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
Montana State University

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.

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

Approved:

PD Skaar
Head, Major Department

Thomas W. Carroll
Co-Chairman, Examining Committee

Stephen R. Chapman
Co-Chairman, Examining Committee

Henry L Parsons
Graduate Dean

MONTANA STATE UNIVERSITY
Bozeman, Montana

December, 1972

ACKNOWLEDGMENT

I would like to acknowledge the assistance of the following:

Dr. Thomas W. Carroll, for serving as co-chairman of my doctoral committee, for taking an interest in me, guiding my work, encouraging me, being patient with my faults, obtaining financial aid for me so that I was able to pursue my research, and for teaching me a great deal.

Dr. Stephen R. Chapman, for serving as co-chairman of my doctoral committee, guiding my work, taking an interest in me, encouraging me, being patient with my faults, and for teaching me a great deal.

Dr. P. D. Skaar, for bringing me to Montana State University, obtaining financial aid for me, giving me counsel and advice, and for keeping me out of the clutches of the Selective Service.

Dr. David G. Cameron, for giving me the chance to supervise a Genetics teaching laboratory, and for obtaining financial aid for me.

Dr. E. A. Hockett, Dr. J. R. Schaeffer, Dr. S. J. Rogers, and Dr. R. E. Moore for taking the time to serve on my doctoral committee, and for giving me advice and encouragement.

Mr. Robert LaRue for technical assistance.

The Department of Zoology and Entomology, the Montana Agricultural Experiment Station and the National Science Foundation for employment and financial assistance.

And last, but always first, my wife Debra, for typing above and beyond the call of duty, tremendous patience, encouragement, and love and affection.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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 F_2 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 S_4 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 F_2 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.

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