Biological and Serological Characterization of Three Montana RMV-Like Isolates of Barley Yellow Dwarf Virus

S. K. Z. BRUMFIELD, Laboratory Technician, and T. W. CARROLL, Professor, Department of Plant Pathology, Montana State University, Bozeman 59717; and S. M. GRAY, Agricultural Research Service, U.S. Department of Agriculture, Department of Plant Pathology, Cornell University, Ithaca, NY 14850

ABSTRACT

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Three Montana (MT) RMV-like isolates of barley yellow dwarf virus (BYDV) collected from Choteau (MT-RMV-C), Fort Ellis (MT-RMV-FE), and Valier (MT-RMV-V) were characterized on the basis of transmission, virulence, serology, and cross-protection studies. Aphid transmission characteristics of MT RMV-like isolates were determined and compared with those of New York (NY) RMV, SGV, RPV, PAV, and MAV isolates. Aphid vectors tested were *Rhopalosiphum maidis*, collected at four locations in Montana, and the NY biotypes of *R. maidis*, *Schizaphis graminum*, *R. padi*, and *Sitobion avenae*. Two different populations of each MT *R. maidis* karyotype, $2n=8$, which is predominant on corn, and $2n=10$, which colonizes barley, were used as vectors in this study. Although both karyotypes were efficient vectors of MT RMV-like isolates, there were differences in transmission efficiency between each of the four populations. Unlike NY-RMV, which is transmitted efficiently only by *R. maidis*, all MT RMV-like isolates were transmitted efficiently by *R. maidis* and *S. graminum*, two were occasionally transmitted by *R. padi*, and none were transmitted by *S. avenae*. MT RMV-like isolates were similar to NY-RMV isolates in that they reacted with NY-RMV immunoglobulin but not with NY-SGV, NY-RPV, NY-PAV, or NY-MAV immunoglobulins in enzyme-linked immunosorbent assay (ELISA). MT RMV-like isolates were more virulent in oats than either NY-RMV or NY-SGV. MT-RMV-C and MT-RMV-V isolates were each used individually in cross-protection experiments to determine if either could cross-protect against NY-SGV in oats. Little or no cross-protection was found.

In 1983, Yount and Carroll (19) described some Montana (MT) barley yellow dwarf virus (BYDV) isolates transmitted most efficiently by the corn leaf aphid, *Rhopalosiphum maidis* (Fitch), but some were also transmitted occasionally by *Schizaphis graminum* Rondani and/or *Rhopalosiphum padi* L. These isolates did not react in double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) with polyclonal rabbit immunoglobulins specific for the New York (NY) BYDV isolates of RMV, RPV, MAV, and PAV. These tests revealed BYDV isolates with unusual aphid transmission patterns and antigenic properties different from previously characterized isolates. Although these isolates were not saved, it was our intention to find similar isolates and obtain more thorough transmission and serological data.

Prior to 1981, it was believed that all *R. maidis* biotypes had $2n=8$ karyotypes, but while confirming the identity of *R. maidis* samples collected in Montana, V. F. Eastop and R. L. Blackman (British Museum of Natural History, London) determined that these aphids had $2n=10$ karyotypes. Collections of *R. maidis* samples from Montana in 1985 revealed that the 12 found on barley had karyotype $2n=10$ and the three found on corn had karyotype $2n=8$. Additional *R. maidis* samples from Montana and Idaho collected in 1986 and 1987 confirmed the association between specific karyotype and selective host colonization (1). Subsequently, in 1988, Brown and Blackman (2) reported that three karyotypes ($2n=8$, $2n=9$, and $2n=10$) were present in populations of *R. maidis* in North America. The NY biotype of *R. maidis*, which is the most efficient vector of the NY-RMV isolate of BYDV, was not karyotyped until 1987 and was found to have a $2n=8$ karyotype. Because all previous transmission data were collected using the NY *R. maidis* biotype, none were available using the $2n=10$ vector with which to compare transmission efficiencies of MT RMV-like isolates and/or the NY-RMV isolate.

The apparent antigenic uniqueness and different transmission phenotypes of some of the MT RMV-like isolates, plus the unexpected karyotype of the MT *R. maidis* samples, prompted us to study additional RMV-like isolates and samples of *R. maidis* from Montana.

In this paper, we report the further characterization of MT RMV-like isolates of BYDV in terms of their transmission and serological properties. Preliminary work in 1988 (20) indicated that, unlike NY-RMV, which is transmitted efficiently only by *R. maidis*, three of the isolates were transmitted efficiently by *R. maidis* from both Montana and New York and by *S. graminum* from New York. Also, two of the three MT RMV-like isolates were detected with the NY-RMV immunoglobulin in DAS-ELISA.

MATERIALS AND METHODS

Virus isolates and aphid vectors. Three MT isolates of BYDV that were obtained in 1986 from fields near Choteau, Fort Ellis, and Valier were designated MT-RMV-C, MT-RMV-FE, and MT-RMV-V, respectively (Fig. 1). These isolates were recovered from *R. maidis* collected on spring barley infected with BYDV. After their initial transmission to oats (*Avena sativa* L. 'California Red' or *A. byzantina* K. Koch 'Coast Black'), they were maintained through serial transfers to oats by means of laboratory-reared aphids, including the NY biotypes of *R. maidis* and NY *S. graminum* and four MT *R. maidis* biotypes, both $2n=8$ and $2n=10$ karyotypes, in an attempt to keep from selecting certain virus biotypes via the vector. The transmission of MT isolates was then compared with the five characterized NY isolates of BYDV (RMV, SGV, RPV, PAV, and MAV) (9–11) obtained from W. F. Rochow (USDA-ARS, Cornell University, Ithaca, NY) in 1986 and S. M. Gray in 1987 and 1988. The NY isolates were maintained in oats by serial transfers using the NY biotypes of *R. maidis*, *S. graminum*, *R. padi*, and *Sitobion avenae* (Fabricius).

R. maidis samples were collected in 1986 and 1987 at four field locations near Bozeman, MT. An individual colony from each collection site was started with several first-instar nymphs and maintained as nonviruliferous aphids on barley (cv. Klages) as described by Rochow (11). MT colonies *R. maidis* B2 and B4, collected on spring barley, had karyotype $2n=10$, whereas colonies *R.*

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maidis C5 and C12, collected on corn (*Zea mays* L.), had karyotype $2n=8$ (1). In addition, nonviruliferous colonies of the NY biotypes *R. maidis*, *S. graminum*, *R. padi*, and *S. avenae* (also supplied by W. F. Rochow) were maintained as previously described (9).

Virus transmission tests. Nonviruliferous aphids were allowed a 2-day acquisition access period (AAP) on detached oat leaves infected with BYDV followed by a 5-day inoculation access period (IAP) on healthy oat seedlings. Transmission studies were begun by using one, 10, and 25 nymphs of MT *R. maidis* B2 or C12 or NY *R. maidis*, previously given an AAP on oats infected with either MT-RMV-C or NY-RMV. Because transmission of the MT RMV-like isolate and NY-RMV was more successful with 25 nymphs of *R. maidis*, transmission studies were conducted with different combinations of aphid vectors and isolates using 25 nymphs of *R. maidis*, *R. padi*, and *S. avenae*. Twenty nymphs of *S. graminum* were used in transmission studies because 25 nymphs caused too much toxin damage during feeding. Viruliferous aphids were caged on individual oat seedlings (two- to three-leaf stage) and maintained in a growth chamber or isolation room at 18 C in constant light (10,000 lux). After the IAP, the infested plants were fumigated with Vapona (dichlorvos) for 2 hr, placed in the greenhouse, and assessed daily for symptom expression during a 6-wk growth period.

Transmission efficiency was calculated as the number of symptomatic oats divided by the number of oats infested. Data from isolates transmitted by the same aphid vectors were pooled over a 2.5-yr period (110 different transmission tests). Transmission specificity and efficiency were analyzed for significant differences using CATMOD within the SAS statistical package (14). The replication aspect of the data was not taken into account because different combinations of aphid vectors and virus isolates, as well as different numbers of plants, were inoculated in each replication and the procedure would be unduly complicated. Consequently, the CATMOD analysis may be somewhat optimistic but could still be used to determine statistical differences. Individual analysis between each vector/isolate combination (including NY *S. graminum*), as well as combinations of $2n=8$ and $2n=10$ karyotype/isolate combinations, were analyzed.

Cross-protection experiments. Because NY *S. graminum* transmitted both MT RMV-like isolates and the NY-SGV isolate of BYDV, assays were conducted to determine if either the MT-RMV-C or MT-RMV-V isolate and the NY-SGV isolate could induce cross-protection against each other in oats. Oats (three-leaf stage) were inoculated with the MT-RMV-C or MT-RMV-V isolate vectored by MT *R. maidis* C5. Two weeks later, the same oats were inoculated with the NY-SGV isolate vectored by the NY biotype of *S. graminum*. Oats were similarly inoculated with the same isolates by the same vectors in a reverse order. Numbers of aphids and experimental conditions were identical to those described earlier for the transmission experiments. In addition, oats were inoculated simultaneously with the MT-RMV-C or MT-RMV-V isolate plus the NY-SGV isolate using 15 viruliferous nymphs each of MT *R. maidis* C5 and NY *S. graminum*. Oats were also inoculated separately with MT-RMV-C, MT-RMV-V, NY-RMV, and NY-SGV isolates to compare sequential or simultaneous and single inoculations. Symptoms were assessed in the greenhouse every week for 5 wk after the IAP. Leaves from the same plants were harvested 14–20 days and again 34–37 days after the first day of the IAP and sent to Ithaca for serological testing by DAS-ELISA. Leaf samples from cross-protection experiments in which oats were doubly or singly inoculated with MT-RMV-C and NY-SGV isolates were also sent to R. M. Lister and R. E. Klein at Purdue University, West Lafayette, IN, for testing by ELISA.

Serological testing. Leaves of indicator test plants from transmission and cross-protection experiments were analyzed by DAS-ELISA at Ithaca following the procedure described previously (S. M. Gray, *personal communication*). Immunoglobulins from antisera specific for NY-RPV, NY-PAV, and NY-MAV produced in New York (13) and from antisera specific for NY-RMV and NY-SGV produced in Indiana (17) were used to detect and identify isolates. NY-SGV and NY-RMV immunoglobulins detect only homologous BYDV isolates. Leaf extracts were prepared by homogenizing tissue with a Polytron (Brinkman Instruments, Westbury, NY) in 1:4 (w/v) dilution phosphate-buffered saline (PBS), pH 7.4, and 2 volumes of chloroform followed by centrifugation (5,000 *g* for 5 min). Two hundred μl of extract per well was added to duplicate wells in ELISA microtiter plates (Immulon II, Dynatech, Rockville, MD), previously coated with immunoglobulins at 2 μg/ml, and incubated overnight at 4 C. Plates were washed with PBS +

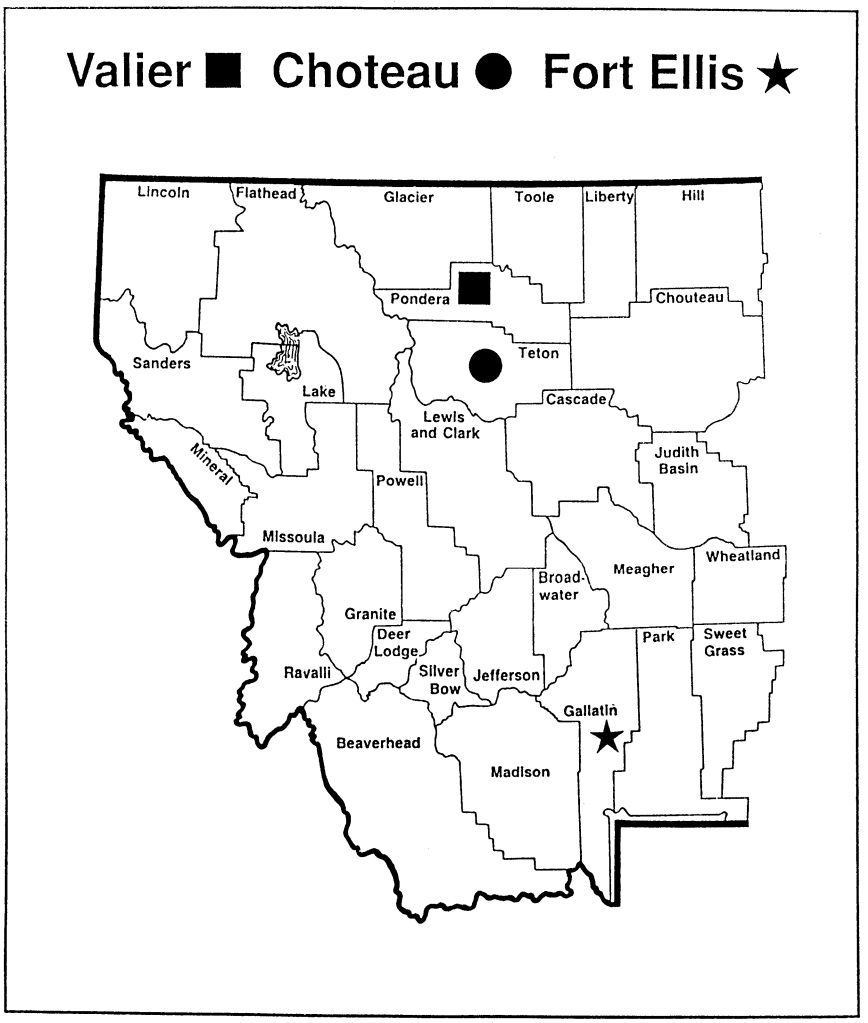

Fig. 1. Locations in central Montana (Valier, Choteau, and Fort Ellis) where MT RMV-like isolates MT-RMV-V, MT-RMV-C, and MT-RMV-FE, respectively, were obtained in 1986.

0.5% Tween 20. Immunoglobulins conjugated with alkaline phosphatase were incubated in the wells for 2–3 hr at 37 C. After additional washing, *p*-nitrophenyl phosphate was added as substrate and absorbance readings were taken at 405 nm. Included as controls on each plate were known concentrations of purified virus diluted in healthy plant sap.

RESULTS

Virus transmission. In a preliminary experiment to determine the optimal number of aphids to use as vectors for MT RMV-like and NY-RMV isolates, one, 10, and 25 inoculative aphids were used in transmission tests. The percent transmission efficiency of the MT-RMV-C isolate vectored by MT *R. maidis* B2, $2n=10$ karyotype, was 5, 50, and 64% when one, 10, and 25 inoculative aphids, respectively, were placed on individual oat seedlings. This MT isolate was transmitted with 11, 56, and 82% efficiency when one, 10, and 25 inoculative aphids of MT *R. maidis* C12 ($2n=8$ karyotype), respectively, were similarly placed on individual test plants. By comparison, the percent transmission efficiency of the NY-RMV isolate by NY *R. maidis* (karyotype $2n=8$) was 12, 64, and 80% with one, 10, and 25 inoculative aphids, respectively.

All three MT RMV-like isolates were transmitted efficiently by MT *R. maidis* of either karyotype and NY *R. maidis* and NY *S. graminum* when groups of aphids were given an IAP on oats (Table 1). MT-RMV-FE was not transmitted by NY *R. padi*, but MT-RMV-C and MT-RMV-V were transmitted inefficiently by this aphid. NY *S. avenae* did not transmit any of the MT isolates. The NY-RMV isolate was transmitted by all four MT biotypes and the NY biotype of *R. maidis*.

Different combinations of MT RMV-like isolates and aphid vectors affected transmission efficiency (Table 1). Transmission efficiencies ranged from 77% when MT-RMV-C was transmitted by MT *R. maidis* C12 to 19% when MT-RMV-FE was transmitted by MT *R. maidis* C5. Transmission percentages of MT isolates vectored by NY *R. maidis* ranged from 27 to 33%, with an average of 29%. The average percent transmission efficiency of the three MT RMV-like isolates was 50, 32, 48, and 70% for MT *R. maidis* B2 ($2n=10$), B4 ($2n=10$), C5 ($2n=8$), and C12 ($2n=8$), respectively. By comparison, the average percent transmission efficiency of the three MT isolates by NY *S. graminum* was 19%.

CATMOD analysis of the transmission data (Table 1) indicated that transmission with each combination of BYDV isolate and aphid vector was different ($P < 0.001$). CATMOD was chosen as the mode of analysis because the sizes of the groups analyzed (number of oat plants infested with aphids) varied but were large enough to allow for the determination of statistical differences. Transmission of the four RMV isolates, MT-RMV-C, MT-RMV-FE, MT-RMV-V, and NY-RMV, by aphids of each MT *R. maidis* colony (B2, B4, C5, and C12) and the NY biotype of *R. maidis* was compared. Aphid colonies transmitted each RMV isolate differently (vertical analysis using Table 1), and each isolate was transmitted differently by each aphid colony (horizontal analysis using Table 1). It also followed that the transmission by each virus isolate/vector

Table 1. Comparative aphid transmission of three Montana (MT) RMV-like isolates of barley yellow dwarf virus (BYDV) on oat plants by four MT colonies and the New York (NY) biotype of *Rhopalosiphum maidis* and *R. padi, Schizaphis graminum*, and *Sitobion avenae* from New York

Virus isolates and controls[a]	Virus transmission (no. of plants infected/no. of plants infested)[b]							
	MT *R. maidis*[c]				NY species			
	$2n=10$ karyotype		$2n=8$ karyotype		$2n=8$ karyotype	ND[d]		
	B2	B4	C5	C12	*R. maidis*	*S. graminum*	*R. padi*	*S. avenae*
MT-RMV-C	69/124 (56)	17/55 (31)	22/58 (38)	82/107 (77)	26/78 (33)	18/72 (25)	3/56 (5)	0/56 (0)
MT-RMV-FE	41/81 (51)	20/75 (27)	14/73 (19)	51/86 (59)	22/77 (29)	8/68 (12)	0/50 (0)	0/73 (0)
MT-RMV-V	34/81 (42)	26/70 (37)	74/98 (76)	51/71 (72)	40/149 (27)	19/92 (21)	3/50 (6)	0/55 (0)
Total	144/286 (50)	63/200 (32)	110/229 (48)	184/264 (70)	88/304 (29)	45/232 (19)	6/156 (4)	0/184 (0)
NY-RMV	41/61 (67)	20/61 (32)	42/57 (74)	62/97 (64)	156/256 (59)	...	...	...
NY-SGV	0/50 (0)	0/51 (0)	0/51 (0)	0/51 (0)	...	88/211 (42)	...	...
NY-RPV	0/50 (0)	0/50 (0)	0/51 (0)	0/52 (0)	...	...	108/121 (89)	...
NY-MAV	0/50 (0)	0/51 (0)	1/51 (2)	0/51 (0)	...	...	...	138/162 (81)
NY-PAV	0/55 (0)	0/62 (0)	0/54 (0)	0.75 (0)	...	...	96/101 (95)	75/132 (57)
Healthy	0/153	0/110	0/154	0/167	0/218	0/195	0/190	0/180

[a] MT-RMV-C, MT-RMV-FE, and MT-RMV-V = three Montana RMV-like isolates of barley yellow dwarf virus. NY-RMV, NY-SGV, NY-MAV, and NY-PAV = New York isolates of barley yellow dwarf virus.

[b] Infested with 25 aphids per species of *R. maidis, R. padi,* and *S. avenae* or 20 aphids of *S. graminum.* Number in parentheses is the transmission percentage of the different BYDV isolate vector combinations. Aphids were allowed a 2-day acquisition access period on detached leaves followed by a 5-day inoculation access period on oat seedlings. Transmission data are a consolidation of 110 replications conducted from September 1986 to June 1989. Analysis of variance using the CATMOD procedure within SAS statistical package showed $P < 0.001$ when combinations of isolates and aphid vectors, as well as MT isolates vectored by the two different *R. maidis* karyotypes, were compared.

[c] B2, B4, C5, and C12 = identification numbers of Montana colonies of *R. maidis* and their respective karyotypes. The karyotype of the NY *R. maidis* is also indicated.

[d] ND = karyotype not determined.

combination was different from every other virus isolate/vector combination. When aphids were grouped according to karyotypes, there was no difference in transmission of an isolate by either karyotype.

The three MT RMV-like isolates differed from the NY-RMV and NY-SGV isolates in virulence. Symptoms caused by the MT RMV-like isolates were much more severe in oats than those caused by the NY-RMV or NY-SGV isolates. Plants infected with MT RMV-like isolates displayed severe symptoms similar to plants infected with the second group of Manitoba isolates reported by Gill (4), but the symptoms caused by the MT RMV-like isolates appeared 12–30 days after aphid infestation. In contrast, symptoms observed on plants infected with NY-RMV or NY-SGV isolates were mild. Although symptoms caused by NY-RMV appeared within the same time frame as those caused by MT RMV-like isolates, those caused by NY-SGV appeared later (20–36 days).

Serological analysis of plants used for comparative aphid transmission. All three MT RMV-like isolates reacted positively with the NY-RMV immunoglobulin in DAS-ELISA (Table 2). No differences in absorbance readings were found between the homologous reaction of NY-RMV and NY-RMV immunoglobulin and the heterologous reaction of MT RMV-like isolates and NY-RMV immunoglobulin in DAS-ELISA. MT-RMV-C and MT-RMV-FE reacted positively to the NY-RMV immunoglobulin if transmitted by groups of either MT *R. maidis* karyotype or NY *S. graminum*. The MT-RMV-V isolate also reacted positively to the NY-RMV immunoglobulin when vectored by MT *R. maidis* colonies (B2, B4, C5, and C12) or NY *R. padi*. Not all symptomatic plants inoculated with MT RMV-like isolates tested positive with the NY-RMV immunoglobulin by DAS-ELISA. Some negative samples were found among plants inoculated with MT-RMV-C vectored by MT *R. maidis* (both $2n=8$ and $2n=10$ biotypes), NY *S. graminum*, and NY *R. padi* as well as MT-RMV-FE and MT-RMV-V vectored by MT *R. maidis* (both karyotypes) and NY *S. graminum*. Even though symptoms were apparent, virus titer may have been lower than detectable limits in these plants because of their advanced age. Montana RMV-like isolates did not react with the NY-SGV immunoglobulin.

Biological and serological analysis of plants used for cross-protection experiments. In reciprocal cross-protection tests between MT-RMV-C and NY-SGV (Table 3), mixed infections of both isolates, as well as single infections of each, were obtained in sets of plants inoculated with one isolate and then challenged 2 wk later with the other. When NY-SGV was used as the protecting virus, a low infection rate (five of 19 compared with 17 of 20 for MT-RMV-C) resulted in fewer mixed infections (four of 19 compared with eight of 20) than when MT-RMV-C was used as the protecting virus. In some cases of mixed infections, tests for SGV were not positive until long after the initial SGV inoculation (five tested positive 34 days after challenge with SGV when MT-RMV-C was the protecting virus, whereas two tested positive 48 days after inoculation when NY-SGV was the protecting virus). The presence of mixed infections indicated a lack of cross-protection.

The DAS-ELISA test results of leaf tissue samples obtained from the same plants used in the cross-protection experiments and processed at Purdue University were mostly in agreement with our serological data. In only five of the samples tested was there noncorrespondence of results. There were also two discrepancies in our results (Table 3). One NY-SGV tested control reacted with NY-RMV immunoglobulin and one NY-RMV control reacted with both NY-SGV and NY-RMV immunoglobulin. Any of these inconsistencies could be attributable to contamination or some

Table 2. Detection by double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) of three Montana RMV-like isolates of barley yellow dwarf virus (BYDV) in leaf samples of representative test plants used for comparative aphid transmission

Virus isolates and controls[a]	Aphid vector species[b]	No. of representative plants tested[c]	No. of plants positive or negative by ELISA[d]	Absorbance at 405 nm[e]	
				Mean	Range
Isolates					
MT-RMV-C	MT *R. maidis*[f]	6	5+	0.408	0.092–0.668
			1−	0.066	···
	NY *S. graminum*	2	1+	0.375	···
			1−	0.039	···
	NY *R. padi*	1	1−	0.063	···
MT-RMV-FE	MT *R. maidis*	6	5+	0.299	0.160–0.569
			1−	0.036	···
	NY *S. graminum*	2	1+	0.394	···
			1−	0.036	···
MT-RMV-V	MT *R. maidis*	9	7+	0.245	0.070–0.462
			2−	0.024	0.020–0.027
	NY *S. graminum*	3	3−	0.026	0.000–0.051
	NY *R. padi*	1	1+	0.075	···
Controls					
NY-RMV	MT *R. maidis*	3	3+	0.373	0.126–0.548
	NY *R. maidis*	4	4+	0.390	0.178–0.719
NY-SGV	NY *S. graminum*	1	1+	0.355	···
NY-RPV	NY *R. padi*	2	2+	0.723	0.641–0.805
NY-MAV	NY *S. avenae*	3	3+	0.402	0.133–0.548
NY-PAV	NY *R. padi*	2	2+	0.404	0.254–0.553

[a] MT-RMV-C, MT-RMV-FE, and MT-RMV-V = three Montana RMV-like isolates of barley yellow dwarf virus. NY-RMV, NY-SGV, NY-RPV, NY-MAV, and NY-PAV = New York isolates of barley yellow dwarf virus used as controls.

[b] *Rhopalosiphum maidis, R. padi, Schizaphis graminum*, and *Sitobion avenae*.

[c] Each leaf sample consisted of one or more expanded leaves collected 29–164 days after the inoculation access period from symptomatic oats grown at Bozeman, MT. One gram of leaf tissue per sample was homogenized with 4 ml of phosphate-buffered saline and 2 ml of chloroform and centrifuged at 7,700 *g*. Two hundred microliters of resulting supernatant was placed in each of two duplicate wells in a microtiter plate. All ELISA tests were conducted at Ithaca, NY.

[d] + = Positive ELISA reaction, − = negative ELISA reaction. A reaction was considered positive only if it was greater than 2× the background reading. Background readings consisted of processed leaf samples from plants previously inoculated by nonviruliferous aphids of each NY species.

[e] Mean absorbance readings for healthy controls were 0.033 for NY *R. maidis* using NY-RMV immunoglobulin, 0.129 for NY *S. graminum* using NY-SGV immunoglobulin, 0.025 for NY *R. padi* using NY-RPV immunoglobulin, and 0.039 for NY *S. avenae* using NY-MAV immunoglobulin, and 0.026 for NY *R. padi* using NY-PAV immunoglobulin. Mean absorbance readings are a consolidation of ELISA results from four transmission tests conducted 18 April, 19 September, and 5 December 1988, along with 20 March 1989. Absorbance readings obtained with immunoglobulin NY-RMV for isolates MT-RMV-C, MT-RMV-FE, MT-RMV-V, and NY-RMV; NY-SGV for isolate NY-SGV; NY-RPV for isolate NY-RPV; NY-MAV for isolate NY-MAV; and NY-PAV for isolate NY-PAV.

[f] MT *R. maidis* is a combination of MT *R. maidis* biotypes B2, B4, C5, and C12. Combining the ELISA data for these colonies did not alter the results because each biotype reacted in a similar manner.

inherent error with the test. In any case, these discrepancies were of little concern because they were so minor.

Reciprocal cross-protection tests between MT-RMV-V and NY-SGV (Table 4) showed results similar to those tests between MT-RMV-C and NY-SGV. The infection rate (11 of 14 compared with seven of 11) was only slightly better when NY-SGV was used as the protecting isolate rather than MT-RMV-V. When NY-SGV was used as the challenging virus, there were fewer mixed infections (one of 11 compared with six of 14) than when MT-RMV-V was used as the challenging virus.

DISCUSSION

The three MT RMV-like isolates differ from the NY-RMV isolate in vector specificity. All MT RMV-like isolates were efficiently transmitted by the NY biotype of *S. graminum* as well as by MT colonies and the NY biotype of *R. maidis*. Some Canadian BYDV strains were previously reported by Gill (5) to also be transmitted efficiently by *R. maidis* and *S. graminum*.

The relatively high transmission efficiency of MT RMV-like isolates by both *R. maidis* and *S. graminum* may have epidemiological significance for Montana because it makes possible the spread of these isolates by two different aphid species. In some years, Montana seems to have an abundance of RMV-like isolates (19) in spring barley, and samples of *S. graminum* collected from diseased fields have transmitted BYDV to oat indicator seedlings in the greenhouse (S. K. Z. Brumfield and T. W. Carroll, *unpublished*). At times, *S. graminum* may be just as important as *R. maidis* in spreading RMV-like isolates.

The MT RMV-like isolates and the NY-RMV isolate were efficiently transmitted by either the $2n=8$ or $2n=10$ karyotype of *R. maidis*. The transmission efficiency of each virus isolate/vector combination was different from that of every other virus isolate/vector combination. These transmission differences may be attributed to the RMV isolates and/or aphid biological types themselves. When the transmission of the RMV isolates by $2n=10$ karyotypes (B2 and B4) was compared with the $2n=8$ karyotypes (C5, C12, and NY) of *R. maidis*, the differences among individual virus isolate/vector combinations overshadowed any differences there may have been between genotypes.

Efficient transmission of RMV isolates by *R. maidis* of the $2n=10$ karyotype is very important, especially in Montana where barley acreage far outnumbers that of corn. Interestingly, transmission studies of the NY-RMV isolate have been with the NY biotype of *R. maidis*, which has a $2n=8$ karyotype. The $2n=8$ karyotype will selectively colonize corn, whereas the $2n=10$ karyotype prefers barley using eupanicoid grasses (*Panicum*, *Setaria*, and *Echinochloa*) as alternate hosts (1,2). In Montana, the $2n=10$ karyotype of *R. maidis* would be the most important vector of RMV-like isolates in barley. The $2n=8$ karyotype may be epidemiologically important in the spread of RMV within corn but will not colonize barley outside of caged greenhouse conditions.

Unlike those MT RMV-like isolates found in 1983 (19), these isolates reacted only with antiserum elicited by NY-RMV and appear to be antigenically similar to the NY-RMV isolate. Normally homologous immunoglobulin for each NY isolate is quite specific for its respective isolate, and although cross-reactions between MAV and SGV are fairly common, they are far less common between RMV and RPV (R. E. Klein, *personal communication*). No cross-reactions between RMV and SGV have been reported. Our transmission and the serological tests, as well as those of Hazelwood and Gray (7), confirm the serological likeness of these MT isolates to RMV. There has been no indication of a mixed infection with SGV in serial aphid transmission tests, and there has been no reaction with SGV immunoglobulin in DAS-ELISA.

Barley yellow dwarf virus isolates have been separated into two groups on the basis of their serological (13) and cytopathological properties (6), dsRNA profile (3), nucleic acid hybridization properties (16), and their ability to cross-protect (8,18). Group I isolates include MAV, PAV, and SGV, whereas group II isolates comprise RPV and RMV. MT RMV-like isolates are more similar to the other RMV and group II isolates based on their positive reaction to RMV immunoglobulins and the lack of cross-protection by NY-SGV.

Because no cross-protection could be observed between MT RMV-like isolates Choteau or Valier and NY-SGV, these MT isolates appear to be biologically similar to other RMV isolates in group II and distantly related to SGV and other isolates in group I. The amount of cross-protection between isolates of BYDV is proportional to the biological similarity of the isolates, and isolates in one group will not protect the plant against further infection from isolates of the other group. MT-RMV-C did not protect against further NY-SGV infection because eight of 20 plants reacted with

Table 3. Biological and serological assay results[a] of cross-protection experiment in oats using the Choteau, MT RMV-like isolate and the New York SGV isolate of barley yellow dwarf virus

Protecting virus or control[b]	Challenging virus or control[c]	No. diseased/ no. inoculated or control[d]	No. positive by ELISA[e]/no. of representative plants tested using the immunoglobulin(s)		
			NY-RMV + NY-SGV	NY-RMV	NY-SGV
MT-RMV-C	NY-SGV	19/20	8/20	9/20	2/20
NY-SGV	MT-RMV-C	19/20	4/19	14/19	1/19
MT-RMV-C+ NY-SGV	MT-RMV-C+ NY-SGV	20/20	17/20	2/20	1/20
NY-SGV		2/20	0/14	1/14	2/14
	NY-SGV	6/19	0/10	0/10	7/10
MT-RMV-C		5/11	0/10	4/10	0/10
	MT-RMV-C	5/10	0/10	6/10	0/10
NY-RMV		12/20	1/10	9/10	0/10
	NY-RMV	14/20	0/10	10/10	0/10
Nonviruliferous aphids		0/15	0/6	0/6	0/6
	Nonviruliferious aphids	0/15	0/6	0/6	0/6

[a] Each leaf sample consisted of one or more expanded leaves collected 7–8 wk and 8–10 wk after the inoculation access period (date of infestation) from symptomatic oats grown at Bozeman, MT. One gram of leaf tissue per sample was homogenized with 4 ml of phosphate-buffered saline and 2 ml of chloroform and centrifuged at 7,700 *g*. Two hundred microliters of the resulting supernatant was placed in each of two duplicate wells in a microtiter plate. All ELISA tests were conducted at Ithaca, NY.

[b] MT-RMV-C = Montana RMV-like isolate Choteau. NY-SGV = New York SGV isolate. Infested with aphids on 1 June 1989.

[c] Infested with aphids on 15 June 1989.

[d] Twenty-five nymphs of MT *R. maidis* C5 were used to inoculate MT-RMV-C and 20 nymphs of *S. graminum* were used to inoculate NY-SGV. Fifteen nymphs of each species were used to inoculate MT-RMV-C and NY-SGV simultaneously.

[e] ELISA results were considered positive if absorbance readings at 405 nm were at least 2× greater than the mean absorbance of healthy controls. The mean absorbance ratings of healthy controls tested with immunoglobulins specific for the detection of NY-RMV and NY-SGV were 0.072 and 0.044, respectively.

immunoglobulin to both BYDV isolates. The inefficient transmission of NY-SGV on 1 June 1989 would account for the lower number of doubly infected plants, four of 19, when NY-SGV was used as the protecting isolate and exclude the possibility of protection by NY-SGV after challenge by MT-RMV-C. It was more difficult to determine if MT-RMV-V offered some protection against NY-SGV because only one of 11 plants was doubly infected when MT-RMV-V was used as the protecting isolate against NY-SGV. The lower infection rate by NY-SGV on 10 November 1989 may have influenced the amount of protection contributed to the MT-RMV-V isolate and a larger sample size was needed to determine if protection occurred. NY-SGV may also replicate more slowly and not reach a high concentration of virus particles within the plant, thus influencing the ability of the ELISA test to determine its presence or to induce cross-protection. NY-SGV did not protect against MT-RMV-V because six of 14 plants became doubly infected.

The separation of BYDV isolates into groups I and II is not based on their aphid vector specificities. Vector specificity is not absolute for any BYDV isolate, and an efficient vector of one isolate can also be an efficient vector of another isolate. *R. padi* is a good example of an aphid vector that will efficiently transmit PAV and RPV. It is not surprising that *S. graminum* will efficiently transmit MT RMV-like isolates (group II) while still being the most efficient vector of the SGV isolate (group I). Aphid vector specificity had been linked to an interaction between the viral coat protein and complimentary attachment sites within the aphid vector (12). Recently, Vincent et al (15) compared sequence similarities and differences among BYDV coat proteins in different isolates and found similarities in their serological relationships but dissimilar amino acid sequences among those luteovirus transmitted by the same aphid vectors. They believe it unlikely that these coat proteins are involved in aphid specificity.

Montana isolates appear to have some unusual and unique aphid transmission properties when compared with the NY isolate of RMV. NY-RMV is inefficiently transmitted by *S. graminum*, whereas MT RMV-like isolates are efficiently transmitted by *S. graminum*. On the other hand, these MT RMV-like isolates are very similar to the NY-RMV isolate, unlike those found previously (19), in that they react serologically with immunoglobulin elicited by NY-RMV and little or no cross-protection with SGV was detected. The high transmission efficiency of the MT RMV-like isolates by *S. graminum*, as well as their spread by $2n=10$ karyotypes of *R. maidis*, could drastically increase the spread of these isolates and economically affect Montana growers in years where epidemic conditions exist.

Table 4. Biological and serological assay results[a] of a cross-protection experiment in oats using the Valier, MT RMV-like isolate and the New York SGV isolate of barley yellow dwarf virus

Protecting virus or control[b]	Challenging virus or control[c]	No. diseased/ no. inoculated or control[d]	No. positive by ELISA[e]/no. of representative plants tested using the immunoglobulin(s)		
			NY-RMV + NY-SGV	NY-RMV	NY-SGV
MT-RMV-V	NY-SGV	14/20	1/11	6/11	4/11
NY-SGV	MT-RMV-V	18/20	6/14	3/14	5/14
MT-RMV-V+ NY-SGV	MT-RMV-V+ NY-SGV	20/20	9/13	4/13	0/13
NY-SGV		15/20	0/11	0/11	10/11
	NY-SGV	13/18	0/11	0/11	5/11
MT-RMV-V		14/20	0/12	7/12	0/12
	MT-RMV-V	13/20	0/11	11/12	0/11
NY-RMV		20/20	0/13	13/13	0/13
	NY-RMV	20/20	0/12	12/12	0/12
Nonviruliferous aphids		0/28	0/6	0/6	0/6
	Nonviruliferous aphids	0/30	0/6	0/6	0/6

[a]Each leaf sample consisted of one or more expanded leaves collected 7–8 wk and 8–10 wk after the inoculation access period (date of infestation) from symptomatic oats grown at Bozeman, MT. One gram of leaf tissue per sample was homogenized with 4 ml of phosphate-buffered saline and 2 ml of chloroform and centrifuged at 7,700 *g*. Two hundred microliters of the resulting supernatant was placed in each of two duplicate wells in a microtiter plate. All ELISA tests were conducted at Ithaca, NY.

[b]MT-RMV-V = Montana RMV-like isolate Valier. NY-SGV = New York SGV isolate. Infested with aphids 25 or 27 October 1989.

[c]Infested with aphids 7 or 10 November 1989.

[d]Twenty-five nymphs of MT *R. maidis* C5 were used to inoculate MT-RMV-V and 20 nymphs of *S. graminum* were used to inoculate NY-SGV. Fifteen nymphs of each species were used to inoculate MT-RMV-V and NY-SGV simultaneously.

[e]ELISA results were considered positive if absorbance readings at 405 nm were at least 2× greater than the mean absorbance of healthy controls. The mean absorbance ratings of healthy controls tested with immunoglobulins specific for the detection of NY-RMV and NY-SGV were 0.063 and 0.053, respectively.

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