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Barley Stripe Mosaic Virus: Its Economic Importance and Control

Fig. 1. Foliage symptoms in Atlas barley caused by Montana isolate-1 of barley stripe mosaic virus.

Currently, the planting of virusfree seed is recommended for control of barley stripe mosaic virus (BSMV) in Montana. This method depends on seed testing by the Seed Laboratory at Montana State University. Seed technologists conduct tests using antisera, virus preparations, and expertise supplied by the staff of the Plant Virology Laboratory at the university. Through the Montana Agricultural Experiment Station and the Montana State University Extension Service, barley growers are encouraged to plant either certified seed or seed known to be free from BSMV. Growers who wish to have seed tested for the virus may submit samples to the Seed Laboratory.

The control program has markedly reduced the incidence and severity of BSMV in Montana. The virus has by no means been eliminated from the state, however, and probably never will be, since the planting of virusfree seed is not obligatory in Montana. In 1974, barley was grown on 8,332 farms in Montana but only 269 seed lots were tested. Assuming that one seed lot was planted per farm and that tested seed lots were among those planted, a mere 3.2% of the total planted statewide had been tested. In addition, records indicate that only about 50% of the seed lots tested are certified virusfree. Thus, some known virus-infected lots may have been among those planted, despite the recommendation not to plant lots with seedborne BSMV. The actual disposition of the tested seed lots has never been determined by state survey. Nevertheless, BSMV has been brought to a reasonable level of control in Montana. All barley cultivars released by the Montana Experiment Station since the late 1950s have had little or no seedborne BSMV, and tested certified and noncertified seed lots supposedly free of the virus have been planted widely in the state.

First Awareness of BSMV

In the late 1940s, various foliar symptoms were noted in some spring

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barleys grown in Montana. Because of the high incidence and severity of these symptoms in Glacier barley, Eslick (5) began a study in 1947 to ascertain their possible effect on yield.

The cause of the symptoms was initially thought to be genetic and/or environmental factors, but later a virus described by McKinney (9) was suspected of having some involvement. Samples of seed from the affected Glacier barley plots in Montana were sent by Eslick to McKinney at Beltsville, Maryland, and it was determined that the resulting progeny plants were, indeed, infected by a virus. The virus was seedborne and mechanically transmissible and could be isolated from both symptomatic and asymptomatic plants. Symptoms induced by the virus in Glacier barley only vaguely resembled those McKinney had observed earlier for the seedborne virus that caused false-stripe symptoms in Chevron barley (10). Because the brown striping symptom caused by the virus was very similar to that caused by the barley stripe fungus, *Helminthosporium gramineum* Rabh., the virus disease was named barley false-stripe. When the virus isolated from Glacier barley was inoculated onto healthy Glacier seedlings, chlorotic markings appeared on the foliage of some, but not all, of the seedlings. When the virus from Glacier barley was inoculated onto healthy Chevron seedlings, only mild symptoms of false-stripe were visible. By contrast, when the false-stripe virus isolated from Chevron barley was inoculated onto healthy Glacier seedlings, mosaic and striping symptoms typical of the false-stripe disease were produced (10).

For a while, the abnormal foliar symptoms in Glacier barley were confusing. Then McKinney (10) discovered that the mild chlorosis induced by the virus isolate from Glacier barley was often obscured by nonparasitic chlorotic and/or necrotic flecks and spots peculiar to both infected and healthy Glacier plants. Further work with the virus from Glacier barley revealed its identity. When highly susceptible seedlings of Atsel barley were mechanically inoculated with the Glacier isolate, typical symptoms of false-stripe occurred (11). McKinney concluded from this observation and other information that the virus was, in fact, false-stripe virus of barley (11). Eventually, the virus was named barley stripe mosaic virus.

Symptoms of BSMV

Symptom expression of BSMV in barley depends on the strain, host cultivar, and environmental interaction. Latent, mild, moderate, and severe strains of the virus have been described. Low temperatures (<18 C) and low light intensities (≃5,000 lux) mask symptoms of mild strains. Field symptoms caused by a moderately severe isolate of BSMV include notable necrosis of some leaves (Fig. 1). Typical symptoms, frequently observed in older leaves of barley in field

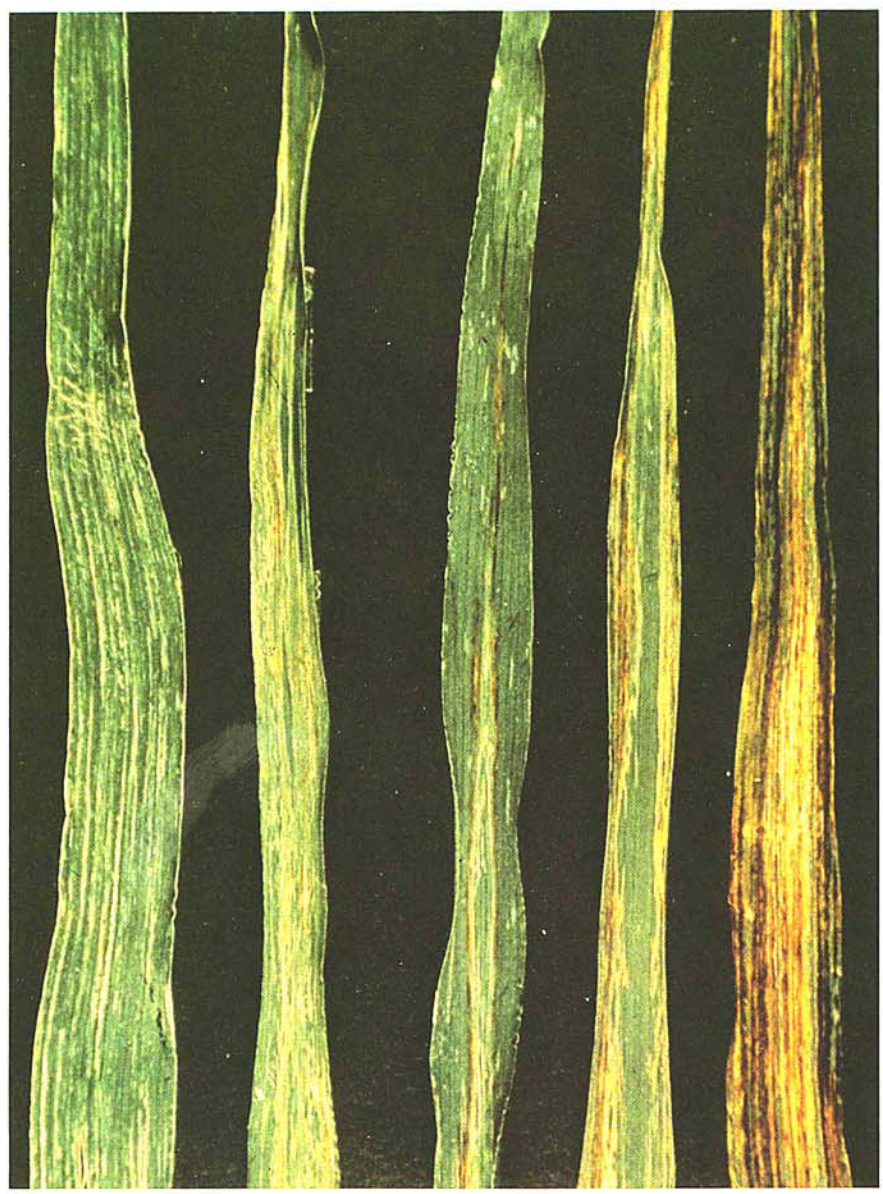


Fig. 2. Leaf symptoms in barley due to barley stripe mosaic virus. Different combinations of virus strain, barley cultivar, and environmental factors produce symptom variations such as striping, streaking, chlorosis, and necrosis.

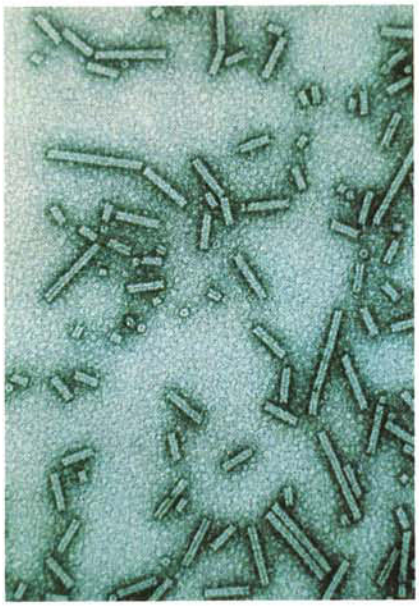


Fig. 3. Electron micrograph of a negatively stained preparation of barley stripe mosaic virus showing different particle lengths.

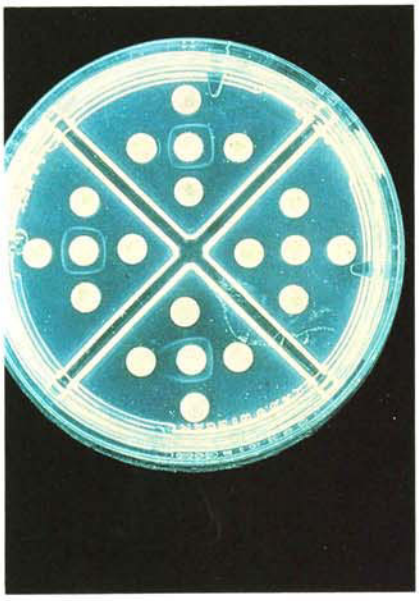


Fig. 5. Sodium dodecyl sulfate (SDS) disk test. Immunoprecipitin lines occur only between infected embryos on the peripheral disks and the antisera in the center disks.

Fig. 4. Spikes of Atlas barley. The two short ones on the right have few plump kernels because of flower sterility induced by barley stripe mosaic virus. The two long spikes on the left with many plump kernels are from virusfree plants.

plantings, include striping, streaking, chlorosis, and necrosis (Fig. 2). In mature plants of some barleys, V-shaped chevrons often occur on the leaves. Usually only one chevron is found per leaf. The two diagonal stripes of the chevron extend outward from the culm and meet at an angle to form a point about 20 cm or more away from the culm.

BSMV is a multicomponent virus, as shown by biochemical and biophysical studies. This means that each strain of the virus has more than one type of nucleoprotein particle, and two or more particle types are required for maximum infectivity. Most strains have three types of particles, but a few have either two or four. Presumably, each particle type contains a single strand of RNA. BSMV particles of different lengths are shown in Figure 3.

Studies of Possible Yield Loss

The first field study conducted by Eslick to determine the possible yield loss caused by the abnormal foliar symptoms in Glacier barley was during 1947–1951. A replicated plot technique was used with irrigated plants grown at Bozeman, Montana. Barley plantings over 90% affected yielded 35–40% less than "disease-free" plantings; the average yield reduction was 31% (5). Furthermore, the test weight of barley from affected plants was reduced significantly (>25%).

Virus-induced sterility of both pollen and ovules is largely responsible for the yield losses in barley. Infected plants of highly susceptible cultivars often have shorter spikes (heads) and more sterile florets than uninfected plants (Fig. 4); the absence of plump seed at each rachis node is evident on the short spikes from infected plants.

Results of the first yield trial plus the knowledge that several barley cultivars recommended for Montana showed the abnormal symptoms led Eslick to conclude that the disease posed a serious threat to barley production in the state (5). So, additional nursery trials of diseased barley were undertaken from 1954 to 1956 at Bozeman, using Betzes, Compana, and Vantage cultivars. All barley plantings were irrigated. Yield

comparisons were made between certified disease-free plantings and plantings that were 100% diseased due to manual inoculation. Asymptomatic plants were rogued. Yield reduction was 31% for Betzes, 24% for Compana, and 35% for Vantage. Rainfed or dryland barleys appeared to have about the same percentages of crop loss as irrigated barleys.

In the spring of 1954, the influence of time of infection with BSMV on symptoms, plant yield, and seed infection of barley was investigated by Eslick and Afanasiev (6). Their findings indicated that with Titan and Compana cultivars, yield reduction was greatest with plants mechanically inoculated 1–3 wk before heading. Furthermore, early inoculations on 20 May and 1 June produced the fewest symptomatic plants, even though later assays of seed from such plants revealed seedborne virus. All plants inoculated after 10 June produced symptoms. Plants inoculated 10 days before heading yielded the most seedborne BSMV.

Survey of Barley Seed

At the urging of Eslick, a seed survey was started during the winter of 1954 and the spring of 1955 by Afanasiev (1) to determine how much seedborne BSMV was present in the leading commercial barleys grown in Montana. A seedling test was used to assay 231 lots of barley seed. Most of the lots consisted of Compana barley, which at the time constituted at least 90% of the barley acreage planted in the state. Samples of 100 seeds per lot were planted in flats in the greenhouse, and seedlings were observed for symptoms of barley stripe mosaic. Of the 219 samples of Compana, 120 (54.8%) were infected. Of the 120 infected samples, 59 (49.2%) showed low levels of seed infection, ie, from trace to 5%. The average level of infection in all Compana samples was 9.2%.

The seed survey was continued during the winter of 1955 and the spring of 1956 by MacWithey et al(8), using the seedling test reported by Afanasiev (1). Again, most of the seed lots assayed were of Compana barley. In addition, a limited number of seed lots of Titan, Moravian, Vantage, Sultana, Spartan, Moore, Glacier, Wolf, and Huskey barleys were examined. Of the 482 Compana seed samples, 246 (51%) contained BSMV. About 74%, or 182, of the infected samples showed a trace to 5% of seed infection. Also included in the survey were 101 seed samples from Compana plants from seed lots found to be free from BSMV during the first survey. Surprisingly, virus symptoms were observed in the seedlings from 36 (35.6%) of these samples, suggesting that the seedling test of Afanasiev (1) had not detected low levels of infection in the original seed lots. The level of infection in the seed samples of the other barley cultivars did not exceed 5%.

During 1957 and 1960, the final years of the seed survey, only 110 Compana seed lots were tested. Unpublished results indicated that over 50% of the samples were still infected by the virus.

On the basis of the seed survey and the experimental determinations of yield loss in barley due to BSMV, a routine barley seed testing program was established in 1960 by the plant pathologists (at that time in the Department of Botany and Bacteriology) at Montana State College. The method of choice was the seedling test of Afanasiev (1).

Later, during 1963 and 1964, a drill box survey was conducted in Montana under the supervision of Afanasiev and Eslick. The three leading counties in barley production were covered. Seed samples of Compana barley were collected from the drill boxes of commercial barley growers and tested by the seedling method of Afanasiev (1). Results of the survey revealed that 85% of 183 samples were infected and that 75 of those samples had infection levels of 11–25%.

Approaches to Control of BSMV

Initially, two approaches were chosen to control BSMV in Montana. The first was to breed for resistance. In 1956, Eslick crossed Modjo, a resistant cultivar, with several susceptible commercial cultivars of barley. The following year, Hockett assumed the responsibility for developing resistant barleys. At first, resistance to mechanical inoculation was

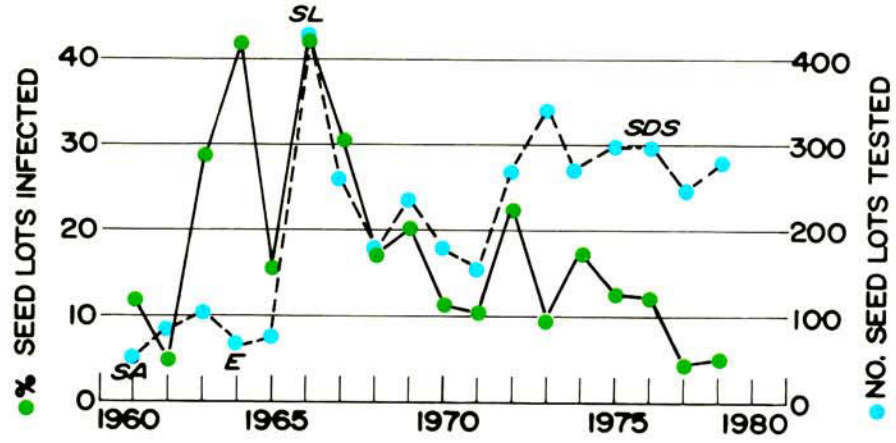


Fig. 6. Barley seed lots tested for barley stripe mosaic virus in Montana. SA = seedling assay method, E = embryo method, SL = Seed Laboratory, SDS = sodium dodecyl sulfate method.

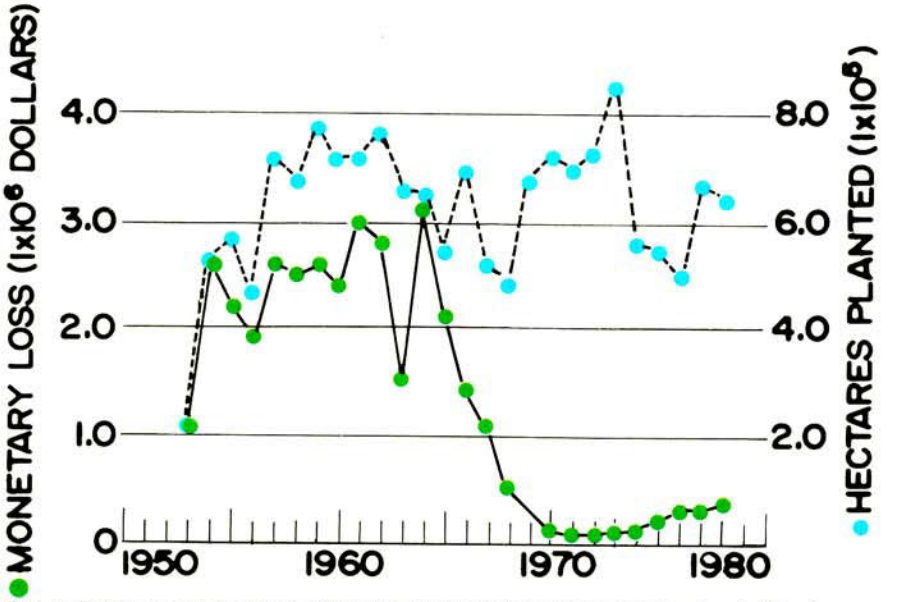


Fig. 7. Estimates of yield loss in barley due to barley stripe mosaic virus in Montana.

sought; later, resistance to seed transmission was pursued. By 1963, lines of each hybrid barley developed for resistance contained seven doses of the commercial cultivars, obtained by backcrossing. In 1973, working with the F_4 generation, Carroll and Hockett determined that only the lines of Betzes barley showed any promising resistance to seedborne BSMV. Although Carroll et al (3) showed that a single recessive gene conditioned a useful resistance to the seed transmission of the MI-3 isolate of BSMV when Modjo was the resistant parent, testing of the advanced generations of the Betzes lines revealed they were resistant to two Montana isolates of the virus but not to 14 different field isolates used in a composite inoculum. At present, six F_{11} lines are being evaluated for resistance to seed transmission of the three isolates of BSMV common in Montana. Hopefully, a useful resistance will be discovered in one or more of these lines.

The second approach was to plant virusfree seed. Lots of both certified and noncertified seed were originally checked for virus freedom by the seedling assay method of Afanasiev (1). This method, however, was laborious and slow and failed to detect latent or masked infections. During the winter, symptoms were especially weak on seedlings grown in the greenhouse under the low light intensity of incandescent lamps. Then, in 1964 Hamilton (7) developed a rapid and accurate serological method for detecting BSMV in barley embryos that could be applied to large-scale screening of seed lots. This method was used successfully for 2 yr in the Plant Virology Laboratory at Montana State University to check seed lots, then responsibility for routine testing was transferred to the Seed Laboratory at the university. Davis and Wallace (4) reported on this testing in 1967.

In 1976, the serological procedures for the embryo test were improved. Virus treated with sodium dodecyl sulfate (SDS) and its homologous antiserum (the antiserum elicited by SDS-treated virus) is now used in a double-diffusion system in agar gel (2). Briefly, the SDS test consists of filter paper disks soaked with seroreactants that are placed on an agar gel medium amended with SDS. The peripheral disks contain crushed barley embryos and the central disks contain the antiserum. An immunoprecipitin line forms only between infected embryos and the antiserum depot (Fig. 5). Since 1976, the SDS procedure has been used for all routine testing for BSMV by the Montana State Seed Laboratory.

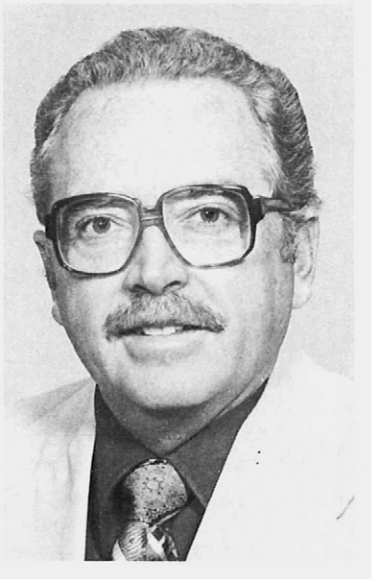
A summary of data from the barley seed lots tested for BSMV in Montana during 1960–1978 is shown in Figure 6. At the beginning of the testing program, fewer than 100 seed lots were analyzed by the seedling assay method. In 1964, fewer than 100 seed lots were tested by Hamilton's embryo method in the Plant Virology Laboratory. In 1966, more than 400 seed lots were tested by the embryo method as a new routine service provided by the Seed Laboratory. The SDS procedure was used by the Seed Laboratory to test 299 seed lots in 1976, 255 lots in 1977, and 277 lots in 1978. Initially, a 5% level of virus-infected seed was tolerated for each certified seed lot. The tolerance level was lowered to 3% in 1968 and to 0% in 1972.

The percentage of infected seed lots has declined appreciably from the early 1960s to the present. Perhaps changes in the number of samples tested per cultivar of barley and the origin of each cultivar are partly responsible for the wide variation seen in the percentage of seed lots infected during the early 1960s.

Losses Due to BSMV

The serious losses attributed to BSMV in commercial plantings of spring barley from 1953 to 1970 are shown in Figure 7. Yield losses are estimates based on the seed and drill box surveys and on the yield trials described earlier. Loss estimates are also based on field surveys and state agricultural statistics from the Montana Crop and Livestock Reporting Service, Helena. At first, the monetary loss followed the same trend as the number of hectares planted in the state. From 1953 to 1966, Compana and Unitan barleys comprised over 50% of the total barley planted. Because over 50% of all commercial seed lots of Compana tested were infected with BSMV, yield losses to these two barleys were estimated at 215.6 kg/ha (4 bu/acre) for rainfed or dryland culture. From 1967 to 1970, yield losses in Compana and Unitan averaged 107.8 kg/ha (2 bu/acre). From 1970 to the present, the estimated yield loss with infected cultivars of barley was about 26.9 kg/ha (0.5 bu/acre).

Losses in barley yield are influenced by the cultivar infected, the level of infection, the infecting virus isolate, and environmental conditions. The total loss in barley due to the virus exceeded $30 million during 1953–1970. The peak year for crop loss was 1964, when BSMV caused over $3.1 million damage. Monetary loss due to the virus has since declined significantly. The serological test has played a major role in the control of BSMV in Montana.



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Dr. Carroll is professor in the Plant Pathology Department, Montana State University, Bozeman. In addition to research and teaching duties in plant virology, he has technical responsibility for the routine test used by the Seed Laboratory at Montana State University to detect barley stripe mosaic virus in barley seed. This involves the production and supply of seroreactants for the current test and evaluation of other testing methods that show promise for future application. He received his Ph.D. at the University of California, Davis, in 1965.

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