Feeding behavior of rice bug *Leptocorisa chinensis* (Dallas) (Heteroptera: Alydidae) nymphs on rice panicles and rice plant extract

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**Abstract**

The feeding behavior of the rice bug *Leptocorisa chinensis* (Heteroptera: Alydidae) on the panicles of *Oryza sativa* rice was observed. The nymphs showed typical behavioral phases towards the rice panicles: antennation, extension of the rostrum, repeated dabbing with the labium, rostrum placing on the plant surface, stylet penetration and continuous sucking. The nymphs also showed similar behavioral phases except for continuous sucking on filter paper treated with a methanol extract of rice panicles. The frequency of bugs showing antennation and repeated dabbing was significantly higher on filter paper with the rice extract than on the control. These results suggest that there are chemical cues in rice panicles which stimulate behavior such as antennation and repeated dabbing by rice bugs.

**Key words:** Heteroptera; dabbing behavior; chemical cues; *Oryza sativa*

**INTRODUCTION**

The number of plant species that are available for food for phytophagous hemipterans are limited (Chapman, 1982; Bernays and Chapman, 1994; Panizzi, 1997). These bugs have to first find a host plant and land on it, then examine the plant closely using sensory apparatus such as antennae to judge whether the plant is of a preferable species or quality (Miller and Strickler, 1984). The feeding behavior and mechanisms of feeding in plant-sucking hemipterans have been studied in various species (Miles, 1958; Sogawa, 1982; Montllor, 1991). Plant acceptance behaviors in phytophagous hemipterans after landing on a plant are divided into three steps (Backus, 1988). The first step is plant surface exploration. In this behavioral phase, the insect walks about, antennating and/or dabbing with the labium tip at the surface of the plant. The next step is stylet probing. In this phase, the insect inserts its stylet into the plant and probes to find a good feeding position. After stylet probing, the third stage, ingestion, begins.

Several methods of behavioral analysis have been conducted thus far to quantify feeding behavior, mainly with respect to probing or ingestion. The methods are effective for studying hemipterans that perform sap-feeding from stems and leaves. Electronic monitoring methods of feeding behavior (McLean and Kinsey, 1964) have been developed for indirect measurement of the frequency and duration of probing, and ingestion behavior in such hemipterans as aphids, whiteflies, mirid and stink bug species (Bonjour et al., 1991; Montllor, 1991; Pickett et al., 1992; Walker and Perring, 1994; Cline and Backus, 2002). Bioassays using a thin membrane to test the probing behavior in response to plant extracts kept under the membrane have been performed in aphids and planthoppers, and various chemical stimulants of probing and ingestion behavior have been investigated (Kim et al., 1985; Montllor, 1991; Pickett et al., 1992; Adjei-Afriyie et al., 2000a, b). Thus, various studies of hemipterans have been conducted on stylet probing, or ingestion behavior, but plant surface exploration behavior, despite being the first step in the sequence of plant acceptance behavior, has not been thoroughly investigated.

The rice bug *Leptocorisa chinensis* is an important agricultural pest in Japan which is known to attack rice panicles and cause abortive or stained grains (Shimizu, 1991; Hayashi, 1997). Little un-
derstanding has been gained to date on the feeding process of phytophagous stink bug species, beginning from plant surface exploration and ending in ingestion. In the present study, therefore, we describe the behavioral sequence of *L. chinensis* from plant surface exploration to ingestion, and compare the behavioral response to the intact rice plant (*Oryza sativa*) with the response to a methanol extract of rice panicles.

**MATERIALS AND METHODS**

Insects and plants. The rice bugs used, *L. chinensis*, were descendants of bugs collected from gramineous fields in Tsukuba, Japan (36°01'N, 140°06'E). To rear the insects, rice panicles cropped at the milk and dough ripening stage, which had been kept in a freezer at −30°C, were used. Nymphs were reared in plastic Petri dishes (9 cm in diameter×2 cm in height) with rice panicles and water at 25°C, 16L:8D (06:00 lights on). Approximately ten to twenty nymphs (depending on the sizes of the nymphs) were reared in each dish. The rice panicles in the containers used for rearing were replaced every week.

Plant extract. For the rice plant extracts, fresh rice panicles in the milk stage (cv. Koshihikari) were prepared on the day of use. Twenty grams of panicles were soaked in 200 ml of methanol (99.8%, Wako Pure Chemical Industries, Ltd.) at room temperature for 6 h, and the extract was kept in a −30°C freezer until use.

Preliminary observation of feeding behavior of *L. chinensis* on rice panicles. The behavioral responses of *L. chinensis* to the intact rice panicles were observed. Five individuals at each nymph stage were used for observation. Rice panicles (cv. Koshihikari) at the milk ripening stage were used for the experiments. Fresh rice panicles were cut 5 cm under the neck node, and the stems were inserted into glass vials (32 mm in diameter×75 mm in height) containing water. The insects were starved for 24 h with water under the rearing conditions prior to observation. An individual was released onto a rice panicle, and its behavior observed until it started to suck the panicle.

Apparatus for feeding response test. Figure 1 shows a diagram of the apparatus used to test the nymphs’ behavior. A plastic dish (3.5 cm in diameter×1 cm in height) was filled with quartz sand (Wako Pure Chemical Industries, Ltd.) to make an arena. Water was poured around the arena to prevent the insects from escaping. Two plastic cylinders (4 mm in diameter×2 cm in length) were set on the quartz sand vertically, and inserted to a depth of 0.5 cm into the sand. The distance between the two cylinders was 3 cm. One cylinder was used as a releasing point for the test insects, and the other was used as a stand for either a rice panicle or paper strip cut into 3 mm×10 cm (filter paper for chromatography, Toyo Roshi Kaisha Ltd.) treated with plant extract.

Observation of feeding response on rice panicle or filter paper with rice extract. A rice panicle was cut to 10 cm length and set on the plastic cylinder of the apparatus (Fig. 1). An individual insect (third instar nymph) was released at the releasing point on the arena. After the insect touched the panicle, its behavioral response on the panicle was recorded. Observation was continued for 5 min after the individual first touched the rice panicle. The behavioral sequences starting within this 5 min period were recorded until the insect ceased the behavioral sequence by withdrawing its rostrum; that is, if the behavioral sequence was still going on after 5 min, recording continued until the end of the sequential behavior. Inactive insects that did not move from the place of release within 2 min were omitted from the assay.

The behavioral responses of nymphs on the filter paper soaked in rice extract were also observed.
Filter paper strips (3 mm × 10 cm) were treated with methanol extract, which was not concentrated or diluted, and 400 μL of extract was spread all over the paper strip. The filter papers were air dried for 30 min before the test to evaporate the solvent. The behavioral phases of the insect on the filter paper were recorded using the same procedure as with the fresh panicles. The plastic cylinder holding the filter paper was changed for each experiment, as was the filter paper. Filter papers treated with methanol were used as a control. Each test was conducted at 25°C and 50–60% r.h., during 15:00–18:00.

Statistics. Each behavioral step is numbered for descriptive purposes: behavioral step 0 indicates that the insect touched the filter paper or panicle, while behavioral steps 1–6 correspond to behaviors A–F described in Results and Discussion. For each treatment t (intact rice panicle, rice plant extract and methanol), the number of individuals N(t, i) exhibiting behavioral step i were counted. For each behavioral step i (i = 1 to 6), a ratio R(t, i) = N(t, i)/N(t, i−1) was calculated. To analyze the effect of treatments, a G-test (with Bonferroni-corrected p value) was conducted. Fisher's exact test with Bonferroni correction was conducted post-hoc to analyze each behavioral step i of the insects on the rice plant extract.

RESULTS AND DISCUSSION

Sequence of feeding behavior

*L. chinensis* nymphs released onto a rice plant showed a series of typical behavioral phases described below.

(A) Antennation: The insect curved its antennae near the plant surface when it climbed up the stem. Upon reaching the panicle, the antennae were bent at right angles. The tips of the antennae lightly touched the plant surface.

(B) Extension of rostrum: The insect lightly touched the plant surface with the labium.

(C) Repeated dabbing: The insect repeatedly dabbed the plant surface with the rostrum tip. As the insect walked slowly forwards, it changed the angle and site at which it dabbed the plant surface with the rostrum tip. The antennae were bent during this behavior.

(D) Rostrum placing: The insect stopped dabbing, and the rostrum was kept motionless on the plant surface. The antennae were straightened.

(E) Stylet penetration: The stylet penetrated the panicle.

(F) Continuous sucking: After a large part of the stylet was inserted into the plant during behavior E, the insect continued sucking by slightly and repeatedly moving the stylet in and out.

The time from first antennation until the start of each behavioral phase of the insect on the rice panicle is shown in Table 1. Behaviors A–C often started almost simultaneously. Sometimes the insect showed only behavior A or behaviors A and B while changing its exploring position. Behavior C continued several minutes before proceeding to D. Behaviors D and E lasted several minutes or more. The duration of behavior F, continuous sucking, was not measured because this phase lasted several hours before withdrawal of the stylet. When the insect interrupted the sequence at one behavioral phase and did not proceed to the following phase, the insect moved to another position on the panicle and restarted from phase A.

### Table 1. Starting time of behavior exhibited by *L. chinensis* nymphs on rice panicles

<table>
<thead>
<tr>
<th>Instar</th>
<th>Time from antennation&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Extension of rostrum</td>
</tr>
<tr>
<td>1st</td>
<td>2.0±4.5</td>
</tr>
<tr>
<td>2nd</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>3rd</td>
<td>5.6±12.5</td>
</tr>
<tr>
<td>4th</td>
<td>0.2±0.4</td>
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<tr>
<td>5th</td>
<td>0.2±0.4</td>
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<sup>a</sup>Five samples for each nymphal stage.

<sup>b</sup>Mean (s)±SD.
The behavioral phases of (A) antennation, (B) extension of the rostrum and (C) repeated dabbing correspond to “plant surface exploration”, and (D) rostrum placing and (E) stylet penetrating correspond to the “stylet probing stage” described by Backus (1988).

The behavioral process and pattern was the same among the different stadia of insects. Third instar nymphs were used for the experiments described below because they are large enough for details of their feeding behavior to be observed, and are easier to handle than later instar insects, which move faster.

**Feeding behavior on rice panicles and methanol extract of rice**

First, we confirmed the response of nymphs to rice panicles using the apparatus in Fig. 1. A rice panicle was inserted into the plastic cylinder instead of the filter paper used in Fig. 1. The insect released at the releasing point walked and climbed up the rice panicle spontaneously and showed behavioral phases. On the rice panicles, all the nymphs showed behavioral phases from A to E, and 87% of the nymphs showed behavioral phase F (Fig. 2, open circles, n=15).

Next, the behavioral sequence of *L. chinensis* on filter paper treated with methanol extract from rice panicles or methanol (control) was observed (Fig. 2, n=30). The insects that climbed up the filter paper showed a pattern of behavior similar to those on rice panicles; 73% of individuals exhibited behaviors A-C in response to filter paper treated with the extract of rice panicles.

We preliminarily observed that nymphs seldom showed feeding behavior on leaves and stems. In addition, methanol extract of leaves did not stimulate feeding behavior in the preliminary experiment. Therefore, we considered that some stimulants for feeding behavior are contained not in leaves but in panicles.

There was a difference between the frequency of behavior shown by nymphs on the rice extract and those on intact rice. Figure 3 shows the fraction of individuals exhibiting each behavioral step after the previously exhibited step, which was counted from data in Fig. 2. The percentage of nymphs on the rice extract who exhibited behavior D after exhibiting behavior C was significantly lower than that on intact rice (Fig. 3; p<0.01, Fisher’s exact test with Bonferroni correction), which resulted in a much lower frequency of D–F on the rice extract than on intact rice. One possible explanation for this difference is that a chemical factor stimulates behavior D, rostrum placing, but this chemical factor could barely be extracted under these extraction conditions. Another possibility is that non-chemical factors, such as physical ones on the plant surface structure, can affect behavior. It is also possible that filter paper is inadequate as a substrate for eliciting complete feeding behavior in response to chemical cues in the extract.

When filter paper treated with rice extract and control filter paper were compared, the percentage of insects exhibiting behaviors A and C on the rice extract was significantly higher than that on the control (Fig. 3; p<0.01, Fisher’s exact test with Bonferroni correction). The result suggests that some chemicals in the rice panicles stimulated behaviors A and C. The difference between the frequency of behavior in response to the rice extract and control was largest in behavior C (Fig. 2).

We demonstrated in the present study that this
Feeding Behavior of Rice Bug

Fig. 3. Percentage of individuals exhibiting behavioral steps that resulted in the next behavioral step. The fraction of individuals exhibiting each behavioral step after the previously exhibited step was counted. Open bar: intact rice panicles (n=15), hatched bar: methanol extract of rice (n=30), closed bar: methanol as control (n=30). Percentages with different letters at each behavioral step are significantly different (p<0.05, Fisher's exact test with Bonferroni correction).

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