Evidence for Infectivity of Maize Chlorotic Dwarf Virus and for a Helper Component in Its Leafhopper Transmission

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ABSTRACT

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The infectivity of maize chlorotic dwarf virus (MCDV) was demonstrated indirectly by in vivo neutralization of infectivity in its leafhopper vector, *Graminella nigrifrons*, and directly by leafhopper transmission of purified virus acquired through parafilm membranes. In neutralization of infectivity tests, transmission of MCDV by viruliferous leafhoppers was reduced (more than eightfold) if they were allowed to feed through parafilm membranes on a suspension containing MCDV antiserum or its IgG fraction compared to control leafhoppers fed on preimmune serum. Leafhoppers given access to membranes containing suspensions of crude, clarified, or concentrated extracts of MCDV or purified virus did not transmit virus to healthy maize plants. However, transmission of a purified isolate (MCDV-white stripe) acquired by membrane feeding occurred if leafhoppers had previously fed on plants infected with another isolate (MCDV-mild), but not if they had first fed on healthy plants or plants infected with maize dwarf mosaic virus. Reversing the feeding sequence, when leafhoppers first fed on membranes containing MCDV-white stripe and then on plants infected with MCDV-mild, they did not transmit MCDV-white stripe. These experiments strongly suggest that a helper component produced in MCDV-infected plants is necessary for the transmission of MCDV by its leafhopper vectors. Use of the term "transitory" by some authors to describe the semipersistent transmission of MCDV and the related rice tungro viruses is discussed.

Additional key words: virus-vector relationships.

Although the maize chlorotic dwarf (MCD) disease was discovered more than 16 years ago, our understanding of the etiology of the disease is incomplete (18,19). A putative virus particle, maize chlorotic dwarf virus (MCDV), has been isolated from MCD-diseased plants and extensively characterized, but Koch's postulates, as modified for viruses (2), have not been fulfilled. Attempts to transmit purified MCDV particles by mechanical inoculation have been unsuccessful (14). Because MCDV is semipersistently transmitted by its leafhopper vectors, *Graminella nigrifrons* (Forbes) and *G. sonora* (Ball) (36), hemocoelic injection of purified virus has not and is not likely to result in transmission. Transmission of purified MCDV fed to vectors through membranes, a proven technique for transmission of certain aphid-borne viruses (37 and references therein), was not achieved in previous attempts with leafhoppers (Nault and Gingery, *unpublished*). Thus, we sought other ways to establish the infectivity of MCDV particles.

Neutralization of infectivity of viruses by virus-specific antiserum has been a valuable serological method to establish relationships among viruses and virus strains that are circulative in their vector(s) and not mechanically transmitted (1,6–9,17,37–41). In these reports, virus was neutralized (rendered noninfective or less infective) either in vitro (e.g., a virus preparation was mixed with antiserum and then fed to vectors through membranes or injected into their hemocoel) or in vivo (e.g., antiserum was injected into vectors before or after virus acquisition). In the present study, we used an in vivo neutralization method applicable to semipersistently transmitted viruses in which antiserum is fed to viruliferous vectors in attempts to neutralize previously acquired virus.

Previous attempts to transmit purified MCDV may have failed because, like the aphid-borne potyviruses and caulimoviruses (20–22,31), a helper component may be required for its transmission. This possibility was first suggested for MCDV by Harrison and Murant (25) who noted similarities in the ultrastructures of infected plants, attachment sites in the foreguts of vectors, and vector transmission patterns between MCDV and the aphid-borne, semipersistently transmitted anthriscus yellows virus.

This report describes the in vivo neutralization of MCDV in its vector and the transmission of purified preparations of an MCDV isolate by leafhoppers previously exposed to plants infected with another isolate. In so doing, we have demonstrated the infectivity of MCDV particles and provided indirect evidence for a helper component essential for the transmission of MCDV.

MATERIALS AND METHODS

MCDV isolates and leafhopper maintenance. The MCDV-mild (MCDV-M) isolate, used for the neutralization of infectivity experiment, was originally obtained from johnsongrass (*Sorghum halepense* (L.) Pers.) near Portsmouth, OH, in 1972 (36). The isolate was maintained in the inbred maize (*Zea mays* L., Oh-28) by serial leafhopper transmission. Symptoms observed were diagnostic mild chlorotic striping of tertiary veins (18) and moderate overall stunting.

MCDV-M and a more severe isolate, MCDV-white stripe (MCDV-WS), were used for the helper component study. The severe isolate, supplied by J. K. Knoke (USDA Agricultural Research Service, Wooster, OH), was obtained from infected field

corn and maintained by serial leafhopper transmission in Oh-28 maize. Symptoms include severe stunting, extensive leaf tearing, and intense vein clearing giving the appearance of white stripes.

Before being used in experiments, *G. nigrifrons* were reared in cages containing oats (*Avena sativa* L.) and sweet corn (Aristogold Evergreen Bantam) or Oh-28. The leafhopper colony was started from adults collected from grasses on the campus of the Ohio Agricultural Research and Development Center, Wooster. Voucher specimens of *G. nigrifrons* used in this study are deposited in The Ohio State University collection of insects and spiders. Rearing cages and tube cages used for acquisition-access periods (AAP) or inoculation-access periods (IAP) were similar to those described by D'Arcy and Nault (5). Adults used in all experiments were 1–3 wk old. Virus-infected source plants, inoculated 10–14 days previously, and test plants (2–3-leaf stage) used in all experiments were Oh-28 maize.

Membrane-feeding systems. The membrane-feeding system was modified from that of Mitsuhasi (32). The system was assembled by stretching a Parafilm membrane to four times its original size and placing it over the opening of a 6-dram vial. Then 200 μl of feeding solution was applied to the membrane surface. A second membrane four times its original size was stretched over the first. Vials were fit snugly into circular holes (receptacles) cut through 2.5-cm foam-rubber sheets. The bottom of the foam sheet was covered with dacron organdy. Leafhoppers were anesthetized with CO_2, placed into the receptacles, and covered with vials. Usually, leafhoppers recovered within 60 sec and began probing the membrane within 10–30 min.

Virus purification. The following procedure, modified from that described by Gingery et al (16), was used to purify MCDV-WS for membrane feeding. The modifications simplified purification and resulted in greater virus yields as determined by photometric scans of sucrose density gradients and by electron microscopic examination (Hunt, *unpublished*).

Sixty grams of MCDV-infected leaves (midribs removed) was ground in 240 ml 0.3 M potassium phosphate buffer, pH 7.0, containing 0.5% 2-mercaptoethanol. The extract was squeezed through two layers of cheesecloth, clarified by emulsifying with $CHCl_3$ (1/3 volume), and centrifuged at low speed (7,700 *g* for 15 min). Virus was pelleted from the clarified extract by centrifugation at 100,000 *g* for 1 hr. The pellet was resuspended in 20 ml 0.01 M potassium phosphate buffer, pH 7.0, with gentle agitation for 3–12 hr at 4 C. This lower molarity buffer caused MCDV to precipitate. Virus was collected by centrifugation (7,700 *g* for 20 min). The pellet was resuspended overnight in 3 ml 0.3 M potassium phosphate, pH 7.0, at 4 C, and contaminates were eliminated by centrifugation (7,700 *g* for 15 min). The supernatant was distributed onto six density gradients. Gradients were prepared in tubes by successively layering 1.8, 3.6, 3.6, and 2.0 ml of 10, 20, 30, and 40% sucrose in 0.3 M potassium phosphate, pH 7.0 (w/w), respectively. Layers were allowed to diffuse overnight at 4 C. Gradients were centrifuged in a Beckman SW 41 rotor at 40,000 rpm for 1.5 hr at 5 C and then scanned photometrically at 254 nm with an ISCO Model 640 Density Gradient Fractionator and a Model UA-5 Ultraviolet Analyzer (Instrumentation Specialties Co., Lincoln, NE). Virus-containing zones were collected, diluted fivefold with 0.3 M potassium phosphate buffer, pH 7.0, and centrifuged at 100,000 *g* for 1 hr. The virus pellet was resuspended in 4.5 ml 0.01 M potassium phosphate, pH 7.0, containing 10% sucrose and fed immediately to leafhoppers as described previously. See "Results" section for explanation of use of buffer concentration. The suspensions contained 37–40 μg/ml of viral protein as determined by Coomassie Brilliant Blue G250 protein assay (42).

Antiserum production. Antiserum was produced according to methods described by Gordon and Nault (18). Preimmune serum was obtained from rabbits before MCDV injections.

Preparation of partially purified extracts for membrane feeding. Crude extract was prepared by grinding leaves infected with MCDV-M in 0.01 M ethylenediaminetetraacetic acid (EDTA), pH adjusted to 7.0 with NaOH (1:4 g/ml), and squeezing the sap through two layers of cheesecloth. Clarified extract was prepared from crude extract by emulsification with chloroform (1/3 volume). The aqueous phase was recovered after centrifugation at 7,700 *g* for 15 min and held for 1 hr at 30 C to allow the dissolved chloroform to evaporate. Concentrated extract was prepared from clarified extract by centrifugation at 100,000 *g* for 1 hr. Pellets were resuspended (1/50 original volume) in 0.01 M potassium phosphate, pH 7.0, by gentle overnight agitation at 4 C. Each preparation was supplemented with 10% sucrose for membrane feeding.

A total of 1,500 *G. nigrifrons* (10 per membrane) were given an 8-hr AAP to the crude, clarified, and concentrated preparations (500 per preparation). Leafhoppers were then transferred to 100 test plants (five per plant) per treatment for a 48-hr IAP. Plants were then sprayed with a pyrethroid insecticide, held in a greenhouse, and rated for symptoms at 7, 14, and 21 days after inoculation.

Neutralization of infectivity. Leafhoppers were given a 96-hr AAP on MCDV-infected plants and then were transferred directly from source plants to test plants to measure transmission efficiency (control group A); fed preimmune serum through parafilm membranes to measure loss of transmission efficiency with time and exposure to serum (control group B); or fed MCDV antiserum or the IgG fraction of MCDV antiserum through parafilm membranes to measure additional loss of transmission efficiency due to the effect of MCDV antibodies. For control group A, two leafhoppers from the acquisition population were placed in each of 10 tube cages containing a single test plant. After a 48-hr IAP, leafhoppers were removed, and test plants were sprayed with a pyrethroid insecticide to kill any residual (overlooked) leafhoppers and placed in the greenhouse. For membrane-feeding treatments, groups of 10 leafhoppers from the acquisition population were placed in the foam receptacles. Leafhoppers were given a 6-hr AAP to the solutions and then transferred to test plants (two leafhoppers per plant, 10 plants per treatment). After a 48-hr IAP, leafhoppers were removed, and test plants were sprayed with a pyrethroid insecticide and placed in the greenhouse. Plants were rated for MCDV symptoms at 7, 14, and 21 days after inoculation.

Transmission of purified MCDV-WS. The procedure was similar to those used to provide evidence for the role of helper component in the transmission of potyviruses and caulimoviruses (28,31).

Leafhoppers from a single population were given a 96-hr AAP to healthy corn, corn infected with MCDV-M, or corn infected with maize dwarf mosaic virus, strain A (MDMV-A), included to determine if the helper component produced by this aphid-borne potyvirus could assist in the transmission of MCDV-WS. Leafhoppers given access to corn infected with MCDV-M were then either transferred to healthy plants or given a 5-hr AAP on membranes containing either MCDV-WS in 10% sucrose, or 10% sucrose alone (both in 0.01 M potassium phosphate, pH 7.0). Similarly, leafhoppers given access to healthy plants were given a 5-hr AAP on membranes containing either MCDV-WS or sucrose. Leafhoppers exposed to plants infected with MDMV-A were transferred only to membranes containing MCDV-WS. Following the AAPs on membranes, all leafhoppers were transferred to healthy test plants (three leafhoppers per plant) for a 48-hr IAP. Then the leafhoppers were manually removed, and plants were sprayed with a pyrethroid insecticide and placed in a greenhouse to await symptom development. Symptoms were rated 21 days after inoculation. The experiment was replicated four times.

To test if the order of presentation of plants infected with MCDV-M and membranes containing MCDV-WS affected virus transmission, the following experiment was conducted. One group of leafhoppers was given a 96-hr AAP on plants infected with MCDV-M, followed by a 5-hr AAP on membranes containing MCDV-WS. A second group was given a 5-hr AAP on membranes containing MCDV-WS, followed by a 5-hr AAP on maize infected with MCDV-M. Three leafhoppers were placed on each test plant for a 48-hr IAP. Then they were manually removed, and plants were sprayed with a pyrethroid insecticide and placed in a greenhouse to await symptom development. Ratings were made 21 days after inoculation. The experiment was replicated three times.

RESULTS

Membrane feeding—preliminary observations. Preliminary experiments were done to determine the effect of sucrose, buffer, and antibody concentration on membrane-feeding behavior and survival. By direct observation methods similar to those of Harris et al (24), groups of 6–12 leafhoppers were observed for 2 hr feeding through membranes containing 2, 5, 10, and 15% sucrose in water. Based on the tendency to probe, secrete salivary sheaths, and remain in feeding positions for sustained periods, leafhoppers preferred the 10% sucrose solution. Treating the outer surface of the membrane with a sucrose solution to promote probing (24) was not necessary. Tests were done to determine the effects of phosphate buffers, EDTA, mercaptoethanol, and preimmune serum (serum in glycerol, 1:1) on leafhopper survival. Solutions containing concentrations of greater than 0.01 M potassium phosphate, 0.01 M EDTA, or 5% preimmune serum (all in 10% sucrose) seemed to inhibit membrane feeding or affect survival as compared to 10% sucrose alone. Mercaptoethanol (0.5% in 10% sucrose) seemed to inhibit feeding.

Neutralization of infectivity. Transmission of MCDV by viruliferous leafhoppers was significantly reduced after they fed on membranes containing MCDV antiserum or the IgG fraction of MCDV antiserum compared to leafhoppers that fed on membranes containing preimmune serum (Table 1), or to leafhoppers transferred directly from acquisition plants to test plants. This loss of transmission by antiserum-treated or IgG-treated leafhoppers can be attributed to natural loss of virus while leafhoppers were on membranes (as measured by the difference in transmission between controls A and B) as well as to in vivo neutralization of virus in vectors (as measured by the difference in transmission between control B and the antiserum or IgG treatment).

Transmission of purified MCDV. No MCDV transmission occurred by 500 leafhoppers each given access to membranes containing crude, clarified, or concentrated extracts of MCDV-M. However, leafhoppers transmitted both MCDV-M and MCDV-WS when they were first exposed to plants infected with MCDV-M and then membranes containing a suspension of purified MCDV-WS (Table 2). Leafhoppers previously exposed to healthy plants or to plants infected with MDMV-A and then to membranes containing MCDV-WS failed to transmit MCDV-WS. Leafhoppers exposed to plants infected with MCDV-M and then to either membranes containing sucrose or healthy maize for 5 hr transmitted MCDV-M. *G. nigrifrons* transmitted MCDV-WS from membranes only when first exposed to plants infected with MCDV-M but not when the order of exposure was reversed (Table 3).

DISCUSSION

This study and another on the rice tungro viruses (27) are the first to report in vivo neutralization of infectivity of semi-persistently transmitted plant viruses in their leafhopper vectors.

TABLE 1. Transmission of maize chlorotic dwarf virus (MCDV) by *Graminella nigrifrons*[a]

Treatment	Replicate 1	2	3	4	5	6	Mean arcsin p[b]	Transmission rate[c]
Control A[d]	3/10[e] (0.163)[h]	8/10 (0.553)	4/10 (0.225)	6/10 (0.368)	6/10 (0.368)	NT[f]	0.6103 a[g]	0.33
Control B[i]	1/10 (0.051)	4/10 (0.225)	2/10 (0.106)	4/10 (0.225)	3/10 (0.163)	4/10 (0.225)	0.4099 b	0.16
Antiserum[j]	0/10 (0.0)	0/10 (0.0)	1/10 (0.051)	0/10 (0.0)	3/10 (0.163)	1/10 (0.051)	0.1455 c	0.02
IgG[k]	NT	NT	0/10 (0.0)	2/10 (0.106)	1/10 (0.051)	0/10 (0.0)	0.1399 c	0.02

[a] *G. nigrifrons* given a 96-hr acquisition-access period on MCDV-infected plants, followed by a 6-hr acquisition-access period on membranes containing preimmune serum, MCDV antiserum, or IgG fraction of MCDV antiserum.
[b] Probability of transmission values for each replicate were arcsin-square-root transformed (not shown) before computing the transformed mean. Transformed data were analyzed with analysis of variance.
[c] Mean transmission rates for single *G. nigrifrons* were calculated by back transforming the mean arcsin-square-root values.
[d] Insects transferred directly from acquisition plants to test plants.
[e] Number of plants infected per number exposed.
[f] Not tested.
[g] Means followed by the same letter are not significantly different according to Duncan's Bayesian least significant difference procedure ($P = 0.05$).
[h] Probability of transmission by a single insect, $p = 1 - (1 - I)^{1/k}$ where I = proportion of 10 test plants infected and k = number of vectors per plant ($k = 2$ for all percentages).
[i] Membranes contained 5% preimmune serum and 10% sucrose.
[j] Membranes contained 5% antiserum and 10% sucrose.
[k] Membranes contained 5% IgG fraction of antiserum and 10% sucrose.

TABLE 2. Effects of prior exposure of *Graminella nigrifrons* to plants infected with maize chlorotic dwarf virus (MCDV) mild isolate (MCDV-M), maize dwarf mosaic virus, strain A (MDMV-A), or healthy plants on membrane transmission of purified MCDV-white stripe isolate (MCDV-WS)

First treatment: 96-hr access to maize plants	Second treatment: 5-hr access to membranes	No. plants infected/no. exposed[a] Replicate 1 MCDV-M	Replicate 1 MCDV-WS	Replicate 2 MCDV-M	Replicate 2 MCDV-WS	Replicate 3 MCDV-M	Replicate 3 MCDV-WS	Replicate 4 MCDV-M	Replicate 4 MCDV-WS	Total percentage infected plants MCDV-M[b]	Total percentage infected plants MCDV-WS
MCDV-M	MCDV-WS[c]	17/62	5/62	32/66	11/66	31/68	8/68	30/70	9/70	41.4 (0.163)[d]	12.4 (0.043)
Healthy	MCDV-WS	0/67	0/67	0/70	0/70	0/69	0/69	0/68	0/68	0	0
MDMV-A	MCDV-WS	...	...	0/69	0/69	0/67	0/67	0/70	0/70	0	0
MCDV-M	Sucrose	27/63	0/63	40/64	0/64	49/73	0/73	29/74	0/74	52.9 (0.222)	0
Healthy	Sucrose	0/67	0/67	0/69	0/69	0/70	0/70	0/68	0/68	0	0
MCDV-M	5-hour hold[e]	48/71	0/71	32/62	0/62	28/70	0/70	36/68	0/68	53.1 (0.223)	0

[a] For all tests, three *G. nigrifrons* were used to inoculate each plant.
[b] Because MCDV-M symptoms are masked in plants also infected with MCDV-WS, percentage transmission for MCDV-M is a conservative estimate.
[c] Virus was resuspended in 0.2 ml 0.01 M potassium phosphate buffer (pH 7.0) containing 10% sucrose.
[d] Probability of transmission by a single insect, $p = 1 - (1 - I)^{1/k}$ where I = proportion of total test plants infected and k = number of vectors per plant ($k = 3$ for all percentages). For treatment sequences where MCDV-WS was also transmitted, the MCDV-M probability estimate is conservative (see footnote b).
[e] Leafhoppers were held on healthy maize.

This approach indirectly demonstrates the involvement of viruses in disease etiology and may be generally useful for semipersistently transmitted viruses that are not mechanically transmitted. Other such viruses include the aphid-borne closteroviruses and several whitefly and mealybug transmitted viruses that have closterovirus-like properties (see 10,12,30,35 for candidate viruses). In vivo neutralization might also be useful for determining relationships among semipersistently transmitted viruses and virus strains, as the technique has for circulative viruses such as barley yellow dwarf and beet western yellows viruses (38,40).

Direct evidence for the infectivity of MCDV was shown by the transmission of MCDV-WS from purified preparations acquired through membranes. However, the direct evidence presented here does not unequivocally prove that the MCDV particle alone is sufficient for infection. Because leafhoppers required prior access to plants infected with a second MCDV isolate, the possibility exists that a second, as yet unrecognized, agent may be required. This question will remain unresolved until transmission and disease can be achieved solely with purified agents.

While a second agent acquired from MCDV-infected plants may be required for MCDV transmission by leafhoppers, the most parsimonious explanation for results reported here is that a virus-induced helper component is involved in the transmission of MCDV by *G. nigrifrons*. The methods used and results obtained in this study are similar to those in studies that first suggested the requirement of a helper component in aphid transmission of potyviruses (28) and cauliflower mosaic virus (CaMV) (31). Kassanis and Govier (28) found that aphids could transmit purified potato aucuba mosaic virus (PAMV) from membranes only if they had previously fed on leaves infected with potato virus Y (PVY). They further demonstrated that the assistance in transmission of PAMV was provided not by PVY, but by a "protein" extracted from PVY-infected leaves (20,21). Since these early studies, PVY and tobacco vein mottling virus (TVMV) helper components have been purified and shown to be virus-coded proteins (46). (See Murant et al [35] and Harrison and Murant [25] for reviews of this subject.) Similarly, CaMV seems to depend on a virus-coded protein for its aphid transmission (48). Although we cannot exclude the possibility that the MCDV-M virion serves as the helper for transmission of MCDV-WS, or that the helper component is an infection-induced plant product, it seems most likely that the MCDV helper component is a virus-coded protein, functionally similar to that involved in the transmission of potyviruses and CaMV. Failure of *G. nigrifrons* to transmit MCDV-WS from membranes after access to plants infected with aphid-borne potyvirus MDMV-A indicates that the putative helper component associated with MDMV-A cannot assist in transmission of MCDV.

A helper component produced as a result of infection by rice tungro spherical virus (RTSV), a probable member of the MCDV group (15), seems to be required for transmission of rice tungro bacilliform virus (RTBV) (26,27). RTBV can be transmitted only from plants dually infected with RTSV and RTBV or from plants singly infected with RTBV if leafhoppers are given previous access to RTSV-infected plants (26). Furthermore, RTSV-infected leafhoppers held 2–3 days on healthy plants so that their ability to transmit RTSV was lost were still able to acquire and transmit RTBV 4–5 additional days. In a second study (27), leafhoppers carrying RTSV and then fed anti-RTSV IgG had impaired transmission of RTSV but could acquire and transmit RTBV. These results suggest that a helper component other than the RTSV virion is responsible for transmission of RTBV.

Failure of *G. nigrifrons* to transmit MCDV and failure of *Nephotettix virescens* (Distant) to transmit RTSV (27) solely from membranes containing crude, clarified, concentrated, or purified preparations may reflect a lack of or insufficient titre of putative helper component in these preparations. The methods used to prepare and isolate MCDV and RTSV from plants may exclude or inactivate helper component. Consistent with such a hypothesis, maintaining the biological activity of PVY and TVMV helper components requires different purification procedures than does maintaining the PVY and TVMV virions (21,46). Contrary to Hibino's (26) results, Galvez (13) reported a 20% transmission rate for leafhoppers fed purified RTSV through membranes. Galvez's report warrants confirmation because the helper component may have been present in sufficient quantities in his preparations.

The requirement of a helper component for semipersistently aphid-transmitted and leafhopper-transmitted viruses suggests similarities in their transmission by the two insect families. A patchwork of evidence tends to support this idea. First, plant cells infected by either CaMV, anthriscus yellows virus (AYV), MCDV, or RTSV contain viruslike particles embedded in dense granular inclusions (3,33,43,49). Second, similar virus retention sites in the foregut of vectors have been reported for AYV and MCDV (23,34; E. D. Ammar and Nault, *unpublished*). Viruslike particles observed at these sites are embedded in a matrix material similar in appearance to the granular inclusion matrix found in plant inclusions. Finally, aphid-transmitted AYV and leafhopper-transmitted RTSV assist in the transmission of parsnip yellow fleck virus and RTBV, respectively (11,26). It may be that all semipersistently transmitted viruses, regardless of homopteran vector family, require a virus-coded helper component for their transmission. We agree with Harrison and Murant (25) that closteroviruses would bear examination for the presence of a helper component.

The characteristics of uptake, inoculation, and persistence of the rice tungro viruses and MCDV by their leafhopper vectors prompted Sylvester (45) and Nault et al (36) to classify these transmission relationships as "semipersistent." Previously, this term had been used only for the aphid-borne closteroviruses, whose transmission characteristics were intermediate between the aphid-borne viruses classified as nonpersistent and those classified

TABLE 3. Effect of order of presentation to *Graminella nigrifrons* of plants infected with maize chlorotic dwarf virus (MCDV) mild isolate (MCDV-M) and of membranes with a purified MCDV-white stripe isolate (MCDV-WS) on virus transmission

First treatment	Second treatment	No. plants infected/no. inoculated[a]						Total percentage transmission	
		Replicate 1		Replicate 2		Replicate 3			
		MCDV-M	MCDV-WS	MCDV-M	MCDV-WS	MCDV-M	MCDV-WS	MCDV-M[b]	MCDV-WS
96-hr access to plants infected with MCDV-M	5-hr access to MCDV-WS[c] membranes	31/70	17/70	42/69	11/69	31/69	21/69	50.0 (0.206)[d]	23.6 (0.086)
5-hr access to MCDV-WS membranes	5-hr access to plants infected with MCDV-M	49/69	0/69	37/69	0/69	41/69	0/69	59.9 (0.263)	0.0

[a] For all tests, three *G. nigrifrons* were used to inoculate each plant.
[b] Because MCDV-M symptoms are masked in plants also infected with MCDV-WS, percentage transmission for MCDV-M is a conservative estimate.
[c] Virus was resuspended in 0.2 ml 0.01 M potassium phosphate buffer, pH 7.0, containing 10% sucrose.
[d] Probability of transmission by a single insect, $p = 1 - (1 - I)^{1/k}$ where I = proportion of total test plants infected and k = number of vectors per plant ($k = 3$ for all percentages). For treatment sequences where MCDV-WS was also transmitted, the MCDV-M probability estimate is conservative (see footnote b).

as persistent. Ling and Tiongco (29) and Choudhury and Rosenkranz (4) disagreed with the use of "semipersistent" to describe leafhopper-transmitted viruses, preferring instead the term "transitory." Their case for applying a new term to these leafhopper-transmitted viruses was twofold. They argued that "semipersistent" is a comparative term to be considered only in the context of nonpersistent and persistent; thus, it cannot be applied to leafhopper-transmitted viruses where there are no examples of nonpersistent transmission. Also, they contended that, because aphids and leafhoppers belong to different groups (families), the virus-vector interactions may be different (29) and terms used for one insect family should not be used for the other.

We disagree with their rationale and prefer to retain "semipersistent" to describe transmission for both insect families for the following reasons. First, when Watson and Roberts (47) coined the terms "nonpersistent" and "persistent," they referred not only to those viruses transmitted by aphids, but also to those vectored by jassids (leafhoppers), thrips, and tingids (lace bugs). With this precedent, "semipersistent" (44,45) can logically be applied to describe the transmission of viruses by any of those vector groups, including leafhoppers. Second, although we do not argue that transmission of semipersistently transmitted aphid-borne and leafhopper-borne viruses is identical, our discovery of a putative helper component for leafhopper transmission of MCDV and the suggestion that a helper component is required for aphid transmission of AYV (25) clearly suggests potential similarities in these virus-vector interactions. Finally, even if the above similarities were not known, the system of classifying transmission is based on virus persistence in the vector and *does not require* that virus-vector interactions be identical or even similar. For example, "persistent" validly describes transmission of viruses that are circulative and do not replicate in their vectors as well as those that are propagative and do multiply in their vectors. It is ironic that those who argue for different terms to describe intermediate retention times for aphids and leafhoppers (4,29) retain the same term, "persistent," to describe lengthy retention times in both insect families.

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