Electronic Journal of Polish Agricultural Universities is the very first Polish scientific journal published exclusively on the Internet, founded on January 1, 1998 by the following agricultural universities and higher schools of agriculture: University of Technology and Agriculture of Bydgoszcz, Agricultural University of Cracow, Agricultural University of Lublin, Agricultural University of Poznan, Higher School of Agriculture and Teacher Training Siedlce, Agricultural University of Szczecin, and Agricultural University of Wroclaw.

ELECTRONIC JOURNAL OF POLISH AGRICULTURAL UNIVERSITIES

2006
Volume 9
Issue 1
Topic
BIOLOGY

URBAńSKA A. , LESZCZYńSKI B. , MATOK H. 2006. HOW DOES GRAMINE AFFECT PROBING BEHAVIOUR OF GRAIN APHID? **Electronic Journal of Polish Agricultural Universities**, Biology, Volume 9, Issue 1.
Available Online http://www.ejpau.media.pl/volume9/issue1/art-02.html

HOW DOES GRAMINE AFFECT PROBING BEHAVIOUR OF GRAIN APHID?

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ABSTRACT

Probing behaviour of the grain aphid, *Sitobion avenae* (F.) on agarose-sucrose gels containing gramine at concentrations between 0.01 mM and 100 mM was examined using the EPG, electrical penetration graph technique.

The following effects were recorded: the decrease in the number of probes, extension of the pathway phases, representing the penetration of leaf tissues by the aphid stylets and subsequent probing before reaching phloem and a strong reduction of the phases E_1, E_2 and G reflecting the activities of the stylets within the phloem and xylem elements as the consequence of increase in the gramine content in the gels.

Accordingly, gramine appears to be the 'anti-feedant' compound towards the grain aphid. Its role as a probing stimulant for the aphid is discussed.

Key words: gramine, grain aphid, *Sitobion avenae*, probing behaviour, EPG, agarose-sucrose gel.

INTRODUCTION

Alkaloids are very diverse plant secondary metabolites with regard to structure, distribution, metabolism, and biochemical role in ecosystems [1]. Effect of the alkaloids, mainly quinolizidines, pyrrolizidines, pyrrolidines, pyridines and indoles towards aphids are argued in terms of toxic, repellent, deterrent, neutral and stimulative activity depending upon the specific aphid-plant relations [2-14].

Gramine, 3-N,N-dimethylaminomethylindole occurs in *Poaceae*, essentially in *Hordeum*. In the barley seedlings gramine is located in the mesophyll parenchyma and epidermal tissue [1, 15]. There is no data on presence of gramine in phloem and xylem exudates.

According to the major part of the available literature, gramine was recognized as an important resistance factor of cereals towards aphids, because of deterrent properties and, consequently, the decrease in reproduction and survival of *Schizaphis graminum* (ROND), *Rhopalosipum maidis* (F.), and *R. padi* (L.)[6, 8, 11, 12, 13, 15]. However it was suggested that gramine was not a strong deterrent and that primary role in resistance of the cereal plants to aphids was played rather by phenolic compounds and hydroxamic acids, especially DIMBOA, 2,4-dihydroxy-7-methoxy-

1,4-benzoxazin-3-one [16, 17, 18, 19, 20]. Thus, the role of gramine in plant-aphid relationship remains not specified.

In this paper we studied the effect of various concentrations of gramine incorporated into sucrose-agarose gels on the probing behaviour of the grain aphid *S. avenae* with the aid of EPG, electrical penetration graph technique.

MATERIAL AND METHODS

Chemicals. Agarose, sucrose and gramine were purchased from Sigma Chemical Co., (St. Louis, MO, USA).

Aphids. The grain aphid *S. avenae* used in the experiment was reared on the ten-day-old seedlings of Polish winter wheat cultivar 'Sakwa' in insectary chamber at L 16 : D 8 photoperiod and 22°C.

Effect of gramine on aphid probing behaviour. Apterous adults were wired and after being connected to an EPG amplifier, they were allowed to probe for 4hrs into 1.25% w/v agarose gels prepared in plastic rings and covered by parafilm ^{TM} membranes. The gels contained 30% w/v sucrose plus gramine at the following concentrations: 0.01 mM, 0.1 mM, 1 mM, 10 mM and 100 mM. Each gel offered to the aphids contained only one the tested concentrations of gramine. Control gels contained no gramine. The recorded EPG-s from ten aphids probing into the gels were stored on computer hard disc using STYLET 2.0 software and analysed in terms of number and duration of the EPG waveforms, as classified by Tjallingii [21].

Statistics. The data denied from the EPG recordings were subjected to the analysis of variance followed by Duncan's test.

RESULTS

Activity of the aphid stylets in agarose-sucrose gels.

Except for the F waveform, which reflects mechanical difficulties during tissue penetration into plants, all other waveforms relating to plant tissues penetration by aphids were shown in the agarose control gels, containing sugar but no gramine: namely, (1) pathway phase A, B, C waveforms, i.e. intercellular stylet penetration *via* epidermis and mesophyll parenchyma (2) E_1 waveform, phloem phase including puncturing and salivation into sieve elements, (3) waveform E_2, phloem phase representing sap ingestion from sieve elements and (4) waveform G, reflecting xylem 'drinking'.

The presence of 0.01 mM of gramine had no effect on the occurrence of various waveforms as compared to the control. The increase in gramine up to 0.1 mM caused the 'disappearance' of the waveforms E_2 and G. 1 mM of gramine and higher, in the gels, limited the aphid stylets activity to pathway phase only (Table 1).

Table 1. Appearance of waveforms during penetration in the agarose-sucrose gels by grain aphid

Concentration of gramine	Waveform*
None, control	path, E_1, E_2 & G
0.01 mM	path, E_1, E_2 & G
0.1 mM	path & E_1
1 mM, 10 mM, 100 mM	path

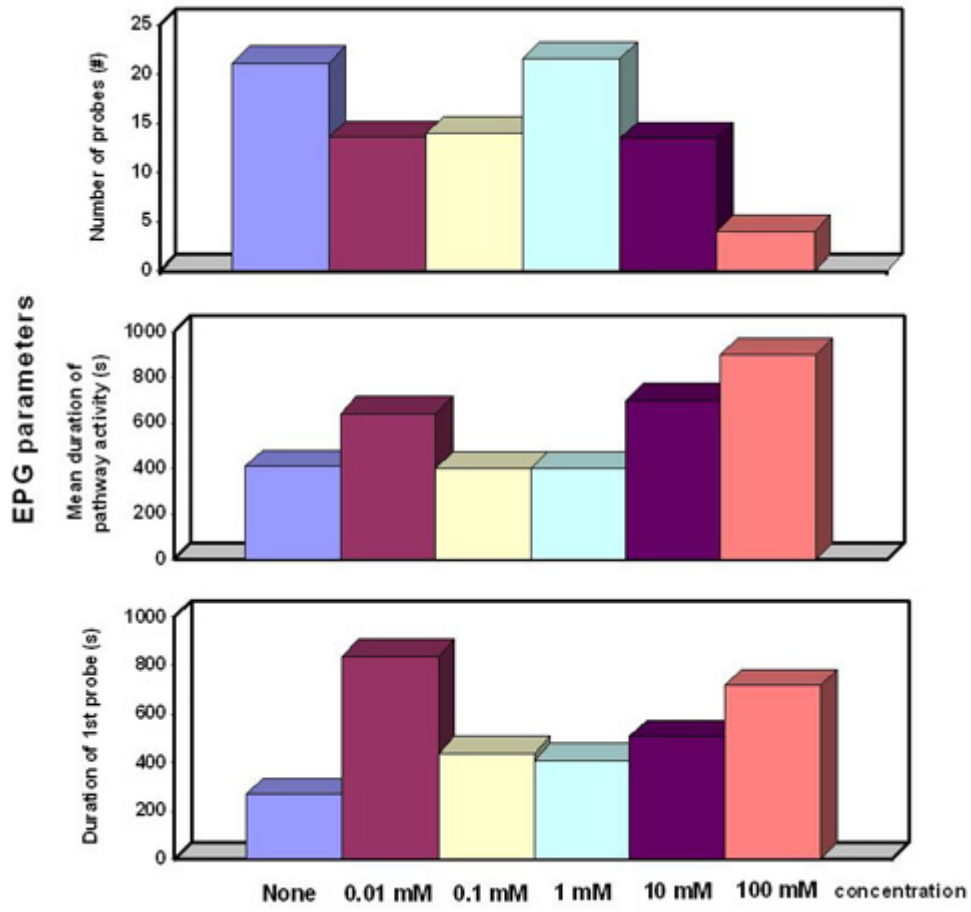
*path = EPG waveforms A,B and C reflecting stylet penetration activities outside phloem;
E_1 = EPG waveform, reflecting salivation into sieve elements;
E_2 = EPG waveform, reflecting ingestion of phloem sap;
G = EPG waveform, reflecting ingestion of xylem sap

Influence of gramine on the grain aphid's probing and feeding.

In general, the aphids that probed into agarose-sucrose gels containing gramine within the range between 0.01 mM and 100 mM showed more intense probing activity in peripheral tissues and reduced feeding activity.

In details, a much longer duration was shown both, for the first and next probes resulting in extension of mean duration of pathway phase. However, all tested gramine concentrations except that for 1 mM caused a strong decrease of number of probes. The extreme effect in that aspect was noticed at concentration as high as 100 mM (Figure 1).

Fig. 1. Effect of gramine on the probing behaviour of *S. avenae*, according to EPG recordings from agarose-sucrose gels during 4hrs



In the matter of effect of the gramine added into agarose-sucrose gels on the aphid phloem and xylem phases (waveforms E_1, E_2 and G), an essential decrease in the duration and frequency was found at concentrations as low as 0.01 mM and 0.1 mM (Table 2).

Table 2. Effect of gramine on the feeding behaviour of *S. avenae*, EPG recordings from agarose-sucrose gels during 4 hrs

EPG parameters	Gramine concentration		
	none	0.01 mM	0.1 mM
Duration of 1stE_1(s)	60.6 a	92.2 a	9.4 b
Total duration of E_1(s)	216.8 b	361.8 a	77.0 c
Number of E_1 (#)	7.0 a	1.8 b	1.4 b
Duration of 1stE_2(s)	63.0 a	23.2 b	-
Total duration of E_2 (s)	106.6 a	84.6 b	-
Number of E_2 (#)	0.6 a	0.6 a	-
Duration of 1stG(s)	1602.0 a	719.0 b	-
Total duration of G (s)	2147.0 a	719.0 b	-
Number of G (#)	2.2 a	1.0 b	-

Values in rows not followed by the same letter are significantly different at the 0.05% level (Duncan's test)

DISCUSSION

Two essential effects were shown as regards the modification of the probing and feeding of the grain aphid, namely (1) gramine prolonged (stimulated) pathway phase (stylet penetration); (2) gramine strongly reduced (inhibited) phases E_1, E_2 representing sieve element salivation and ingestion, and phase G corresponding with 'drinking' of xylem sap. The studied aphids ingested the diet with gramine at concentration relatively low, only 0.01mM.

However, the EPGs of the aphids inserting their stylets into the gels with the higher amounts of gramine, i.e. 1.0mM, 10mM and 100mM showed only pathway phase. Thus, no ingestion from these 'diets' was noted. Moreover, the number of the stylet penetrations decreased whereas their duration became longer as a consequence of increase of the gramine concentration within the diets. Then the results suggest that this obvious 'anti-feedant' can be considered as a probing stimulant in relation to the grain aphid.

Role of alkaloids in probing behaviour of aphids in available literature is described in various terms i.e. toxic, detterent, repellent, ineffective and stimulative [2-14]. Smith [2] demonstrated that quinolizidine alkaloid sparteine was a strong feeding stimulus to *Acyrthosiphon spartii* and Wink *et. al.* [5] reported that quinolizidine alkaloids from *Fabaceae* were involved in resistance to *Aphis cytisorum*. Gramine from *Hordeum* was recognized as a chemical 'disturbing' the probing and feeding activities of cereal aphids e.g. decreased the artificial diet intake by *S. graminum* at concentration 2.1 mM similar to that found in the barley plant leaves [9]. Whereas, at concentration as low as 0.5 mM showed detterent activity on *S. graminum* [6]. Moreover, toxicant or deterrent action of gramine towards *R. padi*, *R. maidis*, *S. graminum* and *S. akeviae* was observed by adding it to artificial diets at concentrations between 0.003 and 0.1% [6, 9, 12, 13, 22].

Gramine is one of the best described examples of secondary chemicals involved in resistance of plants towards cereal aphids i.e. *S .graminum*, *R. maidis*, *R. padi* [6, 9, 11, 12, 13]. There was proposed that gramine acted as deterrent during the penetration of leaves by aphid stylets and subsequent probing before contact with the phloem food source [15]. There are no available data concerning gramine-aphid relations obtained using the EPG technique, that provide insight into the complete feeding mechanism of these insects. However, in 2002 we reported [23] on the role of the cereal *o*-dihydroxyphenolics in the aspect concerned here. It was proposed to distinguish for aphids and similarly feeding insects the 'anti-feeding' activity of chemicals from their 'anti-probing' activity. Now, when we analyse the EPG parameters obtained for gramine that idea seems to be quite reasonable.

In the matter of the 'anti-feeding' action of gramine that is rather obvious according to the results. However, there are reports that aphids could feed on the barley seedlings with very high amounts of gramine [17]. Gramine as a probing stimulus seems to be a matter of discussion because the studied aphids might have 'prolonged' pathway phase to avoid gramine ingestion. In addition, Kadwa and Lohar [24] stated that gramine might act as a resistant component to *S. graminum* due to the increase in probing, duration of salivation and a decrease of ingestion period detected by electronic monitoring method by Mclean and Kinsey [25].

It seems that *Aphididae* 'apply' a strategy of 'avoidance' of alkaloids because their stylets penetrate *via* epidermal and mesophyll parenchyma tissues and vascular sheaths intercellularly passing over the vacuoles, that are sites of accumulation of alkaloids and reach phloem which rather does not contain these chemicals.

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