Barley Yellow Dwarf: Dependent Virus Transmission by Rhopalosiphum maidis from Mixed Infections

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Cooperative Investigation, Northeast Region, Agricultural Research Service, U.S. Department of Agriculture; and Cornell University Agricultural Experiment Station. Supported in part by NSF Grant GB-35242X.

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I am grateful for the excellent technical assistance of Irmgard Muller.

Accepted for publication 1 August 1974.

ABSTRACT

Rhopalosiphum maidis very rarely transmitted the MAV isolate of barley yellow dwarf virus from MAV-infected plants, but it often transmitted MAV, together with the RMV isolate, from plants doubly infected by RMV and MAV. This dependent transmission of MAV by *R. maidis* occurred from barley and oats, from oats at intervals from 2 and 6 weeks after inoculation of the doubly infected source plants, and from oats infected by either of the virus isolates 12 days before the other was introduced. Serological tests confirmed the identity of MAV transmitted dependently by *R. maidis*. No dependent virus transmission occurred when *R. maidis* first fed on plants infected by one virus isolate before feeding on plants infected by the other virus isolate. Although the dependent transmission of MAV by *R. maidis* is similar to the previously studied dependent transmission of MAV by *R. padi* (in the presence of RPV), there are two main differences. Mixed infections are not maintained indefinitely in serial transfers by *R. maidis,* as they are by *R. padi,* and the phenomenon has not occurred to date in tests of purified preparations made from plants infected by RMV and MAV. The helper viruses were specific for each vector—*R. padi* did not transmit MAV from plants infected by RMV and MAV, nor did *R. maidis* transmit MAV from plants infected by RPV and MAV.

Phytopathology 65:99-105

Dependent virus transmissions by aphids are known for a variety of diseases of crops from parsnips to peanuts (5). In dependent transmissions, aphids can transmit a virus only when it occurs in the source plant together with a specific second virus. One system of such dependent transmission has previously been studied for vector-specific isolates of barley yellow dwarf virus (BYDV). *Rhopalosiphum padi* (L.) does not regularly transmit the MAV isolate of BYDV from singly-infected plants, but it often transmits MAV, together with the serologically unrelated RPV isolate, from plants doubly infected by MAV and RPV. As shown by previous studies (5, 6) of this system, transcapsidation (genomic masking) explains the transmission of the dependent virus (MAV) in the presence of the helper virus (RPV).

This paper describes work during 4 years on a second system of dependent transmission of the MAV isolate of BYDV. The vector is *R. maidis* (Fitch); the helper virus is the RMV isolate of BYDV. Differences between this second case of dependent transmission, and the previously studied interaction of RPV and MAV, are discussed.

MATERIALS AND METHODS.—Stock colonies of the same clone of each aphid species used in previous studies were maintained on barley (*Hordeum vulgare* L. 'Catskill' or 'Hudson') by special precautions (4). The aphid species used were *R. padi, Macrosiphum avenae* (Fabricius), and *R. maidis*. Some aphids from each group used in every experiment were always tested as controls. The isolates of BYDV were maintained by serial transmissions to oats (*Avena byzantina* C. Koch 'Coast Black'), the test plant used in most experiments. The virus isolates are differentiated by their relative vector specificity (4). MAV is transmitted specifically by *M. avenae*; RPV is transmitted specifically by *R. padi*; and RMV is transmitted specifically by *R. maidis*. RPV and MAV can also be differentiated serologically (1, 8). Antiserum specific for RMV has not yet been produced because it is more difficult to purify RMV than the other isolates.

Concentrated virus preparations were made by differential and sucrose-gradient centrifugation of clarified juice from infected oat plants (8). The aphid injection procedure was like that described previously (3), except that most needles were made by means of an automatic micropipette puller. For membrane-feeding tests, starved aphids fed through stretched Parafilm M on virus inocula mixed with sucrose (8).

The basis for identification of the virus isolates in most experiments was the transmission pattern in a comparative test, using opposite halves of a detached leaf. One half of the leaf was infested with *M. avenae,* the opposite half with *R. maidis*. Aphids were given a 2-day acquisition feeding period at 15 C in the dark. The inoculation test feeding period in a growth chamber at 21 C was 5 days. Groups of about 10 aphids were placed on each of three seedlings in a 10-cm (4-inch) diameter pot for each treatment. Severity of symptoms observed during 4 weeks in the greenhouse aided identification of the isolates because symptoms of Coast Black oats infected by RMV are milder than are those of plants infected by MAV (4).

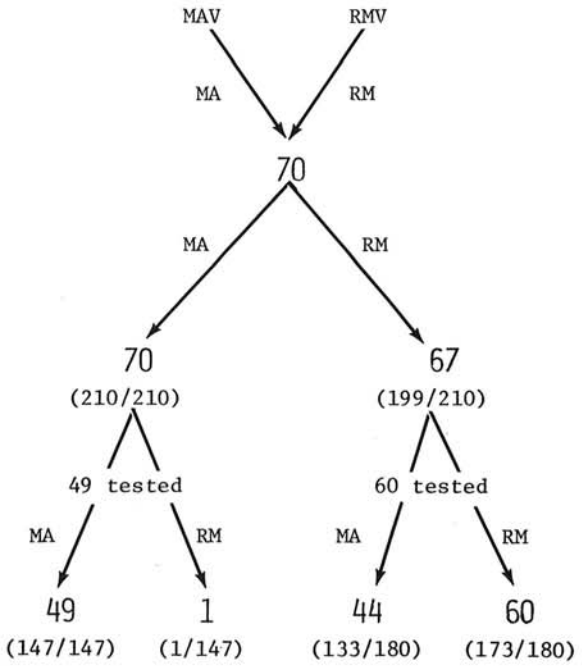

Fig. 1. Schematic summary of data from five experiments of dependent transmission of MAV by *R. maidis* from a total of 70 oat plants inoculated with both the RMV and MAV isolates of barley yellow dwarf virus. Whole numbers are numbers of plants from which virus was recovered in parallel tests with *R. maidis* (RM) and *M. avenae* (MA). In fractions, numerator is number of test plants that became infected; denominator is number of plants infested in tests of the number of plants shown. None of 60 plants infested as controls became infected.

RESULTS.—*Transmissions from doubly infected plants.*—Failure of *R. maidis* to transmit MAV from single infections has been conspicuous in all previous work (4). For example, each time stock cultures of the virus isolates were transferred to new plants, a comparative test was made with four aphid species to attempt detection of any major change in an isolate. In more than 100 such tests during more than 10 years, *R. maidis* did not effect a single transmission of MAV to any of 258 test plants. In the same tests, however, *R. maidis* regularly transmitted the RMV isolate, and *M. avenae* transmitted MAV to almost all the test plants infested.

Tests were made for possible dependent transmission of MAV by *R. maidis,* because symptoms of oats doubly infected by MAV and RMV resembled symptoms of plants doubly infected by RPV and MAV, the system studied previously. In both instances, plants infected by two viruses developed symptoms more severe than those caused by either virus alone.

In each of five early experiments on the transmission of virus from mixed infections of RMV and MAV, *R. maidis* often transmitted MAV, together with RMV, from doubly-infected oat plants (Fig. 1). From 67 of 70 plants originally inoculated with both RMV and MAV, *R. maidis* recovered virus. When opposite halves of a single leaf from 60 of these 67 plants were used in further comparative transmission tests, both *M. avenae* and *R. maidis* transmitted virus from 44 of them. Thus, at least 44 of the 67 plants that became infected following acquisition feeding of *R. maidis* on the doubly-infected source plants were infected by both MAV and RMV. In contrast, only MAV was transmitted from the original double infections by *M. avenae;* no evidence for dependent transmissions by *M. avenae* occurred (Fig. 1).

Dependent transmissions of MAV from mixed infections also occurred from doubly-infected barley plants (*Hordeum vulgare* 'Black Hulless'). In two experiments with barley, both *R. maidis* and *M. avenae* transmitted virus from 14 of 15 doubly-inoculated plants. Tests were made of 12 plants to which *R. maidis* had transmitted virus from the mixed infection. *R. maidis* alone transmitted virus from six of them, but both *R. maidis* and *M. avenae* recovered virus from the other six, which then proved to contain both RMV and MAV. In contrast, 10 plants infected by means of *M. avenae* that acquired virus from the original doubly-infected plants were found to contain only MAV. None of 48 plants infested as controls became infected.

The possible importance of the interval between inoculation of the doubly-infected plants and virus

transmission from them by *R. maidis* was studied in another experiment. At five weekly intervals after inoculation of oats with MAV alone, or with both MAV and RMV, comparative tests were made with both *R. maidis* and *M. avenae* from the two groups of plants. Only *M. avenae* recovered virus from the MAV-infected plants; none of the 135 plants infested with *R. maidis* became infected, additional evidence for the inability of *R. maidis* to transmit MAV alone (Table 1). In tests at each of the five intervals, both aphids transmitted virus from almost all of the doubly-inoculated plants. Subsequent studies of plants that became infected in these tests showed that *R. maidis* had transmitted both RMV and MAV from 25 of 41 doubly-infected plants (Table 1). Dependent transmissions of MAV by *R. maidis* occurred at all of the intervals examined. From the original doubly-infected plants, *M. avenae* transmitted only MAV. In tests of 39 plants, *M. avenae* transmitted virus to 117 of 117 plants; *R. maidis* transmitted virus to 1 of 117 plants.

Another series of experiments showed that dependent transmission of MAV by *R. maidis* occurred from doubly-inoculated oats, not only when plants were inoculated simultaneously with the two viruses, but also when one virus had been introduced by the vector 12 days before the other. In these tests, comparative transmissions by the two vectors were made from three groups of oats: inoculated simultaneously, inoculated with MAV 12 days before inoculation with RMV, and

TABLE 1. Virus transmission by *Rhopalosiphum maidis* (RM) and *Macrosiphum avenae* (MA) from opposite halves of oat leaves at five weekly intervals after the source plants had been doubly inoculated with the RMV and MAV isolates of barley yellow dwarf virus, and results of comparative transmissions by both vectors from plants that became infected in tests of the doubly-inoculated plants

Weeks after inoculation of source plants	No. of plants (of nine tested) from which virus was recovered by aphid species shown after feeding on oats singly or doubly inoculated[a]				No. of plants from those at left infected by means of RM that were found to contain the virus isolate(s) shown			Transmission[b] by aphid species shown in tests to identify virus isolates(s) at left	
	MAV alone		MAV + RMV						
	RM	MA	RM	MA	RMV only	MAV only	RMV+ MAV	RM	MA
2	0	9	8	9	4	0	4	23/24	12/24
3	0	9	8	9	1	0	7	23/24	21/24
4	0	9	8	9	4	0	4	24/24	12/24
5	0	9	8	9	4	0	4	24/24	12/24
6	0	9	9	8	3	0	6	27/27	18/27

[a] Results for MAV-infected plants are based on transmission by *R. maidis* to 0 of 135 test plants, and by *M. avenae* to 134 of 134 plants. Results for doubly-infected plants are based on transmissions by *R. maidis* to 121 of 135 test plants, and by *M. avenae* to 132 of 135 test plants. None of 30 plants infested as controls became infected.

[b] Numerator is number of plants that became infected; denominator is number infested with about 10 aphids for a 5-day inoculation test feeding period following a 2-day acquisition feeding. None of 66 plants infested as controls became infected.

TABLE 2. Virus transmission by *Rhopalosiphum maidis* (RM) and *Macrosiphum avenae* (MA) from opposite halves of leaves of oat plants, originally inoculated simultaneously with both the RMV and MAV isolates of barley yellow dwarf virus, or with one isolate 12 days before the other, and results of comparative transmissions by both vectors from plants that became infected in tests of the doubly infected plants

Order of inoculation of source plants	No. of plants tested and found to be infected by virus isolate(s) shown				Transmission[a] by aphid species shown in tests to identify virus isolate(s) at left		No. of plants found to be infected by virus isolate(s) shown after transmission from doubly-infected plant (at left) by *R. maidis*				Transmission[a] by aphid species shown in tests to identify virus isolate(s) at left	
	No. tests	RMV only	MAV only	RMV+ MAV	RM	MA	No. tests	RMV only	MAV only	RMV+ MAV	RM	MA
MAV before RMV	15	0	0	15	45/45	45/45	15	5	0	10	44/45	30/45
RMV before MAV	15	0	0	15	45/45	45/45	15	3	0	12	45/45	36/45
RMV + MAV simultaneously	8	0	0	8	24/24	24/24	8	2	0	6	23/24	18/24

[a] Numerator is number of plants that became infected; denominator is number infested with about 10 aphids for a 5-day inoculation test feeding period following a 2-day acquisition feeding on portions of detached leaves. None of 33 plants infested as controls became infected.

TABLE 3. Comparison of the MAV isolate of barley yellow dwarf virus recovered from doubly-infected oat plants by *Rhopalosiphum maidis* (RM) with MAV recovered from single or double infections by *Macrosiphum avenae* (MA)

Virus isolate(s) in source plants	Aphid species used for recovery of virus	Virus preparation incubated with antiserum or phosphate buffered saline (PBS)	Transmission[a] after feeding by *M. avenae* on incubated sample	Transmission[b] by aphid species shown in tests of plants infected by means of *M. avenae* at left		
				RP	MA	RM
RMV + MAV	RM	MAV-As	4/12	2/24	24/24	0/24
		PBS	12/12	0/12	12/12	0/12
RMV + MAV	MA	MAV-As	2/12	0/6	6/6	0/6
		PBS	12/12	1/15	15/15	0/15
MAV	MA	MAV-As	3/12	0/9	9/9	0/9
		PBS	12/12	0/6	6/6	0/6

[a]Numerator is number of plants that became infected; denominator is number infested with about 10 *M. avenae* that acquired virus by feeding through membranes.

[b]Numerator is number of plants that became infected; denominator is number infested with about 10 aphids for a 5-day inoculation test feeding following a 2-day acquisition feeding on detached leaves. In addition to *R. maidis* and *M. avenae*, *R. padi* (RP) was used in these comparative tests. None of 24 plants infested as controls became infected.

TABLE 4. Virus transmission by *Rhopalosiphum maidis* fed first on oat leaves that were healthy (H), infected by RMV, or infected by MAV before a second feeding on one of the three kinds of leaves

First feed	Second feed	Transmission[a]	No. of plants tested and found to be infected by virus isolate(s) shown				Transmission by aphid species shown in tests to identify virus isolate(s) shown at left[b]	
			No.	RMV only	MAV only	RMV+ MAV	RM	MA
RMV	MAV	57/78	57	57	0	0	168/171	0/171
MAV	RMV	55/78	55	55	0	0	157/165	0/165
RMV	H	54/78	8	8	0	0	24/24	0/24
H	RMV	55/78	8	8	0	0	23/24	0/24
MAV	H	0/78						
H	MAV	0/78						
H	H	0/30						

[a]Numerator is number of plants that became infected; denominator is number infested with five *R. maidis* for a 5-day inoculation test feeding period following sequential 2-day acquisition feedings.

[b]Numerator is number of plants that became infected; denominator is number infested with about 10 aphids for a 5-day inoculation test feeding period following acquisition feeding of 2 days. None of 42 plants infested as controls became infected.

TABLE 5. Virus transmission by *Rhopalosiphum padi* (RP), *Macrosiphum avenae* (MA), and *R. maidis* (RM) from leaves of oat plants doubly infected with the RPV and MAV isolates or with the RMV and MAV isolates of barley yellow dwarf virus in tests of specificity of dependent transmission from mixed infections

No. of plants tested, and virus(es) with which plants had been inoculated	Transmission[a] by aphid species shown in tests of source plants at left			No. of plants tested and found to be infected by virus isolate(s) shown after transmission by indicated aphid species from doubly-inoculated or control source plant							Transmission[a] by aphid species shown in tests to identify virus isolates at left		
	RP	MA	RM	Aphid species	No. tests	RPV only	MAV only	RMV only	RPV+MAV	RMV+MAV	RP	MA	RM
23 RPV + MAV	69/69	69/69	0/69	RP	8	0	0	0	8	0	24/24	24/24	0/18
				MA	2	0	2	0	0	0	0/6	6/6	–
23 RMV + MAV	3/69	69/69	68/69	RM	10	0	0	0	0	10	1/30	30/30	29/30
				RP	3	0	1	2	0	0	0/9	3/9	6/9
				MA	4	0	4	0	0	0	0/12	12/12	0/12
4 RPV alone	12/12	0/12	0/12										
4 RMV alone	2/12	0/12	11/12	RP	2	0	0	2	0	0	4/6	0/6	5/6
				RM	2	0	0	2	0	0	0/6	0/6	6/6
6 MAV alone	2/18	18/18	0/18	RP	2	0	2	0	0	0	0/6	6/6	–
				MA	2	0	2	0	0	0	1/6	6/6	0/6

[a]Numerator is number of plants that became infected; denominator is number infested with about 10 aphids for a 5-day inoculation test feeding period. None of 54 plants infested as controls became infected.

inoculated first with RMV and then with MAV. From each group, *R. maidis* transmitted both viruses in most instances (Table 2). Of the 38 plants tested, 28 proved to be doubly infected following transmissions from the various source plants by *R. maidis*. Thus, *R. maidis* had transmitted MAV in the presence of RMV from at least 28 original source plants.

In most experiments, the identity of MAV and RMV was based on biological properties, the transmission pattern by two aphid species, and the relative severity of symptoms. Some experiments were carried out to study serological properties of the MAV isolate after transmission by *R. maidis* from doubly-infected plants. Attempts were made to see if MAV transmitted dependently was different from MAV transmitted specifically by *M. avenae*. In one experiment, three concentrated preparations of MAV were compared. One preparation was made from plants (200 g of tissue) infected by means of *R. maidis* that had acquired virus from doubly-infected source plants. A second was made from a parallel group of plants (151 g of tissue) infected by means of *M. avenae* that acquired virus from the same doubly-infected plants. The third preparation was made from plants to which *M. avenae* had transmitted MAV from the singly-infected controls (166 g of tissue). The concentrate made from each kind of tissue was divided into two portions for testing by the technique based on serological blocking of virus transmission by aphids that acquire virus by feeding through membranes (8). One part of each preparation was incubated with antiserum specific for the MAV isolate, and the other with phosphate-buffered saline as control. Virus transmission from all three preparations was much reduced by incubation with the MAV antiserum (Table 3). Further tests of infected plants revealed no differences for the MAV transmitted dependently by *R. maidis* from the MAV transmitted selectively by *M. avenae* (Table 3).

In another kind of serological test, a concentrate was made from oats (363 g of tissue) infected by means of *R. maidis* that fed on doubly-infected source plants. A parallel preparation was made from oats infected by means of *M. avenae* that acquired virus from the same doubly-infected source (269 g of tissue). Each final preparation was suspended in about 0.3 ml of buffer and used in micro-agar double diffusion tests. (1). A third preparation of MAV was included in the tests as a control. Precipitation lines in agar occurred for all three samples in reactions with both MAV-antiserum and with antiserum for the serologically related PAV isolate, but reactions were weak because of the low concentration of the virus. Biological and serological tests all indicated that MAV transmitted from mixed infections by *R. maidis* was "ordinary" MAV.

Lack of virus interaction within the vector.—A significant feature of the dependent transmission of MAV by *R. padi* in the presence of RPV is the apparent importance of virus interaction within the doubly-infected plant and lack of interaction within the aphid vector (6). Some tests were carried out with *R. maidis* to study whether the critical virus interaction in this instance occurs in the plant, in the aphid, or in both. In five separate experiments *R. maidis* was exposed sequentially to the separate isolates of BYDV. In each experiment, some aphids were allowed to feed for 2 days on RMV-infected leaves, and then given a second acquisition feeding on MAV-infected leaves before start of the 5-day inoculation test feeding. Other aphids fed on MAV-infected leaves before feeding on RMV-infected ones. Each kind of infected leaf was used also as a control in combination with feeding on healthy leaves (Table 4). Not a single case of dependent transmission of MAV by *R. maidis* was detected in any of the 112 plants that became infected following the sequential exposure to both viruses. The results agree with more extensive experiments on the dependent transmission of MAV by *R. padi* (6).

Comparison of dependent transmission by R. maidis and R. padi.—Dependent transmission of MAV by *R. maidis* in the presence of RMV was compared with the previously studied system of dependent transmission of MAV by *R. padi* in the presence of RPV. Some experiments were carried out to determine whether or not each of the helper viruses was specific for a vector species. Leaves from each kind of doubly-infected plant were used in comparative transmission tests with three aphid species instead of two. In studies of 23 plants infected by RPV and MAV, virus was recovered by *R. padi* and by *M. avenae,* but not by *R. maidis* (Table 5). Tests of some of the plants that became infected showed that *R. padi* had transmitted both viruses and that *M. avenae* had selectively transmitted MAV, as in all previous tests. In similar studies of plants doubly infected by RMV and MAV, virus was transmitted consistently by *M. avenae* and by *R. maidis,* but from only three plants by *R. padi* (Table 5). Tests of 17 plants that became infected showed that *R. maidis* had effected the expected dependent transmission of MAV, that *M. avenae* had effected the expected selective transmission of MAV, that *R. padi* had transmitted RMV from two plants and MAV from one. The three transmissions by *R. padi* thus represented examples of the relative nature of the BYDV-vector specificity (4), and did not involve dependent transmission of MAV (Table 5). These data show that dependent transmission of MAV by each of the *Rhopalosiphum* species is specific for the helper virus normally transmitted efficiently by each aphid species. RPV serves as a helper virus for dependent transmission of MAV by *R. padi,* but not by *R. maidis*. Similarly, RMV is a helper virus only for dependent transmissions of MAV by *R. maidis*.

In many of these experiments, further comparative tests were made beyond the steps needed to determine which viruses had been transmitted from a doubly-infected plant. These tests suggested that the dependent transmission of MAV by *R. maidis* and that by *R. padi* differ in the ability of the vector to maintain the mixed infections through serial transfers, and thus to continue the dependent transmissions. Therefore, direct comparison was made of the ability of each aphid species to maintain the appropriate mixed infections through 10 serial transfers. When *R. padi* fed on 10 different plants infected by both RPV and MAV, all plants tested through serial transmissions during 12 months proved to be doubly infected by both viruses. In tests of these plants, *R. padi* transmitted virus to 300 of 300 test plants, and *M. avenae* transmitted virus in parallel to all 300 plants. In contrast, *R. maidis* transmitted both MAV and RMV

through a maximum of eight serial transfers in only 4 of 14 cases studied. The number of plants found to be doubly infected by RMV and MAV in each of the 10 serial transfers from 14 original doubly-inoculated plants was as follows: 14, 14, 8, 6, 6, 6, 5, 4, 0, and 0. Thus, in many instances *R. maidis* maintained the mixed infection only through a few serial transfers. In tests of the plants inoculated by means of *R. maidis* in these experiments, virus was transmitted to 247 of 255 plants by *R. maidis* and to 182 of 255 plants by *M. avenae*. None of 120 plants infested as controls became infected.

In the experiments on maintenance of mixed infections by *R. maidis,* the severity of symptoms often varied among the three test plants in each pot of any one test. The probability of maintaining the mixed infections in serial transfers was increased by always selecting the plant with the most severe symptoms for the virus source in the next transfer. During the experiment, 19 comparisons were made by sampling from the same pot a plant with severe symptoms and a plant with mild symptoms. From all 19 plants with severe symptoms, both RMV and MAV were transmitted. *R. maidis* transmitted virus to 50 of 57 test plants; *M. avenae* transmitted virus to 56 of 57 plants. From the 19 plants with mild symptoms, both viruses were recovered only four times. In these four instances, *R. maidis* transmitted virus to 12 of 12 plants; *M. avenae* transmitted virus to 11 of 12 plants. From the other 15 plants, only RMV was recovered. In these tests, *R. maidis* transmitted virus to 43 of 45 plants, but *M. avenae* transmitted virus to 0 of 45 plants. Thus, the mixed infections of MAV and RMV in the experiment discussed above were maintained for a longer time than would have occurred if source plants merely had been randomly selected for acquisition feeding by *R. maidis*.

Another difference between the two systems for dependent transmission of MAV is the usefulness of concentrated virus preparations in studies of the phenomenon. Studies of the mechanism of dependent transmission of MAV by *R. padi* have been possible because the phenomenon occurs, not only when doubly-infected plants are used, but also when virus preparations made from such plants are tested. Attempts to study dependent transmission of MAV by *R. maidis,* by using similar preparations made from doubly-infected plants, however, have not yet succeeded. In 11 separate experiments, concentrates made from doubly-infected plants were either injected into aphids or aphids were allowed to acquire virus from them by feeding through membranes. Only 22 of 324 plants infested with *R. maidis* exposed to such inocula became infected. Tests of all 22 plants showed that only RMV had been transmitted. *R. maidis* transmitted virus to 63 of 66 test plants, but *M. avenae* transmitted virus to 0 of 66. In parallel tests with *M. avenae* and the same inocula, 179 of 198 test plants became infected. Tests of 51 of these infected plants showed that *M. avenae* had transmitted only MAV. Serological tests in some of these experiments showed that MAV from doubly-infected plants was the same as MAV from MAV-infected plants.

The failure of *R. maidis* to transmit both RMV and MAV from preparations made from doubly-infected tissue is not surprising. Previous experience with purification of RMV and with the use of *R. maidis* in membrane or injection assays usually has given inconsistent results and little virus transmission. Perhaps the same factors that make "in vitro" tests so difficult with RMV itself affect use of preparations made from doubly-infected plants. Random tests of plants used as source material for the purification work showed that most of the plants were doubly infected; there is no reason to doubt the integrity of the source material. Moreover, total virus titer in some of the preparations was much higher than the virus titer in many successful experiments with the RPV-MAV interaction. The mechanism for dependent transmission of MAV by *R. maidis* may be the same as that suggested by current evidence for the similar transmission of MAV by *R. padi*. If so, then a possible explanation for the lack of transmission from these virus preparations is that the heterologously encapsidated particles are simply less stable in the purification process than the homologously encapsidated ones, especially those of MAV.

DISCUSSION.—Elucidation of this second system for dependent transmission of MAV from mixed infections is significant for at least three reasons. First, the occurrence of a second system shows that the previously studied interaction of RPV and MAV is more than an isolated laboratory curiosity. It reinforces the potential importance of dependent transmission as a factor that influences spread of viruses in the field.

Second, differences between the two systems of dependent transmission of MAV suggest an important variation that could influence function and study of the interactions in the field. The fact that *R. maidis* usually maintains mixed infections of MAV and RMV through only a few serial transfers, in contrast with the indefinite maintenance of mixed infections of RPV and MAV by *R. padi,* focuses on problems of identification of such dependent transmissions if they do occur in the field. Some dependent transmissions could involve only one serial transmission and thus might not be detected in later tests of field-collected plants. If dependent virus transmissions occur in nature only from one original doubly-infected source, they would be difficult or impossible to detect.

Third, the role of RMV as a helper virus in the dependent transmission of MAV could be significant in epidemiology of BYDV. Viruses similar to RMV are found during most growing seasons in New York, and they have been the predominating type in Manitoba in some seasons (2). A recent 6-year study in New York has shown that mixed infections of isolates of BYDV are more common in winter wheat and winter barley than in spring oats (7). In all our tests of field-collected winter grains, one component of every mixed virus infection was RMV. Thus, a virus isolate known to serve as a helper virus in the dependent transmission of MAV occurs in mixed infections in the field. Whether the mixed infections also function in the field as sources of virus for dependent transmission remains to be studied.

LITERATURE CITED

1. AAPOLA, A. I. E., and W. F. ROCHOW. 1971. Relationships among three isolates of barley yellow dwarf virus. Virology 46:127-141.
2. GILL, C. C. 1971. Prevalence of aphids and barley yellow dwarf virus on barley in Manitoba in 1970. Plant Dis. Rep. 55:797-801.
3. MULLER, I. 1965. Aphid injection, an aid in the study of

barley yellow dwarf virus. Cornell Plant. 20:68-71.
4. ROCHOW, W. F. 1969. Biological properties of four isolates of barley yellow dwarf virus. Phytopathology 59:1580-1589.
5. ROCHOW, W. F. 1972. The role of mixed infections in the transmission of plant viruses by aphids. Annu. Rev. Phytopathol. 10:101-124.
6. ROCHOW, W. F. 1973. Selective virus transmission by Rhopalosiphum padi exposed sequentially to two barley yellow dwarf viruses. Phytopathology 63:1317-1322.
7. ROCHOW, W. F., and I. MULLER. 1974. Mixed infections of barley yellow dwarf virus isolates in winter grains. Plant Dis. Rep. 58:472-475.
8. ROCHOW, W. F., A. I. E. AAPOLA, M. K. BRAKKE, and L. E. CARMICHAEL. 1971. Purification and antigenicity of three isolates of barley yellow dwarf virus. Virology 46:117-126.