DISTRIBUTION OF BARLEY STRIPE MOSAIC VIRUS IN MANITOBA IN 1970[1]

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Abstract

An intensive survey for barley stripe mosaic virus (BSMV) was made throughout most of the barley growing region in southern Manitoba. The virus was transmitted from field samples to 'Black Hulless' barley and subsequently identified by its reaction with BSMV antiserum. BSMV was detected in 22 and 4%, respectively, of the 2- and 6-row barley fields sampled. The incidence of diseased plants in these fields varied from a trace to about 50%. No evidence was obtained of widespread masked infection in barley. However, in a few fields where the disease was detected, some symptomless plants were also infected.

Attempts to differentiate field-collected BSMV isolates using five barley varieties and 'Clintland' oats were unsuccessful.

Introduction

Although barley stripe mosaic (BSM) was not reported to be caused by a seed-borne virus until 1951 (10), the disease was probably observed in Manitoba as early as 1924 (3, 5). Since then, reports of its presence in Manitoba and elsewhere in Canada have appeared frequently (2). Some of these reports, however, suggest that the disease is mainly of consequence in experimental barley plots and that its occurrence in farmers' fields is relatively rare. By contrast, the disease has previously been reported to occur quite commonly in several regions of the United States (1,6,8,13,15). In North Dakota, Timian and Sisler (15) observed BSM in 93% of the barley fields examined in 1954. In 1954 and 1955 BSM was not reported in Manitoba barley fields and annual Canadian plant disease survey reports (2), generally indicate that the incidence of the disease in this province has never remotely approached that in North Dakota. The reason for this apparent disparity is not clear since climates in the two regions are similar and no effort has been made to control the disease in Manitoba. Furthermore, although varietal tolerances vary considerably, all major commercial barley varieties grown in Manitoba are susceptible to barley stripe mosaic virus (BSMV) (Chiko, unpublished).

In Montana, Eslick (4) observed wide annual variation in BSM symptoms in 'Glacier' barley plants derived from infected seed that was continually obtained from the previous year's crop. He also noted that relatively high yield losses occurred during a year when symptoms were almost nonexistent. A latent strain of BSMV was subsequently isolated and described by McKinney and Greeley (12).

The possible masking of BSM symptoms, the former apparent disparity in BSM incidence between North Dakota and Manitoba, and the apparent lack of any previous systematic survey for BSM in Manitoba prompted the survey work reported here. The objectives of this survey were (1) to determine the possible occurrence of masked BSMV infection and (2) to estimate the frequency and distribution of symptomatic BSMV infection in Manitoba barley fields. Preliminary results of attempted varietal differentiation of field-collected BSMV isolates are also reported.

Materials and methods

Survey routes and sampling procedures - The perimeter of the BSMV survey was delimited by a route similar to that described by McDonald et al. (9) for barley disease loss surveys in Manitoba. This route passes through crop districts in which over 75% of the Manitoba barley crop is grown. Several routes within this perimeter were also surveyed and a total of approximately 1600 miles was covered between July 6 and July 23, 1970. Fields of barley in the early tillering to boot stages were inspected and sampled at preselected intervals of about 4-12 miles, the interval generally depending on the length of the particular survey route. Near points of intersecting survey routes, intervals were occasionally shorter than 4 miles, and they were sometimes considerably longer than 12 miles due to the absence or inaccessibility of barley fields.

In each field sampled, regardless of the presence or absence of plants with suspected BSM symptoms, leaves were collected from 10 apparently healthy barley plants. Beginning 20 paces in from one edge of a field, leaves from five healthy plants were sampled at five pace intervals along two traverses 10 paces apart and perpendicular to the edge of the

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field. Two to four apical leaves were selected from one tiller of each plant sampled. When plants with suspected BSM symptoms were observed, leaves from about 10 of these plants were also sampled. Each sample was placed in a tightly sealed polyethylene bag and stored in a cooler with ice-packs until delivered to the laboratory and assayed for infectivity (1-3 days after sampling).

Infectivity assay - Each sample of leaves was ground in a mortar with 2 ml of distilled water and the extract was filtered through cheesecloth. 'Black Hulless' barley (Hordeum vulgare L.) was used as an indicator plant for BSMV. Seedlings in the 1-2 leaf stage were dusted with corundum and sample extract was applied to leaves by a finger-wiping method. Each leaf was rubbed five times and 11-16 indicator plants were inoculated with each sample extract. The presence or absence of symptoms was recorded 7-12 days after inoculation.

Serological procedures - An isolate of BSMV was obtained from Dr. C.C. Gill, Research Station, CDA, Winnipeg. 'Fergus' barley (H. distichum L.), inoculated at about the 2-leaf stage, was used as a propagation host for the virus. Infected leaves were harvested 10-14 days after inoculation and the leaf extract was clarified by the chloroform-charcoal method of Timian and Savage (14), except that the extract was cooled to 0-1 C only at the start of the procedure and was not centrifuged before adding chloroform. The virus was subsequently purified by two cycles of high (70,000 g, 2 hr) - low (5,000 g, 15 min) speed centrifugation. Prior to the final low speed centrifugation, the virus pellet was resuspended in a volume of 0.01 M phosphate buffer, pH 6.5, equal to 1/20 the volume of chloroform-charcoal clarified extract. Virus preparations purified by this procedure were infectious and exhibited strong anisotropy of flow.

A rabbit was given four weekly intravenous injections of 0.5 ml of freshly purified BSMV suspension. Serum obtained from the rabbit 2 weeks after the final injection reacted with chloroform-charcoal clarified extract from BSMV-infected, but not from healthy, 'Fergus' barley. The titer of the BSMV antiserum was 1/512.

Serological reactions were determined using a slide precipitation test. Two drops each of test antigen preparation and antiserum were delivered from Pasteur capillary pipettes into a well of a serological slide plate and incubated 15 min on a platform rotator at 120 rpm. The presence of a precipitate was detected by viewing the slide plate in a dark room under a stereoscopic microscope with indirect light at a magnification of 50X. All dilutions for serological tests were made with normal saline (0.14 M NaCl).

Isolates from the field which induced chlorotic symptoms in 'Black Hulless' barley were each transferred to about 15 seedlings of this variety. After 11-13 days, extract from each group of infected plants was clarified by the chloroform-charcoal method. Each extract was tested undiluted against BSMV antiserum diluted 1/16.

BSMV isolate differentiation - Isolates of BSMV collected from widely separated areas in Manitoba were maintained in 'Black Hulless' barley. 'Clintland' oats (Avena sativa L.) and five barley varieties in the 1-2 leaf stage were inoculated with each isolate as previously described. Three of the barley varieties tested ('Conquest' (H. vulgare), 'Herta' (H. distichum), and 'Fergus') are currently grown commercially in Manitoba. Test plants were grown in the greenhouse under supplemental fluorescent light (15 hr photoperiod) at a mean daily temperature of 25.0 ± 1.4 C.

Results and discussion

Each field-collected isolate which induced chlorotic symptoms in 'Black Hulless' barley also reacted with BSMV antiserum. Chloroform-charcoal clarified extract from uninoculated 'Black Hulless' plants (control) did not react with BSMV antiserum.

BSMV was detected most commonly in southeastern Manitoba where it was distributed fairly consistently throughout the range of 2-row barley (Fig. 1). Although symptoms were most pronounced in 6-row barley, few fields with infected plants were encountered.

BSMV was detected in 22% and 4%, respectively, of the 2- and 6-row barley fields sampled (Table 1). The approximate incidence of plants with BSM symptoms in these fields was' as follows: trace - 4 fields; I-5% - 5 fields; 25% - 1 field; and 50% - 1 field. It should be emphasized that this was a systematic survey. Therefore, the number of fields indicated as having BSMV-infected plants present should not be construed as the total number of fields in

Table 1. Occurrence of barley stripe mosaic virus in fields of 2- and 6-row barley in southern Manitoba in 1970

Type of barley	Fields No. sampled	Fields No. with BSMV*	Fields % with BSMV
2-row	41	9	22.0
6-row	50	2	4.0
2- and 6-row	91	11	12.1

* Transmitted to 'Black Hulless' barley and reacted with BSMV antiserum.

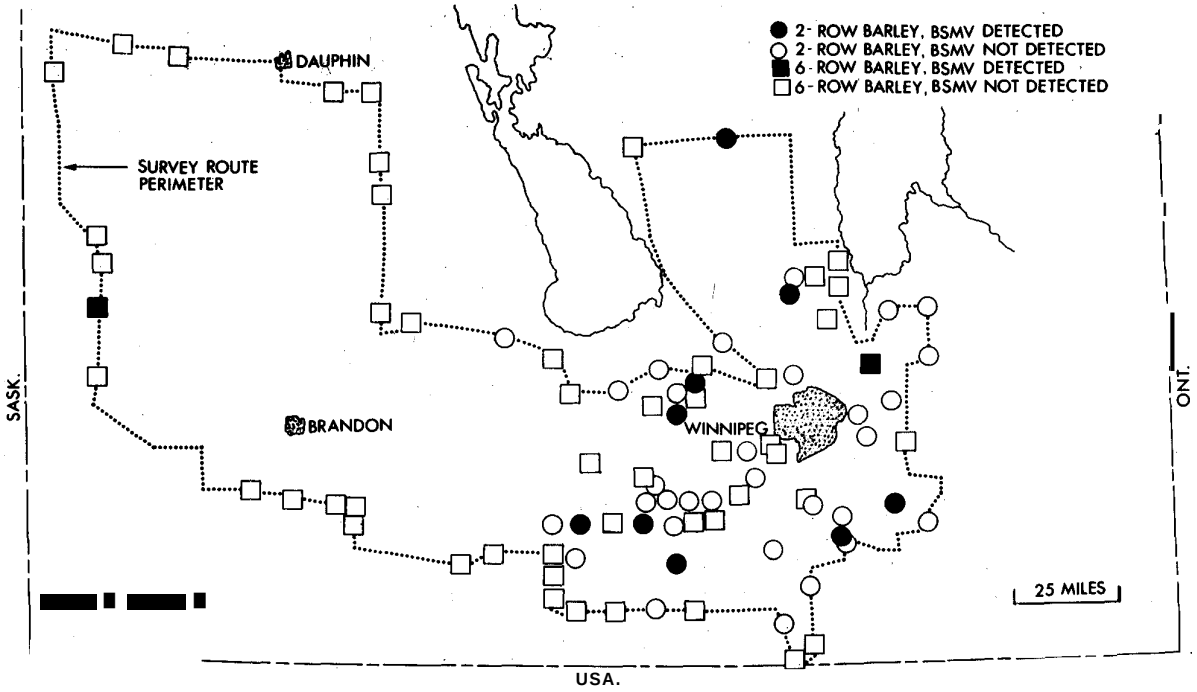

Figure 1. Distribution of barley stripe mosaic virus in fields of 2– and 6– row barley in southern Manitoba in 1970.

Table 2. Symptom severity indices of five barley varieties inoculated with 10 field isolates of barley stripe mosaic virus

Variety	Symptom severity index* for isolate: 1	2†	3	4	5	6†	7	8	9	10	Avg severity index for all isolates	Apparent transmission††
6-row												
Black Hulless	3.8	3.5	3.9	3.7	4.0	4.0	3.7	3.0	3.0	4.1	3.7	115/143
Conquest	2.2	2.5	2.8	2.1	1.7	1.9	1.7	1.4	2.5	2.0	2.1	125/150
2-row												
Herta	1.0	2.0	1.5	1.0	1.0	1.3	**1.4**	1.0	1.5	1.2	1.3	58/150
Fergus	2.0	1.4	2.2	1.6	1.2	1.3	1.7	1.0	1.0	1.2	1.5	68/148
Betzes	1.8	1.5	1.7	1.8	1.3	1.4	1.4	1.0	2.0	1.3	1.5	93/150

* Avg rating for each variety 13 days after inoculation of 11–15 plants with each isolate. Individual plants were rated as follows: 1, 2, 3, and 4 = 1, 10, 25, and 50% or more chlorosis, respectively, on systemically infected leaves and 5 = dead or dying. Plants without symptoms (rated 0) were not used in computing the index.

† These isolates were from 6–row barley; all other isolates were from 2-row barley.

†† Total no. plants with symptoms/total no. plants inoculated.

which the disease was observed. For instance, near one sampling point five fields were observed in each of which the incidence of diseased plants was about 50%. Similar but less extreme situations were noted near most other sampling points where the disease was detected.

BSMV was not isolated from any of the leaf samples obtained from 80 fields in which plants with BSM symptoms were not observed. It thus seems unlikely that any widspread masked or latent form of the virus was present in fields of barley in Manitoba. The virus was, however, detected in symptomless leaf samples from 2 of 11 fields in which plants with BSM symptoms were observed. Both samples were from 2–raw barley.

Symptoms of BSM in 2–row barley were often very mild or inconspicuous. Chlorosis and mosaic were generally absent, the most

apparent symptoms being brown stripes on lower leaves. The length, frequency and color intensity of the stripes varied widely. In several fields, symptoms were so mild that the disease could easily be overlooked and at time of heading the brown stripes might be interpreted as natural senescence.

The symptomatic reactions of five barley varieties to 10 BSMV isolates, each of which induced symptoms in the field, are summarized in Table 2. Each isolate generally incited milder symptoms in the 2-row varieties than in the 6-row varieties. Some differences in varietal response to different isolates were occasionally observed but these were not sufficiently characteristic or uniform to serve as a basis for isolate differentiation. Symptoms induced in the five barley varieties by two isolates from symptomless 2-row barley (data not shown in Table 2) were generally similar to those incited by field-collected isolates obtained from plants with BSM symptoms. The percentage of 2-row barley plants that failed to develop symptoms (51%) in this test was considerably higher than the percentage of symptomless 6-row plants (18%). Therefore, a number of inoculated 2-row plants of the varieties 'Fergus' and 'Herta' were individually assayed for BSMV on 'Black Hulless' barley. The virus was transmitted from 49 of 50 'Herta' and 46 of 46 'Fergus' barley plants showed symptoms ranging from doubtful to severe. In addition, the virus was also transmitted from 32 of 70 (46%) 'Herta' and 24 of 71 (34%) 'Fergus' barley plants which showed no apparent symptoms of infection.

Two attempts were made to differentiate field-collected BSMV isolates using 'Clintland' oats. In one test, 3 of 11 isolates were transmitted to oats but the percentage of plants infected by each of these isolates was low (20% or less). When the test was repeated with the same isolates, only one isolate was transmitted to oats but this isolate was not one of those transmitted in the first test. Although 'Cherokee' oat plants have previously been reported for differentiating BSMV strains (13), 'Clintland' oats did not appear to have any similar value.

The results of this survey suggest that BSM is more common in 2-row barley fields in Manitoba than most previous surveys (2) indicate. Although the frequency of the disease was considerably less than that previously reported in North Dakota (15), the estimate for the percentage of fields with infected plants in Manitoba is considered to be conservative. This is because only a small portion of each field was inspected and trace infections might easily have been overlooked. No explanation can presently be advanced for the large differences encountered in the percentages of 2- and 6-row barley fields with BSM.

Masking of BSM symptoms under greenhouse conditions can probably be attributed to inadequate light intensities (6,7,11). Whether or not the same factor is responsible for masked infection in the field is not known. The extent of symptomless infection would have to be estimated to obtain meaningful yield loss data for BSMV in farmers' fields.

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