Increased Virulence of Barley Stripe Mosaic Virus for Wild Oats: Evidence of Strain Selection by Host Passage

Arthur W. Chiko

Agriculture Canada Research Station, Winnipeg, Manitoba R3T 2M9. Present address: Saanichton Research and Plant Quarantine Station, Sidney, British Columbia, V8L 1H3, Canada.
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ABSTRACT

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Only one of four isolates (C4) of barley stripe mosaic virus (BSMV) was transmitted (airbrush method of inoculation) from barley (*Hordeum vulgare* 'Black Hulless') to wild oats (*Avena fatua*). Initially, the proportion of inoculated wild oat plants systemically infected by isolate C4 was low, and in some plants only localized infections developed in inoculated leaves. However, when isolate C4 was subsequently transferred from infected to healthy wild oats, the proportion of inoculated plants infected was high and the infection was invariably systemic. These observations and several additional lines of evidence indicated that this pattern of transmission was due to strain selection during systemic passage of isolate C4 through wild oats. Complete separation of strains comprising this isolate, however, apparently did not occur until three successive passages of the virus through wild oats. After this, the "selected strain" from barley systemically infected almost all wild oat plants that were inoculated. In immunodiffusion tests with crude extracts from infected barley, no antigenic differences were detected among the four isolates of BSMV or between isolate C4 and the selected strain. The possible significance of some of these findings in the epidemiology of barley stripe mosaic is discussed.

Earlier workers (13,14) observed that isolates of barley stripe mosaic virus (BSMV) from barley were difficult to transmit mechanically to oat (*Avena sativa* L.) plants, but subsequently were readily transmitted from infected to healthy oats. I later described a similar pattern of transmission (7) with a barley isolate of BSMV and wild oats (*A. fatua* L.). These corresponding results were not surprising, since oats and wild oats belong to the same biological species (17). My results with wild oats, however, are probably more closely related to the epidemiology of barley stripe mosaic (BSM), since evidence indicates that this weed can serve as a source of infection for barley crops (6,8).

McKinney and Greeley (14) attributed the pattern of BSMV transmission in the barley-oat system to "strain substitution." Similarly, I suggested that the pattern of BSMV transmission in the barley-wild oat system was due to "strain selection" (7), believing this term to be equivalent in meaning to, but more widely used than, "strain substitution."

Results of preliminary work regarding the transmissibility of certain isolates of BSMV to wild oats (7) are presented here in greater detail. Also, results of additional tests designed to more critically evaluate the aforementioned strain selection hypothesis are reported, and the possible relevance of some of these findings to the epidemiology of BSM is discussed.

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MATERIALS AND METHODS

Virus isolates. Isolates C1, C2, C3, and C4 of BSMV, previously referred to as "strains" (5), were maintained by periodic transfers from infected to healthy plants of barley (*Hordeum vulgare* L. emend. Lam. 'Black Hulless' [CI 666]).

Transmission trials. Unless mentioned otherwise, systemically infected leaves of Black Hulless barley or wild oats harvested 20–25 days after inoculation were used as a source of inoculum. Inoculum was prepared by grinding leaves in a sterile mortar, filtering the juice through cheesecloth, and diluting it 1/5 with distilled water.

Each inoculum applied to wild oat plants was also checked for infectivity using Black Hulless barley. Groups of about 10 wild oat or barley test plants were grown in a mixture of soil, sand, and peat moss (3:1:1, v/v) in 15-cm-diameter peat pots. In each trial, at least 20 wild oat and 10 barley plants at the 2–3½ and 2–2½ leaf stages, respectively, were inoculated per isolate by using an artist's airbrush (Paasche Airbrush Co., Chicago, IL 60614). Inoculum containing 2% corundum was sprayed on test plants using a flow rate of 10–12

ml/min and a pressure of 4.2 kg/cm^2. Ten horizontal passes were made on each of two opposing sides of each group of test plants with the airbrush nozzle held about 2 cm from the leaves. Virus source and test plants were maintained in a greenhouse under supplemental fluorescent light (from 0600 to 2100 daily) at about 27 C. Final examinations of test plants for symptoms were made 20–25 days after inoculation.

In each trial, 10–30 control plants of wild oats and/or barley were sprayed similarly to inoculated plants but with diluted extract from leaves of appropriate healthy plants.

Serology. Isolates C1, C2, C3, and C4, each propagated in Black Hulless barley, were purified as described previously (4), except that low speed centrifugation was done at 10,000 *g* for 10 min. About 1 mg of each purified virus isolate, suspended in 1 ml of 0.01 M phosphate buffer, pH 6.5, was emulsified with 1 ml of Freund's incomplete adjuvant (Difco, Detroit, MI 48232) and each emulsion was injected intramuscularly into a different rabbit. Each rabbit was given a second, similar injection with the same isolate 8 wk later. Antisera used in this study were obtained from bleedings made 2, 6, and 14 wk after the first injection (designated 2-, 6-, and 14-wk antisera, respectively) and were stored at −20 C until used.

All serological tests were done by the immunodiffusion method in a medium consisting of 0.5% Ionagar No. 2 and 0.2% sodium azide.

Antigenic relationships (ie, the presence or absence of spurs in agar gel immunodiffusion tests) among selected isolates of BSMV were evaluated using crude undiluted extracts from systemically infected leaves (10 days after inoculation) and 2-, 6-, and 14-wk antisera to isolates C1, C2, C3, or C4. Each 2-wk antiserum was tested at dilutions of 1/1 (undiluted), 1/2, and 1/4; whereas 6- and 14-wk antisera were tested at dilutions of 1/1, 1/4, and 1/16.

Virus end points in extracts used to prepare certain inocula of BSMV were determined by testing twofold serial dilutions of each extract against 6-wk antiserum to isolate C4 diluted 1/4. All dilutions were made with 0.14 M sodium chloride.

A number of individual inoculated wild oat plants were assayed serologically for systemic infection by BSMV. For these assays, undiluted extract from the fourth or fifth leaf of each plant, extracted with a plier-type press, was tested against undiluted antiserum to isolate C4. Final readings of all serological tests were made 7 days after initiation.

Relative infectivity (ID_{50}). With certain extracts used to prepare inocula of BSMV, 20 Black Hulless barley plants were inoculated at each of a series of twofold dilutions and the number of plants infected at each dilution was recorded 20 days later. The ID_{50} (dilution estimated to result in infection of 50% of the inoculated plants), used as a measure of relative infectivity of BSMV inoculum, was calculated by the method of Reed and Muench (18).

RESULTS

Certain inocula of BSMV induced symptoms only in inoculated leaves of wild oats. Therefore, unless indicated otherwise, the term "transmission" refers to situations in which inoculated plants developed systemic symptoms.

In a single trial, none of the wild oat plants inoculated with isolates C1, C2, or C3 developed symptoms, and serological tests indicated that none of these plants was symptomlessly infected. The same inoculum of each of these isolates infected all barley plants that were inoculated.

Isolate C4 from barley induced three types of reaction in wild oats: (i) only eyespots in inoculated leaves (Fig. 1), (ii) eyespots in inoculated leaves and systemic symptoms, and (iii) only systemic symptoms. The latter usually consisted of a few conspicuous pale green or chlorotic spindle-shaped stripes confined to one or two leaves. In five trials with isolate C4 from barley, 1–31% (avg 11%) of the inoculated wild oat plants developed systemic symptoms. In four of these trials, 2–40% (avg 15%) of the wild oat plants showed

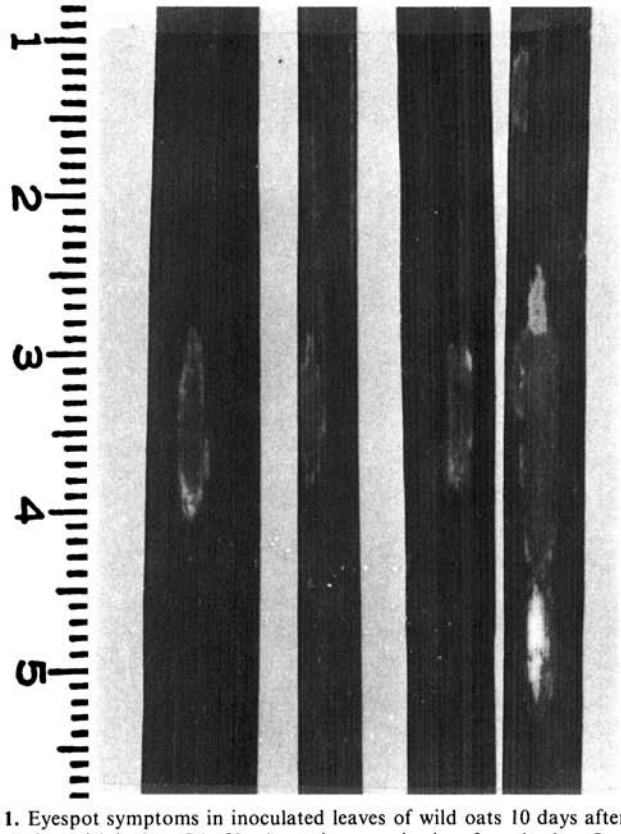

Fig. 1. Eyespot symptoms in inoculated leaves of wild oats 10 days after inoculation with isolate C4 of barley stripe mosaic virus from barley. One division on the scale = 1 mm.

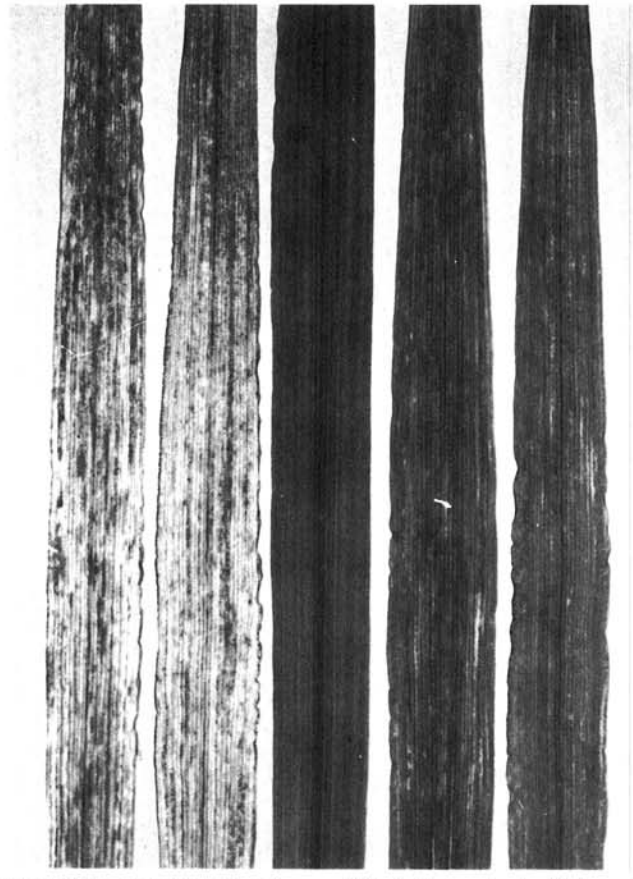

Fig. 2. Systemic symptoms in leaves of Black Hulless barley 10 days after inoculation with barley stripe mosaic virus isolate C4 from barley (two leaves on the left) or subisolate C4-W1 from wild oats (two leaves on the right). C4-W1 was obtained from systemically infected leaves of plants inoculated with C4 from barley. At the center is a corresponding leaf from a healthy control.

only eyespots in inoculated leaves. In five corresponding trials with isolate C4 from wild oats, 60–100% (avg 88%) of the inoculated wild oat plants developed symptoms. These invariably consisted of elongated necrotic stripes in inoculated leaves and mottling, accompanied by numerous faint chlorotic streaks or spindle-shaped stripes, in most or all systemically infected leaves.

Systemic symptoms induced in wild oat plants by isolate C4 from barley or wild oats initially appeared, on the average, 16 and 7 days after inoculation, respectively. In inoculated leaves of wild oats, eyespots induced by the former isolate generally appeared 5–8 days after inoculation, whereas the necrotic stripes induced by the latter isolate usually appeared 9–10 days after inoculation.

Six wild oat plants, each with a single eyespot on the uppermost inoculated leaf (third leaf) and with no other apparent symptoms, were assayed for infectivity 20 days after inoculation with isolate C4 from barley. Extracts were prepared from excised eyespots, from remaining tissue of the third leaf, and from the entire fifth leaf of each plant by grinding each tissue sample in a small volume of distilled water. Each extract was used to manually inoculate five corundum-dusted Black Hulless barley plants. BSMV was recovered from each excised eyespot that was assayed, but was only recovered from one of the third leaves from which eyespots had been excised, and was not recovered from any of the fifth leaves that were assayed. Several wild oat plants inoculated with isolate C4 from barley and showing only eyespot symptoms were observed periodically until they reached the heading stage. No systemic symptoms developed in any of these plants.

To facilitate the description of additional transmission trials, the symbols used for isolate C4 from barley and for certain derivatives (subisolates) of this isolate from systemically infected leaves of wild oats or barley were defined as follows: C4 = parent isolate from barley (ie, isolate C4 not previously passed through wild oats); C4-W1, C4-W2, and C4-W3 = subisolates from wild oats after one, two, or three consecutive systemic passages, respectively, of isolate C4 through this species; C4-W1-B, C4-W2-B, and C4-W3-B = subisolates from barley after one, two, or three previous consecutive systemic passages, respectively, of isolate C4 through wild oats. Two or more of these subisolates are sometimes subsequently referred to collectively as "subisolates of C4." C4-es refers to subisolates from eyespots excised from wild oat plants inoculated with isolate C4.

In each of three preliminary trials, barley plants inoculated with subisolate C4-W1 developed considerably milder symptoms than those induced by the parent isolate (Fig. 2). In two of these trials, a subsequent virus transfer from these plants also produced mild symptoms in barley. In the third trial, however, a similar transfer to barley produced severe symptoms essentially identical to those induced by the parent isolate. Differences in symptom severity in barley were confined to systemically infected leaves above the third leaf.

Barley plants inoculated with C4-es subisolates generally developed only slightly milder symptoms than those induced by the parent isolate. Subsequent transfers of virus from these plants to barley usually produced symptoms indistinguishable from those induced by the parent isolate.

Results of a more thorough series of trials on the transmissibility of isolate C4 and of certain subisolates of C4 to wild oats are given in Table 1. Localized or systemic infection of wild oat plants inoculated with isolate C4 was extremely rare and occurred only in the first of these trials. As noted previously, subisolates from wild oats were transmitted to most or all wild oat plants that were inoculated and invariably produced much milder symptoms in barley than those induced by the parent isolate. The overall efficiency of transmission of subisolates from barley to wild oats

TABLE 1. Transmission of isolate C4 of barley stripe mosaic virus and its derivatives (subisolates) from barley or wild oats to wild oats by airbrush inoculation

Trial no.[a]	Isolate or subisolate[b]	Virus source and symptom severity[c]	Passage history[d]	Inoculum titer		No. wild oat test plants inoculated	Test plants with symptoms (%)	
				Virus end point[e]	ID_{50}[f]		Localized[g]	Systemic
1	C4	B,S	0,3	···	···	100	2	1
2	C4	B,S	0,4	···	···	60	0	0
	C4-W1	WO	1,0	···	···	20	0	85
3	C4	B,S	0,5	64	911	60	0	0
	C4-W1-B	B,M	1,1	64	1,186	60	8	40
	C4-W2	WO	2,0	8	·241	60	0	100
4	C4	B,S	0,6	64	1,245	60	0	0
	C4-W2-B	B,M	2,1	32	658	60	0	98
	C4-W3	WO	3,0	8	239	60	0	100
5	C4	B,S	0,7	···	···	20	0	0
	C4-W1-B	B,M	1,3	···	···	20	0	5
	C4-W2-B	B,M	2,2	···	···	20	0	90
	C4-W3-B	B,M	3,1	···	···	20	0	100
6	C4	B,S	0,8	···	329	30	0	0
	C4-W1-B	B,M	1,4	···	944	40	18	10
	C4-W2-B	B,M	2,3	···	557	30	0	90
	C4-W3-B	B,M	3,2	···	456	30	0	100
7	C4	B,S	0,10	···	···	30	0	0
	C4-W1-B	B,M	1,6	···	···	30	23	3
	C4-W2-B	B,S	2,5	···	···	30	13	0
	C4-W3-B	B,M	3,4	···	···	30	0	97

[a] Trials were conducted consecutively in ascending numerical order.
[b] In each trial, inoculum of each isolate or subisolate was tested for infectivity by inoculating at least 20 Black Hulless barley plants. Each inoculum infected 100% of the barley plants inoculated, except that of C4-W3, which infected 95%.
[c] B = Black Hulless barley, WO = wild oats, S = severe symptoms, M = mild symptoms; symptom severity is designated only for barley source plants.
[d] Number of consecutive systemic passages of isolate C4 through wild oats (first digit) and then through barley (second digit) prior to preparing inoculum from systemically infected leaves of source plants. For this series of trials, lyophilized infected barley leaves were the initial source of isolate C4.
[e] Reciprocal of highest inoculum dilution to react with antiserum to isolate C4 (diluted 1/4) in immunodiffusion tests.
[f] Reciprocal of inoculum dilution estimated to result in infection of 50% of the inoculated barley plants.
[g] Eyespots confined to inoculated leaves.

increased with the number of times (one to three) the parent isolate had been previously passed through wild oats; C4-W3-B was transmitted to all but one of the wild oat plants inoculated. With each successive transfer, subisolates C4-W1-B and C4-W3-B produced mild symptoms in barley. In the first three transfers, subisolate C4-W2-B also produced mild symptoms in barley. In a fourth transfer, however, this subisolate produced severe symptoms in barley, identical to those induced by the parent isolate. This reversion in symptom severity was accompanied by a sharp decline in the transmission of subisolate C4-W2-B to wild oats.

There was no apparent relationship between inoculum titer, estimated by either infectivity or serological assays, and the transmissibility of isolate C4 and various subisolates of C4 to wild oats (Table 1). Because growing conditions probably varied from one trial to another, titers of different inocula should only be compared within individual trials. On this basis, the relative infectivity (ID_{50}) of subisolates which induced mild symptoms in barley generally exceeded that of the parent isolate which induced severe symptoms in barley. An exception to this occurred in the fourth trial (Table 1) in which the relative infectivity of isolate C4 was about twice that of subisolate C4-W2-B.

Symptoms induced in wild oats by subisolates C4-W1, C4-W2, C4-W3, C4-W2-B, and C4-W3-B were generally similar to those described previously for isolate C4 from wild oats. Symptoms in most wild oat plants infected with subisolate C4-W1-B, and in a small proportion of those infected with subisolate C4-W2-B, were similar to those described previously for isolate C4 from barley.

Seventy wild oat plants showing systemic symptoms after inoculation with isolate C4 or subisolates C4-W1, C4-W2-B, or C4-W3-B were tested serologically for infection by BSMV; the virus was detected in 68 (97%) of these plants. In similar tests with 20 symptomless wild oat plants inoculated with isolate C4 or subisolates C4-W1 or C4-W2-B, there was no evidence of infection by BSMV.

No symptoms developed in numerous wild oat or barley control plants observed throughout this study. Many wild oat control plants were tested serologically for BSMV but there was no evidence of infection in any of these plants.

Antigenic differences among isolates C1, C2, C3, and C4 were not detected in immunodiffusion tests with 2-, 6-, or 14-wk antiserum to each of these isolates. With each dilution of each antiserum tested, every possible combination of two of these isolates, placed in adjacent antigen depots, resulted in confluent bands of precipitate. In similar tests with antiserum to isolate C4, no antigenic differences were detected between isolate C4 and subisolate C4-W3-B.

DISCUSSION

Collectively, the following results provide strong evidence that isolate C4 (parent isolate from barley) had changed during systemic passage through wild oats: (i) symptoms induced in wild oats by the parent isolate and by certain subisolates of C4 from either wild oats or barley differed, (ii) the parent isolate was either not transmitted or was inefficiently transmitted to wild oats, whereas some subisolates of C4 from either wild oats or barley were transmitted to most or all wild oat plants inoculated, (iii) although the concentration of virus in wild oats infected with particular subisolates of C4 was considerably lower than that in barley infected with the parent isolate, these subisolates were transmitted much more efficiently to wild oats than was the parent isolate, (iv) the parent isolate induced severe symptoms in barley but subisolates of C4 from both wild oats and barley generally induced mild symptoms in barley; this difference in symptom severity was not attributable to a lower concentration of infectious virus in plants with mild symptoms, since the relative infectivity of virus from these plants generally exceeded that of the parent isolate.

Evidence provided by others suggests that two types of host-mediated selection of virus strains can occur. Either a mixture of strains in the original host is partly or completely separated by passage through a different kind of host (11,21) or, following transmission of a particular strain from one kind of host to another, a new strain is derived from the original strain which partly or completely replaces the latter in the new host (2,10,12). Because of the variation in symptoms induced by isolate C4 in wild oats and because this isolate had not been previously "purified" by passage through a local lesion host, isolate C4 was probably originally a mixture of strains. Therefore, it is believed that the changes encountered with this isolate were due to the former type of strain selection.

Assuming that isolate C4 was originally a mixture of a predominantly wild-oat-incompatible strain and traces of a wild-oat-compatible strain, infrequent transmission of the virus from barley to wild oats might be attributed to an initially low concentration of the latter. However, in wild oats the compatible strain presumably multiplies more rapidly and/or is more invasive than the incompatible strain and, consequently, reaches relatively high concentrations in this species. Hence, transmission of the virus from infected to healthy wild oats occurs readily. The efficiency with which subisolates from barley were transmitted to wild oats (Table 1) suggested that complete replacement of the incompatible by the compatible strain did not occur until after three successive passages of isolate C4 through wild oats. Major declines in transmissibility of subisolates C4-W1-B and C4-W2-B to wild oats were possibly due to the incompatible strain regaining its predominance in barley. Although a concomitant reversion in symptom severity (mild to severe) occurred only with subisolate C4-W2-B, symptoms induced in barley by some strain mixtures (eg, C4-W1-B) might not be indicative of the predominant strain. In a study with two strains of tobacco mosaic virus in tobacco, Cohen et al (9) found that systemic symptoms were usually, but not always, determined by the predominant strain.

The nature of symptoms induced in barley by BSMV from excised eyespots initially suggested that this symptom might represent a resistant reaction of wild oats to infection solely by the incompatible strain. However, it is difficult to reconcile this hypothesis with the failure to obtain any eyespot symptoms in inoculated wild oat plants in six successive trials with isolate C4 (Table 1). Alternatively, eyespots may have been induced by an additional minor strain in inocula of isolate C4, varying widely in concentration with time, and possibly eventually eliminated from this isolate during successive passages through barley. In a similar manner, the compatible strain may also have been eliminated from inocula of isolate C4, resulting in the ultimate failure of this isolate to systemically infect any inoculated wild oat plants.

Ohmann-Kreutzberg (15) transmitted BSMV from barley to wild oats by manual inoculation, but other workers (3,7,19,20) failed to do so. My success in infecting wild oats with one isolate of BSMV from barley was probably partly because the airbrush method of inoculation is a more efficient means of transmitting the virus (7,16). Nevertheless, contact transmission of BSMV from a commercial barley cultivar to wild oats has been demonstrated in field tests (8).

Wild oats naturally infected with BSMV have been reported only in the Canadian prairies. Initially, such plants were believed to be rare (6), but in one field they were subsequently found to be common (8). In this region, wild oats are the most common weed in cultivated soils (1) and, therefore, a high incidence of BSMV-infected wild oats could seriously threaten commercial barley production by favoring a high incidence of BSM in this crop. My results, and those of others, suggest that wild oats are probably resistant to most strains of BSMV from barley. However, it has been shown here that a strain in barley with a high degree of virulence for wild oats could be obtained by previous passage through this weed. Under some circumstances, strains in barley virulent to wild oats might evolve similarly in nature and, via contact transmission, ultimately lead to a high incidence of BSMV-infected wild oats. Because of this possibility, in some regions efforts to breed barley cultivars with resistance to BSMV are probably warranted.

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