EFFECT OF GRAMINE ON THE FECUNDITY, LONGEVITY AND PROBING BEHAVIOUR OF THE GREENBUG, SCHIZAPHIS GRAMINUM (RONDANI)

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Several secondary metabolities are thought to protect plants against herbivores. Hydroxamic acids from Gramineae have been suggested to protect wheat and maize against the European corn borer, *Ostrinia nubilalis* and the greenbug, *Schizaphis graminum* (Klun et al., 1967 and Argandona et al., 1982).

Indole alkaloids have also been reported as toxic to aphids (Corcuera 1984) and several of these alkaloids are present in various species of Gramineae, Leguminosae and other plant families (Culvenor 1973). Addition of indole alkaloids in artificial diets have decreased the survival of aphids and are therefore considered as feeding deterrents (Corcuera 1984). It is also reported that gramine a simple indole alkaloid is present in several barley cultivars (Hanson et al., 1981) and it is responsible for the resistance of barley seedlings to the aphids *S. graminum* and *Rhopalosipum padi* (Zuniga et al., 1985 and Zuniga and Corcuera 1986).

We report the effects of gramine on reproduction, survival and feeding behaviour of the S. graminum.

MATERIAL AND METHODS

Aphids : The apterous virginoparous adults of S. graminum were collected from a field colony and maintained at 20° C in a 16L : 8D photoperiod regime on barley seedling var. Kikaihadaka.

Preparation of diet: The artificial diet for *S. graminum* was prepared as described by Cress and Chada (1971). Gramine was diluted in alcohol and added to the artificial diet to obtain various concentrations.

Fecundity and longevity : One-day old adults of S. graminum were kept individually on barley seedling in a test tube $(20 \times 2 \text{cm dia.})$ or in small vials $(4 \times 0.5 \text{cm dia.})$ The vial contained artificial diet (control) or various concentrations of gramine containing diet between two layers of Parafilm M. The food was changed daily and the number of nymphs in all treatments was estimated daily by dislodging the aphids with a fine

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brush into a gridded petri-dish and counting them under a dissecting microscope.

Probing behaviour : The electronic system used for monitoring the probing behaviour of S. graminum was similar to that described previously (Lohar and Kawada 1987). Before their probing was monitored, aphids were tethered to 15μ m gold wire starved for 30 min and placed individually on Parafilm containing either artificial diet or artificial diet treated with various gramine concentrations. After initiation of the first probe, probing behaviour was recorded for 60 min in all treatments at room temperature ($26\pm 2^{\circ}$ C). The parameters of the aphid probing behaviour tested between gramine-treated diet or control diet were the total number of probes, duration of salivation and duration of ingestion. The data was analysed by Dankans multiple range test using a Hewlet 9845A Computer.

RESULTS

Effect of gramine on the fecundity and longevity of S. graminum

The results presented in Table 1 show the relative number of nymphs of *S. graminum* collected on barley seedlings and artificial diet containing gramine. The fecundity was counted daily until the death of adults. The highest mean number of nymphs was recorded from the barley seedlings (71.2) followed by the mean number of nymphs on artificial diet (10.2), and on diet containing 0.01% (4.4), 0.025% (2.7) and 0.05% (1.2) gramine.

Analysis of the data show that the number of nymphs collected on barley seedlings was significantly higher than that on the artificial diet or diet containing 0.01%, 0.025% and 0.05% gramine (P<0.01). Similarly, the number of nymphs counted on artificial diet medium was significantly higher than on any gramine-containing diet (P<0.01). The number of nymphs was smallest on the diet containing the highest concentration (0.

Diet medium		Mean fecundity	Mean longevety ±S. D.
	No. aphids	±S. D.	
Barley plant	35	71.2±12.1c	30.1±5.3e
Artificial diet	35	$10.2\pm$ 9.0b	8.1±5.3d
Gramine*			
0.01% 35		4.4± 4.1a	$6.3 \pm 2.9c$
0.025%	35	$2.7\pm$ 2.4a	$4.0 \pm 1.4 ab$
0.05%	35	$1.2 \pm 1.6 a$	$3.4 \pm 1.3a$

TABLE 1 Effect of gramine on the fecundity and longevity of S. graminum.

* : Gramine concentrataion added to artificial diet

Note : The means indicated by the same letters are not significantly different

05%) of gramine.

The data given in Table 1 also represent the longevity of the adult aphid on barley seedling, artificial diet and artificial diet containing gramine. The results reveal that aphids lived significantly longer on barley seedlings than on the artificial diet or artifical diet containing 0. 01% or 0.025% or 0.05% gramine (P<0.01) with mean adult life being 30. 1, 8.1, 6.3, 4.0 and 3.4 days, respectively. Similarly, the mean adult life was longer on the artificial diet than on a diet containing 0.01% (P<0. 01) or 0.025% (P<0.01) or 0.05% (P<0.01) gramine.

2. Probing behaviour of S. graminum on artificial diet or gramine containing diets

The data presented in Table 2, Fig. 1 and 2 show the results of various probing parameters monitored for S. graminum feeding on

 TABLE 2
 Feeding behaviour of S. graminum on Parafilm membrane containing artificial diet medium and gramine during a 60-min period.

Diet medium	No. aphids	Mean no. probes ±S. D.	Salivation min. \pm S. D.	Ingestion min. ±S. D.
А	21	4.9±3.1	4.6±4.4	45.7±11.3
В	22	8.9±4.3**	10.3 ± 5.0 **	20.8± 8.6**
С	13	12.4±5.0**	17.3±5.3**	$8.6 \pm 5.2^{**}$

A : Artificial diet

B: Artificial diet containing 0.025% gramine

C: Artificial diet containing 0.05% gramine

** : Significantly different from artificial diet at 1% level

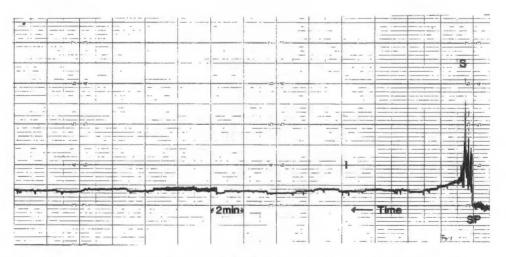


FIG. 1 Typical record of the feeding activity of the greenbug on artificial diet. S : salivation I : ingestion SP : indicates starting point

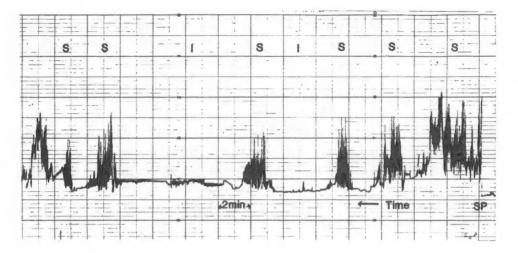


FIG. 2 Typeical record of the feeding activity of the greenbug on a diet containing 0.05% gramine. S : salivation I : ingestion SP : indicates starting point

artificial diet medium with or without various gramine concentrations. The results indicate that the higer mean number of probes (12.4) during one hour was made by aphids probing on a higher concentration of 0.05% gramine during the electronic monitoring time. The fewest probes (4.9) were recorded when aphids were fed on artificial diet alone. The mean number of probes made on the diet containing 0.05% gramine was significantly greater than that recorded on the diet containing 0.025% gramine or the artificial diet (P<0.01). The results also indicate that there was a significant difference in the duration of salivation made by the aphids S. graminum when fed the artificial diet and graminecontaining diets. The mean duration of salivation during a one hour period was shortest for aphids feeding on the artificial diet (4.6 min) as compared to those feeding on a diet containing 0.025% (10.3 min) and 0.05% (17.3 min) gramine. The aphids salivated significantly more on the diet containing a high concentrations (0.05%) of gramine than on that containing a low concentration (0.025%) of gramine or artificial diet alone (P<0.01). The most pronounced difference in the feedindg behaviour of S. graminum was found in the duration of ingestion (Table 2). The aphids during a one hour period ingested significantly more on the artificial diet (45.7 min) than on the diet containing 0.025% (20.8 min) or 0.05% (8.6 min) gramine.

DISCUSSION

It is well known that indole alkaloids are present in many species of Gramineae, Leguminosae and oter plant families (Culvenor 1973). Several plant secondary metabolites have been observed to protect plants against aphids (Argandona et al., 1982 and Long et al., 1977). Gramine and related indole alkaloids present in plant families are thought to be responsible for their toxicity to ruminants (Gallaghar et al., 1964) and aphids (Corcuera 1984).

In this study it was observed that gramine incorporated with artificial diet reduced the fecundity and longevity of the S. graminum (Table 1). In addition when the S. graminum was fed on the artificial diet containing gramine, there was a greater change in its probing behaviour (Table 2 and Fig. 1 and 2). The reduction in the ingestion period or increase in the number of probes and salivation period in gramine fed aphids are thought to affect the amount of food ingested by the aphids. Corcuera (1984) and Zuniga et al., (1985) reported that gramine caused feeding deterrence and decrease in reproduction rate of the S. graminum reared on the artificial diet. They further described that the gramine concentration in barley is sufficiently high to cause toxicity and feeding deterrence to aphids. Similarly, Zuniga and Corcuera (1986) described that the barley cultivars lacking gramine were most susceptible to the aphid, R. padi and that gramine incorporated with artificial diet medium decreased the period of survival, amount of diet ingested and reproduction of the same aphid.

During the present studies the abnormality in the feeding behaviour of *S. graminum* observed on gramine-treated artificial diets is related to the probing behaviour of *S. graminum* recorded on non-host plants (Campbell et al., 1982). The decrease in the ingestion period of *S. graminum* observed (Table 2) is thus correlated with the lack of food nutrients which might have effected the fecundity and longevity of the aphid. Therefore, gramine may play an important role in protecting the barley plants against aphid attack.

SUMMARY

Gramine incorporated in artificial diets significantly decreased the fecundity and longevity of the aphid, *Schizaphis graminum* (Rondani). In addition the electronic monitoring of the probing behaviour indicated that the aphids probing on artificial diets containing gramine showed a significant increase in the number of probes, increase in the duration of salivation and decrease in the duration of ingestion. Thus, gramine may plays a role in the resistance of barley plants to aphids.

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REFERENCES

- Argandona, V. H., Pena, G. F., Niemeyer, H. M. and Corcuera, L. J. 1982. Effect of cysteine on stability and toxicity to aphids of a cyclic hydroxamic acid from Gramineae. Phytochemistry 21: 1573-1574.
- Campbell, B. C., McLean, D. L., Kinsey, M. G., Jones, K. C. and Dreyer, D. L. 1982. Probing behaviour of the greenbug (*Schizaphis graminum*, biotype C) on resistant and susceptible varieties of sorghum. Entomol. Exp. Appl. 31: 140-146.
- Corcuera, L. J. 1984. Effects of indole alkaloids from Gramineae on aphids. Phytochemistry 23: 539-541.
- Cress, D. C. and Chada, H. L. 1971. Development of a synthetic diet for the greenbug, *Shizaphis graminum*. 1. Greenbug development on two synthetic diets. Ann. Entomol. Soc. Am. 64 : 1237-1240.
- Culvenor, C. C. 1973. Chemistry and biochemistry of harbage. 375 pp. Academic press, London.
- Gallagher, C. H., Koch, J. H., Moore, R. M. and Steel, J. D. 1964. Toxicity of *Phalaris* tuberosa for sheep. Nature 204 : 542-545.
- Hanson, A. D., Traynor, P. L., Ditz, K. M. and Reicosky, D. A. 1981. Gramine in barley forage-Effects of genotype and environment. Crop Sci. 21 : 726-730.
- Klun, J. A., Tipton, C. L. and Brindley, T. A. 1967. 2, 4-dihydroxy-7-methoxy-1, 4benzoxazin-3-one (DIMBOA), on active agent in the resistance of maize to the European corn borer. Jour. Econ. Entomol. 60 : 1529–1533.
- Lohar, M. K. and Kawada K. 1987. Probing bahaviour of the aphids, Schizaphis graminum (Rondani), Rhopalosiphum maidis (Fitch) and Longiunguis sacchari (Zehntner) on resistant and susceptible sorghum plants. Ber. Ohara Inst. landw. Biol., Okayama Univ. 19 : 137-144.
- Long, B. J., Dunn, G. M., Bowman, J. S., and Routley, D. G. 1977. Relationship of hydroxamic acid content in corn and resistance to the corn leaf aphid. Crop Sci. 17: 55-58.
- Zuniga, G. E. and Corcuera, L. J. 1986. Effect of gramine in the resistance of barley seedlings to the aphid *Rhopalosiphum padi*. Entomol. Exp. Appl. 40 : 259-262.
- Zuniga, G. E., Salgado, M. S. and Corcuera, L. J. 1985. Role of an indole alkaloid in the resistance of barley seedlings to aphids. Phytochemistry. 24 : 945-947.