

Phylogenetic Relatedness of Maize Chlorotic Dwarf Virus Leafhopper Vectors

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ABSTRACT

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Twenty-five leafhopper (Cicadellidae) species from 13 genera representing three tribes (Deltocephalini, Euscelini, and Macrostelini) in the subfamily Deltocephalinae were tested as vectors of the semipersistently transmitted maize chlorotic dwarf virus (MCDV). Vectors and their estimated percent transmission by single insects when maize served as the virus source and inoculation test plant were: *Graminella nigrifrons*, 35.9%; *Amblysellus grex*, 24.8%; *Stirellus bicolor*, 13.7%; *Planicephalus flavocostatus*, 12.9%; *Exitianus exitiosus*, 12.6%; *G. sonora*, 10.5%; *Macrostes severini*, 1.9%; and *Endria inimica*, 1.5%. Leafhopper species that did not transmit MCDV from maize to maize were *Baldulus tripsaci*, *Cicadulina mbila*, nine *Dalbulus* species, *Euscelidius variegatus*, *G. fitchii*, *G. oquaka*, *M. fascifrons*, *Ollarianus strictus*, and *Psammotettix lividellus*. When johnsongrass rather than maize was used as a virus source and test plant, *G. oquaka* transmitted MCDV. When the relationship between

transmission rate of MCDV by a leafhopper species and its phylogenetic (evolutionary) relatedness to *G. nigrifrons* (the principal field vector) was evaluated by Kendall's tau correlation analysis, there was a significant positive relationship for phylogeny with one ($P < 0.05$), but not a second ($P > 0.10$) proposed phylogeny, when all leafhopper species used in this study were considered. When grass-specializing leafhoppers whose developmental hosts that do not include maize were excluded from the analysis, both phylogenies were significantly correlated ($P < 0.01$) with MCDV transmission. From this study, it can be predicted that leafhopper species from the tribes Deltocephalini or recent (advanced) Euscelini that use maize as a feeding and breeding host have a high probability of being MCDV vectors, whereas leafhoppers from those taxa that do not feed well on maize or those from the primitive Euscelini or Macrostelini, even if maize is a preferred host, have a low probability of being vectors.

Additional keywords: semipersistent transmission, vector specificity, *Zea mays*.

Vector specificity has long been regarded as an important characteristic in the transmission of plant viruses. In his discussion of the criteria available for classifying plant viruses, Matthews (16) considers the type of arthropod vector as well as the mechanism of transmission to be stable characters in delineating major groups or subgroups of viruses. Vector specificity is well documented at the family level for homopteran-transmitted viruses. However, specificity at intrafamily levels for these viruses seldom has been seriously considered or explored.

An examination of current information compiled on leafhopper-transmitted plant viruses illustrates the difficulty in recognizing intrafamily vector specificity (3,17). Of the approximately 60 recognized Cicadellidae subfamilies (23), only the Deltocephalini, with 16 vector genera, and the Agalliinae, with three vector genera, are known to have species that transmit plant viruses (17). This apparently narrow spectrum of known vectors among the Cicadellidae may not reflect the true situation in nature, since failure of leafhoppers from other subfamilies to transmit plant viruses has been inadequately documented. Several reasons account for the current limited knowledge of leafhopper vectors. Of 21 well-characterized plant viruses transmitted by leafhoppers, 16 have hosts exclusively among the Gramineae (17). This explains why the Deltocephalinae, a leafhopper group abundant on the grasses (33), is the best represented vector subfamily among the leafhoppers. Experimental factors also contribute to the paucity of knowledge of leafhopper virus vectors. For example, investigators seldom test leafhopper species not found on virus-infected crop plants for their ability to transmit viruses because they recognize that candidate leafhopper vectors must be able to feed on the phloem of a plant in order to transmit phloem-restricted viruses (17). This contrasts with many aphid-borne, nonpersistently

transmitted viruses that are acquired or inoculated during superficial test probes in the epidermis (18), thereby allowing aphids that do not feed long or breed on the virus host plant to be tested as vectors. A final problem in testing leafhopper vectors is that considerable time, space, and manpower are needed to maintain laboratory colonies of large numbers of species.

Previously, *Graminella nigrifrons* (Forbes), a common species in maize (*Zea mays* L.) fields (6,7,25,28), was identified as an efficient vector of the maize chlorotic dwarf virus (MCDV) and considered to be the primary vector in the field (20). The region of MCDV occurrence in the United States is defined by the overlapping area of frequent occurrences of johnsongrass, *Sorghum halepense* (L.), the MCDV overwintering host, and *G. nigrifrons* (9). Nault et al (20) showed that *G. sonora* Ball (formerly *Deltocephalus*) is an experimental vector of MCDV but *Dalbulus maidis* (DeLong and Wolcott), *D. elimatus* (Ball), and *Macrostes fascifrons* (Stål) do not transmit the virus. The latter three species belong to a different tribe than does *Graminella*, suggesting that specificity of MCDV transmission might be defined at the tribal, if not the generic level. The objective of this study was to test a wide spectrum of leafhopper species as vectors of MCDV to determine if vector specificity can be described according to phylogenetic relatedness of leafhoppers.

MATERIALS AND METHODS

Collection and rearing of leafhoppers. Leafhoppers selected for this study were grass-feeding species from the tribes Deltocephalini, Euscelini, and Macrostelini. Voucher specimens of these leafhopper species were deposited in The Ohio State University Collection of Insects and Spiders. Data with voucher specimens include the locality, time of collection, and the collector of specimens used to start laboratory colonies. Whenever possible, maize was used as an ovipositional and developmental host for

leafhoppers even for species not normally found in the field on this plant (Table 1). Four species (*Endria inimica* (Say), *Graminella fitchii* (Van Duzee), *G. oquaka* DeLong, and *Psammotettix lividellus* (Zetterstedt)) could not be reared on maize. Cages and procedures used to rear leafhoppers were described previously (5).

Transmission of MCDV by leafhoppers. Two to four virus-infected source plants were placed in a rearing cage with 50–150 adult insects. Source plants were inoculated with MCDV-inoculative *G. nigrifrons* 12–14 days before the test (11,20). Lower leaves not showing MCDV symptoms were removed from virus source plants to maximize the opportunity for leafhoppers to feed on infected tissues. Leafhoppers were given a 48-hr acquisition access period (AAP) on source plants, then survivors were placed five per plant (three per plant for *G. nigrifrons*) on Oh 28 maize at the two- to three-leaf stage for a 48-hr inoculation access period (IAP). All species that use maize as a developmental host were observed to settle and feed on maize during the AAP and IAP. The four species that do not use maize as a developmental host did not settle as readily during the AAP and IAP. After the IAP, leafhoppers were removed manually with an aspirator, the test plants were sprayed with a pyrethroid insecticide to kill any leafhoppers that escaped manual removal, and the plants were placed in a greenhouse to await symptom development. Symptoms appeared as early as 5 days after inoculation and were recorded 14 days after inoculation.

Leafhopper species were tested for MCDV transmission in three to seven trials, depending on the availability of adults. A minimum

of 300 adults of each species were tested for ability to transmit MCDV, thus assuring detection of species capable of transmitting virus to 1% or more of test plants, $P = 0.05$ (27). In all leafhopper transmission trials (except for *G. fitchii* and *G. oquaka*), transmission by *G. nigrifrons* was included as a check.

In a separate trial to test for transmission of MCDV by *G. oquaka*, johnsongrass was used as a virus source and test plant. Thirty MCDV-inoculative *G. nigrifrons* were used to inoculate two johnsongrass seedlings. Both johnsongrass plants showed MCDV symptoms 14 days later. Sixty adult *G. oquaka* were given access to the infected plants for 48 hr (all survived) and then transferred 12 per plant to five healthy johnsongrass seedlings for a 48-hr IAP. Similar tests were not performed for the remaining three leafhopper species that could not be reared on maize (*E. inimica*, *G. fitchii*, and *P. lividellus*) since they also do not feed on johnsongrass. Transmission rates for single leafhoppers were estimated with the procedure described by Swallow (29).

Leafhopper phylogeny and analysis of the data. Two similar phylogenies were used to compare relatedness of leafhoppers to transmission rate of MCDV. One phylogeny and the placement of species into tribes (Fig. 1A) was based on comments by Nielson (21–23), who considers the Macrostelini to be the most primitive and Deltocephalini to be the most advanced among the three Deltocephalinae tribes used in this study. A second phylogeny and placement of species (Fig. 1B) was based on comments and characters provided by Oman (24), Linnavuori (14), Wagner (32), and Whitcomb et al (33). The tribal placements of *P. lividellus* and

TABLE 1. Field and laboratory hosts for 25 leafhopper species from the subfamily Deltocephalinae, tested for ability to transmit maize chlorotic dwarf virus (MCDV)

Leafhopper species	Field hosts ^a	Laboratory hosts ^b	Transmission by ^c Test species		Transmission by ^c <i>Graminella nigrifrons</i> check	
			<i>N</i>	<i>p</i>	<i>N</i>	<i>p</i>
<i>Amblysellus grex</i>	grass and forb generalist	<i>Avena sativa</i> , <i>Zea mays</i>	335	0.248(0.058)	234	0.453(0.190)
<i>Balduhus tripsaci</i>	<i>Tripsacum dactyloides</i>	<i>T. dactyloides</i> , <i>Z. mays</i>	310	0	234	0.246(0.020)
<i>Cicadulina mbila</i>	grass generalist	<i>Z. mays</i>	310	0	180	0.190(0.017)
<i>Dalbulus charlesi</i>	<i>Tripsacum</i> spp.	<i>Z. mays</i>	300	0	177	0.317(0.076)
<i>D. chiapensis</i>	<i>Tripsacum</i> spp.	<i>T. dactyloides</i> , <i>Z. mays</i>	300	0	180	0.367(0.059)
<i>D. elimatus</i>	<i>Zea</i> spp.	<i>Z. mays</i>	315	0	240	0.435(0.190)
<i>D. gelbus</i>	<i>Zea</i> & <i>Tripsacum</i> spp.	<i>Z. mays</i>	300	0	264	0.284(0.066)
<i>D. guevarai</i>	<i>Zea</i> & <i>Tripsacum</i> spp.	<i>Z. mays</i>	300	0	177	0.317(0.076)
<i>D. longulus</i>	<i>Zea</i> & <i>Tripsacum</i> spp.	<i>Z. mays</i>	300	0	177	0.211(0.005)
<i>D. maidis</i>	<i>Zea</i> spp.	<i>Z. mays</i>	300	0	180	0.481(0.260)
<i>D. quinquenotatus</i>	<i>Tripsacum</i> spp.	<i>Z. mays</i>	300	0	264	0.197(0.021)
<i>D. tripsacoides</i>	<i>Tripsacum</i> spp.	<i>T. maizar</i> , <i>Z. mays</i>	310	0	237	0.224(0.014)
<i>Endria inimica</i>	pooid grasses	<i>A. sativa</i> , <i>Secale cereale</i>	415	0.015(0.008)	240	0.347(0.063)
<i>Euscelidius variegatus</i>	grass and forb generalist	<i>S. cereale</i> , <i>A. sativa</i> , <i>Z. mays</i>	335	0	237	0.278(0.054)
<i>Exitianus exitiosus</i>	grass generalist	<i>A. sativa</i> , <i>Z. mays</i>	325	0.126(0.028)	240	0.427(0.192)
<i>Graminella fitchii</i>	<i>Leersia oryzoides</i>	<i>L. oryzoides</i>	300	0	... ^d	... ^d
<i>G. nigrifrons</i>	grass generalist	<i>A. sativa</i> , <i>Z. mays</i>	761	0.359(0.046)	NA ^e	NA
<i>G. oquaka</i>	<i>Panicum virgatum</i>	<i>P. virgatum</i>	300	0	... ^d	... ^d
<i>G. sonora</i>	grass generalist	<i>A. sativa</i> , <i>Z. mays</i>	305	0.105(0.008)	177	0.264(0.071)
<i>Macrosteles fascifrons</i>	grass and forb generalist	<i>A. sativa</i> , <i>Z. mays</i>	310	0	237	0.278(0.047)
<i>M. severini</i>	grass and forb generalist	<i>A. sativa</i> , <i>Z. mays</i>	600	0.019(0.009)	240	0.469(0.144)
<i>Ollarianus strictus</i>	grass and forb generalist	<i>Z. mays</i>	315	0	237	0.336(0.82)
<i>Planicephalus flavocostatus</i>	grass generalist	<i>A. sativa</i> , <i>Z. mays</i>	300	0.129(0.032)	310	0.454(0.273)
<i>Psammotettix lividellus</i>	pooid grasses	<i>A. sativa</i> , <i>S. cereale</i>	300	0	417	0.291(0.038)
<i>Stirellus bicolor</i>	grass generalist	<i>S. cereale</i> , <i>A. sativa</i> , <i>Z. mays</i>	345	0.137(0.037)	234	0.322(0.091)

^a Field hosts from Whitcomb et al (33) or authors' observations.

^b When two or more hosts listed, the first host species noted is favored for oviposition and development.

^c Leafhoppers were tested in from three to seven trials to total number of insects indicated. After a 2-day acquisition access period (AAP) on MCDV-infected maize, test species were placed five per plant on maize test plants for a 2-day inoculation access period (IAP). *G. oquaka* was a vector when johnsongrass rather than maize was used as a virus source and test plant. *G. nigrifrons* was used as a check to be certain that a given batch of source plants contained virus and that other conditions were conducive to MCDV transmission. *G. nigrifrons* were given the same AAP and IAP but placed three per plant on test plants. N = number leafhoppers tested, p = transmission rate for single insects, $1 - (1 - I)^{1/k}$ in which I is the proportion of infected plants and k is the number of insects per plant (29). Number in parentheses is the standard error of p .

^d No simultaneous checks were tested.

^e Not applicable.

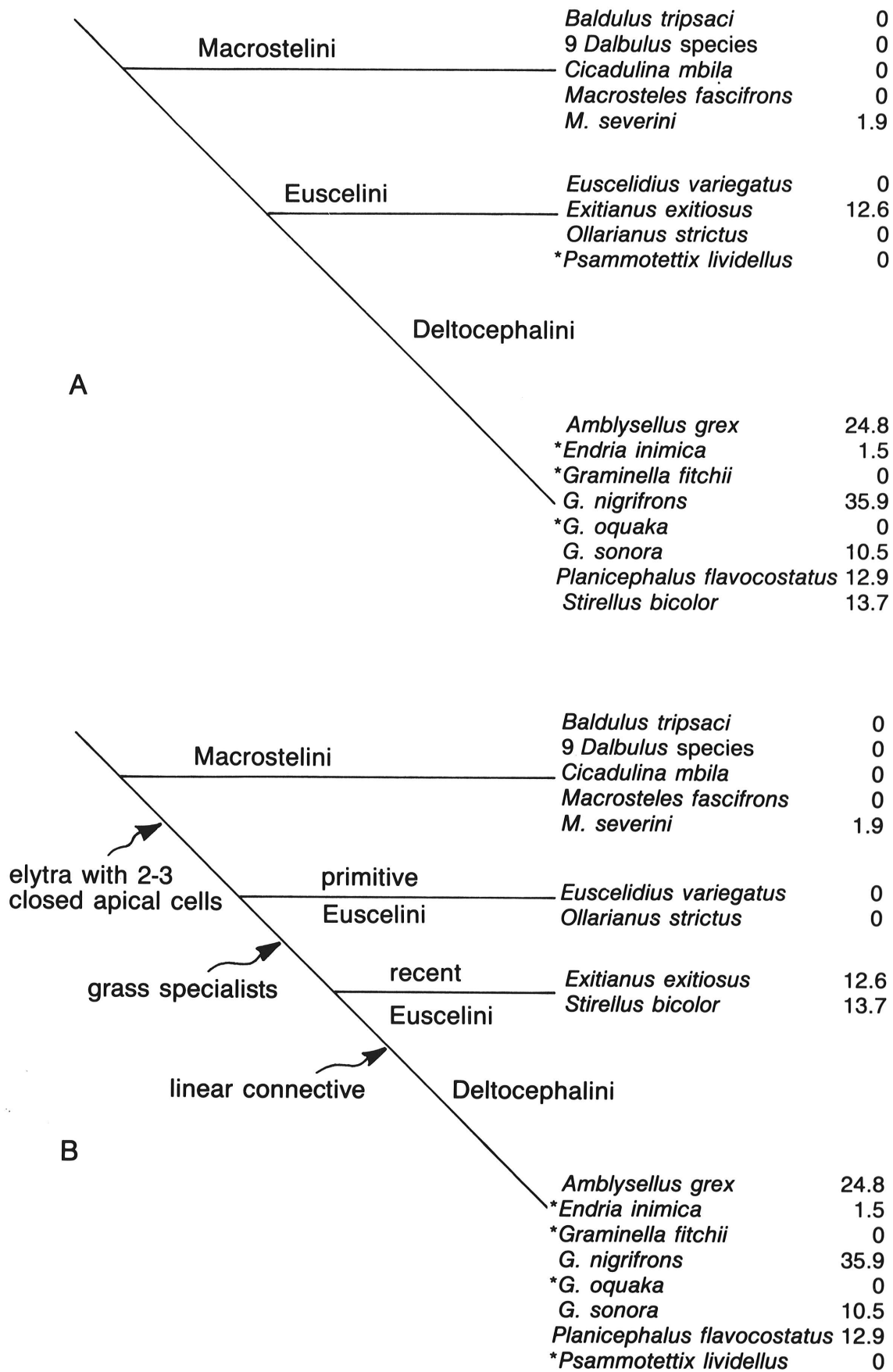


Fig. 1. Phylogenetic relationships of leafhopper species tested as vectors of maize chlorotic dwarf virus. Species that are grass specialists and whose host range do not include maize are preceded by an asterisk. Leafhopper tribes are arranged from most primitive (Macrostelini) to most advanced (Deltocephalini). Numbers after species names are estimated percent transmission of MCDV (from maize to maize) by single insects taken from Table 1. *G. oquaka* is a vector when johnsongrass rather than maize is used as a virus source and test plant. **A**, Phylogeny and placement of species based on Nielson (21–23). **B**, Phylogeny and placement of species based on Oman (24), Linnavuori (14), Wagner (32), Whitcomb et al (33) and Blocker (*personal communication*). Ancestral species have elytra with one closed apical cell, feed on forbs and/or grasses and have a Y-shaped connective.

S. bicolor differ in the two phylogenies. Both phylogenies are based on the assumption that the Deltocephalini is a monophyletic group (i.e., has single common ancestor). The relationship between transmission rate of MCDV by a leafhopper species and its phylogenetic distance from *G. nigrifrons* or the status of maize as a host plant was evaluated using Kendall's tau correlation, a nonparametric analog of the traditional Pearson's product-moment correlation (8). In this analysis, all leafhopper species from the Deltocephalini were considered most closely and equally related to *G. nigrifrons*, leafhoppers from the Macrostelini most distantly related, and those from the Euscelini intermediate between the other tribes. It should be noted that the phylogenies used in this analysis are based on a suite of morphological and ecological characters and should be regarded as preliminary.

RESULTS AND DISCUSSION

The efficient vector, *G. nigrifrons*, was tested simultaneously with other species; it transmitted MCDV in all tests (Table 1). Of 24 additional leafhopper species tested, seven transmitted MCDV from maize to maize (Table 1, Fig. 1). The rate of transmission for *G. nigrifrons* was higher than for all other vector species. The eight vector species were from seven genera and all three Deltocephalinae tribes tested. When all leafhopper species tested were considered, there was a significant correlation with one of the two phylogenies and transmission rate of MCDV (Table 2). When leafhopper species considered as grass specialists (33) and whose developmental host ranges do not include maize were excluded from the analysis, the relationship of the remaining leafhoppers from both phylogenies were significantly correlated with MCDV transmission rate. There was no significant correlation between MCDV transmission rate and leafhopper species whose developmental host do or do not include maize (Table 2). This is because species whose developmental hosts include maize varied tremendously in transmission rate (0–35.9%). Although analysis of host status did not significantly correlate with MCDV transmission, the importance of host is clearly illustrated by results with *G. oquaka*. When maize was used as both a virus source and test plant, none of 300 leafhoppers transmitted MCDV, but when *S. halepense* was used as a virus source and test plant, groups of 12 *G. oquaka* transmitted MCDV to four of five test plants resulting in an estimated transmission rate by single leafhoppers of 12.6% (29). Johnsongrass, a panacoid, is taxonomically closer to *Panicum virgatum* L., the field host of *G. oquaka* (33) than it is to maize, an andropogonoid. It is speculated that the reason *G. oquaka* does not transmit MCDV from maize is that it does not feed at all or only briefly from maize phloem or vascular parenchyma, whereas it feeds from these tissues in johnsongrass. The ability of a leafhopper species to transmit MCDV likely

depends on its ability to feed in the phloem of its host, as does *G. nigrifrons* in maize and johnsongrass (31), where MCDV mainly occurs (1).

Previous studies on two other leafhopper-borne viruses suggest that the vectors of maize streak virus (MSV) but not maize rayado fino virus (MRFV) may be phylogenetically related. MSV, a circulatorily transmitted geminivirus, is transmitted by nine *Cicadulina* species (4,26) but not by seven other Deltocephalinae species from other genera (10). The propagatively transmitted MRFV is transmitted by some but not all *Dalbulus* species (19; Nault, unpublished). Other Macrostelini tested were *B. tripsaci*, that was a MRFV vector, and *M. fascifrons* that was not. The Deltocephalini *G. nigrifrons* and the Euscelini *S. bicolor* were also MRFV vectors.

In another study where a phylogenetically diverse array of potential vectors were selected, Zettler (34) demonstrated that aphids from the subfamily Aphidinae were better vectors of bean common mosaic virus (BCMV) than were aphids from the subfamilies Callaphidinae or Chaitophorinae. Differences in transmission of BCMV were attributed, in part, to differences in probing behavior between Aphidinae and non-Aphidinae species.

Recent findings that explain how MCDV is transmitted by its vectors offer insight as to why phylogenetic relatedness may be important for MCDV transmission. Hunt et al (11) demonstrated that *G. nigrifrons* can transmit purified MCDV acquired through Parafilm membranes if they are first fed on plants infected with another MCDV isolate, but not if they fed first on healthy plants or plants infected with the aphid-borne maize dwarf mosaic virus. These results are similar to those reported in earlier studies with aphid-borne potyviruses (12) and cauliflower mosaic virus (15) that suggested these viruses require a virus-coded helper component for their transmission. The helper components, probably glycoproteins (30), most likely serve to bind virus reversibly to attachment sites on the stylets or foreguts of aphid vectors (2). In a separate study of MCDV, Ammar et al (1) observed viruslike particles embedded in a dense matrix attached to the cuticular linings of the precibarium, cibarium, pharynx, and esophagus of MCDV-inoculative *G. nigrifrons* but not in non-MCDV-exposed control leafhoppers. The viruslike particles and densely staining material observed in vectors are similar to virus inclusions seen in MCDV-infected plants (1). We postulate that the physical and chemical properties of the linings of the foreguts are more similar in closely related leafhoppers than to those of distantly related ones and that helper component and virus attachment to the foregut depends on these cuticular properties.

Although seven additional leafhopper species were identified in this study as vectors of MCDV, none are thought to contribute significantly to the field spread of MCDV in maize. In the region of the United States where MCDV is endemic, populations of the most common of these species (*E. exitiosus*, *S. bicolor*, and *P. flavocostatus*) in maize or nearby grasses are low compared with *G. nigrifrons* and the non-MCDV vector, *M. fascifrons* (7,25). However, the grass generalist, *A. grex*, that ranked second in transmission efficiency to *G. nigrifrons* would be of special concern if MCDV were introduced to the western United States. Because this Deltocephalini species, along with johnsongrass, is abundant in California and other Western states (13), the potential for spread exists.

One benefit of knowing that the leafhopper vectors of MCDV are phylogenetically related is the ability to predict the spread of MCDV should it be accidentally introduced into a new region. For example, if MCDV were introduced to the African continent, it would not be expected that *C. mbila* or any other *Cicadulina* species would be vectors, despite the fact that they are abundant grass generalists (26). Rather, this study suggests that common grass-feeding Deltocephalini or recent Euscelini species would be important field vectors.

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TABLE 2. Kendall tau correlation (8) between transmission rate of maize chlorotic dwarf virus (MCDV) and phylogenetic relatedness of leafhoppers or the status of maize as a host plant for leafhoppers

Trait correlated with MCDV transmission rate ^a	Observations	Correlation	Significance (P)
Phylogeny 1, includes all leafhopper species	17	0.48	<0.05
Phylogeny 1, does not include selected grass specialists ^b	13	0.70	<0.01
Phylogeny 2, includes all leafhopper species	17	0.37	>0.10
Phylogeny 2, does not include selected grass specialists ^b	13	0.68	<0.01
Host status of maize ^c	17	0.30	>0.20

^a All leafhoppers and their transmission rates included in analysis, except that the nine *Dalbulus* species were lumped together for a 0% transmission rate to avoid overly weighting the Macrostelini. Phylogeny 1 is from Figure 1A, Phylogeny 2 is from Figure 1B.

^b Does not include transmission rates from grass specialists whose host range does not include maize.

^c Comparison of leafhopper species that use maize as a developmental host with those that use maize only as a short term feeding host (see Figure 1).

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