Dependent Transmission by Aphids of Barley Yellow Dwarf Luteoviruses from Mixed Infections

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ABSTRACT

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Dependent virus transmission by aphids occurred from seven of 14 double infections among five barley yellow dwarf luteoviruses in tests with four aphid species in 70 experiments. The RPV isolate was the best helper virus, enabling *Rhopalosiphum padi* to transmit RMV, MAV, and SGV, together with RPV, from most mixed infections. The PAV isolate was equally effective in enabling *R. padi* to transmit MAV, but somewhat less effective in enabling *R. padi* to transmit RMV. The RMV isolate was a helper virus in transmissions of RPV and MAV by *R. maidis*. Four of these seven systems involved interaction of serologically distinct viruses; three involved serologically related virus pairs. Dependent virus transmission did not occur from seven other double infections that included MAV, SGV, and RMV in tests with *R. maidis, Macrosiphum* (=*Sitobion*) *avenae,* and *Schizaphis graminum*. Enzyme immunosorbent assays were especially useful to identify viruses in the mixed infections.

Additional key words: virus vectors, heterologous encapsidation, vector specificity.

Dependent virus transmission from mixed infections is a special feature of plant virus transmission by aphids. In dependent virus transmission, aphids transmit one virus (dependent virus) only in the presence of a second virus (helper virus). Examples of this phenomenon are known for nonpersistent, semipersistent, as well as for persistent virus-aphid systems (3,4,7,10). Two different mechanisms currently seem to explain dependent virus transmission for the different systems. For most nonpersistent and semipersistent systems, dependent virus transmissions occur, not only following aphid probing on doubly infected plants, but also following sequential probing first on plants infected by the helper and then on plants infected by the dependent one. Action of some "accessory factor" seems to be involved (3,4). In contrast, dependent virus transmission of persistent viruses seems to occur only when aphids feed on doubly infected plants, apparently because interactions (transcapsidation or genomic masking) during simultaneous virus replication provide the basis for the phenomenon (2,3,10). Only persistent systems will be considered here.

Two main kinds of dependent virus transmission are known for luteoviruses, small isometric viruses confined to plant phloem tissue and transmitted in the persistent, circulative manner by aphids. Some complex diseases involve a luteovirus only as one component of a mixed infection; the other virus may be mechanically transmissible alone, but is transmitted by aphids only in the presence of the luteovirus helper. Examples discussed in recent reviews (10,14) include lettuce speckles, tobacco mottle, carrot mottle, groundnut rosette, and tobacco yellow vein. In the second kind, illustrated by mixed infections of isolates of barley yellow dwarf virus (BYDV), both components of the mixture are luteoviruses. These viruses undergo altered vector specificity as a result of the mixed infection. The best known example is interaction of the MAV and RPV isolates of BYDV (6,8,10,15). *Rhopalosiphum padi* (L.) does not transmit MAV from singly infected plants, but regularly transmits MAV, together with the serologically distinct RPV isolate, from plants doubly infected by MAV and RPV. Similarly, *Rhopalosiphum maidis* (Fitch) is unable to transmit MAV from singly infected plants, but can transmit MAV from plants also infected with the RMV isolate (9). I study these systems of dependent virus transmission because they are useful approaches both to understanding mechanisms of virus-aphid specificity, and to learning how virus interactions could affect spread of luteoviruses by aphids in the field.

This paper describes efforts in recent years to evaluate systematically the occurrence of dependent virus transmission from mixed infections among the five characterized luteoviruses that cause barley yellow dwarf (5,13). The work was made possible by using the enzyme-linked immunosorbent assay (EIA) technique, and was stimulated by the finding that the five viruses fall into two serological groups in EIA tests (11,13). One focus of this study was the question of whether or not serological relatedness of interacting viruses is correlated with the occurrence of dependent virus transmission.

MATERIALS AND METHODS

Stock colonies of the same clone of each of the four aphid species used in previous work were maintained on barley as described (5). The five isolates of BYDV were maintained by serial transmissions to oats (*Avena byzantina* Koch 'Coast Black'), the test plant used in all experiments. The relative vector specificity of each isolate is illustrated by accumulated results of about six comparative transmissions made in each of the 10–22 years since each virus was originally obtained from a field-collected plant (Table 1).

In a typical experiment, groups of about 12 plants were inoculated with one or the other of two virus isolates alone, or with a mixture of the two, by infesting 6-day-old seedlings with one or two of the appropriate viruliferous aphid vectors. Following a 5-day inoculation test feeding period, plants were grown in the greenhouse for about 4 wk. A leaf was then removed from each infected plant, cut in half, and each half infested with the specific aphid vector of one virus or the other to begin a comparative transmission test. From each half-leaf, about 10 aphids were transferred to each of three seedlings. Results of tests of leaves from singly infected plants were compared with results of further tests of virus transmitted in parallel from doubly infected plants. Since the helper virus was transmitted by its specific vector from almost every

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doubly infected plant, further tests were always needed to determine whether or not they also transmitted the dependent virus. Viruses transmitted from a doubly infected plant were identified in one of two ways. In some experiments, virus identification was based on a further comparative transmission test with the two appropriate aphid species from opposite halves of a single detached leaf. In these experiments, aphids were permitted a 2-day acquisition feeding at 15 C in the dark, and a 5-day inoculation test feeding at 21 C in a growth chamber. Three plants were used for each sample, and at least three were infested with aphids as controls in each test. In other experiments, virus or viruses transmitted from the doubly infected plants were identified by the EIA procedure. In these tests 2-g samples of tissue from each infected plant were tested with globulins from at least two of the virus-specific antisera (11,13). Parallel aphid transmission tests and EIA tests were done in some experiments. Depending on the particular pair of viruses, each experiment involved comparisons of virus transmitted from the double infection by both aphid species with each of the appropriate parallel transmissions from singly infected plants. However, with some pairs of viruses, only one aphid species could be used for comparison because the second aphid species was a vector of both viruses from single infections. In other words, I only studied combinations in which the vector used effected no consistent transmission of the test virus from single infections.

Because of the specificity of the EIA procedure for the four viruses studied, there was no reason to question the validity of the technique for detecting one virus in the presence of another (11,13). Nevertheless, I made several different tests to evaluate whether or not the presence of one virus had any influence on detection of another in the EIA procedure. In one such experiment, for example, clarified juice from plants infected by each of four viruses was mixed together in all possible combinations with the other three viruses and used in EIA tests with four virus-specific globulins. Neither the homologous nor heterologous reactions of any of the four viruses was affected by the presence of any other virus. For example, the homologous reaction of RPV (A_{405nm}) was 0.95, 1.05, 0.99, and 0.95 when diluted with preparations of healthy, MAV-, PAV-, or RMV-infected plants, respectively. In other tests, I made direct comparisons of virus prepared from known singly and doubly infected plants. Again, the presence of one virus had no effect on the reactions of the other. Although I do not yet have a homologous antiserum for the SGV isolate, identification of SGV in EIA tests was often possible by means of weak, but consistent heterologous reactions with antiserum for PAV and MAV (11,13). For a recently prepared batch of labeled PAV and MAV globulins, however, this reaction was less reliable and identifications of SGV in some cases were based mainly on comparative aphid transmission tests. Controls in every EIA test included preparations of healthy plants, preparations of each singly infected plant, and buffer (13).

RESULTS

Special efforts were made to study the interaction between RPV and RMV because there were preliminary indications that this system differs from those studied previously. In all past work, dependent virus transmission of isolates of BYDV occurred in only one direction with only one vector. Thus, MAV is transmitted by *R. padi* in the presence of RPV, and MAV is transmitted by *R. maidis* from mixed infections with RMV, but neither RPV nor RMV is transmitted from the same mixed (or single) infections by *M. avenae* (9,10). In contrast, both RPV and RMV were transmitted in a dependent manner from mixed infections by both *R. padi* or *R. maidis* (Table 2). The RPV isolate was clearly a very effective helper virus for dependent transmission of RMV by *R. padi*. Although *R. padi* occasionally transmitted RMV from single infections in six of 11 experiments, it regularly transmitted RMV from most of the doubly infected plants in all 11 experiments (Table 2). From RMV-infected plants, *R. padi* transmitted virus from only 15 of 97 plants (to 18 of 291 test plants). In all but three cases, each of these 15 transmissions involved infection of only one of the three test plants infested. In parallel tests of the doubly infected plants, however, *R. padi* transmitted RMV (in the presence of RPV) from 106 of 124 plants (Table 2). From the same doubly infected plants, *R. maidis* transmitted RPV in the presence of RMV in 10 of the 11 experiments, but the percentage of transmission was low; dependent transmission occurred from only 31 of 123 plants (Table 2). This level of dependent virus transmission is significant, however, compared with that of parallel tests of RPV-infected plants in which not a single transmission by *R. maidis* occurred from any of 98 plants (Table 2). Thus, none of nearly 3,000 *R. maidis* transmitted RPV alone to any of 294 test plants!

The first six experiments with these RPV and RMV interactions were done several years ago only with aphids before the EIA procedure became available. Agreement between results of this early work and those of the more recent work done with the serological assay illustrates the stability and reproducibility of the phenomenon (Table 2).

Some of the experiments with mixtures of RPV and RMV were

TABLE 1. Comparative transmission tests with four aphid species and five isolates of barley yellow dwarf virus in serial transfers during 10–22 yr[a]

Virus isolate	Number of plants that became infected over number infested in tests with aphid species shown			
	Phopalosiphum padi	*Macrosiphum* (=*Sitobion*) *avenae*	*Rhopalosiphum maidis*	*Schizaphis graminum*
RPV	454/461	3/412	1/365	132/342
RMV	43/360	9/358	355/399	42/348
MAV	10/400	464/467	0/366	4/357
SGV	2/192	1/192	1/192	153/192
PAV	309/309	239/306	3/309	101/308
Aphid controls	0/414	0/414	0/360	0/411

[a]During 10–22 yr since each virus was originally obtained from a field-collected plant.

TABLE 2. Results of tests for dependent virus transmission from mixed infections of the RPV and RMV isolates of barley yellow dwarf virus

Virus tested in role shown		Aphid vector	Number of plants from which dependent virus was transmitted over number tested for each kind of infection		Method used to identify viruses transmitted from double infection[a]
Dependent	Helper		Single	Double	
RMV	RPV	*R. padi*	0/2	8/11	Aphids
			0/2	10/10	Aphids
			2/6	7/10	Aphids
			5/17	12/16	Aphids
			1/6	10/12	Aphids
			0/10	8/10	Aphids
			2/12	10/13	EIA
			4/12	13/13	EIA
			0/10	11/11	EIA
			1/10	9/9	EIA
			0/10	8/9	EIA
RPV	RMV	*R. maidis*	0/2	0/10	Aphids
			0/2	2/10	Aphids
			0/6	2/10	Aphids
			0/18	2/16	Aphids
			0/6	5/12	Aphids
			0/10	3/10	Aphids
			0/12	4/13	EIA
			0/12	5/13	EIA
			0/10	4/11	EIA
			0/10	1/9	EIA
			0/10	3/9	EIA

[a]Tests with aphids were based on parallel transmissions with *Rhopalosiphum padi* and *R. maidis* as described in text. Virus identifications in enzyme immunosorbent assays (EIA) were based on parallel tests with virus-specific antisera for RMV and RPV (13).

based on transmissions from singly and doubly infected plants 2, 4, and 6 wk after inoculation. Since results were similar for all three time intervals studied, data of two such experiments were grouped together in Table 2. Further comparative transmission tests showed that *R. padi* readily maintained mixed infections of RMV and RPV through successive serial transfers. In six of 10 tests, for example, *R. padi* transmitted both viruses through all five serial transfers studied. In another case, the mixture was maintained by *R. padi* through all four serial transfers studied. In contrast, serial transmission by *R. maidis* from the mixed infections usually resulted in loss of the dependent virus (RPV), as transfers were continued, so that only RMV remained. These results agree with those of earlier studies with the same vectors and two other dependent virus transmission systems—mixed infections of RPV and MAV were maintained essentially indefinitely in transfers by *R. padi,* but mixed infections of RMV and MAV were not similarly maintained in successive transfers by *R. maidis* (9).

TABLE 3. Results of tests for dependent virus transmission from mixed infections of five pairs of isolates of barley yellow dwarf virus

Virus tested in role shown		Aphid	Number of plants from which dependent virus was transmittted over number tested for each kind of infection		Method used to identify viruses transmitted from double infection[a]
Dependent	Helper	vector	Single	Double	
MAV	RPV	*R. padi*	1/10	11/11	EIA
MAV	RMV	*R. maidis*	0/10	7/9	EIA
			0/10	5/11	EIA
RPV	MAV	*M. avenae*	0/10	1/11	EIA
RMV	MAV	*M. avenae*	0/10	0/9	EIA
			0/10	0/11	EIA
SGV	RPV	*R. padi*	0/5	3/4	EIA
			0/5	3/3	EIA
			0/12	5/10	EIA
			0/12	10/10	EIA and Aphids
			0/12	14/16	EIA

[a] Virus identifications in enzyme immunosorbent assays (EIA) were based on parallel tests with at least two virus-specific antisera (13). The aphid transmission, results of which were in agreement with those of EIA, was based on parallel comparisons with *Rhopalosiphum padi* and *Schizaphis graminum* as described in text.

TABLE 4. Results of tests for dependent virus transmission from mixed infections of PAV with the MAV or RMV isolates of barley yellow dwarf virus

Virus tested in role shown		Aphid	Number of plants from which dependent virus was transmitted over number tested for each kind of infection		Method used to identify viruses transmitted from double infection[a]
Dependent	Helper	vector	Single	Double	
MAV	PAV	*R. padi*	0/11	11/12	EIA and Aphids
			0/12	16/16	EIA
			0/10	12/12	EIA
			3/12	15/16	EIA and Aphids
			0/12	9/17	EIA
			1/12	16/16	EIA
RMV	PAV	*R. padi*	2/12	5/11	EIA and Aphids
			2/12	6/12	EIA
			1/12	5/11	EIA
			0/10	2/6	EIA
			0/10	2/9	EIA
			0/10	2/9	EIA
PAV	RMV	*R. maidis*	1/12	1/11	EIA
			0/12	2/12	EIA
			1/12	1/11	EIA
			1/10	2/6	EIA
			0/10	0/9	EIA
			2/10	3/9	EIA

[a] Tests with aphids were based on parallel transmissions with *Rhopalosiphum padi* and *Macrosiphum* (=*Sitobion*) *avenae* or with *R. padi* and *R. maidis* as described in text. The enzyme immunosorbent assays (EIA) were done with at least two virus-specific antisera (13). When both methods were used, results were in agreement.

A few tests were done with these two previously studied systems to evaluate use of the EIA procedure and the experimental plan used here. Data were in agreement with those of many past experiments in which RPV was a helper virus for the dependent transmission of MAV by *R. padi*, and RMV was a helper virus for the dependent transmission of MAV by *R. maidis* (Table 3). As in the past, *M. avenae* transmitted only MAV from both mixed infections (Table 3).

The RPV isolate also proved to be an effective helper virus in the dependent transmission of SGV by *R. padi* (Table 3). This mixture is an example of those that can be tested with only one vector because *Schizaphis graminum* (Rondani) is a fairly efficient vector of RPV, but *R. padi* almost never transmits SGV (Table 1). In these five experiments, *R. padi* did not transmit SGV alone from any of 46 plants, but did transmit SGV together with RPV from 35 of 43 doubly infected plants (Table 3).

In a newly discovered role, PAV was an effective helper virus for the transmission of MAV by *R. padi*. In six experiments, *R. padi* consistently transmitted MAV (from 79 of 89 plants) in the presence of PAV, even though it was unable to transmit MAV regularly from parallel single infections (Table 4). The PAV isolate was a consistent, although not especially effective helper virus for the dependent transmission of RMV by *R. padi* in each of six experiments. In contrast, there was no clear pattern of dependent virus transmission of PAV in parallel tests made with *R. maidis* from the same mixed infections of PAV and RMV (Table 4).

Although several new cases of dependent virus transmission were identified in this work, the studies also show that not all of these luteoviruses interact in this way. The complete lack of dependent virus transmission was striking in transmissions from mixtures of SGV and MAV as well as from mixed infections of RMV and SGV (Table 5). In all 19 experiments, only occasional transmissions by the "nonvector" occurred, whether the aphids fed on singly or doubly infected plants.

DISCUSSION

Dependent virus transmission by aphids occurred in seven of the 14 virus interactions studied (Table 6). A pattern of serological relationship was not associated with the occurrence of dependent transmission. Four of the seven cases (Table 6) involve interactions between viruses that are serologically distinct in EIA tests (13). But the dependent transmission of MAV in the presence of PAV results from interaction of two serologically related luteoviruses. It may be significant that the one example of reciprocal dependent virus transmission with both vectors and both viruses also occurs between two serologically related ones, RPV and RMV. Perhaps a different kind of heterologous encapsidation during simultaneous virus replication occurs for interactions of serologically related viruses than for distinct ones.

Studies of the interaction between RPV and MAV have provided evidence that transcapsidation (genomic masking) is the kind of heterologous encapsidation that explains dependent transmission by *R. padi* of MAV from mixed infections (6,10). During simultaneous replication of the two viruses some virions apparently are formed that contain nucleic acid of MAV encapsidated with RPV protein. A similar type of virus interaction apparently occurs in other mixed infections in which luteoviruses are the helper (2,3,10). Perhaps the reciprocal dependent virus transmission with RMV and RPV results from phenotypic mixing rather than transcapsidation. Thus, atypical virions in mixed infections of

TABLE 5. Results of tests for dependent virus transmission from mixed infections of SGV and two other isolates of barley yellow dwarf virus

Virus tested in role		Aphid vector	No. of plants from which dependent virus was transmitted over no. tested for each kind of infection		Method used to identify viruses transmitted from double infection[a]
Dependent	Helper		Single	Double	
SGV	MAV	*M. avenae*	0/5	0/3	Aphids
			0/3	0/5	Aphids
			0/11	0/12	EIA and Aphids
			0/7	0/16	Aphids
			0/7	0/10	Aphids
MAV	SGV	*S. graminum*	0/5	0/3	Aphids
			0/2	0/5	Aphids
			3/12	2/12	EIA and Aphids
			0/6	1/16	EIA and Aphids
			1/8	0/10	Aphids
SGV	RMV	*R. maidis*	0/5	1/4	Aphids
			0/5	0/10	Aphids
			0/7	0/11	EIA
			0/10	0/7	EIA
			0/10	0/8	Aphids
RMV	SGV	*S. graminum*	1/5	0/4	Aphids
			0/10	2/11	EIA
			5/10	2/7	EIA
			2/10	0/8	Aphids

[a] Tests with aphids were based on parallel transmissions with *Macrosiphum* (=*Sitobion*) *avenae* and *Schizaphis graminum* or with *Rhopalosiphum maidis* and *S. graminum* as described in text. The enzyme immunosorbent assays (EIA) used at least two virus-specific antisera in parallel (13). When both methods were used, results were in agreement.

RPV and RMV may contain a mosaic of proteins of both viruses. This possibility is consistent with results of studies with animal viruses that show phenotypic mixing to be common among serologically related viruses and transcapsidation to be a likely kind of heterologous encapsidation for unrelated viruses (1). Further work should make it possible to distinguish between these possibilities and may also shed additional light on mechanisms of luteovirus-aphid interactions.

Three (RPV, PAV, and RMV) of the five isolates of BYDV studied can serve as helper viruses in dependent virus transmission systems (Table 6). Each of these three helper viruses has its own potentially practical aspects. The RPV isolate, which is the most effective helper virus, is the isolate of BYDV most closely related serologically to beet western yellows virus (14). Perhaps RPV interacts in the field with other luteoviruses and other aphid vectors to change the vector range of viruses and blur distinctions now often made among virus diseases thought to be distinct (10). The discovery that PAV can also serve as a helper virus in at least two systems is especially significant because BYDV isolates similar to PAV have been the most common ones in many areas of the USA in recent years (12). These PAV-like isolates generally cause more severe symptoms than do the others. Although RMV is the least effective of the three helper viruses, it could also be important in the field because mixed infections of small grains found consistently in New York in recent years almost always contain viruses similar to RMV as one component of the mixture (11,12).

TABLE 6. Summary of tests of 14 barley yellow dwarf luteovirus double infections for dependent virus transmission by aphids with a helper virus

Tested as dependent virus	Presence (+) or absence (−) of dependent virus transmission in tests with helper virus and aphid vector shown[a]				
	RPV *Rhopalosiphum padi*	RMV *Rhopalosiphum maidis*	PAV *Rhopalosiphum padi*	MAV *Macrosiphum* (=*Sitobion*) *avenae*	SGV *Schizaphis graminum*
RPV	0	+	0	−	0
RMV	+	0	+	−	−
PAV	0	−	0	0	0
MAV	+	+	+	0	−
SGV	+	−	?	−	0

[a] A 0 indicates combinations not available for study because the aphid species involved is a vector of the potential dependent virus. The role of PAV as a helper virus for SGV also was not studied because of lack of a reliable way at present to identify SGV in the presence of PAV. The lines separate the two serological groups of viruses.

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