Perennial Wheat Germ Plasm Lines Resistant to Eyespot, Cephalosporium Stripe, and Wheat Streak Mosaic

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ABSTRACT

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A perennial wheat cropping system on the Palouse Prairie of eastern Washington may provide an alternative to the Federal Conservation Reserve Program and reduce soil erosion while providing a harvestable crop for growers. Twenty-four perennial wheat germ plasm lines resulting from crosses between wheat and wheatgrass were evaluated under controlled environment conditions for resistance to *Wheat streak mosaic virus* (WSMV), *Cephalosporium gramineum*, and *Tapesia yallundae* (anamorph *Pseudocercosporella herpotrichoides* var. *herpotrichoides*). Perennial wheat lines SS452, SS103, SS237, MT-2, and PI 550713 were resistant to all three pathogens. Eight lines (33%) were resistant to WSMV at 21°C and 25°C; AT3425 was resistant to WSMV at 21°C but not at 25°C. Thirteen lines (54%) were highly to moderately resistant to *C. gramineum*. Thirteen lines (54%) were resistant to *T. yallundae* in each experiment, but the reactions of four lines differed between experiments. The wheatgrasses *Thinopyrum intermedium* (PI 264770) and *Thinopyrum ponticum* (PI 206624) are reported as new sources of resistance to *T. yallundae*. Perennial wheat must have resistance to these diseases in order to be feasible as a crop in the Pacific Northwest.

The Palouse Prairie of eastern Washington was identified in the 1997 USDA National Resources Inventory as one of the most highly erodible areas in the United States. It is estimated that 10% of the original topsoil in eastern Washington has been lost since cultivation began in the region in the late 1800s (32). Furthermore, runoff from agricultural land reduces water quality in streams and rivers (34), and dust is the leading air pollutant in Washington (33).

Healthy stands of perennial grasses like those established under the Federal Conservation Reserve Program (CRP) reduce soil erosion (17) and competition from weeds (8), restore soil nutrients (9), increase soil quality (11), and increase wildlife habitat (24). Over 29 million acres of highly erodible agricultural land in the United States are currently enrolled in CRP with over 2 million acres located in Washington, Oregon, and Idaho. However, there are more acres of land considered highly erodible than can be enrolled in the current program. Thus, alternative solutions for soil erosion control are needed that are ecologically and economically sustainable.

Perennial wheat has the potential to convert unprofitable land back into production, save state and federal money, conserve agricultural land and natural resources, and provide a profitable crop for growers.

Past efforts to develop perennial wheat for grain production were begun in the 1920s (30,31). Sando hybridized domestic wheat with wheatgrass (then called *Agropyron* spp.) and selected for perennial habit (36), as did Suneson (28) and Suneson and Pope (29). The best perennial lines of those programs yielded only 60% of the grain of the best annual varieties and the project was abandoned because yield was the ultimate goal (29).

The loss of topsoil that results in non-point source water pollution and reduces soil productivity over time has resulted in a renewed interest in perennial wheat. Cultural practices such as modification of seeding date and tillage that are used to reduce disease in conventional and no-till wheat production systems are of limited value in a perennial wheat crop. Inoculum of many pathogens is capable of surviving from crop to crop in senescent tissue, in live root and crown tissue, and in perennial plants (4). Perennial wheat must contain appropriate disease resistance genes for locally important diseases for it to be feasible. Furthermore, resistance must be durable and suppress disease below an economic threshold to reduce external inputs and enhance the profitability of perennial wheat (37). It is possible that combining genotypes and pyramiding re-

sistance genes from wild relatives of wheat that durable resistance can be achieved within a perennial wheat cropping system.

Perennial wheat is essentially a no-tillage system with persistent crowns and residue in the soil and as such, could potentially serve as a reservoir for pathogens that survive in plant debris (4). Eyespot, Cephalosporium stripe, and wheat streak mosaic (WSM) caused by *Tapesia yallundae* Wallwork & Spooner and *T. acuformis* (Boerma, Pieters & Hamers) Crous (anamorphs *Pseudocercosporella herpotrichoides* (Fron.) Deighton var. *herpotrichoides* and var. *acuformis*, respectively), *Cephalosporium gramineum* Nisikado & Ikata, and *Wheat streak mosaic virus* (WSMV), respectively, are among the most significant diseases in the intermediate- and low-rainfall regions of Washington for which perennial wheat is being developed (12). The fungi that cause eyespot and Cephalosporium stripe survive in crop residues (38) and thus, may be favored in a perennial wheat cropping system. Likewise, annual wheat escapes infection by WSMV in most years because it dies and does not overlap with potential reservoir hosts. Perennial wheat, by virtue of its regrowth after harvest, may become infected by WSMV resulting in less productivity, shorter stand duration, and it may serve as an inoculum reservoir for nearby annual small grain crops.

Several wheatgrasses are resistant to many of the diseases that infect annual wheat, which include Cephalosporium stripe, WSM, stripe rust, leaf rust, and barley yellow dwarf (6,7,13,15). Despite efforts by breeders to exploit the genetic resources of the wheatgrasses and other members of the Triticeae, winter wheat cultivars adapted for production in the Pacific Northwest with highly effective resistance to Cephalosporium stripe and/or WSM are not available (13). Control of these diseases has relied on cultural practices such as elimination of volunteer wheat to prevent carryover of virus and vectors from spring to winter crops, 3-year rotations between susceptible wheat crops, destruction of residue by tillage or burning, and delayed seeding in the autumn (23,38). Eyespot is controlled largely with disease-resistant cultivars and foliar fungicides (10,22,23). Cultural practices such as seeding date and tillage for disease control will not be feasible in a perennial wheat crop-

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ping system and, therefore, breeding for combined resistance to eyespot, Cephalosporium stripe, and WSM is critical for the successful development of commercially acceptable perennial wheat cultivars.

The objective of this study was to identify perennial breeding lines [wheatgrass (*Thinopyrum* spp.) × wheat] with individual or combined resistance to eyespot, Cephalosporium stripe, and WSM for use as parents in development of perennial wheat.

MATERIALS AND METHODS

Perennial wheat germ plasm. Twenty-four perennial wheat germ plasm lines were evaluated. Eighteen of the 24 lines originated from the Sando and Suneson collections of annual wheat cultivars (*Triticum* spp.) hybridized with *Agropyron elongatum* (synonym *Thinopyron ponticum* Barkworth & Dewey, $2n = 10x = 70$) or *A. intermedium* (synonym *Thinopyron intermedium* (Host) Barkworth & Dewey, $2n = 6x = 42$). It should be noted that the taxonomy of the wheatgrasses is in flux. Currently accepted names based on chromosome number are used in this paper except where consistency with previous work is intended. These lines, designated "SS", represent advanced yet heterogeneous genotypes with varying chromosome constitutions from 42 to 56 (data not presented). AT3425 (Agrotriticum No. 3425; wheat × *Thinopyron ponticum*), PI 550713 (wheat × *Thinopyron ponticum*: 14 *Thinopyrum* chromosomes + 3 wheat-*Thinopyrum* translocated chromosomes) and PI 550715 lines are perennial amphiploids with 56 chromosomes; Montana-2 (MT-2; *Triticum durum* x *Thinopyron intermedium*) is not stable (14). The perennial wheatgrasses *Thinopyron intermedium* (PI 264770) and *Thinopyron ponticum* (PI 206624) were also included in this study.

Wheat streak mosaic. Stephens winter wheat (CI 17596) is highly susceptible to WSMV. It was used as a susceptible control and was mock-inoculated with buffer + diatomaceous earth for the negative control. WSMV was cultured in Daws winter wheat (CI 17419) and used as the inoculum source.

Seeds of each genotype were imbibed at 4°C for 4 days on moist filter paper in petri dishes to synchronize germination and then sown five per pot in 8.9-cm-square pots filled with Thatuna silt loam (TSL)/vermiculite/sand mix (90:5:5, wt/wt/wt), pH 4.5. Experimental and source plants were grown in a growth chamber at 21 to 22°C with a 12-h photoperiod. Plants were fertilized every 2 weeks by adding a solution of 300 mg/liter Nutriculture (20-20-20 N-P-K, Plant Marvel Lab, Chicago, IL) to each pot. Plants were thinned to three per pot prior to inoculation.

Pots were arranged in a randomized complete block design with five replicates and the experiment was repeated twice.

The third experiment differed from the first two in that (i) only lines identified as resistant from the first two experiments were included, (ii) the temperature in the growth chamber was raised to 25°C 18 days after the second inoculation, and (iii) plants were grown for another 2 weeks after raising the temperature.

Inoculum preparation and inoculation. The WSMV strain used throughout this study was the Washington/Idaho isolate registered in GenBank (Accession No. AF034415, courtesy of P. McCarthy and P. Berger, University of Idaho). Inoculum was extracted from infected source plants by grinding young, symptomatic leaves with a mortar and pestle in 0.01 M sodium phosphate buffer (1:10, wt/vol), pH 7.4, and diatomaceous earth (0.01 g/ml). Test plants were inoculated at the two- to three-leaf stage by sandwiching the second leaf in cheesecloth saturated with inoculum between the thumb and forefinger, and rubbing the blade once from base to tip (18). The third leaf of each plant was inoculated 8 to 10 days after the first inoculation.

Disease evaluation. Plants were visually evaluated for the presence or absence of WSM symptoms prior to harvest. Eighteen days after the second inoculation the youngest leaves of all plants in each pot were harvested (inoculated leaves were avoided) and tested for the presence of WSMV by indirect enzyme-linked immunosorbent assay (ELISA) (3). Plant tissue was cut into 1- to 3-cm pieces, frozen in liquid nitrogen, and ground with a mortar and pestle. Carbonate coating buffer at a concentration of 0.02 M, pH 9.5 was added (1:10, wt/vol) and the tissue was further ground until the liquid turned dark green. To individual wells of a 96-well, flat bottom, ELISA plate (Corning Glass Works, Corning, NY), 200 μl of each treatment sample was added. Plates were incubated at room temperature for 2 h, and then rinsed with ELISA wash (0.8 g KH_2PO_4, 0.8 g KCl, 4.6 g anhydrous $NaPO_4$ dibasic, 32.0 g NaCl, 2.0 ml Tween 20, and 4.0 ml tap water; pH 7.3) three times for 3 min each. Two-hundred microliters of purified anti-WSMV IgG (1 μg/ml) in V/C buffer (20 g Sigma polyvinyl-pyrrolidone, 2 g ovalbumin, 1 liter ELISA wash) was added to each well and incubated at room temperature for 2 h, and then washed as described above. The IgG was purified by mixing 6.5 ml of phosphate-buffered saline (PBS) and 7.5 ml of saturated ammonium sulfate to 1.0 ml of WSMV antiserum and allowed to sit on ice for 1 h. The mixture was centrifuged at 10,000 rpm for 10 min and resuspended in 1.0 ml of PBS. Goat antirabbit IgG-alkaline phosphatase conjugate (1:3,200 in V/C buffer; Sigma Chemical Co., St. Louis, MO) was then added to each well and incubated for another 2 h, washed as above, and *p*-nitrophenyl phosphate (PNP) (1 mg/ml in diethanolamine

buffer, pH 9.8) was added. Plates were incubated in the light at room temperature for 30 min and then optical density at 405 nm was measured with a Molecular Devices E-max microplate reader (Molecular Devices, Menlo Park, CA). Triplet wells of each treatment, a carbonate buffer control, a mock-inoculated control (healthy Stephens), and an inoculated control (Stephens) were included on each plate. Peripheral wells of all plates remained empty.

ELISA readings were considered positive if absorbance readings were at least three times greater than the healthy control. Preliminary studies indicated that ELISA values were positive when one out of three or four plants per pot were symptomatic. Statistical analyses were not conducted due to the qualitative nature of the assay. A line was considered susceptible if a positive reading was expressed in one or more replicates or resistant if all readings were negative.

Cephalosporium stripe. Perennial wheat lines were screened for resistance to *C. gramineum* under controlled environment conditions. Madsen (PI 511673) and Stephens winter wheat cultivars (both susceptible), WA7437 (moderately resistant), and AT3425 (highly resistant) were included as controls. Seeds were imbibed in petri dishes on moist filter paper at 4°C for 4 days to synchronize germination, then sown into sand-filled galvanized steel flats and placed in a growth chamber at 15°C with 12 h of light. Plants were pulled from the sand when most of the seedlings were at the two-leaf-stage of growth and then transplanted four per pot, into 15.2-cm-diameter pots filled with Thatuna silt loam soil mix (described above). Prior to planting, each pot was weighed and filled with 1,315 g oven-dry equivalent of soil. Pots were drenched with water once a week to maintain gravimetric soil water content at 40% (approximately –0.01 MPa) (1). Soil moisture was maintained with regular watering between drenches. Plants were fertilized every 2 weeks by adding 50 ml of 300 mg/liter Nutriculture to each pot.

Inoculum preparation and inoculation. A wild-type isolate of *C. gramineum* (Cg84-30) originally isolated from Stephens winter wheat was used. Ten-mm-diameter plugs of corn meal agar colonized by Cg84-30 were removed from petri dishes with a sterile dissecting needle and added one or two at a time to 50 ml of sterilized potato dextrose broth in 250-ml flasks. Liquid cultures were incubated at room temperature on a rotary shaker (70 to 90 rpm). After 5 to 8 days of growth the cultures were strained through a double layer of sterile cheesecloth to remove the mycelium from the suspension. The suspension was centrifuged for 15 to 20 min at 6,300 rpm (~5,468 g) at room temperature. The supernatant was discarded and the pellet was resuspended in distilled

water and centrifuged a second time. Spore concentration was calculated with the aid of a hemacytometer and adjusted to 1×10^6 spores per g of soil with tap water.

Immediately after transplanting, plants were inoculated by pouring 150 ml of a conidial suspension on the soil surface of each pot (26). Plants were reinoculated with 50 ml of conidia prepared in the same manner 1, 2, and 3 weeks after the initial inoculation, for a total of four inoculations over a 3-week-long prevernalization period. Fresh inoculum was prepared for each inoculation.

After the last inoculation, plants were vernalized at 4°C for 6 weeks with a 10-h photoperiod. Plants were then moved to a greenhouse and allowed to grow until shortly after anthesis, about 6 to 8 weeks, when disease was evaluated.

Disease evaluation. Plants were rated visually for disease expression during the early stages of kernel development. Individual stems in each pot were rated on a 0 to 5 scale where 5 = a dead stem (white head); 4 = chlorotic stripes and or necrotic vascular bundles on the flag leaf; 3 = chlorotic stripes and or necrotic vascular bundles on the leaf below the flag leaf; 2 = chlorotic stripes and or necrotic vascular bundles on the second leaf below the flag leaf; 1 = chlorotic stripes and or necrotic vascular bundles on the third leaf below the flag leaf; and 0 = no symptoms in the uppermost four leaves (27). Disease incidence was expressed as the percentage of symptomatic stems per pot and disease severity was calculated as the mean rating of symptomatic stems. Disease index was calculated by multiplying disease incidence by disease severity and dividing by five to obtain a value ranging from 0 to 100 (27).

Experimental design and data analysis. Pots were arranged in a randomized complete block design with five replicates per line. The experiment was repeated. Data were analyzed with analysis of variance (ANOVA) using SAS statistical analysis software (SAS Institute, Cary, NC). Means were separated using Fisher's protected LSD ($P = 0.05$). A line was considered susceptible if its mean disease index was significantly greater or resistant if it was not significantly greater than the resistant controls (AT3425 and WA7437).

Eyespot. Perennial lines were screened for resistance to eyespot under controlled environment conditions using a modified method of de la Peña and Murray (5). Madsen and VPM-1 were included as resistant controls and Eltan as a susceptible control.

Seeds were imbibed on moist filter paper in petri dishes at 4°C for 4 days to synchronize germination. Two seeds of each genotype were sown in 5-cm-diameter pots in a Thatuna silt loam and covered with approximately 2 cm of 2:1 vermiculite/TSL. Plants were grown in a growth chamber at 15°C with 12 h of light

and 98 to 100% relative humidity. Pots were arranged in flats (up to 50 pots per flat) and approximately 2 to 3 liter of water was added to each flat once a week. Plants were fertilized every 2 weeks by adding a solution of 300 mg/liter Nutriculture to the flats after seedling emergence.

Inoculum preparation and inoculation. A β-D-glucuronidase (GUS)-transformed strain of *T. yallundae* (tPhmon8) was used as inoculum (5). Cultures on potato dextrose agar were cut into small pieces, macerated in sterile water, spread on water agar plates, and incubated under continuous UV light at 16°C for 2 to 3 weeks to induce sporulation. Plates were periodically agitated to maintain moisture across the agar surface, and sterile water was added as necessary to avoid drying. Conidia were harvested by scraping the agar plates with a bent glass rod and counted with a hemacytometer. They were then added to a water agar slurry to give a final concentration of 1×10^5 conidia per ml (5).

A drinking straw approximately 3.2 cm long × 6.0 mm wide and split along its length was placed around the coleoptile of each plant near the soil line. When plants reached the 1.5- to 2-leaf-stage of growth, 250 µl of the inoculum slurry was placed in the straw collar of each plant (5). Plants were reinoculated the next day using the same volume and slurry.

Disease evaluation. Plants were evaluated for disease severity 6 weeks after inoculation. The shoots of each plant were clipped approximately 1.5 cm above the straws and discarded. The stem bases were removed from the pots, the roots discarded, and the lowermost 4 cm of each plant was washed briefly with tap water to remove soil debris. Each plant was then rated visually for disease severity using a 1 to 4 scale modified from de la Peña and Murray (5) where: 1 = no disease; 2 = a lesion on the first sheath and/or brown flecking (tiny lesions) on the first or second sheath (hypersensitive reaction); 3 = a well-defined lesion(s) on the first and second sheaths; 4 = lesion(s) girdling or covering over two-thirds of the area of the first and second sheaths. Individual stems were wrapped in paper towels and frozen at –20°C until further analyses.

Colonization was further quantified by measuring GUS produced by the transformed pathogen during colonization of the plant. GUS activity was determined by the conversion of 4-methylumbelliferyl β-D-glucoside (MUG) to 4-methylumbelliferone (MU) by GUS (5). GUS activity was expressed as nm of MU produced per sample. Each plant (1 to 3 tillers) was ground in 2.5 ml of extraction buffer (50 mM NaHPO4, pH 7.0, 5 mM dithiothreitol, 10 mM Na₂EDTA, 0.1% sodium lauryl

Table 1. Reaction to *Wheat streak mosaic virus* (WSMV) of perennial wheat lines in three experiments under controlled environment conditions

| Line | Number of positive plants per total[a] | | | Phenotype[d] |
	Experiment 1[b]	Experiment 2[b]	Experiment 3[c]	
Noninoculated control	0/5	0/5	0/5	…
MT-2	0/5	0/5	0/5	R
PI 206624	0/5	0/5	0/5	R
PI 264770	0/5	0/5	0/5	R
PI 550713	0/5	0/5	0/5	R
PI 550715	0/5	0/5	0/5	R
SS103	0/5	0/5	0/5	R
SS237	0/5	0/5	0/5	R
SS452	0/5	0/5	0/5	R
AT3425	0/5	0/5	5/5	S
SS191	0/5	1/5	4/5	S
SS679	0/5	5/5	…	S
SS49	5/5	5/5	…	S
SS56	5/5	5/5	…	S
SS60	5/5	5/5	…	S
SS241	3/5	5/5	…	S
SS259	2/5	3/5	…	S
SS364	3/5	4/5	…	S
SS365	2/5	5/5	…	S
SS485	5/5	5/5	…	S
SS498	5/5	5/5	…	S
SS524	5/5	5/5	…	S
SS744	5/5	5/5	…	S
SS767	3/5	4/5	…	S
SS791	3/5	5/5	…	S
Inoculated control	5/5	5/5	5/5	S

[a] Enzyme-linked immunosorbent assay (ELISA) absorbance readings were considered positive (susceptible) if values were threefold greater than those of the healthy check.
[b] Plants were tested for WSMV by ELISA 18 days after the second inoculation.
[c] Only plants resistant in experiments 1 and 2 were evaluated. The temperature was raised to 25°C 18 days after the second inoculation and plants were tested using ELISA 2 weeks later.
[d] A line was considered susceptible (S) if a positive reading was expressed in one or more replicates, or resistant (R) if all readings were negative.

sarcosine, 0.1% Triton X-100) and the extract was poured into 1.2 ml microfuge tubes and frozen at –20°C until analyses.

GUS determinations were conducted by adding 40 μl of MUG substrate to 50 μl of sample in 1.2-ml plastic tubes and then incubating them at 37°C for 60 min. Adding 1.0 ml of 0.2 M sodium carbonate to each tube stopped the reaction. Two hundred microliters of each sample was then transferred into individual wells of black microtiter plates, and fluorescence determined immediately with a fluorimeter (Dynatech Laboratories, Inc., Chantilly, VA). Each plate included two MUG, extraction buffer and stop buffer checks, and four MU standards of different concentration.

Experimental design and data analyses. Pots were arranged in a randomized complete block design with six replicates. The experiment was repeated. The second experiment differed from the first in that disease evaluation and GUS activity were determined only from the main stem rather than all stems on a plant. Both visual and GUS data were analyzed by ANOVA using SAS statistical analysis software. GUS scores were transformed logarithmically due to the nonnormal distribution of the means and variances. Means were separated using Fisher's protected LSD ($P = 0.05$). Individual lines were considered

susceptible if the GUS score was significantly greater or resistant if the GUS score was not significantly different from the resistant control (Madsen).

RESULTS

Wheat streak mosaic. All samples with visible symptoms of WSM reacted positively for the presence of WSMV in the ELISA assay. Individual lines varied in their reaction to WSMV in repeated experiments (Table 1), but all inoculated controls reacted positively and all mock-inoculated healthy controls reacted negatively for the presence of WSMV as determined by ELISA.

Eleven of the 24 perennial lines were resistant in the first experiment (Table 1) and nine of those 11 lines were resistant in the second experiment. AT3425 was resistant in the first two experiments, but tested positive in the third experiment after the temperature was raised to 25°C and was considered susceptible.

Cephalosporium stripe. There were significant ($P < 0.05$) differences among perennial lines for disease incidence and severity in both experiments and, therefore, disease index was used to compare lines (Table 2). Homogeneity of variance did not differ significantly between experiments, and thus, the data were combined for analysis. Disease did not develop on PI

206624, PI 264770, or SS452 in either experiment; disease indices for eight other perennial lines were less than 6.0 when averaged across experiments, and all were considered resistant. By comparison, average disease indices for Madsen and Stephens, the susceptible controls, were 31.5 and 35.3, respectively. The average disease indices of 10 perennial lines were the same or significantly greater than Madsen and Stephens and were considered susceptible.

Eyespot. Homogeneity of variance differed significantly for disease index but not for GUS score between experiments and thus, they were analyzed separately. GUS scores differed significantly among perennial lines in the first experiment ($P = 0.0001$) (Table 3). GUS scores for MT-2 and PI 206624 were significantly ($P \leq 0.05$) less than Madsen, and 11 other perennial lines had GUS scores that were not statistically different from Madsen; all were considered resistant. All other perennial lines had GUS scores that were significantly greater than Madsen and were considered susceptible. Disease indices ranged from 1.0 to 3.5 and, with the exception of SS744, was consistent with GUS scores in assigning phenotype (Table 3).

GUS scores likewise differed significantly among lines in the second experiment ($P < 0.05$) (Table 3). Fourteen perennial lines had GUS scores that were not statistically different from Madsen and were considered resistant. Disease indices ranged from 1.3 to 3.2 and with the exception of SS237 and SS452 was consistent with GUS score in assigning phenotype.

Four perennial lines differed in their reaction between experiments. Lines SS679 and SS744 were resistant in the first experiment and susceptible in the second. In contrast, lines SS241 and SS56 were susceptible in the first experiment but resistant in the second.

DISCUSSION

Lines MT-2, PI 550713, PI 550715, SS103, SS237, and SS452 were resistant to Cephalosporium stripe, wheat streak mosaic, and eyespot based on our tests. The wheatgrasses *Thinopyrum intermedium* (PI 264770) and *Thinopyrum ponticum* (PI 206624) were likewise resistant to these three diseases. Genes from the wheatgrasses confer perennial habit and are also the probable source of resistance to these diseases. Lines SS49, SS56, SS60, SS485, SS498, SS524, and SS791 were susceptible to all three pathogens and although the objective of this work was to identify potential sources of resistance for development of perennial wheat cultivars, these susceptible lines may be useful as controls in future studies and as parental lines in genetic studies.

Lines SS452, SS679, MT-2, PI 206624, and PI 264770 did not develop symptoms of Cephalosporium stripe in the first

Table 2. Cephalosporium stripe disease index for perennial wheat lines determined in two controlled environment experiments

Line	Experiment 1[a]	Experiment 2	Mean	Phenotype[b]
PI 264770	0	0	0	R
PI 206624	0	0	0	R
SS452	0	0	0	R
MT-2	0	1.7	0.9	R
PI 550713	0.5	2.0	1.3	R
SS191	0.9	2.9	1.9	R
SS103	2.4	2.3	2.4	R
SS679	0	4.7	2.4	R
SS364	3.7	2.5	3.1	R
AT3425 (control)	0.2	7.3	3.8	R
SS237	8.0	2.7	5.4	R
SS365	4.5	12.8	8.7	R
SS259	0.6	21.5	11.1	R
WA7437 (control)	7.1	16.9	12.0	R
PI 550715	9.1	20.6	14.9	R
Madsen (control)	26.0	36.9	31.5	S
SS791	22.9	41.4	32.2	S
SS524	30.3	36.3	33.3	S
SS241	29.2	41.0	35.1	S
Stephens (control)	37.9	32.6	35.3	S
SS744	27.5	43.4	35.5	S
SS485	34.6	50.9	42.8	S
SS60	50.6	35.2	42.9	S
SS498	47.6	49.0	48.3	S
SS56	39.6	62.1	50.9	S
SS49	63.8	38.7	51.3	S
SS767	48.2	57.6	52.9	S
LSD[c] ($P = 0.05$)	13.7	15.1	10.8	

[a] Disease index is determined by multiplying the percent infected stems by the average disease severity (rated on a 0 to 5 scale where 0 = no symptoms in the uppermost four leaves and 5 = a dead stem), and dividing by 5 to obtain a value ranging from 0 to 100.

[b] A line was considered susceptible (S) if its mean disease index was significantly greater or resistant (R) if it was not significantly different than the resistant controls (AT3425 and WA7437).

[c] LSD = Fisher's least significant difference. Values within columns represent the mean of five replicates with four plants per replicate.

greenhouse experiment and had low or no disease in the second experiment (Table 2). Although Cephalosporium stripe developed to some extent on SS237, SS259, and SS365, these lines were considered resistant since the mean disease index for each was statistically equal to AT3425, the resistant control. Similar results were obtained from a field plot near Ritzville, WA that was naturally infested with *C. gramineum* (*unpublished data*). Previous work also demonstrated effective resistance to Cephalosporium stripe in *A. elongatum* and *A. intermedium* (20). Mathre and Johnston (19) showed that movement of *C. gramineum* was slower through roots and especially in crown tissues of *A. elongatum* and *A. intermedium* than in wheat lines. Resistance to *C. gramineum* has been transferred from AT3425 to wheat and is being used for the development of winter wheat cultivars. Some of these lines have been grown in field plots for more than 5 years now and the resistance has remained effective (Jones and Murray, *unpublished*).

Eleven lines were resistant to WSMV in the first experiment, nine were resistant in the second experiment, and all but one of these nine was resistant at the higher temperature in the third experiment (Table 1). PI 206624 and PI 264770 were also resistant to WSMV, which agrees with previous work (6,21). Mite resistance, another approach for limiting the spread of WSMV, was first identified by Andrews and Slykhuis (2) in hybrids derived from crosses of wheat x *A. elongatum* and *A. intermedium*. Resistance gene *Wsm1* from *A. intermedium* confers resistance to WSMV and to wheat curl mite colonization (6).

It should be noted that some lines had a variable reaction to WSM in these experiments. Specifically, not all plants of lines SS191, 241, 259, 364, 365, 767, and 791 reacted positively in ELISA tests in one or more experiments. It is possible that these lines are heterogeneous for resistance to WSM or still segregating for resistance. Indeed, subsequent work with fluorescent in situ hybridization (FISH) has indicated that plants within some SS lines (e.g., 241) carry unequal numbers of telocentric chromosomes that may carry genes conferring WSM resistance (Li and Murray, *unpublished*). For the sake of simplicity and because our goal was to identify potential parents for a breeding program, we elected to classify plants as susceptible or resistant.

Anecdotal observations (Murray and Jones, *unpublished*) indicated that AT3425 was susceptible to WSM under field conditions, but it did not develop symptoms and tested negative for presence of WSMV by ELISA in the first two experiments. The temperature was increased to 25°C after inoculation in the third experiment, and plants were allowed to grow 2 weeks longer than in the first two experiments. Three days after raising the temperature, chlorotic spots and streaks were visible on

the young leaves of some plants of AT3425. Two weeks after the temperature was increased, mosaic was evident on all plants of AT3425 and ELISA readings were positive.

The absence of symptoms and positive ELISA reactions for AT3425 in the first two experiments may be the result of genetic instability (14) but is more likely due to a temperature sensitive resistance. Seifers et al. (25) showed that when certain *A. intermedium*-wheat translocation lines are inoculated 11 days following planting at 20°C under controlled conditions they are resistant to WSM, but not at 27°C. These lines carried only the short arm of the *A. intermedium* chromosome 4Ai-2. Although WSM resistance in these lines was temperature sensitive, it appeared stable enough to protect against disease under field conditions. AT3425 is currently being used as a parent in the development of new adapted perennial lines, but the usefulness of resistance to WSM derived from it under field conditions needs further investigation in light of these data.

Based on GUS and visual data from the first experiment, PI 264770, PI 206624, and MT-2 are more resistant to *T. yallundae* than Madsen. Resistance to *T. yallun-*

dae exists in other wild wheats (18), but has not been reported previously in the genus *Thinopyrum*. It would be worthwhile to characterize resistance in these species to further broaden the genetic base of resistance genes available to wheat breeding programs and to facilitate the incorporation of this resistance into perennial lines.

Eyespot resistance in this study was determined using an isolate of *T. yallundae* (formerly W pathotype). However, recent data indicates that *T. acuformis* (formerly R pathotype) has become more prevalent in the Pacific Northwest during the last 10 years (Douhan and Murray, *unpublished*). Resistance to *T. acuformis* has not been evaluated fully in wheat and its relatives, and recent evidence suggests that different genes may condition resistance to this species (35). Further work is needed to characterize resistance to *T. acuformis* given its increased prevalence and to ensure eyespot resistance in perennial wheat is effective against both pathogen species.

Of the perennial amphiploids that were resistant to all three diseases in this study, MT-2 has been used as a parent in perennial wheat and forage grass breeding programs elsewhere, although sterility is high and progeny lines are unable to sustain

Table 3. Eyespot disease severity and β-D-glucuronidase (GUS) scores for perennial wheat lines determined in two experiments.

	Experiment 1			Experiment 2		
Line	DS[a]	GUS score[b]	Phenotype[c]	DS	GUS score	Phenotype
MT-2	1.0[d]	0.56	R	1.5	0.68	R
PI 206624	1.1	0.68	R	1.3	0.85	R
PI 264770	1.1	0.72	R	1.5	0.97	R
VPM (control)	1.4	0.82	R	1.5	1.04	R
PI 550715	1.3	0.84	R	1.5	0.74	R
PI 550713	1.7	0.91	R	1.8	0.98	R
AT3425	1.5	0.93	R	1.4	0.87	R
Madsen (control)	1.7	1.00	R	1.5	1.00	R
SS 259	1.7	1.01	R	1.6	0.90	R
SS 679	1.6	1.03	R	2.4	1.40	S
SS 237	2.2	1.08	R	2.1	1.25	R
SS 452	1.6	1.09	R	2.2	1.32	R
SS 103	2.2	1.23	R	1.5	1.14	R
SS 744	2.3	1.23	R	2.2	1.70	S
SS 767	2.0	1.25	R	1.9	1.12	R
SS 191	2.3	1.30	S	2.8	1.95	S
SS 364	2.8	1.38	S	2.27	1.36	S
SS 241	2.3	1.44	S	1.3	0.63	R
SS 498	2.3	1.45	S	2.8	2.02	S
SS 365	2.5	1.46	S	1.8	1.36	S
Eltan (control)	2.6	1.55	S	3.1	1.96	S
SS 49	2.9	1.55	S	2.9	1.94	S
SS 485	2.9	1.60	S	2.9	1.97	S
SS 56	2.7	1.61	S	2.0	1.07	R
SS 524	3.0	1.66	S	3.2	2.16	S
SS 791	3.0	1.70	S	3.1	1.74	S
SS 60	3.5	1.89	S	2.5	1.40	S
LSD[e] (P=0.05)	0.6	0.29	…	0.4	0.35	…

[a] DS = average disease severity of symptomatic stems on a 0 to 4 scale, where 1 = healthy and 4 = lesions covering over two-thirds of the stem base.

[b] β-D-glucuronidase (GUS) scores are expressed as the ratio of the accession to the resistant control (Madsen) plus one [$\log_{10}$ (X/Madsen) + 1)]. Lines more resistant than the control have values less than one and lines more susceptible than the control have values greater than one.

[c] Individual lines were considered susceptible (S) if the GUS score was significantly greater or resistant (R) if the GUS score was not significantly different from the resistant control.

[d] Values within columns represent the mean of five replicates with two plants each.

[e] LSD = Fisher's least significant difference.

yield over time (14). Relative to wheat, most of the amphiploids are difficult to thresh, their spikelets are smaller, and the heads are less compact. For example, the average seed weight of MT-2 is almost three times greater than *A. intermedium*, but less than half that of a typical annual wheat cultivar (16). In related field studies, the amphiploids appeared vigorous in field plots located near Ritzville and Kahlotus, WA, once they were established. MT-2 emerged poorly, but was the most vigorous of all amphiploids tested once established (Murray and Jones, *unpublished*).

Resistance to eyespot, Cephalosporium stripe, and WSM was identified in several perennial wheat amphiploids using growth chamber tests in this study. Further work is needed to transfer resistance to these diseases into adapted perennial and annual wheat lines and test it under field conditions before it is known how useful they will be on a commercial scale.

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