The impact of host variation on the host-parasite relationship between the type strain of barley stripe mosaic virus and three cultivars of barley, Hordeum vulgare L.

by Donald Wayne Fenbert

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Genetics
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Abstract:
The impact of genetic variation in the host on the host-parasite relationship between barley stripe mosaic virus (BSMV) and barley (Hordeum vulgare L.), was studied at Bozeman, Montana. Four characters were examined: susceptibility, symptom expression score (SES), embryo infection (EI), and seed transmission (ST).

Seeds from two-forced self-pollinated single plant generations, and an open-pollinated base generation of each of three cultivars of barley ('Atlas', C.I. 4118; 'Black Hulless', C.I. 666; 'Hypana', C.I. 11772) and F2 generations from crosses among the cultivars were planted in a nursery, Single plants were completely randomized within blocks. All plants in the nursery were inoculated with the type strain of BSMV (A.T.C.C. #69).

Symptom expression was scored on the plants, at heading time, based on the distribution of leaf symptoms on the individual plants. Progeny from plants infected by inoculation were assayed for the presence of BSMV using Hamilton's embryo test and Afanasiev's seedling test. The analysis of variance was used to test for genetic differences among the generation means.

All lines studied were highly susceptible to BSMV. The percentage of non—infected plants varied among the generations and this was due to genetic differences among them. Studies on plant progeny revealed that any resistance to mechanical inoculation in the generations studied is inherited in a complex manner.

The pattern of genetic variation for SES indicates that low SES is dominant. Hypana and Atlas cultivars are genetically similar for SES and exhibit significantly lower SES values than those for Black Hulless. The single plant S4 generation of Atlas which had been forced-selfed for four generations exhibited significantly higher SES than the open pollinated base generation of Atlas, indicating that the base generation was genetically heterogeneous for this character.

When individual plant data for ST or EI were pooled within generations, the amount of ST among all generations studied was not significantly different. The amount of EI in the BH X HY F2 generation was significantly higher than in all the other generations studied. Over all plants tested, the amount of EI (3.31%) was significantly higher than the amount of ST (1.99%). The low percentages of EI and ST may have been due to low temperatures in the field during the growing season.

The low correlations (based on single plant values) between ST and EI, between ST and SES, and between EI and SES, suggest that each of these characters is controlled by a different genetic system. A high correlation of SES for the generations suggest that these characters are controlled by the same genetic system.

When EI or ST of individual plant progeny were compared within generations, significant individual

plant heterogeneity was found within some of the generations. This heterogeneity reflects genetic variation among plants of the same generation.

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A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree

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Genetics

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ABSTRACT

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The low correlations (based on single plant values) between ST and EI, between ST and SES, and between EI and SES, suggest that each of these characters is controlled by a different genetic system. A high correlation of SES for the generations suggest that these characters are controlled by the same genetic system.

When EI or ST of individual plant progeny were compared within generations, significant individual plant heterogeneity was found within some of the generations. This heterogeneity reflects genetic variation among plants of the same generation.

INTRODUCTION AND LITERATURE REVIEW

Barley stripe mosaic virus (BSMV) is the only virus definitely known to be seed-borne in grasses (27). The disease, barley stripe mosaic (BSM), caused by the virus has produced serious yield losses in barley, *Hordeum vulgare* L. Eslick (12) reported that over a five year period BSM reduced the yield in 'Glacier' barley by 31%. The maximum yield reduction in one year was 41%.

BSMV is maintained in barley, year after year, only through the mechanism of seed transmission. BSMV has been found in cultivated barley acreage in North America, Europe, and Asia. BSMV was probably unintentionally introduced into commercially planted cultivars by some barley breeders who maintained BSMV in their breeding stocks, unaware that the symptoms of BSM were caused by a seed-borne virus.

Until 1951, in the United States, BSM was believed to be a physiological disorder of barley. The disorder was called "False Stripe" because the leaf symptoms of the disorder occasionally mimicked those of the disease caused by the fungus *Helminthosporium gramineum* Rabh. In 1951, McKinney (25) demonstrated that the false stripe disorder of barley was caused by a seed-borne virus.

In Japan, BSM was originally believed to be a genetic defect of barley (21). The major consequence of the defect was semi-sterility. After many genetical experiments had yielded confusing results,

Inouye (21), in 1956, finally demonstrated that the semi-sterility was caused by BSMV.

BSMV has not been shown to be vectored by insects, mites, nematodes, or fungi. In barley plants, BSMV may reach the "seed" (botanically the caryopsis, grain, or fruit) of an infected plant through either the pollen (14) or the ovule (21). In field plantings, BSMV, which has been seed transmitted, can enter the leaves of healthy plants if they rub against leaves of infected plants (contact transmission). BSMV is readily transmitted by mechanical inoculation.

BSMV is a short rigid rod about 20 to 30 nanometers (nm.) in diameter, and from 20 to 280 nm. in length (14,23). Harrison, Nixon, and Woods (17) have shown, for the isolate of BSMV with which they worked, that the most common length of the particles was 128 nm. Particles of this isolate of BSMV less than 111 nm. in length were not infectious. The term "isolate" refers to a population of BSMV which has been isolated from infected plants growing in the field. McKinney (26) has stated that these "isolates" are always comprised of at least two different strains of BSMV. Atabekov and Novikov (2) have shown that BSMV, like most plant viruses, has its genome encoded in single stranded RNA.

Because of the economic damage caused by BSMV, attempts were made to find an effective method to eliminate it in cultivated barley. A search was made through part of the world barley collection by

Timian and Sisler (35) for a barley variety resistant to mechanical inoculation with BSMV. They worked with three isolates of BSMV, and found resistance to the California E isolate in 'Modjo' (C.I. 3212). They did not report the age of the host at the time of inoculation. Sisler and Timian (32) determined that the resistance was produced by a pair of unlinked recessive genes. In more recent studies, three more genes for resistance to BSMV have been reported by Vasquez, Peterson, and Timian (37). A single dominant gene in Modjo and a single recessive gene in 'Moreval' determine resistance to the ND 50 strain of BSMV. A single recessive gene in Modjo determines resistance to the ND 1 strain of BSMV. In Japan, Inouye (21) screened 2,200 varieties of barley for resistance to mechanical inoculation with a Japanese isolate of BSMV. He found two varieties that were resistant, 'Wien' and 'Imperial'. A single dominant gene in Wien and a single recessive gene in Imperial determine the resistance to BSMV.

The studies made on genetic resistance to BSMV do not include any study on the genetics of the seed transmissibility of BSMV. There are so many strains and isolates of BSMV that it may be very difficult to find a small number of genes that can provide resistance to infection from all strains and isolates. Resistance to mechanical inoculation with BSMV has not been used as a control method for BSMV in commercially grown barley.

BSMV is a problem in barley only if infected seeds are planted. The most practical method of controlling BSMV is to plant virus-free seeds. To accomplish this, infected seed lots must be detected and eliminated.

The first practical method to screen seeds for the presence of BSMV was developed by Afansiev (1). He planted seeds in flats in the greenhouse and visually examined the emerged seedlings for symptoms of BSM. This seedling test has three disadvantages to its use in Bozeman, Montana. First, it can only detect BSMV in seedlings that show symptoms. Secondly, it takes two to three weeks to run the test, and finally, it requires a great deal of greenhouse space if large numbers of seed lots are to be tested.

In 1964, Hamilton (15) perfected a serological test which could detect the presence of BSMV coat protein. The test employed the Ouchterlony double-diffusion technique. The test was first used to detect the presence of BSMV in leaf sap. Later, Hamilton (16) demonstrated that his serological test could detect BSMV in barley embryos. When embryos are used for BSMV detection, the disadvantages of Afanasiev's seedling test are overcome. No greenhouse space is required, it takes only 48 hours to run the serological test, and, since the serological test detects the virus, symptoms of BSM (which are not present in barley embryos) are not required. Furthermore, the serological test

can be used to detect BSMV in leaf sap of seedlings which do not show symptoms of BSM. When Hamilton compared the results of the seedling test and the embryo test on the same infected seed lot, he obtained 34% seed transmission and 32% embryo infection. This ratio, approximately 1:1, demonstrated that infected embryos give rise to infected seedlings.

The serological test for eliminating infected seed lots can be used only where qualified personnel and adequate facilities are available. In underdeveloped countries where barley is grown, facilities and personnel may be inadequate. A more practical solution in those countries may be to grow barley through which BSMV is incapable of being seed transmitted. This solution may be simpler than breeding for resistance to mechanical inoculation, which may involve numerous genes to condition for resistance to a wide array of viral isolates, while a mechanism which could block BSMV seed transmission in barley might block all strains.

Seed transmission is a phenomenon vital to the distribution of BSMV. The amount of seed transmission is affected by three factors: (1) The environment; (2) The strain or isolate of BSMV; (3) The host barley plant. The picture is further complicated by potential interactions among these factors.

The effect of temperature on BSMV seed transmission was studied by Singh, Arny, and Pound (31). They obtained 0 to 3% seed transmission in the greenhouse when the air temperature was maintained at 16%C. When the temperature was maintained at 20 or 24°C, seed transmission increased from 7 to 28%, depending on the barley variety examined. Carroll (8) has shown that the type strain of BSMV in 'Atlas' barley grown in the greenhouse is seed transmitted at a level of about 40% in the summer and 10% in the winter. Also, it has been demonstrated that different strains and isolates of BSMV are seed transmitted at different rates in the same barley variety under the same environmental conditions. Hamilton (16), McKinney and Greeley (27), Shivanathan (30), and Carroll (7) have shown that the type strain of BSMV was seed transmitted in Atlas barley while the NSP (non-seed-passage) strain was not. Furthermore, Carroll (9) discovered that in Atlas barley grown in the greenhouse during the winter, a Montana isolate of BSMV was seed transmitted 5 times as much as the type strain.

The effect of host age at the time of mechanical inoculation with BSMV on the amount of seed transmission has been widely studied. There are two conflicting viewpoints in this area of research.

Crowley (10) working with the "Manchurian" isolate of BSMV in 'Mars' and 'Compana' barley, Timian (36) working with an "isolate of

the type culture" of BSMV in 'Manchuria' and 'Kindred' barley, and Singh, Arny, and Pound (31) working with an isolate of BSMV in 'Oderbrucker' barley, all agree that early inoculation with BSMV gives the greatest amount of seed transmission. Eslick and Afanasiev (13), working with an isolate of BSMV in Compana and 'Titan' barley, and Inouye (21) working with a Japanese isolate of BSMV in 'Hakata No. 2', 'Golden Melon No. 1', and 'Chevalier' barley, agree that late inoculation (10 to 21 days prior to heading) gives the greatest amount of seed transmission. Timian (36) and Singh, Arny, and Pound (31) reported that in their experiments no seed transmission of BSMV occurred when the plants were inoculated after flowering. By contrast, Inouye (21) and Crowley (10) obtained low levels of seed transmission, 1.1% and 1.6% respectively, from plants which were inoculated after flowering. When Eslick and Afansiev (13) inoculated barley plants in the hard dough stage, they obtained 14% seed transmission. If all fertilization had taken place prior to inoculation, the fact that BSMV was still seed-transmitted could only be due to direct invasion of the barley embryo by the virus. Carroll (7) hypothesized that seed transmission attributed to direct invasion of the embryo by BSMV may have been caused by pollen or ovule transmission in florets where fertilization had not yet occurred at the time the plants were inoculated. The hypothesis was based on the observation that barley spikes on the

same plant may vary greatly in terms of developmental stage, and developmental stages can vary within a spike. To test his hypothesis, Carroll (7) inoculated Atlas barley with the type strain of BSMV after fertilization had occurred in the older spikes. At the time of inoculation, spikes in all developmental stages were coded, and, on some of the older spikes which mainly bore developing kernels, all unfertilized florets were removed. Later, all spikes were harvested at maturity, and the embryos of their ripened seeds were assayed for BSMV using Hamilton's (16) embryo test. None of the embryos was infected from florets which were fertilized at the time of inoculation, while 12.7% of the embryos from the unfertilized control florets were infected.

The discrepancy can be resolved by considering the effects of environment, virus strain or isolate, and the host plant on seed transmission. The variation in amount of seed transmission may be due to different experimental conditions.

A prerequisite to studying the effect of host age on the seed transmission of BSMV is barley lines which respond identically to BSMV if all variables are kept constant. The reports of scientists who studied the effect of host age on seed transmission imply that all plants of a variety studied responded similarly to the virus, since individual plant data are not discussed.

Carroll and Chapman (6) compared the seed transmissibility of the type strain of BSMV in Atlas and 'Hypana' barley. They detected significant and differential heterogenity between the cultivars, and suggested the heterogenity was due to genetic variation.

The present research is an expansion of the work of Carroll and Chapman. The purpose of this study was to investigate the host-parasite relationships between the type strain of BSMV and three cultivars of barley in terms of four important economic characteristics of the relationship: percent field infection, level of field symptoms, percent seed transmission, and percent embryo infection. The extent and impact of genetic variation on these characteristics was also assayed.

This study deals with one part of the host-parasite-environment relationship. To study accurately variation in the host, it is necessary to be able to treat the parasite and the environment as constants. In order to meet, in part, the assumptions of least square analyses, plants were individually randomized over the planting area. This effectively permits treating the environment as a constant. The type strain of BSMV was selected and used because the method chosen to isolate it and its biological behavior support the hypothesis that it is a genetically uniform stain and can be treated as a constant. The type strain of BSMV was selected and used because the method chosen

to isolate it and its biological behavior support the hypothesis that it is a genetically uniform strain and can be treated as a constant.

Three cultivars of barley were used in the experiment, Atlas and Hypana, also studied by Carroll and Chapman (6), and 'Black Hulless', a hypersensitive host for BSMV. Selection of single plant lines was made from stock populations of the cultivars to investigate whether or not the lines, and the stock populations would respond the same way to the type strain of BSMV. Crosses were made between the cultivars to investigate genetic differences among the cultivars.

MATERIALS AND METHODS

I. Barley Stocks

Three cultivars of barley (*Hordeum vulgare*, L.) were used in this study. 'Atlas' (AT), C.I. 4118 (38), is a six row, spring habit, hulled barley. 'Black Hulless' (BH), C.I. 666 (35), is a six row, spring habit, naked barley. 'Hypana' (HY) C.I. 11772 (18), is a two row, spring habit, hulled barley. The stock seed lots of AT and HY were the same stock seed lots used by Carroll and Chapman (6) and were obtained from them. The BH seeds were obtained from the U.S.D.A. station at Aberdeen, Idaho, supplied through the kindness of Dr. J. G. Moseman.

II. Virus Stock

The type strain (American Type Culture Collection #69) of BSMV was used throughout this experiment. The origin of this strain has been described by McKinney and Greeley (27). The type strain was isolated from a single local lesion, which is a method used to separate a single virus strain from a multiple strain isolate (24). The type strain of BSMV was obtained from Dr. T. W. Carroll and has been maintained in the Montana State University greenhouse at a temperature range of 15° to 30°C in AT barley since 1968.

III. Development of Genetic Stocks of Barley

In an attempt to eliminate the heterogeneity reported by Carroll and Chapman (6) in the stocks of AT and HY barley, bags were placed over the spikes of the barley plants to insure self-pollination. The bags used were glassine, 1.8 cm. in width, cut to a length of approximately 20 cm. They were obtained from the U.S.D.A. through the kindness of D. E. A. Hockett. To treat all cultivars uniformly, bagging was also done to the BH plants.

Although barley is normally self-fertilizing, up to 2% outcrossing is possible (19). One dose of 2% outcrossing is not enough to explain the heterogeneity reported by Carroll and Chapman (6). However, over several generations this much outcrossing could easily make a "pure line" cultivar quite heterogeneous. AT was developed as a "pure line" selection from Coast, the original barley introduced by the Spanish settlers to California (38). It was released in 1924 and has had forty-four years to acquire heterogeneity. HY is a recently developed variety released in 1964, and was developed from the cross Glacier by Compana and developed through selfing beginning in 1941 (18). It has had only five years of potential outcrossing to increase heterogeneity. BH is a variety not grown commercially, and it is probably heterogeneous because of many years for potential outcrossing.

The development of seeds used in the field experiment took place in the greenhouse from January 1970 to May 1971. All plants grown in the greenhouse were seeded in steam sterilized soil in 20 cm. plastic pots. The AT and BH stock seeds were planted to yield two plants per pot, and the HY stock seeds were planted to yield four plants per pot. HY plants are smaller in the greenhouse, and therefore twice as many could be grown per pot. Twenty pots were used for each cultivar. When the plants headed, the spikes were bagged. When the seeds matured, they were harvested and all the bagged seeds harvested from plants of the same cultivar were bulked. The plants from which the stock seeds were obtained were probably not homozygous based on the previously discussed assumptions about the cultivars. The stock seed was designated S_0 and the first generation of bagged seeds is designated S_1. Seeds were randomly selected from the bulked S_1 seeds and grown following the previously described procedure. The procedure was repeated to produce bulked lots of S_2, S_3, and S_4 seeds for each of the three cultivars. Stock S_0 seeds, and bulked S_1, S_2, S_3, and S_4 seeds were replanted so that single plant lines of S_1, S_2, S_3, S_4, and S_5 seeds could be obtained. Greenhouse produced, forced selfed seeds from single plants were produced from January 1971 to May 1971. The S_1 seeds developed from this replanting came from a single S_0 plant, the S_2 seeds from a single S_1 plant grown from a single seed from the

bulked S_1 seeds, and similarly for S_3 and S_4. Because all the single plant lines were developed at the same time, it is unlikely that a pedigree relationship exists among single plant lines of the same cultivar, since each line came from a different bulked seed lot. The development of the S generations is presented in Figure 1 (page 15).

When the plants grown from the bulked S_3 seeds headed, a diallel cross, including reciprocals, was attempted among the cultivars. Four of the six crosses were successful. Following conventional procedure, the seed parent in a cross is written first. One F_1 seed was obtained from the cross BH X AT, five F_1 seeds from HY X AT, seven seeds from HY X BH, and six F_1 seeds were obtained from the reciprocal cross, BH X HY. The F_1 plants were checked to verify cross fertilization using the following marker traits: (1) Two row vs. six row, (2) Hulled vs. naked seed, (3) Rough vs. smooth awn, (4) Black vs. white pericarp. There were no common parents in any of the crosses. Where more than one F_1 plant was grown, the F_2 seeds from the F_1 plants were bulked within the cross. Since there was only one plant from the BH X AT cross, the F_2 seed obtained is of a single plant line. The F_1 plants were treated in the same manner as the other greenhouse grown plants.

Stock S_0 seeds* ---------> Single S_0 plant ----------> S_1 seeds

↓

S_0 plants

↓

Bulked S_1 seeds ----------> Single S_1 plant ----------> S_2 seeds*

↓

S_1 plants

↓

Bulked S_2 seeds ----------> Single S_2 plant ----------> S_3 seeds

↓

S_2 plants

↓

Bulked S_3 seeds ----------> Single S_3 plant ----------> S_4 seeds*

↓

S_3 plants

↓

Bulked S_4 seeds ----------> Single S_4 plant ----------> S_5 seeds

↓

S_4 plants

↓

Bulked S_5 seeds

* - Planted in the field, 1971

Figure 1

Scheme Showing the Development of the S Generations
Used in the Field Experiment

For the field experiment, the S_0, S_2, and S_4 seeds for each of the three cultivars were planted. The S_2 and S_4 seeds were from the single plant lines. Each of the four F_2 populations was also used.

IV. The Field Experiment

Thirteen treatments were set up as follows:

1. AT S_0	6. BH S_4	10. BH X AT F_2
2. AT S_2	7. HY S_0	11. HY X AT F_2
3. AT S_4	8. HY S_2	12. HY X BH F_2
4. BH S_0	9. HY S_4	13. BH X HY F_2
5. BH S_2		

The field design was a randomized complete block, with four blocks, each containing 190 plants, for a total of 760 plants. The seeds were planted (in a nursery) at the Montana State University Horticultural Research Farm at the west end of the Montana State University campus. The soil type is Huffine Silt Loam. In the 1970 growing season the nursery area was not used for research purposes. Peas were grown as a cover and green manure crop. No fertilizer was applied to the nursery during the study. Prior to planting, the seeds from treatments 10-13 were refrigerated at approximately 5°C for three days in an attempt to enhance germination. All the seed planted was treated

with Ceresan M to control covered smut. Eslick (12) has reported that Ceresan M has no appreciable effect on BSMV.

Individual plants (seeds which became plants) of each of the 13 treatments were randomized within each block. The seeds were planted in a 30.5 cm. grid. Each block had 19 rows, with ten spaces per row. The blocks were separated by alleys 1.2 m. wide. On the north, east, and west borders of the nursery, corn was planted as a buffer against the wind. Blocks 1 and 2 were planted May 26, 1972, and blocks 3 and 4 were planted the following day.

A total of 755 seeds was planted. Ten seeds each of treatments 1-9 and 25 seeds each of treatments 10-13 were planted. Due to error, 11 seeds of treatment 6 and only 24 seeds of treatment 11 were planted in block one. An accident during planting caused a loss of seeds of treatment 9 resulting in five plants instead of 10 being planted in block 4. The spaces for the other 5 plants of treatment 9 in block 4 were left empty.

On June 14, all emerged plants were counted and inoculated with the type strain of BSMV. No plants emerged after June 14. The inoculum was prepared by grinding leaf blades from infected AT plants in a mortar with a pestle. Corundum (Al_2O_3) powder and distilled water were added to make the grinding more efficient. Each plant was dusted with Corundum powder prior to inoculation. The inoculum was

rubbed on all leaf blade surfaces using Johnson and Johnson (New Brunswick, N.J.) swabs. At the time of inoculation, the plants were in the 2 to 6 leaf stages of development.

On July 11, the bagging of spikes, to prevent outcrossing was started. Three spikes were bagged on each of the six row barley plants, and four spikes on each of the two row plants. Bagging was continued over the growing season until all plants which headed had the proper number of bags. Some plants were not bagged because they were so severely infected with BSMV that they failed to head.

On July 12 and July 13, all the plants were examined for symptoms of BSM. All plants failing to show symptoms were checked for viral presence using the serological test described later in this section. This test verified viral presence or absence in symptom free plants.

All plants were read individually, twice, for viral induced symptom expression, and the data from the second reading used in this thesis. The first reading was made from July 30 to August 3, the second from August 16 to August 21. On the basis of symptom distribution, the individual plants were scored on a scale of 0 to 3 as follows:

0-The plant did not show symptoms of BSM.

1-Some lower leaves of the plant showed visible symptoms of

BSM, but less than 1/3 of the flag leaves of the plant showed symptoms.

2-Some lower leaves of the plant showed symptoms, and 1/3 to 2/3 of the flag leaves of the plant showed symptoms.

3-Some lower leaves of the plant showed symptoms and more than 2/3 of the flag leaves of the plant showed symptoms, or the infection was so severe, the plant failed to head.

Typical field symptoms of BSM in the field are shown in Figures 3, 4, and 5 (pages). In addition, plant heights were determined by measuring each plant from its crown at ground level to the tip of the awns of its tallest spike. Also, the foliage was read for field symptoms of BSM in terms of degree of chlorosis and necrosis. Any plant showing symptoms of BSM was considered susceptible. To check on whether symptomless field plants were genetically resistant, or represented merely escapes from mechanical inoculation, progeny of 7 symptomless plants were grown in the greenhouse and mechanically inoculated with BSMV. Progeny from plants of treatments 3, 4, 9, and 10 were tested. A sample of 30 progeny from each plant was used. A sample of healthy progeny, from infected plants of these treatments, was also inoculated.

The plot was irrigated weekly to field capacity from the middle of June until the last week in July. On August 5, the entire nursery was sprayed with Thiodan-2 (150 ml. of Thiodan-2 in 57 liters of water) to control aphids. The plants were individually harvested from September 13 to September 16.

V. Assays for Seed Transmission and Embryo Infection

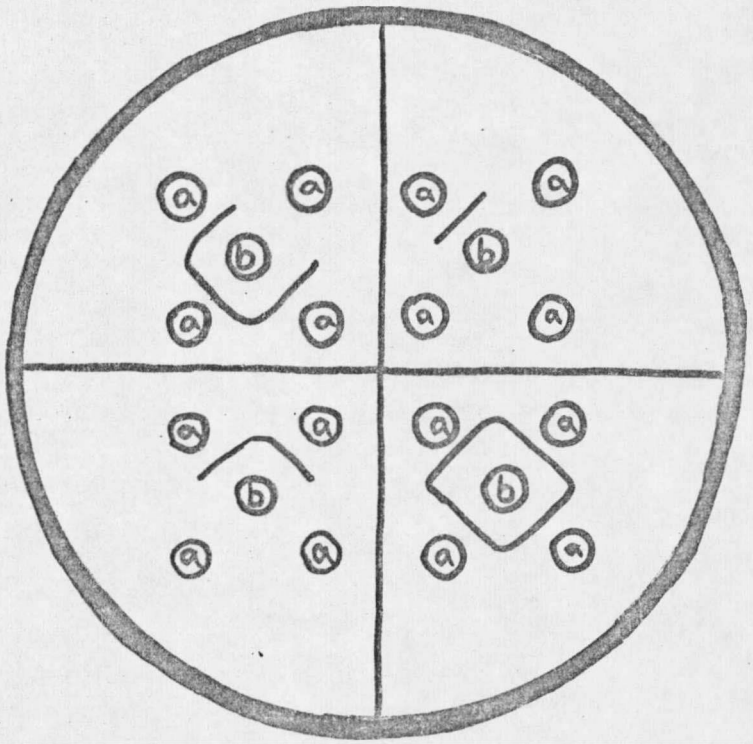
The progeny of plants which were infected with BSMV by mechanical inoculation in the field were assayed for BSMV seed transmission and embryo infection. The progeny of each individual plant were assayed separately. The progeny of the field plants used in the tests were from spikes which had been bagged. The progeny of an S_0, S_2, or S_4 plant represent the S_1, S_3, or S_5 generations respectively. The progeny of the individual F_2 plants represent F_3 families. Two assays were done, the seedling test of Afanasiev (1) which measures seed transmission and the embryo test of Hamilton (16) which detects embryo infection.

The seedling test was done in the greenhouse in metal flats approximately 50 cm. long, 35 cm. wide, and 7.5 cm. deep, filled with steam sterilized soil. Approximately 60 seeds were planted per flat. The flats were usually divided in half, so two samples of 30 seeds each could be tested. Each 30 seed sample was made up of 30

individual progeny from a single field plant. The seeds planted in the flats and the emerged seedlings were read at the 3 leaf stage for symptoms of BSM. As a check for the presence of virus, samples of seedlings which did and did not show symptoms of BSM, were tested for the presence of BSMV using the serological test of Hamilton (15). Progeny sample sizes used in the seedling test were either 30 or 60 seeds. To check the effect of bagging spikes on BSMV seed transmission, samples of unbagged seed were tested using the seedling test. The samples were from plants from which bagged material had previously been tested. Genetic analyses are based on data from bagged material exclusively.

The serological test used was a modification of the Ouchterlony double-diffusion technique devised by Hamilton (16). The serological assay can detect virus in leaf sap and embryos. For the embryo test, a progeny sample of 25 embryos was taken from each field plant selected for analysis. The seeds were soaked in distilled water for 24 hours to allow the palea and lemma to be easily removed. The embryo was dissected from the seed and rinsed in PSB (0.01 M potassium phosphate buffer, pH 7.0 containing 0.85% sodium chloride) and rabbit antiserum to BSMV. The rinse was to remove any virions which might be contaminating the surface of the embryo. The embryo was then placed on a 6 mm. diameter disc of filter paper, and squashed in a press to

release intracellular contents. The press has a capacity of 100 individual embryos. The squashed embryos were then placed in a quadrant petri plate. Each section of the plate contained 1 ml. of medium containing 1% purified agar, 0.5% leonyl sulfonate, and 0.02% sodium azide in PSB. The leonyl sulfonate is a detergent which breaks down the viral antigen into rapidly migrating fractions. It allows the plates to be read in 24 hours. The sodium azide is a preservative which prevents contamination of the plates by bacteria or fungi. The discs on which the embryos were placed were arranged at the corners of a square and a disc containing antiserum to the virus was placed in the center of the square. The discs are placed so their centers were 12 mm. apart. Known concentrations of virus antigen, normal rabbit serum, and healthy embryos were used as a control to check each batch of media, and to make certain that positive reactions would be obtained if all the samples to be tested were negative. If the embryo contained BSMV in a concentration of 0.008 mg./ml. or higher, a precipitation line formed in the zone of equivalence between the discs. In order to detect the presence of BSMV in leaf sap, a disc saturated with the sap is placed in the plate where the disc containing an embryo would have gone. (A diagram of a serological plate can be found in Figure 2, page 23).



a - A disc containing leaf sap or a squashed embryo

b - A disc containing antiserum to BSMV

The precipitation lines between a and b indicate the presence of BSMV in a.

The distance from the center of a to the center of b is 12 mm.

Figure 2

A Diagram of a Serological (Ouchterlony Double-Diffusion) Plate

The antiserum to the virus was crude, undiluted rabbit serum. It was prepared by giving intramuscular injections of 2 mg. of BSMV weekly to a rabbit until its sera could detect BSMV at a concentration of 0.030 mg./ml. in the double-diffusion plate. The BSMV injected was partially purified virus in a solution of citrate buffer (0.1 M citric acid-sodium citrate buffer, pH 6.1). Prior to injection, 1 ml. of BSMV solution was mixed with 1 ml. Freund's incomplete adjuvant.

The inject antigen was prepared following a scheme developed by Shepard (29). Infected Atlas barley leaves were harvested and cut into short pieces. The leaves were macerated in a solution of 0.5 M sodium borate-boric acid buffer, pH 8.2 (1 gm. leaf tissue to 1 ml. buffer). The leaf macerate was filtered through cheesecloth to remove large debris. The filtered leaf extract was then clarified by adding cold chloroform (1 part chloroform to one part extract) and shaking for at least two minutes. The mixture was then centrifuged at low speed (for ten minutes at 12,000 g.). The aqueous phase was collected and centrifuged at high speed (for 90 minutes at 78,000 g.). The supernate was discarded and the pellets were resuspended in a 4% sodium chloride solution (2.5 ml. solution per pellet). All the pellets were combined and stirred until they went into solution. The mixture was then centrifuged at low speed. Polyethylene glycol (M. W. 6000) was then added to the supernate to a final concentration of 7 gm./ml. The

polyethylene glycol was stirred in for 20 minutes in the refrigerator, using a mechanical stirrer. The mixture was centrifuged at low speed. The supernate was discarded and each pellet was resuspended in 10 ml. of Tris buffer (0.05 M Tris-HCL, pH 6.5). The pellets were combined, and the mixture was stirred in the refrigerator for 60 minutes. The mixture was then centrifuged at low speed. The supernate was collected and combined with the previously collected supernatant fractions. The supernate was centrifuged at high speed. The supernate was discarded, and the pellets were now resuspended, each, in 1.5 ml. of citrate buffer. The mixtures were combined and centrifuged at low speed. The supernate was collected and centrifuged at high speed. The partially purified virus pellet was then resuspended in a small volume (about 5 ml.) of citrate buffer and the amount of BSMV determined. For the low speed centrifugations, a Sorvall RC 2-B centrifuge was used. For the high centrifugations, a Beckman Model L ultracentrifuge was used.

The amount of BSMV was determined using a spectophotometer. Two optical density readings were taken. A reading at 260 nm. is used to measure the nucleic acid component of BSMV. A reading at 320 nm. is used to measure light scattering by the virions. The 320 nm. reading is subtracted from the 260 reading and the result is used to calculate the amount of BSMV present. Until it was needed for injection, the purified BSMV was stored in a freezer.

VI. Statistical Methods Used to Analyze the Data

All statistical methods follow Snedecor and Cochran (33) except for Duncan's New Multiple Range Test (NMRT). The NMRT follows Steel and Torrie (34).

The basic field design of the experiment allows the use of the randomized complete block (RCB) analysis of variance. The RCB analysis was used to analyze emergence, susceptibility and symptom expression score data. Analyses of the results of the seedling test and the embryo test follow a completely random (CR) design.

The analysis of variance is basically used to test the hypothesis that all treatment means came from the same population. When the RCB design is used, the hypothesis that all block means come from the same population can also be tested. An F test is used to test these hypotheses.

If the calculated F value is significant, the only information obtained is that at least two of the means differ. When the RCB design was used, the NMRT can be used to test the equality of all means. In all analyses of variance and mean separations, p .05 is used as the level of statistical significance. The Least Significant Difference (LSD) was selected to compare means when the CR design was used because of unequal sample sizes among the 13 treatments.

The purpose of the symptom expression score-measurements were (1) to classify the plants phenotypically, and (2) to determine if the

level of seed transmission could be predicted from symptoms. All possible correlations between the embryo test, the seedling test, and symptom expression score readings were made. All correlations were done for each treatment and for all plants, where individual plant data were available. Each correlation coefficient (r) was tested to determine if it was significantly different from zero. The coefficient of determination (r^2) was calculated to determine how much of the variation in the dependent variable is due to difference in the independent variable.

In order to test the hypothesis that the patterns of seed transmission and embryo infection were the same for all plants, Heterogeneity Chi-square techniques were used. Heterogeneity Chi-square tests the hypothesis that all individual samples (single plant progeny) fit a common ratio. If the calculated Chi-square value is significant, the progeny of all plants do not fit the same ratio; they are heterogeneous. Significant heterogeneity in individual F_2 populations reflects genetic variation for the amount of seed transmission or the amount of embryo infection.

RESULTS AND DISCUSSION

Field grown plants were randomized to meet, in part, the assumptions of the analysis of variance concerning random error. The virus stock is assumed to be a stable, uniform strain. Thus, significant differences among treatment means then must reflect, in part, genetic differences among the treatments.

The emergence data for the field experiment are presented in Table 1 (page 29). The analysis of variance on untransformed percentages indicates that at least two of the treatment means differ from each other. The mean of BH S_0 differs from all other treatment means. Only the seeds of the S_0 generations were machine thrashed, and only the BH seeds are naked. The most probable reason for the low level of emergence of the BH S_0 is not genetic variation but is damage to the naked seeds of BH S_0 in thrashing.

The susceptibility data of the emerged plants to the type strain of BSMV are summarized in Table 2 (page 30). A plant susceptible to BSMV in the field showed symptoms of BSM on its foliage. Serological tests on leaf tissue from symptomless field plants indicated that the type strain of BSMV did not cause latency of symptoms. All plants which did not show symptoms of BSM did not contain virus as determined by serology.

The analysis of variance on untransformed percentages for susceptibility indicates that at least two of the treatment means are

Table 1

Emergence of Barley Plants in the Field Experiment
Summer 1971

Cultivar or Cross	Generation	No. of Seed Planted	No. of Plants Emerged	Percent Emerged
AT	S_0	40	37	92.50 A[1]
AT	S_2	40	38	95.00 A
AT	S_4	40	39	97.50 A
BH	S_0	40	16	40.00 B
BH	S_2	40	39	97.50 A
BH	S_4	41	41	100.00 A
HY	S_0	40	39	97.50 A
HY	S_2	40	36	90.00 A
HY	S_4	35	33	94.29 A
BH X AT	F_2	100	97	97.00 A
HY X AT	F_2	99	98	98.99 A
HY X BH	F_2	100	97	97.00 A
BH X HY	F_2	100	99	99.00 A
Total		755	709	93.91

[1] Means followed by the same letter are not significantly different from each other at the 5% level by Duncan's NMRT.

Table 2

Susceptibility of Barley Plants
to BSMV in the Field - 1971

Cultivar or Cross	Generation	Plants Showing Symptoms	Plants Not Showing Symptoms	Mean Percent Showing Symptoms	
AT	S_0	35	2	94.44	ABC[1]
AT	S_2	33	5	86.66	ABCD
AT	S_4	34	5	87.50	ABCD
BH	S_0	15	1	96.42	AB
BH	S_2	39	0	100.00	A
BH	S_4	40	1	97.72	A
HY	S_0	35	4	89.72	ABCD
HY	S_2	32	4	88.54	ABCD
HY	S_4	27	6	78.89	D
BH X AT	F_2	81	16	83.45	BCD
HY X AT	F_2	92	6	93.82	ABC
HY X BH	F_2	80	17	82.31	CD
BH X HY	F_2	93	6	94.00	ABC
Total		636	73	89.70	

[1] Means followed by the same letter are not significantly different from each other at the 5% level by Duncan's NMRT.

significantly different from each other. Duncan's NMRT indicates that within each cultivar the means are the same.

The genetic interpretation of this is, since single plant lines did not differ from the stock population, the plants of a cultivar were uniform genetically with respect to susceptibility to BSMV within each cultivar.

The HY S_4 treatment mean differs significantly from the AT S_0 and the three BH treatment means. The differences reflect true genetic differences between the HY S_4 single plant line and BH, and the HY S_4 and AT S_0. Since HY generations do not differ, it is suggested that the HY S_4 differences reflect the selection of a plant line which differs significantly from AT S_0 and all BH generations.

An examination of the hybrid treatments is difficult since the parental lines were not included in the field study. Since there are no significant differences within the cultivars for susceptibility, the best estimator of the parental mean should be the cultivar mean. The cultivar means for susceptibility are: $\overline{AT}$ = 89.53 (ABCD), $\overline{BH}$ = 98.05 (A), $\overline{HY}$ = 85.72 (BCD). The letter or letters following the cultivar means indicate mean groupings following Duncan's NMRT, p .05. The BH X AT F_2 treatment mean is not significantly different from $\overline{AT}$, but is significantly lower than $\overline{BH}$, and $\overline{AT}$ and $\overline{BH}$ are not significantly different from each other. The difference between $\overline{BH}$ and BH X AT F_2

may be explained in terms of two near homozygous, but different genotypes ($\overline{BH}$ and $\overline{AT}$) having the same phenotype, but which in the F_2 yield segregants which are more resistant to BSMV than $\overline{BH}$. The HY X AT F_2 treatment mean is the same as $\overline{AT}$ and $\overline{HY}$. It would seem that susceptibility to the virus is determined by the same genes in both parents.

The HY X BH F_2 and BH X HY F_2 means are not significantly different. $\overline{HY}$ is not significantly different from HY X BH F_2 and BH X HY F_2, but $\overline{BH}$ is significantly more susceptible than HY X BH F_2. This could be due to a maternal effect, or might be due to genetic differences in the parents since the parents were different for each cross.

When progeny of symptomless field plants were checked for susceptibility to viral infection due to mechanical inoculation, they were highly susceptible. When healthy progeny of infected field plants were checked for susceptibility to viral infection due to mechanical inoculation, the progeny were highly susceptible. The non-random pattern of symptomless field plants reflects genetic differences among the treatments. Therefore, the progeny check results lead to the conclusion that susceptibility to viral infection due to mechanical inoculation with BSMV is probably controlled by a complex genetic mechanism.

The results of the symptom expression score (SES) readings are presented in Table 3 (page 33). Generation means included symptomless

Table 3

Symptom Expression Score Means
of Emerged Field Plants - 1971

Cultivar or Cross	Generation	Number of Plants Read	Mean Score
AT	S_0	37	1.95 CD[1]
AT	S_2	38	2.23 BC
AT	S_4	39	2.45 AB
BH	S_0	16	2.60 A
BH	S_2	39	2.49 AB
BH	S_4	41	2.69 A
HY	S_0	39	1.76 DE
HY	S_2	36	1.73 DE
HY	S_4	33	1.51 EF
BH X AT	F_2	97	1.88 D
HY X AT	F_2	98	1.87 D
HY X BH	F_2	97	1.40 F
BH X HY	F_2	99	1.89 D

[1] Means followed by the same letter are not significantly different from each other by Duncan's NMRT, at the 5% level.

plants (0 score), because not all of these plants were verified as escaping infection by mechanical inoculation or being genetically resistant. The conclusions from the experiment do not change if the zeros are excluded. The reasons for measuring the SES values were to determine the association between the SES and seed-transmissibility, and to examine the treatments to see if the pattern of SES varied among them. If a strong association existed between SES and seed-transmissibility, BSMV could be eliminated by roguing those plants with a high SES.

With the type strain of BSMV, all leaves showing symptoms do contain serologically detectable virus. Carroll (7) has shown that the type strain of BSMV is seed transmitted in AT barley because the virus readily moves from the vegetative to the reproductive structures of the plant, although the lower foliage of AT plants infected with the NSP (non-seed-passage) strain exhibit chronic symptoms of BSM. The blade of the flag leaf is the closest part of the barley plant to the reproductive structures where visible chronic symptoms of BSM can be easily seen. The type strain of BSMV is capable of inducing symptoms of BSM in all three cultivars and the hybrids. However, the extent to which the symptoms were distributed over all the plants varied. Some plants had leaf symptoms only on their lower foliage; some had symptoms only on one half of their flag leaves and lower leaves; and others had symptoms on all the leaves, in continuum. Since, for the

type strain, symptoms indicate the presence of virus, and invasion of the reproductive structures is necessary for seed transmission, the presence or absence of symptoms on the flag leaf blades was chosen as the main criterion for the SES. The readings for degree of chlorosis and necrosis were not used because of the great difficulty involved in the analysis of these symptoms, due to the complex distribution of the symptoms within and among plants. The plant heights were not analyzed because a sufficient number of healthy plants was not available for a control.

The type of symptoms induced by the type strain of BSMV did not differ among the treatments. Color photographs illustrating the syymptoms of the type strain of BSMV in the field, with descriptions are presented on pages 59-61 of the Appendix.

The analysis of variance of the SES data indicates that at least two of the treatment means differ significantly from each other. The analysis of variance also indicates that at least two of the block means differ significantly from each other. There is no apparent explanation for the block effect, but it has been removed from error and does not affect the differences in treatment means.

The AT S_0 and AT S_4 treatment means are significantly different. Genetically this would indicate that chance selection had occurred for a higher SES in developing the single plant lines, and that

AT S_0 was genetically variable for SES. The treatment means of HY and BH are the same within the cultivars; no apparent selection has taken place for SES level in developing the single plant lines. All the BH treatment means are significantly higher than all the HY treatment means. Significant differences also exist between AT means and means of the other two cultivars, but not for all means. Genetic differences exist between BH and HY, and selection from stock AT S_0 which has been shown to be heterogeneous might allow the development of lines similar to either HY or BH.

Because of the genetic variation within AT S_0, and selection in developing the generations studied, the mean of the three AT treatments probably does not reflect the true mean of AT. AT S_0 is assumed to better represent the unselected mean of all AT, and the AT S_0 mean will be used to represent the value of the AT parents used to make the hybrids. Since the variation within HY and BH generations is not significant, the mean of the three S generations will be used to represent the value of the parents used to make the hybrids. The parental means and letters representing the mean groupings for the NMRT are: $\overline{AT}$ - 1.95 (CD), $\overline{BH}$ = 2.59 (AB), $\overline{HY}$ = 1.67 (DEF). The mean of BH X AT F_2 is not significantly different from $\overline{AT}$ but is significantly less than $\overline{BH}$. The genetic interpretation of this is that a lower level of SES is dominant and was inherited from the $\overline{AT}$ parent. The mean of

HY X AT F_2 is not significantly different from $\overline{AT}$ or $\overline{HY}$, suggesting that the parents were probably similar genetically. A significant reciprocal difference exists between HY X BH F_2 and BH X HY F_2. $\overline{HY}$ is not significantly different than either HY X BH F_2 or BH X HY F_2, but $\overline{BH}$ is significantly higher than both of them. The dominance of $\overline{HY}$ to $\overline{BH}$ is similar to the dominance of $\overline{AT}$ to $\overline{BH}$, supporting the hypothesis that $\overline{AT}$ and $\overline{HY}$ are similar genetically. $\overline{BH}$ is closer to the mean of BH X HY F_2 and it appears that the difference in the hybrids could be due to a maternal effect, or to genetic differences in the parents.

The results of the seedling test and the embryo test are summarized in Table 4 (page 38). The tests were performed on the progeny of randomly selected field plants. The field plants whose progeny were tested were known to have been infected by mechanical inoculation. The analysis of variance for seed transmission based on untransformed percentages indicates that none of the treatment means differ from each other. The analysis for embryo infection indicates that at least two of the treatment means differ significantly from each other. The LSD indicates that the BH X HY F_2 mean differs from all the other means. This should be due to the genetic differences, and a significant reciprocal difference is present and is similar to the reciprocal difference present in the susceptibility and SES data. There is a twofold difference between HY X BH F_2 and BH X HY F_2 with respect to the percent of

Table 4

A Summary of Seed Transmission and Embryo Infection of the Sample of Field Plants Whose Progeny Were Assayed for These Properties

Cultivar or Cross	Generation	No. of Field Plants Assayed	Percent Seed Transmission	Percent Embryo Infection
AT	S_0	20	2.11	2.40
AT	S_2	10	4.21	3.60
AT	S_4	10	2.32	0.40
BH	S_0	5	1.43	2.40
BH	S_2	10	1.43	2.40
BH	S_4	10	2.85	2.00
HY	S_0	10	1.44	0.80
HY	S_2	10	0.67	2.00
HY	S_4	10	2.44	1.60
BH X AT	F_2	30	0.72	3.60
HY X AT	F_2	30	2.18	2.80
HY X BH	F_2	30	1.21	2.80
BH X HY	F_2	30	2.60	8.24 *

* - Significantly different at the 5% level by the LSD

seed transmission, but the difference is not statistically significant. Both the seed transmission and embryo infection data were based on the progeny of individual field plants, but individual plant data were pooled within treatments for the analysis of variance. The individual plant data are presented in Appendix Table 1 (pages 51-58).

Because only seeds from bagged spikes were used in the assays of seed-transmissibility, samples were taken of unbagged seed to use as controls to check the effect of the bag. Bagged and unbagged seed samples were taken from the same plants and assayed using the seedling test. Ten of 381 seeds (2.63%) from bagged spikes were infected, while 15 of 356 (4.22%) from unbagged spikes were infected. The means of these two samples do not differ significantly, implying that the bagging of spikes had no effect on the amount of seed transmission.

When all the assays for seed transmission were pooled, 1.99% of the emerged progeny seedlings were infected, while 3.31% of the embryos tested contained detectable virus. These percentages are extremely low when compared to the 40% seed transmission of the type strain in AT in the greenhouse reported by Carroll (8), but the difference between the overall mean level of seed transmission and the overall mean level of embryo infection is statistically significant. The low transmission of the type strain in the field may be explained by the temperature data for the 1971 growing season in Bozeman (3),

summarized in Table 5 (page 41). Singh, Arny, and Pound (30) reported that at 16°C, the "Manchurian" isolate of BSMV was seed transmitted at a maximum of 3%. The field temperatures were highly variable in Bozeman and the mean temperature for the growing season was 17.7°C, which probably was the cause of the low seed transmission.

Since the individual samples for the assays were drawn at random from the same pool for each of the two tests, about 40% of the seeds with infected embryos (based on serology) failed to give rise to infected seedlings. Hamilton (16) reported about a 1:1 ration between infected embryos and infected seedlings. Inouye (22) reported that 87% of infected embryos gave rise to infected seedlings. At least two possibilities exist to explain the failure of the virus to be transmitted from infected embryos. Either some mechanism in the germinating embryo can inactivate the virus, or the amount of virus present in the embryo was insufficient for the passage of the virus to the seedling. The present study did not allow separation of these alternatives.

In order to pool the results of the seedling test with those of the embryo test, a high correlation between the two tests was required. Table 6 (page 42) presents correlation coefficients based on the correlation of the individual plant data for seedling and embryo tests. The correlation of the individual plant data is presented for each treatment and for all plants over treatments. The overall

Table 5

The Mean Centigrade Temperatures
For the Growing Season in
Bozeman, Montana 1971

Month	Mean High Temperature	Mean August Temperature	Mean Low Temperature
June	22.0	14.4	6.8
July	26.8	18.4	9.9
August	30.7	21.6	12.5
September[1]	24.1	14.9	5.6

[1] Through September 15, 1971

Table 6

Correlation Coefficients Comparing the Seedling Test (ST), the Embryo Test (ET), and the Symptom Expression Score (SES) Results

Cultivar or Cross	Generation	ST and ET	ST and SES	ET and SES
AT	S_0	.54 *	.71 *	.35
AT	S_2	.69 *	.41	.41
AT	S_4	.10	.00	.00
BH	S_0	.88 *	.99 *	.92 *
BH	S_2	.90 *	.32	.55
BH	S_4	.22	.04	.15
HY	S_0	.61	.21	.58
HY	S_2	.97 *	.53	.55
HY	S_4	.59	.22	.11
BH X AT	F_2	.16	.28	.26
HY X AT	F_2	.49 *	.24	.17
HY X BH	F_2	.27	.44 *	.04
BH X HY	F_2	.45 *	.42 *	.43 *
All Plants		.43 *	.34 *	.22 *

* - Significant at the 5% level

correlation coefficient is .43, and the coefficient of determination (r^2) is .18, indicating that probably two distinct genetic systems, one controlling embryo infection, the other controlling seed transmission are operative, or that sampling error has given rise to the low r value.

Another non-genetical explanation might be the sensitivity of the sera used to detect the infected embryos. The antisera used could detect partially purified virus down to the concentration of 0.008 mg./ml. It is suspected that Hamilton's antisera could only detect partially purified virus down to a concentration of 0.030 mg./ml. These alternatives might be resolved by studies with an isolate of BSMV which gives fairly high (above 50%) seed transmission in the field.

In the S generations the correlations tend to become lower through selfing. The variation observed is probably due to random genetic drift. Thus, if both genetic systems were heterogeneous in the S_0 population, they may have been highly correlated by chance. Since heterozygosity decreases 50% each generation with selfing, the increasing homozygosity could have reduced the correlation especially if many are genes are involved. In the hybrid populations the variation introduced by the crosses has caused the low r values.

One of the reasons for measuring the SES values was to find out if a relationship existed between SES and seed-transmissibility. Since the r value comparing the results of the seedling and embryo test results for all plants was too low to permit pooling the data, the result of each test and the SES result was correlated using individual plant values and presented in the same way as the correlations coefficients for the embryo and seedling test. The r values are presented in Table 6 (page 42). Because of the low r values obtained for the correlations between the two tests and the SES for all plants, the conclusion is that SES is not controlled by the same genetic system as seed transmission or embryo infection and the SES value can not be used to predict seed-transmissibility in the material studied. McKinney and Greeley (26) have reported that viral strains which cause the severest infections often are not seed transmitted at a high level. The r values for the S generations show a similar random drift pattern as those for the correlation of embryo infection and seed transmission, supporting the hypothesis of the separate genetic systems. The difference in the r values obtained for SES and embryo infection in the reciprocal cross most likely reflect genetic differences between the BH or HY parents for embryo infection, or a maternal effect.

The pattern of variation in the hybrids between all the cultivars is similar for susceptibility and SES. The r value obtained when

the treatment means of the S generations for susceptibility and SES were correlated was .71. Because of the variation in the AT generations for SES, the r value was recalculated using only the BH and HY treatment means. The r value increased to .91. This high correlation indicates that the same gene or set of genes control susceptibility and SES, and the patterns in the hybrids would tend to confirm this.

In most of the research on the seed-transmissibility of BSMV, only an analysis of pooled data is presented (7,10,13,16,19,30,35). Information based on single plant analysis is not presented. To analyze the single plant data, the Heterogeneity Chi-square test was used to look for variation within treatments. The results of the Heterogeneity Chi-square analysis are presented in Table 7 (page 46).

Significant heterogeneity within a treatment should be due to genetic differences among individual field plants and reflected in progeny. For seed transmission, the AT S_0 and S_2 populations are significantly heterogeneous, but the AT S_4 is not. A possible explanation for the homogeneity of the AT S_4 is that it was produced by four generations of selfing and by the use of a single plant line. If non-linked genes are involved, selfing decreases heterozygosity by 50% each generation. The BH S_0 population is not significantly heterogeneous, but the sample contained only five plants (all BH S_0 plants with enough progeny to analyze). The possibility exists that the

Table 7

Heterogeneity Chi-square Values and Goodness of Fit Probabilities for the Amount of Seed Transmission and Embryo Infection of the Progeny of Individual Infected Field Plants

Cultivar or Cross	Generation	Seed Transmission		Embryo Infection	
		X^2 value	Probability[1]	X^2 value	Probability[1]
AT	S_0	36.78	.010 - .005	25.30	.250 - .100
AT	S_2	25.54	<.005	24.09	<.005
AT	S_4	11.04	.500 - .250	9.23	.500 - .250
BH	S_0	3.27	.750 - .500	5.47	.250 - .100
BH	S_2	22.19	.025 - .010	14.36	.250 - .100
BH	S_4	19.65	.025 - .010	9.18	.500 - .250
HY	S_0	16.59	.100 - .050	18.23	.050 - .025
HY	S_2	19.90	.025 - .010	29.59	<.005
HY	S_4	8.87	.500 - .250	11.21	.500 - .250
BH X AT	F_2	34.44	.250 - .100	49.22	.025 - .010
HY X AT	F_2	67.94	<.005	65.15	<.005
HY X BH	F_2	68.59	<.005	41.59	.100 - .050
BH X HY	F_2	148.81	<.005	90.70	<.005

[1] The Chi-square value is significant if the probability is less than .050

BH S_2 and BH S_4 populations, which are significantly heterogeneous, may have developed from genotypically heterozygous, unrelated S_0 plants. These explanations can account for the heterogeneity or homogeneity of the S generations for seed transmission or embryo infection. The heterogeneity in the hybrid population reflects genetic differences between the parents. In the crosses where no significant heterogeneity occurred, it can be assumed that no genetic differences existed between the parents for the trait. In the cases of the BH X AT F_2 and HY X BH F_2 populations where one of the two traits is homogeneous, and the other heterogeneous, the conclusion may be made that two different genetic systems are involved.

The results of both the seedling test and the embryo test contain individual plants with higher embryo infection and seed transmission than those in the other treatments. This is probably due to some form of heterosis.

The possible maternal effect from BH is worthy of a speculative attempt at an explanation. Some plastid functions are known to be regulated by nuclear genes. Imai (20) has presented genetic evidence that a gene producing albino plastids is only maternally inherited in barley. Carroll (5) has observed BSMV virions closely associated with plastids in barley cells. Pring and Timian (27) have demonstrated that BSMV reduces the amount of chlorophyll in the cells. Since

chlorophyll is in the plastids, the virus associates with the plastids, and some 'genes' controlling plastid characters are maternally inherited, it is not unreasonable to assume that BH plastids are more susceptible to BSMV than those of other barleys, making it a hypersensitive host. Inouye (19) has demonstrated in reciprocal crosses that higher seed transmission is obtained when the maternal plant is infected. The higher seed transmission may have been due to the association of the virus with plastids. The BH X HY F_2 population had significantly higher embryo infection than any other treatment, possibly due in part to a maternal effect.

This research sought the answers to two questions: (1) Did genetic variation exist for the four economic characters studied? (2) Did individual plants of the same cultivar type respond uniformly to BSMV?

Genetic variation was found for the four economic characters studied. Lower susceptibility and lower symptom expression scores appear to be dominant. Individual plant variation within a cultivar was found for symptom expression score, seed transmission, and embryo infection. Although treated as a constant, the environment for the growing season had the effect of lowering the seed transmission and embryo infection of BSMV from what had been expected.

Much work is to be done if the genetic variation reported here is to be fully explained. More suitable systems must be found so that Mendelian separations can be readily observed, but from the data the possibility exists for the development of barley in which BSMV would not be seed transmitted.

An effective method to further this research would be to find a stable BSMV strain or isolate which gives high (greater than 50%) seed transmission in the field in one cultivar and low seed transmission in another. The plants should be screened to eliminate heterogeneity for the studied characters and to determine accurately parental genotypes for crossing.

APPENDIX

Appendix Table 1

Results of the Seedling Assay and Embryo Test on a Sample of the Progeny of Individual Infected Field Plants and the Symptom Expression Score of the Field Plants Tested

Field Plant Number	Progeny Seed Planted	Progeny Seedlings Emerged	Progeny Seedlings Infected	Progeny Embryos Infected	Symptom Expression Score
		Treatment 1 - AT S_0			
5- 5-3	60	47	3	1	2
5-17-2	60	48	2	0	2
7- 6-1	60	43	0	0	2
6- 9-1	60	42	0	0	1
3-11-2	60	41	0	0	2
9-10-2	60	45	0	0	1
6- 6-1	60	36	2	1	3
9- 6-1	61	59	0	0	1
7-19-1	60	47	5	2	3
9- 7-2	60	23	2	1	3
2- 4-2	31	31	0	0	2
10-11-2	30	27	0	2	1
2- 5-2	30	29	0	0	2
10- 6-2	30	28	0	0	2
3-17-1	30	28	0	0	2
7-17-1	30	29	0	0	1
9- 4-2	30	29	1	3	2
5-18-4	30	27	0	0	1
3-11-4	30	30	1	1	3
3-13-4	30	27	0	1	2

Appendix Table 1 (continued)

Field Plant Number	Progeny Seed Planted	Progeny Seedlings Emerged	Progeny Seedlings Infected	Progeny Embryos Infected	Symptom Expression Score
		Treatment 2 - AT S_2			
1- 5-1	30	21	0	0	3
1- 4-1	30	27	0	1	3
3-19-1	30	26	3	0	3
1- 3-2	30	14	0	0	2
9- 9-1	30	24	0	0	2
9- 2-2	30	28	0	1	3
4- 9-1	30	27	5	5	3
10-10-2	30	25	3	1	3
1-12-2	30	22	0	0	2
2- 1-2	30	24	0	1	3
		Treatment 3 - AT S_4			
9-18-2	31	31	1	1	3
6-19-1	30	28	0	0	3
6- 2-2	30	30	3	0	3
1-10-2	30	28	0	0	3
2- 3-1	30	29	0	0	3
3-15-1	31	31	1	0	3
6-11-1	30	27	0	0	3
9-16-1	30	25	0	0	3
10- 3-2	30	29	1	0	3
9-16-2	30	30	1	0	3

Appendix Table 1 (continued)

Field Plant Number	Progeny Seed Planted	Progeny Seedlings Emerged	Progeny Seedlings Infected	Progeny Embryos Infected	Symptom Expression Score
		Treatment 4 - BH S_0			
2- 9-3	30	30	0	0	2
5-12-2	30	30	1	2	3
7- 5-1	30	29	0	0	2
10- 9-3	30	30	0	0	2
6-10-1	30	26	1	1	3
		Treatment 5 - BH S_2			
9- 2-1	30	26	0	0	1
7-13-1	30	30	0	1	3
4- 5-2	30	29	0	0	2
5- 2-2	30	30	0	1	3
9- 9-2	30	30	0	0	2
7-10-1	30	30	0	0	1
9-10-1	30	29	0	0	2
3-12-1	30	28	3	3	3
6- 8-2	30	28	1	1	2
4-17-1	30	29	0	0	3
		Treatment 6 - BH S_4			
8-16-1	30	30	0	2	3
3-10-1	30	29	2	1	3
4- 4-1	30	30	0	1	2
2- 1-1	30	25	3	1	2
6- 3-2	30	28	0	0	2
7- 8-2	30	28	0	0	2
3-11-1	30	30	0	0	3
2-11-1	30	29	0	0	3
8- 6-2	30	30	0	0	2
8- 3-1	30	29	2	0	3

Appendix Table 1 (continued)

Field Plant Number	Progeny Seed Planted	Progeny Seedlings Emerged	Progeny Seedlings Infected	Progeny Embryos Infected	Symptom Expression Score
		Treatment 7 - HY S_0			
2-15-2	30	30	2	2	3
4- 5-1	30	30	0	0	1
2-10-2	30	28	0	0	1
3- 1-2	30	30	0	0	3
8-14-1	30	27	0	0	2
4- 7-1	30	30	0	0	2
3-18-2	30	28	0	0	1
7- 3-3	30	26	2	0	1
8- 9-1	30	27	0	0	1
4-10-1	30	27	0	0	1
		Treatment 8 - HY S_2			
7- 1-1	30	28	0	0	2
2- 9-2	30	30	2	4	3
6-14-2	30	30	0	0	1
8- 6-1	30	30	0	0	3
7- 3-1	30	29	0	0	2
3- 8-2	30	28	0	0	1
4-11-1	30	29	0	0	1
6- 3-1	30	30	0	0	2
7-16-1	30	30	0	1	2
5- 3-1	30	30	0	0	1
		Treatment 9 - HY S_4			
1-15-2	30	30	2	2	2
2- 5-1	30	29	0	0	2
2-15-1	30	30	0	0	1
1-13-4	30	29	1	0	2
3-16-2	30	29	0	1	2
8-15-1	30	29	0	0	2
2-11-2	30	25	1	0	3
9- 4-1	30	30	1	0	1
2-19-2	30	29	2	1	2
5-11-1	30	29	0	0	2

Appendix Table 1 (continued)

Field Plant Number	Progeny Seed Planted	Progeny Seedlings Emerged	Progeny Seedlings Infected	Progeny Embryos Infected	Symptom Expression Score
		Treatment 10 - BH X F_2			
4- 6-1	30	19	1	0	3
8-11-1	30	29	0	2	3
5- 7-2	30	28	0	0	1
1- 2-2	30	30	0	0	1
10-14-1	30	29	0	0	3
8-18-2	30	29	2	3	3
5-17-1	30	28	0	2	2
6- 5-2	30	30	0	0	2
8- 5-2	31	31	2	0	2
4-15-1	31	31	0	0	2
5- 9-1	30	30	0	0	2
8-17-1	30	29	1	2	3
4-13-2	30	30	0	0	2
9- 8-1	30	28	0	4	2
9-15-2	30	21	0	0	1
9-17-1	30	28	0	0	1
6- 7-2	30	20	0	1	2
10- 1-2	30	26	1	3	3
7-12-2	30	27	1	0	2
8-13-1	30	14	0	0	3
10- 8-2	30	29	1	3	1
1-14-2	30	28	2	0	2
3-12-2	30	30	0	2	3
8- 5-1	30	28	0	1	3
9- 1-2	30	30	0	2	2
10- 8-1	32	32	2	1	3
1-15-1	30	29	0	1	2
8- 8-2	30	30	0	0	2
8- 2-1	30	30	0	0	2
9-13-1	30	30	0	0	2

Appendix Table 1 (continued)

Field Plant Number	Progeny Seed Planted	Progeny Seedlings Emerged	Progeny Seedlings Infected	Progeny Embryos Infected	Symptom Expression Score
		Treatment 11 - HY X AT F_2			
1-16-1	30	30	0	0	2
1-18-1	30	30	0	0	2
1- 6-2	30	29	0	0	1
9-13-2	30	27	1	0	2
4- 3-1	30	30	1	0	1
1-10-1	30	30	0	0	2
5-12-1	30	30	0	0	2
1- 8-1	30	30	0	1	2
6-16-1	30	28	1	1	1
10- 9-1	30	29	0	0	3
8- 3-2	30	30	0	0	1
3-13-1	30	28	0	0	1
4- 2-2	30	30	0	0	2
9-15-1	30	30	0	0	2
7-19-2	30	30	0	1	3
8-11-2	30	30	0	1	2
2- 7-1	30	30	0	0	3
2-14-2	30	29	0	1	1
1- 7-1	30	30	0	0	2
10-18-1	30	29	0	1	1
3-17-2	30	28	4	5	3
3-10-2	30	30	0	0	1
5- 3-2	30	29	0	1	2
9-17-2	31	31	2	3	3
5-15-2	32	32	1	0	2
6-18-1	30	29	0	0	1
6-15-1	30	30	2	0	3
6-13-2	30	30	0	4	1
2-19-1	30	29	3	1	1
8- 1-2	30	29	4	1	2

Appendix Table 1 (continued)

Field Plant Number	Progeny Seed Planted	Progeny Seedlings Emerged	Progeny Seedlings Infected	Progeny Embryos Infected	Symptom Expression Score
		Treatment 12 - HY X BH F_2			
5- 4-2	30	30	1	2	1
5- 6-1	30	28	1	0	3
1- 4-2	30	30	0	3	2
5-11-2	30	28	0	0	2
3- 3-2	30	30	0	1	2
2-17-1	30	30	0	0	2
2- 8-2	30	30	0	0	2
7- 4-1	30	28	0	2	3
5-10-1	30	26	0	0	1
6- 7-1	30	30	0	0	2
5- 9-2	30	25	0	0	1
9-12-1	30	30	0	0	2
1-13-1	30	29	0	1	2
5-16-1	30	29	1	2	3
8- 4-1	30	28	0	0	2
7-15-1	30	30	0	2	1
3- 5-2	30	30	0	0	1
1- 8-2	30	29	1	1	3
6-18-2	30	28	2	0	2
5-16-2	30	26	4	2	3
2-12-2	30	29	0	1	1
10- 5-1	30	29	0	0	1
1-19-2	30	30	0	0	2
4-14-1	30	30	0	0	2
10-12-1	30	29	0	1	2
5-14-1	30	30	0	0	1
1-16-2	30	30	0	0	2
7- 9-1	30	27	0	0	2
10-10-1	30	30	0	3	1
5-19-2	30	30	0	0	2

Appendix Table 1 (continued)

Field Plant Number	Progeny Seed Planted	Progeny Seedlings Emerged	Progeny Seedlings Infected	Progeny Embryos Infected	Symptom Expression Score
		Treatment 13 - BH X HY F_2			
10-18-2	30	29	0	8	3
6-11-2	30	29	0	0	1
3- 7-1	30	30	0	0	1
8-17-2	31	31	1	5	3
8-18-1	30	28	0	0	2
7-11-1	31	31	0	1	2
7-15-2	30	29	0	0	2
1-18-2	30	30	0	4	1
1- 3-3	30	24	0	3	1
4- 8-1	30	28	2	3	3
2-19-3	30	30	1	3	2
2- 2-2	30	28	4	2	2
10-15-1	30	30	2	1	2
2-17-2	30	29	0	2	3
3- 6-1	30	30	0	4	1
2-10-1	30	30	0	0	2
6- 1-3	30	30	0	0	1
7- 4-2	31	31	0	0	2
4-18-1	30	29	0	0	1
10- 1-1	30	27	0	0	1
5- 8-1	30	30	2	0	3
7-17-2	30	30	1	7	3
6- 4-2	30	30	0	0	2
2- 6-1	30	30	0	0	2
1- 3-1	30	29	0	2	2
6- 1-1	30	29	0	1	1
1- 1-2	30	29	0	2	2
1- 5-2	30	30	0	4	1
8- 9-2	30	27	0	0	2
8-12-1	30	30	10	8	3

Figure 3

Leaf Blade Showing Symptoms of BSM Which Mimic the Disease Caused by *Helminthosporium gramineum* Rabh

Figure 4

Leaf Blade Showing Chronic Striping, and
Chlorosis at the Tip

Figure 5

Leaf Blades Showing Chlorotic and Necrotic Striping

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