A Characteristic Body Posture of the Potato Leafhopper is Correlated with Probing[1]

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ABSTRACT Electronic monitoring was combined with video observation in order to determine whether a characteristic body posture of the potato leafhopper, *Empoasca fabae* (Harris) (Homoptera: Cicadellidae), indicated probing. Stylet probing was strongly correlated with ($r > 0.99$) and could be accurately predicted by a characteristic body posture. We describe a technique whereby video observation, alone, can be used to apply standardized pulses of leafhopper probing injury to plants.

KEY WORDS Electronic monitoring, videomicrography, feeding behavior, alfalfa.

Since the 1960s, electronic monitoring systems have been the most accurate methods of determining when a homopteran is probing into plant tissue (McLean and Kinsey 1964, Backus 1994). This is because visual observation of proboscis contact with a plant has often proven to be unreliable as an indication of stylet penetration. Proboscis contact can occur during labial dabbing to apply saliva to the plant surface for tasting, or even during rest periods (Backus 1988, Miles 1972, Pollard 1968). In contrast, electronic monitoring allows definitive correlation of waveforms with probing behaviors and penetration of specific plant tissues or cells (McLean and Kinsey 1967, Nault and Styer 1972, Tjallingii 1987), or quantification of numbers of probes and waveform durations while examining the effects of leafhopper probing inside plant tissue (Kabrick and Backus 1990). Therefore, electronic monitoring is indispensable in describing the activities of homopteran stylets. However, it can also present difficulties. Only one insect at a time can be electronically monitored on each small area of plant without risk of entangling the gold-wire tethers. Electronic monitoring, as a consequence, has problems as a method of standardizing leafhopper probing, especially for experiments that require many plant tissues which each must receive equally high doses of probing damage in brief periods of time. Furthermore, the process of wiring an insect is time-consuming, and under some circumstances can stress the insect or alter its feeding behavior (Tjallingii 1986, Hardie et al. 1992).

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Because our research on feeding damage from potato leafhoppers, *Empoasca fabae* (Harris) (Homoptera: Cicadellidae), often requires significant numbers of equally treated plants, we needed a method by which leafhopper probing could be easily quantified by direct observation. Herein we describe results from experiments using electronic monitoring and videomicrography (wherein a videocamera is mounted on a stereomicroscope) to test the hypothesis that a characteristic body posture indicates probing.

Materials and Methods

Insect Rearing and Plant Growth. Vegetative clones of alfalfa, cv. 'Ranger', were grown in growth chambers under photoperiod 16:8 (L:D) h at 31°C during the day and 23°C at night. Plants were cut once, then were allowed to regrow to a height of 20 to 30 cm when immature internodes between 1.0 and 2.5 cm in length were chosen for all experiments.

The *E. fabae* colony was maintained in a growth chamber under photoperiod 16:8 (L:D) h at 27°C day and night. Insects were reared on greenhouse-grown broad bean plants (*Vicia faba* L., cv. 'Windsor'). Prior to testing, adult insects, one to seven days post-eclosion, were caged on alfalfa for a conditioning period of five days. Only females were used.

Electronic Monitoring. One channel of a four-channel version of the AC electronic monitoring system (the "Missouri Monitor"; Backus and Bennett 1992) was used to determine when a test insect was probing. Two or three hours prior to each test, several insects were prepared for electronic monitoring. For each insect, one end of a 12.7-µm diameter gold wire (Sigmund Cohn Corp., Mt. Vernon, NY) was attached with silver conducting paint (Ladd Industries, Burlington, VT) to the pronotum and the other end was attached to a copper stub. Insects were placed on alfalfa for at least one hour to acclimate to the tether and were removed from the plant and starved for approximately one hour before being electronically monitored.

The AC electronic monitor produced a 50 mV, 500 Hz signal which was applied through a copper wire embedded in the freshly watered soil of a test plant. The copper stub was connected to the input of the monitor, and probing was detected when the tethered leafhopper inserted its stylets into the plant, thereby completing the electrical circuit. Changes in resistance through the stylets were amplified, rectified, and recorded as voltage changes on a two-channel Servogor 430 strip-chart recorder (BBC Goerz Metrawatt, Broomfield, CO) operating at 1.0 cm/min. Each probe, defined as each continuous duration of stylet insertion, was quantified by measuring the distance along strip-chart paper over which voltage fluctuations were written as waveforms.

Correlation of Videomicrography With Electronic Monitoring. For each leafhopper, a 5.0-mm long segment of a single face of a square, 1 mm wide internode of an alfalfa stem, aligned horizontally, was made available for probing by fitting the stem into the groove formed by two adjacent Plexiglas® plates, each 1.0-mm thick, spaced 1 mm apart on a flat surface. Leaves and regions of stem adjacent to the feeding area were made inaccessible for probing by being covered with cellophane tape over Parafilm®. A Javlin (JE-7242), high-resolution, black-and-white videocamera captured a 38X view of the feeding area through a trinocular Wild M5 Apo® stereomicroscope, and displayed the view on a Javlin black-and-white monitor (all equipment from Adlon Instruments, Inc., St. Louis, MO). Displayed views were not videotaped. One insect at a time was observed and electronically monitored. As each insect became satiated and ceased feeding during a test, it was removed from the plant and given an additional starvation period of approximately 30 min before being returned to the plant. This cycle was repeated as long as the insect would resume feeding or until the tether broke.

Based on preliminary observations, the characteristic probing position was defined as a body posture in which the insect placed the tip of its proboscis on the plant at the angle between 85° and 92° with respect to the plant surface. The angle was measured, from the caudal side of the proboscis to the plant surface in front of the insect, with a protractor placed against the videomonitor screen. Angle of proboscis contact was written on the strip chart paper as each probe was being electronically monitored. Initiation and termination of probing were easily detected by listening for the sound of the deflection of the strip chart recorder pen and were marked by hand on the strip chart recorder paper. In the rare event that an insect faced the camera or its proboscis angle could not be easily determined, a camel's hair brush was used to nudge the insect off the stem, and any probing that had occurred was excluded from the data set. A total of ten insects was electronically monitored and simultaneously viewed with videomicrography during a total of 10 hours of probing, comprising 187 probes, over the course of one month. Because the data generated in preliminary work indicated that a characteristic body posture was consistently associated with probing, we considered this sample size (10 insects) to be sufficient; most electronic monitoring studies that examine much more variable behaviors use a sample size of 20 insects (Backus 1994).

Statistical Analysis. Student's t test (GLM procedure of SAS) (SAS 1985) was used to determine a correlation between frequency of probing as determined by electronic monitoring compared with probing position as determined by videomicrography.

Results

Correlation of Videomicrography With Electronic Monitoring. Stylet probing was strongly correlated with and could be accurately predicted by a characteristic body posture in which the insect's proboscis touched the plant surface at an angle between 85° and 92° (Fig. 1). One hundred percent of the 187 proboscis contacts made in this position resulted in stylet penetration (probing). However, in 17 of those contacts (less than 10%), visual observation of contact occurred slightly before electronically detectable probing began (Table 1). Therefore, if video observation alone had been used to detect probing, we would have incorrectly gained some probing duration. However, it would have been very short. These portions of 17 proboscis contacts contributed only 2.3 min (ranging from 0.02 to 0.50 min each) to the entire experiment. In addition, part of a single proboscis contact, representing only 1.3 min of probing, occurred while the insect was in an uncharacteristic body posture: with the proboscis at an angle of 60°. Thus, only 1.3 min of probing would have

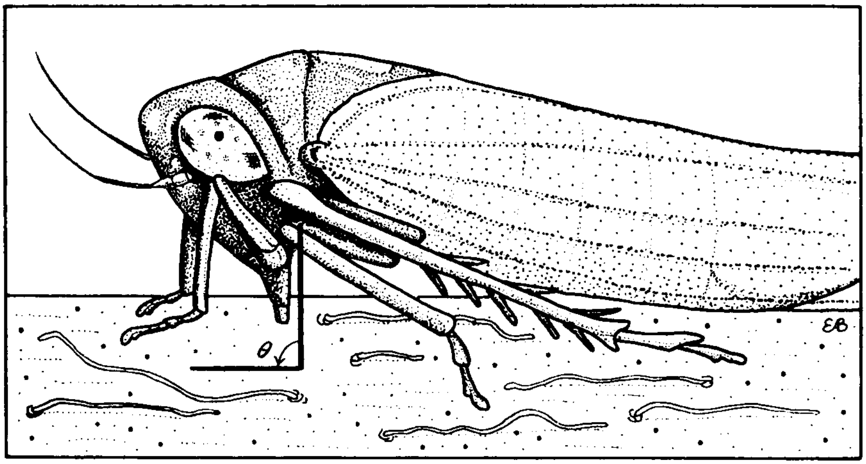

Fig. 1. Characteristic body posture of *E. fabae* when probing. The proboscis is nearly perpendicular to the plant surface (θ = 85° to 92°).

been missed had videotaping alone been used. In such a case, the error in estimating probing duration would have been less than 5.9% within a 95% confidence interval. At the termination of virtually every probe, the insect either moved to a new feeding site, repositioned its proboscis to an angle less than 60°, or jumped off the plant. We found a strong linear correlation between durations of electronic monitoring-measured probing and video-observed proboscis contact in characteristic probing position ($r > 0.99$). The two methods yielded nearly equal values when used to measure probing duration.

Discussion

Horizontal alignment of the plant surface is used by researchers who use electronic monitoring to study *E. fabae* probing (Kabrick and Backus 1990) and is necessary when electronically monitoring highly active leafhopper species, like *E. fabae,* because such insects frequently jump off a vertically aligned feeding substrate and dangle on the delicate, easily broken tether. We believe that horizontal alignment in this study did not affect our results, but allowed us to establish a new videomicrography method for detecting *E. fabae* probing.

Previous research has shown that potato leafhoppers spend little or no time testing the substrate prior to initiating a probe, and that probe termination is equally abrupt (E. A. B. unpub. data). Furthermore, microscopic observations of electronically monitored potato leafhoppers led us to hypothesize that these insects assumed an identifiable posture during probing. Using videomicrography to measure durations of this characteristic body posture, intense, brief pulses of probing damage could be applied using more than one

Table 1. Proboscis contact frequency and duration of adult female potato leafhoppers while feeding on alfalfa stems.

	Contact Frequency*	Percent of Total
Total No. of Proboscis Contacts	187	100
In Characteristic Body Posture**		
Probing†	187	100
Not Probing‡,§	17	9.1
Not in Characteristic Body Posture		
Probing†	1	0.54
	Duration (min)	Percent of Total
Total Duration of Proboscis Contact	601.2	100
In Characteristic Body Posture**		
Probing†	597.6	99.4
Not Probing‡,§	2.3	0.38
Not in Characteristic Body Posture		
Probing†	1.3	0.22

* Values in this column represent number of proboscis contacts or proboscis contact duration, determined by video observation, during which the indicated event was detected by electronic monitoring. Note that more than one behavior may occur during a given proboscis contact.

** Proboscis touching the plant at an angle of 85° to 92° with respect to the plant surface, i.e. making proboscis contact.

† Stylets in plant.

‡ Stylets not in plant.

§ For 17 probes, some non-probing occurred while the insect was in characteristic position, but was always followed by stylet insertion.

insect at a time. Average probing intensities per plant were at least as great as or greater than those which were possible using electronic monitoring.

We found videomicrography to be simpler and more convenient than electronic monitoring to quantify potato leafhopper probing. We prefer videomicrography alone over electronic monitoring for experiments demanding large sample sizes of stems heavily damaged by probing, but not requiring detailed information about the specific waveforms and feeding behaviors occurring during probing. This method of quantifying insect probing avoids potential unknown artifacts caused by tethering the insects. Also, it allows a more natural test environment for insects because they can feed on stems held upright. Therefore, video observation can be substituted for electronic monitoring as an accurate method of quantifying the duration of individual probes by the potato leafhopper. We used a modification of this videomicrography technique to study the effects of probing by *E. fabae* on alfalfa stems (Ecale 1993). This videomicrography technique could be applied to other homopterans, provided that they exhibit a characteristic body posture indicative of probing.

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