An Isolate of Barley Yellow Dwarf Virus Transmitted Specifically by Schizaphis graminum

Rebecca A. Johnson and W. F. Rochow

Graduate Assistant; and Research Plant Pathologist, Plant Science Research Division, ARS, USDA, and Professor of Plant Pathology, respectively, Cornell University, Ithaca, New York 14850.

Cooperative investigation, Plant Science Research Division, ARS, USDA, and Cornell University Agricultural Experiment Station. Based on an M.S. thesis by the senior author. Research supported in part by National Science Foundation Grant No. GB-21013.

Accepted for publication 28 February 1972.

ABSTRACT

An isolate of barley yellow dwarf virus (BYDV), SGV, differed from those previously studied in New York because it was transmitted specifically by *Schizaphis graminum*. Specificity of SGV was relative, not absolute; *Rhopalosiphum padi* was more likely to effect an occasional transmission of SGV than was *R. maidis* or *Macrosiphum avenae*. Rearing aphids on SGV-infected plants increased chances for such occasional transmissions. Specificity of SGV was not altered after occasional transmissions by "nonvector" aphids, by feeding any of the aphid species at 20, 25, or 30 C, by passage of SGV through several varieties of oats, wheat, or barley, or by mixed infections with other BYDV isolates.

Injections of a concentrate of SGV into *S. graminum* and subsequent daily serial transfers of individual aphids showed that SGV has a typical circulative or persistent relationship with its vector. *S. graminum* was more likely to transmit SGV as length of acquisition feedings on detached leaves was increased (1-5 days), and as longer inoculation test feeding periods (1-5 days) were used. The aphid was more likely to transmit SGV when fed on seedlings at 20 or 25 C than at 30 C. A major factor affecting transmission of SGV was the age of *S. graminum*. Individual first- or second-instar nymphs transmitted virus to 56 of 164 plants; only 8 of 164 plants became infected in parallel tests with adults.

Phytopathology 62:921-925.

Additional key words: aphid transmission of virus, vector specificity.

Some isolates of barley yellow dwarf virus (BYDV) can be efficiently transmitted by more than one aphid species, whereas transmission of other isolates is limited to one efficient vector (12). Three previously studied isolates are transmitted specificially by *Rhopalosiphum padi* (RPV), *R. maidis* (RMV), and *Macrosiphum avenae* (MAV), respectively. A fourth isolate, PAV, is transmitted by both *R. padi* and *M. avenae* (11). For 12 years, all BYDV isolates which were recovered from field samples in New York could be grouped into one of four types represented by these four isolates. In tests of field samples collected in New York during 1969 and 1970, however, three isolates of what appeared to be a fifth variant of BYDV were found (15). The isolates, which appear to be similar to those found by Gill (4, 5) in Manitoba in 1965 and 1967, were transmitted specifically by *Schizaphis graminum* in preliminary studies (15).

Some workers have found *S. graminum* to be an efficient vector of BYDV; others have found it to be less efficient than other aphid species that transmit BYDV (12). In our tests, *S. graminum* has generally transmitted the same virus isolates as *R. padi*, but usually in a more erratic manner. Clones of *S. graminum* differ in their ability to transmit BYDV (10), and the species differs from most vectors of BYDV in its production of a potent salivary toxin that discolors the leaf tissue at feeding sites. The importance of the age of *S. graminum* in transmission of several isolates of BYDV has recently been reported by Gill (6) and Halstead & Gill (7).

This is a report on virus-vector relationships and on transmission of one of the new BYDV isolates, designated SGV. Studies focused on the possibility that SGV is a new variant of BYDV for New York, and on comparison with previously characterized virus isolates.

MATERIALS AND METHODS.–The four aphid species used were *Schizaphis graminum* (Rondani), *Rhopalosiphum padi* (Linnaeus), *R. maidis* (Fitch), and *Macrosiphum avenae* (Fabricius). Aphids were maintained on barley (*Hordeum vulgare* L. 'Catskill') using special precautions (11). Some aphids from each colony were always tested as controls. The four isolates of BYDV used for comparison in this study were RPV, MAV, RMV, and PAV (11). The SGV isolate originated from a spring oat plant (*Avena sativa* L.) collected at the Cornell Tailby Farm in 1969 (15). All virus isolates were maintained by serial transmission to oats (*Avena byzantina* K. Koch 'Coast Black') at 6- to 8-week intervals.

Most virus transmission tests were based on use of detached leaves for a 2-day acquisition feeding at 15 C in the dark, and on a 5-day inoculation test feeding in a growth chamber at 21 C (11). Aphids were removed from test seedlings by fumigation with DDVP (o, o-dimethyl 2, 2-dichlorovinyl phosphate), and plants were placed in a greenhouse under supplemental light for observation during a 4-week period.

In most tests, the groups of about 10 aphids placed on each seedling consisted of a mixture of apterous and alate adults and nymphs. For tests on the age of the aphid, instars (first or second) or adults were transferred from the same source leaf to separate test plants. For injection of SGV into *S. graminum*, concentrated virus preparations were made by differential centrifugation of clarified oat juice (14). The injection procedure was similar to that

described by Muller (9), except that needles were made by means of an automatic micropipette puller.

RESULTS.—*Virus-vector relationship.*—Two kinds of experiments were used to study the virus-vector relationship of SGV. Firstly, a concentrated SGV preparation was injected into the hemolymph of *S. graminum.* A total of 14 of 32 plants, each infested with five injected aphids, became infected by SGV. None of the eight control plants became infected. These data reveal the circulative nature of SGV in *S. graminum.*

Secondly, newly emerged nymphs were given a 2-day acquisition feeding on SGV-infected leaves followed by successive 1-day inoculation test feedings singly on each of nine oat seedlings. Transmission patterns by the 9 aphids (of 40 tested) that transmitted virus to more than one plant demonstrated the persistence of SGV in its vector (Table 1). Only two plants became infected after feeding by aphids older than the third instar stage (Table 1). In this serial transfer experiment, few plants became infected, but it is clear that SGV can be retained by the aphid for several days and that the virus is not lost when the aphid molts. The low incidence of infection in transfers from days 6 to 9 (Table 1) may reflect the limited ability of older nymphs and adults to transmit the virus (7).

Transmission of SGV by Schizaphis graminum.—*S. graminum* produces a salivary toxin that discolors leaf tissue at the site of feeding. The importance of feeding damage and the related problem of the amount of transmission were assessed by varying the length of feeding time and the number of aphids used.

When the acquisition feeding on caged source plants was varied from 1 to 5 days in two experiments, the percentage of 88 plants for each treatment that became infected was 7% with 1 day of feeding, 16% with 2 days, 26% with 3 days, 27% with 4 days, and 49% with 5 days. None of 28 control plants became infected.

TABLE 1. Persistence of the SGV isolate of barley yellow dwarf virus within its vector, *Schizaphis graminum*

	Virus transmission after successive 1-day feedings on plant shown[a]								
Aphid no.	1	2	3	4	5	6	7	8	9
1	–	(+)	+	(–)	–	(–)	–	–	(–)
2	–	(–)	+	(–)	+	(–)	–	–	(–)
3	–	(+)	+	(–)	+	(–)	–	–	(–)
4	–	(–)	+	(+)	+	(–)	–	–	(–)
5	–	(+)	–	(–)	–	–	(–)	+	(–)
6	–	–	(+)	–	(+)	–	(+)	–	(–)
7	–	(–)	+	(–)	+	–	(–)	–	(–)
8	–	(+)	+	(+)	+	–	(–)	–	(–)
9	–	(+)	–	(–)	+	(–)	–	–	(–)

[a] Newly emerged nymphs were allowed a 2-day acquisition feeding on SGV-infected leaves before the 1-day inoculation test feeding on each of nine single oat seedlings. None of three plants infested as controls became infected. A plus means that the plant became infected, a minus means that it did not become infected, and the parentheses indicate plants on which the aphid molted.

TABLE 2. Effect of length of inoculation test feeding on transmission of the SGV isolate of barley yellow dwarf virus by *Schizaphis graminum*

No. days of inoculation test feeding	% Plants that became infected after feeding by groups of 5 or 10 aphids on each seedling[a]	
	5	10
1	13	20
2	11	39
3	23	64
4	34	73
5	33	75
Aphid controls	0	0

[a] Data are based on six experiments involving 96 plants for each treatment. Acquisition feeding was for 2 days on detached leaves at 15 C.

When the inoculation test feeding was varied from 1 to 5 days, transmission also increased as longer periods were used (Table 2). Using 10 instead of 5 aphids/plant generally doubled the percentage of plants that became infected. In two additional experiments, using 3 days for both the acquisition and inoculation test feeding, transmission was not increased over that obtained when a 2-day acquisition and a 3-day inoculation test feeding were used.

Despite the local feeding injury, only 4 of 505 plants died. Therefore, death of test plants was not an important factor in these tests. It was usually not difficult to distinguish between BYDV symptoms and feeding damage.

Other varieties of small grains were tested for susceptibility to SGV. In two experiments, parallel inoculations by means of *S. graminum* were made to 18 seedlings of each of seven varieties of oats, barley, or wheat (*Triticum aestivum* L. emend. Thell.). Typical BYDV symptoms were observed on 81% of Coast Black, 94% of Clintland 64, and 83% of California Red oat plants. Stunting, a yellow mottling, and deep leaf serrations were characteristic of symptoms of Hudson barley. Black Hulless barley reacted with severe stunting. In tests with *S. graminum*, virus was recovered from 67% of Hudson and 75% of Black Hulless barley plants, most of which had questionable or no symptoms. Though symptoms were not observed on wheat, virus was recovered from 86% of Genesee and 94% of Yorkstar plants.

When the same seven varieties of oats, barley, and wheat were used earlier (11) to compare the reactions caused by four other virus isolates, all four caused clear symptoms on Coast Black and California Red oats, but not on Genesee or Yorkstar wheat. PAV was unique in causing clear symptoms on Clintland 64 oats and on Black Hulless and Hudson barley. The reactions produced by SGV are therefore similar to those produced by PAV.

The effect of temperature on transmission of SGV by *S. graminum* was studied in three growth

TABLE 3. Influence of temperature on transmission of the SGV isolate of barley yellow dwarf virus by *Schizaphis graminum*

Temperature (C) during 2-day acquisition feeding	No. test plants infested	% Plants that became infected after 5-day inoculation test feeding at temperature (C) shown[a]		
		20	25	30
15	288	13	18	6
20	120	3	8	3
25	120	8	12	4
30	120	3	3	2
Aphid controls	108	0	0	0

[a] Single oat seedlings were infested with groups of 5, 8, or 10 aphids, the number remaining constant within each of nine experiments.

chambers which provided a 14-hr photoperiod, about 800 ft-c of light, and 20, 25, or 30 C, respectively. The 2-day acquisition feeding was at 15, 20, 25, or 30 C; the 5-day inoculation test feeding, at 20, 25, or 30 C. Results of the different combinations are shown in Table 3. The adverse effect of high temperature on transmission of SGV is similar to the effect of temperature on transmission of RPV and PAV by *S. graminum* (11). Since fewer *S. graminum* survived the 5-day inoculation test feeding at 30 C than at 20 or 25 C, the low level of transmission might be a function of poor tolerance of the aphid to this temperature.

Importance of aphid age.—Since nymphs appeared to transmit SGV more frequently than did adults, experiments were carried out to study the importance of aphid age. In three experiments using 1 aphid/plant, nymphs transmitted SGV to a total of 56 of 164 plants, whereas adults transmitted to 8 of 164. All 24 control plants remained healthy. When tests were carried out at three different temperatures, nymphs again transmitted SGV more often than did adults (Table 4). These data confirm similar observations that appeared during the course of our work (6, 7).

Tests were carried out to compare nymphs and adults of *S. graminum* in the transmission of four other isolates of BYDV. Each test was based on two groups of 32 plants, one infested with adults, the other with nymphs. Corresponding plants were each infested with five adults or nymphs that had fed on the same source leaf. None of 144 control plants became infected. In the four tests with PAV, a total of 31 of 128 groups of nymphs transmitted virus; 8 of 128 groups of adults transmitted PAV. These differences were not so striking as for SGV. In three tests with RPV, a total of 35 of 96 plants became infected with virus transmitted by adults; 13 of 96 plants infested with nymphs became infected. Neither nymphs nor adults transmitted MAV in a single test. In one test with RMV, one plant in each group became infected.

Similar tests were carried out to determine if a difference in transmission of SGV occurred between nymphs and adults of *R. padi, R. maidis,* or *M. avenae.* A 2-day acquisition and a 5-day inoculation test feeding with 5 aphids/plant were used. None of the three species transmitted SGV to any of 64 plants; neither nymphs nor adults transmitted virus. None of 36 control plants became infected.

Vector specificity of SGV.—Because long acquisition feedings have been useful in studying specificity of other BYDV isolates (12), a comparison of the ability of the four aphid species to transmit SGV was made in four experiments using 1- or 2-week acquisition feedings on caged source plants. The inoculation test feeding varied from 3 to 6 days, and 10 aphids/plant on a total of 128-184 plants were used for each treatment. Feeding periods were varied to provide the maximum likelihood for transmission. Hudson barley plants infected with SGV were used as source plants for one experiment, since *R. maidis* survives poorly on oats for extended periods.

S. graminum transmitted SGV to 82% of the plants, *R. padi* to 14%, *M. avenae* to 5%, and *R. maidis* to fewer than 1%. The general agreement of these results with those of Gill (5) suggests that SGV is similar to the *S. graminum*-specific isolates he found in Manitoba.

A comparative transmission test using seven of the plants that became infected by means of "nonvectors" showed that selection of a mutant or different virus strain did not occur. Virus was transmitted to 10 of 21 plants by *S. graminum*, and to 0 of 21 plants by each of the other three aphid species. None of 12 control plants became infected. In other comparative transmission tests using three SGV-infected plants of each of the seven varieties of oats, barley, or wheat discussed previously, *S.*

TABLE 4. Effect of temperature on transmission of the SGV isolate of barley yellow dwarf virus by nymphs and adults of *Schizaphis graminum*

Trial	Aphid stage	No. plants that became infected after a 5-day inoculation test feeding at indicated temperature (C)[a]		
		20	25	30
1	Nymph	11	10	2
	Adult	2	1	1
2	Nymph	8	13	0
	Adult	0	0	0
3	Nymph	18	14	5
	Adult	0	0	0
Aphid controls		0	0	0

[a] Data for each treatment are based on 24 plants, each infested with five aphids that had been allowed a 2-day acquisition feeding at 15 C on detached leaves.

TABLE 5. Comparative transmission by four aphid species of five isolates of barley yellow dwarf virus (BYDV) in 12 serial transfers

BYDV isolate[a]	No. test plants that became infected with BYDV (of 36 infested) in parallel tests with *Rhopalosiphum padi* (RP), *Macrosiphum avenae* (MA), *R. maidis* (RM), and *Schizaphis graminum* (SG)[b]			
	MA	RP	RM	SG
MAV	35	2	0	1
RPV	0	36	0	19
RMV	0	5	35	5
SGV	0	2	0	27
PAV	26	36	0	11
None (controls)	0	0	0	0

[a] The four vector-specific isolates, MAV, RPV, RMV, and SGV, are transmitted specifically by *M. avenae, R. padi, R. maidis,* and *S. graminum,* respectively. PAV is characterized by nonspecific transmission by *R. padi, M. avenae,* and *S. graminum.*

[b] Serial transfers for PAV and RPV were from oats infected by means of *R. padi*; transfers for RMV were from oats infected by means of *R. maidis*; those for MAV were from oats infected by means of *M. avenae*; and for SGV from oats infected by means of *S. graminum*. Tests were based on an acquisition feeding of 2 days at 15 C on detached leaves, followed by an inoculation test feeding period of 5 days with about 10 aphids/seedling. Controls were 36 plants for each aphid species.

graminum transmitted virus to 35 of 36 plants. *R. padi, R. maidis,* and *M. avenae* each transmitted virus to 0 of 36 plants. None of 24 control plants became infected. Thus, selective multiplication of a mutant or different virus isolate within the various source plants was not detected, and the relative specificity of SGV is similar to that of other vector-specific isolates of BYDV (11, 12).

Since temperature alters the vector specificity of RMV (11), an attempt was made to determine whether temperature during the inoculation test feeding affected vector specificity of SGV. The aphid species, *R. padi, R. maidis,* and *M. avenae*, were fed at 15 C on SGV-infected leaves and then allowed an inoculation test feeding at 20, 25, or 30 C, using 10 aphids/plant. None of 288 and 216 plants became infected when *R. padi* and *R. maidis*, respectively, were used. Three of 216 plants (one at 25 C and two at 30 C) infested by *M. avenae* became infected. None of 120 plants infested as controls became infected. To determine if an interaction of age and temperature might affect vector specificity of SGV, groups of the three aphid species were separated into nymphs and adults for inoculation test feedings at 20, 25, or 30 C. For each species, none of 72 plants became infected. None of 36 control plants became infected. Temperature did not alter the vector specificity of SGV.

Plants doubly infected with RPV and MAV are often severely stunted, and *R. padi* usually transmits MAV from doubly infected plants but not from plants only infected with MAV (13). Preliminary tests were made to determine if a similar breakdown of vector specificity occurred with SGV in combination with RPV, RMV, or MAV. We obtained doubly infected oat plants by allowing 5 viruliferous *S. graminum* and 5 viruliferous aphids of another species to feed simultaneously on a seedling for 5 days. Leaves from these plants were then used in comparative transmission tests with the two aphid species that had originally infested the plant. Virus was always recovered by both species in the first comparative test using infected leaves from the doubly inoculated plants. In subsequent tests, using infected leaves from the previous test as source, vector specificity remained unchanged except in one case which suggested the possibility that *R. maidis* might be more likely to transmit SGV from plants also infected by RMV than from those infected by SGV alone. Neither severely stunted plants nor pronounced breakdown in vector specificity was observed.

Results of 12 serial transmission tests with the four aphid species made during a 2-year period confirmed the vector specificity of SGV and showed that properties of the isolate remained stable. Each of the tests also included other isolates of BYDV; thus, results provided a direct comparison of transmissibility among the five distinct BYDV isolates that have been found in New York (Table 5).

DISCUSSION.—The biological properties of SGV are sufficiently different from other characterized isolates of BYDV to warrant its designation as a distinct virus isolate. The major differentiating factor is specific transmission by *S. graminum.*

SGV shares many features with other isolates of BYDV. These include the type of symptoms produced, the persistent or circulative relationship between the aphid and the virus, and the relative (not absolute) nature of the vector specificity. Symptoms caused by SGV are generally mild, and resemble those caused by RPV or RMV (11).

The age of the aphid vector is a key aspect of the transmission of SGV by *S. graminum*. In some previous tests with other vectors of BYDV, no significant differences were found in the relative ability of nymphs and adults to transmit some virus isolates (3, 6, 17). Sana & Schulz (16) reported that adults of *R. padi* were better vectors of BYDV than were instars. Gill (6) and Halstead & Gill (7) found that nymphs of both *R. maidis* and *S. graminum* were better vectors of several BYDV isolates than were adults. These observations are similar to earlier ones for transmission of filaree red leaf virus (1), pea enation mosaic virus (2), potato leaf roll virus (8), and turnip latent virus (8).

Many possibilities have been proposed to explain why nymphs can be better vectors of virus than adults (2, 6). These suggestions include occurrence of a shorter latent period in nymphs than adults (maybe as a result of a fast feeding rate by immature aphids), more rapid accumulation of virus in salivary glands of nymphs than adults, and more rapid diffusion of virus

within nymphs than adults. Another factor that might be involved in understanding the differences between nymphs and adults of *S. graminum* in transmission of SGV is the potent salivary toxin produced by *S. graminum*. Perhaps the toxin is quantitatively or qualitatively different in nymphs and adults. The occurrence of greater SGV transmission by nymphs of *S. graminum* appears to be a function of the specific aphid-virus interaction. The virus alone is probably not responsible, since SGV was not transmitted by nymphs of other aphid species. *S. graminum* nymphs did not transmit all other BYDV isolates more efficiently than did adults.

Compared with previously characterized isolates of BYDV, SGV appears to be rare in nature. This apparent scarcity may be misleading, since extensive tests have been made mainly in regions where populations of *S. graminum* are usually very low. Halstead & Gill (7) also suggested that such virus isolates could be missed in recovery tests because of the poor transmissibility by adults of *S. graminum*. The dramatic changes in predominating variants of BYDV from year to year in any one region illustrate the potential importance of all variants of BYDV in epidemiology (4, 5, 12).

LITERATURE CITED

1. ANDERSON, C. W. 1951. The insect vector relationships of the filaree red-leaf virus, with special reference to a latent-period difference between nymphs and adults in Macrosiphum geranicola (Lambers). Phytopathology 41:699-708.
2. CHAPMAN, R. K., & J. E. BATH. 1968. The latent period of pea enation mosaic virus in three of its aphid vectors with emphasis on adult versus nymph comparisons. Phytopathology 58:494-499.
3. DIZON, R. L. 1968. Seasonal temperature effects on the transmission of barley yellow dwarf virus by four cereal aphids. 97 p. Diss. Abstr. 29:4697-B.
4. GILL, C. C. 1967. Transmission of barley yellow dwarf virus isolates from Manitoba by five species of aphids. Phytopathology 57:713-718.
5. GILL, C. C. 1969. Annual variation in strains of barley yellow dwarf virus in Manitoba, and the occurrence of greenbug-specific isolates. Can. J. Bot. 47:1277-1283.
6. GILL, C. C. 1970. Aphid nymphs transmit an isolate of barley yellow dwarf virus more efficiently than do adults. Phytopathology 60:1747-1752.
7. HALSTEAD, B. E., & C. C. GILL. 1971. Transmission of barley yellow dwarf virus by different stages of the greenbug. Phytopathology 61:749-750.
8. MAC KINNON, J. P. 1962. Comparative studies between the aphid transmission of potato leaf roll and turnip latent viruses. Can. J. Bot. 40:525-531.
9. MULLER, IRMGARD. 1965. Aphid injection, an aid in the study of barley yellow dwarf virus. The Cornell Plantations 20:68-71.
10. ROCHOW, W. F. 1960. Specialization among greenbugs in the transmission of barley yellow dwarf virus. Phytopathology 50:881-884.
11. ROCHOW, W. F. 1969. Biological properties of four isolates of barley yellow dwarf virus. Phytopathology 59:1580-1589.
12. ROCHOW, W. F. 1969. Specificity in aphid transmission of a circulative plant virus, p. 175-198. *In* Karl Maramorosch [ed.]. Viruses, vectors, and vegetation. Intersci. Publ., New York.
13. ROCHOW, W. F. 1970. Barley yellow dwarf virus: phenotypic mixing and vector specificity. Science 167:875-878.
14. ROCHOW, W. F., & M. K. BRAKKE. 1964. Purification of barley yellow dwarf virus. Virology 24:310-322.
15. ROCHOW, W. F., & IRMGARD MULLER. 1971. A fifth variant of barley yellow dwarf virus in New York. Plant Dis. Reptr. 55:874-877.
16. SANA, D. L., & J. T. SCHULZ. 1962. Barley yellow dwarf transmission by instars of Rhopalosiphum fitchii and Toxoptera graminum. 17th Annu. Meeting North Central Branch Entomol. Soc. Amer. Proc. 17:95.
17. TOKO, H. V., & G. W. BRUEHL. 1959. Some host and vector relationships of strains of the barley yellow-dwarf virus. Phytopathology 49:343-347.