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Feeding deterrence of barley seedlings against the migratory locust *Locusta migratoria* (Orthoptera: Acrididae)

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Abstract

Barley seedlings strongly deterred feeding of the migratory locust *Locusta migratoria*. Analysis of feeding-related behavior revealed that approach to barley seedlings and palpation occurred just as with palatable plants, but rejection of the plant occurred immediately after biting. Excision of the maxillary and labial palps had no effect on the rejection of barley seedlings, and feeding of palatable plants occurred as with intact insects. Removal of compounds on the surface of the barley leaves also had no effect, while ethanol extraction of the leaves diminished the deterrence of the barley seedlings. These results indicated that feeding deterrent(s) are localized inside the seedlings. An ethanol extract of barley seedlings exhibited strong deterrence in a feeding assay using a piece of sucrose-impregnated filter paper as the feeding substrate. Fractionation of the ethanol extract revealed that the strongest feeding deterrence was observed with the basic fraction, which contains alkaloids such as gramine. The other fractions also showed weak feeding deterrence. A crude alkaloid extract equivalent to 100 mg of barley leaf inhibited feeding of the locusts by 90%.

Key words: *Locusta migratoria*, feeding deterrent, alkaloid, barley, palpation

INTRODUCTION

In our laboratory, the migratory locust *Locusta migratoria* (Orthoptera: Acrididae) has been routinely reared on wheat seedlings. It was found by chance that a barley seedling, which was accidentally mingled among densely planted wheat seedlings, received many biting attempts but was left uneaten even a few hours after provision to the locusts. It was also discovered that a mature barley plant was eaten, albeit reluctantly. Feeding deterrence of the seedlings against *L. migratoria* is known in several Gramineae plants such as sorghum, maize and fescue (Bernays et al., 1974; Woodhead and Bernays, 1978). Feeding deterrence of sorghum seedlings is ascribed to multiple compounds: hydrocyanic acid glucoside, several phenolic compounds and *p*-hydroxybenzaldehyde (Woodhead and Bernays, 1978; Woodhead, 1982, 1983). However, only the deterrents of a few plant species have been chemically identified, although it has been suggested that feeding deterrents exist in many plant species (Bernays et al., 1974).

In the present study, we first reconfirmed that barley seedlings deter feeding of the migratory locust, and subsequently analyzed at which behavioral step the rejection occurs. We also obtained information on the location and chemical nature of the feeding deterrent(s).

MATERIALS AND METHODS

Locust. The migratory locust, *Locusta migratoria*, used in this study originated from a colony provided by the Insectarium, Tama Metropolitan Zoo. Insects were reared on wheat seedlings at 28°C under a photoregime of 16L–8D. Locusts were kept in a 30×30×30 cm cage at high densities (about 100/cage from the first to third stadia, and 40–50/cage for the fourth and later stadia) under dry conditions, and thus were in the gregarious phase. One to 2 week-old young adults were used for the feeding assay to avoid any influence of reproductive behavior. To verify age, insects were marked on the pronotum with a white marker within 24 h after

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eclosion. Six males and six females were used per assay.

Plants. Barley and wheat seeds were provided by the experimental farm of Tokyo University. The seeds (20 g) were soaked in water overnight, sowed in a plastic container in soil fertilized with 5 g Magamp® K, and kept at 20°C. After germination, the seedlings were placed in a plant growth chamber (25°C, 16L8D) for 1 week and then grown under outdoor conditions (air temperature 20–25°C) for 1 week. Seedlings grown to about 10 cm were subjected to feeding experiments. Some barley plants were grown to maturity in an environmental chamber (20–25°C) with natural sunlight. The maize (white dent corn) used in the feeding assay was grown in the field, and the leaves of plants grown to 50–150 cm height were used for experiments.

Observation of feeding-related behavior. A locust was put in a cage (6 cm in diam. × 12 cm) made from Saran® mesh and starved for 4–5 h. Seedlings of barley and wheat, and leaves of mature barley and maize were cut to lengths of 5 cm, and offered to a locust in a cage to observe feeding-related behavior. After introduction of the plants, the cage was set with its long axis horizontally to facilitate encounter of the locust with the plant, and the following behaviors were observed for 15 min: approach to the leaf, palpation (examining the surface of the plant with maxillary and labial palps), biting and continuous feeding. The time required for the decision to feed or reject, i.e., the time between the first contact with the plant and the start of feeding or rejection, was measured using a stopwatch. In this study, non-continuous biting or nibbling was not regarded as feeding. After the experiment, the test plants were examined to see whether or not they had been eaten.

Palpectomy. Locusts were anesthetized by carbon dioxide, and the distal segment of the palp was excised with fine forceps. These treated insects were allowed to rest for at least 24 h with a supply of their usual food before they were subjected to bioassays.

Removal of leaf surface compounds. Compounds present on the surface of a leaf were removed by immersing a leaf in chloroform for 2 s. Chloroform was then immediately removed from the surface of the leaf using blotting paper. The feeding deterrence of these treated leaves was examined using locusts starved for 4–5 h or for 24 h. Two seconds was chosen as the treatment time, since this is the maximum time for washing off the surface compounds without disrupting the epithelium of the plant. After a longer treatment, exudation of sap from the plant body became noticeable.

Barley seedlings extracted with ethanol. A few barley seedlings of about 10 cm in length were immersed in about 20 times their weight of ethanol overnight, and dried at 40°C for 2 h in a forced convection dryer. After drying, the seedlings were immersed in distilled water for 5 min and placed on blotting paper. Since these treated leaves were very soft, they were bundled with a rubber band and used for feeding experiments.

Ethanol extraction of barley seedlings and fractionation of the extract. Barley seedlings (150 g) were extracted with 3 *l* of ethanol overnight at room temperature. The extract was filtered through glass wool, and concentrated to about 200 ml with a rotary evaporator under reduced pressure. To this concentrate, 150 ml each of distilled water and ethyl acetate were added, and the solution was partitioned conventionally. Partitioning was repeated after changing the pH of the aqueous phase, and three fractions (basic, acidic and neutral) were finally obtained.

Extraction of alkaloids. Alkaloids in the barley seedling were efficiently extracted by the method of Zuniga et al. (1985). In brief, leaves of barley (2–5 g fresh weight) were frozen at −20°C and homogenized in a blender with 40 ml of methanol containing 1% NH_4OH. The homogenate was filtered through glass wool, and the filtrate was dried up *in vacuo* with a rotary evaporator. After the solid residue was dissolved in 20 ml of 0.1 N HCl, the solution was filtered, the pH was adjusted to pH 9.0 with conc. NH_4OH, and the solution was then extracted with chloroform. The concentration of the extract was adjusted to 5 g fresh weight equivalent/ml.

Filter paper bioassay. To evaluate the feeding deterrence of the extracts and fractions, a filter paper bioassay was employed. To stimulate the feeding of the locusts, 300 μl of 5% sucrose in 50% ethanol was applied to a piece of filter paper (No. 51B, 2×8 cm, Toyo Roshi, Tokyo) and the filter paper was completely dried. The extracts or fractions to be tested were applied to the paper, which was then dried again and subjected to feeding by the locusts. As a control for the crude extract, 0.1 N HCl was neutralized with conc. NH_4OH, concentrated using an evaporator and then used for the assay. Chloroform was used as a control for the alkaloid fraction. The filter paper used for each assay weighed about 150 mg, and the sucrose content was 5–6% (g/g).

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 by $Y=(1-X_t/X_c)\times 100$, where X_c and X_t indicate the decrease in weights (mg) of the control and treated filter paper, respectively.

RESULTS

Feeding-related behavior of intact insects

All intact locusts exhibited a series of feeding-related behaviors such as approaching, palpation of and biting all the plants tested (Table 1). While wheat, maize and mature barley were eaten, barley seedlings strongly deterred the feeding of the locusts. After a single biting trial, the locusts usually abandoned the leaf. A similar refusal reaction was observed in some individuals against the leaves of grown barley, but more than half of the locusts accepted the mature plant.

The decision to feed or reject occurred immediately after biting; therefore, the 'time before decision' in Table 1 mostly reflects the time for palpation. The time of palpation was very short in the palatable plants. In other words, biting and feeding occurred soon after contact with the plant (Table 1). On the other hand, in barley, the time for palpation was significantly longer both for the seedling and the mature plant, and biting did not readily follow palpation (Table 1). Refusal behavior took place immediately after biting the barley seedling, but in the case of mature barley, palpation frequently recurred after biting.

Table 1. Feeding-related behavior of intact and palpectomized *Locusta migratoria* toward barley, wheat and maize

	Approach	Palpation	Biting	Feeding[a]	Time before decision[b]
Barley, seedling					
Intact	+	+	+	0/12	3.7±0.6 c
Palpectomized	+		+	0/12	4.0±0.7 c
Barley, mature					
Intact	+	+	+	8/12	2.5±0.7 b
Palpectomized	+		+	6/12	4.0±0.8 c
Wheat, seedling					
Intact	+	+	+	12/12	1.3±0.2 a
Palpectomized	+		+	11/12	5.0±1.0 c
Maize, mature					
Intact	+	+	+	11/12	1.2±0.2 a
Palpectomized	+		+	9/12	4.8±0.6 c

[a] Number of insects that started feeding in 15 min/number of insects tested.
[b] Time (s) between first contact with plant and decision to feed or reject. Mean±SE, 6 insects. Means followed by the same letter are not significantly different by the Tukey-Kramer multiple range test at the level of 5%.

Feeding-related behavior of palpectomized insects

The palpectomized insects showed a series of feeding-related behaviors similar to those of intact insects (Table 1). Although the distal parts of the palps were lost, the insects vibrated the proximal part of the palps, trying to palpate. Barley was rejected after biting, and wheat and maize were eaten just as by intact insects. The time required for acceptance of the plant was much longer than that required by the intact insects; however, the time required for refusal was similar to that of the intact insects (Table 1).

Deterrence of barley seedlings after surface washing or solvent extraction

Locusts starved for 4–5 h did not feed on the surface-washed or non-treated barley seedlings at all, although they touched these plants frequently (Table 2). Not many individuals approached the residual plant body after ethanol extraction, but a few individuals fed on the residue continuously. A majority of the individuals starved for 24 h fed on the residue. In contrast, non-treated or surface-washed barley seedlings were never eaten by the 24-h starved insects, although the insects palpated and bit these seedlings repeatedly.

Feeding deterrence of barley seedling extract

The ethanol extract (1 g eq.) of the barley seedlings exhibited strong deterrence in a feeding assay using a piece of sucrose-impregnated filter paper as the feeding substrate (Fig. 1). The basic, acidic and neutral fractions obtained after partitioning were next tested for feeding deterrence. When the fractions equivalent to 1 g of fresh leaf were applied to the filter paper, the basic fraction, which is considered to contain alkaloids, showed the strongest feeding deterrence against the migratory locust (Fig. 1). The acidic and neutral fractions also showed weaker but significant feeding deterrence.

Feeding deterrence of crude alkaloid extract

The crude alkaloid extract from barley seedlings showed dose-dependent feeding deterrence against the migratory locust (Fig. 2). When compared with the ethanol extract described above, a smaller amount of the alkaloid extract caused a similar feeding deterrence. An aliquot of the extract equivalent to 25 mg of fresh barley leaf showed 50% feeding inhibition, and a 100-mg-equivalent showed more than 90% feeding inhibition (Fig. 2).

Table 2. Feeding tests of intact, ethanol-extracted and surface-washed barley seedlings against *Locusta migratoria*

	Number of insects feeding	
	4–5 h Starved	24 h Starved
Barley seedling	0/12	0/12
After ethanol extraction (C_2H_5OH, 12 h)	3/12	8/12
After surface washing ($CHCl_3$, 2 s)	0/12	0/12

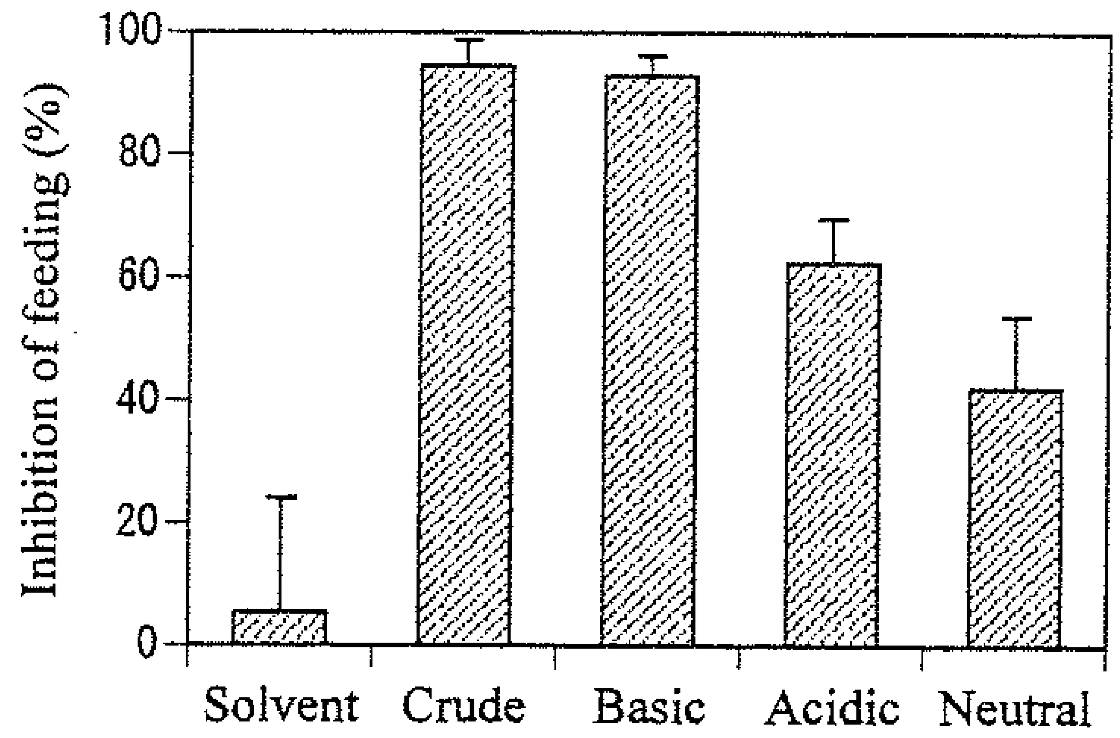

Fig. 1. Feeding deterrence of the crude ethanol extract, and the basic, acidic and neutral fractions of the extract of barley seedlings. A bioassay using a sucrose-impregnated filter paper as the feeding substrate was employed. See text for details. Means and standard errors of 6 replicate experiments are shown.

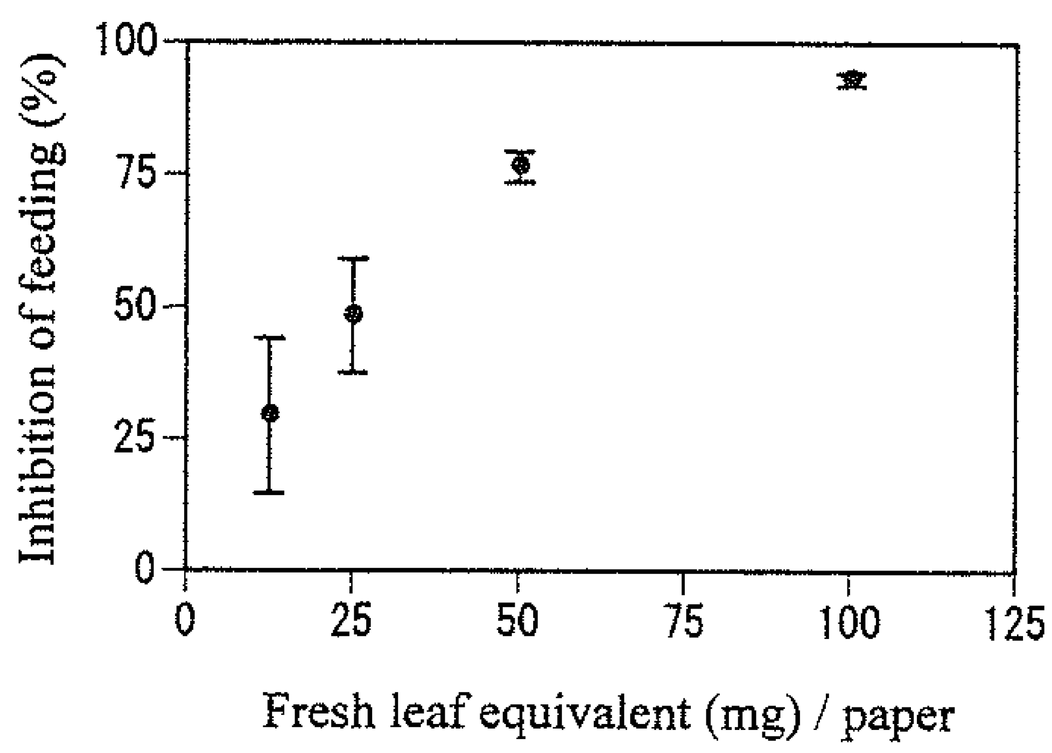

Fig. 2. Feeding deterrence of the crude alkaloid extract from barley seedlings. Bars indicate standard errors of the means.

DISCUSSION

L. migratoria is an oligophagous insect feeding on Gramineae plants. The feeding-related behavior of the migratory locust consists of the following five sequential steps: approach, contacting the plant with forelegs or antennae, palpation, biting and continuous feeding (Chapman, 1990). Olfaction and vision have been considered to be important in approach from a long distance; the odor of the host plant is attractive to the locust (Moorhouse, 1971; Lee et al., 1987). Gustatory organs are known to exist on the palps of the maxilla and labia, and on the tarsi of the forelegs, and these are able to perceive compounds on the surface of the leaf (Blaney and Chapman, 1970; Blaney, 1974, 1975; Blaney and Simmonds, 1990). Biting is considered to be suppressed if strong feeding deterrents on the surface of plants are perceived by palpation (Blaney, 1975). Feeding deterrents contained in the plant, if present, are perceived by sense organs in the mouth parts, and induce refusal behavior (Haskell and Schoonhoven, 1969; Blaney and Simmons, 1990). Information on the behavioral step at which feeding is refused, therefore, helps in estimating the location of deterrents on/in the plant.

The present study confirmed that barley seedlings strongly deter feeding of *L. migratoria*. Analysis of feeding-related behavior revealed that approach to the barley seedling and palpation occurred just as with palatable plants, but rejection of the plant occurred immediately after biting. This finding indicated that factor(s) that induced rejection could be perceived only after biting. Furthermore, 1) excision of the maxillary and labial palps had no effect on rejection of the barley seedlings, 2) removal of compounds on the surface of the leaves did not affect the deterrence of the seedlings, and 3) ethanol extraction of the leaves diminished it, indicate that feeding deterrent(s) are localized inside the seedling.

Since the palpectomized insects required a longer time for the decision to feed on wheat seedlings than the intact insects, and the intact insects required a shorter time to accept wheat than to reject barley seedlings, it is possible that feeding stimulant(s) on the wheat leaf surface helped the intact insects in making an earlier decision. The palpectomized insects were unable to perceive the feeding stimulant(s) on wheat, and were unable to recognize wheat as palatable until they bit the leaf. However, recognition of surface substances is known to be influenced by learning (Blaney and Winstanley, 1982; Blaney and Simmonds, 1985), and thus it cannot be concluded only from this observation that palps sensed the presence of feeding stimulant(s) on the wheat seedlings. The apparent effect of palpectomy might simply be due to loss of the ability to physically detect the existence of plants (Sinoir, 1969).

The ethanol-extracted residue of barley leaves was not preferred by the locust. This is probably because feeding stimulants such as saccharides, as well as deterrents, were removed by the extraction. The locusts starved for 24 h ate larger amounts of the residue. This is possibly due to the increase in demand for water, as moisture is an important factor for inducing feeding (Sinoir, 1966, 1968; Ben Halima et al., 1983).

After fractionation, the strongest feeding deterrence was exerted by the basic fraction, which is thought to contain alkaloids. The other fractions also showed weaker feeding deterrence, indicating that multiple deterrents contained in the barley act in concert. In feeding deterrence, additive effects of many weak feeding deterrents are often observed (Adams and Bernays, 1978).

Gramine is the principal alkaloid in barley, and is contained in high concentrations, particularly at the seedling stage (Euler and Hellstrom, 1933; Argandona et al., 1987). This compound is considered to confer resistance against several aphids to the barley (Zuniga et al., 1985; Zuniga and Corcuera, 1986; Kanehisa et al., 1990), and was once suggested to work as a feeding deterrent against *L. migratoria*. However, in an assay in which wheat flour wafers were used as the feeding substrate, no feeding deterrence of gramine was observed at the concentration that occurs in plants (Bernays and Chapman, 1977). The role of gramine in the feeding deterrence of barley will be discussed in detail elsewhere (Ishikawa and Kanke, 2000).

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