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Electrical Feeding Patterns and Stylet Movement of Rice Brown Planthopper, *Nilaparvata lugens* (Homoptera), in the Rice Tissues

벼멸구의 섭식 패턴과 벼 조직내에서 구침의 이동

Young Nam Youn and Young Duck Chang[1]

尹 泳 南 · 張 英 德[1]

ABSTRACT Feeding behavior of female brown planthoppers, *Nilaparvata lugens* Stål, was examined with an electrical recording technique using DC amplifier and through anatomical observation of stylet movement in the rice plant with electrical recordings. There was six feeding patterns. type P, S, SB, O, X and Ph with the brown planthopper. Type P was a probing pattern during searching the proper feeding site. Type S appeared to be associated with the initial penetration and changing direction through the tissues, and from this type type SB pattern could be distinguished by the regularity of the large potential drops seen, and might be associated with penetration of the phloem sheath and/or salivation in the phloem sheath. The type O pattern shows none of the large voltage drops which were believed to occur when cell walls were being broken down and passed through a relatively thin layer of cells into an air space. The very constant waveform of the type X pattern could be seen during ingestion within the xylem bundle sheath area. The Ph pattern always followed an SB pattern and was associated with a marked negative voltage drop. When this pattern was seen, the brown planthopper might be ingested plant sap from phloem sheath area.

KEY WORDS Electrical feeding pattern, *Nilaparvata lugens*. brown planthopper, stylet movement, rice plant.

초 록 벼멸구의 식이행동을 전기적인 측정 방법을 통하여 관찰하였으며 이들을 각각의 특성에 따라 분류하였고 그 결과 type P, S, SB, O, X. Ph등 6가지로 나누어 볼 수 있었다. Type P는 벼멸구가 식물체에 처음 접근하여 기주를 탐색할 때 볼 수 있었으며, 구침을 조직내에 찔어 넣거나 조직내에서 이동할 때에는 type S를 관찰할 수 있었다. 또한 매우 규칙적인 S패턴은 체관부에 구침을 찔어 넣을때만 볼 수 있어 type SB로 따로이 분류하였다. X와 Ph패턴은 각각 물관부와 체관부에서 흡즙할 때 나타나는 것을 확인하였으며, type O는 기타 다른 조직내에 구침이 있을때 이러한 전기적 패턴을 보여 주었다. 이러한 각각의 전기적 패턴을 확인하기 위하여 원하는 패턴이 나타날 때에 식흔이 있는 벼의 조직을 잘라서 현미경으로 관찰하였으며, 각각의 타입별로 벼멸구 배설물을 측정하였다.

검 색 어 전기적 섭식패턴, 벼멸구, 구침의 이동, 벼

Heavy infestations of planthopper may cause a great loss of the crop due to rapid desiccation of the plants as the insects feed. Typical of the homopteran piercing-sucking mode of feeding, hoppers penetrate the food substrate to a depth of up to 0.7 mm with their modified, interlock-

1 Department of Agricultural Biology, College of Agriculture, Chungnam National University, Taejon, 305-764. Korea.

ing, mandibles and maxillae which constitute the stylet. During stylet penetration hoppers secrete both watery and gelling salive (Miles 1972). Feeding causes a range of injurious effects to the rice plant, giving rise to the symptoms known as 'hopper-burn'. In individual plants, hopperburn is caused by drying out of the foliage as a result of blockage of the vascular tissues and a loss of photosynthetic products.

Detailed studies of the feeding behaviour of homopterans and hemipterans are difficult since it is impossible to determine by simple visual observation whether the stylets have pierced the plant or where they are located within the plant. Simple observation gives no information as to whether the stylets of the piercing and sucking insect have pierced the plant, where they are located within the plant and whether the insect is actively ingesting material from the plant. In 1964, McLean and Kinsey developed an electrical monitoring system to study the feeding mechanism of aphids and variations of this monitoring system have now been used to study a wide range of piercing and sucking hemipterans, including some blood suckers.

The penetration activities of the insect evoke electrical events inside the stylet canals or at the extremities of the stylet, the stylet tips at one end and the cibarial and salivary pump cavities on the other. During penetration and feeding then a series of electrical events are plotted against time, this record of changing electrical activity has been called the electrical penetration graph (EPG) by Tjallingii (1986). Periods of stylet contact with the plant can readily be identified and throughout stylet contact smaller resistance fluctuations occur. The problem is to correlate these changes with the location and activity of the stylets within the plant tissue.

Since the introduction of this method by McLean and Kinsey (1964), the amplifier input specifications have been modified by several workers (Schaefers 1966, Brown & Holbrook 1976, Tjallingii 1978). Also, two methods of recording have been applied, one using alternating currents (AC method) (McLean & Kinsey 1964, Brown & Holbrook 1976), and the other using direct currents (DC method) (Schaefers 1966, Tjallingii 1978, Losel 1987, Youn 1992).

In aphids a good understanding of the relationship between EPG patterns and feeding behaviour has been achieved due to the possibility of sectioning the stylets when the insect is feeding and leaving the stylets in situ. *N. lugens* has proved to the more difficult to study in this way since during recording it has proved impossible to cut the stylets while the insect is feeding, thus leaving a marker in the plant. The stylets are very rapidly withdrawn by the insect when disturbed leaving only the salivary sheath within the plant. Sectioning the plant tissue around the point of stylet penetration has allowed workers to trace the probing activities of the stylets but there is no way of being certain which of the sheath pathways was occupied by the stylets on the point of withdrawal or whether the stylets extended beyond the tip of the salivary sheath when in the plant. Recently, Spiller (1990) has cut the stylets in situ using a laser beam while the insect is feeding and has examined the fine structure of the stylet in position in the plant tissue.

Thus the majority of the work on *N. lugens* using the electrical recording technique has been directed towards understanding mechanisms of resistance or to determining the effect of novel insecticides. There has been no work on the feeding behaviour of the insect over a

period of days, most recordings taking place over a period of 3-4 hours although Kimmins (1989) made 8 hour recordings. In this study, the feeding behaviour of planthoppers over periods up to 14 days was examined using the DC recording technique developed by Youn (1992) with no applied current and a high input impedance amplifier. Although Khan and Saxena (1988) have made some attempt to correlate their waveforms with the position of the insects stylet sheaths, their recording method yields rather different patterns to those of Kimmins and Losel. For this reason a detailed study of the stylet sheath tracts within the rice plant was made in relation to the feeding patterns recorded.

MATERIALS AND METHODS

1. Electrical Recording of Feeding Behaviour

In electrical monitoring of feeding behaviour the insect was attached to a fine gold wire, which formed the recording electrode, and placed on a small whole rice plant with the indifferent electrods in the water surrounding the root of the plant. The electrodes were linked to the differential inputs of a Grass P-16 preamplifer and the amplified signal was displayed on a pen recorder.

Fig. 1 illustrates the arrangement of the equipment employed in electrical monitoring of feeding. With no external power source in the recordings circuit, the recording electrode was connected to the G1 terminal and the indifferent electrode to the G2 terminal of a Grass P-16 preamplifier (input impedence, 2×10^{11} ohms), used in the DC mode.

2. Semithin Sections of Rice Plants Containing Salivary Sheaths

Since it proved impossible to cut the stylet mouth parts while they were still embedded in the rice tissues the insect, with its stylet, was removed from the plant during a given feeding pattern. The feeding mark on the plant was marked with Chinese ink using the T.V. camera with the tele macrolens (Tamron, 90 mm F/2.5) to ensure that the correct feeding mark was located and that piece of the rice plant isolated. In this way salivary sheaths and rice tissues associated with each feeding pattern were examined in turn. A 3.5 mm length of the leaf-sheaths and leaf-blades containing the feeding mark was dissected from the remainder of the rice plant and immersed in 2.5% glutaraldehyde in 0.2 M sodium cacodylate buffer. The plant tissuse were post-fixed for 2 hrs in 1% O_5O_4 in the same buffer, dehydrated in ethanol series and embedded in TAAB resin. Impregnated tissue was placed into individual plastic vials containing fresh TAAB resin and polymerized at 60 ± 3℃ for 48 hours. The rice plant tissues were sectioned to 0.5~1㎛ thickness using a Reichert OM U2 ultramicrotome and mounted on glass slides with water drops. Several sections can be placed on this droplet. Up to a 500 sections could be made in the examination of each feeding site. One hundred and ten feeding sites were sectioned for *N. lugens*. Slides were heated on the hot plate for 5~8 minutes at 85 ℃, stained with 1% toluidine blue in 1% borax, mounted in TAAB resin and viewed under a Zeiss Photomicroscope II at magnifications between 100 and 400 times.

3. Honeydew Excretion

Honeydew excretion was monitored by observing the insect via the video camera and putting a signal on the second channel of the Washington recorder every time a droplet was

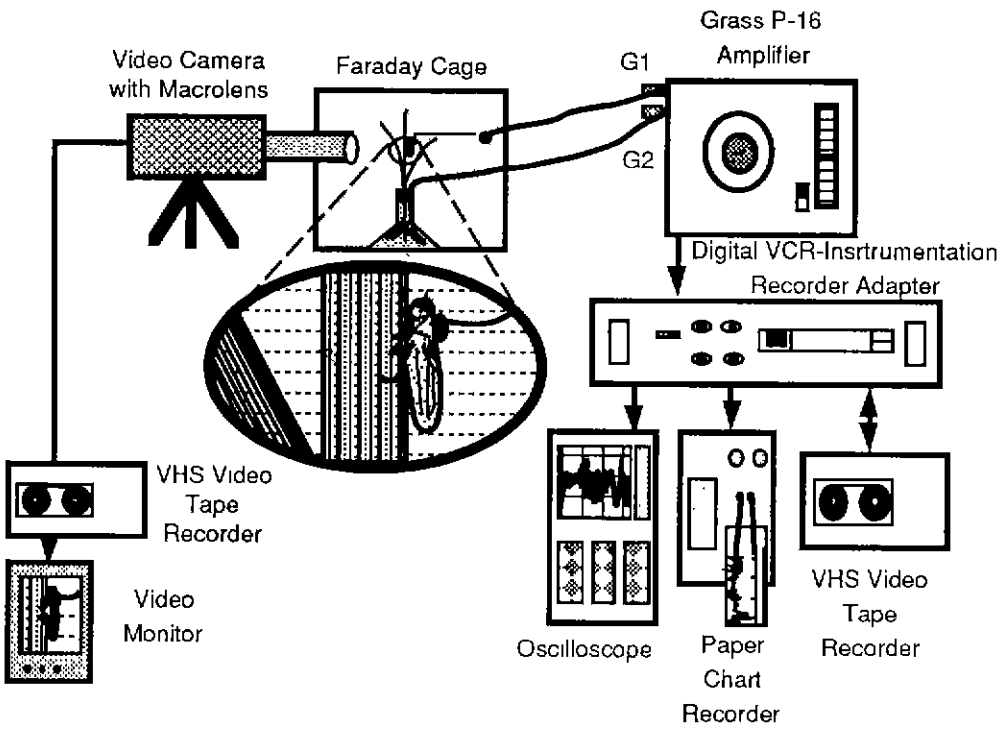

Fig. 1. Diagram of the arrangement of the plant and the brown planthopper for the electrical recording of feeding behaviour (Insect enlarged).

produced.

RESULTS

The feeding behaviour of more than 50 adult, brachypterous or macropterous, female brown planthoppers was examined with an electrical recording technique. After the settling period, the insect began to explore the plant surface, dabbing with its labium which bear mechano- and chemoreceptors (Foster *et al.* 1983). However, no signal was recorded during these movements unless the labium was pressed firmly against the rice plant and the stylets were extended and penetrated the plant epidermis. The signal level drops by some 60~80 mV from the calibrated zero volts non-feeding level. Three to four seconds elapsed between observation of the hopper applying the labium closely to the plant and the sudden, large potential drop suggesting that the hoppers take something of the order of 3~4 seconds to penetrate the epidermis with the stylets. It was observed that re-penetration at the same site did not result in this delay.

1. Patterns observed when the insect is feeding on the leaf-sheath

The signals recorded when the insect is feeding on the leaf-sheath and the leaf-blade can be categorized into six main types (Fig. 2). No signal is recorded unless the stylets penetrate the plant tissue and on the EPG recordings these periods of nonfeeding appear as a straight line separated in level from the remain-

der of the recording.

After exploration of the plant surface with its labium, the insect attempts to penetrate the epidermis of the plant. Penetration is always accompanied by a large voltage drop. During the initial period of beginning to feed on a new plant or a new position on the same plant, the insect can be often seen on the video monitor to move the labium, presumably to find a better probing site. When this happens, the signal rapidly returns to the base line. Another sudden drop in voltage will signal penetration at the new site, and this may happen several times. These brief potential drops appear to signal penetration of the plant and withdrawal to probe at another site. This signal, accompanying initial probing, is termed type P. It was noticable that if the insect was moved to a fresh part of the plant then this type of brief potential drop could be recorded for a long period (30~60mins).

After the initial voltage drop the next signal recorded is always complex, of widely varying frequency and amplitude. It precedes all of the signal patterns seen, and may be interspersed also between other types of pattern. Fig. 2 shows examples of this pattern, which has been called type S, showing its variability. It does not consist of a simple, sinusoidal type of waveform but of repeating sequences of a mixture of signals of different amplitudes and waveforms. Pattern S is so varied that it cannot readily be further subdivided. However there is one distinctive pattern that nearly always precedes one of the remaining patterns, type Ph, that can be distinguished. This pattern, called here pattern SB, is much more regular in frequency and amplitude than other sections of pattern S. It consists of high frequency components, separated by rapid drops in potential, almost equaling to those which are seen at the beginning of the type Ph pattern which it precedes.

The pattern following SB is always preceded by a rapid, negative drop in potential which can range from 50 mV to 200 mV. The signal could remain at this voltage level from a few minutes to over 24 hours. This pattern shows a very regular frequency within the range of 0.15~0.35 Hz and small voltage fluctuations about the mean level of the pattern voltage level of around 3 mV. This pattern is here called pattern type Ph (Fig. 2.A). However, type Ph pattern is hardly seen at a small vein in leaf-blade.

A fourth pattern follows directly from type S pattern, without the intervention of a period of type SB. and with no marked negative potential drop as with type Ph. For this pattern the trace level either remained around zero volts or become slightly more positive. This pattern was also distinguished by being very regular with a signal frequency in the range 3.5~8 Hz and signal amplitudes between 10 and 40 mV. This pattern is nearly sinusoidal in appearance (Fig. 2). It has been designated pattern type X.

A fifth pattern type, very variable in waveform and frequency but on the whole of a small amplitude (10~30 mV) and lower frequencies (2~4 Hz), was designated as type O (see Fig. 2). This pattern was difficult to be distinguished from S in many cases, but it was always during a period of the type O signal that the sixth pattern type was seen.

2. The stylet pathway in the rice plant leaf-sheath

Since it was not possible to cut the insect's stylets while they were in the plant, the insect was removed from the plant during a particular pattern and that portion of the rice plant was sectioned to examine the tract of the salivary sheath left behind. Some sections were made for

the electron microscope. In all 52 salivary sheaths were examined in this way, 30~50 sections being taken to encompass the tracks of each sheath.

Characteristic regular circular feeding marks, formed from the sheath-forming material, were formed by the brown planthoppers around the point of insertion of the stylets in the rice plant surface. The size of the feeding marks was 15~17 μm in diameter and 8~9 μm in height. The point of entry of the stylet into the sheath or leaf was identified by these marks. Sections of the plant containing the sheath material gives information on the course taken by the stylets.

As seen the Fig. 3, the sheaths may be single or branched, straight or curved, and the outer surface is irregularly beaded suggesting intermittent secretion of the sheath material. Overall examination of the sheath pathway in rice tissue showed that the initial probe was made perpendicularly to the epidermis in most cases, but subsequent probes through the same entry always curved away from the initial track. The stylets usually were inserted slantingly into the vascular tissue, approaching the xylem and phloem bundles from the side. If the penetration has taken place at some distance from the vascular tissuse, rather than at the side of a vein, then the stylet sheath makes a sharp change in direction and extends horizontally in the plant, passing through parenchyma cells on its way to the vascular bundle. The majority of the feeding tracks examined had branches created by the planthopper probing in different directions in the plant from a single entry point. Of the sheaths examined, there was a mean of 3.5 branches on the leaf-sheath and 3 on the leaf blade. The mean width of the tracks was 10μm. The mean length of the largest track in each feeding mark was 94 μm in the leafsheath.

When the stylet passed into the parenchyma cell and lacuna, type S and O patterns were occurred. It is suggested that type S and O are salivation and ingestion, respectively. Otherwise when the stylet penetrated into the vascular vandle sheath, type X and Ph patterns were observed. These patterns are ingestion feeding behaviuor in xylem and phloem, respectively.

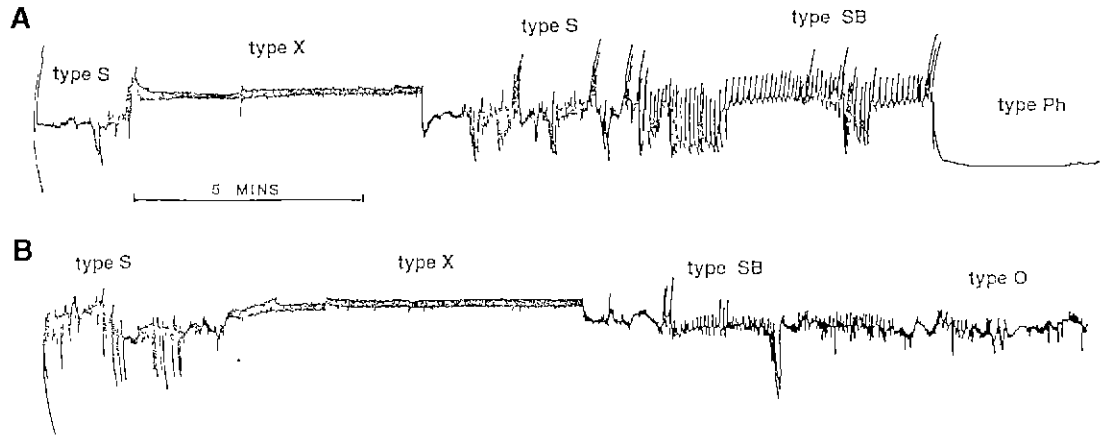

Fig. 2. The trace of *N. lugens* shows pattern types S, X, O, SB and Ph was and was stopped after a period in which the Ph pattern (A) and O Pattern (B) was generated on the rice plant leaf-sheath and leaf-blade, respectively.

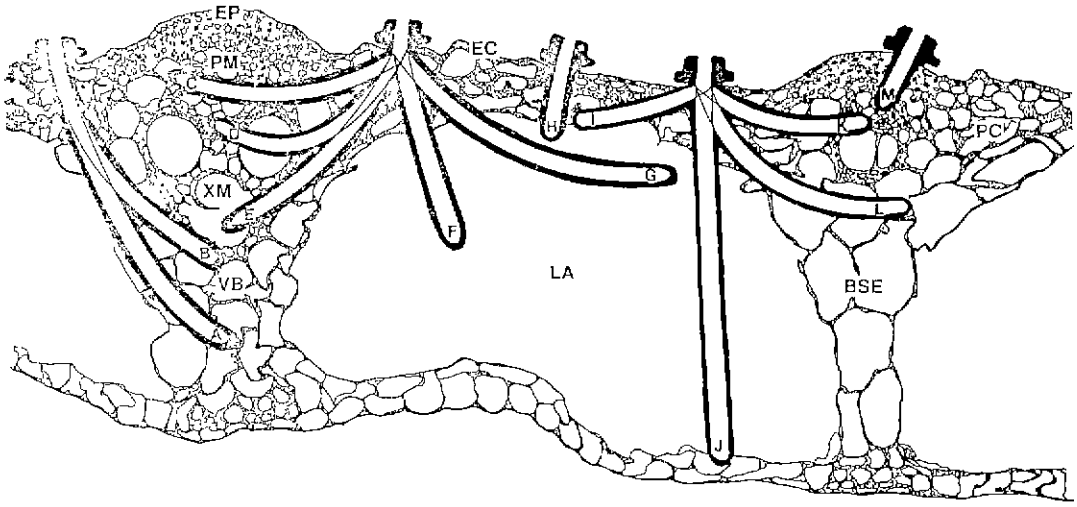

Fig. 3. Diagram illustrating the structure of leaf-sheath and the trace of stylet-sheath. **BSE**: Bundle sheath extension; **EC**: Epidermic cell; **EP**: Epidermal papillae; **LA**: Lacuna; **PC**: Parenchyma cell; **PM**: Phloem; **VB**: Vascular bundle; **XM**: Xylem.

3. Honey dew drops with patterns

In the figure 4. there are example of honey dew drops with continuous recording patterns for 44 minutes. Here, we can see only one honey dew drop during pattern X. It might be suggested that type X pattern is ingestion feeding pattern. Of course, the brown planthopper is phloem feeder. When the continuous type Ph pattern is occurred, honey dews were dropped every 20~30 minutes.

DISCUSSION

The general observations on exploratory and initial probing behaviour observed in this study agree with reports from other workers. In the exploratory behaviour it is noticeable that the insect moves around the plant moving its antennae but also tapping the surface with its front legs as noted by Losel (1987) and Youn (1992). Losel (1987) and Youn (1992) demonstrated the presence of tarsal receptors, although it is not clear whether they are mechanoreceptors, chemoreceptors or a mixture of both. After this initial exploratory period the planthopper begins to explore the plant surface with the labium which bears mechano-and chemoreceptors (Foster *et al.* 1983). Following this test probes into the plant were made. Youn (1992) confirmed that the preferred site of entry was seen to be at the side of a leaf vein on leaf-sheath. The stylets rarely penetrate a vascular bundle directly but curve laterally from the point of entry to pierce the vascular bundle from the side, a strategy reported also by Sögawa (1970). On the leafblade, which is much thinner with less well developed vascular bundles in the smaller veins, probing was distributed evenly over the entire surface of the leaf and direct penetration of veins was quite common. Possibly this is because the epidermis covering the veins is not so thick in the leaf-blade.

Five patterns, S, SB, X, Ph and O were distin-

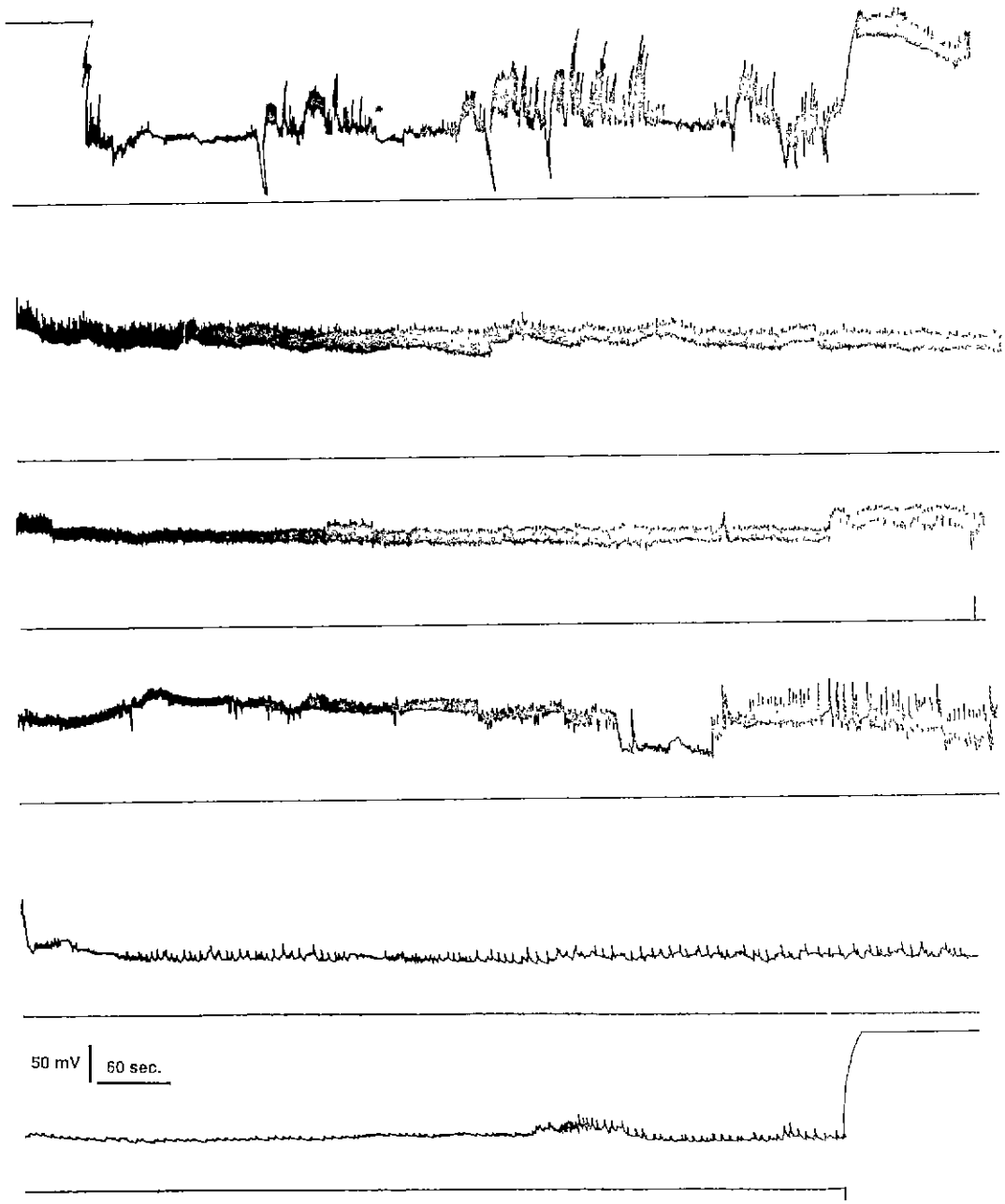

Fig. 4. (A) A recording showing pattern X over a period of 44 minutes. Note the large changes in amplitude in pattern S prior to the large change in potential as pattern X beginnings. After the X pattern there is a period of SB pattern followed by a potential drop, however, the potential produced there is more irregular than the normal Ph pattern, recording more an O pattern. (B) Only one honeydew droplet is produced in the 44 minutes of the X pattern.

guished on the basis of their signal level, frequency and amplitude. A number of workers have made electrical recording of feeding behaviour in *N. lugens* (Khan & Saxena 1984 1988, Velusamy & Heinrichs 1986, Losel 1989, Kimmins 1989, Spiller 1990, Youn 1992).

Velusamy and Heinrichs (1986) used an AC system to monitor the probing behaviour of *N. lugens* on susceptible and resistant rice varieties. They reported three waveforms following initial probing, which they call S, A and I. They give no details of the waveform but S appears to be an irregular signal of large amplitude always following probing, P, but also seen between A and I waveforms. The A waveform consists of large, regular potential changes, somewhat similar to SB in these recordings, which precedes pattern I with a regular low frequency, and a low amplitude signal. Velusamy and Heinrichs reported only one type of ingestion waveform, but their interpretation of waveforms was based upon McLean and Kinsey's (1967) identification of waveforms from the pea aphid, *Acyrthosiphon pisum*. Kawabe and McLean (1980) have shown that these are not the same waveforms as those from other homopteran families.

In Youn's thesis (1992) an attempt was made to correlate the waveforms recorded with the position of the stylets within the plant. Histological preparations which contain only the salivary sheath can not establish the exact position of the insects' stylets at the moment feeding ceases. The insect makes several probes in one position usually, so that a branched salivary sheath is left behind. In addition, it has been suggested that the stylet tips might extend beyond the salivary sheath so that the termination of the sheath will now necessarily give a reliable indication of the termination of the stylet tips. However, Spiller (1990) has managed to amputate the stylets of *N. lugens* while still embedded in the tissue using a laser beam. This EM study has shown that the presence of the stylet tips in sieve cells is accompanied by sheath saliva, suggesting that the sections showing the salivary sheath tip may give a good indication of the position of the tip of the stylets.

A large number of salivary sheaths in rice tissue were sectioned in this study in an attempt to correlate the EPG produced by an insect with the probes it had made in the plant. In spite of the drawbacks of this method, if a sufficiently large number of sheaths are examined, it can provide an evidence that a particular pattern is linked with penetration and/or ingestion in a particular tissue. If, over a large survey, a pattern type never appears unless a branch of the salivary sheath is found in a particular tissue, then this can at least provide a strong indication that the two are associated.

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