Etiology

Leafhopper Transmission and Host Plant Range of Maize Chlorotic Dwarf Waikavirus Strains

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ABSTRACT

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Two strains of maize chlorotic dwarf waikavirus (MCDV), the type strain MCDV-T and the recently isolated MCDV-M1, were compared for leafhopper transmission, vector specificity, and host plant range. Leafhopper species tested as vectors and their estimated transmission rates (by single insects) of MCDV-T and MCDV-M1, respectively, were *Graminella nigrifrons,* 27.0 and 20.4%; *Amblysellus grex,* 17.5 and 6.7%; *Planicephalus flavicostatus,* 7.9 and 1.0%; *Stirellus bicolor,* 5.9 and 1.2%; *G. sonora,* 1.2 and 2.5%; and *Dalbulus maidis,* 0.8 and 1.7%. *Euscelidius variegatus, Macrosteles quadrilineatus,* and *Ollarianus strictus* were tested as well but did not transmit either strain. No vector was found that transmitted only one of the strains; however, *A. grex, P. flavicostatus,* and *S. bicolor* transmitted MCDV-T at rates significantly higher than those for MCDV-M1. Rates of loss of inoculativity of MCDV-T and MCDV-M1 by *G. nigrifrons* after virus acquisition were similar but were slower at 15 C than at 30 C. Estimated retention half-lives at 15 C for MCDV-T and MCDV-M1 were 7.7 and 12.5 h, respectively. The corresponding half-lives at 30 C were 3.4 and 4.8 h, respectively. A sample of grass species representing the major groups of Gramineae was tested for susceptibility to MCDV strains with *G. nigrifrons* as a vector. Of 46 grass species tested, 19 (mostly panicoids and andropogonoids) were found to be susceptible to each strain on the basis of symptomatology and detection assays (dot blot hybridization and Western blots). Two symptomless differential hosts were found: *Sorghastrum nutans* tested positive to MCDV-T but not to MCDV-M1, whereas the reverse was observed for *Schizachyrium scoparium.* Tertiary veinbanding symptoms induced by MCDV-T in susceptible grasses were always more pronounced than those caused by MCDV-M1. The differences in symptom severity and transmission efficiency observed between MCDV-M1 and MCDV-T support previous conclusions that MCDV-M1 should be considered a new MCDV strain.

Additional keyword: virus retention.

Since the discovery of maize chlorotic dwarf waikavirus (MCDV) (6,34), various isolates have been described that differ in severity of tertiary veinbanding and stunting produced on maize (*Zea mays* L.) (17,21,30). Recently, it was found that severe stunting observed in the field can result from a synergistic interaction between the type isolate, MCDV-T, and a second isolate, MCDV-M1 (17). With single infections, either isolate causes no stunting or only mild stunting and veinbanding in maize.

MCDV-M1 can be differentiated from MCDV-T on the basis of serology and the electrophoretic patterns of the coat proteins (17). Capsids of MCDV, like other waikaviruses (36,49), contain three coat protein species (CP1, CP2, and CP3), which arise by cleavage of a polyprotein. Two of the three coat proteins of MCDV-M1 (CP2 and CP3) are larger than the corresponding ones of MCDV-T (17). The CP1s of MCDV-M1 and MCDV-T have similar molecular weights but appear to be serologically distinct. In Western blots (WB), a polyclonal antiserum to MCDV-T reacted to some extent with CP2 and CP3 but not with CP1 of MCDV-M1 (17). A further distinction is that crystals of viruslike particles found in the vacuoles of cells from leaves infected with MCDV-M1 contain a high proportion of "empty" or "partially empty" particles (1). In cells of plants infected with typelike isolates, mainly "full" viruslike particles are observed.

MCDV-M1 has been designated a new MCDV strain on the basis of its reactivity in enzyme-linked immunosorbent assay with an antiserum to the type strain (MCDV-T) and similarities with MCDV-T concerning leafhopper transmission (10), diagnostic symptoms, and particle morphology (17). However, a recent study indicated a low frequency of nucleic acid homology between these two strains, suggesting that they may be ultimately designated distinct viruses (33). To better understand the relationships between them, other criteria, such as biological properties, should be examined further. Work on leafhopper transmission (9,28,31,32) and host plant range (29,30) of MCDV-T has been conducted, yet there is little comparable information on the biological properties of MCDV-M1 (10).

Coat proteins have been shown to affect biological properties

of plant viruses such as vector specificity (3,8,15,16) and symptom development (37,39). Considering the evidence that coat proteins of MCDV-M1 and MCDV-T differ physically and serologically (17), we anticipated that their biological traits might also differ. This study tests this hypothesis by comparing the two strains with respect to host plant range, vector range, and retention (persistence) of inoculativity. New experimental host plants and vectors of these strains are reported, and a discussion of the origin of MCDV is presented.

MATERIALS AND METHODS

MCDV strains and leafhopper rearing. Isolates of MCDV-T and MCDV-M1 were obtained from johnsongrass rhizomes collected near Portsmouth, Ohio, in 1972 (32) and 1988 (17), respectively. Both strains were maintained in maize (*Z. mays* L. 'Early Sunglow' sweet corn) by serial leafhopper transmission.

In addition to the principal field vector, *Graminella nigrifrons* (Forbes), eight Deltocephalinae species that use maize as a developmental host (31) were chosen for the vector range experiment: three from the tribe Deltocephalini (*Amblysellus grex* (Oman), *G. sonora* (Ball), and *Planicephalus flavicostatus* (Van Duzee)); three from Euscelini (*Euscelidius variegatus* (Kirshbaum), *Ollarianus strictus* (Ball), and *Stirellus bicolor* (Van Duzee)); and two from Macrostelini (*Dalbulus maidis* (DeLong & Wolcott) and *Macrosteles quadrilineatus* (=*fascifrons*) Forbes). Voucher specimens for most species, with information on the origin of the laboratory colonies, are deposited in The Ohio State University Collection of Insects and Spiders (31). Voucher specimens were not selected for *P. flavicostatus* and *S. bicolor,* which were collected in Wooster, Ohio, in September, 1990; however, morphological examination of male genitalia showed that specimens of the populations used here were similar to those from populations used by Nault and Madden (31), for which vouchers were selected.

Cages and tubes used in the leafhopper rearing and transmission studies were described previously (11). Hosts used to rear *A. grex, G. nigrifrons, G. sonora, M. quadrilineatus,* and *P. flavicostatus* were oats (*Avena sativa* L.) and maize (cultivar Aristogold Evergreen Bantam or Early Sunglow sweet corn). *E. variegatus* and *S. bicolor* were reared on rye (*Secale cereale* L.) and *D. maidis* and *O. strictus* only on maize. Colonies were kept in a growth room at 26 ± 2 C with a photoperiod of 14 h of light and 10 h of dark.

Virus acquisition and inoculation. Adults used in the experiments were 1–3 wk old. Leafhoppers were placed in rearing cages for a 48-h acquisition access period (AAP) on maize source plants, which had been inoculated with either MCDV-M1 or MCDV-T 10–14 days before the test. Fifty to 80 insects were used per source plant. Viruliferous leafhoppers (the number varied with experiment) then were transferred to test plants for a 48-h inoculation access period (IAP), either in tube cages or in rearing cages. Unless otherwise stated, both AAPs and IAPs were carried out in a walk-in chamber adjusted to 28 ± 2 C (day) and 20 ± 2 C (night) and a photoperiod of 14 h of light and 10 h of dark. After the IAP, the leafhoppers were manually removed, and the test plants were sprayed with a pyrethroid insecticide to eliminate residual insects. Test plants were then placed in the greenhouse, and symptoms were recorded 2 and 3 wk later.

Vector range study. Transmission efficiencies of MCDV-M1 and MCDV-T by each leafhopper species were evaluated in three to six trials. In each trial, 20 maize seedlings (inbred cultivar VA-35) at the two- to three-leaf stage were inoculated by confining five insects per test plant within tube cages. For *G. nigrifrons,* whose transmission rate is higher than for other species (31), only three individuals per plant were used. To detect transmission rates as low as 1%, a minimum of 300 insects were tested in all trials for each vector-virus combination (48). Controls with *G. nigrifrons* were included in all trials to assure that experimental conditions were adequate for transmission.

Estimated transmission rates for single leafhoppers (p) were calculated as described by Swallow (41). Values of p obtained for each vector-virus combination were transformed to $\arcsine\sqrt{p}$ and submitted to a two-way analysis of variance by the Minitab General Linear Model (GLM) procedure (26). Data obtained for nonvectors were not included in the statistical analysis. Least significant difference ($\alpha = 0.05$) was calculated and used for multiple comparisons of the means. Because sample size varied, the least significant difference was estimated on the basis of the harmonic mean ($\tilde{n}$) of the sample sizes of the treatments (40).

Rate of loss of inoculativity. The rate of loss of inoculativity of MCDV-M1 and MCDV-T by *G. nigrifrons* was assessed at 15 and 30 C in growth chambers. Only adult females were used because they are more efficient vectors than males when confined on test plants (9). Maize seedlings (Early Sunglow sweet corn) at the two- to three-leaf stage were placed individually in tube cages for inoculation. In each treatment trial, 20 groups of three viruliferous females were serially transferred to test seedlings for successive 4-h IAPs up to 24 h after the AAP. Thus, transmission rates for each strain and temperature were evaluated at the intervals of 0–4, 4–8, 8–12, 12–16, 16–20, and 20–24 h following virus acquisition. Because inoculativity of plant viruses is usually lost by insect vectors at a logarithmic rate over time (5,14,47), transmission rates for single leafhoppers (p) estimated for each time interval (4-h IAP) were transformed to $\log(p)$. Least-squares linear regression was used to fit the exponential model to the transmission data obtained for each strain and temperature. Transmission rates used in the analyses were means of three and four trials at 30 and 15 C, respectively. Midpoints of each time interval (4-h IAP) were used as independent variables. Slopes of the regression equations were compared by a t test ($P = 0.05$) to detect differences in the rate of loss of inoculativity between treatments. On the basis of the regression slopes, retention half-lives ($t_{1/2}$) for each strain at the two temperatures were estimated by using the formula $t_{1/2} = \log(0.5)/\text{slope}$. This formula also was used to calculate 95% confidence intervals (95% CIs) for the half-lives from 95% CIs estimated for the slopes.

Host plant range study. Forty-six species in thirty-one genera of grasses from the main groups of Gramineae, including oryzoids, festucoids, chloridoids, panicoids, and andropogonoids, were tested for susceptibility to MCDV-M1 and MCDV-T. Test plants were grown in 10-cm-diameter plastic pots and thinned to one to three plants per pot after germination. At the three- to five-leaf stage, 10 plants of each grass species were confined with 130–150 viruliferous *G. nigrifrons* within a rearing cage for a 48-h IAP. Another five to 10 plants not exposed to viruliferous leafhoppers were used as a negative control for each species. As a positive control for the detection assays and to check for the inoculativity of the leafhoppers used in each trial, 10 healthy maize seedlings (Early Sunglow sweet corn) were simultaneously exposed to a sample of these insects. In all trials, transmission to maize was 80–100%.

Test and control plants were placed in the greenhouse and evaluated for diagnostic MCDV symptoms (tertiary veinclearing) at 3 and 4 wk after inoculation by comparing leaves of inoculated and noninoculated plants of the same species. After symptom evaluation, leaf tissue of test and control plants was sampled for detection assays. Dot blot hybridization (DB), which tests samples for the presence of viral nucleic acid, was the main assay used. cDNA clones used to prepare specific MCDV-T and MCDV-M1 probes were MC-23 and #48, respectively. Probe MC-23 was provided by M. McMullen (U.S. Department of Agriculture, Agricultural Research Service, Wooster, OH) and is from the coding region for CP1 in the MCDV-T genome (M. McMullen, *personal communication*). Probe #48 was prepared by C. Mzira, Department of Plant Pathology, Ohio Agricultural Research and Development Center, Wooster. Its location on the MCDV-M1 genome is unknown. Many samples were also tested for the presence of viral coat proteins by electrophoresis followed by electroblot immunoassay (WB) to confirm results obtained with DB. All grass species in which any of the strains could replicate and reach levels detectable by either DB or WB were considered experimental hosts, regardless of the presence or absence of visible

symptoms.

Samples consisted of 5 g of leaf tissue collected from the whole plant. Inoculated test plants showing symptoms were sampled individually, while symptomless ones were pooled in groups of five and a single sample was taken from each group. A pooled sample was also taken from three to five noninoculated plants of each grass species (negative control) and from maize plants inoculated with each strain (positive control). Each sample was extracted in 20 ml of 0.3 M potassium phosphate buffer, pH 7.0, containing 0.5% 2-mercaptoethanol. The extract was filtered through two layers of cheesecloth and clarified by emulsification with a 1/5 volume of $CHCl_3$ followed by low-speed centrifugation (5,900 *g* for 10 min). The supernatant was recovered and centrifuged at high speed (105,000 *g* for 2 h). The resulting pellet was then resuspended overnight in 0.25 ml of 0.3 M sodium phosphate buffer, pH 7.0, with gentle agitation at 4 C. From this volume, 0.1 ml was used for WB and 0.15 ml for DB.

DB. Viral nucleic acid was extracted with a mixture of TE (10 mM Tris-HCl and 1 mM EDTA, pH 7.9)-saturated phenol (AMRESCO Inc., Solon, OH) and chloroform-isoamyl alcohol (25:24:1) according to Sambrook et al (38), except that samples were first mixed with 25 μl of 10% sodium dodecyl sulfate (SDS) and 300 μl of TE-saturated phenol and centrifuged at 16,000 *g* for 3 min. Purified nucleic acid was precipitated with 600 μl of cold 95% ethanol and 0.2 M NaCl at −20 C for at least 24 h. The precipitated nucleic acid was pelleted by centrifugation at 16,000 *g* for 15 min, washed with 70% ethanol, and repelleted by another 15-min centrifugation (38). The pellet was resuspended in 0.1 ml of distilled water, and the samples were applied (10 μl per well) to polyvinylidene difluoride (PVDF) membranes (Imobilon-N; Millipore Corp., Bedford, MA) by using a Bio Dot dot blot apparatus (Bio-Rad Laboratories, Richmond, CA). PVDF membranes previously had been wet in methanol, rinsed in water, and stabilized in 20× SSC (1 × SSC is 0.15 M NaCl plus 0.015 M sodium citrate, pH 7.0) transfer buffer for 15 min. Each group of test samples, including positive and negative controls, was blotted in duplicate to two separate membranes: one to be tested for MCDV-M1 and the other for MCDV-T. After the blotting, membranes were baked at 85 C under vacuum for 2 h for fixation of the nucleic acid. Dry membranes were wet in methanol, rinsed with water, and incubated for at least 3 h in a prehybridization buffer (1 ml of denatured salmon sperm DNA [250 μg/ml], 5× SSC, 1.5× Denhardt's solution, 37.5 mM Tris, pH 8.0, 0.15% SDS, and 7.5 mM EDTA) in heat-sealed plastic bags. Duplicate membranes to be tested for MCDV-M1 and MCDV-T were kept in separate bags. A heat-denatured ^{32}P-labeled cDNA probe was then added, and hybridization was carried out for 18 h. Both prehybridization and hybridization were done at 65 C under gentle agitation. Probes were radiolabeled by random priming by the procedure of Feinberg and Vogelstein (13). Oligodeoxyribonucleotides used were $pd(N)_6$ (hexamers, catalog no. 272166-01) purchased from Pharmacia LKB Biotechnology, Piscataway, NJ. The amount of cDNA probe used in each preparation was 50–100 ng (5 μl; 10–20 ng/μl). After hybridization, the membranes were washed five times in 2× SSC plus 0.1% SDS at 65 C for 5 min per wash followed by another five washes in 0.2× SSC plus 0.1% SDS to eliminate excess probe. Autoradiography of the membranes was done at −70 C for 3–7 days with an X-ray film (X-Omat AR, Eastman Kodak Company, Rochester, NY) and intensifying screen.

WB. Each sample (0.1 ml) was mixed with an equal volume of loading buffer (0.13 M Tris-HCl, pH 6.8, 4% SDS, 20% glycerol, 10% mercaptoethanol, 0.5% sucrose, and 10 ppm bromophenol blue) and boiled for 4 min. Viral coat proteins were then separated by electrophoresis on 12.5% vertical slab polyacrylamide gels in a Mini-Protean II dual slice slab cell (Bio-Rad Laboratories) by the Laemmli system (23). In addition to samples of inoculated grasses, each gel contained negative and positive control samples and prestained standards. After electrophoresis, proteins were transferred from polyacrylamide gels onto PVDF membranes (Imobilon-P; Millipore Corp.) at 30 V for 18 h, as described previously (17). After the transfer, viral coat proteins were visualized by using the immunostaining procedure detailed by Gingery and Nault (17), except that total protein staining was not used. For each membrane, two immunostaining series were done to detect the two strains. In the first, the blot was probed with the primary antibody specific for CP1 of MCDV-M1. Soon after development with the solution of nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate, the membrane was photographed, reblocked, and probed with the primary antibody specific for CP2 of MCDV-T.

RESULTS

Vector range. No vector was found that transmitted only one of the strains. However, analysis of variance indicated differences in transmission efficiency (*p*) among vector species ($F = 44.5$; df = 5; error = 62; $P < 0.001$) and between strains ($F = 9.6$; df = 1; error = 62; $P < 0.001$). *G. nigrifrons,* which was tested simultaneously with all the other species as a control, efficiently transmitted both strains in all trials at a higher rate than other vector species did (Table 1). *A. grex* was also an efficient vector but transmitted MCDV-M1 at a lower rate than that for MCDV-T. Two other species, *P. flavicostatus* and *S. bicolor,* were relatively efficient vectors of MCDV-T but poor vectors of MCDV-M1. *G. sonora* and *D. maidis* were poor vectors of both strains, whereas *E. variegatus, O. strictus,* and *M. fascifrons* failed to transmit virus (Table 1).

There was a statistical interaction between vector species and strains ($F = 2.9$; df = 5; error = 62; $P = 0.019$), which reflects the differential effect of vector species on the transmission efficiency of the two strains. Efficient and relatively efficient vectors (*A. grex, P. flavicostatus,* and *S. bicolor*), except *G. nigrifrons,* transmitted MCDV-T at rates higher than those for MCDV-M1 (Table 1). Inefficient vectors (*D. maidis* and *G. sonora*), however, transmitted both strains at equally low rates.

Rate of loss of inoculativity of MCDV strains. Transmission efficiencies of MCDV-M1 and MCDV-T by *G. nigrifrons* at 15 and 30 C decreased at a logarithmic rate with time after virus acquisition (Fig. 1). Regression equations obtained for the mean transmission rates of the two strains at 15 and 30 C were all statistically significant ($P < 0.05$); R^2 values ranged from 87.1 to 97% (Fig. 1). Comparisons by *t* test ($P = 0.05$) indicated no significant differences between the regression slopes obtained for MCDV-M1 and MCDV-T at either 15 or 30 C (Table 2). This result suggests that, at the same temperature, the two strains have similar rates of loss of inoculativity. However, slopes were affected by temperature. At 30 C, slopes of both strains were significantly steeper than at 15 C (Table 2), demonstrating that virus inoculativity by *G. nigrifrons* declines faster at higher temperatures. As a result, estimated half-lives for MCDV-M1 and MCDV-T were shorter at 30 C than at 15 C (Table 2).

Host plant range. Nineteen grass species were found to be susceptible to each strain on the basis of symptomatology and detection assays (Table 3). Among these, 18 species were common hosts to both strains. Two differential hosts were found, and both were symptomless: *Sorghastrum nutans* was susceptible to MCDV-T but not to MCDV-M1, whereas the reverse was observed for *Schizachyrium scoparium* (Table 3). *S. nutans* was confirmed as susceptible to MCDV-T by DB and WB. *S. scoparium,* however, tested positive to MCDV-M1 by DB but not by WB (Table 3); thus, its susceptibility to MCDV-M1 is uncertain. MCDV strains differed with respect to symptom expression. The diagnostic veinbanding caused by MCDV-T on susceptible grasses was always more pronounced than that induced by MCDV-M1. Furthermore, three susceptible grasses (*Ischaemum rugosum, Panicum miliaceum,* and *Setaria glauca*), which showed veinbanding when infected with MCDV-T, were symptomless when infected with MCDV-M1 (Table 3).

Most species (78.9%) and genera (71.4%) susceptible to MCDV strains were panicoids and andropogonoids. Of 11 festucoid grasses tested, only two (*Muhlenbergia sobolifera* and *Triticum aestivum*) were susceptible (Table 3). While most panicoid and andropogonoid hosts showed clear tertiary veinbanding, these

TABLE 1. Transmission efficiency of maize chlorotic dwarf waikavirus (MCDV) strains MCDV-M1 and MCDV-T by nine leafhopper species from the subfamily Deltocephalinae

Leafhopper species MCDV strain	Transmission by test species[w]		Transmission by *Graminella nigrifrons* checks[w]	
	N	*p*	*N*	*p*
Amblysellus grex				
MCDV-M1	530	0.067 (0.011) b[x]	294	0.184 (0.021)
MCDV-T	520	0.175 (0.037) a	285	0.250 (0.025)
Dalbulus maidis				
MCDV-M1	625	0.017 (0.009) c	378	0.192 (0.031)
MCDV-T	625	0.008 (0.006) c	372	0.273 (0.042)
Euscelidius variegatus[y]				
MCDV-M1	295	0	189	0.141 (0.015)
MCDV-T	305	0	189	0.171 (0.013)
Graminella nigrifrons				
MCDV-M1	780	0.204 (0.022) a	NA[z]	NA
MCDV-T	771	0.270 (0.031) a	NA	NA
G. sonora				
MCDV-M1	360	0.025 (0.003) bc	231	0.176 (0.030)
MCDV-T	370	0.012 (0.007) c	228	0.207 (0.023)
Macrosteles quadrilineatus[y]				
MCDV-M1	520	0	294	0.227 (0.033)
MCDV-T	500	0	284	0.295 (0.038)
Ollarianus strictus[y]				
MCDV-M1	450	0	252	0.190 (0.011)
MCDV-T	445	0	246	0.293 (0.008)
Planicephalus flavicostatus				
MCDV-M1	475	0.010 (0.007) c	294	0.155 (0.015)
MCDV-T	475	0.079 (0.025) b	291	0.201 (0.028)
Stirellus bicolor				
MCDV-M1	400	0.012 (0.008) c	234	0.230 (0.054)
MCDV-T	340	0.059 (0.016) b	234	0.340 (0.083)

[w]Transmission of MCDV strains by each species was tested in three to six trials. Insects were allowed a 48-h acquisition access period on virus source plants and subsequently placed five per plant on maize test plants for a 48-h inoculation access period. Unlike other species, *G. nigrifrons* was placed three per plant on test plants. *G. nigrifrons* was included in all trials to check whether source plants and experimental conditions were adequate for transmission. N = total number of leafhoppers tested in all trials; and p = transmission rates for single insects = $1 - (1 - I)^{1/k}$, in which I is the proportion of infected plants and k is the number of insects placed per plant (41). Number in parentheses is the standard error of p.

[x]Mean transmission rates followed by the same lowercase letter are not significantly different ($P = 0.05$; LSD test preceded by a two-way analysis of variance using transformed transmission rates [arcsine $\sqrt{p}$]).

[y]Nonvectors were not included in the statistical analysis.

[z]Not applicable.

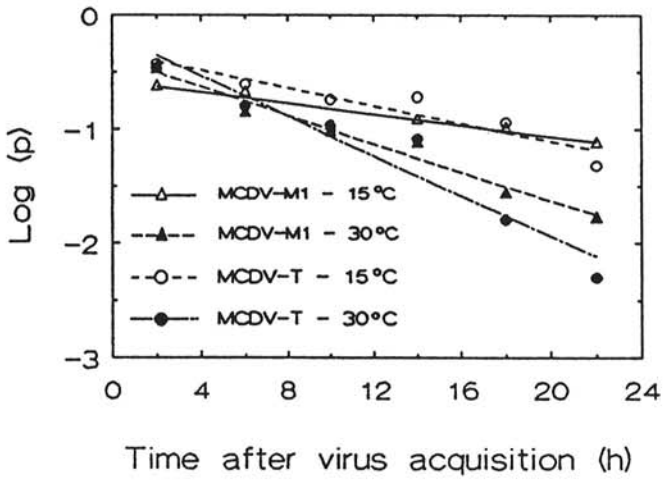

Fig. 1. Observed mean (points) and predicted log-transformed transmission rates, log(p) (lines), of maize chlorotic dwarf waikavirus (MCDV) strains MCDV-M1 and MCDV-T at 15 and 30 C by *Graminella nigrifrons* over time elapsed after virus acquisition (t). Regression equations and associated coefficients of determination (R^2) obtained for MCDV-M1 at 15 and 30 C were, respectively, $\log(p) = -0.584 - 0.024t$, $R^2 = 87.1\%$, and $\log(p) = -0.376 - 0.063t$, $R^2 = 97.0\%$. For MCDV-T at 15 and 30 C, regression equations and associated R^2s were $\log(p) = -0.328 - 0.039t$, $R^2 = 89.1\%$, and $\log(p) = -0.183 - 0.088t$, $R^2 = 92.7\%$, respectively. 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 placed per test plant (41).

TABLE 2. Regression slopes and half-lives of inoculativity of maize chlorotic dwarf waikavirus (MCDV) strains MCDV-M1 and MCDV-T by *Graminella nigrifrons* at 15 and 30 C

Temperature (C)	McDV strain	Regression slope[x]	Half-life[y] (h)	
			Mean	95% CI
15	MCDV-M1	−0.024 (0.005) a[z]	12.5	7.9, 30.0
	MCDV-T	−0.039 (0.007) a	7.7	5.1, 15.0
30	MCDV-M1	−0.063 (0.006) b	4.8	3.9, 6.1
	MCDV-T	−0.088 (0.012) b	3.4	2.5, 5.5

[x]Slopes estimated by least-squares linear regression of log-transformed transmission rates (means of three and four trials at 30 and 15 C, respectively) over time elapsed after virus acquisition. Number in parentheses is the standard error of the slope.

[y]Mean half-life ($t_{1/2}$) estimated on the basis of the slope, $t_{1/2} = \log(0.5)/\text{slope}$. A 95% confidence interval (CI) for mean half-life was estimated from the 95% CI for the slope by using the same formula.

[z]Regression slopes followed by the same lowercase letter are not significantly different ($P = 0.05$; t test).

two festucoid hosts were symptomless. The majority of susceptible species were annuals. Sixteen of 21 annual grasses tested were susceptible, with 14 and 11 species showing symptoms when infected by MCDV-T and MCDV-M1, respectively. In contrast, only four of 24 perennial grasses evaluated were susceptible to any of the strains, and all were symptomless (Table 3).

TABLE 3. Susceptibility of Gramineae species to maize chlorotic dwarf waikavirus (MCDV) strains MCDV-T and MCDV-M1 on the basis of symptomatology and detection assays

Major groups of Gramineae Species[t]	Growth habit[u]	MCDV-T			MCDV-M1		
		Symptoms[v]	DB[w]	WB[x]	Symptoms	DB	WB
Oryzoids							
Leersia hexandra Sw.	P	−	−	N[y]	−	−	N
L. oryzoides (L.) Sw.	P	−	−	N	−	−	N
Festucoids							
Agropryon repens (L.) Beauv.	P	−	−	−	−	−	−
Avena sativa L.	A	−	−	−	−	−	−
Bromus secalinus L.	A	−	−	−	−	−	−
Dactylis glomerata L.	P	−	−	−	−	−	−
Hordeum vulgare L.	A	−	−	−	−	−	−
Lolium perenne L.	P	−	−	N	−	−	N
Muhlenbergia sobolifera (Muhl.) Trin.[z]	P	−	+	+	−	+	+
Phalaris arundinacea L.	P	−	−	−	−	−	−
Poa pratensis L.	P	−	−	−	−	−	−
Secale cereale L.	A	−	−	−	−	−	−
Triticum aestivum L.	A	−	+	N	−	+	N
Chloridoids							
Eleusine coracana (L.) Gaertn.[z]	A	+	+	N	+	+	N
E. indica (L.) Gaertn.	A	−	−	−	−	−	−
Eragrostis cilianensis (All.) E. Mosher[z]	A	−	+	N	−	+	N
Spartina pectinata Link	P	−	−	−	−	−	−
Arundinoid-Danthonoids							
Danthonia pilosa R. Br.	P	−	−	−	−	−	−
Panicoids							
Digitaria decumbens Stent	P	−	−	−	−	−	−
D. ischaemum (Schreb.) Schreb. ex Muhl.[z]	A	+	+	N	+	+	N
D. sanguinalis (L.) Scop.	A	+	+	N	+	+	N
Echinochloa crusgalli (L.) Beauv. var. *crusgalli*	A	+	+	N	+	+	N
Panicum capillare L.	A	−	−	N	−	−	N
P. miliaceum L.	A	+	+	+	−	+	−
P. virgatum L.	P	−	−	−	−	−	−
Paspalum notatum Fluegge	P	−	−	−	−	−	−
Pennisetum americanum (L.) Leeke	A	+	+	N	+	+	N
Setaria faberi Herrm.	A	+	+	N	+	+	N
S. glauca (L.) Beauv.	A	+	+	+	−	+	+
S. magna Griseb.	A	+	+	+	+	+	+
S. viridis (L.) Beauv.	A	+	+	N	+	+	N
Andropogonoids							
Andropogon gerardii Vitm.	P	−	−	−	−	−	−
A. ternarius Michx.	P	−	−	N	−	−	N
A. virginicus L.[z]	P	−	+	+	−	+	−
Heteropogon contortus (L.) Beauv. ex Roem. & Schult.	P	−	−	−	−	−	−
Ischaemum rugosum Salisb.[z]	A	+	+	+	−	+	+
Rottboellia exaltata L. f.[z]	A	+	+	N	+	+	N
Schizachyrium condensatum (Kunth) Nees	P	−	−	N	−	−	N
S. scoparium (Michx.) Nash	P	−	−	−	−	+	−
Sorghastrum nutans (L.) Nash[z]	P	−	+	+	−	−	−
Sorghum sudanense (Piper) Stapf	A	+	+	+	+	+	+
S. bicolor (L.) Moench	A	+	+	N	+	+	N
Tripsacum dactyloides (L.) L.	P	−	−	N	−	−	N
T. lanceolatum Rupr. ex Fourn.	P	−	−	−	−	−	−
Zea perennis (Hitchc.) Reeves & Mangelsd.	P	−	−	−	−	−	−
Z. diploperennis Iltis, Doebley, & Guzman	P	−	−	−	−	−	−

[t] Taxonomic arrangement and species names according to Watson and Gibbs (44) and Terrell (43), respectively.
[u] P = perennial growth habit, and A = annual growth habit.
[v] + = Present, and − = absent.
[w] Dot blot hybridization assay: + = tested positive, and − = tested negative. Range of sensitivity: 0.001–0.0001 ng of RNA per milligram of tissue for the MCDV-T probe (MC-23) and 0.01–0.001 ng of RNA per milligram of tissue for the MCDV-M1 probe (#48).
[x] Western blot assay: + = tested positive, and − = tested negative. Range of sensitivity: 0.01–0.001 ng of protein per milligram of tissue for the antiserum to MCDV-T and 0.1–0.01 ng of protein per milligram of tissue for the antiserum to MCDV-M1.
[y] Assay not carried out.
[z] New previously unreported host of MCDV-T.

DISCUSSION

The rationale for this study was to test the hypothesis that the differences between MCDV-M1 and MCDV-T in serology and molecular weight of coat proteins (17) might translate into substantial differences in their biological properties. The results revealed that the two strains are rather similar in host plant range, vector range, and retention of inoculativity by the vector. However, it was shown that the strains can be distinguished from one another by symptomatology as well as by transmission rate by some vector species. In addition, two differential host plants were identified.

Similarities in the rates of loss of inoculativity of MCDV-T and MCDV-M1 by *G. nigrifrons* with time after virus acquisition suggest that the variations in coat proteins between these strains have no significant effect on their retention in the vector. As has been observed for aphid-transmitted potyviruses and caulimoviruses (19,35), there is evidence that transmission of MCDV by leafhoppers is dependent on nonstructural viral-coded proteins known as helper components (HCs) (10,21), which presumably assist in the binding of virions to retention sites in the vector's foregut (2,4,27). Because a previous study on the putative HC-mediated transmission of MCDV showed that HCs of MCDV-M1 and MCDV-T can be used interchangeably (10), it seems reasonable to assume that the two strains have similar HCs. This may explain why these strains have comparable retention half-lives in the vector.

The higher rates of decay of inoculativity of MCDV-M1 and MCDV-T by *G. nigrifrons* at 30 C compared with those at 15 C are consistent with previous findings that retention times of MCDV-T in *G. nigrifrons* are prolonged at lower temperatures (28). A similar effect of temperature on retention times has been reported for other foregut-borne viruses transmitted by aphids (7,20,22,42) and leafhoppers (24), including rice tungro spherical virus, the type member of the waikavirus group. These findings suggest that the ability of vectors to retain and spread foregut-borne viruses may vary seasonally. Virus spread in the summer, for instance, might be restricted to shorter distances from the inoculum sources than in the spring, because summer temperatures are higher and the rate of loss of inoculativity is expected to be faster.

Results of the vector range study of MCDV strains confirm observations of Nault and Madden (31) that the potential to transmit MCDV among leafhoppers that use maize as a breeding host is correlated with the phylogenetic relatedness of the species. Among the leafhoppers tested here, only species from the Deltocephalini (*G. nigrifrons, A. grex,* and *P. flavicostatus*) and recent Euscelini (*S. bicolor*) were efficient vectors of MCDV-T. MCDV-M1 was efficiently transmitted only by two Deltocephalini species (*G. nigrifrons* and *A. grex*). *D. maidis* was an inefficient vector of both MCDV strains. Previous reports list *D. maidis* as a nonvector (31,32,45). The smaller number of insects tested in previous studies (100–300) may be the reason that the low levels of MCDV transmission by *D. maidis* were not detected before. With 300 insects, the probability of transmission could still be 0.01 when all observed insects failed to transmit (41).

The host plant range comparison between MCDV-M1 and MCDV-T in this study confirms previous observations of Nault et al (30) that most MCDV hosts are nonfestucoid grasses, especially panicoids and andropogonoids. This is consistent with observations that MCDV is more prevalent in warm regions of the southeastern states where nonfestucoid grasses are more abundant and where the primary vector, *G. nigrifrons,* may have evolved (46). In fact, the few grasses in which MCDV has been found in nature are either panicoids or andropogonoids (29,34). This supports the view that a greater number of susceptible experimental hosts are most likely to be found in plant groups containing species that the virus infects naturally (12).

Eight grasses were identified as new experimental hosts of MCDV-T in this research: four andropogonoids (*Andropogon virginicus, I. rugosum, Rottboellia exaltata,* and *S. nutans*), two chloridoids (*Eleusine coracana* and *Eragrostis cilianensis*), one festucoid (*M. sobolifera*), and one panicoid (*Digitaria ischaemum*). Among 11 species not tested to MCDV-T before, six (all perennials) were found not susceptible: three andropogonoids (*Andropogon ternarius, Heteropogon contortus,* and *Schizachyrium condensatum*), one panicoid (*Digitaria decumbens*), one chloridoid (*Spartina pectinata*), and one oryzoid (*Leersia oryzoides*). Three species originally found to be immune to MCDV-T by Nault et al (30) (*A. virginicus, E. cilianensis,* and *I. rugosum*) are reported here as susceptible to this strain. The reverse was observed for two other species, *Eleusine indica* and *Panicum capillare.* Differences in sensitivity between the detection assays (DB and WP) used in this study (Table 3) and by Nault et al (30) (back inoculation by leafhopper transmission, rate-zonal sucrose density gradient centrifugation, and transmission electron microscopy) may be the reasons for these conflicting results. Other possibilities include escape (an inadequate number of test plants and/or inoculative leafhoppers used), misidentification of grass species, and use of different varieties of a species.

A. virginicus, M. sobolifera, S. nutans, and *S. scoparium* are the first native perennial grasses reported to be susceptible to MCDV. Previously, the only perennial species known to be susceptible to this virus was johnsongrass (*Sorghum halepense* (L.) Pers.) (30), an andropogonoid grass introduced from the Mediterranean region early in the 19th century (25). Johnsongrass is considered to be the major overwintering host of MCDV in the United States (18,30). It is possible that MCDV was introduced into the United States from the Mediterranean region. However, MCDV is not seed transmitted (johnsongrass seed, not rhizomes, was introduced to the United States), and there are no reports of any similar diseases of maize outside the United States, thus suggesting that MCDV originated in North America. The discovery of perennial hosts native to the United States supports the hypothesis that MCDV is an indigenous virus. It remains to be learned whether any of these experimental perennial hosts are natural overwintering hosts of MCDV.

The finding that four (including johnsongrass) of the five susceptible perennial grasses are andropogonoids favors the idea that MCDV strains may have evolved in an andropogonoid host. However, only a limited number (12 genera) of native perennial grasses so far have been surveyed for susceptibility to MCDV (this study and that by Nault et al [30]). Moreover, it is puzzling that one of the experimental perennial hosts (*M. sobolifera*) was found among the festucoid grasses, where the concentration of susceptible species is very low and all hosts are symptomless. Considering that the oldest and most evolved host-parasite relationships might be those where least damage is inflicted on the hosts (44), we also cannot rule out the possibility that MCDV first evolved in a festucoid host.

In summary, this study shows that MCDV-M1 and MCDV-T share very similar biological properties but can be distinguished from one another on the basis of severity of symptoms and transmission efficiency by some of the vector species. Overall, these results support the conclusions of Gingery and Nault (17) that MCDV-M1 should be considered a new strain of MCDV. Additional information on genome and coat protein structure of MCDV-M1 and MCDV-T and on the genetic basis for the observed phenotypic variations is necessary for a confirmation of the taxonomic status of MCDV-M1.

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