Vector Relationship of *Graminella nigrifrons* to Maize Chlorotic Dwarf Virus

M. M. Choudhury and Eugen Rosenkranz

Former graduate research assistant, Department of Plant Pathology and Weed Science, Mississippi State University, Mississippi State 39762; and plant pathologist, Agricultural Research Service, U.S. Department of Agriculture, and professor, Department of Plant Pathology and Weed Science, Mississippi State University, Mississippi State 39762. Present address of senior author: EMBRAPA-Centro Nacional de Pesquisa Agropecuaria do Tropico Semi-Arido, Petrolina, Pernambuco, Brazil.

Journal Series Paper 3672 of the Mississippi Agricultural and Forestry Experiment Station.
Accepted for publication 19 November 1982.

ABSTRACT

Choudhury, M. M., and Rosenkranz, E. 1983. Vector relationship of *Graminella nigrifrons* to maize chlorotic dwarf virus. Phytopathology 73:685-690.

In laboratory colonies, where the ratio of males to females was normally 2:1, 34–35% of adult *Graminella nigrifrons* transmitted maize chlorotic dwarf virus (MCDV). There was a statistically significant difference between the rate of MCDV transmission by females (46%) and that by males (29%). While all five nymphal instars transmitted MCDV, the last three instars were as efficient vectors as adults. Noninfective adult leafhoppers acquired MCDV from diseased corn plants in as little as 15 min. The frequency of transmission increased with extension of the acquisition access period (AAP). Viruliferous adult leafhoppers infected corn seedlings during an inoculation access period (IAP) of 15 min. No latent period of MCDV in *G. nigrifrons* could be demonstrated; transmission occurred after 1 hr AAP and 0.5 hr IAP. The minimum incubation period of MCDV in sweet corn was 6 days at a temperature range of 20–32 C. The longest tested retention period of MCDV in *G. nigrifrons* was 37–40 hr, but most transmitters lost their inoculativity earlier. Noninfective leafhoppers recovered MCDV from inoculated corn plants 3–5 days before first symptoms appeared. There was no transovarial passage of MCDV in *G. nigrifrons* reared on infected plants. Inoculative nymphs lost inoculativity immediately upon molting. When MCDV-infective adult *G. nigrifrons* were confined to healthy corn test plants, they retained infectivity for up to 2 days, then lost it and remained noninfective during the next day. After that they began to reacquire the virus from the same test plants. Thus, transmission data from tests involving transfers at intervals of more than 3 days may suggest that MCDV is being transmitted by *G. nigrifrons* in a persistent manner. In reality, all aspects of the transmission process reveal a relationship between MCDV and *G. nigrifrons* that is not persistent and is best described as transient or transitory.

Additional key words: ecdysis, efficiency of transmission, Ohio corn stunt agent, *Zea mays* L.

In 1968, Rosenkranz (10) discovered a new stunting disease of corn (*Zea mays* L.) in Ohio and tentatively named its incitant the Ohio corn stunt agent (CSA-OH). He reported that the agent was vectored by the leafhopper *Graminella nigrifrons* (Forbes) but not by *Dalbulus maidis* (De Long & Wolcott), the principal leafhopper vector of what later came to be known as corn stunt spiroplasma. Subsequently, he found that 35% of individually tested adult *G. nigrifrons* transmitted CSA-OH (Rosenkranz, *unpublished*). In 1969, the same corn stunting agent was isolated, in Mississippi, from naturally infected sorghum (*Sorghum bicolor* (L.) Moench) (11) and from field-grown corn (M. M. Choudhury, *unpublished*). We (2) obtained 34% transmission of the CSA-OH isolate (derived from corn in Mississippi) by randomly selected single adult *G. nigrifrons*. The percentage of transmitters of CSA-OH was higher among female than male *G. nigrifrons* (3).

In 1972, Bradfute et al (1) and Pirone et al (8) reported the isolation from Johnson grass (*Sorghum halepense* Pers.) and stunted corn of a virus that subsequently proved to have biological characteristics surprisingly similar to those of CSA-OH. The following year, the name maize chlorotic dwarf virus (MCDV) was proposed for this virus (7).

The investigations reported here were intended to correct certain misconceptions about the vector-pathogen relationship presented in previous reports (9,10), to offer further evidence that MCDV and CSA-OH are identical pathogens, and to present extensive data on the virus vector relationship between the Mississippi isolate of MCDV (CSA-OH) and its main leafhopper vector, *G. nigrifrons*, both from the same location.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

MATERIALS AND METHODS

All leafhoppers used in this study originated from isolated eggs laid by descendants of several hundred adult *G. nigrifrons* that had been collected on corn and indigenous grasses at Mississippi State. Colonies of the leafhopper were reared on corn, barley, and rice plants in 794-μm (32-mesh) Saran (nylon) screen-covered, wooden-frame cages, 42 × 66 × 92 cm, having a glass top. MCDV-inoculative leafhopper colonies were maintained in the same type of cages on sweet corn plants severely infected with the virus. During inoculation access periods and serial transfers, leafhoppers were confined with test seedlings in cylindrical cages (5 cm in diameter × 22 cm high) made of cellulose butyrate and fitted with 794-μm (32-mesh) Saran screen at one end and four screened windows on the sides. The open end of the cage was placed over the seedlings and pressed into the soil. Adult leafhoppers were used in all experiments except where indicated.

The isolate of MCDV originated from a severely diseased field corn plant collected at Mississippi State, and was maintained in sweet corn. Sweet corn, cultivar Seneca Chief, was also used as the test plant in all experiments. Symptoms of maize chlorotic dwarf (MCD) in that cultivar consist first of a mild yellowish chlorosis along the margins of the youngest leaf, followed by fine chlorotic striping of tertiary veins, wavy or undulate and drooping leaf blades, dull and somewhat rough upper leaf surfaces, and uniformly slower than normal plant growth that eventually results in stunted plants (one-fifth to one-half the size of healthy plants) with partially sterile tassels and small, incompletely filled ears.

The experiments were conducted year-round in a greenhouse with a daily temperature range of 20–32 C and a daily relative humidity range of 70–100%. The test plants grew under the prevailing natural light conditions and received an ample supply of nutrients.

Since the objectives and requirements of each test varied, the detailed procedures used in individual experiments will be described together with the results of each experiment.

TABLE 1. Effect of length of acquisition access and inoculation access periods on the rate of transmission of maize chlorotic dwarf virus by groups of *Graminella nigrifrons*

	Transmission (groups transmitting/groups tested) after (hr)									
	0.25	0.50	1	2	4	8	12	16	20	24
After acquisition access[a]	1/10[b]	2/10	2/10	3/10	6/10	8/10	9/10	9/10	9/10	9/10
After inoculation access[c]	1/10	1/10	3/10	5/10	6/10	6/10	7/10	7/10	9/10	10/10

[a] Each of the 10 groups used for each of the 10 acquisition access periods consisted of 12 leafhoppers (ie, 120 leafhoppers per period, or 1,200 leafhoppers for the entire acquisition experiment).
[b] Following the specified acquisition access period, the 12 leafhoppers in each group were caged with a corn test seedling for 48 hr.
[c] Each of the 10 groups used for each of the 10 inoculation access periods consisted of 12 leafhoppers taken from a viruliferous colony (ie, 120 leafhoppers per period, or 1,200 leafhoppers for the entire inoculation experiment).

TABLE 2. Comparative transmission of maize chlorotic dwarf virus by immature and adult stages of *Graminella nigrifrons* after a 24-hr acquisition access period

Colony no.	Transmission by colony of 10 insects[a]					
	Nymphal instar					
	1st	2nd	3rd	4th	5th	Adult
1	–[b]	+	–	+	+	+
2	+	–	+	+	+	–
3	–	+	+	–	–	+
4	+	–	+	–	+	+
5	–	+	+	+	–	–
6	–	+	+	+	–	+
7	–	–	+	+	+	+
8	+	+	–	+	+	+
9	–	–	+	–	+	–
10	–	–	+	+	+	+

[a] Insects in each colony of all six stages fed on a healthy corn test plant for 7 days.
[b] – = healthy plants; + = diseased plants.

RESULTS

Acquisition threshold of MCDV by *G. nigrifrons*. Tests were conducted to determine the shortest access period during which *G. nigrifrons* could acquire MCDV from experimentally infected corn plants. Leafhoppers from a stock colony were starved for 2 hr before being placed on the virus source plants for acquisition access periods of 0.25, 0.50, 1, 2, 4, 8, 12, 16, 20, and 24 hr. After each of the 10 acquisition access periods, 12 leafhoppers were caged on each of 10 corn test seedlings for an inoculation access period of 48 hr. Thus 120 leafhoppers were tested for inoculativity after each acquisition access period. After the 15-min acquisition access, one of the 10 groups of 12 leafhoppers transmitted MCDV. The frequency of transmission increased progressively with extension of the acquisition access period up to 12 hr, after which the level of transmission remained constant, with nine of 10 leafhopper groups transmitting the virus (Table 1).

Minimum and maximum inoculation access periods of MCDV-infective *G. nigrifrons*. Tests were conducted to determine the shortest access period during which inoculative *G. nigrifrons* could transmit MCDV to healthy corn seedlings, and the shortest inoculation access period that would result in the infection of all 10 test plants used. We used groups of 12 leafhoppers per test plant. Ten such groups each were employed to test inoculation access periods of 0.25, 0.50, 1, 2, 4, 8, 12, 16, 20, and 24 hr. The results of these tests are summarized in Table 1. Transmission of MCDV was effected by one of the 10 groups when test seedlings were exposed to inoculative leafhoppers for 15 min. The frequency of transmission increased progressively with increasing length of the inoculation access period, but the maximum transmission did not occur until the test seedlings had been exposed to the inoculative leafhoppers for 24 hr when all 10 test plants became infected.

To confirm the minimum inoculation access period obtained in the above experiment, and to determine what percent of potential transmitters could actually transmit MCDV during a 15-min feeding period, we tested 23 leafhoppers individually. These leafhoppers were reared on diseased plants and were starved for 4 hr before being placed singly on a test seedling. Each leafhopper was timed separately for a total period of 15 min of actual feeding. Under these conditions, two leafhoppers transmitted MCDV, but the test plant on which one of the two transmitters had fed developed only a mild form of the disease. Since we would have expected eight potential transmitters among the 23 leafhoppers tested, a 15-min inoculation feeding period appears to be only 25% as effective as an inoculation access period lasting 24 hr.

Latent period of MCDV in *G. nigrifrons*. Results from one experiment indicated that the latent period of MCDV in *G. nigrifrons* was less than 5 hr. Two of six groups of 20 leafhoppers each transmitted MCDV after a 4-hr acquisition access and a 1-hr inoculation access. We made a further attempt to determine the minimum latent period by using a shorter acquisition access period. Leafhoppers from a noninfective stock colony were starved for 4 hr and then exposed for 1 hr to a virus source plant. Thereafter, these leafhoppers were caged in colonies of 15 insects per plant on 40 test plants. Ten colonies each (150 leafhoppers) remained on their respective test plants for a 0.50-, 1-, 2-, or 3-hr inoculation access period. Thus, we could test possible latent periods of 1.5, 2, 3, and 4 hr. Transmission of MCDV occurred after each period tested. However, only one of the 10 colonies that were given a 0.5 hr inoculation access period transmitted MCDV, and that was only a mild infection. Of the other three groups of 10 colonies each that had received inoculation access periods of 1, 2, or 3 hr, two colonies each transmitted MCDV, and the resultant infection was severe. Thus, if a latent period is required by *G. nigrifrons* before it can transmit MCDV, this period could be as brief as 1.5 hr.

Transmission of MCDV by immature stages of *G. nigrifrons*. Representatives of all five nymphal instars and adults from stock colonies were given a 24-hr acquisition access to severely infected source plants. Then, leafhoppers from each of the six age classes were placed (10 per plant) on test seedlings for an inoculation access period of 7 days. Ten colonies of 10 leafhoppers of each of the six developmental forms were tested. Table 2 shows that all six stages of *G. nigrifrons* were capable of transmitting MCDV, but first- and second-instar nymphs seemed to be less efficient transmitters than older nymphs or adults. The rate of transmission increased with age of the nymphs up to the third instar, after which the transmission efficiency remained at about the same level. When the chi-square tests of independence and/or association was applied to the data presented in Table 2, the rates of MCDV transmission by first instar and third instar nymphs were significantly different ($\chi^2 = 5.050$, $P = 0.05$–0.025). However, the difference in the rate of MCDV transmission between the first instar nymphs and fourth and fifth instar nymphs as well as adults fell just short of being significant ($\chi^2 = 3.200$, $P = 0.10$–0.05). To be significant, χ^2 would have had to be 3.841 or larger. In view of the obtained results, we used fourth- and fifth-instar nymphs in lieu of adult leafhoppers in

some of our later experiments.

Different rate of transmission of MCDV by male and female *G. nigrifrons*. The experiment on the transmission efficiency of sexed *G. nigrifrons* was performed twice to confirm our previously obtained transmission rate of 34%. In one experiment, 300 adult leafhoppers from a stock colony were confined to a cage containing six MCDV-infected corn plants for 7 days. Then 50 males and 50 females were removed and caged singly on test seedlings for 14 days. In this test, 14 males (28%) and 23 females (46%) transmitted MCDV, giving a total transmission percentage of 37%. A random laboratory population of *G. nigrifrons*, however, is composed, on the average, of two males to one female. Therefore, based on the percentages of transmitters among males (28%) and females (46%) in this experiment, a random population would have had (28 [2/3] + 46 [1/3] =) 34% transmitting leafhoppers.

In another similar experiment, 50 leafhoppers of each sex were singly tested at a different time of the year. In this test, 15 males (30%) and 23 females (46%) transmitted MCDV. The total and adjusted (for sex ratio in random population) percentages of transmitters were 38 and (30 [2/3] + 46 [1/3] =) 35%, respectively.

The chi-square test of independence and/or association applied to the combined data from the two experiments showed that the transmission rate of MCDV for males (29%) and that for females (46%) was significantly different ($\chi^2 = 6.166$, $P = 0.02$–0.01). When the data were pooled from all three experiments we had conducted to date (one is not reported here) and analyzed by the same method, the difference in the rate of MCDV transmission between males (29%) and females (45%) became highly significant ($\chi^2 = 7.564$, $P = 0.01$–0.005).

Retention of MCDV by *G. nigrifrons*. In the first experiment, adult leafhoppers, reared on diseased corn plants, were placed at the rate of 20 per plant on 10 test plants and transferred to fresh sets of test plants every 24 hr for 7 days. None of the 10 groups of initially viruliferous leafhoppers transmitted MCDV to the third set of the 10 test plants on which they had fed for 48–72 hr after having been removed from the virus source.

The second experiment was designed to observe the decrease in MCDV inoculativity of *G. nigrifrons* with shorter intervals in time. Fourth- and fifth-instar nymphs, reared from eggs on MCDV-infected plants, were used to minimize the loss of inoculativity among transmitters from natural mortality. Twenty nymphs were caged on each of 10 corn seedlings and then transferred to fresh test seedlings every 4 hr for 40 hr, which resulted in 10 transfers and 100 inoculated test plants. At the end of the 40-hr period, there were 188 surviving insects, and of these, six had become teneral adults. Some of the transmitters retained their inoculativity throughout the experiment; three of the 10 colonies still transmitted MCDV after the last transfer (Table 3). Whereas only 6% of the 200 nymphs were dead at the time of the last transfer, the inoculativity of the 10 colonies had decreased by 70% after 40 hr.

In still another experiment, when nonviruliferous adult *G. nigrifrons* were given only a 4-hr acquisition access period and transferred, in groups of 20 leafhoppers, to test seedlings every 4 hr, all 12 such groups lost inoculativity before the sixth transfer was made, ie, 24 hr after removal from the virus source.

Minimum incubation period of MCDV in corn plants and recovery of the virus with *G. nigrifrons* from inoculated plants during the incubation period. The length of the incubation period of a disease agent in its host plant may be indicative of its rate of multiplication and movement. Originally, Rosenkranz (9) observed the shortest incubation period of MCDV in sweet corn to be 9 days. In the present study, we observed a minimum incubation period in sweet corn of 6 days following inoculation access for 24 hr and subsequent daily air temperature range in the greenhouse of 20–32 C. In general, the average incubation period was 7–9 days during the warm season and 11–14 days during the cool season.

To obtain additional information on the rate of multiplication and movement of MCDV in corn plants, we wished to determine how soon after inoculation, but before initial symptom expression, *G. nigrifrons* could recover the virus from inoculated test plants. In this experiment we used only female leafhoppers because of their greater efficiency in vectoring MCDV. Fifteen female leafhoppers from an infective colony were caged with each of four corn seedlings for a 24-hr inoculation access period. At the same time the inoculative leafhoppers were removed, 15 nonviruliferous females were placed on each of these four inoculated test plants for an acquisition access period of 24 hr. Then, the survivors from each of the four test plants were transferred, in groups, to each of four healthy indicator corn plants on which they remained for 7 days. This procedure of placing nonviruliferous leafhoppers on the inoculated four test plants for a 24-hr acquisition access and then removing them to fresh indicator plants for a 7-day inoculation access was followed for 9 consecutive days, at which time the first two of the four inoculated source plants developed the first visible symptoms. In this manner, we obtained nine indicator plants, each representing a consecutive 24-hr acquisition access period to which 15 nonviruliferous leafhoppers were subjected, for each of the four inoculated source plants, or a total of 36 indicator plants. It took 9, 9, 10, and 11 days for the inoculated source plants 1, 2, 3, and 4, respectively, to show the first discernible symptoms (Table 4). By comparison, nonviruliferous female *G. nigrifrons* were able to recover MCDV from the same four inoculated, but as yet symptomless, source plants on the fifth, sixth, sixth, and sixth day after the beginning of the inoculation access period. Thus, the difference in time between the recovery of MCDV by the vector and the onset of the first symptoms was 4, 3, 4, and 5 days for the inoculated source plants 1, 2, 3, and 4, respectively (Table 4). Concurrently, we tested for the virus-free state of the colony of *G. nigrifrons* used in the recovery phase of the experiment. None of the nine test plants exposed to a total of 135 female leafhoppers (15 per plant) developed symptoms of the disease. The data presented here indicate that MCDV multiplies and moves in corn plants relatively fast compared to other leafhopper-transmitted viruses.

Loss and reacquisition of MCDV by *G. nigrifrons* during extended inoculation feeding periods. This experiment was conducted to determine how soon *G. nigrifrons* lose inoculativity

TABLE 3. Decrease in infectivity of maize chlorotic dwarf virus in fourth and fifth instar nymphs of *Graminella nigrifrons* after their removal from the virus source

Colony no.	Transmission during stated interval (hr)						
	0–4[a]	5–8	9–12	25–28	29–32	33–36	37–40
1	+(20)[b]	+(20)	+(20)	−(20)	+(20)	+(20)	+(20)
2	+(20)	+(20)	+(20)	+(19)	+(19)	+(19)	+(19)
3	+(20)	+(20)	+(20)	+(19)	+(19)	+(19)	−(19)
4	+(20)	+(20)	+(20)	+(20)	−(20)	−(20)	−(20)
5	+(20)	+(20)	−(20)	−(20)	+(20)	−(20)	−(20)
6	+(20)	+(20)	+(19)	+(18)	−(18)	−(18)	−(18)
7	+(20)	+(20)	−(20)	−(18)	−(18)	−(18)	−(18)
8	+(20)	−(20)	+(20)	+(20)	−(20)	−(20)	−(19)
9	+(20)	+(20)	+(19)	+(16)	+(16)	−(16)	−(16)
10	+(20)	+(20)	+(20)	+(19)	+(19)	+(19)	+(19)

[a] Transfers of the surviving leafhoppers in each colony were made to a fresh healthy corn seedling (in the one-leaf stage) every 4 hr during a period of 40 hr.
[b] − = Healthy plants; + = diseased plants; numbers in parentheses denote numbers of surviving leafhoppers placed on test plants for infectivity assay.

after removal from an MCDV source and then reacquire the virus from the same test plants they inoculated during prolonged inoculation access periods. Twelve adult leafhoppers from a viruliferous colony were caged on each of 70 test plants. Then the leafhoppers from 10 such test plants each were transferred three times to a set of 10 fresh test plants at intervals of 1, 2, 3, 4, 5, 6, or 7 days. This resulted in four series of 10 test plants each for each of the seven transfer intervals. The data from this experiment, involving 840 leafhoppers and 280 test plants, are summarized in Table 5. The 10 subcolonies transferred daily transmitted MCDV only to the first two series of plants (ie, during the 48 hr after removal from MCDV source), but most transmitters ceased transmitting after the first 24 hr. The 10 subcolonies that had been transferred every 2 days transmitted MCDV only to the first set of plants. Similarly, the 10 subcolonies transferred every 3 days transmitted the virus only to test plants of the first series, after which they were rendered noninoculative in the subsequent three transfers. On the other hand, when the 10 subcolonies were transferred every 4 days, the transmitting leafhoppers infected all test plants in the first series, lost their infectivity, and some of them reacquired MCDV from the same test plants they had inoculated, all within the first 96-hr period. However, the amount of virus the transmitters reacquired collectively during the 4-day transfer intervals was only sufficient to infect four test plants in the second, one plant in the third, and none in the fourth series. Transfers made at 5-day intervals allowed some of the vectoring leafhoppers enough time to reacquire a sufficient amount of virus to transmit it to some test plants in each series. The number of subcolonies with leafhoppers transmitting MCDV serially in all transfers increased progressively as the interval of serial transfers was extended to 6 and 7 days. In fact, the subsequent transmission of MCDV, reacquired during 7 days of inoculation access on the first set of test plants, was as efficient as the initial transmission of the virus by the leafhoppers removed directly from the viruliferous stock colony. In both cases, 9 of 10 subcolonies transmitted MCDV. Thus, if the inoculation access period in a serial transmission experiment is extended beyond 3 days, the transmission of MCDV by *G. nigrifrons* may appear to be of the persistent type, which it is not. Also, data from this experiment show that MCDV-inoculative *G. nigrifrons* are rendered nonviruliferous when feeding on healthy corn plants for 48 to 72 hr.

Absence of transovarial passage of MCDV in *G. nigrifrons*. From various aspects of the MCDV-vector relationship studied by us, we assumed that the virus could not pass through eggs to the progeny of *G. nigrifrons*. We wished to provide experimental evidence, however, for this assumption. Six hundred eggs in the advanced stage of development (with distinct eye spots), deposited by females from MCDV-inoculative colonies into MCDV-infected

TABLE 4. Recovery of maize chlorotic dwarf virus (MCDV) with female *Graminella nigrifrons* from inoculated corn plants before expression of symptoms

Inoculated source plant[a]	Day from start of inoculation								Incubation period minus day of first recovery
	4	5	6	7	8	9	10	11	
First symptoms on inoculated source plant									
1	–[c]	–	–	–	–	+/–	+	+	9–5 = 4
2	–	–	–	–	–	+/–	+	+	9–6 = 3
3	–	–	–	–	–	–	+/–	+	10–6 = 4
4	–	–	–	–	–	–	–	+/–	11–6 = 5
Recovery of MCDV from inoculated source plant as expressed in indicator plant[b]									
1	1–4 –[c]	1–5 +	1–6 +	1–7 +	1–8 +	1–9 +	1–10 +		
2	2–4 –	2–5 –	2–6 +	2–7 +	2–8 +	2–9 +	2–10 +		
3	3–4 –	3–5 –	3–6 +	3–7 +	3–8 +	3–9 +	3–10 +		
4	4–4 –	4–5 –	4–6 +	4–7 +	4–8 +	4–9 +	4–10 +		

[a] Source plants consisted of four corn seedlings each having been exposed for 24 hr to 15 female leafhoppers from an infective colony.
[b] In the recovery phase of the experiment, 15 noninfective female leafhoppers were confined to each of the four inoculated source plants for 24 hr, starting immediately after termination of inoculation feeding, and then transferred to a healthy test plant for 7 days. This process was continued for 9 consecutive days.
[c] – = symptomless plants; +/– = plants with first discernible symptoms on specified day; + = diseased plants.

TABLE 5. Loss and reacquisition of maize chlorotic dwarf virus (MCDV) by *Graminella nigrifrons* during progressively extended inoculation access periods

Interval of serial transfers (days)[a]	Transmission by aggregate colonies per transfer:				Total transmission
	1	2	3	4	
1	9/10 (120)[b]	2/10 (115)	0/10 (112)	0/10 (109)	11/40[c]
2	9/10 (120)	0/10 (115)	0/10 (110)	0/10 (101)	9/40
3	9/10 (120)	0/10 (114)	0/10 (96)	0/10 (84)	9/40
4	10/10 (120)	4/10 (113)	1/10 (99)	0/10 (85)	15/40
5	10/10 (120)	6/10 (104)	3/10 (93)	1/10 (79)	20/40
6	10/10 (120)	7/10 (109)	3/10 (91)	2/10 (75)	22/40
7	9/10 (120)	9/10 (108)	3/10 (87)	3/10 (59)	26/40

[a] Interval of serial transfers in days equals the length of the inoculation access period.
[b] Fraction expresses aggregate number of transmitting colonies (numerator) over number of colonies tested; number in parentheses denotes total number of survived leafhoppers per 10 colonies placed on 10 test plants in each serial transfer (initially each colony consisted of 12 leafhoppers derived from a viruliferous stock colony).
[c] Fraction expresses total number of MCDV-infected test plants in four serial transfers per transfer interval (numerator) out of possible 40 plants on which a leafhopper colony fed.

corn leaf tissue, were excised and placed on moist filter paper, 30 eggs per seedling, around the base of corn seedlings that were in the one-leaf stage. A cage was then placed over each of two such seedlings growing in a 15-cm-diameter clay pot, so that we had a total of 20 test plants growing under 10 cages. After 4 wk, the cages were removed, the emerged nymphs were counted, and the test plants were observed for an additional 6 wk. Of the 600 eggs isolated, 286 nymphs developed, but none of the 20 test plants colonized by these nymphs (25–30 nymphs per two plants) developed symptoms of maize chlorotic dwarf. Based on these results, and our earlier finding that first-instar nymphs of *G. nigrifrons* are able to transmit MCDV, we conclude that MCDV is not transmitted through eggs to the progeny of female *G. nigrifrons* that are continuously exposed to this virus.

Effect of molting of inoculative *G. nigrifrons* on MCDV transmission. We selected 100 fifth-instar nymphs from MCDV-inoculative stock colonies and confined them singly for 8 hr on an equal number of newly emerged corn seedlings. To prevent the natural decline of inoculativity with time, each nymph was then transferred to a separate test tube containing MCDV-infected corn leaf tissue for an 8-hr period of virus reacquisition. This process of alternate 8-hr inoculation feeding and 8-hr reacquisition feeding was continued until all 100 nymphs had molted, which took 144 hr. Leafhoppers that molted on the test plant were transferred at the end of the 8-hr inoculation feeding period to another test plant for a 24-hr inoculation access period. Leafhoppers that molted on the infected tissue during reacquisition were transferred immediately after molting to a test plant for a 24-hr inoculation access period, which was followed by another transfer to a fresh test plant for an additional 24-hr inoculation access period.

Examination of the young adults at the end of the experiment showed that 71 of the tested nymphs had been females and 29 males. Based on this sexual composition of the test population, knowing that fifth-instar nymphs and adults have the same transmission efficiencies, and assuming that 46% of the females and 29% of the males are able to transmit MCDV, we would have expected 41 transmitters. In this experiment, 40 of the 100 fifth-instar nymphs transmitted MCDV before molting and none transmitted the virus after molting (Table 6). The remaining 60 nymphs did not vector MCDV before or after molting. Of the 40 transmitting nymphs, 29 infected test plants in the first series (during first 8 hr after removal from MCDV source), six nymphs transmitted MCDV after the first 8-hr reacquistion period (16–24 hr after removal from virus source), and the remaining five nymphs transmitted the virus only after two or more 8-hr periods of reacquisition. Several nymphs transmitted MCDV intermittently.

TABLE 6. Transmission pattern of maize chlorotic dwarf virus (MCDV) by single immature *Graminella nigrifrons* before and after molting

Insect no.	Reaction of test plants (TP) between reacquisition (RA)[a]															
	TP_1	RA_1	TP_2	RA_2	TP_3	RA_3	TP_4	RA_4	TP_5	RA_5	TP_6	RA_6	TP_7	RA_7	TP_8	TP_9
1	+[b]	E[c]	–[d]		–											
2	+	E	–		–											
3	+	E	–		–											
4	+	E	–		–											
5	+		E–[c]		–											
6	+		E–		–											
7	+		E–		–											
8	+		E+		–											
9	–		E+		–											
10	+		+	E	–		–									
11	+		+	E	–		–									
12	+		+	E	–		–									
13	–		+	E	–		–									
14	+		+		E–		–									
15	–		+		E–		–									
16	+		+		+	E	–		–							
17	+		+		+	E	–		–							
18	+		+		+	E	–		–							
19	+		+		+	E	–		–							
20	+		+		+	E	–		–							
21	+		+		+	E	–		–							
22	+		–		+	E	–		–							
23	+		+		+		E–		–							
24	+		+		+		E–		–							
25	+		+		+		E+		–							
26	+		–		+		E+		–							
27	+		–		–		E+		–							
28	+		+		+		+	E	–		–					
29	–		–		+		+	E	–		–					
30	–		–		–		+	E	–		–					
31	+		+		+		+		E–		–					
32	+		+		–		+		E–		–					
33	–		+		+		+		E–		–					
34	–		+		+		+		E–		–					
35	+		+		+		+		+		E–		–			
36	+		+		+		+		–		E–		–			
37	–		–		–		–		+		E+		–			
38	–		–		–		–		+		+	E	–		–	
39	–		–		+		+		–		–		+	E	–	–
40	–		+		+		+		+		–		+	E	–	–

[a] Individual leafhoppers spent alternately 8 hr on test plants (TP_1, TP_2, etc.) and 8 hr on MCDV-infected leaf tissue for reacquisition (RA_1, TA_2, etc.).
[b] + = test plants infected with MCDV; – = healthy test plants.
[c] E = molting occurred either on test plants or on infected leaf tissue during reacquisition. When molting took place on a test plant, the latter either remained healthy (E–) or it became infected (E+), depending on when molting occurred during the 8-hr period.
[d] After molting (E), individual leafhoppers were given either one 24-hr inoculation access period (when molting occurred on a test seedling) or two such successive periods (when molting occurred on infected tissue during reacquisition) on indicator corn seedlings.

DISCUSSION

Compared to most other leafhopper-transmitted viruses, MCDV has an unusual relationship to its vector, *G. nigrifrons*. Rice tungro virus is the only other one that has a similar relationship to its leafhopper vector (4). MCDV in *G. nigrifrons* and rice tungro virus in *Nephotettix virescens* (previously known as *N. impicticeps*) can complete the transmission process in 2 hr, after 1 hr of acquisition access and 1 hr of inoculation access. No definite latent period could be demonstrated for either virus in its vector. Molting caused the loss of rice tungro virus and MCDV in their respective leafhopper vectors. The two viruses differ, however, in the length of time they can be retained by their vectors. The maximum retention period of rice tungro virus in *N. virescens* is 5 days (4), that of MCDV in *G. nigrifrons* is 2 days. More recent work showed that *N. virescens* can retain rice tungro virus for up to 6 days at 32 C and up to 22 days at 13 C (5). Also, a recent report indicated that at below-ambient temperature *G. nigrifrons* retained MCDV up to 4 days (6).

Most of our data on MCDV (CSA-OH) and *G. nigrifrons* from Mississippi agree with those of Nault et al (7) on MCDV and the same leafhoppers species from Ohio. There are, however, differences in the efficiency of MCDV transmission when single *G. nigrifrons* were used. Nault et al (7) obtained MCDV transmission by 40.0–41.4% of the *G. nigrifrons* that were tested. In a later experiment, 21.2% of singly tested *G. nigrifrons* transmitted MCDV (6). Our tests consistently yielded transmission rates of 34–35%. The differences in the percentage of transmitters obtained by Nault and co-workers and by us may be explained in terms of different biotypes or different male:female ratios of *G. nigrifrons* existing in the two laboratories. A more notable discrepancy is found in the data on the separate efficiencies of transmission by male and female *G. nigrifrons*. Nault et al (7) found no evidence for a significant difference in MCDV transmission between males and females, whereas we obtained a 50% greater efficiency of MCDV transmission by female than by male leafhoppers. The percentages of transmitters in our three experiments (one not presented in this report) were 30, 28, and 30% for males and 42, 46, and 46% for females. There was, however, a difference in procedure: we worked with single, sexed leafhoppers, whereas Nault et al (7) used five leafhoppers of same sex per test plant.

We established 15-min acquisition and inoculation threshold periods for MCDV with *G. nigrifrons*. The minimum inoculation access period was determined with leafhoppers that had been exposed to MCDV for many days. These minimum periods, however, are insufficient to complete the transmission process when starting with virus-free leafhoppers. The shortest time during which the transmission cycle could be completed was 90 min, after a 60-min acquisition access period and a 30-min inoculation access period. More reliable MCDV transmission was obtained after a 60-min duration of each acquisition access and inoculation access.

When MCDV-inoculative *G. nigrifrons* were confined to healthy corn test plants for an extended time, they retained inoculativity for varying periods not exceeding 48 hr. During the next 24 hr inoculativity could not be detected in the vectoring leafhoppers. This "dark period" was followed by reacquisition of MCDV by the active transmitters from the test plants they had inoculated a few days earlier. Therefore, if the inoculation access period is allowed to continue for more than 3 days, as was done by the junior author in his early work (9,10), the transmission of MCDV by *G. nigrifrons* may appear to be of a persistent nature. The present report corrects this misconception by establishing the relationship between MCDV (CSA-OH) and *G. nigrifrons* as being not persistent.

A successful acquisition of MCDV by *G. nigrifrons* in 15 min, a positive inoculation also within 15 min, the lack of a demonstrable latent period in the vector, a retention period of only 48 hr (at ambient temperature), and the loss of inoculativity after molting indicate a nonpersistent association between MCDV and *G. nigrifrons*. In fact, the relationship between MCDV and *G. nigrifrons* is the least persistent one for any leafhopperborne virus known so far. However, to avoid applying the term nonpersistent to leafhopper-transmitted viruses in the same sense as it is applied to aphid-transmitted viruses (which are styletborne), the relationship between MCDV and *G. nigrifrons* may best be described as being "transient" or "transitory" (analogous to some virus disease symptoms that disappear with time). We support the recommendation made by Ling and Tiongco (5) that rice tungro virus be classifed as a transitory, rather than semipersistent, leafhopper-transmitted virus.

Consequently, we do not agree with the description of MCDV as having a semipersistent relationship to its leafhopper vector *G. nigrifrons*. Nault et al (7) based their proposal for designating MCDV as a semipersistent leafhopperborne virus on the classification of aphidborne viruses by Sylvester (12). Since aphids and leafhoppers do not have identical interactions with the viruses they transmit, and there are no known nonpersistent (sensu styletborne) leafhopper-transmitted viruses, the terms transient or transitory more aptly describe the relationship between MCDV and *G. nigrifrons*. Applying "semipersistent" to leafhopperborne viruses presupposes the existence of both persistent and nonpersistent viruses, on the one hand, and implies a parallel virus-vector relationship between aphids and leafhoppers, on the other hand. At present, both assumptions are unjustified.

LITERATURE CITED

1. Bradfute, O. E., Louie, R., and Knoke, J. K. 1972. Isometric viruslike particles in maize with stunt symptoms. (Abstr.) Phytopathology 62:748.
2. Choudhury, M. M., and Rosenkranz, E. 1971. *Graminella nigrifrons* as a vector of corn stunt agent. (Abstr.) Phytopathology 61:888.
3. Choudhury, M. M., and Rosenkranz, E. 1973. Differential transmission of Mississippi and Ohio corn stunt agents by *Graminella nigrifrons*. Phytopathology 63:127-133.
4. Ling, K. C. 1966. Nonpersistence of the tungro virus of rice in its leafhopper vector, *Nephotettix impicticeps*. Phytopathology 56:1252-1256.
5. 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. 644 pp.
6. Nault, L. R., and Knoke, J. K. 1981. Maize vectors. Pages 77–84 in: Virus and Viruslike Diseases of Maize in the United States. D. T. Gordon, J. K. Knoke, and G. E. Scott, eds. South. Cooperative Ser. Bull. 247. 210 pp.
7. 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. 66:1271-1273.
8. Pirone, T. P., Bradfute, O. E., Freytag, P. H., Lung, M. C. Y., and Poneleit, G. C. 1972. Viruslike particles associated with a leafhopper-transmitted disease of corn in Kentucky. Plant Dis. Rep. 56:652-656.
9. Rosenkranz, E. 1969. Two types of corn stunt in the USA. (Abstr.) Phytopathology 59:1047.
10. Rosenkranz, E. 1969. A new leafhopper-transmissible corn stunt disease agent in Ohio. Phytopathology 59:1344-1346.
11. Rosenkranz, E. 1970. Corn stunt agent isolated from sorghum. (Abstr.) Phytopathology 60:1311.
12. Sylvester, E. S. 1969. Virus transmission by aphids—A viewpoint. Pages 159–173 in: Viruses, Vectors, and Vegetation. K. Maramorosch, ed. Interscience Publishers, New York. 666 pp.