Identification of Winter Wheat Cultivars and Experimental Lines Resistant to Wheat Spindle Streak Mosaic Virus

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ABSTRACT

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Five commercial cultivars and eight experimental lines of soft white and soft red winter wheat, selected from hundreds of cultivars and lines screened in field plots, were rated for their reactions to wheat spindle streak mosaic virus (WSSMV) under field and growth-chamber conditions. Ratings were based on symptom severity and virus particle counts determined by immunosorbent electron microscopy. Two of the cultivars and all of the experimental lines showed some resistance to WSSMV. Virus was not detected in leaves from three of the experimental lines. Resting spores of the fungal vector *Polymyxa graminis* were found in roots from susceptible and resistant cultivars, indicating that resistance to WSSMV may be due to resistance to virus infection or multiplication.

Wheat spindle streak mosaic (WSSM) has been prevalent in Michigan winter wheat fields during the past several growing seasons. In the past, diagnosis of WSSM was based on characteristic foliar symptoms during cool spring weather. Recently, immunosorbent electron microscopy (ISEM) with decoration (1,2) has been used to confirm the identity of the causal agent, wheat spindle streak mosaic virus (WSSMV).

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Considerable variation in WSSM symptom expression has been observed in many wheat cultivars and experimental lines in field plots rated for resistance to WSSMV (2). During the same season, symptoms among the different lines have ranged from a mild mottle only on lower foliage to a severe mosaic up to the flag leaf. Similarly, several other workers (3,5,8,9) have observed a wide range in symptom severity among different wheat cultivars and numbered entries. The purpose of this study was to identify winter wheat cultivars and lines potentially resistant to WSSMV on the basis of ratings for symptom expression and virus titer in infected leaves.

MATERIALS AND METHODS

Field studies. Field plots were planted in mid-October of 1980–1983 and rated for symptoms in May of the following year. Symptoms were rated on a scale of 0–2, where 0 = plants without symptoms, 1 = plants with either indistinct symptoms or symptoms of questionable origin, and 2 = plants with distinct, characteristic symptoms. Plants were located in advanced yield trial breeding nurseries containing five-row plots of 30 entries that were replicated three times. Plants chosen for virus titer studies that were performed during the 1982–1983 and 1983–1984 growing seasons were collected from plots, transferred to flats, and placed in a growth chamber at 10 C before sampling for virus. To conserve growth-chamber space, leaves from eight experimental lines chosen for further study were collected and immediately frozen at −20 C. For sampling for virus particle counts, lower leaves from randomly chosen plants from the growth chamber were harvested. Fresh and frozen leaves were ground in liquid nitrogen, and buffer was added in a 1:5 (tissue/buffer) final dilution to extract virus as described previously (1). For virus particle counts, ISEM was performed also as described previously (1). Three virus particle counts were made each year (1983 and 1984) for each cultivar or line. Each count consisted of the average number of particles found on 10 randomly chosen grid squares (GS) per 300-mesh grid.

Cold-frame studies. Cold-frame studies were initiated to provide stable environmental conditions for promotion of uniform infection and to compare disease reactions with those from field-grown plants. Five commercial cultivars and eight experimental lines of winter wheat (*Triticum aestivum* L.) were selected for further study on the basis of field symptoms during the 1980–1984 growing seasons and virus titer studies during the 1982–1983 and 1983–1984 growing seasons. These cultivars included Ionia, Augusta, and Tecumseh, soft white wheat developed at Michigan State University; Genesee, a soft white cultivar from New York; and S-76, a soft red cultivar from Pioneer. Six of the eight experimental lines represented soft white wheat, whereas the remaining two (accession numbers B7321 and B9028) were soft red wheat. All of the experimental lines except 12724 were developed through the Michigan State University breeding program and have pedigrees of diverse genetic origin (Table 1). Seed of these cultivars and lines were planted in flats and germinated in infested soil. The flats were then placed in an outdoor cold frame for 2 mo and transferred to a growth chamber at 10 C as described previously (1). Twelve rows of seed were planted per flat, two rows per cultivar or line, each of which was replicated three times among different flats.

WSSM disease ratings were based on severity of symptoms and virus particle counts in infected leaves determined by ISEM. Symptoms were rated as for field plants with a scale of 0–2, except a rating of 1 was assigned to plants bearing indistinct or mild symptoms. Symptom ratings were made 6 wk after flats were transferred to the growth chamber. Leaf tissue was prepared and virus particle counts were performed as described for field material. Plants were checked for virus biweekly from the day flats were transferred from the cold frame to the growth chamber until heading (10 annual counts).

Examination of roots for *Polymyxa graminis*. Roots from all cultivars and lines from cold-frame-treated plants were examined for the presence of the fungal vector *P. graminis*. Roots were cleared and stained according to a modification of Phillips and Hayman (6). After excision from infected plants, roots were rinsed in distilled water and placed in 10% KOH at 95 C for 1 hr. After an additional distilled water rinse, roots were acidified for 1 hr in 0.1 M HCl, stained with 0.05% trypan blue in lactophenol, and examined with a Wild light microscope at ×600.

RESULTS

Although symptoms on cold-frame plants were not quite as well defined as those found on field plants, field and cold-frame symptom ratings were not significantly different for either the commercial cultivars or experimental lines (Tables 1 and 2). Similarly, virus particle counts from cold-frame plants were somewhat lower than counts obtained from field plants, but this difference also was not significant (Table 2). Virus counts reported from cold-frame plants were averages of 12 particle counts (120 GS) per cultivar or line taken during the weeks of peak virus titer, about 2 mo after placement of flats in the growth chamber. Particle counts for both field and cold-frame plants ranged from 0 to 400 particles per grid square. Because counts for each cultivar and line consistently fell within a narrow range, four groups of particle counts were arbitrarily chosen to represent each range: 0, 1–20, 21–70, and >70 particles per grid square. Cultivars and lines were assigned WSSM susceptibility ratings based on the two criteria of symptom severity and virus particle count groups (Tables 1 and 2).

Three of the commercial cultivars selected (Ionia, Genesee, and Augusta) were rated susceptible to WSSMV. Ionia leaves showed bright and distinct symptoms and frequently yielded virus particle counts as high as 400/GS (Table 2). Augusta and Genesee were also considered susceptible to WSSMV, although particle counts from leaves of these cultivars were somewhat lower (70–200/GS). Less severe symptoms were found on leaves from Tecumseh than on leaves from susceptible cultivars, and particle counts were much lower (15–50/GS). Tecumseh was therefore considered only moderately susceptible to WSSMV. Symptoms on S-76 leaves were even milder and less distinct than those found on Tecumseh leaves, and particle counts were low (0–25/GS). Thus S-76 was rated moderately resistant to WSSMV.

The eight experimental lines selected from field studies (Table 1) failed to develop distinct WSSM symptoms in either cold-frame or field tests, although a light mottle was occasionally found on leaves from field plants of accession number B4145. Virus particles were not found in leaves from three of the experimental lines (B6018, B9028, and 12724); these lines were rated resistant to WSSMV. Fewer than five particles per grid square were found in leaves from three of the other lines (B2231, B7321, and B7322), whereas particle counts from the remaining two lines (B4135 and B4145) were slightly higher (5–20/GS). These five lines therefore were rated moderately resistant to WSSMV.

Resting spores or zoosporangia of *P. graminis* were found in about the same concentration in roots of all cold-frame-grown plants regardless of their disease susceptibility rating. Resting spores and zoosporangia of the parasitic fungus *Olpidium brassicae* were found in about 15% of the samples examined.

Table 1. Wheat spindle streak mosaic virus particle counts of eight wheat lines showing no symptoms in field or cold-frame studies

Accession number	Origin	Particle count[a] (cold frame)	Disease rating[b]
B2231	United States	2	MR
B4135	United States, Japan	8	MR
B4145	United States, New Zealand	15	MR
B6018	United States, Japan	0	R
B7321	Russia	4	MR
B7322	Russia	2	MR
B9028	Yugoslavia, Mexico	0	R
12724	United States	0	R

[a] Numbers are ISEM averages of 120 grid squares for each individual cold-frame-grown wheat line.
[b] MR = moderately resistant and R = resistant. Disease rating is based on symptom severity rating and virus particle counts per entry.

Table 2. Winter wheat commercial cultivar reactions to wheat spindle streak mosaic

Cultivar	Symptom expression[a]		Particle count[b]		Disease rating[c]
	Field	Cold frame	Field	Cold frame	
Ionia	2.0	1.8	350	325	S
Augusta	1.8	1.8	180	150	S
Genesee	1.8	1.5	160	130	S
Tecumseh	1.2	0.8	40	30	MS
S-76	0.8	0.5	15	6	MR

[a] Numbers represent averages of six symptom ratings each for field and cold-frame wheat cultivars. Differences between field and cold-frame ratings were not significant at the 0.05 level of confidence based on the Student's *t* test.
[b] Numbers are the ISEM averages per grid square (GS) obtained by examining 120 GS for individual field and cold-frame wheat cultivars. Differences between field and cold-frame ratings were not significant at the 0.05 level of confidence based on the Student's *t* test.
[c] S = susceptible, MS = moderately susceptible, MR = moderately resistant, and R = resistant. Overall disease rating is based on symptom severity rating and virus particle counts per entry.

DISCUSSION

Resistance to WSSMV was present in two of five commercial soft white or red winter wheat cultivars and in all eight breeding lines examined. As in earlier work by Jackson et al (3), our disease susceptibility ratings were based not only on symptom severity but also on pathogen titer, which affords a better definition of genetically resistant cultivars. A direct correlation between WSSM symptom severity and virus titer was evident; cultivars and lines showing moderate to severe symptoms had high virus particle counts, whereas cultivars and lines showing less severe or no symptoms had few to no particles. To study the inheritance of resistance to WSSM, three of the commercial cultivars and four of the experimental lines were chosen as parents and crossed in a diallel mating design (7). Results of this study, which will be published elsewhere, indicated that resistance was dominant and conferred by one or two genes.

Little is known about yield loss caused by WSSMV because uninfected controls are difficult to run without resorting to extreme measures like soil removal or fumigation (5). We hope that isogenic lines representing genotypes susceptible, moderately susceptible, moderately resistant, and resistant to WSSMV will help determine accurate yield-loss data.

It was interesting that *P. graminis* resting spores were found not only in roots from cultivars susceptible to WSSMV but also in roots from resistant lines. This indicates that resistance to WSSMV may be due to resistance to virus infection or multiplication rather than to resistance to the fungal vector. Similar results were obtained by Larson et al (4) for soilborne wheat mosaic virus.

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