Proc. 6th Auchen. Meeting, Turin, Italy, 7-11 Sept. 1987, 285-292.

THE EFFECT OF NITROMETHYLENE HETEROCYCLES ON THE FEEDING OF THE RICE BROWN PLANTHOPPER NILAPARVATA LUGENS (STÅL).

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ABSTRACT

The effects of sub-lethal concentrations of a nitromethylene heterocycle (NMH) insecticide compound on the feeding biology of the Brown Planthopper Nilaparvata lugens has been investigated using an electrical recording technique which measures potentials of biological origin generated whilst the insect is feeding. The effect of the compound on honeydew excretion has also been determined. While having no apparant repellent action, the compound reduced the total time spent feeding as well as altering the proportion of time spent feeding from phloem, xylem and mesophyll tissues of the rice plant.

KEY WORDS

Electrical recording, feeding behaviour, honeydew excretion, insecticide.

INTRODUCTION

There is considerable interest in identifying physical and chemical factors which reduce feeding activity of brown planthoppers which are major vectors of rice viral diseases such as Rice Ragged Stunt Virus and Rice Grassy Stunt Virus, transmitted via the hoppers' salivary glands (Foster, 1983).

Homopteran feeding has been assessed experimentally in two main ways. Firstly by measurements of the length of time for which the insect's mouthparts are in contact with the plant, either visually or employing various electronic techniques, and secondly, using estimates of the amount of honeydew excreted.

Working on the cabbage aphid, Brevicoryne brassicae, Wensler (1962) used visual estimates of probing time to investigate the effect of sinigrin extracts on the feeding behaviour of this insect on Vicia faba. An electrical recording device was first employed by McLean and Kinsey (1964), to measure feeding of the pea aphid Acrythosiphon pisum. Various forms of this method have since been used to investigate feeding behaviour of a range of Auchenorryncha including Sogatella furcifera (Kahn and Saxena, 1984), Nephotettix cincticeps (Kawabe and McLean, 1980), and

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Zyginidea scutellaris Marion-Poll (1987). The technique has also been used successfully to study the effects of resistant rice strains on feeding of the leafhopper Nephotettix virescens (Kahn and Saxena, 1985) and for the Brown Planthopper Nilaparvata lugens (Kimmins et al., 1986).

Honeydew excretion by the Brown Planthopper has been measured directly by volume taken up in graduated micro-pipettes (Pathak et al. 1982). Paguia et al., (1980) have used the amino acid indicator ninhydrin to detect honeydew, while bromocresol green indicator was used by Fisk (1980) to investigate the effects of various phenolic compounds on the feeding behaviour of the planthopper Peregimus maidis on Sorghum bicolor.

A modified electrical recording technique combined here with simultaneous video-filming of the insect, has been used to correlate the electrical signals with its behaviour to investigate the response of N. lugens to a new group of insecticides, the nitromethylene heterocycles (Shell Research Limited). The effect of these compounds on honeydew excretion is assessed using the bromocresol green indicator method.

MATERIALS AND METHODS

Electrical Recordings

Figure 1 shows a schematic representation of the apparatus used. Brachypterous female brown planthoppers collected from a culture maintained in the insectary at 28°C, 70% RH were anaesthetised in a slow stream of carbon dioxide to allow one end of a 30mm long, 25µm diameter gold wire lead to be attached to the dorsum of the hopper, anterior to the base of the wings, by means of silver-loaded conductive paint. The other end of the gold wire was connected to the recording electrode input terminal of a Grass P-16 microelectrode amplifier (input impedence $2X10^{11}$ Ohms), with shielded cable. The hopper, wire and cable were supported on a micromanipulator with which the insect could be positioned on a 3 to 4 week old rice seedling. The seedling was connected to the recording

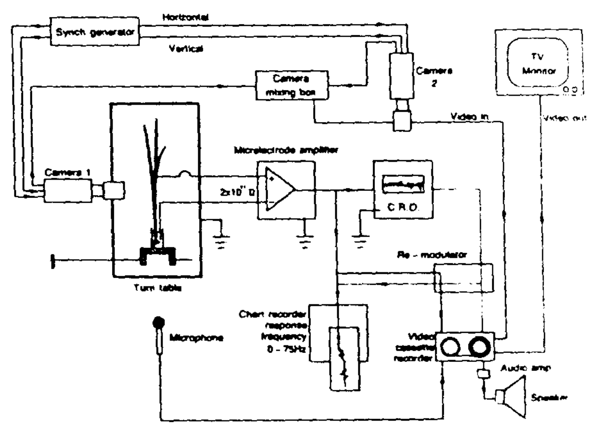

Figure 1 - Plan diagram of the electrical recording apparatus.

circuit through a chloride coated silver wire electrode standing in water surrounding the roots of the rice seedling. By adjusting the offset voltage, the amplifier was 'balanced' only when the hopper made internal contact with the rice seedling. The currents generated whilst the insect was feeding were then displayed on a cathode ray oscilloscope, fitted with a storage screen and single sweep facilities, and also on a chart recorder with a frequency response range of 0–75Hz.

The behaviour of 'wired' insects feeding on rice seedlings was filmed with a monochrome video-camera fitted with a macro lens (f=90mm 1:2,5) on 80mm extension tubes, while another video-camera was used to film the oscilloscope screen trace. The cameras were synchronised to allow the merged picture from both cameras to be recorded and displayed together on the T.V. monitor. A control on the video mixing box allowed the relative strength of the video input from either channel to be varied. The low frequency feeding pattern signals were converted to an amplitude modulated signal with a high frequency carrier acceptable to the narrow band width of the video recorder audio amplifier, and this signal could then be recorded on one of the two sound tracks. A microphone was used to record experimental observations on the other sound track.

Hoppers were allowed to feed on untreated rice seedlings for approximately two hours before being transferred to an insecticide-treated test seedling for a recording period of around four hours except in cases where field concentrations of pesticide were being used, where knock-down occurred before this period had elapsed. Following a 4 hour exposure to a sub-lethal pesticide dose, the hoppers were transferred to an untreated rice seedling for a further 3 hour recording period.

Honeydew Excretion Experiments

Bromocresol green indicator powder (B.D.H.) was dissolved in ethanol at a concentration of 20mg/ml, and acidified with a few drops of acetic acid to give an orange coloured solution. The indicator solution was applied to 120mm discs of filter paper using an aerosol sprayer. The test seedlings were placed in the centre of the filter paper disc, and were covered with a 250mm high, 100mm diameter acetate sheet cylinder. Ten adult female planthoppers were then introduced into the cylinder and the open end closed off with a perspex petri dish.

Test seedling preparation

Single rice seedlings were removed from a soil-based growing medium and the roots rinsed to remove soil. Single plants were placed in 10ml of tap water, which was sufficient to cover the roots, in 19ml glass vials, and held in place by a collar of foam rubber. NMH compound WL 108477 (Harris et al., 1986) dissolved in distilled water with 10% acetone/ wetting agent mixture was applied as a foliar spray. The deposit was allowed to dry for 2 hours before exposure to the insects.

RESULTS

Feeding Behaviour

On all plant substrates, the sequence of events was the same. Initially, periods of walking were punctuated by stationary periods during which rapid dabbing of the plant surface by the planthopper's tarsi occurred. Particularly marked was the movement of the fore tarsi which were extended fully in front of the insect, and then dragged backwards, scraping the leaf surface. Concurrent with the tarsal tapping, slower labial dabbing of the leaf surface occurred.

Electrical feeding patterns

Figure 2 shows a typical chart recorder trace of the initial phases of the brown planthopper feeding pattern. The traces were analysed in terms of five types of response, non-feeding, probing, 'mesophyll' feeding, 'xylem' feeding and 'phloem' feeding, defined by the following characteristics:

(i) Non-feeding: Periods of no contact between the hopper mouthparts and the surface of the leaf.
(ii) Stylet probing: Brief periods of labial contact with the leaf lasting less than 1.5 minutes.
(iii) Mesophyll feeding: A very variable signal, both in terms of frequency and amplitude, which always precedes patterns iv. and v. The signal varied in size between approximately 3 and 20mV.
(iv) Xylem Feeding: This signal consisted of a very regular 5 to 6Hz wave form, with an amplitude of 5 to 25mV. Of all the signals identified, the potential of this signal was the most positive. The duration of this signal varied from around one minute to over an hour.
(v) Phloem feeding: The most negative voltage values occurred during this pattern, which usually had a frequency of 3 to 4 Hz with a signal amplitude in the range 2 to 5mV. Occasionally however, the signal consisted of a strong 0.25Hz pattern with a higher frequency pattern, having an amplitude of less than 1mV, superimposed upon it.

Figure 3a shows the proportion of the total recording time during which brachypterous females were engaged in each of the above described types of behaviour, when feeding off untreated rice. Figures 3b and 3c show the feeding behaviour of brown planthopper feeding off rice treated with a foliar deposit of sub-lethal doses of the nitromethylene heterocycle WL 108477 at concentrations of 0.001 and 0.0001% active ingredient respectively, and subsequent 'recovery' on untreated rice. There is a highly significant decrease in the total period of ingestion ($t=7.317$ df21, $P<0.001$), (electrical patterns, iii, iv and v) for insects feeding on plants treated with the higher sub-lethal dose, from the control level. Of the individual feeding patterns, the most

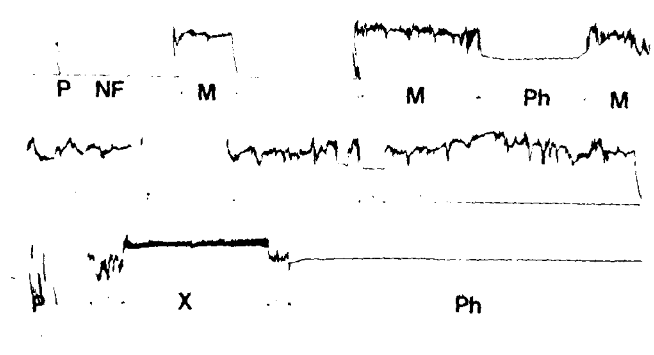

Figure 2 - Initial stages of a brown planthopper electrical feeding pattern. N.F.= non-feeding, P= probing, M='mesophyll' feeding, X= 'xylem' feeding, Ph= 'phloem' feeding.

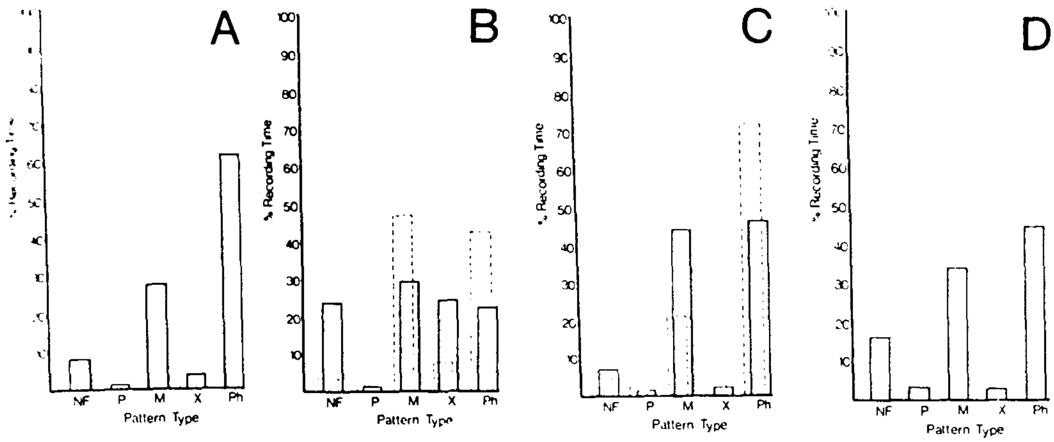

Figure 3 - Proportions of the total electrical recording time for which each of the five patterns occurred, for hoppers on rice with the following treatments: (A) untreated, (B) 0.001% NMH spray deposit, (C) 0.0001% NMH spray deposit, (D) NMH lethal, field dose rate; 0.05%. For (B) and (C) the dotted line represents feeding of post-treatment insects on untreated rice

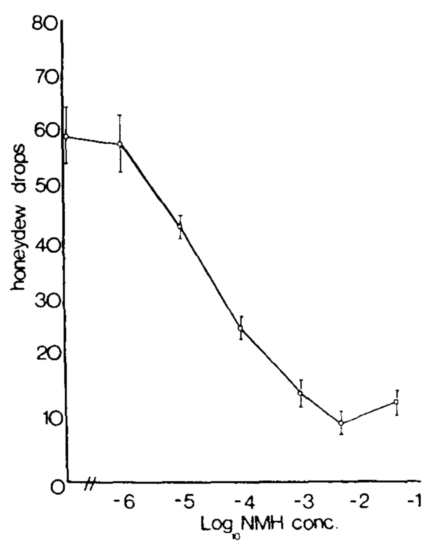

Figure 4 - Effect of NMH spray deposit concentration, on honeydew excretion by 10 female planthoppers, allowed to feed from treated rice for a 3 hour period.

noticable change is the significant reduction in phloem feeding ($t = 5.525$, df21 $P < 0.001$). Both mesophyll and xylem feeding increased, but these did not differ significantly from the control level. On being returned to the untreated rice plant, there were increases in both mesophyll and phloem feeding. Although still lower than the control level of phloem feeding, this difference was not significant ($t=0.90$, df17, $P > 0.05$). For the lower sub-lethal insecticide concentration, there was a significant increase in mesophyll feeding ($t=2.291$, df17, $P < 0.05$) accompanied by a decrease in the level of phloem feeding. The decrease in phloem feeding was, however, not significant at the 5% probability level ($t=1.831$, df17). During the 3 hours of recording on untreated rice plants, following the 4 hour recording period on the low, sub-lethal NMH concentration, no significant changes from the control levels in relative proportions of time spent on each of the recording patterns were noted. In the post-treatment period the feeding time of the insects increased significantly ($t=3.135$, df17, $P < 0.01$) above that of the controls (Fig. 3c). Figure 3d shows the distribution of time over the five types of response for brown planthoppers feeding on rice seedlings treated with a lethal concentration of the compound (0.05% active ingredient) corresponding to the field dose rate. The insects were irreversibly damaged within one hour of coming into contact with the spray deposit, and were thus unable to remain attached to the rice seedling. In the period before knock-down occurred, the proportions of non-feeding, probing, mesophyll feeding and xylem feeding did not differ significantly from the control level. There was however a significant decline in the amount of phloem feeding ($t=2.246$, df25, $P < 0.05$).

Honeydew Excretion Experiment

Figure 4 shows the effect of increasing NMH spray deposit concentration on the number of honeydew drops released by brown planthoppers feeding on the treated rice seedlings. For all pesticide concentrations of 0.0001% active ingredient and below, there were significant decreases in the quantity of honeydew drops produced. Despite levels of mortality of over 80% and therefore a reduction in total feeding time, there is a slight increase in honeydew excretion for hoppers exposed to the highest NMH concentration.

DISCUSSION

Idenfification of the various feeding patterns described above is based on descriptions of patterns with similar characteristics which have been obtained on a wide range of sucking insects, by various electronic recording devices. Unlike the majority of electrical recording methods, where either an alternating or direct current is applied to the insect and the plant on which it is feeding, the potentials described here are generated entirely within the preparation. They therefore correspond to the 'e.m.f. component' of Tjallingii's (1978) recordings on the aphid *Brevicoryne brassicae* , which are a combination of insect muscle electrical activity and electrical activity of plant tissues. The mesophyll feeding pattern bears a close resemblance to the waveforms recorded by Marion-Poll *et al*. (1987) for the mesophyll feeding planthopper *Zyginidea scutellaris*. These authors suggest that intracellular penetration occurs in the mesophyll cells of the leaf, which causes 'injury potentials' to spread through the tissue, (Goldsworthy, 1983). The relatively higher concentration of cations transported in the xylem sap, and the much more negative potential existing in the phloem sieve element help to explain the large difference in potential between these two regular and prolonged waveforms.

The main effect of applying the NMH compound at sub-lethal concentrations, appears to be to cause reductions in the time spent feeding, and in the volume of honeydew excreted. Working on aphids, Sassen (1982) similarly found the pyrethroids deltamethrin and permethrin to have marked antifeedant effects. While the reductions in total feeding times, as recorded by the electrical recording method are only fairly small, the reduction in quantity of honeydew produced is large. The analysis of the feeding patterns shows that most of the reduction in feeding occurs due to a decrease in feeding from the phloem. The amount by which the phloem feeding was reduced was greatest for those insects feeding on plants treated with the higher, sub-lethal NMH concentration. This suggests that the volume ingested, and therefore the amount excreted also, depends very much on the duration of phloem feeding. Mittler (1957) showed that phloem sap enters the aphid under pressure generated by the sieve element and suggested, that this is also the main force driving the sap through the gut. It seems likely therefore that a similar situation pertains to the planthopper's feeding.

How the insect is able to detect the presence of the pesticide deposit on the surface of the leaf, remains unclear as there were no observable changes in behaviour. While chemical and physical features of the leaf surface will stimulate feeding, the increased mesophyll feeding by hoppers on sub-lethal NMH treated plants indicates a greater degree of stylet relocation and thus more unsettled feeding in response to the compound. Since it is unlikely that the NMH as a spray deposit,could have penetrated deeply into the leaf, where detection by cibarial receptors (Foster, 1983) during ingestion would be possible, the chemoreceptors of the labium (Foster et al., 1983) and tarsi seem likely sites of NMH detection. The fact that for the short recording period of insects exposed to a lethal dose, the relative proportions of time spent on each of the feeding patterns did not differ from the control levels, suggests however that this inhibitory effect is weak, compared to the stimuli promoting feeding when the brown planthopper first encounters the rice plant.

In the light of work by Scheller and Shukle (1986) on Barley Yellow Dwarf Virus transmission by the aphid Sitobion avenae on oats, which showed that the chance of virus transmission increased with increased phloem feeding, the sub lethal effects of NMH compounds would seem to make them well suited to the purpose of protection against insect-borne viral plant pathogens.

ACKNOWLEGEMENTS

The above work was funded by the Science and Engineering Research Council and Shell Research Limited. I am indebted to my supervisors, Dr. L.J. Goodman (Q.M.C.) and Mr. R.N. Price (Shell Research Ltd.), for their suggestions and encouragement in the course of this work.

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