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Increase in gramine content in barley infested by the aphid *Schizaphis graminum* R

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Abstract

The kinetics of accumulation of gramine were measured in three aphid infested and non-infested cultivars of barley (*Hordeum vulgare;* cvs. Frontera, Libra and Acuario). After 6 days of infestation gramine content increased 10-fold in the cvs. Libra and Acuario, while in the cv. Frontera it only doubled. The maximum level found in infested cv. Acuario was 50% of the level observed in non-infested plants of the cv. Frontera. Therefore, in the cvs. Libra and Acuario gramine content is mainly an inducible response, while it is mainly constitutive in the cv. Frontera. This increase in gramine may be of significance in resistance to aphids. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Hordeum vulgare*; *Schizaphis graminum*; Aphids; Barley; Gramine

1. Introduction

Infestation of barley (*Hordeum vulgare*) by aphids produces physiologic alterations, which result in a significant loss of productivity (Miller & Haile, 1988; Cabrera, 1994). Among the secondary metabolites of barley which are toxic to aphids the indole proto-alkaloid gramine is one of the most abundant (Corcuera, 1984; Gröger, 1980). In previous laboratory experiments and field observations, it has been demonstrated that the susceptibility of barley to aphids is negatively correlated with gramine content in the leaves (Kanehisa, Tsumuki, Kawada & Rustamani, 1990; Rustamani, Kanehisa, Tsumuki & Shiraga, 1992; Zuñiga, Salgado & Corcuera, 1985). The abundance of this alkaloid varies significantly among different barley cultivars. Various factors such as photoperiod, age and temperature were shown to affect the content of gramine (Hanson, Ditz, Singletary & Leland, 1983; Salas & Corcuera, 1991), which has been found in mesophyll parenchyma and in epidermis but not in the vascular bundles (Argandoña, Zuñiga & Corcuera, 1987). A small fraction of gramine is, however, located on the leaf surface (Yoshida, Tsumuki, Kanehisa & Corcuera, 1993). In feeding experiments with holidic diets, it has been demonstrated that this compound is toxic to aphids, decreasing fecundity, longevity and feeding in *Schizaphis graminum* Rondani and *Rhopalosiphum padi* L. (Zuñiga et al., 1985; Zuñiga & Corcuera, 1986; Zuñiga, Varanda & Corcuera, 1988). These results suggest that the gramine in the leaves of barley is a major constitutive determinant of its resistance to aphids (Corcuera, 1993). In this paper, we show that the content of this alkaloid can be increased by aphid infestation.

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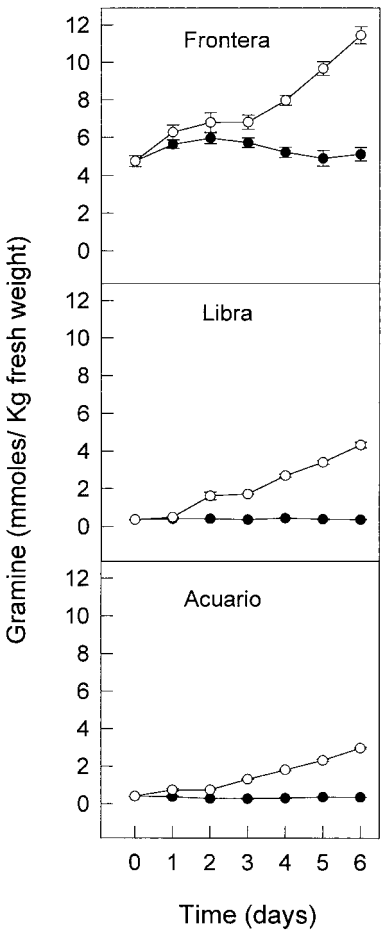

Fig. 1. Gramine content in barley leaves infested by the aphid *S. graminum*. Plants of cultivars Frontera, Libra and Acuario were infested with 40 nymphs/plant and gracontent was measured. Non-infested plants (●); infested plants (○). Values are the average of six samples ± S.E. When S.E. are not shown, it is because they are smaller than the symbols.

2. Results and discussion

Ten-day-old plants showed a progressive increase in gramine content when they were infested with aphids, while no significant changes were observed in non-infested plants (Fig. 1). The accumulation of gramine in the three investigated cultivars showed two phases of increase. The first increase was observed before the second day of infestation. The second increase occurred after 3–4 days and continued until the end of the experiment. Gramine content was always smaller in the cvs. Libra and Acuario than in cv. Frontera. The levels of gramine in non-infested plants after six days of infestation were 0.36 mmol/kg fr. wt in cvs. Libra and Acuario and 5.3 mmol/kg fr. wt in cv. Frontera. The increment of gramine in cv. Frontera was 123%, in cv. Acuario 720% and in the cv. Libra 1080%. These increments with respect to the control plants were highly significant ($P < 0.01$)

The dependence of the accumulation of gramine on aphid density was studied only in cv. Libra, because it was the cultivar that showed the highest change in gramine content (Fig. 2). The alkaloid accumulation showed a direct correlation with aphid density ($r^2 = 0.95$, $P < 0.01$ from 2nd to 4th day). The two phases of accumulation were observed in all cases, as the amount of initial aphids was increased, the phases occurred earlier. However, these direct correlations between gramine increments and aphid density held only until the 4th day of infestation. Afterwards, the vigour of the plants decreased significantly.

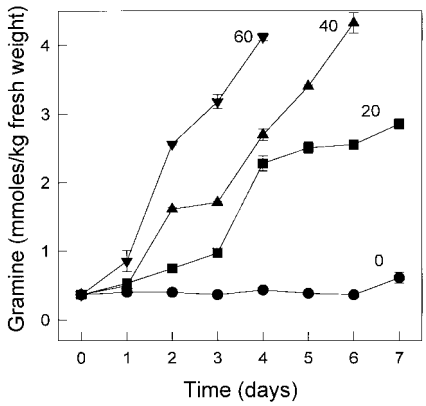

Fig. 2. Effect of aphid density on gramine accumulation in barley leaves. Plants of cv. Libra were infested with 0, 20, 40 and 60 aphids/plant. Numbers on top of the lines indicate the amount of initial aphids per plant. Values are averages of three samples ± S.E. When S.E. are not shown, it is because they are smaller than the symbols.

These results agree with previous studies with respect to the inverse correlation between gramine content and susceptibility of barley leaves to aphids (Kanehisa et al., 1990; Rustamani et al., 1992; Zuñiga et al., 1985). Thus, cv. Frontera, the more resistant cultivar, showed a higher level of gramine than the more susceptible cvs. Libra and Acuario (Casaretto, 1996). When the percentages of increment in gramine content upon infestation were compared, the more resistant cultivar Frontera had very little increase compared to the more susceptible cultivars.

The biological significance of the increase in gramine accumulation upon infestation is not clear at present. It is likely that after infestation with aphids the more susceptible cultivars reached levels of gramine in their leaves that could be toxic to the aphids. Our group has shown earlier that concentrations of gramine higher than 1 mM applied in artificial diets result in death of more than 50% of the aphid *S. graminum* (Zuñiga et al., 1985). The mechanisms by which the aphids induce gramine accumulation are still unknown. Elucidation of this mechanism may be of relevance for the selection of varieties that are able to respond to aphid infestation by increasing some of their defence compounds. It is likely that the accumulation of gra-

mine, in addition to affecting aphids, could also protect barley against other insects and pathogens.

3. Experimental

3.1. Plants and growth conditions

Ten-day-old *Hordeum vulgare* seedlings of cvs. Frontera, Libra and Acuario were used for the infestation experiments. These cultivars show different levels of susceptibility to the aphid *Schizaphis graminum*, cv. Frontera being the less susceptible and Acuario the most susceptible (Casaretto, 1996). Seeds were sown in pots with vermiculite and grown at 25°, in a 14/10 h (light/dark) photoperiodic cycle under a photosynthetic active radiation of 70 μmol photons m^{-2} s^{-1}. The plants were kept in a hydroponic medium with Hoagland solution.

3.2. Aphids and infestation

Individuals of the aphid *Schizaphis graminum* were reared on barley cv. Acuario. Plants and aphids were kept in a growth chamber at 25° under constant light. Plants of the three cultivars were infested with 40 individuals (third instar) for 6 days and were kept under the same conditions described above for a grown plant. In order to evaluate the effect of aphid density on gramine accumulation, plants from cv. Libra were infested with 20, 40 and 60 aphids per plant. The experiments had three replicates of six plants each and were repeated twice.

3.3. Plant extracts for gramine analysis by HPLC

Fresh leaves (three samples of 1 g fr. wt. each) from non-infested and infested plants were taken every 24 h. The leaves were cut into small pieces and extracted in 15 ml of methanol (three times for 24 h). The methanolic extracts were combined and evapd. The residue was dissolved in 10 ml of the eluent used for HPLC analysis. The insoluble material was removed by centrifugation (10,000*g* for 5 min). Recovery of gramine, measured by adding known amounts of gramine to the crude extracts, was 95.7%. Reported values in this paper are uncorrected. The HPLC analysis was carried with a RP18 column (Supersphere 4 μm, Merck, 119 × 4 mm i.d.). Elution was performed isocratically with a mixture of H_2O:CH_3CN:EtOAc:H_3PO_4 (97:3:2.5:1.5) with a flow rate of 1.5 ml/min. Peaks were detected at 275 nm. The R_t for gramine was 2.2 min. The collected fractions from this peak had the same UV spectrum as gramine. Gramine was also isolated from the samples and identified by ^{1}H NMR and mass spectroscopic analysis, respectively.

3.4. Statistics

Differences in gramine accumulation with infestation were tested using a one-way ANOVA ($P < 0.01$) followed by a Tukey test (software Systat 6.01 for Windows). Standard errors (SE) are usually shown, unless they are smaller than the symbols.

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