Electronic Monitoring of Feeding and Oviposition Behavior of Rice Planthoppers and Its Application in Plant Resistance Study

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电子记录稻飞虱取食和产卵行为及其在植物抗虫性研究中的应用

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摘　要:采用AC型电子记录系统(EMS)对褐飞虱在非寄主植物稗草和具有抗性基因 *bph4* 的水稻品种 Babawee 上的取食行为进行了比较研究。结果表明，褐飞虱口针到达稗草韧皮部以前停止取食，这揭示了在稗草非韧皮部组织中存在拒食剂。而在抗虫品种上，口针到达韧皮部以后取食行为终止。因此，可以清楚地识别两类具有不同抑制取食抗性因子的抗性植物。分析白背飞虱产卵行为的电子记录表明，在大多数情况下，就旺盛生长的水稻植株的下半部分而言，白背飞虱产卵器插入植物组织中但不产卵。这表明旺盛生长的植株能诱导白背飞虱的产卵行为，但由于某些拒避性因素使整个产卵过程不能完成。因此，这一系统可以有效地识别具有产卵抗性的植物上行为序列中受到破坏的步骤。

关键词:褐飞虱；白背飞虱；电子记录系统；取食行为；产卵行为；抗虫性；稗草；水稻

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Abstract: Feeding behaviors of *Nilaparvata lugens* on a non-host plant, the barnyard grass *Echinochloa crus-galli* var. *oryzicola*, and resistant rice Babawee (*bph4*) were compared by using AC-eletronic monitoring system (EMS). Waveform data obtained by EMS showed that *N. lugens* usually stopped feeding activities before stylets reach the phloem in barnyard grass which revealed to contain an antifeedant in the non-phloem, whereas feeding was interrupted after stylets reached the phloem in resistant rice. Thus, EMS analysis clearly distinguished two types of resistant plants with different resistant factors against planthopper feeding. EMS analysis of *Sogatella furcifera* oviposition behavior on the lower part of the leaf sheath of fully-grown rice demonstrated that in most cases the ovipositor was inserted into the plant but pulled out without depositing any eggs. It suggested that oviposition behavior itself was normally induced on the fully-grown rice, but the entire process was not completed due to some deterring factors. Thus, this system is also effective in identifying a disrupted step in the behavioral sequence on a plant resistant to oviposition.

Key words: *Nilaparvata lugens*; *Sogatella furcifera*; electronic monitoring system; feeding behavior; oviposition behavior; insect resistantce; *Echinochloa crus-galli*; rice

1 Introduction

AC electronic monitoring system (AC-EMS) was originally developed to study feeding behavior of aphids[14]. Subsequently, DC system was also devised[18] and improved[22]. The EMS has become the most accurate and direct method by which the probing activities of piercing-sucking insects within the plant tissue can be monitored[1]. It has been used as a tool in the study of plant resistance to aphids and other homopteran insects, to identify the mechanisms of resistance, antixenosis, antibiosis and tolerance[9,17,24]. Currently, this technique was also applied for analyzing oviposition behavior of the brown planthopper (BPH), *Nilaparvata lugens* (Stål), which lacerates plant tissue with its saw-like ovipositor and deposits eggs[5].

BPH and the whitebacked planthopper (WBPH), *Sogatella furcifera* (Horváth)are recognized as serious pests in Asia, including Japan and China. So far, rice varieties with each of three resistant genes to BPH have been released, but have often broken down under severe population pressure. A breeding approach by combining or pyramiding major resistant genes is important for producing high levels of resistant varieties[3]. For this purpose, efficient methods for evaluating various kinds of resistance will be required.

In this study, it was confirmed that the EMS is a useful tool in elucidating mechanisms underlying plant resistance to feeding of piercing-sucking insects and oviposition of lacerating-depositing insects. In this paper, the correlations between various waveforms and behavioral events of BPH feeding and oviposition were first described and then waveform outputs were compared between BPH and WBPH. On the basis of the waveform interpretation, feeding behavior of BPH on a non-host plant, whose resistant factor was determined chemically, was recorded by using the EMS, and its

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waveform profiles were compared with those of a resistant variety of rice. Furthermore, behavior of WBPH on a plant unsuitable for oviposition was also measured to identify the step in which oviposition sequence was interrupted.

2 AC electronic monitoring system (EMS)

The AC-EMS records changes in electrical resistance of the insect-plant interface, while the DC-system is able to record resistant changes and electromotive force of the insect and plant[1]. However, for collection of data on salivation and phloem ingestion, AC and DC systems are equally useful[16]. In this study, AC voltage (500 Hz, 0.5 V) was introduced into the planthopper-plant interface. When a wired insect penetrates its stylets or ovipositor into the plant, the circuit is completed and then waveforms characterized by amplitude, frequency and voltage level are recorded, depending on specific activities of the insect during feeding and oviposition. Stylets and ovipositor activities including penetrating and ejecting saliva or eggs evoke electrical events, resulting in characteristic waveform types.

2.1 Characteristic waveforms correlated with particular behavioral events of feeding

Rice planthoppers, such as BPH and WBPH are vascular feeders and target almost exclusively phloem, and rarely xylem as sucking sites (phloem feeders)[10,19]. The feeding process of BPH consists of two major behavioral steps: exploratory probing with the secretion of coagulable sheath material (salivation), and sucking from phloem sieve element (phloem ingestion)[20]. Out of several waveforms observed during feeding activities of BPH, three waveforms, S, A, and I are distinctive and particularly important (Fig. 1). The S waveform with high amplitude that appeared immediately after stylet penetration may represent salivation of the stylet sheath accompanied with the movements of the stylets and samplings of plant fluid. The I waveform associated with the phloem ingestion can be confirmed from analysis of honeydew droplets and cut stylets. Sequential spots of honeydew droplets (ca. 25/h) collected on a filter paper during sustained I waveform (over 30 min) always showed high ninhydrin and positive for sugar. Furthermore, cut stylets nearly always exuded droplets of phloem sap if they were cut during sustained I waveform. The I waveform always immediately followed a series of the A waveform. It is reported that during the A waveform the terminal locations of feeding track ended in phloem[11]. Waveforms observed in BPH during feeding activities strongly resembled those in WBPH, although the A waveform continued for a shorter period and its voltage level was relatively unstable (Fig. 2). Therefore, the interpretation of waveform in BPH will be applicable for the WBPH's.

2.2 Difference between feeding behavior of BPH on a non-host and a resistant host plant

The feeding activity of BPH was recorded on the barnyard grass, *Echinochloa crus-galli* var. *oryzicola*, and on a resistant variety of rice, Babawee (*bph4*), by using the EMS[6]. The barnyard grass is regarded as a model plant for analyzing feeding interruption process, because this species has been proven to contain (E)-aconitic acid, an antifeedant for BPH[12] in non-phloem tissue of leaf sheath[8]. Comparison of the feeding pattern of BPH on two resistant plants allowed clear differentiation of probing profiles. On the barnyard grass, the planthoppers performed many probes, but the mean duration of a probe per insect was shorter than that on the resistant and susceptible rice (Fig. 3). Furthermore, the percentage of probes that consisted only of S waveform (salivation) were higher on the barnyard grass than that on the resistant rice, accounting for more than 50% of total probes (Fig. 4). On the other hand, on the resistant rice, the I waveform was observed as frequently as it was on the susceptible rice. However, its duration on the resistant rice was much shorter than that on the susceptible one (Fig. 5).

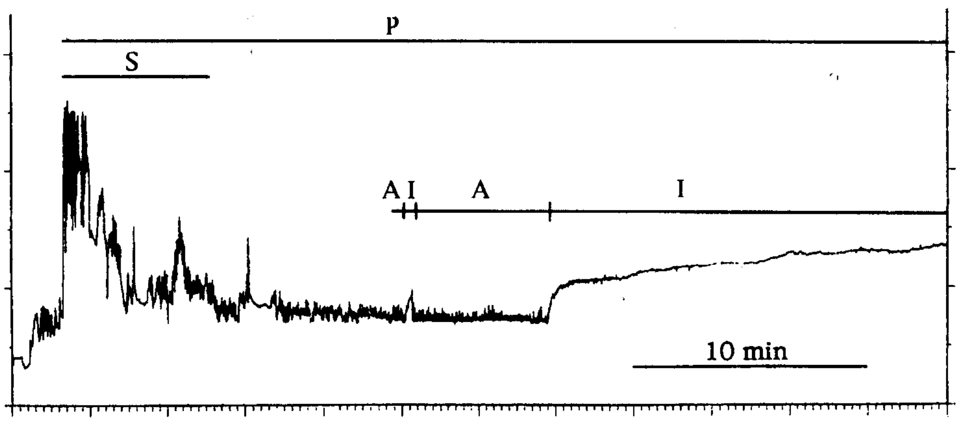

Fig. 1. Waveforms recorded during feeding of BPH on susceptible rice.
Chart to be read from left to right.

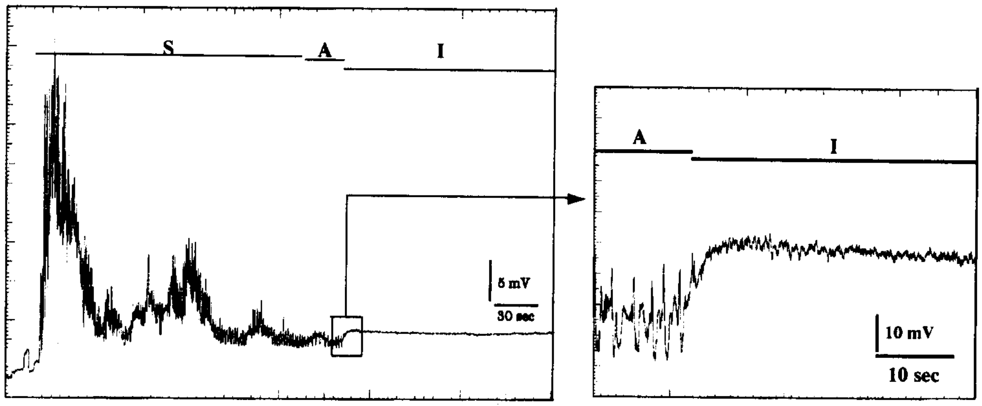

Fig. 2. Waveforms recorded during feeding of WBPH on rice.

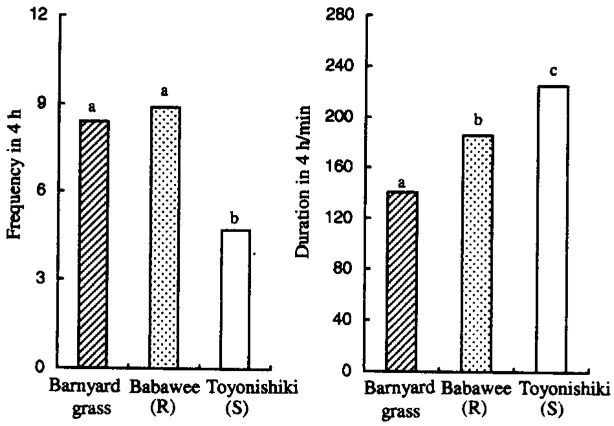

Fig. 3. Frequency and duration of probes by BPH on three different plants.

Bars with the same letter are not significantly different ($P < 0.05$).

Feeding patterns were summarized with respect to BPH feeding on three plants with different characteristics in Fig. 6. On the barnyard plant, the insect frequently made short probes with only salivation. As a result, duration of non-probing increase and successful accessing into the phloem became rare; namely, interruption of probing mainly occurs during salivation in non-phloem tissue in which the antifeedant exists. As gustatory receptors are located in the small passage way leading from the food duct to cibarium[4], the insect seemed to take up some fluid during salivation. In aphids probing on non-host plants, an absence of phloem ingestion, but occurrence of non-phloem ingestion has been frequently observed[2,15]. In these cases, however, the presence or location of chemicals that could deter accessing to the phloem or ingestion was not determined.

On the resistant rice, the planthopper made the phloem access without difficulty as on the susceptible rice, unlike on the barnyard grass. However, ingestion was interrupted in

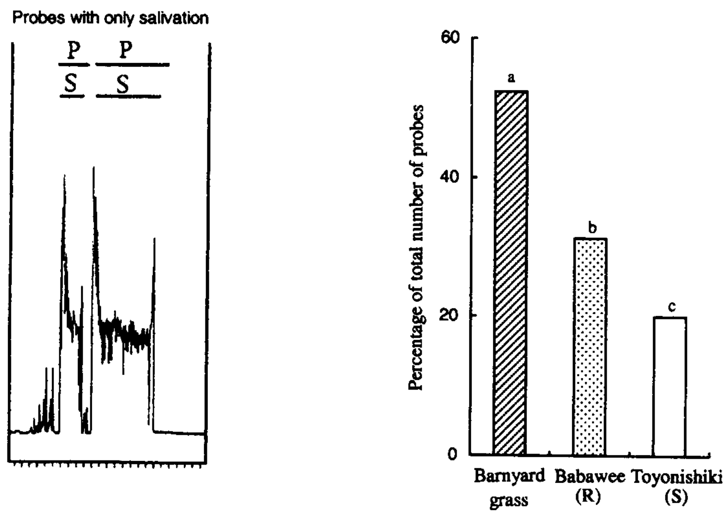

Fig. 4. Duration of S waveform and percentage of probes consisting of only S waveform during feeding by BPH on three different plants.

Bars with the same letter are not significantly different ($P < 0.05$).

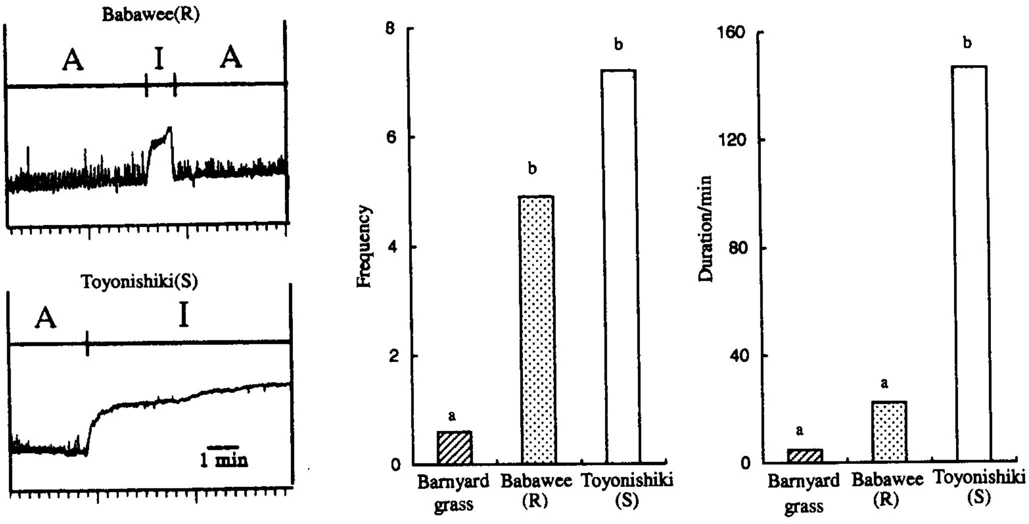

Fig. 5. Frequency and duration of I waveform during feeding by BPH on resistant and susceptible rice.
Bars with the same letter are not significantly different ($P<0.05$).

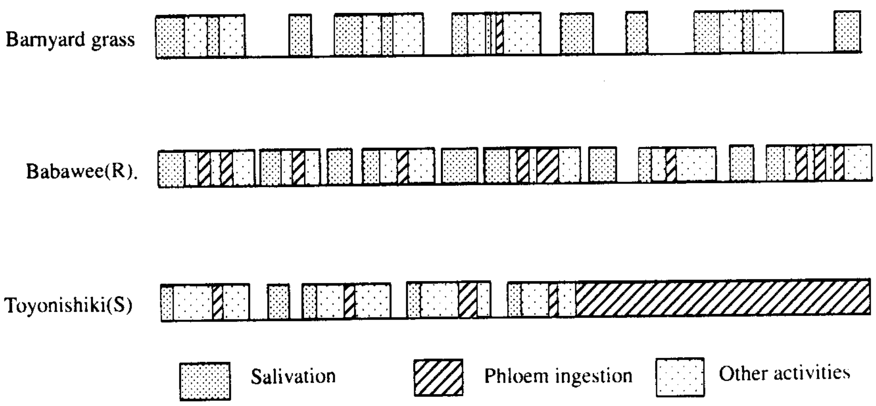

Fig. 6. Schematic illustration of feeding pattern of BPH on three different plants.

the phloem in a short time. Such a feeding interruption in BPH was also observed on other resistant rices by using AC or DC systems[11,13,23].

Thus, EMS analysis clearly distinguished two types of resistant plants with different resistance factors against planthopper feeding.

2.3 Characteristic waveforms correlated with particular behavioral events of oviposition

The EMS, combined with simultaneous TV camera monitoring was used to explore the correlation between characteristic waveforms and particular behavioral events of BPH on rice[5]. Gravid females were released on a half longitudinally sectioned leaf sheath to observe eggs inserted into the plant tissue from the back during the movement of the ovipositor. Observation and recording of WBPH behavior revealed that they exhibited oviposition in a manner basically similar to that of BPH[5] with regard to sequence, and homologous waveforms produced during oviposition (Fig. 7).

St waveform indicating initiation of stylet penetration was always recorded a few minutes prior to ovipositor penetration. The voltage level increased rapidly with the ovipositor penetration and reached up to 8 times as high as that during stylet penetration only. During this high voltage level three waveforms Si, E, and pW, correlating to specific behavioral events were recorded. The Si waveform synchronized with the forward-backward motion of the ovipositor, cutting into the plant tissue. The E waveform was characterized by the shape of the letter S with a rapid succession of very low amplitude pulses, which corresponded to the short reciprocating motion of the ovipositor to release an egg. The pW waveform with a small peak on which low amplitude pulses were superimposed, occurred during part withdrawal of the ovipositor. Repetitions of these behavioral events resulted in a mass of several eggs in a row. After the release of the final egg of an egg mass, the ovipositor was completely withdrawn (cW waveform) from the plant and the voltage dropped back to the initial level. From a sequence of waveform patterns, it was possible to determine the number of eggs and egg masses

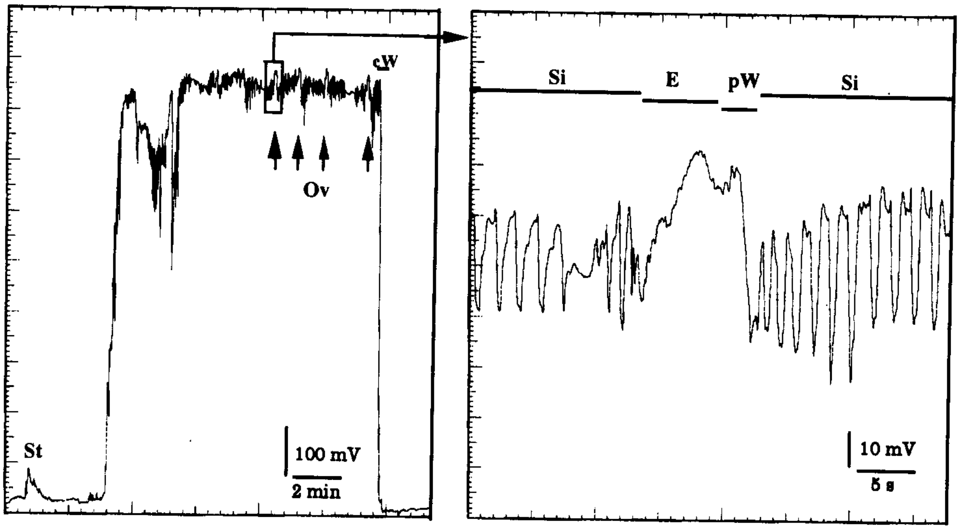

Fig. 7. Waveforms recorded during oviposition by WBPH on rice.

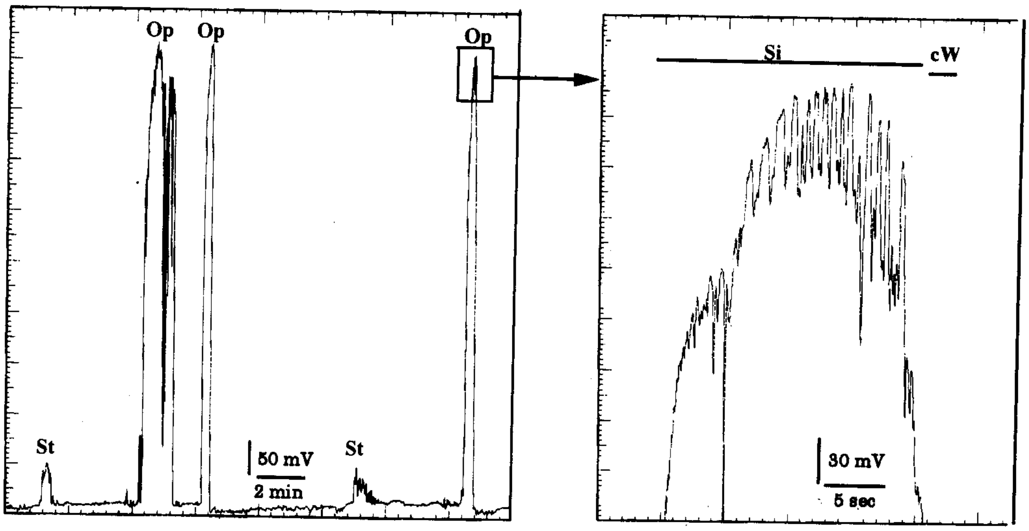

Fig. 8. Waveforms recorded during oviposition by WBPH on the leaf sheath of fully-grown rice.

laid inside the plant tissue.

2.4 Oviposition behavior of WBPH on a grown-up rice plant

It was reported that WBPH did not prefer laying eggs on the lower part of leaf sheath in the fully-grown rice[7,21]. Therefore, ovipositional sequences of gravid females on rice 60-70 days after seeding were observed by using EMS. Fig. 8 shows that a female tried to lay eggs three times in two different sites. Although, Si and cW waveforms representing penetration and complete withdrawal of the ovipositor were recorded, no E waveform corresponding to the release of the egg was observed. This suggested that oviposition behavior itself was induced, but the full process was not completed due to some deterring factors. Thus, the EMS was effective in identifying a disrupted step in the behavioral sequence on less suitable plants or sites for oviposition. The system will be useful in analyzing disruption of the behavioral sequence for oviposition on resistant plants.

3 Conclusion

The EMS is an effective tool in elucidating mechanisms underlying resistance to feeding by piercing-sucking insects and oviposition by lacerating-depositing insects in plants. This technique will constitute a further step, in combination with other methods in measuring the relative degree of resistance in plants, screening for multiple resistant varieties and checking virulence level of biotype.

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