Role of gramine in the feeding deterrence of barley against the migratory locust, *Locusta migratoria* (Orthoptera: Acrididae)

Yukio Ishikawa* and Tôru Kanke

Laboratory of Applied Entomology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo 113-8657, Japan

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**Abstract**

To examine whether gramine has an important role in the feeding deterrence of barley against the migratory locust, *Locusta migratoria* (Orthoptera: Acrididae), a bioassay using sucrose-impregnated filter paper as the feeding substrate and a feeding assay on barley lines with different contents of gramine were conducted. Gramine deterred feeding of the locust dose dependently in a range of 50–500 µg/150 mg paper in the filter paper assay. On the whole, there was a good correlation between gramine content in mature plants and feeding deterrence against *L. migratoria*; barley lines with gramine content of more than 100 µg/g deterred feeding of the locust, while those with less than 50 µg/g of gramine were susceptible to feeding by the locust. However, when the developmental changes of gramine content in barley and the changes of feeding deterrence against the locust were investigated, a barley line was found in which the young plant was susceptible to feeding by the locust despite a high gramine content. Therefore, the level of gramine alone cannot account for the deterrence of barley against the migratory locust. The feeding deterrence of barley against *L. migratoria* must be due to multiple feeding deterents, one of which is gramine.

**Key words:** Barley, feeding deterrence, gramine, *Locusta migratoria*

**INTRODUCTION**

In a previous study (Ishikawa and Kanke, 2000), we found that barley seedlings strongly deterred feeding of the migratory locust, *Locusta migratoria*. Analysis of an ethanol extract of barley showed that the alkaloid fraction had strong deterrence against feeding by the locust, while the acidic and neutral fractions had weaker deterrence.

Gramine, 3-(dimethylaminomethyl)-indole, is the principal alkaloid contained in barley (Zuniga et al., 1985), and it, as well as other alkaloids, was shown to exhibit feeding deterrence against a polyphagous grasshopper, *Melanoplus bivittatus*, at a high concentration (Harley and Thorsteinson, 1967). Barley lines resistant against aphids (e.g., *Rhopalosiphum padi*, *R. maides*, *Schizaphis graminum* and *Sitobion akeviae*) have been shown to contain high concentrations of gramine (Corcuera, 1984; Zuniga and Corcuera, 1986). A negative correlation was found between the gramine concentration in barley and the population size of aphids on plants in the field (Kanehisa et al., 1990; Rustamaini et al., 1992b; Moharramipour et al., 1996). The growth of aphids, *S. graminum* and *R. padi*, was retarded when they were reared on an artificial diet containing gramine (Zuniga et al., 1988; Kawada and Lohar, 1989). Hence, gramine has been considered as an important factor that confers resistance against aphids in barley.

Our previous findings and the above-mentioned findings on the effect of gramine on *Melanoplus* and the role of this compound in the aphid resistance of barley suggest that gramine confers feeding deterrence against the migratory locust in barley plants. In the present study, a correlation between the content of gramine and deterrence against the locust was investigated using barley lines differing in gramine content and resistance against aphids. Since gramine content in barley is known to

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*To whom correspondence should be addressed.*
change greatly with development (Argandona et al., 1987; Kanehisa et al., 1993), the changes in gramine content in the leaf during its growth were examined and compared with the changes of feeding deterrence against *L. migratoria*.

**MATERIALS AND METHODS**

**Barley.** The barley lines used in this study were obtained from the Research Institute for Bioresources, Okayama University. Four aphid-susceptible and three aphid-resistant barley lines were used for the experiments (Table 1). Barley was grown from September to December in a temperature-controlled greenhouse (25°C in the day and 20°C in the night). Three to five barley plants were grown in a Wagner pot (16 cm diam. ×20 cm), and the leaves were cut and offered to the locusts. ‘Young’ denotes those plants grown to about 20 cm in height with five or six leaves, and the third leaf counted from the top was cut and subjected to analysis. ‘Mature’ plants were those about 40 cm in height and near the earing stage. The second and third leaves of mature plants were used. To obtain seedlings, barley seeds (5 g) were sown on soil fertilized with 5 g of Magamp® K in a plastic container (10 × 15 × 6 cm). After 2 weeks, the first leaf grown to a height of about 10 cm was subjected to bioassay and quantification of gramine.

**Locusts.** Locusts were reared in our laboratory as previously described (Ishikawa and Kanke, 2000). One- to two-week-old adults were used for the bioassay. For determining the age of individual locusts, a white mark was painted on the pronotum within 24 h after eclosion.

**Filter paper bioassay.** To evaluate the feeding deterrence of gramine, a filter paper bioassay was employed. To stimulate feeding of the locusts, 300 µl of 3% sucrose in 50% ethanol was applied to a piece of filter paper (No. 51B, 2 × 8 cm, about 140 mg, Toyo Roshi, Tokyo) and dried completely. The content of sucrose in the filter paper was about 6% by weight. Gramine solution in chloroform was then applied to the paper, which was dried again and subjected to feeding by the locusts. Chloroform was used as a control.

Five pieces of pre-weighed filter paper, including a control, were hung from the ceiling of a cage (30 × 30 × 20 cm), in which 20 male and 20 female adults starved for 4–5 h were housed. The height of the filter paper was adjusted so that the lower end of the sheet touched the bottom of the cage. After 2 h, the weights of the filter papers were measured to within 0.1 mg with an electronic balance (AEX-180, Shimadzu, Kyoto). Feeding deterrence (Y%) was defined as \[ Y = \left(1 - \frac{X_{c}}{X_{i}}\right) \times 100 \], where \( X_{c} \) and \( X_{i} \) indicate the decrease in weight (mg) of the control and treated filter papers, respectively.

**Feeding test.** A locust was put in a cage (6 cm in diam. ×12 cm) made from Saran® mesh and starved for 4–5 h. A leaf of mature barley was cut to a length of 5 cm, and presented to a locust in a cage; feeding behavior was observed for 15 min. After introduction of plants, the cage was set with its long axis horizontally to facilitate encountering of the plant by the locust. Feeding was recorded only when the leaf was eaten. Mere nibbling or biting was not considered as feeding.

**Extraction and quantification of gramine.** Gramine in the barley was extracted by the method of Zuniga et al. (1985). In brief, barley leaves (2–5 g fresh weight) were frozen at –20°C and homogenized in a blender with 40 ml of methanol containing 1% NH₄OH. The homogenate was filtered through glass wool, and the filtrate was dried *in vacuo* with a rotary evaporator. After the solid residue was dissolved in 20 ml of 0.1 N HCl, the solution was filtered, adjusted to pH 9.0 with conc. NH₄OH,
and then extracted with chloroform. The concentration of the extract was adjusted to 1 g fresh weight equivalent/ml.

The extract (20 μl per spot, 20 mg leaf eq.) and standard gramine (Nacalai Tesque, Kyoto) were spotted on Silica gel TLC plates (60F254, Wako Pure Chemical Industry) and developed using a mixture of methanol, chloroform and conc. NH4OH (11 : 100 : 1 v/v). The plates were sprayed with van Urk-Salkowski reagent (Ehmann, 1977) and heated at 110°C for 10 min. The density of the purple spots (Rf 0.11) was quantified using a TLC-scanner (CS-920, Shimadzu) at 550 nm. The gramine concentration in the extract was calculated using a simultaneously obtained calibration curve.

RESULTS

Feeding deterrence of gramine

The feeding deterrence of gramine against L. migratoria was examined using sucrose-impregnated filter paper as the feeding substrate (Fig. 1). Gramine deterred feeding of the locusts dose dependently in a range of 50-500 μg/paper. Fifty percent feeding inhibition was obtained at a dose of about 150 μg, and 500 μg/paper was necessary to inhibit feeding by 90%.

Correlation between gramine content and feeding deterrence

The gramine content and feeding deterrence of seven barley lines were examined at the mature plant stage (Fig. 2). On the whole, there was a good correlation between the gramine content in the plants and their feeding deterrence against L. migratoria; barley lines with a gramine content of more than 100 μg/g deterred feeding of the locust, while those with a gramine content of less than 50 μg/g were susceptible to feeding by the locust (Fig. 2). The content of gramine necessary to induce 50% inhibition appeared to be about 110 μg/g leaf.

Among the seven barley lines, the aphid-resistant wild species H. spontaneum-4969 (No. 7) contained as much as 830 μg gramine/g leaf, and was not eaten at all by L. migratoria. Two other aphid-resistant lines (Nos. 5 and 6) contained 150-290 μg gramine/g, and were only slightly eaten. Moreover, two aphid-susceptible lines (Nos. 2 and 4), which contained 125-135 μg gramine/g, also showed strong deterrence against L. migratoria. In contrast, two other aphid-susceptible lines (Nos. 1 and 3), which contained less than 50 μg gramine/g, were susceptible to feeding by the locust.

Developmental changes of gramine content and changes in feeding deterrence

The developmental changes of gramine content in the barley plants were examined using four barley lines (Nos. 1, 2, 3 and 6) which showed little or moderate feeding deterrence against the locust at the mature plant stage. The content of gramine in the barley plant tended to decrease with growth; however, the profile of reduction was very different among the lines (Fig. 3). At the seedling stage, all four lines had
Fig. 3. Developmental changes of the gramine content in four barley lines which showed little or moderate feeding deterrence against the migratory locust at the mature plant stage. Vertical lines on top of the bars indicate standard errors of the means. See Table 1 for description of the barley lines.

A gramine content of more than 150 μg/g leaf, regardless of the aphid resistance. The gramine content in an aphid-susceptible line (No. 1) decreased sharply to as low as 65 μg/g by the five- to six-leaf (young) stage. The decrease in the other lines was not as conspicuous. The gramine content of line No. 3 at the seedling stage was the lowest, but this line did not show any decrease in gramine content at the young plant stage. At the mature plant stage, the gramine contents of lines No. 1 and No. 3 were decreased to less than 50 μg/g, while high gramine contents (>120 μg/g) were maintained in aphid-resistant line No. 6 and in aphid-susceptible line No. 2.

In the feeding assay against Locusta migratoria, all four lines at the seedling stage completely averted feeding of the locust, regardless of deterrence potential at the mature plant stage (Table 2). As the barley grew to the young stage, however, the immunity of the plants against feeding by the locust tended to become weaker. Barley lines No. 1 and No. 3 became susceptible to feeding by the locust, while lines No. 2 and No. 6 remained moderately deterrent against the locust. No large changes in deterrence were observed in any of the barley lines between the plants at the young stage and the mature plant stage. The most notable finding in this experiment was that the young plants of line No. 3 were susceptible to feeding by the locust despite a high content of gramine (Fig. 3, Table 2).

DISCUSSION

When a wheat flour wafer is used as the feeding substrate, a gramine concentration as high as 3% (dry weight basis) is necessary to inhibit feeding of the migratory locust (Bernays and Chapman, 1977). In the present study, 150 μg gramine/paper, which was equivalent to about 0.1% on a dry weight basis, inhibited feeding of the locust by 50%. This large difference in the effective concentration may be attributable to differences in moisture, the number and amount of feeding stimulants in the feeding substrate, and the physical properties of the substrate, as well. Certainly, sucrose-impregnated filter paper was not a highly preferred substrate. Because of this lower preference, however, the assay used in the present study was very sensitive for detecting feeding deterrence of test compounds.

There was a good correlation between the

<table>
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<tr>
<th>Stage</th>
<th>Height (cm)</th>
<th>Barley line</th>
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<td></td>
<td></td>
<td>No. 1</td>
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<tr>
<td>Seedling</td>
<td>10</td>
<td>100</td>
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<td>Young</td>
<td>20</td>
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<tr>
<td>Mature</td>
<td>40</td>
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a Twelve young adults were individually used in each test.

b Grown in a greenhouse at 20-25°C under natural sunlight. Seedling, 2 weeks after sowing; young, 5 weeks after sowing; mature, 12-14 weeks after sowing.
Gramine as a Feeding Deterrent against *L. migratoria*

Although epicuticular wax does not appear to contribute to the deterrence against *L. migratoria* (Ishikawa and Kanke, 2000), the effect of aconitic acid against the locust should be examined.

The gramine contents in the barley lines examined in this study were higher than those reported by Kanehisa et al. (1990). It is known that the gramine content in barley is affected by temperature (Salas and Corcuera, 1991). In the present study, barley was grown at 20–25°C, at which temperature the content of gramine is maximized. Moreover, the barley in this study was grown under highly fertilized conditions, which may also have contributed to an increase of gramine. Kanehisa et al. (1990) used barley grown in the field, and thus it is not unlikely that the level of gramine concentration differed in the two studies.

In conclusion, the feeding deterrence of barley against *L. migratoria* must be due to multiple feeding deterrents, one of which is gramine.

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REFERENCES


