Spread of Seed-Borne Barley Stripe Mosaic Virus and Effects of the Virus on Barley in California

S. A. Slack, R. J. Shepherd, and D. H. Hall

Current address of senior author: Department of Plant Pathology, University of Wisconsin, Madison 53706.
Department of Plant Pathology, University of California, Davis 95616.

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ABSTRACT

Barley was planted at two locations in California which corresponded to two different growing seasons, to determine the spread of barley stripe mosaic virus (BSMV) and its effect on yield. Greater spread of the virus and higher percentage of seed transmission occurred in spring-seeded barley than when the same cultivars were sown in the fall. Spread was effected by leaf contact transmission, but not by infected pollen dispersal. The possibility that BSMV can be reduced or eliminated from spring-seeded barley by producing seed sources in fall-seeded areas is suggested.

BSMV infection of barley cultivar CM67 resulted in the production of significantly fewer and smaller seed. BSMV was found to have a detrimental effect both on male and female gametes of CM67 barley. Fertilized ovules from infected plants of this cultivar set approximately 10% less seed than ovules from healthy plants. Plants of infected cultivars CM67 and Firlbecks III produced less pollen per anther and a smaller percentage of apparently mature pollen grains (as determined by iodine stainability) than comparable healthy plants. Early infection of CM67 barley with BSMV resulted in reduced seed set and germinability and 38-45% seed transmission; in contrast, inoculation at heading produced no detectable effects compared to uninoculated controls.

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Spring barley is grown as a fall-planted crop in the relatively warm interior valleys and coastal regions of California and as a spring-seeded crop in the cooler higher elevations of northern California. Barley stripe mosaic virus (BSMV) is present throughout these areas, but is more prevalent in the spring-seeded crops (13).

The incidence of a virus disease in a crop or growing area is dependent upon the efficiency of its transmission under the prevailing conditions. Since BSMV is believed to spread mainly or wholly by leaf contact (4, 8, 10) and seed transmission (6, 9), without participation of any vector or external agent, it seems likely that the virus would be relatively independent of environmental or other external factors and perhaps more dependent upon host cultivar or the virus-strain/host-cultivar combination. This appears to be true for BSMV as suggested by the fact that seed transmission is highly dependent upon the virus strain (1, 10) or host cultivar (15). In fact, Timian (15) has established that a compatible strain-cultivar combination is a critical factor in the successful perpetuation of BSMV in barley. For example, in many artificially produced combinations in which barley was highly resistant or, alternatively, highly susceptible, the virus survived in seed for only one or two generations.

Highly susceptible reactions to BSMV infection are distinguished by the production of little or no seed. Floret sterility (8, 11) contributes to such a response. Inouye (8), for example, suggested that sterility was due to abnormal anther and pollen development which reduced the amount of pollen produced and released. Some of these effects may be caused by virus-induced genetic damage. Sandfaer (12) observed chromosome fragments and chromosome damage associated with BSMV infection in barley, and demonstrated that BSMV induced aneuploids and triploids.

This investigation was undertaken to determine the spread of seed-borne BSMV in the fall- and spring-seeded barley crops in California, and to assess the effects of the virus on individual plants.

MATERIALS AND METHODS.—Two cultivars of barley (*Hordeum vulgare* L.) were selected for study. Firlbecks III (C.I. 10086), a spring-seeded two-rowed barley grown in the Klamath Basin of northern California and southern Oregon, was selected because high levels of seed transmission in commercial seedlots have been demonstrated (13), and CM67 (C.I. 13782), a six-rowed barley, was chosen since it is one of the principal fall-seeded cultivars grown in California. Field studies were conducted on fall plantings at Davis and on spring plantings at Tulelake, California.

A commercial lot of Firlbecks III naturally infected with BSMV and CM67 which was infected with a selected strain of the virus (13) were the seed-borne inoculum sources for field studies. These seedlots were planted in blocks consisting of three 6-m rows at Tulelake in 1973. Seed harvested from Tulelake was used for plantings at Davis in 1973, and at Tulelake in 1974. A portion of the infected Firlbecks III seed planted at Tulelake in 1974 was diluted tenfold with virus-free seed to obtain an additional level of seed transmission. Blocks consisted of three and four 3-m rows at Davis and Tulelake, respectively. Blocks were separated by 1.2 m and outside rows were not included in yield data. Virus-free seed to be planted in blocks as healthy controls were produced by increasing the progeny from 10 seedlings of each cultivar found by serological tests (13) to be free of BSMV.

RESULTS.—*The effect of BSMV on spring-seeded and fall-seeded barley.*—The percentage of field-infected plants and seed transmission in harvested seed increased over the percentage in planted seed in spring-seeded Firlbecks III barley, but there was essentially no change in initial and final levels of seed transmission in CM67 (Table 1). In contrast, there was no field spread and a

decrease in seed transmission of BSMV in fall-seeded barley of both cultivars. Since the same seed lot of Firlbecks III (49% seed transmission) was used at Davis and at Tulelake in 1974, the effect of difference in environment could be determined. At Davis, only 43% of the field-assayed plants and 20% of the harvested seed were infected. At Tulelake, however, 65% of the field-assayed plants and 68% of the harvested seed were infected. These data indicate that the environment during the growing season can have a profound effect on virus spread and the resulting seed transmission of BSMV.

Visual scoring of Firlbecks III for symptoms of BSMV at Tulelake in 1974 indicated lower infection levels than were indicated by serological tests. The lower visual readings were due to poor symptom expression in seed-borne infected plants.

The two virus-strain/barly-cultivar combinations reacted differently to BSMV infection. Infected CM67, but not Firlbecks III, exhibited significant floret sterility and produced significantly smaller kernels at both locations. These factors were associated with a significant reduction in yield in spring-seeded CM67 barley.

Field spread.—Spread of BSMV from a point source was determined at both Davis and Tulelake. Each treatment was composed of 10 healthy plants spaced at 5 cm intervals in the row with an infected source plant at one end of the row. Barley was direct-seeded at Davis, and plants in the two- to three-leaf stage were transplanted to the field at Tulelake. Control plants were arranged in the same manner, but none of the plants was initially infected. Each treatment was isolated by 1.2 m of bare soil.

Virus spread in CM67 grown at Davis was not detected until April following a 7-day warm period in mid-March when maximum temperatures were between 19-25 C, or about 14 C higher than earlier March temperatures (Table 2). However, virus spread at Tulelake occurred as soon as interplant contact was established. No control plants became infected.

Anthesis started about April 1 in CM67 at Davis, but plants infected with BSMV reached anthesis 4-7 days later than healthy plants. Anthesis of Firlbecks III was about 7 days later than CM67, and occurred at the same time in diseased and healthy plants. The mean temperature during anthesis and early stages of seed development was 13 C.

At Tulelake, BSMV spread in Firlbecks III to six or seven successive plants in a row or a distance of 30-35 cm from the inoculum source. Only two CM67 plants, both adjacent to infected plants, became infected in the same period. However, CM67 matured much earlier and was not as vigorous as Firlbecks III. Periodic examination of transplanted Firlbecks III plants revealed that plants from infected seed never exhibited marked symptoms, but plants infected by leaf contact during the current season showed severe symptoms. In the same environment, however, all CM67 plants infected with BSMV expressed severe symptoms.

Germination of seed samples from infected plants at both locations showed that most contained BSMV in the progeny. Control plants at both locations produced healthy progeny.

Effect of temperature on transmission.—The difference in plant-to-plant spread of BSMV in field plantings at Tulelake and Davis suggested that the phenomenon might be affected by temperature. This hypothesis was tested by growing plants in environmental chambers for 60 days following inoculation of CM67 and Firlbecks III source plants with the same virus culture and observing the amount of contact spread. Ten healthy plants were grown in a circle with a radius of 5 cm around each source plant. Leaf agitation was produced by fans and temperatures were set to correspond to the mean temperatures encountered at Davis or Tulelake, respectively, during different months of the two growing seasons.

Virus spread occurred when the mean temperature was 12 or 18 C, but not at 7 C (Table 3). In addition, more Firlbecks III plants than CM67 plants became infected. Since the same virus strain was used for both cultivars in this test, Firlbecks III apparently was more susceptible to infection and plant-to-plant spread than CM67. Approximately 30 days were necessary for symptom development in plants held at an average of 7 C. At the higher temperatures, symptoms developed in 7-8 days. CM67 inoculated at 7 C developed a chlorotic flecking

TABLE 1. Spread of barley stripe mosaic virus in barley grown at two locations in California and the effect of the virus on yield

Location and year	Time seeded	Cultivar	Infection (%) in			Yield[a]	
			Planted seed[b]	Field plants[c]	Harvested seed[b]	kg/ha	Healthy control (%)
Davis							
1973	Fall	CM67	34	19	3	6,742	102
1973	Fall	Firlbecks III	49	43	20	3,348	80
Tulelake							
1973	Spring	CM67	36	35	34	2,395	54**
1973	Spring	Firlbecks III	19	90	49	5,748	90
1974	Spring	Firlbecks III	5	44 (30)[d]	17	6,991	97
1974	Spring	Firlbecks III	49	65 (23)[d]	68	6,881	96

[a]Mean of six replications at Davis in 1973 and Tulelake in 1974, and of three replications at Tulelake in 1973. ** = significant difference, $P = 0.01$.

[b]Minimum of 300 serological assays with an equal number of 'healthy' controls negative.

[c]Minimum of 100 serological assays with an equal number of 'healthy' controls negative except for one positive in Firlbecks III at Davis.

[d]Number in parentheses represents percent infection in same samples determined by visual symptoms.

TABLE 2. Spread of barley stripe mosaic virus from an infected plant down a row of healthy plants at two locations in California

Location, cultivar and date of assay	Replication and plant number																																		
	I											II											III											IV			
	1	2	3	4	5	6	7	8	9	10	11	1	2	3	4	5	6	7	8	9	10	11	1	2	3	4	5	6	7	8	9	10	11	1	2	3	4	5	6	7	8	9	10	11
Davis[a]																																											
CM67																																											
5 March	S[b]	–	–	–	–	–	–	–	–	–	–	S	–	–	–	–	–	–	–	–	–	–	S	–	–	–	–	–	–	–	–	–										
11 April	S	+	–	–	–	–	–	–	–	–	–	S	–	–	–	–	–	–	–	–	–	–	S	+	+	–	–	–	–	–	–	–										
2 May	S	+	+	–	–	–	–	–	–	–	–	S	–	–	–	–	–	–	–	–	–	–	S	+	+	–	–	–	–	–	–	–										
Tulelake[a]																																											
CM67																																											
30 May	S	–	–	–	–	–	–	–	–	–	–	S	–	–	–	–	–	–	–	–	–	–	S	–	–	–	–	–	–	–	–	–	S	–	–	–	–	–	–	–	–	–
28 June	S	+	+	–	–	–	–	–	–	–	–	S	+	+	–	–	–	–	–	–	–	–	S	+	+	–	–	–	–	–	–	–	S	–	+	–	–	–	–	–	–	–
24 July	S	+	+	–	–	–	–	–	–	–	–	S	+	+	–	–	–	–	–	–	–	–	S	+	+	–	–	–	–	–	–	–	S	+	+	–	–	–	–	–	–	–
Firlbecks III																																											
30 May	S	–	–	–	–	–	–	–	–	–	–	S	–	–	–	–	–	–	–	–	–	–	S	–	–	–	–	–	–	–	–	–	S	–	–	–	–	–	–	–	–	–
28 June	S	+	+	–	–	+	+	–	–	–	–	S	+	+	+	–	–	–	–	–	–	–	S	+	+	+	+	–	–	–	–	–	S	+	+	+	–	–	–	–	–	–
24 July	S	+	+	+	+	+	+	–	–	–	–	S	+	+	+	+	+	+	–	–	–	–	S	+	+	+	+	+	–	+	–	–	S	+	+	+	+	+	+	–	–	–

[a]Mean monthly temperatures (C) for 1974: February = 9, March = 12, and April = 13 at Davis; and May = 12, June = 17, and July = 19 at Tulelake.

[b]S = infected source plant (source plants were inoculated in two-leaf stage at Davis, and seedlings from infected seed were transplanted in the two- to three-leaf stage at Tulelake), + = infected plants, and – = healthy plants. Ten virus-free plants were arranged 5 cm apart in a row with an infected source plant located at one end of the row.

that was unrelated to virus infection. Thus, it appears that BSMV will not spread from plant to plant when the plants have been maintained at low temperatures.

Mechanical transmission by pollen.—Since BSMV is known to occur in pollen (1, 5, 6), it was postulated that the virus might spread by contamination of leaf surfaces with infected pollen which could result in contact transmission through wounds sustained by leaf agitation. Pollen collected from healthy and infected barley in the field at Davis was rubbed onto or dusted over healthy

TABLE 3. Spread of barley stripe mosaic virus from a point source of inoculum in two barley cultivars in a controlled environment[a]

Barley cultivar	Temperature (C)		Plants infected/ plants tested	Mean plant height (cm)
	Maximum	Minimum		
CM67	10	3	0/40	38
Firlbecks III			0/30	23
CM67	18	6	1/30	76
Firlbecks III			13/20	56
CM67	29	7	2/40	56
Firlbecks III			14/20	46

[a]Environmental chambers with 12-hour daylength, fan-induced air turbulence, and temperatures controlled to correspond to average temperatures during growing seasons at Davis and Tulelake, California. Ten healthy plants equally spaced in a circle (5-cm radius) around each source plant were exposed for 60 days following source plant inoculation.

TABLE 4. Effect of barley stripe mosaic virus on the male and female gametes of CM67 barley as determined by intercrosses between healthy and infected plants

Female	Male	Plants[a]	Florets[b]	Seed set (%)	Seed germinated (%)	Seed infected (%)
Healthy	Healthy	20	299	96	85	0
Healthy	Infected	20	289	93	53	3
Infected	Healthy	20	324	82	72	66
Infected	Infected	20	320	84	67	70

[a]One spike per plant of CM67 barley grown in the field at Davis, California.
[b]Florets were emasculated before anthers matured. Field-collected pollen was used for cross-pollination.

TABLE 5. Effect of barley stripe mosaic virus on pollen development in two field-grown barley cultivars at Davis, California[a]

Barley cultivar	Treatment	Pollen grains (no.)	Stained pollen[b] (%)	Stained pollen/anther[c] (% of healthy)
CM67	Healthy	2,954	93	100
CM67	Infected	2,611	52	49
Firlbecks III	Healthy	3,467	66	100
Firlbecks III	Infected	2,624	53	61

[a]Each treatment consisted of pollen collected from 200 anthers (10 anthers per head, 20 heads).
[b]Staining blue-black with an aqueous solution of iodine was used as an index of maturity.
[c]Determined as follows: (number of pollen grains) × (water dilution) × (1/200 anthers). The resulting number was divided by the number calculated for the appropriate healthy control and recorded as percentile.

TABLE 6. Effect of barley stripe mosaic virus on seed set, germination, and transmission in CM67 barley when infected at different stages of growth in the greenhouse

Stage infected	Plants (no.)	Heads (no.)	Florets (no.)	Seed set (%)	Seed germinated (%)	Seed infected (%)
Seed-borne	12	20	663	37	53	45
Two-leaf	20	20	741	61	44	38
Tillering	10	20	588	48	71	44[a]
Heading[b]	64	105	2,782	66	70	0
Uninoculated	17	20	537	77	80	0

[a]Percentage infected seed was determined using 195 instead of 200 for the number germinated since five plants were albino.
[b]Composite of plants that were 0-3 days before or after anthesis.

plants of the same cultivar without inducing infection in CM67. Two of 20 Firlbecks III plants rubbed with pollen from infected sources became infected when leaves had been previously dusted with 22-μm (600-mesh) Carborundum. However, a similar number of tests were negative when Carborundum was not employed. Control plants treated with pollen from healthy sources did not become infected.

Field experiments were also designed to test natural dispersion of infected pollen. Two 3-m rows were planted with infected seed 1.2 m on either side of a 75 cm row of healthy CM67 at Davis or Firlbecks III at Tulelake. The virus-free CM67 seed was planted 1 month later than infected seed at Davis to delay maturity and maximize any expression of mechanical infection from a pollen source. Three replications were made at each site and 40 randomly selected heads from an equal number of early- and late-maturing tillers were collected from each replication and germinated in the greenhouse to check for seed transmission. None of the plants in the field developed symptoms of BSMV infection, and greenhouse tests for seed transmission were negative (> 1,200 CM67 seedlings per replicate and > 400 Firlbecks III seedlings per replicate, three replications each). These trials indicate that BSMV is rarely, if ever, disseminated in infected pollen in these cultivars.

The effect of BSMV on barley gametes.—Intercrosses between healthy and infected CM67 plants used reciprocally as males and females were made in the field at Davis. Florets were emasculated when the anthers were immature, covered with glassine bags, and pollinated 2-4 days later.

BSMV was seed-transmitted primarily through ovules of infected CM67 barley (Table 4). Florets of crosses between infected pollen donors and healthy ovules set 93% seed of which only 53% germinated and 3% of the progeny were infected. Crosses using healthy and infected pollen and ovules on infected plants showed 66 and 70% seed transmission, respectively. The seed transmission results are similar to reports on other cultivars infected with BSMV (6, 8, 14). In addition, a detrimental effect on maternal development was indicated by about a 10% reduction in seed set from infected florets regardless of pollen source.

The effect of BSMV on the number of pollen grains developed per anther and its stainability by iodine (used as an indicator of maturity) was studied in collections from healthy and infected plants of each cultivar at Davis (10 anthers from each of 20 heads per treatment). The anthers from each head were pooled and gently macerated with dissecting needles in a few drops of an equal volume of glycerin and an aqueous solution of iodine (1 g potassium iodide and 0.3 g iodine in 100 ml glass-distilled water). After a few minutes, the stained pollen grain suspension was made up to a known volume (usually 50 ml) in glass-distilled water, a portion removed, and pollen grains observed with a light microscope (×50) using a 1-ml counting chamber.

The number of pollen grains per anther, and the percent stainable pollen grains were diminished by BSMV- infection (Table 5). Infected CM67 and Firlbecks III plants produced 51 and 39% less stainable pollen grains, respectively, than healthy plants. The decrease in stainable pollen in CM67 might be related to the sterility observed. Also pollen from three times as many infected heads as from healthy heads was required to make a comparable number of crosses.

The effect of BSMV infection at different stages of development.—Reports (2, 3, 7, 8, 14) have differed concerning whether barley inoculated at the time of flowering will result in seed transmission of BSMV, but all reports indicated that seed transmission was reduced when infection occurred at boot stage or later. Therefore, CM67 tillers with flowers were checked for stage of development, tagged for later collection, and either the leaves or heads were mechanically inoculated.

Plants from infected seed were the most severely affected by BSMV as judged by the marked reduction in seed set and germination (Table 6). Seed set of plants inoculated at tillering and germination of seed from plants inoculated at the two-leaf stage were less than uninoculated controls. Seed germinated from these treatments produced 38-45% BSMV-infected plants. No detectable reduction in set or germination of seed resulted from inoculation at heading, and no seed transmission was noted from 1,289 germinated seeds.

Tillers on which only heads were inoculated did not express symptoms, but regrowth which developed while the tagged heads matured showed severe symptoms. The fact that BSMV can translocate readily to regrowth or late tillers could affect BSMV perpetuation to future generations. This would be true in an area such as Tulelake where regrowth is common.

DISCUSSION.—BSMV virus-strain/barley-cultivar combinations found in nature usually result in mild or moderate reactions (15). In our tests, Firlbecks III reacted in a tolerant manner to naturally occurring strain(s) of BSMV, and there was good survival of the virus through two successive summer generations. Only moderate decreases in seed yield resulted from infection, and high rates of seed transmission were maintained. Thus, this virus-cultivar pair would appear to fit the tolerant category of Timian (15) which favors continued survival of both host and virus. In contrast, a selected strain of BSMV in spring-seeded CM67 barley caused significant yield loss and maintained a high rate of seed transmission. It seems likely that this cultivar would soon succumb to the disease, and could be considered very susceptible under these conditions. Conversely, when CM67 was fall-seeded there was no yield loss and a tenfold decrease in seed transmission occurred. Since the virus would probably not persist under these conditions, CM67 would be considered resistant. Hence, environment may be equally as important as virus strain or host cultivar in affecting the ultimate outcome on virus or host survival.

BSMV infection of barley affected both male and female gametes as shown by reduced seed set and seed germination and appeared to be enhanced by early infection. Infection of BSMV prior to anthesis was required for seed transmission.

Field spread of BSMV apparently resulted from leaf contact as indicated by new infections originating in plants adjacent or nearly adjacent to previously infected plants and the absence of infection in nearby, but spatially separated, healthy controls. High rates of seed transmission and efficient field spread by contact transmission are probably essential for survival of BSMV in barley. Although some virus-strain/host-cultivar

combinations might exhibit more than a 50% rate of seed transmission, this is not common. Under conditions in which there is less than 50% seed transmission, a progressively lower proportion of infected plants would result in successive host generations if the virus were solely dependent upon seed transmission for survival. Thus, it appears that contact transmission is an important factor in the epidemiology of BSMV and that host cultivar, virus strain, and environment influence contact transmission as well as seed transmission. In fact, the results indicate that one of the most significant effects of low temperature on BSMV infection in fall-seeded barley is to slow or prevent systemic movement and/or contact transmission which results in lower levels of seed transmission in harvested seed and relatively little reduction in yield. Therefore, it is possible that BSMV can be reduced or eliminated from areas like the spring-seeded northern California environment by producing foundation seed sources in another region such as the fall-planted areas of California.

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