Interference Between Two Luteoviruses in an Aphid: Lack of Reciprocal Competition

W. F. Rochow, Irmgard Muller, and F. E. Gildow

Research plant pathologist, Agricultural Research Service, U.S. Department of Agriculture, and professor, Department of Plant Pathology, Cornell University, Ithaca, NY 14853; research support specialist, Department of Plant Pathology, Cornell University, Ithaca, NY 14853; and assistant professor of plant pathology, University of California, Berkeley 94720, respectively.

Cooperative investigation, Agricultural Research Service, U.S. Department of Agriculture, Cornell University Agricultural Experiment Station, and California Agricultural Experiment Station. Supported in part by NSF Grant PCM 8007852 to Cornell and by grants from The Rockefeller Foundation to both Cornell and the University of California.

Mention of a trademark, proprietary product, or specific equipment does not constitute a guarantee or warranty by the U.S. Department of Agriculture, and does not imply approval to the exclusion of other products that also may be suitable.

We are grateful to Laura Tufford, Dawn Smith, Mary Ballinger, and Kit Galvin for excellent technical assistance.

Accepted for publication 19 January 1983.

ABSTRACT

Rochow, W. F., Muller, I., and Gildow, F. E. 1983. Interference between two luteoviruses in an aphid: Lack of reciprocal competition. Phytopathology 73: 919-922.

Transmission rate of the PAV isolate of barley yellow dwarf virus was consistently reduced compared to that of controls when clones of the aphid vector (*Sitobion* (=*Macrosiphum*) *avenae* from either New York or California) acquired the MAV isolate before PAV. In one test, transmission of the PAV isolate was reduced, even though it had been acquired before the MAV. No corresponding consistent interference by PAV in the aphid transmission of MAV was detected when the interaction was tested four ways in the reverse order. Transmission rate of MAV was not less than that of the controls in any of 13 experiments when *S. avenae* fed first on PAV-infected plants before acquiring MAV. Similarly, when MAV was injected into vectors in 10 other experiments, consistent interference in transmission of MAV by PAV did not occur. Despite lack of reciprocal interference between MAV and PAV in the vector, study of this luteovirus interaction remains a useful approach to understanding mechanisms of circulative virus transmission by aphids.

In previous papers (5,6), we described transmission interference between two serologically similar isolates of barley yellow dwarf virus (BYDV), designated MAV and PAV, in an aphid vector *Sitobion* (=*Macrosiphum*) *avenae* (F.). Fewer aphids transmitted PAV if they had first acquired MAV; this phenomenon was observed consistently in many experiments over a wide range of conditions (5,6). Study of this interaction (MAV-PAV) has helped us refine previous concepts of luteovirus-aphid interactions and has contributed to a better understanding of transmission mechanisms for these circulative, persistently transmitted luteoviruses. A current working hypothesis is that circulative luteovirus transmission by aphids is regulated by interactions between virus capsid protein and specific receptor sites in accessory salivary glands of the vector (3,4). We think MAV reduces transmission of PAV by occupying specific cellular receptor sites in the glands necessary for PAV virions first to attach, then enter the glands, and be transmitted when aphids feed in phloem tissue of plants.

A useful test of the hypothesis would be to examine the possibility of interference between the two luteoviruses within *S. avenae* in the reverse order (PAV-MAV). When we began the earlier studies, we lacked a method to study possible interference in the reverse order because we could not detect MAV in the presence of PAV in infected plants by available biological transmission tests. Now the enzyme-linked immunosorbent assay (EIA) procedure has made it possible to study this PAV-MAV interaction, and to make more precise assays of the previously studied MAV-PAV sequence as well. Although these two isolates of BYDV are serologically related, they can readily be distinguished in EIA procedures, both in single and mixed infections, which often occur in these studies (5,11).

The main purpose of this work was to evaluate possible interference in transmission of MAV by PAV by exposing aphids first to PAV before they acquired or were injected with MAV. Another purpose was to evaluate the consistency of the MAV-PAV interference by testing it in a different geographic location with different clones of the vector.

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

MATERIALS AND METHODS

The aphid vectors, the BYDV isolates, and the methods used in this study have been described (5,9,10). The MAV isolate of BYDV is transmitted specifically by *Sitobion* (=*Macrosiphum*) *avenae* (F.), but not by *Rhopalosiphum padi* (L.) from plants infected only by MAV. The PAV isolate is transmitted by both aphid species. Acquisition feedings of 3–48 hr were on detached leaves in plastic dishes at 15 C. For acquisitions lasting >2 days, aphids were caged on oat plants in a growth chamber at 21 C (5,9,10). When virus was injected into aphids, about 0.02 μl of a purified preparation was the inoculum (6,8). Unless specified otherwise, inoculation test feedings were on seedlings of *Avena byzantina* Koch 'Coast Black' for 5 days at 21 C in a growth chamber.

Every experiment included some aphids fed only on healthy plants or leaves as controls. Other controls usually included aphids exposed only to either of the two viruses separately.

Since *S. avenae* transmits both MAV and PAV, it was necessary to test each plant to determine which virus or viruses had been transmitted. These tests of individual plants were done by means of EIA. Parallel tests were made with virus specific antisera for MAV and PAV as described previously (11), with two exceptions. First, the reaction in each well was evaluated by means of a Dynatech Micro-ELISA reader model 2-580 at 405 nm. Second, sensitivity of this reader permitted greater dilution of the globulins than had been used previously (11); precoating was at 10 μg of globulin per milliliter and conjugated globulin was used at a dilution of 1:800 of stock. Although MAV and PAV are serologically related, the homologous reactions in these EIA assays are so much stronger than the heterologous ones that differentiation between the two viruses is easy (11). Controls in each EIA plate included preparations made from healthy oats, and homologous virus controls containing 0.5 μg of each virus per well.

RESULTS

PAV-MAV sequence. When *S. avenae* fed first on PAV-infected plants or leaves, as many aphids transmitted MAV following a second acquisition as those previously fed on healthy tissue or on oats infected with either RMV or RPV, two BYDV isolates serologically unrelated to PAV (Table 1). No evidence of interference by PAV in transmission of MAV occurred in any of eight experiments that included different time intervals for the two acquisition feedings as well as inoculation test feeding periods of 3–6 days. Under similar conditions, *S. avenae* exposed first to MAV was much less likely to transmit PAV. In six previous experiments, for example, transmission of PAV by 168 aphids fed first either on healthy, on RMV-infected, or on MAV-infected oats was 55, 45, and 10%, respectively (5).

In a second series of experiments, *S. avenae* were reared on healthy or infected plants before they were given a second, relatively short, acquisition feeding on MAV-infected leaves. We thought that PAV-MAV interference might not be demonstrable when the second acquisition feeding was 1–2 days (Table 1). During this relatively long time, an early interaction might be obscured by later saturation of the system with "excess" MAV. But, even with short acquisition feedings on MAV-infected tissue (8–12 hr), aphids previously exposed to PAV were just as likely to transmit MAV as were controls previously grown on healthy or RPV-infected plants (Table 2).

In a similar feeding experiment, *S. avenae* were reared on either healthy or PAV-infected oats. Aphids were then allowed to feed for an additional 3 or 6 hr on healthy oat leaves or on MAV-infected leaves. Aphids were placed individually on 7-day-old oat seedlings for a 5-day inoculation feeding. Of 20 aphids reared on PAV-infected plants and then fed for 3 or 6 hr on healthy oats, 13 and 15, respectively, transmitted PAV. Of 20 aphids reared on healthy oats and then fed for 3 or 6 hr on MAV-infected oats, 11 and 16, respectively, transmitted MAV. Aphids reared on PAV-infected plants and then fed for 3 or 6 hr on MAV-infected leaves transmitted PAV to 15 and 16 plants, respectively; they also transmitted MAV to 13 and 15 of the plants, respectively. No interference in MAV transmission occurred following prior acquisition of PAV, even though aphids had access to MAV-infected leaves for only 3 hr.

The third kind of experiment was based on aphids receiving MAV by injection rather than by feeding. In these tests *S. avenae* were reared on healthy, on RPV-infected, or on PAV-infected oats. Some aphids from each group were injected with partially purified MAV. In two experiments, fewer of the injected aphids from PAV-infected plants transmitted MAV than did those of controls from the RPV-infected plants or the healthy ones (Table 3); in three similar experiments, however, no differences in MAV transmission occurred among the three groups of aphids. If any PAV-MAV interference occurs, these data show that it is not as consistent as the MAV-PAV interaction.

In the fourth kind of experiment, *S. avenae* were injected with inocula containing a mixture of different amounts of MAV and PAV. In four of five tests, four inocula were used. One contained only MAV, the second contained the same amount of MAV with a "low" amount of PAV, the third contained the same amount of MAV with a "high" amount of PAV, and the fourth contained only PAV. In one test, only the "high" level of PAV inoculum was used. Plants that became infected by means of these "doubly" injected aphids were then tested to determine how many aphids had transmitted MAV following injection. In one experiment, fewer aphids transmitted MAV when the inocula also contained 100 μg/ml of PAV than did those that received MAV with only 10 μg/ml of PAV or those that received no PAV (Table 4). But when this experiment was repeated, no differences in MAV transmission among the three groups of aphids occurred. Similarly, there were no clear differences in three other experiments involving varying concentrations of PAV (Table 4). None of these experiments yielded consistent evidence for interference in MAV transmission by PAV.

MAV-PAV sequence. Interference of PAV transmission by prior acquisition of MAV had been studied in only one clone of *S. avenae* maintained under controlled conditions at Ithaca, NY (5,6). To

TABLE 1. Transmission of MAV by single *Sitobion avenae* allowed a first acquisition feeding on healthy or infected oat leaves or plants, before a second acquisition feeding on MAV-infected leaves

Aphids tested in each group (no.)	No. of aphids that transmitted MAV to oats following first acquisition on tissue infected by the isolate of barley yellow dwarf virus shown[a]		
	RMV or RPV	PAV	None
12	7	8	8
10	10	9	8
20	12	12	14
20	16	13	13
24	9	11	11
24	9	5	7
24	10	7	9
18	12	11	13
Totals 152	85	76	83
MAV transmission (%)	56	50	55

[a] Except for the two control groups, transmission of MAV was determined in enzyme immunosorbent assays of individual plants. For 116 plants inoculated by aphids exposed to PAV before MAV, 25 were found to be infected only by MAV, 51 by both viruses, and 40 only by PAV. Among the eight experiments, the first acquisition feeding varied from 2–10 days; the second from 1–2 days. Inoculation test feedings were from 3–6 days. None of 60 plants infested as controls became infected.

TABLE 2. Transmission of MAV by single *Sitobion avenae* reared on healthy or infected plants, before given a second, short acquisition feeding on MAV-infected leaves

Hours of acquisition feeding on MAV-infected leaves	Percentage of 96 aphids that transmitted MAV to oats after first feeding on kind of plant shown[a]		
	RPV-infected	PAV-infected	Healthy
8	23	40	20
10	9	19	9
12	86	93	86

[a] For the two control groups data are based on numbers of plants that developed symptoms; 15 were tested in enzyme-linked immunosorbent assays (EIA) and found to contain only the MAV isolate of barley yellow dwarf virus. For groups exposed first to the PAV isolate, data are based on EIA of 42 plants per group. Of 64 found to be infected by MAV, 36 also contained PAV; 63 were infected by PAV alone. Inoculation test feedings were two days except for the third (12-hr) experiment, which was 5 days. None of the about 120 aphids tested as controls transmitted virus.

TABLE 3. Transmission of MAV to oats by single *Sitobion avenae* injected with MAV after being reared on one of three kinds of oat plants

No. of aphids injected from each group	Percentage transmission of injected MAV by aphids reared on kind of plants shown[a]		
	RPV-infected	PAV-infected	Healthy
36	31	8	39
48	23	38	42
15	33	33	27
48	15	4	19
48	90	77	92

[a] Data for the two control groups were based on numbers of plants that developed symptoms; when 13 of them were tested in enzyme-linked immunosorbent assays (EIA), all were found to contain only the MAV isolate of barley yellow dwarf virus. For the aphids reared on PAV-infected plants, data are from EIA assays of 156 infected plants; 34 contained only MAV, both viruses were found in 31, and 91 contained only PAV. None of 320 control aphids transmitted virus.

determine whether or not this interference occurred in other populations of *S. avenae*, a colony was initiated from a single apterous parthenogenetic female collected at Berkeley, CA. The California *S. avenae* were allowed a 2-day acquisition feeding on either healthy or MAV-infected oats, followed by a second 1-day acquisition feeding on PAV-infected oats or on healthy oats as controls. Individual third-instar nymphs were then placed on 7-day-old seedlings of cultivar California Red oats (*A. sativa* L.) for a 5-day inoculation test feeding. Each seedling was later tested to determine which aphids had transmitted PAV. Of 24 aphids in each treatment, 20 transmitted PAV after feeding first on healthy oat plants, but only nine of 24 transmitted PAV after feeding first on MAV-infected ones. None of four plants infested with 20 aphids that had fed on healthy plants became infected. These results indicate that transmission interference between the MAV and PAV isolates previously characterized (5,6) can occur in dissimilar clones of *S. avenae*.

In another experiment, we studied the possibility that MAV might interfere with PAV transmission even if PAV was acquired first. To test this idea, *S. avenae* were allowed a 2-day acquisition feeding first on PAV-infected oats or on healthy ones. Then they were given a second 2-day acquisition feeding on MAV-infected or healthy oats. Single aphids were next allowed a 2-day inoculation test feeding on one set of seedlings and then were transferred individually to a second set of oat seedlings for an additional 2-day test feeding. We thought there might be a difference in transmission of PAV to the two groups of test plants. When infected plants were later tested to determine which viruses had been transmitted, however, a 50% reduction in transmission frequency of PAV occurred for both groups of test seedlings. Aphids fed first on healthy oats and then on MAV-infected ones transmitted MAV to 22 and 20 of 28 plants during the first and second inoculation test feedings, respectively. Of 28 aphids fed on PAV-infected oats and then on healthy ones, 20 transmitted PAV during each of the two feedings. When aphids were fed first on PAV and then on MAV-infected oats, they transmitted MAV to 22 and 21 plants, but they transmitted PAV to only 10 plants in each of the two successive groups of seedlings. None of the 16 plants infested with 80 aphids as controls became infected. These results showed no interference in MAV transmission by prior acquisition of PAV, but they did show that MAV reduced transmission of PAV even when MAV was not acquired first.

TABLE 4. Summary of MAV transmission by single *Sitobion avenae* injected with MAV alone or mixed with "high" or "low" amounts of PAV

Virus concentration (μg/ml) in inocula		Virus transmission to oats[a]	Percentage of injected aphids that transmitted MAV[b]
MAV	PAV		
5	100	8/28	11
5	10	12/28	43
5	0	12/28	43
5	100	14/28	32
5	10	9/28	32
5	0	9/28	32
5	75	19/20	95
5	0	14/20	70
2	36	11/30	23
2	18	5/28	14
2	0	10/31	32
2	36	15/32	28
2	18	17/36	42
2	0	13/32	41

[a] Numerator is number of plants that became infected; denominator is number infested with an injected aphid. None of 310 noninjected aphids tested as controls transmitted virus. For controls injected only with PAV, 30 of 111 aphids transmitted virus.

[b] Transmission of the MAV isolate of barley yellow dwarf virus was determined by enzyme-linked immunosorbent assays (EIA) of 110 infected plants; 54 were found to be infected with only MAV, 33 with both viruses, and 23 only with PAV. Controls in the EIA tests included plants infected by means of aphids injected only with one of the two viruses; in all 14 such tested cases the EIA result confirmed the identity of the virus involved.

DISCUSSION

These data show that interaction between MAV and PAV within *S. avenae* is not reciprocal. No consistent evidence for interference in transmission of MAV by PAV was found in any of four different types of experiments. In contrast, previous and current studies showed a consistent reduction in transmission of PAV by MAV in many experiments done under a range of conditions in two laboratories. This lack of reciprocity does not invalidate the hypothesis that the MAV-PAV interaction within the aphid is based on competition between the two viruses for virus-specific receptor sites on aphid salivary glands.

Many studies with animal viruses have shown the importance of virus attachment to cellular receptor sites as a first step of virus entry into animal cells (2,7,12). The lack of reciprocal virus interaction between MAV and PAV described here is similar to some interference patterns among characterized animal cell-virus systems. For example, cross-interference patterns among chick retrovirus subgroups have been studied by preinfecting chick embryo fibroblasts with one virus before challenge with a second virus (12). A reciprocal interference between subgroups B and D occurred; prior infection with one of the viruses prevented establishment of the second. But the interference between subgroups B and D with subgroup E was not reciprocal. Prior treatment with B or D interfered with establishment of E, but prior treatment with E did not interfere with subsequent establishment of B or D. A possible explanation for these data was that subgroups B and E utilized the same cellular receptor sites, but that subgroup B had a much stronger affinity for the sites and therefore displaced subgroup E (12).

The same concept of affinity for receptor sites is suggested by the interactions of MAV and PAV described here. The model serves as an especially plausible explanation for results of the experiment in which MAV interfered with PAV transmission even though PAV was acquired first by *S. avenae*. Perhaps MAV displaces PAV at the salivary gland receptor sites because of its stronger affinity for these sites.

Two differences between MAV and PAV probably are involved in the lack of reciprocal interaction between the viruses in the vector. One is their serological relationship; the other is the relative efficiency with which *S. avenae* transmits each virus. Although MAV and PAV are serologically related, they are not identical. One comparison between the two viruses, based on homologous and heterologous titers, suggested that most of the determinants on PAV produce antibodies that also react with MAV; only a small proportion are PAV-specific (1). In comparison with PAV, MAV has a lower proportion of determinants in common with PAV; a higher proportion are specific for MAV (1). A similar relationship might occur between the two viruses in relative numbers of virus attachment determinants that react with the receptor sites on salivary glands. Perhaps MAV virions contain more reactive determinants than PAV. Thus, MAV would be more likely to occupy sites and perhaps even displace PAV virions previously attached to membranes, as suggested in the last experiment described.

The relationship of relative numbers of virus attachment determinants in proteins of the two viruses may also be a factor in the difference in transmission efficiencies. In many experiments over the years, we have found that *S. avenae* transmits MAV much more efficiently than PAV. When single aphids were tested for ability to transmit each virus at four different acquisition feeding temperatures, in combination with three different temperatures during inoculation test feeding, *S. avenae* was more likely to transmit MAV than PAV over the whole range studied (9). This difference also suggests that MAV is more likely than PAV to overcome whatever barriers limit virus circulation through the aphid.

It is possible that PAV can interfere in the transmission of MAV

within *S. avenae*; one or two experiments reported here suggested this. Perhaps we simply did not have the right conditions in most of these experiments for the PAV-MAV interference to occur at a measurable level. However, the level of any such PAV-MAV interference clearly is different from that of the MAV-PAV sequence, in which the interference has occurred every time we tested for it.

LITERATURE CITED

1. Aapola, A. I. E., and Rochow, W. F. 1971. Relationships among three isolates of barley yellow dwarf virus. Virology 46:127-141.
2. Crowell, R. L. 1976. Comparative generic characteristics of picornavirus-receptor interactions. Pages 179–202 in: Cell Membrane Receptors for Viruses, Antigens, and Antibodies, Polypeptide Hormones, and Small Molecules. R. F. Beers, Jr. and E. G. Bassett, eds. Raven Press, New York. 540 pp.
3. Gildow, F. E. 1982. Coated vesicle transport of luteoviruses through salivary glands of *Myzus persicae*. Phytopathology 72:1289-1296.
4. Gildow, F. E., and Rochow, W. F. 1980. Role of accessory salivary glands in aphid transmission of barley yellow dwarf virus. Virology 104:97-108.
5. Gildow, F. E., and Rochow, W. F. 1980. Transmission interference between two isolates of barley yellow dwarf virus in *Macrosiphum avenae*. Phytopathology 70:122-126.
6. Gildow, F. E., and Rochow, W. F. 1980. Importance of capsid integrity for interference between two isolates of barley yellow dwarf virus in an aphid. Phytopathology 70:1013-1015.
7. Lonberg-Holm, K., and Philipson, L. 1974. Early interaction between animal viruses and cells. Monographs in Virology, Vol. 9. S. Karger, Basel, Switzerland. 148 pp.
8. Muller, I. 1965. Aphid injection, an aid in the study of barley yellow dwarf virus. Cornell Plant. 20:68-71.
9. Rochow, W. F. 1969. Biological properties of four isolates of barley yellow dwarf virus. Phytopathology 59:1580-1589.
10. Rochow, W. F., Aapola, A. I. E, Brakke, M. K., and Carmichael, L. E. 1971. Purification and antigenicity of three isolates of barley yellow dwarf virus. Virology 46:117-126.
11. Rochow, W. F., and Carmichael, L. E. 1979. Specificity among barley yellow dwarf viruses in enzyme immunosorbent assays. Virology 95:415-420.
12. Weiss, R. A. 1981. Retrovirus receptors and their genetics. Pages 185–202 in: Virus Receptors, Part 2, Animal Viruses, Receptors and Recognition, Series B. Vol. 8. K. Lonberg-Holm and L. Philipson, eds. Chapman and Hall with Methuen, Inc., New York. 217 pp.