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Leafhoppers on Leaves: An Analysis of Feeding Behavior Using Conditional Probabilities

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Feeding behavior of four deltocephaline leafhoppers, Graminella nigrifrons, G. oquaka, Amblysellus grex, *and* Dalbulus maidis *on maize and johnsongrass was analyzed using an electronic monitoring device. Five distinct waveform patterns were identified: secretion of sheath saliva (salivation), nonvascular probing, nonsieve element ingestion, x-waveform, and phloem ingestion. Waveforms were associated with feeding activities by correlation with light microscopic examination of salivary sheath termination points in leaf tissue and analysis of honeydew excreted by monitored leafhoppers. In previous studies x-waveforms have been reported to occur only when the stylets of homopterans are in contact with the phloem; the function of x-waveforms is poorly understood. There were no differences in time spent salivating or ingesting from nonsieve elements among* G. nigrifrons, G. oquaka *and* A. grex *on either plant.* D. maidis *differed from other species in phloem probing and feeding behavior; only a small proportion produced x-waveforms, although those that did spent significantly more time in this behavior than other species. Also,* D. maidis *spent more time than other leafhoppers ingesting from tissues other than sieve elements. Kinetic diagrams of transition probabilities show that probing activities of all species were not random regarding the sequence of behaviors culminating in phloem ingestion. Thirty-five percent of* G. nigrifrons *x-waveforms were followed by nonsieve element ingestion. This was consistent with observations showing that salivary sheaths of leafhoppers producing x-waveforms sometimes do not terminate in the phloem, but rather in nearby cells. Phloem ingestion was always preceeded by x-waveforms. The quantitative differences in probing behavior are discussed*

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in relation to ability of these leafhoppers to transmit the phloem-associated maize chlorotic dwarf waikavirus.

KEY WORDS: feeding behavior; electronic monitoring; virus transmission; *Graminella nigrifrons*; *G. oquaka*; *Amblysellus grex*; *Dalbulus maidis*.

INTRODUCTION

Leafhoppers and other homopterans feed by inserting stylets into plant tissues where they feed on plant sap. Because feeding occurs beneath plant epidermal layers, stylet tips and associated feeding behavior cannot be observed. The electronic feeding monitor (McLean and Kinsey, 1965; Backus and Bennett, 1992) has allowed researchers to learn many details of homopteran feeding *in situ*. Compared to aphids (McLean and Kinsey, 1967; Campbell *et al.*, 1982; Dorschner *et al.*, 1990), only a few leafhopper species have been studied using the alternating current electronic monitoring system (AC-EMS). These include a xylem feeder (Crane, 1970), a mesophyll feeder (Hunter and Backus, 1989; Wayadande and Backus, 1989), and four species that feed primarily from the phloem (Kawabe and McLean, 1978, 1980; Triplehorn *et al.*, 1984; Rapusas and Heinrichs, 1990). Several probing behaviors have been associated with AC-EMS waveforms produced by phloem feeders. These are (1) secretion of sheath saliva, (2) ingestion from phloem, and (3) ingestion from nonvascular tissue and xylem. The behaviors associated with two other waveforms, the R-waveform and x-waveform, are not well understood. The R-waveform (Rapusas and Heinrichs, 1990) has been associated with probing of nonvascular tissue, but it is not known whether salivation or ingestion occurs during the probe. The x-waveform (McLean and Kinsey, 1965, 1967; McLean, 1977) is associated with penetration of phloem sieve elements and always precedes ingestion from the phloem. Previously, the AC-EMS has been used to study host plant selection and crop resistance to leafhoppers. However, the system has not been applied to understand better plant virus transmission or the feeding behavior of vector leafhoppers as it has for aphids (McLean, 1977; Scheller and Shukle, 1986).

Maize chlorotic dwarf waikavirus (MCDV) is a semipersistently transmitted, foregut-borne (Nault and Ammar, 1989), phloem-associated virus (Ammar *et al.*, 1987) transmitted in the field by the black-faced leafhopper, *Graminella nigrifrons* (Forbes) (Gordon and Nault, 1977). The virus has been experimentally transmitted by 9 of 24 deltocephaline leafhopper species (Nault and Madden, 1988). Most Deltocephalinae leafhoppers from the tribe Deltocephalini and the morphologically advanced Eucelini were efficient MCDV vectors, provided that the virus test plant, maize, was a developmental host for leafhoppers. Interestingly, a congener of the black-faced leafhopper, *G. oquaka* DeLong, failed to transmit MCDV when maize was used as a source and test plant. However,

the species transmitted MCDV when johnsongrass was used as a virus source and test plant. Nault and Madden (1988) speculated that the reason *G. oquaka* failed to transmit MCDV from maize is that it does not feed at all or only briefly from maize phloem or vascular parenchyna, whereas it feeds from those tissues in johnsongrass. Most leafhoppers from the tribe Macrostelini failed to transmit MCDV or were poor vectors, including the maize specialist and pest, *Dalbulus maidis* (DeLong and Wolcott), a species known to acquire but not transmit the virus (Ammar and Nault, 1991), thus factors associated with inoculation feeding offer the best explanation for MCDV vector specificity (Wayadande and Nault, 1993).

In this study we used the AC-EMS to look for quantitative differences in probing behavior among four leafhopper species, *G. oquaka*, *D. maidis*, and two efficient vector species, *G. nigrifrons* and *Amblysellus grex* (Oman).

MATERIALS AND METHODS

Leafhoppers and Plants

Leafhoppers were reared in organdy covered cages (D'arcy and Nault, 1982) in a room held at 27 ± 2°C, under a 16:8 L:D photoperiod. *G. nigrifrons*, collected from Wooster in 1988, and *A. grex*, collected from near Provo, Utah, in 1988, were reared on oats, *Avena sativa* (variety unknown). *G. nigrifrons* was collected from grasses near Wooster and *A. grex* from grasses in Utah County, Utah. *G. oquaka* and its host plant, *Panicum virgatum* L., were collected in 1987 near Brewster, OH. Each year laboratory colonies of *G. oquaka* were supplemented with field-collected specimens. *G. oquaka* was reared on mature *P. virgatum* grown from rhizomes. The *D. maidis* colony was started from adults collected on maize near Tepexpan, Mexico, in 1982, and was reared on sweet corn (variety 'Aristogold Evergreen Bantam'). Maize and johnsongrass seedlings were grown in a greenhouse and used in experiments after reaching the five- to six-leaf stage.

Experimental Procedures

G. nigrifrons, *G. oquaka*, and *A. grex* were electronically monitored on maize and johnsongrass. *D. maidis* was monitored on maize only. Adult females 1–3 weeks posteclosion were used in all tests. Leafhoppers were caged on the recording host for a 24-h acclimation period prior to electronic monitoring. A 2.5-cm segment of 12-μm-diameter gold wire tether (Sigmond Cohn Inc., Mt. Vernon, NY) was attached with silver conductive paint (Ladd Industries, Burlington VT) to the pronotum of adults immobilized on a stage by a gentle vacuum. Tethered leafhoppers then were placed onto the abaxial surface of a severed leaf of the recording host for a 1-h acclimatation period before recording began.

Five- to six-leaf maize or johnsongrass plants were severed at their bases and placed in a glass vial containing water and the voltage input electrode. Leaves were laid flat onto a Plexiglas holder so that leafhoppers could feed on the abaxial surface. An alligator clip on the holder held a 2.5-cm copper stub glued to the gold-wire tethered leafhopper. Leafhoppers were monitored electronically with the Insect Feeding Monitor [IFM; Electronic Instruments Laboratory, University of Missouri, Columbia, MO (Backus & Bennett, 1992)] for 3 h. The IFM is a differential amplifier with two input electrodes; noise from the reference electrode is automatically subtracted from the signal of the insect electrode. A 70-mV current with a carrier frequency of 125 Hz was applied to the plant by the input electrode. Because of the low voltage, it was not necessary to modify the signal by logarithmic scaling. After amplification, the signal was sent to a strip-chart recorder (Servagor 430, ABB Metrawatt, Bloomington, CO) operated at 100-mV sensitivity and a chart speed of 3 cm/min. Eighteen or 19 leafhoppers were monitored for each species/host combination using a completely randomized design.

Waveform patterns on strip charts were identified and measured using a metric ruler. Differences in the number of leafhoppers producing specific patterns were determined using chi-square analysis. Differences in probe number and duration of salivation, x-waveform behavior, phloem ingestion, nonvascular probing, nonsieve element ingestion, and total probing were tested using analysis of variance (Minitab, Inc.). Means were compared with the least significant difference mean separation test if there was a significant F value. Only leafhoppers which produced patterns were included in the analyses. When necessary, data were subjected to square root or log transformation to stabilize heterogeneity of variance.

To describe changes in behavior during probing, transitional matrices were constructed in which each cell in the matrix (N_{ij}) was the number of times behavior i was preceded by behavior j. First-order transitions were tested for randomness by the G test statistic (Sokal and Rohlf, 1969) applied to a 2×2 collapsed table around each cell (Hancock *et al.*, 1989). Transition probabilities >0.02 were used to construct kinematic diagrams for each species–host combination. Specific transitions were compared among species–host combinations by chi-square analysis of 2×2 collapsed tables around cells containing the transition being analyzed (Paynter *et al.*, 1990).

To associate waveform patterns with probing behaviors in specific plant tissues, *G. nigrifrons* and *D. maidis* leafhoppers producing specific waveforms were interrupted and plant tissues examined for sheath saliva termination points. Plant tissues (2×2 mm) were excised and fixed in 0.1 M phosphate buffer containing 3% gluteraldehyde, 2% paraformaldehyde, and 1.5% acrolein for a minimum of 3 days, then dehydrated in increasing percentages of ethanol and tertiary-butyl alcohol (30–100%). Tissues were then infiltrated with Paraplast,

embedded, sectioned at 12 μm, and stained with safranin and fast green for examination under the light microscope at 100 to 400×. Salivary sheaths and xylem vessels stain red and other tissues stain green.

To relate further waveforms to probing behavior, honeydew pH and rate of droplet production were studied. Honeydew pH was determined by collecting droplets with a glass microsyringe pulled by a Micropipette Puller (Model M1; Industrial Science Associates, Ridgewood, NY) and spotting them onto pH indicator paper (Micro-Essential Laboratories, Brooklyn, NY). In some cases, leafhoppers were allowed to excrete directly onto the indicator paper. Buffers of known pH were used as standards. Honeydew droplets excreted by leafhoppers and planthoppers that feed from phloem have a neutral to basic pH, whereas those that feed from xylem are acidic (Auclair *et al.*, 1982; Kimmins, 1989). Honeydew droplet excretion rates were calculated by counting droplets and dividing by the number of minutes in the observation period.

RESULTS

Waveform Descriptions and Associated Probing Activities

G. nigrifrons, *A. grex*, *G. oquaka*, and *D. maidis* produced five distinct waveform patterns when feeding on maize or johnsongrass. Similar waveforms have been reported for other leafhopper species (Kawabe and McLean, 1978, 1980; Rapusas and Heinrichs, 1990) and *G. nigrifrons* (Triplehorn *et al.*, 1984). In this paper we use the same terminology to describe salivation, phloem ingestion, and nonsieve element sap ingestion waveforms. We also use the term x-waveform to describe the stereotypic pattern which preceeds phloem ingestion (McLean and Kinsey, 1967; Triplehorn *et al.*, 1984) and refer to it as x-waveform behavior. The term nonvascular probing is used for the pattern previously called the "R" waveform (Sogawa, 1973; Rapusas and Heinrichs, 1990). The rationale for this is explained in the discussion. All leafhopper species produced identical salivation, nonvascular probing, and nonsieve element ingestion waveforms. Representative patterns associated with these behaviors are shown in Fig. 1. X-waveforms and phloem ingestion patterns were similar among *G. nigrifrons*, *A. grex*, and *G. oquaka* but were different qualitatively from those produced by *D. maidis* (Figs. 2 and 3).

Interpretation of behaviors associated with waveforms is supported by salivary sheath termination points (Table I), honeydew excretion rates, and droplet pH (Table II). When leafhoppers produced salivation waveforms (Fig. 1), salivary sheaths were observed in plant tissue that terminated in nonvascular tissues (mesophyll parenchyma and bundle sheath cells). *D. maidis* and *G. nigrifrons* infrequently produced honeydew droplets when the salivation waveform was recorded (Table II). When nonvascular probing was recorded, salivary

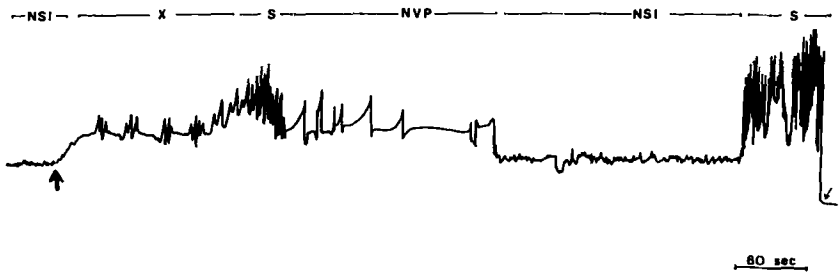

Fig. 1. Electronically recorded patterns of *Graminella nigrifrons* probing maize. S = salivation; NSI = nonsieve element ingestion; NVP = nonvascular probing; X = x-waveform. Waveforms are read right to left; small arrow denotes probe initiation; bar = 60 s. Note the dropoff in amplitude of the NSI (large arrow) relative to the x-waveform. Compare this to the midamplitude phloem ingestion waveform following the x-waveform in Fig. 2.

sheaths for both leafhoppers species usually terminated in the mesophyll or bundle sheath (Table I) and the behavior infrequently was associated with the excretion of honeydew (Table II). Phloem and nonsieve element sap ingestion for *G. nigrifrons* and *D. maidis* were associated with honeydew droplets of neutral to basic pH (Table II). Although the droplet rate for *G. nigrifrons* was stable at 0.7/min after 1 h of sustained phloem ingestion, nonsieve element

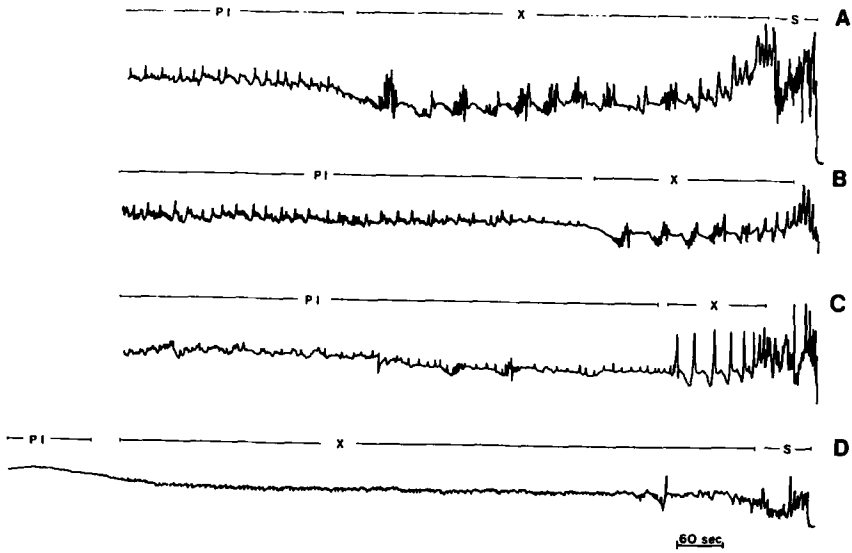

Fig. 2. Comparison of representative x-waveforms and phloem ingestion of A. *Graminella nigrifrons*, B. *Amblysellus grex*, C. *Graminella oquaka*, and D. *Dalbulus maidis* electronically monitored on maize. S = salivation; x = x-waveforms; PI = phloem ingestion. Waveforms read right to left; bar = 60 s.

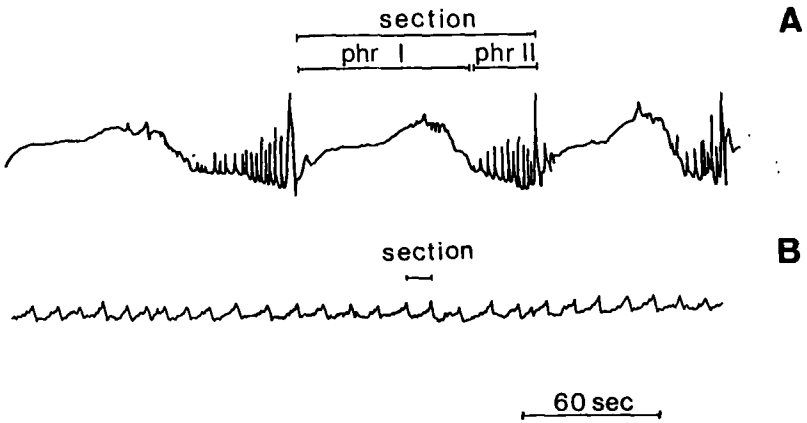

Fig. 3. Center sections of x-waveform sequences of A. *Graminella nigrifrons* and B. *Dalbulus maidis* electronically monitored on maize. Phr I = phrase I (smooth phrase) and Phr II = phrase II (spiking phrase). Bar = 60 s.

ingestion rates were more difficult to measure because a series of droplets rarely was produced during brief (avg., 6.1-min) bouts of nonsieve element ingestion.

Although there was no unique waveform pattern associated with xylem ingestion, *G. oquaka* occasionally produced 6–10 (pH 4–5) droplets/min during the nonsieve element ingestion pattern. One leaf tissue containing the salivary sheath of a leafhopper producing rapid, low-pH droplets was examined and the

Table I. Association of Electronically Monitored Waveform Patterns[a] with the Distal End of the Salivary Sheaths of *Graminella nigrifrons* and *Dalbulus maidis* in Maize Tissue

			Salivary sheath termination points in maize tissues				
	Waveform pattern	*N*	Xylem	Phloem	Mesophyll or bundle sheath	Collenchyma	Unclear or not found
G. nigrifrons	Salivation	10	0	0	7	0	3
	X-waveform	15	0	7	3	1	4
	Phloem ingestion	12	0	8	0	0	4
	Nonvascular probing	29	0	1	14	0	14
	Nonsieve element ingestion	17	0	1	10	0	6
D. maidis	Salivation	10	0	0	6	0	4
	X-waveform	4	0	2	0	0	2
	Phloem ingestion	3	0	3	0	0	0
	Nonvascular probing	20	0	1	10	0	9
	Nonsieve element ingestion	25	0	0	14	0	11

[a]Leafhopper feeding was interrupted by removing leafhoppers during indicated behavior, then leaf tissue on which the leafhopper was feeding was excised and processed for observation by thick section light microscopy.

Table II. Excretion Rate and pH of Honeydew Droplets Produced During Salivation, X-Waveforms, Phloem Ingestion, Nonvascular Probing, and Nonsieve Element Ingestion by Electronically Monitored *Graminella nigrifrons* and *Dalbulus maidis* on Maize

	Waveform pattern	No. of times behavior was recorded	No. of leafhoppers which excreted honeydew	Mean no. drops/ min ± SD	Mean pH ± SD
G. nigrifrons	Salivation	32	5[a]	—	—
	X-waveform	28	0	—	—
	Phloem ing.	19	19	0.70 ± 0.31	7.02 ± 0.15
	Nonvasc. prob.	10	5[a]	—	—
	Nonsieve element ingestion	21	14	0.53 ± 0.37	6.70 ± 0.24
D. maidis	Salivation	15	1[b]	—	—
	X-waveform	10	0	—	—
	Phloem ing.	9	9	0.62 ± 0.38	6.90 ± 0.23
	Nonvasc. prob.	14	1[b]	—	—
	Nonsieve element ingestion	22	9	0.38 ± 0.25	6.50 ± 0.71

[a]Five times a single droplet was excreted, but only when following periods of ingestion from phloem or nonsieve element tissue.
[b]One time a single droplet was excreted but this was following a period of ingestion from nonvascular tissue.

sheath was found terminated in the xylem. *G. nigrifrons*, *A. grex*, and *D. maidis* never produced rapid, low-pH droplets during ingestion waveforms in this study. However, in another study *G. nigrifrons* produced rapid, acidic droplets on young maize seedlings during the nonsieve element ingestion pattern (three- to four-leaf stage) (Wayadande 1991) and was thought to be xylem ingestion.

X-waveforms were produced in sequences. We adopted the terminology used by Heady and Denno (1991) for acoustic signals of planthoppers for describing the x-waveform sequences of these insects. *G. nigrifrons* (Fig. 2A), *A. grex* (Fig. 2B), and *G. oquaka* (Fig. 2C) sequences consisted of 5–25 repeated sections. Each section (1 section = 1 waveform) was comprised of two phrases, a smooth phrase and a spiking phrase (Fig. 3A). Section duration averaged 60 s and increased with each successive section culminating in either phloem ingestion or transition to another pattern. *D. maidis* x-waveform sequences (Fig. 2D) consisted of 80–120 repeated single phrase sections, each approximately 5–10 s, with one to three intermittent spikes throughout the sequence.

Two ingestion patterns followed x-waveforms. The phloem ingestion pattern of *G. nigrifrons*, *A. grex*, and *G. oquaka* consisted of a midamplitude, level waveform with regularly occurring spikes, one every 20–30 s (Figs. 2A–C). Between these spikes were four to eight smaller spikes. The *D. maidis* phloem ingestion pattern was also midamplitude but flat, without spikes or other characteristics (Fig. 2D). Unlike phloem ingestion, which was always preceded by x-waveforms, nonsieve element ingestion was preceded by salivation, x-waveforms, or nonvascular probing. Nonsieve element ingestion was distin-

guished from the phloem ingestion pattern by its low amplitude relative to the x-waveform midline (Fig. 1, see arrow).

Comparison of Probing Behavior on Maize and Johnsongrass

G. nigrifrons, *A. grex*, *G. oquaka*, and *D. maidis* displayed little difference in total probing duration during the 180-min access period ($F = 2.89$, df = 6,121; NS) regardless of host. *D. maidis* probed more frequently than other species on maize. Because of the high rate of probing, the average duration of *D. maidis* probes was correspondingly shorter than for other species ($F = 3.60$, $P < 0.005$). All leafhoppers salivated during probing (Table III), however, salivation duration was different among species. *A. grex* salivated significantly longer when feeding on maize than on johnsongrass and salivated longer than any other species (Table IV).

Significantly fewer *D. maidis* produced x-waveforms than *G. nigrifrons* (Table III). Host plant did not affect total duration of x-waveform behavior, however, there were differences among the four species probing maize. *G. oquaka* x-waveform sequences were shorter than *G. nigrifrons* sequences, whereas average *D. maidis* x-waveform sequences were longer (Table IV). Because leafhoppers often initiated more than one x-waveform sequence in the 180-min recording period, individual x-waveform sequences were analyzed separately. When examined individually, *D. maidis* x-waveform sequences were longer than those of *G. nigrifrons*, *A. grex*, and *G. oquaka* on maize (Fig. 4).

Phloem was the predominant ingestion site for *G. nigrifrons*, *A. grex*, and *G. oquaka*. On maize, phloem ingestion comprised 66% or more of all ingestion and more than 75% of ingestion on johnsongrass for these species. Not all leafhoppers ingested from phloem; but of those that did, there were significantly fewer *D. maidis* that ingested from phloem, compared to *G. nigrifrons* (Table III). Fewer *A. grex* and *G. oquaka* ingested from phloem than *G. nigrifrons*, however, these differences were not significant. The duration of phloem ingestion did not differ between species monitored on maize compared to johnsongrass, nor were there differences between the two hosts for each species (Table IV).

Frequency and duration of nonvascular probing did not differ between leafhopper species or between hosts. Of the leafhoppers monitored on maize, *D. maidis* ingested significantly longer from nonsieve element tissues than did other species. Only *G. nigrifrons* showed a host response by ingesting from nonsieve element tissues longer on maize than on johnsongrass (Table IV).

Feeding Transitions During Probing

Kinematic diagrams illustrating conditional probabilities of behavioral transitions were used to follow the sequence of behaviors during the 180-min recording period (Figs. 4–7). Leafhoppers behaved in a similar manner, thus a general

Table III. Number of *Graminella nigrifrons, Amblysellus grex, Graminella oquaka*, and *Dalbulus maidis* Leafhoppers Which Produced Salivation, X-Waveform, Phloem Ingestion, Nonvascular Probing, and Nonsieve Element Ingestion Patterns When Electronically Monitored for 180 min on Maize and Johnsongrass

Species	Host	*N*	Number of leafhoppers producing pattern*				
			Salivation	X-waveform	Phloem ingestion	Nonvascular probing	Nonsieve element ingestion
G. nigrifrons	Maize	19	19a	19a	18a	12a	17a
G. nigrifrons	Johnsongrass	18	18a	15ab	15a	14a	16a
A. grex	Maize	18	18a	12ab	11ab	16a	18a
A. grex	Johnsongrass	18	18a	16ab	12ab	16a	18a
G. oquaka	Maize	18	18a	14ab	10ab	14a	17a
G. oquaka	Johnsongrass	18	18a	14ab	12ab	15a	14a
D. maidis	Maize	19	19a	6 b	5 b	18a	17a

*Frequencies in columns followed by different letters are significantly different, confidence interval test, $\chi^2 = 12.590$, df = 6, $P < 0.05$.

Table IV. Average Time Probing, Salivating, Producing X-Waveforms, Ingesting from Phloem, Probing Nonvascular Tissues, and Ingesting from Nonsieve Element Tissues for *Graminella nigrifrons*, *Amblysellus grex*, *Graminella oquaka*, and *Dalbulus maidis* When Electronically Monitored for 180 min on Maize (M) and Johnsongrass (J)

			Mean ± SE (min) (*n*)[a]					
Species	Host	N^b	Total probing	Salivation	X-waveform	Phloem ingestion	Nonvascular probing	Nonsieve element ingestion
G. nigrifrons	M	19	171.1 ± 2.5a* (19)	14.3 ± 1.7a (19)	15.3 ± 1.7c (19)	96.9 ± 11.5abc (18)	30.0 ± 9.3a (12)	33.3 ± 8.4b (17)
G. nigrifrons	J	18	168.0 ± 3.4a (18)	12.2 ± 4.1a (18)	13.7 ± 1.6c (15)	127.5 ± 8.3c (15)	39.7 ± 13.3a (14)	10.3 ± 2.7a (16)
A. grex	M	18	155.8 ± 5.9a (18)	42.5 ± 4.3c (18)	14.5 ± 1.6bc (12)	64.7 ± 14.8a (11)	47.4 ± 10.6a (16)	19.4 ± 2.9ab (18)
A. grex	J	18	146.2 ± 8.6a (18)	31.5 ± 5.4b (18)	14.4 ± 2.8c (15)	89.1 ± 14.0ab (12)	35.4 ± 9.2a (16)	11.4 ± 2.7a (18)
G. oquaka	M	18	165.2 ± 4.8a (18)	30.3 ± 6.3b (18)	9.2 ± 2.1ab (13)	89.9 ± 19.3abc (10)	74.6 ± 15.0a (14)	20.7 ± 2.7b (17)
G. oquaka	J	18	151.1 ± 7.0a (18)	18.2 ± 4.6a (18)	8.0 ± 1.8a (13)	93.4 ± 15.2abc (12)	55.0 ± 12.0a (15)	14.4 ± 3.7ab (14)
D. maidis	M	19	163.5 ± 2.8a (19)	18.5 ± 2.8a (19)	27.7 ± 5.3d (6)	54.5 ± 13.5a (5)	58.7 ± 8.0a (18)	66.2 ± 7.9c (19)

[a] Number of insects recorded.

[b] Number of insects from which behavior was recorded.

*Means in columns followed by different letters are significantly different; least significant differences mean separation test. Total probing $F = 2.96$, df 6,121, $P < 0.05$; salivation $F = 6.06$, df = 6,121, $P < 0.000$; x-waveform $F = 4.91$, df 6.86, $P < 0.000$; phloem ingestion $F = 2.55$, df = 6.76, $P < 0.027$; nonvascular probing $F = 1.75$, df = 6,98, NS; nonsieve element ingestion $F = 14.80$, df = 6,112, $P < 0.000$.

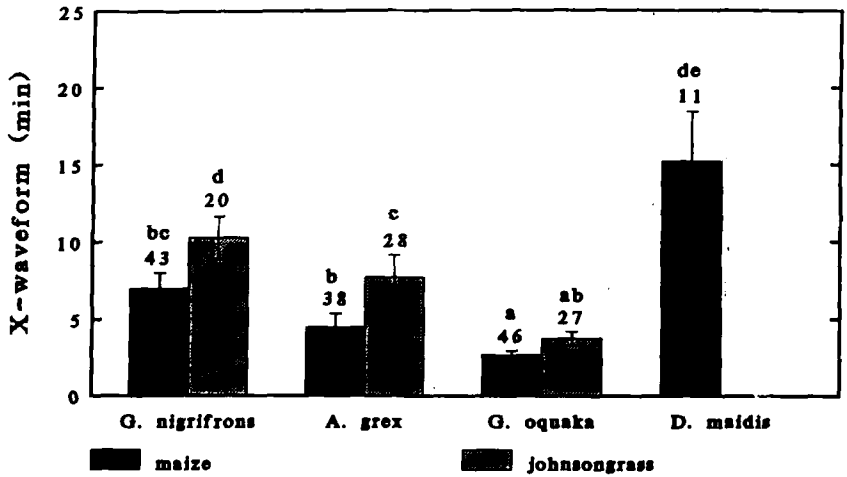

Fig. 4. Average time of x-waveform sequences (summed x-waveform durations/number of sequences performed) of *G. nigrifrons*, *A. grex*, *G. oquaka* and *D. maidis* electronically monitored for 180 min on maize and johnsongrass. *D. maidis* was monitored on maize only. Vertical lines indicate standard error of the mean, numbers indicate sample size, and means with different letters are significantly different ($F = 13.42$, df = 6,217, $P < 0.05$), least significant differences mean separation test.

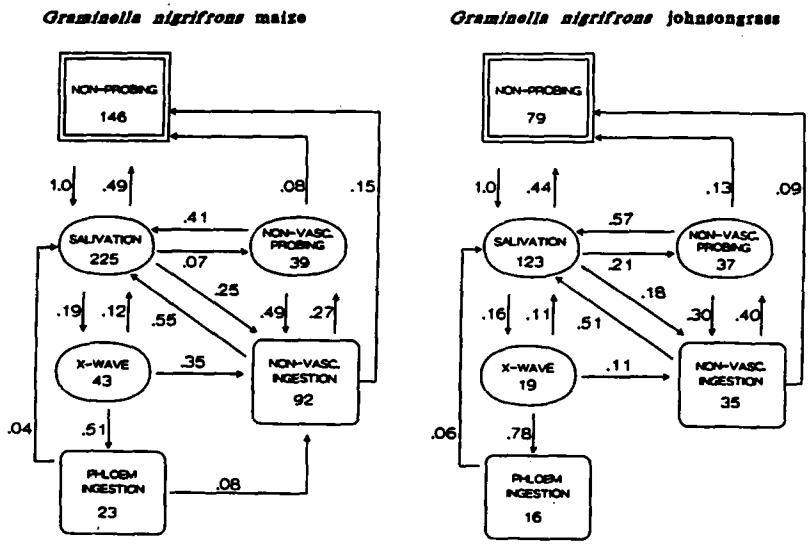

Fig. 5. Kinematic diagram of behavior transitions for *Graminella nigrifrons* electronically monitored for 180 min on maize ($N = 19$ leafhoppers) and johnsongrass ($N = 18$ leafhoppers). Values enclosed in circles and boxes are the number of times a behavior was recorded. Numbers by arrows are the proportion of insects changing from one behavioral state to another indicated by arrows. Transitions from nonprobing state (double-lined box) to probing state always began with salivation.

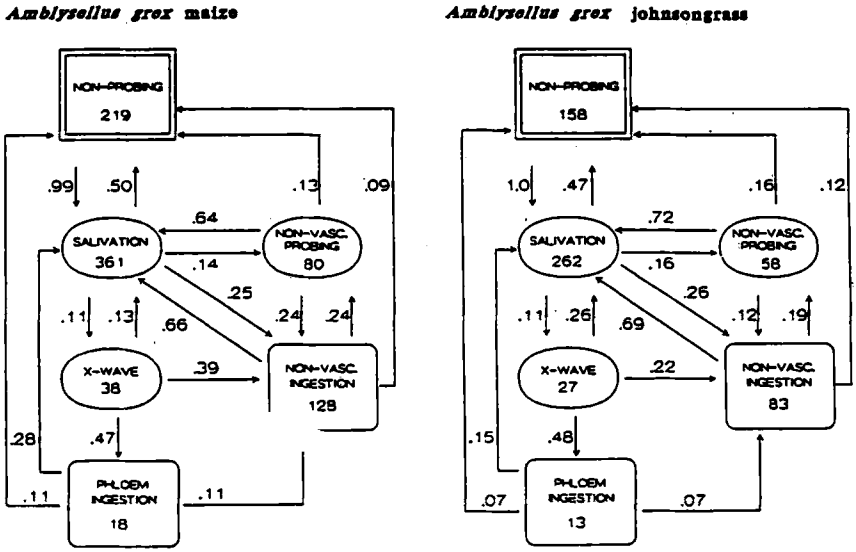

Fig. 6. Kinematic diagram of probing behavior transitions for 18 *Amblysellus grex* electronically monitored for 180 min on maize and johnsongrass. See the legend to Fig. 4 for more information.

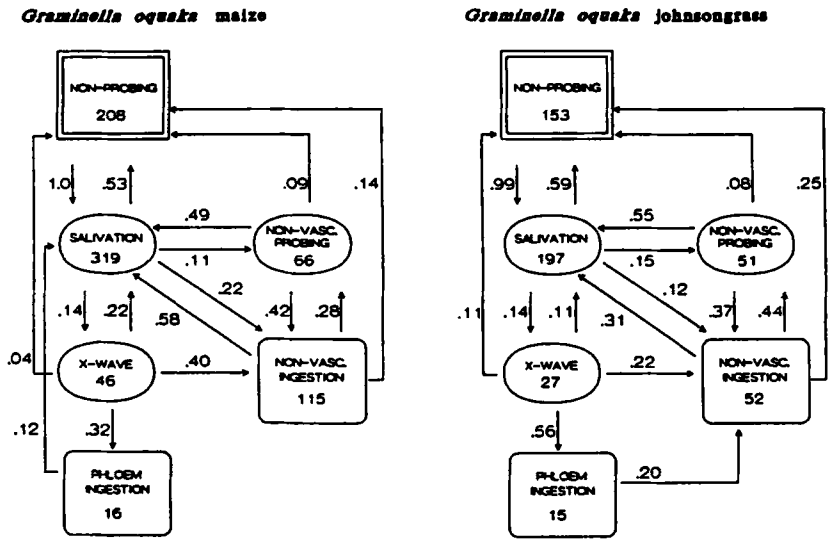

Fig. 7. Kinematic diagram of probing behavior transitions for 18 *Graminella oquaka* electronically monitored for 180 min on maize and johnsongrass. See the legend to Fig. 4 for more information.

description of transitions is applicable to all four species. For many leafhoppers, sustained phloem ingestion lasted several hours. If more than 30 min of sustained phloem ingestion was recorded, it was considered a terminal behavior. The type and order of behavior preceding x-waveform behavior were variable. Probes (stylet insertion into plant tissue) always began with salivation followed by nonvascular probing, nonsieve element ingestion, x-waveform behavior or by stylet withdrawal. Transitions between nonvascular probing and nonsieve element ingestion were common. Although probes may begin with a high degree of behavioral switching, the final sequence of behaviors culminating in sustained phloem ingestion was stereotypic: salivation always preceded x-waveforms, which always preceded phloem ingestion. However, phloem ingestion did not always follow x-waveform sequences. A significant proportion of transitions from x-waveforms was to nonsieve element ingestion or salivation for all species. The probability of changing from one behavior to another was about the same for most of the possible transitions for all species–host combinations (Figs. 5–8). However, there were some differences between species on the same host, as well as differences between hosts, especially for phloem-associated probing. Once an insect salivated, the probability of changing to x-waveform behavior was the same on maize and johnsongrass for *G. nigrifrons*, *G. oquaka*, and *A.*

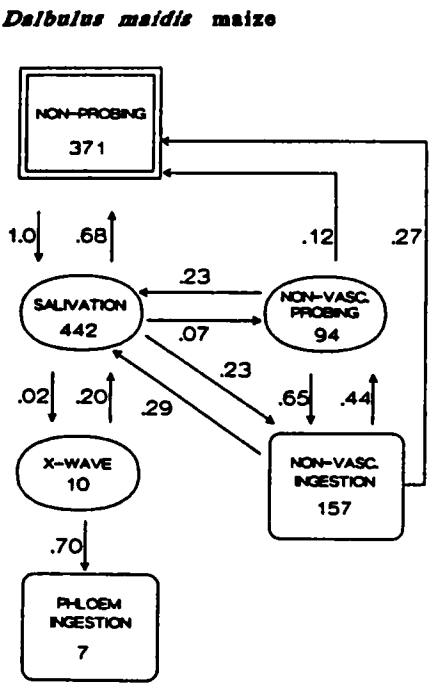

Fig. 8. Kinematic diagram of probing behavior transitions for 19 *Dalbulus maidis* electronically monitored for 180 min on maize. See the legend to Fig. 4 for more information.

grex (Figs. 5–7). However, *D. maidis* was much less likely to produce x-waveforms (Fig. 8) than *G. nigrifrons* on maize ($\chi^2 = 55.34$, df = 1, $P < 0.001$). *G. nigrifrons* feeding on johnsongrass was more likely to follow x-waveform behavior with phloem ingestion than when feeding on maize ($\chi^2 = 4.23$, df = 1, $P < 0.05$) (Fig. 5). There were no differences in x-waveform to phloem ingestion probabilities between maize and johnsongrass for *A. grex* and *G. oquaka*. There also were no differences between maize and johnsongrass in the probability of phloem ingestion continuing for more than 30 min for each species (e.g., *G. nigrifrons*-maize compared to *G. nigrifrons*-johnsongrass) or between *G. nigrifrons* and *D. maidis* on maize ($\chi^2 = 0.126$, df = 1, $P = 0.05$).

DISCUSSION

Previous electronic monitoring studies have shown that most sheath-feeding leafhoppers produce identical or nearly identical waveform patterns associated with salivation, phloem ingestion, or nonsieve element ingestion (Crane, 1970; Kawabe and McLean, 1978, 1980; Triplehorn *et al.*, 1984; Rapusas and Heinrichs, 1990). The leafhoppers in this study also produced similar patterns associated with these behaviors. In early studies with leafhoppers *Hordnia circellata* (Baker) (=*Graphocephala atropunctata*) (Crane, 1970) and *Macrosteles fascifrons* (=quadrilineatus) Stål (Kawabe and McLean, 1978) workers did not report x-waveforms. Thus, at first it was thought that, unlike aphids, leafhoppers did not produce x-waveforms prior to phloem ingestion. Later, this behavior (Xip) was described for *Nephotettix cinciteps* (Kawabe and McLean, 1980) and *G. nigrifrons* (Triplehorn *et al.* 1984). In our study, we show that *G. oquaka*, *A. grex*, and *D. maidis*, also produce x-waveforms.

The x-waveform was first described by McLean and Kinsey (1965) as a repeating pattern with an unknown associated probing behavior(s). Salivary sheaths of aphids interrupted during x-waveforms always terminated in phloem. McLean and Kinsey (1967) and McLean (1977) postulated that, during x-waveforms, aphids secrete enzyme-bearing watery saliva to prevent callose formation and/or to taste small quantities of phloem sap. The termination of stylets or salivary sheaths in the phloem suggests that x-waveform behavior is produced in response to phloem contact by aphids (Scheller and Shukle, 1986; McLean and Kinsey, 1967; Nault and Styer, 1972). In this study of leafhoppers, some of the salivary sheaths excised during *G. nigrifrons* x-waveforms did not terminate in the phloem, but rather in nearby cells. Also, not all x-waveforms were followed by phloem ingestion (Figs. 5–8). Thirty-five percent of *G. nigrifrons* x-waveforms were followed by nonsieve element ingestion, suggesting that the x-waveform pattern is not associated exclusively with phloem probing. Nevertheless, without exception, phloem ingestion was always preceded by

x-waveforms. We propose that x-waveform behavior is produced in response to chemical and mechanical stimuli received from sieve elements and perhaps these stimuli occasionally are present in nonvascular cells located near sieve elements.

The nonvascular probing waveform was described previously as a pattern of unknown behavioral activity by Kawabe and McLean (1978, 1980) and Sogawa (1973). This waveform was designated the "R" pattern by Rapusas and Heinrichs (1990) and interpreted as probing without ingestion because *N. virescens* did not excrete honeydew when the pattern was recorded. Similarly, we found that *G. nigrifrons* and *D. maidis* rarely produced honeydew when the pattern was recorded. We refer to the pattern as nonvascular probing because it reflects stylet position in plant tissues during probing without inferring whether or not ingestion or salivation occurs. This same pattern was indistinguishible among the four species in our study and from 10 other sheath-feeding leafhoppers and planthoppers (Kawabe and McLean, 1978; Kimmins, 1989; Wayadande, 1991). This pattern strongly resembles the I_b pattern produced by the mesophyll-feeding leafhopper, *Empoasca fabae* Harris (Wayadande and Backus, 1989). The I_b pattern was correlated with puncturing and draining of individual parenchyma cells by *E. fabae* feeding on faba bean leaves (Hunter and Backus, 1989). However, in our study, examination of cells in plant tissues penetrated by stylets during nonvascular probing were not damaged significantly, suggesting that puncturing and ingesting of cell contents did not occur.

Plasticity in Leafhopper Probing Behavior

Prior to electronic monitoring studies, little was known about leafhopper probing activity, other than what could be inferred from transmission of tissue-specific plant pathogens (Purcell, 1979; Tonkyn and Whitcomb, 1987, and references therein) and from light microscopic examination of plant tissues containing salivary sheaths (Smith and Poos, 1931; Day *et al.*, 1952; Alivizatos, 1982). The prevailing view that homopterans were specific with respect to tissue selection served as the basis for erecting three feeding guilds among the Homoptera: phloem feeders, xylem feeders, and mesophyll feeders (Tonkyn and Whitcomb, 1987). Although this classification may be useful for ecologically separating the Homoptera, it suggests that homopterans may be inflexible in their choice of probing sites. The present study shows that leafhoppers may ingest from several tissues but prefer one site over others. For example, *G. nigrifrons*, *G. oquaka*, and *A. grex*, ingested more from phloem than from other tissues, whereas *D. maidis* did not. Although the number of *D. maidis* that ingested from phloem was low and the proportion of probes that contacted phloem was also low, the likelihood of sustained ingestion was high ($P = 0.70$). It is possible that *D. maidis* requires a longer settling time than for the other species and that the 180-min access period we used was not long enough to show this.

Analysis of behavioral transitions has been used to describe insect courtship behavior (Phelan and Baker, 1990; Birch *et al.*, 1989; Hancock *et al.*, 1989) and host acceptance (Drost and Carde, 1989; Paynter *et al.*, 1990). Ullman and McLean (1988) were the first to apply conditional probabilities to homopteran feeding with pear psylla. They showed that although overall ingestion duration was the same from phloem, xylem, or nonvascular tissues, the psyllid showed a higher proability of sustained ingestion when probing from vascular tissues. Our analysis confirms speculation that homopteran tissue site selection is a nonrandom process (Ullman and McLean, 1988), and not the hit-or-miss energy strategy proposed by Day and McKinnon (1951). As a way to describe leafhopper feeding, conditional probabilities showed that there is a pattern of successive behaviors leading to phloem ingestion. No such pattern was apparent with nonsieve element ingestion. Furthermore, analysis of transitions showed that leafhoppers can probe and ingest from more than one tissue during the same probe, indicating that leafhoppers have flexibility in their choice of tissues during a probe. Flexibility allows leafhoppers to exploit several tissues and may be especially important when the insect is feeding on a suboptimal host (Khan and Saxena, 1985; Kimmins, 1989; Rapusas and Heinrichs, 1990).

Probing Behavior and Its Relationship to Vector Competency

There were no major differences in probing on maize and johnsongrass among the three MCDV vectors, including the *Panicum virgatum* specialist, *G. oquaka*. This result was surprising since *G. oquaka* transmitted MCDV from johnsongrass to johnsongrass but not maize to maize. Because *G. oquaka* survived well on johnsongrass but not maize, Nault and Madden (1988) speculated that failure of this leafhopper to transmit MCDV to maize was because it did not probe maize phloem. Probing frequency, total probing duration, and ingestion, specifically phloem ingestion, are often used as measures of host suitability for homopterans (Backus 1985). This study showed that maize and johnsongrass were acceptable experimental, short-term feeding hosts for *G. oquaka*. On johnsongrass, *G. oquaka* made fewer x-waveforms than on maize, but a higher proportion of these waveforms was followed by phloem ingestion, however, the difference was not significant (Fig. 7). Because these leafhoppers were monitored for only 180 min, extrapolation of these results to the 24-h acquisition and inoculation access periods used by Nault and Madden (1988) might not be appropriate. It is possible that forced long-term feeding on maize (48 total h) might have impaired the phloem-finding or feeding capacity of this species. Nevertheless, results from this study do not support the hypothesis that failure of *G. oquaka* to transmit MCDV from maize to maize is because the species fails to probe maize phloem.

MCDV is a phloem-associated virus (Ammar *et al.*, 1987) and it is likely that phloem-associated probing by leafhopper vectors is necessary for transmis-

sion. Most leafhopper species probe phloem (Tonkyn and Whitcomb, 1987), but not all phloem feeders are vectors; MCDV vector species are found primarily in the leafhopper tribe Deltocephalini and morphologically advanced Eucelini (Nault and Madden, 1988). Among the experimental vectors, including *A. grex* and *G. oquaka*, transmission efficiency varied but none were as efficient as *G. nigrifrons* (Nault and Madden, 1988). In another study, *A. grex* were 20% less likely to transmit MCDV from maize and johnsongrass than *G. nigrifrons* (Wayadande, 1991). In this study, we showed that 40% fewer *A. grex* and 50% fewer *G. oquaka* than *G. nigrifrons* located and ingested from phloem sieve elements (Table III). Although these differences were not statistically significant, they may be biologically important and suggest that fewer phloem contacts by these two leafhoppers species might result in less MCDV acquisition or inoculation compared to *G. nigrifrons*.

D. maidis contacted and ingested from phloem less often than *G. nigrifrons*. Lower phloem probing frequency or ingestion duration is unlikely to explain vector specificity, however, because this leafhopper transmits other phloem-limited pathogens, including maize rayado fino marafivirus (Gamez, 1988) and two corn stunting mollicutes (Nault, 1980). Moreover, *D. maidis* acquires and retains MCDV on the same attachment sites in the maxillary food canal and foregut as do vector species (Ammar and Nault, 1991). Thus, factors other than the ability to probe phloem and acquire virus is determining vector specificity. In another study we have shown that the x-waveforms of *D. maidis* and four other nonvector species are qualitatively distinct from those of five vector species (Wayadande and Nault, 1993). We speculate that a behavior associated with x-waveforms, extravasation (the expulsion of contents of the leafhoppers precibarium back through the maxillary food canal) may be infrequent or absent in nonvectors such as *D. maidis*. Extravasation is a behavior prerequisite to inoculation of foregut-borne viruses such as MCDV.

REFERENCES

Alivizatos, A. S. (1982). Feeding behavior of the spiroplasma vectors *Dalbulus maidis maidis* and *Eucelidius variegatus in vivo* and *in vitro*. *Ann. Inst. Phytopath. Benaki* **13:** 128–144.

Ammar, E. D., Gordon, D. T., and Nault, L. R. (1987). Ultrastructure of maize chlorotic dwarf virus infected maize and viruliferous leafhopper vectors. *Phytopathology* **77:** 1743 (abstract).

Ammar, E. D., and Nault, L. R. (1991). Maize chlorotic dwarf virus-like particles associated with the foregut in vector and non-vector leafhopper species. *Phytopathology* **81:** 444–448.

Auclair, J. L., Baldos, E., and Heinrichs, E. A. (1982). Biochemical evidence for the feeding sites of the leafhopper *Nephotettix virescens* within susceptible and resistant rice plants. *Insect Sci. Appl.* **3:** 29–34.

Backus, E. A. (1985). Anatomical and sensory mechanisms of planthopper and leafhopper feeding behavior. In Nault, L. R., and Rodriguez, J. G. (eds.), *The Leafhoppers and Planthoppers*, Wiley, New York.

Backus, E. A., and Bennett, W. H. (1992). New AC electronic insect feeding monitor for fine-structure analysis of waveforms. *Ann. Entomol. Soc. Am.* **85:** 437–444.

Birch, M., Lucas, C. D., and White, P. R. (1989). The courtship behavior of the cabbage moth, *Mamestra brassicae* (Lepidoptera: Noctuidae), and the role of male hair pencils. *J. Insect Behav.* **2:** 227–239.

Campbell, B. C., McLean, D. L., Kinsey, M. G., Jones, K. C., and Dreyer, D. L. (1982). Probing behavior of the greenbug, (*Schizaphis graminum*, Biotype C) on resistant and susceptible varieties of sorghum. *Entomol. Exp. Appl.* **31:** 140–146.

Crane, P. S. (1970). *The Feeding Behavior of the Blue-Green Sharpshooter*, Hordnia circellata *(Baker) (Homoptera: Cicadellidae)*, Ph.D. dissertation, University of California, Davis.

D'Arcy, C. J., and Nault, L. R. (1982). Insect transmission of plant viruses and mycoplasmalike and rickettsialike organisms. *Plant. Dis.* **66:** 99–104.

Day, M. F., and McKinnon, A. (1951). A study of some aspects of the feeding of the jassid *Orosius. Aust. J. Sci. Res.* **4:** 125–135.

Day, M. F. H. Irzykiewicz, and McKinnon, A. (1952). Observation on the feeding of the virus vector *Orosius argentatus* (Evans) and comparisons with certain other jassids. *Aust. J. Sci. Res.* **5:** 128–142.

Dorschner, K. W., and Baird, C. R. (1990). Electronically monitored feeding behavior of *Phorodon humuli* (Homoptera: Aphididae) on resistant and susceptible hop genotypes. *J. Insect Behav.* **2:** 437–447.

Drost, Y. C., and Carde, R. T. (1990). Influence of experience on the sequential and temporal organization of host-acceptance behavior in *Brachymeria intermdia* (Chalcidae), an endoparasite of gypsy moth. *J. Insect Behav.* **3:** 647–661.

Gamez, R., and Leon, P. (1988). Maize rayado fino and related viruses. 1988. In Koenig, R. (ed.), *The Plant Viruses*, Plenum, New York.

Gordon, D. T., and Nault, L. R. (1977). Involvement of maize chlorotic dwarf virus and other agents in stunting dieases of *Zea mays* in the United States. *Phytopathology* **67:** 27–36.

Hancock, R. G., Foster, W. A., and Yee, W. L. (1989). Courtship behavior of the mosquito, *Sabethes cyaneus* (Diptera: Culicidae). *J. Insect Behav.* **3:** 401–416.

Heady, S. E., and Denno, R. F. (1991). Reproductive isolation in *Prokelisia* planthoppers (Homoptera: Delphacidae): Acoustic differentiation and hybridization failure. *J. Insect Behav.* **4:** 367–390.

Hunter, W. B., and Backus, E. A. (1989). Mesophyll-feeding by the potato leafhopper, *Empoasca fabae*, (Homoptera: Cicadellidae): Results from electronic monitoring and thin-layer chromatography. *Environ. Entomol.* **18:** 465–472.

Kawabe, S., and McLean, D. L. (1978). Electronically recorded waveforms associated with salivation and ingestion behavior of the aster leafhopper *Macrosteles fascifrons* Stal (Homoptera: Cicadellidae). *Appl. Entomol. Zool.* **13:** 143–148.

Kawabe, S., and McLean, D. L. (1980). Electronic measurement of the probing activities of the green leafhopper of rice. *Entomol. Exp. Appl.* **27:** 77–82.

Khan, Z. R., and Saxena, R. C. (1985). Mode of feeding and growth of *Nephotettix virescens* (Homoptera: Cicadellidae) on selected resistant and susceptible rice varieties. *J. Econ. Entomol.* **78:** 583–587.

Kimmins, F. M. (1989). Electrical penetration graphs from *Nilaparvata lugens* on resistant and susceptible rice varieties. *Entomol. Exp. Appl.* **50:** 583–587.

McLean, D. L. (1977). An electrical measurement system for studying aphid probing behavior. In Harris, K. F., and Maramorosch, K. (eds.), *Aphids as Virus Vectors*, Academic Press, New York.

McLean, D. L., and Kinsey, M. G. (1965). Identification of electronically recorded curve patterns associated with aphid salivation and ingestion. *Nature* **205:** 1130–1131.

McLean, D. L., and Kinsey, M. G. (1967). Probing behavior of the pea aphid *Acyrthosiphon pisum*. I. Definitive correlation of electronically recorded waveforms with probing activities. *Ann. Entomol. Soc. Am.* **60:** 400–406.

Nault, L. R. (1980). Maize bushy stunt and corn stunt: A comparison of disease symptoms, pathogen host ranges, and vectors. *Phytopathology* **70:** 659–662.

Nault, L. R., and Ammar, E. D. (1989). Leafhopper and planthopper transmission of plant viruses. *Annu. Rev. Entomol.* **34:** 503–530.

Nault, L. R., and Madden, L. V. (1988). Phylogenetic relatedness of maize chlorotic dwarf virus leafhopper vectors. *Phytopathology* **78:** 1683–1687.

Nault, L. R., and Styer, W. E. (1972). Effects of sinigrin on host selection by aphids. *Entomol. Exp. Appl.* **15:** 423–437.

Paynter, Q. E., Anderbrant, O., and Schlyter, F. (1990). Behavior of male and female spruce bark beetles, *Ips typographus*, on bark of host trees during mass attack. *J. Insect Behav.* **3:** 529–543.

Phelan, P. L., and Baker, T. C. (1990). Comparative study of courtship in twelve Phycitine moths (Lepidoptera: Pyralidae). *J. Insect Behav.* **3:** 303–326.

Purcell, A. H. (1979). Leafhopper vectors of xylem-borne plant pathogens. In *Leafhopper Vectors and Plant Disease Agents*, Academic Press, New York.

Rapusas, H. R., and Heinrichs, E. A. (1990). Feeding behavior of *Nephotettix virescens* (Distant) on rice varieties with different levels of resistance. *Environ. Entomol.* **19:** 594–602.

Scheller, H. V., and Shukle, R. H. (1986). Feeding behavior and transmission of barley yellow dwarf virus by *Sitobion avenae* on oats. *Entomol. Exp. Appl.* **40:** 189–195.

Smith, F. F., and Poos, F. W. (1931). The feeding habits of some leafhoppers in the genus *Empoasca*. *J. Agr. Res.* **43:** 267–285.

Sokal, R. F., and Rohlf, F. J. (1969). *Biometry*, W. F. Freeman, San Franscisco.

Sogawa, K. (1973). The rice brown planthopper: Feeding physiology and host-plant interaction. *Annu. Rev. Entomol.* **27:** 49–74.

Tonkyn, D. W., and Whitcomb, R. F. (1987). Feeding strategies and the guild concept among vascular feeding insects and microorganisms. In Harris, K. F. (ed.), *Current Topics in Vector Research, Vol. 4*, Springer-Verlag, New York.

Triplehorn, B. W., Nault, L. R., and Horn, D. J. (1984). Feeding behavior of *Graminella nigrifrons* (Forbes). *Ann. Entomol. Soc. Am.* **77:** 102–107.

Ullman, D. E., and McLean, D. L. (1988). The probing behavior of the summer-form pear psylla. *Entomol. Exp. Appl.* **47:** 115–125.

Wayadande, A. C., and Backus, E. A. (1989). Feeding behavior of the potato leafhopper, *Empoasca fabae* (Homoptera: Cicadellidae) on chlordimeform- and phosmet-treated alfalfa. *J. Econ. Entomol.* **82:** 766–772.

Wayadande, A. C. (1991). *Studies of Leafhopper Probing Behavior and Its Role in Maize Chlorotic Dwarf Virus Transmission*, Ph.D. dissertation, Ohio State University, Columbus.

Wayadande, A. C., and Nault, L. R. (1993). Leafhopper probing behavior associated with maize chlorotic dwarf virus transmission to maize. *Phytopathology* **83:** 522–526.