cause various deleterious effects on mammals. They cause acute toxicity and death of ruminants [4], decrease food palatability and intake by lambs [5]. Gramine-fed meadow voles (*Microtus pennsylvanicus*) developed kidney lesions, glycosuria and lower weight gains than control animals [6].

Although total alkaloid content varies between 0.02–1.19% of dry wt in *Phalaris arundinacea* [7] and gramine concentration may reach up to 7 mmol/kg fr. wt in barley [8], the importance of these alkaloids to the plants is unknown. Several plant secondary metabolites are thought to protect plants against insects and pathogens. For example, hydroxamic acids from Gramineae have been suggested to protect wheat and maize against the European corn borer, *Ostrinia nubilalis* [9] and the aphids *Metopolophium dirhodum* [10], *Schizaphis graminum* [11] and *Rhopalosiphum maidis* [12, 13]. Flavonoids and several aromatic compounds are feeding deterrents to *S. graminum* and *Myzus persicae* [14]. In this paper toxicity and feeding deterrency of various alkaloids to the aphids *R. maidis* and *S. graminum* are described.

RESULTS AND DISCUSSION

Toxicity and feeding deterrency of gramine

Aphid nymphs were exposed to diets containing 0–8 mM gramine. Survival of *S. graminum* was measured



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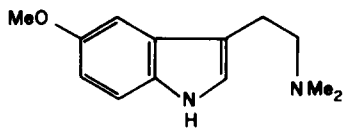

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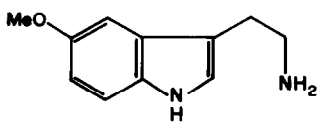

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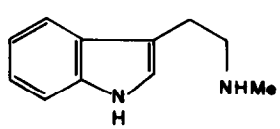

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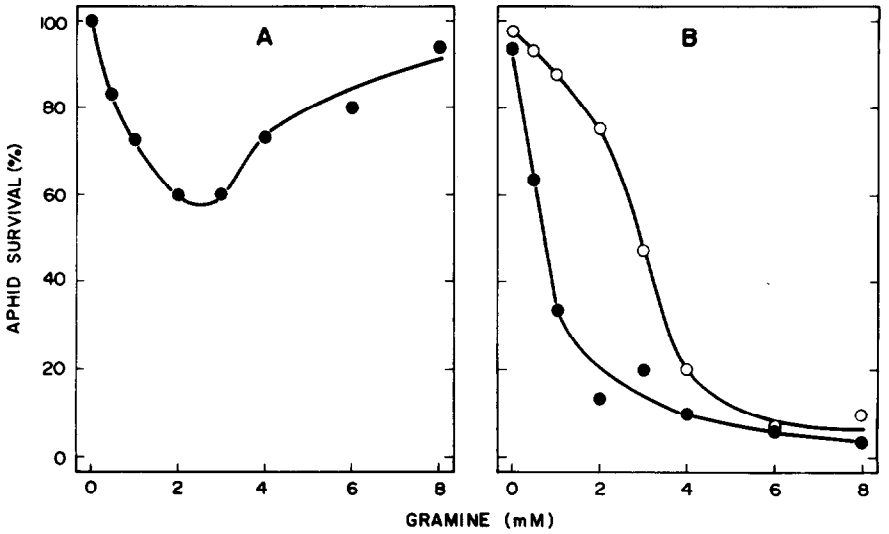

Fig. 1. Effect of gramine on survival of *Schizaphis graminum* (●) and *Rhopalosiphum maidis* (○) reared on a synthetic diet. Survival of nymphs was measured after 10 hr (A) and 48 hr (B) of feeding. Each point is the average of three samples of 10 aphids each. Since standard errors were usually less than 10%, they were omitted for simplicity.

after 10 hr of feeding (Fig. 1A). The lowest survival was observed at 2–3 mM gramine, suggesting that this compound had feeding deterrent activity at the highest concentrations, thus limiting ingestion of the compound. At lower concentrations feeding deterrency would be lower and, possibly, more compound would be ingested causing toxic effects. When aphids fed on the diets for 48 hr, survival was lower at the highest concentrations with an LD_{50} of 0.7 and 2.9 mM for *S. graminum* and *R. maidis*, respectively (Fig. 1B).

Also, nymphs of *S. graminum* and *R. maidis* were exposed to diets containing gramine and the fraction of aphids that were feeding on the diet was determined. The proportion of aphids feeding was decreased by gramine (Fig. 2). Feeding deterrency of gramine towards *S. graminum* and *R. maidis* is equivalent to that of hydroxamic acids from cereals [15, 16] and that of flavonoids and related phenolics [14].

Toxicity and feeding deterrency of indole alkaloids

Aphid nymphs of *R. maidis* were exposed to diets containing various concentrations of indole bases (Table 1). All compounds tested decreased survival and had feeding deterrent activity, gramine and MOT being the most active. Since indole alkaloids cause acute toxicity and decrease palatability of fodder plants to sheep and cattle, it has been proposed that alkaloid content of various Gramineae be reduced by plant breeding [17]. Results in this paper show that indole alkaloids are toxic and cause feeding deterrency to both insect species tested at concentrations below those found in some plants, suggesting a role of these compounds in protection of the plant against aphids. Although the role of these alkaloids in plant resistance to aphids remains to be established, plant breeders should be aware that decreasing alkaloid content of some pastures may be convenient for feeding various animals, but it may also increase susceptibility of the plants to insects.

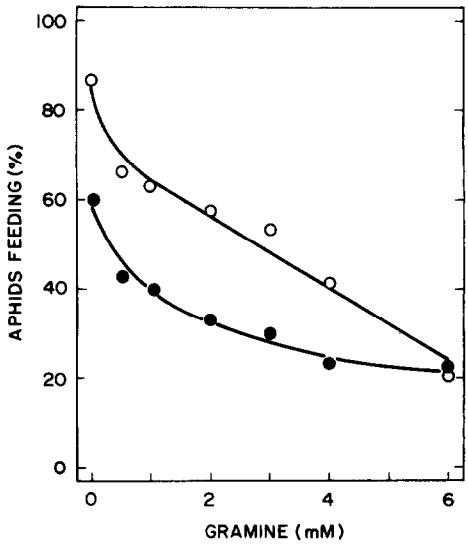

Fig. 2. Effect of gramine on feeding of nymphs of *Schizaphis graminum* (●) and *Rhopalosiphum maidis* (○). Aphids feeding (%) was the proportion of aphids that were stationed on the diet after 5 hr of feeding. Each point is the mean of three samples of 10 aphids each. Aphids were initially over the diet. The diet represented about 8% of surface available to the aphids.

EXPERIMENTAL

Aphids. Individuals of *S. graminum* and *R. maidis* were collected from naturally infested barley and maize, respectively, and allowed to reproduce on barley plants kept inside a nylon net under continuous light in the laboratory.

Feeding assay. Aphids fed from a diet soln were placed between two layers of Parafilm [18]. The diet contained 35 mg cysteine, 100 mg alanine, 400 mg arginine, 50 mg aspartic acid, 200 mg glutamic acid, 100 mg serine, 150 mg threonine, 100 mg histidine,

Table 1. Toxicity and feeding deterrency of indole alkaloids on *Rhopalosiphum maidis*

Compound		Survival* (%)	LD^{*}_{50} (mM)	Aphids feeding† (%)
None	—	99	—	87
MT	(4)	47	3.8	53
MOM_2T	(2)	40	3.5	52
Gramine	(1)	20	2.9	41
MOT	(3)	13	2.3	51

*Survival with 4 mM compound and LD_{50} were measured in a diet after 48 hr of feeding. Numbers represent average of three samples of 10 aphid nymphs each. Standard errors were usually less than 10%.

†Determined as % of aphids stationed on the diet containing 4 mM compound after 5 hr of feeding. Aphids were initially placed over the diet. The diet represents about 8% of surface available to the aphids.

200 mg leucine, 100 g methionine, 10 mg i-inositol, 250 mg choline chloride, 200 mg KH_2PO_4, 200 mg $MgSO_4$, 35 g sucrose and 100 ml water. The pH was adjusted to 6.0 with 0.1 N HCl. On this diet, nearly all aphids survive for more than 48 hr.

Compounds. Gramine and 5-methoxy-*N*,*N*-dimethyl-tryptamine were obtained from Sigma; *N*-methyltryptamine and 5-methoxytryptamine were obtained from Aldrich and Fluka, respectively.

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