Leafhopper Probing Behavior Associated with Maize Chlorotic Dwarf Virus Transmission to Maize

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ABSTRACT

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Maize chlorotic dwarf virus (MCDV) is a semipersistently transmitted, foregut-borne virus, transmitted in the field by the leafhopper, *Graminella nigrifrons*. When MCDV-inoculative *G. nigrifrons* were given access periods from 15 min to 4 h on maize test plants, there was a positive correlation between longer access and higher transmission. An electronic insect feeding monitor was used to evaluate components of leafhopper probing associated with MCDV inoculation and to compare the probing behaviors of five MCDV-vector and five nonvector leafhopper species. MCDV transmission occurred only when monitored *G. nigrifrons* produced x-waveforms (this waveform was recorded from all 16 monitored leafhoppers that transmitted MCDV). This characteristic waveform always was recorded prior to ingestion from phloem by leafhoppers. Furthermore, a positive association was found between longer x-waveform patterns and higher transmission rates. Time spent by these leafhoppers ingesting from phloem following x-waveforms did not increase transmission rate. Of 42 viruliferous leafhoppers that failed to transmit MCDV when monitored, 20 produced x-waveforms. Thus, phloem contact and x-waveform behaviors by inoculative leafhoppers does not always result in MCDV transmission. Cluster analysis of components of the complex x-waveform patterns of five MCDV vector species showed that they are similar to one another and distinct from the less complex waveforms produced by five leafhopper species that do not transmit MCDV. Extravasation (the expulsion of contents of the leafhopper's precibarium back through the maxillary food canal to the plant) is the behavior associated with leafhopper x-waveform that is thought to be responsible for inoculation of MCDV. We propose that *Dalbulus maidis* (previously shown to acquire, but not transmit MCDV) and other nonvector species fail to transmit MCDV because extravasation is qualitatively or quantitatively different from vector species or perhaps absent from the repertoire of behaviors associated with their x-waveforms.

Most leafhopper-borne plant viruses are persistently transmitted and have a circulative or propagative relationship with their vectors (22). In contrast, the maize chlorotic dwarf virus (MCDV) is one of only three semipersistent, foregut-borne viruses transmitted by leafhoppers (4,17,22,25). The principal field vector of MCDV is *Graminella nigrifrons* (Forbes) (10,25), but several other leafhopper species are experimental vectors. Nault and Madden (23) tested 25 species from the subfamily Deltocephalinae and found that nine transmitted MCDV. Moreover, they discovered that vector species were more closely related to one another than they were to nonvectors. Most Deltocephalinae leafhoppers from the tribe Deltocephalini and the morphologically advanced Eucelini were efficient vectors, provided that the virus test plant, maize (*Zea mays* L.), was a developmental host for leafhoppers. Deltocephalinae species from the less closely related, primitive Eucelini and Macrostelini were inefficient MCDV vectors or did not transmit the virus. Two hypotheses were proposed to explain these results. First, some leafhoppers, such as *G. fitchii* (Van Duzee), may not feed from the phloem where virus inclusions are found (1). Second, MCDV may not bind to attachment sites in the foreguts of nonvector species such as *Dalbulus maidis* (DeLong & Wolcott) that do feed in the phloem and transmit other phloem-limited viruses, e.g., maize rayado fino marafivirus (MRFV) (22).

Ammar and Nault (2) later found that binding is not the reason why *D. maidis* fails to transmit MCDV. They examined the foreguts of MCDV-exposed leafhoppers from three vector species and *D. maidis*. In all four species they found viruslike particles (VLPs) embedded in a densely staining matrix attached to the lining of the food canal, precibarium, cibarium, and pharynx. The VLPs were similar in size and shape to maize chlorotic dwarf virions. VLPs were not seen in leafhoppers exposed to MRFV-infected or healthy plants. The densely staining matrix is thought to be the putative helper component needed for leafhopper transmission of MCDV (13). These results demonstrated that virus acquisition and binding occur in *D. maidis* as they do in vector species and, therefore, other factors, perhaps those associated with inoculation feeding, might explain failure of this species to transmit MCDV.

Our previous work demonstrated that *D. maidis* and three MCDV vector species, *G. nigrifrons*, *G. oquaka* DeLong, and *Amblysellus grex* Oman, feed from nonvascular tissues as well as the phloem (31). Penetration of the phloem by the stylets was associated with the recording of x-waveforms, a characteristic, repeating pattern that always precedes phloem ingestion by aphids (5,19,20,24), leafhoppers (27,29), and planthoppers (16,30). We also reported that the x-waveform of *D. maidis* was different from that of the three vector species and suggested that perhaps behavior(s) associated with the x-waveform could explain vector specificity.

In this study, we present evidence that MCDV is transmitted to maize by inoculative *G. nigrifrons* when x-waveforms are recorded. We report that longer periods of phloem-associated, x-waveform probing result in higher transmission rates, and that x-waveforms of five MCDV leafhopper vector species are qualitatively similar to but are distinct from *D. maidis* and four other leafhopper species that do not transmit MCDV. Finally, we propose that the x-waveform behavior associated with MCDV inoculation is extravasation (21) and suggest that this behavior in vector species may be distinct from or may be absent in *D. maidis* and other nonvector species.

MATERIALS AND METHODS

Leafhopper and virus maintenance. Leafhoppers were reared in organdy-covered cages in a room held at 27 ± 2 C with a 16:8 L:D photoperiod. *G. nigrifrons, G. sonora* Ball, *Amblysellus grex*, and *Macrosteles quadrilineatus* Forbes, were reared on *Avena sativa* L., *G. oquaka* on *Panicum virgatum* L., and *Euceli-dius variegatus* (Kirschbaum) and *Stirellus bicolor* Van Duzee on *Lolium multiflorum* Lam. *D. maidis, D. quinquenotatus* DeLong and Nault, and *Ollarianus strictus* Ball were reared on maize (sweetcorn cultivar Aristogold Evergreen Bantam). Origin of leafhopper colonies was discussed previously (23). Voucher specimens of species used in this study are deposited at the Ohio State Collection of Insects and Spiders.

The isolate of the MCDV-type strain was originally obtained from johnsongrass rhizomes collected in 1972 (25). Virus was maintained in sweetcorn by inoculating three to four leaf seedlings every 2 wk with MCDV-exposed *G. nigrifrons*. Infected plants were used as a virus source 12–16 days after inoculation.

Inoculation access and MCDV transmission. To test for the relation of inoculation access time of *G. nigrifrons* to transmission rate of MCDV, *G. nigrifrons* females were placed on MCDV-infected source plants for a 48-h acquisition access period. Leafhoppers then were transferred individually to 5- × 15-cm tube cages placed over three to four leaf seedling corn, inbred OH28, for each of 1-, 2-, 4-, 8-, 16-, and 24-h inoculation access periods (IAP). For 15- and 30-min IAPs, leafhoppers were placed directly on plants and individually observed and timed before removal. Plants were placed in the greenhouse and rated for symptoms after 12–14 days. Twenty leafhoppers were tested for each time interval except for the first replication of 15 and 30 min, where only 10 were tested. The experiment was repeated three times. Percent transmission was calculated and correlated with IAP duration using Pearson's product moment correlation coefficient.

Electronic monitoring of leafhopper probing. Adult female *G. nigrifrons* were caged on MCDV-infected maize plants for a 48-h acquisition access period, then prepared for electronic monitoring. Leafhoppers were immobilized with a gentle vacuum and tethered to a 12-μm-diam, 2.5–3.0-cm-long gold wire glued to the pronotum with silver conductive paint (Ladd Industries, Burlington, VT). Leafhoppers were immediately placed on three or four leaf maize seedlings and electronically monitored using an insect feeding monitor (IFM; Scientific Instruments Laboratory, University of Missouri, Columbia). The IFM is an alternating current device that uses a differential amplifier in which the signal from the reference electrode (background noise) is subtracted from the insect electrode before amplification. A 70-mV input voltage with a 125-Hz carrier frequency was applied to the plant via an electrode embedded in the soil. Leafhoppers were recorded during an IAP for approximately 45 min or until a specific waveform(s) was produced. After recording the IAP, tethers were removed and leafhoppers were placed immediately on a second three to four leaf maize plant for a second 48-h IAP to determine if they were viruliferous. The test plants on which inoculation feeding was monitored and the second test plant from the nonmonitored feeding were placed in a greenhouse and rated for symptoms 12–14 days later. Behaviors associated with waveforms were determined previously by observing in which plant tissues salivary sheaths terminated and by recording the pH and rate of excretion of honeydew of IFM-monitored leafhoppers (31). Our results were consistent with those reported by others for leafhoppers (14,15, 27,29) and planthoppers (16,30).

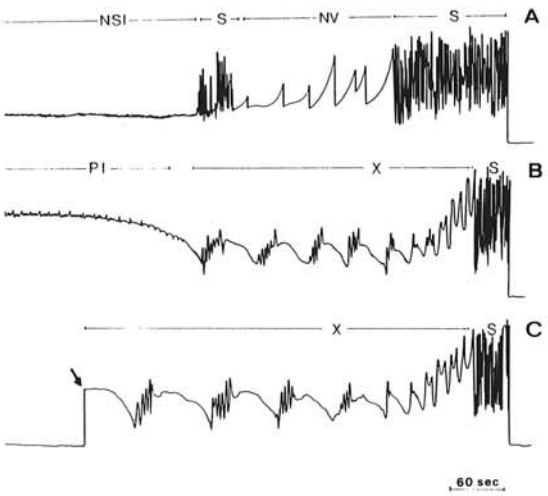

Fig. 1. Waveforms from *Graminella nigrifrons* leafhoppers recorded while feeding on maize. Waveforms are produced from right to left, bar insert = 60 sec. **A,** Leafhopper probe begins with salivation (S), then nonvascular probing (NV), then more salivation, and finally nonsieve element ingestion (NSI); **B,** leafhopper probe begins with salivation, then x-waveforms (X), and finally phloem ingestion (PI); **C,** leafhopper probe begins with salivation, then x-waveforms. Probe interrupted (arrow) before phloem ingestion could commence.

For analysis, leafhopper probing behaviors were placed into three groups: 1) probing that included penetration of and ingestion from nonvascular or xylem tissue (Fig. 1A); 2) probing that included phloem penetration (x-waveforms) and phloem ingestion (Fig. 1B); and 3) probing that included phloem penetration, but not phloem ingestion (Fig. 1C). Probe number and time of salivation, x-waveform behavior, phloem ingestion, and total probing duration of vectors and nonvectors were compared using Student's *t* tests. Probing behavior and its association with transmission was analyzed with chi-square contingency tests. Only leafhoppers that transmitted MCDV during the recorded IAP or second IAP were classified as viruliferous and included in the analyses.

X-waveform patterns were recorded from 10 deltocephaline leafhopper species. *D. maidis, O. strictus*, and *D. quinquenotatus* were recorded on maize. *G. sonora* and *M. quadrilineatus* were recorded on maize or oats. *E. variegatus* and *S. bicolor* were recorded on ryegrass, whereas *G. nigrifrons, G. oquaka,* and *A. grex* were recorded on maize or *Sorghum halapense* L. Since the x-waveform pattern for each leafhopper species does not differ from one host to another (31), patterns produced on different hosts can be compared. Terms to describe x-waveform patterns are taken from those used by workers for interpreting oscillographic waveforms produced by insect acoustic signals (12). An x-waveform sequence (Fig. 1B,C) typically has repeated sections. The following section characters were identified for each species: 1) sections with one or two phrases (Fig. 2A,B); 2) section duration increasing or not increasing in time later in the x-waveform sequence; 3) duration of the last complete section; 4) presence of major and/or minor spikes (Fig. 2A); 5) number of spikes per section, and 6) amplitude (< or > 10 mV) of the waveform around the midline (Fig. 2A,B). Although x-waves were infrequently produced by several of these species, at least six sequences from a minimum of three insects per species were included in the analysis. Euclidean distances from standardized means were calculated and used to form a complete-linked hierarchal dendogram (cluster tree) using the Cluster option in Systat, Inc. (33).

RESULTS

Inoculation access and MCDV transmission. *G. nigrifrons* transmitted MCDV at all time intervals with transmission rate increasing with longer IAPs (Fig. 3). Based on these results, a 30- to 60-min IAP was selected for subsequent studies for electronically monitored IAPs. Longer periods were not considered because of the large number of insects needed to obtain an adequate sample of recorded inoculative leafhoppers. Also, longer feeding periods resulted in more switching from one feeding behavior to another, e.g., from phloem feeding to mesophyll feeding and then back to phloem feeding, making it difficult to know which behavior was associated with MCDV inoculation.

Probing behavior associated with MCDV transmission. Fifty-eight of 148 *G. nigrifrons* tested in this study transmitted MCDV

either during the monitored IAP or during the second, unmonitored 48-h IAP. Fourteen leafhoppers transmitted MCDV during both the monitored and unmonitored IAP. As indicated earlier, only data from viruliferous insects were considered in the analyses (Table 1). We did not notice qualitative differences in feeding behavior of the 90 leafhoppers excluded from the study compared to the 58 included in our analysis.

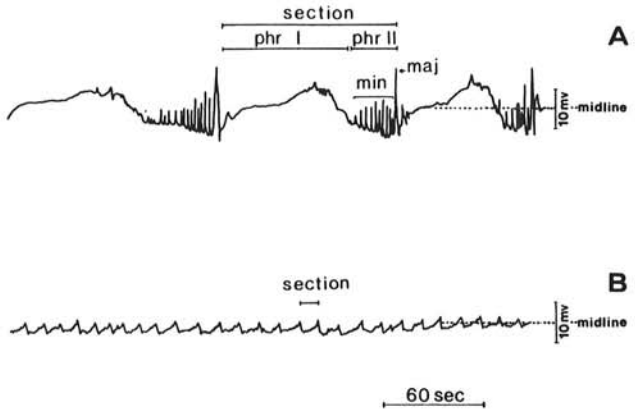

Fig. 2. Middle sections of x-waveform sequences of **A,** *Graminella nigrifrons* and **B,** *Dalbulus quinquenotatus* electronically monitored on maize. Patterns are produced from right to left, bar insert = 60 sec, Phr I = smooth phrase, Phr II = spiking phrase, min = minor spikes, and maj = major spike. Note amplitude around section midline and number of phrases and spikes per section.

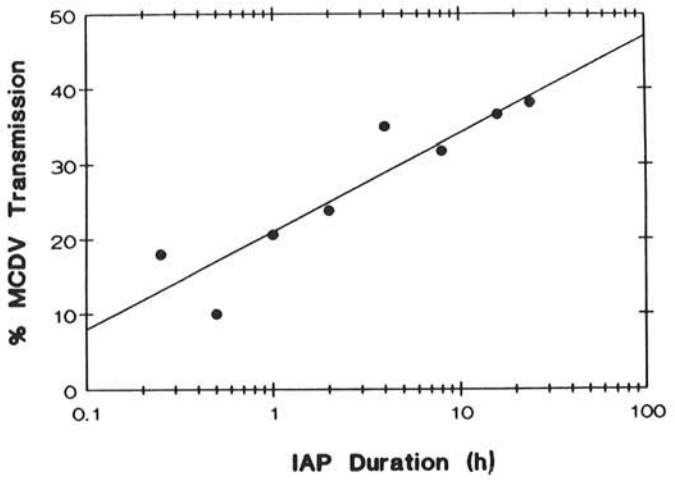

Fig. 3. Mean transmission rate of single *Graminella nigrifrons* females exposed to MCDV-infected maize for 24 h followed by a 0.25-, 0.50-, 1-, 2-, 4-, 8-, 16-, and 24-h inoculation access period on maize test plants. $N = 50$ for 0.25 and 0.50 h; $N = 60$ for all others. Pearson's product moment, $R = 0.91$, $P < 0.05$.

Sixteen of the 58 viruliferous *G. nigrifrons* transmitted MCDV during the monitored IAP, all of which probed phloem, indicating that MCDV inoculation was dependent on phloem contact ($X^2 = 12.53$, df = 1, $P = 0.001$). Twenty leafhoppers that produced x-waveforms did not transmit MCDV. All 22 leafhoppers which salivated and probed nonvascular tissues or xylem but not phloem failed to transmit virus during the monitored IAP. X-waveform duration of the 16 monitored transmitters was significantly longer than that of the 20 viruliferous, monitored nontransmitters, but there were no differences in mean probe number, time of salivation, or phloem ingestion between the two groups (Table 1).

Half of the 16 leafhoppers that transmitted MCDV were interrupted before the x-waveform sequence was completed and before phloem ingestion could begin. These leafhoppers were compared to leafhoppers allowed to continue to probe and ingest from phloem. Transmission rate by interrupted leafhoppers did not differ from those allowed to ingest from the phloem ($X^2 = 0.36$, df = 1, $P = 0.20$). There was no difference in x-waveform duration between interrupted leafhoppers ($n = 8$, mean = 14.7 min) and those allowed to ingest from phloem ($n = 8$, mean = 13.4 min) ($F = 0.15$, $P = 0.70$), thus, the x-waveform durations of viruliferous leafhoppers that did and did not transmit virus when monitored were pooled for further comparisons. The 36 insects that produced x-waveforms were grouped by duration of x-waveforms (0–5 min, 5–10 min, 10–15 min, and > 15 min) and correlated with transmission rate. Transmission rates for these groups were 1 of 8, 3 of 12, 8 of 10, and 4 of 6, respectively. Linear trend analysis (8) showed that longer x-waveform patterns were associated with higher transmission rates ($X^2 = 6.83$, df = 1, $P < 0.01$).

Analysis of MCDV vector and nonvector x-waveforms. Representative 3-min middle segments of x-waveform sequences from five leafhopper species that transmit MCDV (*G. nigrifrons, G. oquaka, G. sonora, A. grex,* and *S. bicolor*) and five species that do not (*D. maidis, D. quinquenotatus, M. quadrilineatus, E. variegatus,* and *O. strictus*) are illustrated in Figure 4. MCDV vector species all produced x-waveforms with multiple high-amplitude (>10 mV), biphrasic sections with two or more major spikes per section (mean = 6.28; SE = 0.44 min). X-waveform sequences varied between 5 and 15 min, and the number of sections per sequence ranged from 4 to 20. Section time mean was 1.21 (SE = 0.28) min, and section duration increased in length over time. In contrast, for species that do not transmit MCDV, x-waveforms typically were low-amplitude (<10 mV), repeated monophrasic sections without spikes. Section time (mean = 0.31, SE = 0.32 min) was shorter than for vector species, and section duration did not increase in length over time. The two *Dalbulus* species produced similar x-waveform patterns (Fig. 4); however, *D. quinquenotatus* produced longer sequences composed of 3–4× the number of monophrasic sections than did *D. maidis*. Some *D. quinquenotatus*, but not *D. maidis*, sequences lasted over 1 h and contained several hundred sections. Generally, *O. strictus* and *M. quadrilineatus* x-waveform sequences consisted of short, repeated humps or peaks, which lasted several seconds to several minutes. *E. variegatus* x-waveforms shared few characteristics

TABLE 1. Number of probes, salivation, x-waveform behavior, phloem ingestion, and total probing time of electronically monitored, viruliferous *Graminella nigrifrons* that did or did not transmit maize chlorotic dwarf virus (MCDV) during inoculation test feeds on maize seedlings of up to 45 min

Type of *Graninella nigrifrons*	Leafhoppers (no.)	Probes (mean no.)	Mean (min) ± SE (*N*)* Salivation	X-waveform	Phloem ingestion	Total probing duration
Transmitters	16	6.5 ± 1.0[y] (16)[z]	9.0 ± 1.3a (16)	14.3 ± 1.6a (16)	19.5 ± 4.3 a (8)	42.9 ± 2.4 a (16)
Nontransmitters	42	4.6 ± 0.6a (42)	8.3 ± 0.9a (42)	7.8 ± 1.1b (20)	15.2 ± 3.0a (8)	34.8 ± 1.8 b (42)
t value, *P*		1.78 0.08	0.38 0.70	3.23 0.003	0.72 0.48	2.49 0.016

[y] Means in columns followed by different letters are significantly different, Student's *t*-test.
[z] Number of leafhoppers performing behavior.

with those produced by other species (Fig. 4). Cluster analysis of the six x-waveform characteristics showed significant differences between the MCDV vectors, *G. nigrifrons, G. sonora, A. grex, G. oquaka,* and *S. bicolor*, and the nonvectors, *D. maidis, D. quinquenotatus, M. quadrilineatus, O. strictus,* and *E. variegatus*. Vectors clustered into one major branch and nonvector species into another (Fig. 5).

DISCUSSION

Results from timed inoculation-feeding periods for *G. nigrifrons* were similar to an earlier report by Choudhury and Rosenkranz (7) that showed that longer inoculation access by vectors resulted in higher MCDV-transmission rates. However, we report higher transmission rates for 15- and 30-min inoculation access. The probable reason for this is that we used single *G. nigrifrons* and recorded inoculation access only when leafhoppers were in contact with plants. Choudhury and Rosenkranz (7) used groups of leafhoppers on caged plants. They did not observe whether insects settled immediately on plants or elsewhere in the cage. This may explain why they reported such low transmission rates for IAPs under 30 min. The increase in transmission rate with increase in inoculation access feeding is typical for what has been reported for semipersistently transmitted foregut-borne closteroviruses, badnaviruses, and caulimoviruses (17,18,26). It is generally assumed that longer inoculation-feeding periods increase the probability of phloem contact and, hence, transmission rate (18).

In this study we showed that *G. nigrifrons* must probe phloem to transmit MCDV but that not all inoculative leafhoppers that contact the phloem transmit virus. Phloem-associated probing consists of two major components, x-waveform behavior and phloem ingestion (31). Our results show that the behaviors associated with x-waveform production are essential to transmission of MCDV and that inoculation of this virus does not occur during ingestion from the phloem. To our knowledge, this is the first report in which the significance of separating x-waveform behaviors from phloem ingestion is recognized relative to transmission of phloem-limited viruses. For example, the aphid *Sitobion avenae* (F.) transmitted barley yellow dwarf luteovirus more often when it penetrated two to three sieve elements compared to a single phloem contact (28). Although barley yellow dwarf luteovirus is a circulative virus and is transmitted when aphids salivate in the phloem, these authors did not specify whether phloem ingestion was excluded in the analysis of the data.

Behaviors associated with homopteran x-waveforms are largely unknown. McLean and Kinsey (20) speculated that aphid x-waveforms represent bouts of salivation and fluid uptake. Aphids secrete salivary enzymes, some of which may deactivate P proteins responsible for callose formation in the sieve plate. X-waveform peaks are thought to be associated with salivation, whereas the plateau (smooth) region may be uptake of plant sap through the food canal (19,20). Imbibed plant sap is sampled when it passes over the precibarial chemosensilla (3,32), and in leafhoppers, sensory cues received during x-waveform may inform the leafhopper that its stylets have penetrated to the phloem (31). At present, there is no direct evidence that x-waveform behavior consists of salivation and sampling of phloem sap.

The inoculation of MCDV during the x-waveform is evidence

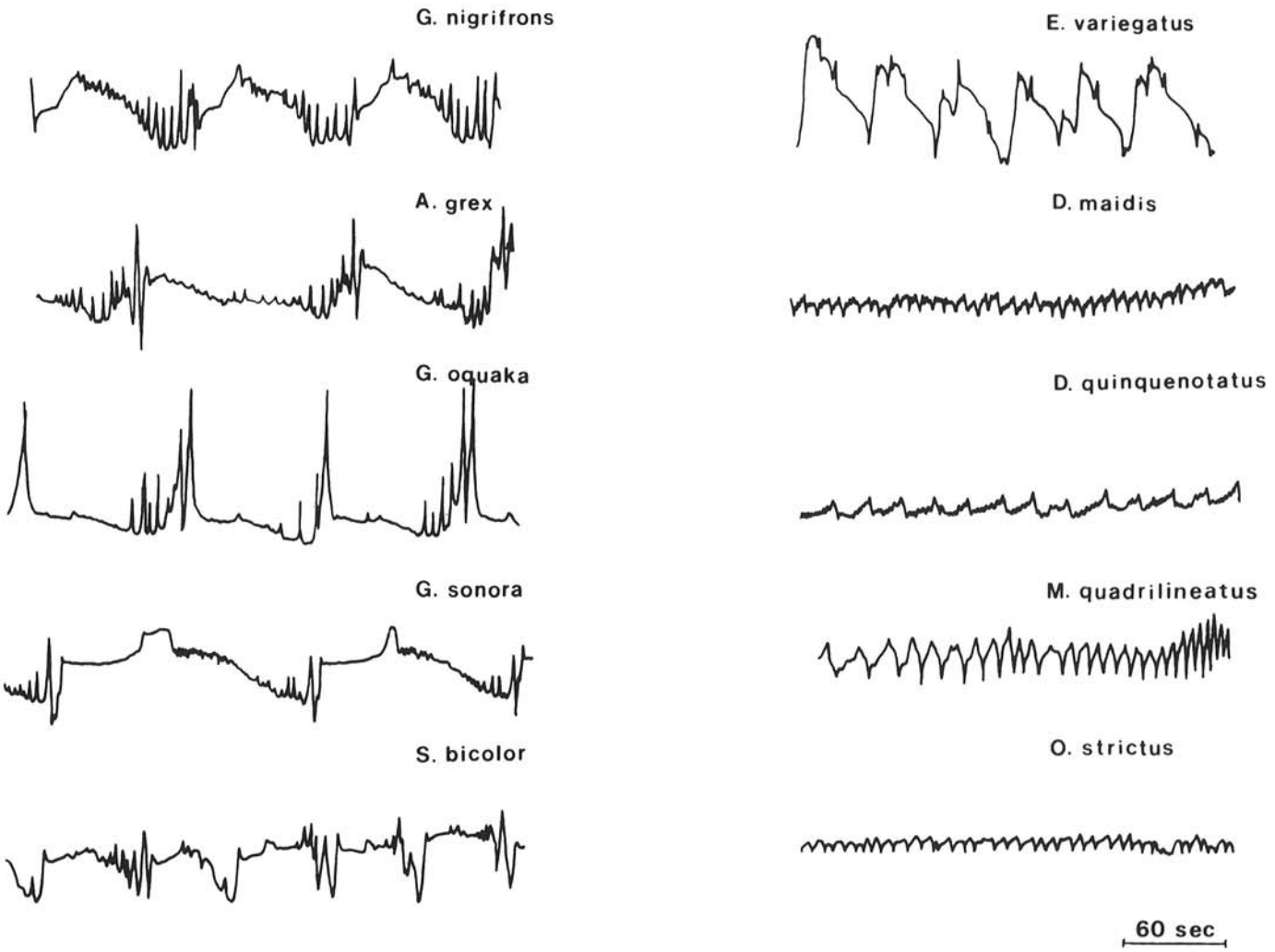

Fig. 4. Representative excerpts of center sections from x-waveform sequences of electronically monitored vector leafhoppers, *Graminella nigrifrons, Amblysellus grex, Graminella oquaka, Graminella sonora, Stirellus bicolor*, and nonvector leafhoppers *Eucelidius variegatus, Dalbulus maidis, Dalbulus quinquenotatus, Macrosteles quadrilineatus*, and *Ollarianus strictus*. Waveforms are produced from right to left, bar insert = 60 sec.

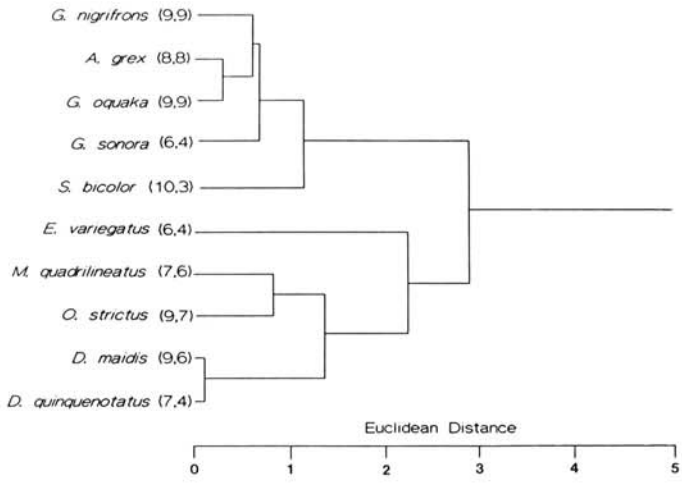

Fig. 5. Hierarchal dendrogram of MCDV vectors *Graminella nigrifrons, Amblysellus grex, Graminella oquaka, Graminella sonora, Stirellus bicolor*, and nonvectors *Dalbulus maidis, Dalbulus quinquenotatus, Euscelidius variegatus, Ollarianus strictus*, and *Macrosteles quadrilineatus* based upon cluster analysis of x-waveform characteristics. First number in parenthesis is number of sequences analyzed, second number is number of leafhoppers that produced sequences.

that extravasation is a behavior associated with the waveform. Extravasation is defined as the expulsion of contents from the precibarium and food canal (21). The mechanics of extravasation are not understood, but it is thought to be caused when the cibarium (sucking pump) collapses and the precibarial valve piston opens, expelling fluid within the buccal and food canal through the stylets and back into plant tissue. For MCDV inoculation to occur, virions attached to the foregut cuticle and food canal (2,6) must first detach and then be expelled by extravasation (9,11,21).

Analysis of 10 leafhopper species shows that the x-waveforms of MCDV vectors are more complex and differ significantly from those of leafhoppers that fail to transmit MCDV. Thus, underlying behaviors represented by the x-waveforms produced by these two groups are likely to be different. Vector species of the semipersistently transmitted, rice tungro spherical virus, *Nephotettix virescens* (Distant) and *N. cincticeps* (Uhler), produce biphrasic waveforms similar to those of MCDV vectors, suggesting that the same behaviors are responsible for transmission of both viruses. We propose that the reason that *D. maidis* and perhaps the other four nonvector leafhopper species we studied do not transmit MCDV is because extravasation is qualitatively or quantitatively different in these species or perhaps missing from the repertoire of behaviors associated with their x-waveforms. This hypothesis awaits further experimentation.

LITERATURE CITED

1. Ammar, E. D., Gordon, D. T., and Nault, L. R. 1987. Ultrastructure of maize chlorotic dwarf virus infected maize and viruliferous leafhopper vectors (Abstr.). Phytopathology 77:1743.
2. 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.
3. Backus, E. A. 1985. Anatomical and sensory mechanisms of leafhopper and planthopper feeding behavior. Pages 163-194 in: The Leafhoppers and Planthoppers. L. R. Nault and J. G. Rodriguez, eds. Wiley Press, New York.
4. Brunt, A., Crabtree, K., and Gibbs, A. 1990. Viruses of Tropical Plants. CAB International, Redwood Press, Whiltshire, UK.
5. Campbell, B. C., McLean, D. L., Kinsey, M. G., Jones, K. C., and Dreyer, D. L. 1992. Probing behavior of the greenbug (*Schizaphis graminum*, biotype C) on resistant and susceptible varieties of sorghum. Entomol. Exp. Appl. 31:140-146.
6. Childress, S. A., and Harris, K. F. 1989. Localization of virus-like particles in the foreguts of viruliferous *Graminella nigrifrons* leafhoppers carrying the semipersistent maize chlorotic dwarf virus. J. Gen. Virol. 70:247-251.
7. Choudhury, M. M., and Rosenkranz, E. 1983. Vector relationship of *Graminella nigrifrons* to maize chlorotic dwarf virus. Phytopathology 73:685-690.
8. Cochran, W. G. 1954. Some methods for strengthening the common X^2 tests. Biometrics 10:417-441.
9. Garrett, R. G. 1972. Non-persistent aphid-borne viruses. Pages 476-492 in: Viruses and Invertebrates. A. J. Gibbs, ed. North-Holland Publishing Co., Amsterdam.
10. Gordon, D. T., and Nault, L. R. 1977. Involvement of maize chlorotic dwarf virus and other agents in stunting diseases of *Zea mays* in the United States. Phytopathology 67:27-36.
11. Harris, K. E., Treur, B., Tsai, J., and Toler, R. 1981. Observations of leafhopper ingestion-egestion behavior: Its likely role in the transmission of noncirculative viruses and other plant pathogens. J. Econ. Entomol. 74:446-453.
12. Heady, S. E., and Denno, R. F. 1991. Reproductive isolation in Prokelesia planthoppers (Homoptera: Delphacidae): Acoustic differentiation and hybridization failure. J. Insect Behav. 4:367-390.
13. Hunt, R. E., Nault, L. R., and Gingery, R. E. 1988. Evidence for infectivity of maize chlorotic dwarf virus and for a helper component in its leafhopper transmission. Phytopathology 78:499-504.
14. Kawabe, S., and McLean, D. L. 1978. Electronically recorded waveforms associated with salivation and ingestion behavior of the aster leafhopper, *Macrosteles fascifrons* Stål (Homoptera: Cicadellidae). Appl. Entomol. Zool. 13:143-148.
15. Kawabe, S., and McLean, D. L. 1980. Electronic measurement of the feeding activities of the green leafhopper of rice. Entomol. Exp. Appl. 27:77-82.
16. Kimmins, F. M. 1989. Electrical penetration graphs from *Nilaparvata lugens* on resistant and susceptible rice varieties. Entomol. Exp. Appl. 50:69-79.
17. Ling, K. C., and Tiongco, E. R. 1979. Transmission of rice tungro virus at various temperatures: A transitory virus-vector interaction. Pages 349-366 in: Leafhopper Vectors and Plant Disease Agents. K. Maramorosch and K. F. Harris, eds. Academic Press, New York.
18. Matthews, R. E. F. 1991. Plant Virology, 3rd ed. Academic Press, New York.
19. McLean, D. L. 1977. An electrical measurement system for studying aphid probing behavior. Pages 277-290 in: Aphids as Virus Vectors. K. F. Harris and K. Maramorosch, eds. Academic Press, New York.
20. 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.
21. McLean, D. L., and Kinsey, M. G. 1984. The precibarial valve and its role in the feeding behavior of the pea aphid, *Acyrthosiphon pisum*. Bull. Entomol. Soc. Am. 30:26-31.
22. Nault, L. R., and Ammar, E. D. 1989. Leafhopper and planthopper transmission of plant viruses. Annu. Rev. Entomol. 34:503-530.
23. Nault, L. R., and Madden, L. V. 1988. Phylogenetic relatedness of maize chlorotic dwarf virus leafhopper vectors. Phytopathology 78:1683-1687.
24. Nault, L. R., and Styer, W. E. 1972. Effects of sinigrin on host selection by aphids. Entomol. Exp. Appl. 15:423-437.
25. Nault, L. R., Styer, W. E., Knoke, J. K., and Pitre, H. N. 1973. Semipersistent transmission of leafhopper-borne maize chlorotic dwarf virus. J. Econ. Entomol. 66:1281-1273.
26. Raccah, B., Loebenstein, G., and Bar-Joseph, M. 1976. Transmission of citrus tristeza by the melon aphid. Phytopathology 66:1102-1104.
27. Rapusas, H. R., and Heinrichs, E. A. 1990. Feeding behavior of *Nephotettix virescens* (Homoptera: Cicadellidae) on rice varieties with different levels of resistance. Environ. Entomol. 19:594-602.
28. Scheller, H. V., and Shukle, R. N. 1986. Feeding behavior and transmission of barley yellow dwarf virus by *Sitobion avenae* on oats. Entomol. Exp. Appl. 40:189-195.
29. Triplehorn, B. W., Nault, L. R., and Horn, D. J. 1984. Feeding behavior of *Graminella nigrifrons* (Forbes). Ann. Entomol. Soc. Am. 77:102-107.
30. Velusamy, R., and Heinrichs, E. A. 1986. Electronic monitoring of feeding behavior of *Nilaparvata lugens* (Homoptera: Delphacidae) on resistant and susceptible rice cultivars. Environ. Entomol. 15:678-682.
31. Wayadande, A. C. 1991. Studies of leafhopper probing behavior and its role in maize chlorotic dwarf virus transmission. Ph.D. dissertation. The Ohio State University, Columbus.
32. Wensler, R. J., and Filshie, B. K. 1969. Gustatory sense organs in the food canal of aphids. J. Morphol. 129:473-492.
33. Wilkinson, L. 1989. Systat. Evanston, IL. Systat, Inc.