Identifying New Sources of Resistance to Eyespot of Wheat in *Aegilops longissima*

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Abstract

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Eyespot, caused by *Oculimacula yallundae* and *O. acuformis*, is an economically important disease of wheat. Currently, two eyespot resistance genes, *Pch1* and *Pch2*, are used in wheat breeding programs but neither provides complete control or prevents yield loss. *Aegilops longissima* is a distant relative of wheat and proven donor of genes useful for wheat improvement, including disease resistance. Forty *A. longissima* accessions and 83 *A. longissima* chromosome addition or substitution lines were evaluated for resistance to eyespot. Among the 40 accessions tested, 43% were resistant to *O. yallundae*, 48% were resistant to *O. acuformis*, and 33% were resistant to both. Addition or

substitution lines containing chromosomes 1S¹, 2S¹, 5S¹, and 7S¹, and a 4S¹7S¹ translocation were resistant to *O. yallundae*. Chromosomes 1S¹, 2S¹, 4S¹, and 5S¹ contributed to resistance to *O. acuformis* more than others. Chromosomes 1S¹, 2S¹, 5S¹, and 7S¹ provided resistance to both pathogens. This is the first report of eyespot resistance in *A. longissima*. These results provide evidence that genetic control of eyespot resistance is present on multiple chromosomes of the S¹ genome. This research demonstrates that *A. longissima* is a potential new source of eyespot resistance genes that could broaden the genetic diversity for wheat improvement.

Eyespot is an economically important disease of winter wheat in the U.S. Pacific Northwest (PNW) and other areas of the world with cool, wet autumn and winter weather (39). Winter wheat occupies about 80% of the total PNW wheat acreage. Yield losses of up to 50% as a result of eyespot can occur in commercial wheat fields when disease is severe (39).

Eyespot is caused by the soilborne fungi *Oculimacula yallundae* (Wallwork & Spooner) Crous & W. Gams (syn: *Tapesia yallundae* Wallwork & Spooner) and *O. acuformis* (Boerema, R. Pieters & Hamers) Crous & W. Gams (syn: *T. acuformis* Boerema, R. Pieters & Hamers) (11). These fungi were known as the W and R types of *Pseudocercospora herpotrichoides* (Fron) Deighton, respectively, prior to identification of the teleomorph and their separation into distinct species (34). Both species affect the stem base of wheat and produce indistinguishable elliptical lesions that result in reduced grain fill and lodging. Foliar fungicides have played a major role in control of eyespot. However, the limited selection of registered fungicides in the PNW as well as resistance in *Oculimacula* spp. to benzimidazole fungicides have resulted in the need for alternative disease management strategies (38). Planting disease-resistant cultivars is the most economical and effective strategy to control eyespot.

Currently, two resistance genes, *Pch1* and *Pch2*, are used in wheat breeding programs. *Pch1* was transferred from *Aegilops ventricosa* Tausch (2n = 28, DDM'M') into the breeding line VPM-1 (17) and is a single dominant gene located near the distal end of chromosome 7DL (51). *Pch1* is very effective in limiting eyespot development but does not protect wheat completely (28). *Pch2*, from the French 'Cappelle Desprez', acts as a single partially dominant gene (47). Law et al. (30) found that chromosomes 1A, 2B, 5D, and 7A of Cappelle Desprez influenced eyespot re-

sistance, with a major component on chromosome 7A. de la Peña et al. (15) mapped this gene to the distal portion of chromosome 7AL.

Several wheat cultivars containing *Pch1* have been developed. 'Madsen' was released in 1988 (1) and was widely grown in the PNW for about two decades (39). Cappelle Desprez contains *Pch2* and was grown extensively from the 1950s to 1970s in the United Kingdom; its resistance has been transferred to many other wheat cultivars (24). Although the eyespot resistance of Cappelle Desprez has been durable, *Pch2* is less effective than *Pch1* (27). More resistance genes are desired for incorporation into wheat cultivars to improve overall effectiveness and broaden the genetic diversity of disease resistance.

The common wheat gene pool contains little resistance to soilborne pathogens (33). Wild relatives of wheat have been sources of resistance genes for many diseases, and genes conferring numerous traits have been transferred from wild species into bread wheat (26,28,45). *Pch1* was the first successful example of an alien gene for eyespot resistance in commercial wheat (1). Murray et al. (40) found resistance to *O. yallundae* on chromosome 4V of *Dasypyrum villosum* (L.) Candargy (syn. *Haynaldia villosa* L.) (2n = 14, VV). Yildirim et al. (52) mapped a single dominant gene to the distal portion of *D. villosum* chromosome 4VL with restriction fragment length polymorphism (RFLP) markers; however, this gene has not yet been transferred into wheat cultivars. Other sources of resistance to eyespot have been identified in *Triticum tauschii* (syn. *A. squarrosa*, 2n = 14, DD) (53), *T. monococcum* (2n = 14, AA) (5,6), *T. dicoccoides* (2n = 28, AABB) (20), *A. kotschy* (2n = 28, UUS'S') (48), *Thinopyrum ponticum* (2n = 70, JJJJ'S') (10,31), and *T. intermedium* (2n = 42, StJJ'S') (10,32).

A. longissima Schweinf. & Muschl. (2n = 14, S'S') is a diploid species in the section *Sitopsis* of *Aegilops* (50) that has been used as a donor of numerous genes for wheat improvement, including disease resistance (21). Ecker et al. (18) identified *A. longissima* accessions that were highly resistant to Septoria glume blotch of wheat. Powdery mildew resistance gene *Pm13* was mapped to the short arm of chromosome 3S¹ of *A. longissima* and transferred into 'Chinese Spring' wheat (7,8). Anikster et al. (2) reported that *A. longissima* carried genes for resistance to stripe rust, leaf rust, and stem rust of wheat. Prior to this study, *A. longissima* had not been examined for resistance to eyespot.

The objectives of this research were to identify potential new sources of genetic resistance to *O. yallundae* and *O. acuformis* from *A. longissima* and find genomic locations associated with resistance to

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these pathogens. Because the responses of *A. longissima* accessions to *O. yallundae* and *O. acufiformis* were different in preliminary experiments (*data not shown*), the pathogen species were tested separately to determine whether the genes that confer resistance to *O. yallundae* and *O. acufiformis* in *A. longissima* are independent.

Materials and Methods

Plant materials. Forty *A. longissima* accessions obtained from the United States Department of Agriculture National Small Grains Collection were screened for eyespot resistance; all were winter habit and collected from central Israel, with the exception of plant introduction (PI) 542196 from Izmir, Turkey and PI 330486 from an unknown source. Eighty-three *A. longissima* addition or substitution lines in a Chinese Spring or 'Selkirk' background were obtained from the Wheat Genetics and Genomic Resources Center at Kansas State University. The *A. longissima* addition lines include 16 Chinese Spring disomic additions (DAs), 13 Chinese Spring ditelosomic additions (DtAs), and 7 Selkirk DAs. The *A. longissima* substitution lines include 21 Chinese Spring disomic substitutions (DSs), 17 Chinese Spring ditelosomic substitutions (DtS), and 9 Chinese Spring double ditelosomic substitutions (dDtS). *A. longissima* accession TA1910, which is the parent of TA7515-7528, was included in experiments with the genetic stocks. The donor of other lines was an Israeli accession, *A. longissima* TL20, that was not available for testing. Seven wheat cultivars were used as controls. Madsen and Cappelle Desprez, which contain *Pch1* and *Pch2*, respectively, are eyespot-resistant winter wheat cultivars. The winter wheat 'Hill 81' and spring wheat Chinese Spring are susceptible to eyespot. Selkirk and 'Opata' spring wheat were tested to confirm their susceptibility to eyespot. The resistant breeding line VPM-1 containing *Pch1* was also included in these experiments.

Growth chamber experiments. *A. longissima* accessions and addition or substitution lines were evaluated for differential resistance to *O. yallundae* and *O. acufiformis* by inoculating them with the respective pathogens in separate experiments. Due to limited seed availability, *A. longissima* addition and substitution lines were tested only once with *O. acufiformis* but all other experiments were repeated. Experiments were arranged in a randomized complete block design with six blocks and 12 plants per line for *A. longissima* accessions, and three blocks and 6 plants per line for *A. longissima* addition or substitution lines. Fifty pots (6.4 by 6.4 cm) were randomly arranged in a plastic tray without drain holes (54 by 27 by 6 cm) as a block.

Seed were imbibed on moist filter paper in petri dishes for 4 days at 4°C to synchronize germination and then kept at room temperature for 2 to 3 days. Sprouted seed were planted into 6.4-cm² plastic pots (McConkey Co.) with commercial Sunshine Potting Mix number 1/LC1 (SunGro Horticulture) and fertilized with Osmocote (14-14-14, wt/vol; The Scotts Company LLC). Two seeds were planted in each pot as subsamples. Trays were placed in the growth chambers at 15 and 13°C (day and night, respectively) with a 12-h photoperiod. Relative humidity was maintained from 98 to 100%. All plants of one experiment were kept in one growth chamber. Trays were rotated within chambers every 2 to 3 days to minimize variation in relative humidity.

Inoculation. One week after planting, a 3.3-cm-long split drinking straw was put around the coleoptile of each plant at the soil surface. Seedlings were inoculated when the second leaf was half the size of the first leaf, at about 2 weeks old. Four *O. yallundae* isolates (tph8934-5-61, tph8934-5-62, tph8934-5-68, and tph8934-5-70) and five *O. acufiformis* isolates (tph98-1-54AA, tph98-1-54SS, tph98-2-34D, tph98-2-34E, and tph98-2-34M) transformed with the β -glucuronidase (GUS) reporter gene were used in all experiments. The method to test eyespot severity was modified following the protocol developed by de la Peña and Murray (14).

Inoculum was produced by growing mycelia of *O. yallundae* and *O. acufiformis* on potato dextrose agar (PDA) (Difco Laboratories) plates for 6 weeks at room temperature before inoculation. PDA plugs (1 cm²) containing mycelia were chopped into small

pieces and spread onto 1.5% water agar (WA) (Sigma Life Science) plates, and 3 ml of sterilized distilled water was added. The plates were sealed with Parafilm (Pechiney Plastic Packaging) and placed in an incubator with near UV light at 13°C for at least 2 weeks to produce conidia. On the day of inoculation, conidia were collected by scraping WA plates with a bent glass rod and counted with a hemacytometer. A slurry was prepared by blending fresh 1.5% WA and water. Conidia of *O. yallundae* or *O. acufiformis* were added to give a final concentration of 2 to 3 $\times 10^5$ conidia/ml of slurry. During inoculation, 250 μ l of the slurry was pipetted into the straw collar around each stem base. The same amount of inoculum was added again 1 to 2 days later.

Disease evaluation and analysis of GUS activity. Eight weeks after inoculation at growth stage 23 to 25 (55), a 3-cm section of the stem around the inoculation site was removed and briefly washed with tap water to remove soil. Visual disease ratings were performed on a 0-to-4 scale (54), where 0 = no symptoms (healthy), 1 = a lesion only on the first leaf sheath, 2 = a lesion on the first leaf sheath and a small lesion on the second leaf sheath, 3 = a lesion covering the first leaf sheath and up to half of the second sheath, and 4 = a lesion covering the first and second sheaths (nearly dead). Only the main tiller of wheat controls and genetic stocks was evaluated. All tillers ($n = 2$ to 4) of each *A. longissima* plant were evaluated as a whole due to their small size. Stem segments were then wrapped with paper towels and frozen at -20°C until the GUS assay.

GUS activity in stems was used as a surrogate measurement of fungal colonization. Sap was extracted from frozen stems by placing them in a leaf squeezer (Ravenel Specialties Company) and adding 2.5 ml of GUS extraction buffer (50 mM NaH₂PO₄, pH 7.0; 5 mM dithiothreitol; 10 mM Na₂EDTA; 0.1% sodium lauryl sarcosine; and 0.1% Triton-100) to each sample. About 1 ml of sap was collected in an Eppendorf tube placed on ice and frozen at -20°C until GUS activity was measured. GUS activity was determined by adding 50 μ l of extract with 40 μ l of 10 mM fluorescent substrate 4-methylumbelliferyl β -D-glucoside (Sigma Life Science) in a 1.2-ml polypropylene microtube (USA Scientific), and then incubated at 37°C for 1 h. The reaction was stopped by adding 1 ml of stop buffer (0.2 M sodium carbonate) to each tube. Then, 200 μ l of each sample was transferred to a 96-well black microtiter plate (Greiner Bio-One). Two wells were used for each sample. Methylumbelliferone (MU) standards (Sigma Life Science) and samples from inoculated Madsen were included on each plate. The fluorescence intensity of MU was measured in a Molecular Devices SpectraMax M2 microplate reader (Molecular Devices Co.).

GUS scores were expressed as the log₁₀ transformed ratio [$\log_{10}(x/\text{resistant control}) + 1$] of GUS activity of an individual accession (x) compared with the activity of the resistant control (Madsen). Accessions were classified as resistant if GUS scores were less than or not significantly ($P > 0.05$) greater than Madsen. Accessions were classified as susceptible if GUS scores were significantly ($P < 0.05$) greater than Madsen.

Statistical analysis. Homogeneity of variances of the repeated tests was evaluated using the F ratio of the larger to the smaller error variance (22). Variances of visual ratings and GUS scores did not differ ($P < 0.05$) between experiments based on the F -ratio test. Therefore, experiments were combined for all subsequent analyses. Statistical analysis was conducted with SAS (version 9.2; SAS Institute Inc.). Analysis of variance of individual or combined experiments and standard deviation of each accession were carried out using PROC GLM on the visual disease rating and GUS score. Pearson correlation coefficients between the visual disease rating and GUS score of combined experiments were estimated with PROC CORR. Dunnett's t test was used to compare the least squares mean (LS-mean) of each accession with the LS-mean of Madsen (resistant control) at the 95% significance level.

Results

Reaction of *A. longissima* accessions to *O. yallundae*. *A. longissima* accessions differed significantly ($P < 0.0001$) in re-

sistance to *O. yallundae* based on GUS scores (Fig. 1) and visual ratings (Table 1). GUS scores were significantly correlated ($r = 0.87$, $P < 0.0001$) with visual ratings. Visual ratings of the 40 *A. longissima* accessions ranged from 1.3 to 3.6 and GUS scores ranged from 0.9 to 1.7. In all, 22 accessions (55%) had visual ratings less or not significantly ($P > 0.05$) greater than Madsen (≤ 2.4), and 18 accessions (45%) had GUS scores less or not significantly ($P > 0.05$) greater than Madsen (≤ 1.2). Seventeen accessions (43%) were classified as resistant to *O. yallundae* based on both GUS score and visual rating. One accession, PI 604134, had a visual rating that was significantly greater than Madsen but a GUS score that did not differ; therefore, it was categorized as intermediate. Five accessions (PI 604105, PI 604107, PI 604123, PI 604135, and PI 604143) had visual ratings that did not differ from Madsen but GUS scores that were significantly greater and were classified as susceptible.

Reaction of *A. longissima* accessions to *O. acuformis*. Reactions among *A. longissima* accessions to *O. acuformis* were significantly different ($P < 0.0001$) for both GUS scores (Fig. 1) and visual ratings (Table 1). GUS scores and visual ratings were significantly correlated ($r = 0.83$, $P < 0.0001$). Visual ratings of the 40 *A. longissima* accessions ranged from 1.7 to 3.7 and GUS scores ranged from 1.0 to 1.6. In all, 23 accessions (58%) had visual ratings equal or not significantly ($P > 0.05$) greater than Madsen (visual ratings ≤ 2.4) and 21 (53%) had GUS scores less or not significantly ($P > 0.05$) greater than Madsen (GUS scores ≤ 1.3). Nineteen accessions (48%) were classified as resistant to *O. acuformis* because they had both GUS scores and visual ratings that were not significantly ($P > 0.05$) different from Madsen. Two accessions, PI 604131 and PI 604134, had significantly ($P < 0.05$) greater visual ratings than Madsen but GUS scores that did not differ; therefore, they were categorized as intermediate. Four accessions (PI 604105, PI 604107, PI 604108, and PI 604115) had visual ratings that did not differ from Madsen but GUS scores that were significantly greater and were classified as susceptible.

Differential reaction of *A. longissima* accessions to *O. yallundae* and *O. acuformis*. Reaction of VPM-1, Madsen, Chinese Spring, and Selkirk were consistent between *O. yallundae* and *O. acuformis* (Table 1). However, Cappelle Desprez had less severe visual ratings for *O. acuformis* than *O. yallundae*, Opata was susceptible to *O. yallundae* but resistant to *O. acuformis*, and Hill 81 was highly susceptible to *O. yallundae* but its reaction to *O. acuformis* was inconsistent between GUS score and visual rating. On average, *A. longissima* accessions had the same GUS scores (1.3) and visual ratings (2.4) for both pathogens. Based on the GUS scores, 13 accessions (33%) were resistant to both *O. yallundae* and *O. acuformis* (Table 1, bold), 15 accessions (38%) were

susceptible, and 10 accessions (25%) reacted differently. Accessions PI 604108, PI 604109, PI 604114, and PI 604128 were resistant to *O. yallundae* by both GUS scores and visual ratings but were susceptible to *O. acuformis*. In contrast, accessions PI 604103, PI 604130, PI 604135, PI 604138, PI 604139, and PI 604144 were susceptible to *O. yallundae* by both assessments but were resistant to *O. acuformis*.

Reaction of *A. longissima* addition or substitution lines to *O. yallundae*. Disease reactions of the 83 lines to *O. yallundae* were significantly different ($P < 0.0001$) for both GUS score and visual rating (Table 2). GUS scores were significantly correlated ($r = 0.63$, $P < 0.0001$) with visual ratings. Visual ratings of the 83 genetic stocks ranged from 1.4 to 3.6 and GUS scores ranged from 1.2 to 1.9. In all, 43 (52%) lines had visual ratings that were not significantly ($P > 0.05$) different from Madsen (≤ 2.8) and 29 (35%) had GUS scores not significantly ($P > 0.05$) different from Madsen (≤ 1.5). Twenty-three (28%) lines were classified as resistant to *O. yallundae* based on both GUS scores and visual ratings. The other six lines were categorized as intermediate because their visual ratings were significantly greater than Madsen. The remaining 54 lines were considered susceptible. The donor accession TA1910 was resistant to *O. yallundae*. Among the 23 resistant genetic stock lines, five contain *A. longissima* chromosome 1S¹, two contain 2S¹, five contain 5S¹, five contain 7S¹, two contain the 4S¹/7S¹ translocation, and four contain unknown *A. longissima* chromosomes (Fig. 2). Reaction of two DtA lines and four DtS lines to *O. yallundae* revealed that resistance was controlled by genes on the long arm of chromosomes 1S¹, 5S¹, and 7S¹. TA7524 and TA7528, whose donor parent is TA1910, contain the resistance to *O. yallundae* on the long arms of 5S¹ and 7S¹, respectively.

Reaction of *A. longissima* addition or substitution lines to *O. acuformis*. There were significantly different disease reactions to *O. acuformis* among the 83 lines for GUS score ($P = 0.0024$) and visual rating ($P = 0.0008$). GUS scores were significantly correlated ($r = 0.61$, $P < 0.0001$) with visual ratings. Visual ratings of the 83 genetic stocks ranged from 1.2 to 3.5 and GUS scores ranged from 1.0 to 1.7 (Table 2). In all, 50 (60%) lines had visual ratings not significantly ($P > 0.05$) different from Madsen (≤ 2.4) and 27 (33%) had GUS scores not significantly ($P > 0.05$) different from Madsen (≤ 1.3). Twenty-six lines (31%) were classified as resistant to *O. acuformis* based on GUS scores and visual ratings. Because its visual rating was significantly greater than Madsen, TA6506 was categorized as intermediate. The remaining 57 lines were considered susceptible. The donor accession TA1910 was resistant to *O. acuformis*. Among the 26 resistant genetic stock lines, 5 lines contain *A. longissima* chromosome 1S¹, 7 lines contain 2S¹, 1 line contains 3S¹, 4 lines contain 4S¹, 4 lines contain 5S¹,

Fig. 1. Reaction of 40 *Aegilops longissima* accessions to *Oculimacula yallundae* and *O. acuformis*. Black bars represent accessions that are not significantly ($P > 0.05$) greater than 'Madsen' and white bars are accessions that are significantly ($P < 0.05$) greater than Madsen based on Dunnett's *t* test. The β -glucuronidase (GUS) score of each line was the mean of 24 plants in two experiments.

2 lines contain 6S¹, 2 lines contain 7S¹, and 1 line contains an unknown *A. longissima* chromosome (Fig. 2). Reactions of four DtAs and six DtS lines to *O. acufomis* revealed that resistance was controlled by genes on the long arm of chromosomes 1S¹, 2S¹, 5S¹, and 7S¹ and the short arm of 2S¹, 3S¹, 4S¹, and 6S¹. TA7516, TA7528, and TA7525, whose donor parent is TA1910, contain the resistance to *O. acufomis* on the long arms of 1S¹ and 7S¹ and short arm of 6S¹, respectively.

Differential reaction of *A. longissima* addition or substitution lines to *O. yallundae* and *O. acufomis*. Reaction of Madsen was similar to both pathogens (Table 2). GUS scores for Hill 81, Chinese Spring, and Selkirk were larger when inoculated with *O. yallundae* than *O. acufomis*. *A. longissima* addition or substitution lines had mean GUS scores of 1.6 and 1.4 and mean disease ratings

of 2.8 and 2.3 for *O. yallundae* and *O. acufomis*, respectively. There were 62 lines with GUS scores for *O. yallundae* greater than for *O. acufomis* but only 9 lines with higher GUS scores for *O. acufomis*. The parental accession TA1910 also had a higher GUS score for *O. yallundae* than for *O. acufomis*. Based on GUS scores, 8 lines (10%) were resistant to both *O. yallundae* and *O. acufomis* (Table 2, bold); 32 lines (39%) reacted differently to *O. yallundae* and *O. acufomis*, including 15 lines resistant to *O. yallundae* alone and 17 lines resistant to *O. acufomis* alone; and 37 lines (45%) were susceptible to both pathogens. Lines resistant to both pathogens collectively contain *A. longissima* chromosomes 1S¹, 2S¹, 5S¹, and 7S¹ (Fig. 2) and genes conferring resistance are located on the long arms of 1S¹, 5S¹, and 7S¹, with the exception of chromosome 2S¹. Resistance to *O. acufomis* alone is conferred by

Table 1. β -Glucuronidase (GUS) scores and visual disease ratings of 40 *Aegilops longissima* accessions inoculated with *Oculimacula yallundae* or *O. acufomis* under controlled environment conditions^a

Accessions ^b	<i>O. yallundae</i>			<i>O. acufomis</i>		
	GUS	Ratings	Reaction	GUS	Ratings	Reaction
PI 604140	0.9	1.3	R	1.2	2.0	R
PI 604116	1.0	1.3	R	1.3	2.0	R
PI 604108	1.1	1.3	R	1.4*	2.3	S
PI 604136	1.1	1.5	R	1.2	2.3	R
PI 604137	1.1	1.6	R	1.1	1.7	R
PI 604128	1.1	2.4	R	1.4*	2.9*	S
PI 542196	1.2	1.7	R	1.3	2.0	R
PI 604126	1.2	1.8	R	1.3	2.2	R
PI 604114	1.2	1.9	R	1.5*	2.9*	S
PI 604119	1.2	1.9	R	1.0	2.0	R
PI 604109	1.2	2.0	R	1.4*	2.7*	S
PI 604112	1.2	2.0	R	1.2	2.3	R
PI 604125	1.2	2.1	R	1.2	2.3	R
PI 604133	1.2	2.1	R	1.3	1.9	R
PI 604141	1.2	2.1	R	1.3	2.1	R
PI 604127	1.2	2.2	R	1.3	2.1	R
PI 604104	1.2	2.3	R	1.2	1.9	R
PI 604134	1.2	2.6*	I	1.2	2.5*	I
PI 604105	1.3*	2.0	S	1.4*	1.9	S
PI 604142	1.3*	2.7*	S	1.5*	3.3*	S
PI 604118	1.3*	2.8*	S	1.4*	2.8*	S
PI 604138	1.3*	2.8*	S	1.1	2.2	R
PI 604131	1.3*	3.3*	S	1.2	2.5*	I
PI 604143	1.4*	2.1	S	1.5*	2.8*	S
PI 604107	1.4*	2.3	S	1.4*	1.8	S
PI 604123	1.4*	2.3	S	1.5*	2.9*	S
PI 604135	1.4*	2.4	S	1.3	1.7	R
PI 604110	1.4*	2.7*	S	1.4*	2.5*	S
PI 604106	1.4*	2.8*	S	1.5*	3.4*	S
PI 604144	1.4*	2.8*	S	1.2	2.0	R
PI 604103	1.4*	3.0*	S	1.3	2.1	R
PI 604122	1.4*	3.0*	S	1.4*	2.8*	S
PI 604129	1.4*	3.2*	S	1.5*	2.8*	S
PI 604130	1.4*	3.2*	S	1.2	2.1	R
PI 604115	1.5*	2.7*	S	1.4*	2.3	S
PI 604139	1.5*	3.3*	S	1.3	2.4	R
PI 604124	1.6*	3.0*	S	1.4*	2.7*	S
PI 604111	1.6*	3.3*	S	1.5*	3.0*	S
PI 330486	1.6*	3.6*	S	1.5*	3.7*	S
PI 604117	1.7*	3.1*	S	1.6*	3.2*	S
Wheat						
VPM-1 (R)	0.9	0.4	R	0.9	0.5	R
'Madsen' (R)	1.0	0.5	R	1.0	0.9	R
CD (R)	1.1	1.6	R	1.0	0.7	R
'Hill 81' (S)	1.7*	3.7*	S	1.3	2.6*	I
CS (S)	1.6*	3.2*	S	1.4*	2.6*	S
'Opata'	1.5*	3.3*	S	1.2	1.8	R
'Selkirk'	1.8*	3.6*	S	1.5*	3.1*	S
MSD _{0.05}	0.23	1.91	...	0.32	1.5	...

^a Data sorted by GUS score of *O. yallundae*. R = resistant; GUS scores and visual ratings are not significantly greater than Madsen ($P > 0.05$) based on Dunnett's t test. S = susceptible; GUS scores are significantly ($P < 0.05$) greater than Madsen. I = intermediate; GUS scores are not significantly ($P > 0.05$) greater than Madsen but visual ratings are significantly greater. Accessions in bold are resistant to both pathogens. The GUS score and visual rating of each line was the mean of 24 plants in two experiments; * indicates lines that are significantly different than Madsen.

^b PI = plant introduction, CD = 'Cappelle Desprez', CS = 'Chinese Spring', and MSD_{0.05} = minimum significant difference at $P = 0.05$ using Dunnett's t test to compare the least squares mean (LS-mean) of each accession with the LS-mean of Madsen.

genes on the short arm of chromosomes 2S¹, 3S¹, 4S¹, and 6S¹. Overall, 62% of the lines with *A. longissima* chromosome 1S¹, 50% with 2S¹, 10% with 3S¹, 49% with 4S¹, 78% with 5S¹, 25% with 6S¹, and 56% with 7S¹ were resistant to eyespot.

Discussion

Among the 40 *A. longissima* accessions tested in this study, more than 40% were statistically equal to Madsen, the eyespot-resistant control, and, therefore, considered resistant to either *O. yallundae* or *O. acuformis*. Therefore, genetic diversity for eyespot resistance exists within *A. longissima* even though the majority of the accessions were collected from the same geographic area. This is the first report that genes in *A. longissima* confer eyespot resistance. Although *A. longissima* was not previously known to be resistant to eyespot, it was reported to be resistant to Septoria glume blotch, powdery mildew, and rust diseases (2,8,18). Thus, *A. longissima* is a potential source of resistance to eyespot as well as these other diseases for wheat improvement programs.

In all, 10 of 40 *A. longissima* accessions and 32 of 83 *A. longissima* addition or substitution lines responded differently to *O. yal-*

lundae than to *O. acuformis*. These results support the hypothesis that genetic control of resistance to *O. yallundae* and *O. acuformis* is different in some lines. Uslu et al. (49) first reported differential genetic control of resistance to the eyespot pathogens in *D. villosum*. Recently, Burt et al. (5) demonstrated that 4 of 22 *Triticum monococcum* lines had significantly different reactions to *O. yallundae* and *O. acuformis*. It was reported that W-type isolates (*O. yallundae*) were more virulent to wheat than to rye, whereas R-type isolates (*O. acuformis*) were almost equally virulent to both rye and wheat (12). Although these pathogens coexist in the same fields and cause similar symptoms, their infection processes are slightly different. Daniels et al. (13) observed different patterns of infection for *O. yallundae* and *O. acuformis* isolates on the same host; *O. yallundae* isolates infected wheat faster and caused more severe lesions than *O. acuformis* but there was little difference in severity between them by the end of the season (3,23). Poupard et al. (42) confirmed that *O. acuformis* colonized coleoptiles and leaf sheaths of wheat more slowly than *O. yallundae*. Uslu et al. (49) tested *O. yallundae* and *O. acuformis* separately when they investigated resistance in *D. villosum* and found that the resistance was

Table 2. β -Glucuronidase (GUS) scores and disease ratings of 83 *Aegilops longissima* (2n = 14, S¹S¹) addition or substitution lines in 'Chinese Spring' (CS) or 'Selkirk' background inoculated with *Oculimacula yallundae* or *O. acuformis* under controlled environment^a

Lines ^b	Description ^c	<i>O. yallundae</i>			<i>O. acuformis</i>		
		GUS	Rating	Reaction	GUS	Rating	Reaction
TA3716	Selkirk DA ?S ¹	1.2	1.6	R	1.5*	1.7	S
TA3710	Selkirk DA ?S¹	1.3	2.5	R	1.3	1.7	R
TA3576	DA 4S ¹ /7S ¹	1.3	2.2	R	1.6*	2.5*	S
TA3717	Selkirk DA ?S ¹	1.3	1.4	R	1.7*	2.0	S
TA6519	DS 7S¹ (7D)	1.4	2.6	R	1.2	1.8	R
TA6504	DS 2S ¹ (2A)	1.4	3.2*	I	1.3	2.2	R
TA3599	DA ?S ¹	1.4	1.9	R	1.4*	1.8	S
TA3579	DA 7S ¹ /4S ¹	1.4	2.4	R	1.4*	2.5*	S
TA3466	DS 5S ¹ (5B)	1.4	2.3	R	1.5*	3.0*	S
TA6523	DtS 1S ¹ /L (1B)	1.4	2.0	R	1.7*	2.8*	S
TA6503	DS 1S¹ (1D)	1.5	2.8	R	1.2	1.5	R
TA7528	DtA 7S¹/L	1.5	2.2	R	1.3	2.0	R
TA6513	DS 5S¹ (5A)	1.5	2.4	R	1.3	2.2	R
TA6540	DtS 5S¹/L (5D)	1.5	2.5	R	1.3	2.0	R
TA7544	DA 2S¹	1.5	2.7	R	1.3	2.2	R
TA6603	dDtS 1S¹ (1D)	1.5	2.8	R	1.3	1.2	R
TA6506	DS 2S ¹ (2D)	1.5	3.0*	I	1.3	3.0*	I
TA7550	DA 2S ¹	1.5	2.3	R	1.4*	2.2	S
TA7543	DA 1S ¹	1.5	2.6	R	1.4*	2.3	S
TA7524	DtA 5S ¹ /L	1.5	2.8	R	1.4*	2.8*	S
TA6512	DS 4S ¹ (4D)	1.5	2.9*	I	1.4*	3.0*	S
TA6518	DS 7S ¹ (7B)	1.5	2.5	R	1.5*	2.7*	S
TA6543	DtS 7S ¹ /L (7B)	1.5	2.6	R	1.5*	2.3	S
TA6510	DS 4S ¹ (4A)	1.5	3.1*	I	1.5*	2.5*	S
TA7547	DA 5S ¹	1.5	2.3	R	1.6*	2.3	S
TA3709	Selkirk DA ?S ¹	1.5	3.3*	I	1.6*	2.8*	S
TA6502	DS 1S ¹ (1B)	1.5	2.4	R	1.7*	2.5*	S
TA6542	DtS 7S ¹ /L (7A)	1.5	2.4	R	1.7*	2.7*	S
TA3635	DA ?S ¹	1.5	3.3*	I	1.7*	3.5*	S
TA7548	DA 6S ¹	1.6*	3.4*	S	1.0	1.2	R
TA6505	DS 2S ¹ (2B)	1.6*	3.1*	S	1.1	1.8	R
TA7551	DtA 2S ¹ /L	1.6*	2.8	S	1.2	2.2	R
TA7525	DtA 6S ¹ /S	1.6*	3.2*	S	1.2	2.4	R
TA6529	DtS 2S ¹ /S (2D)	1.6*	3.5*	S	1.2	1.8	R
TA6537	DtS 4S ¹ /S (4B)	1.6*	2.8	S	1.3	2.0	R
TA6545	DS 2S ¹ (2B)	1.6*	2.8	S	1.4*	2.7*	S
TA6517	DS 7S ¹ (7A)	1.6*	2.8	S	1.4*	1.5	S
TA6528	DtS 2S ¹ /L (2B)	1.6*	2.8	S	1.4*	1.8	S
TA6610	dDtS 6S ¹ (6B)	1.6*	2.8	S	1.4*	1.7	S
TA6508	DS 3S ¹ (3B)	1.6*	3.0*	S	1.4*	2.3	S

(continued on next page)

^a Data sorted by GUS scores of *O. yallundae*. R = resistant; GUS scores and visual ratings are not significantly ($P > 0.05$) greater than Madsen based on Dunnett's t test. S = susceptible; GUS scores are significantly ($P < 0.05$) greater than Madsen. I = intermediate; GUS scores are not significantly ($P > 0.05$) greater than Madsen but visual ratings are significantly greater. Lines in bold are resistant to both pathogens. Data for *O. yallundae* are the mean of 12 plants per line in two experiments and *O. acuformis* is the mean of 6 plants in one experiment; * indicates lines that are significantly different than Madsen.

^b MSD_{0.05} = minimum significant difference at $P = 0.05$ using Dunnett's t test to compare the least squares mean (LS-mean) of each line with the LS-mean of Madsen.

^c DA: disomic addition; DS: disomic substitution; DtS: ditelodisomic substitution; DtA: ditelodisomic addition; dDtS: double ditelodisomic substitution.

more effective to *O. yallundae* than to *O. acuformis*. The resistant controls also reacted differentially to the eyespot pathogens. VPM-1 and Madsen were highly resistant to both pathogens but Madsen was slightly less resistant to *O. acuformis* than to *O. yallundae*. In contrast, Cappelle Desprez was more resistant to *O. acuformis* than *O. yallundae*. Burt et al. (5) also found that *Pch2* in Cappelle Desprez was significantly more effective against *O. acuformis* than *O. yallundae*. Differential resistance to *O. yallundae* and *O. acuformis* in these *A. longissima* accessions supports assertions that *O. yallundae* and *O. acuformis* should be tested separately when screening wild relatives of wheat for resistance to eyespot.

Madsen was included in this study as a standard of eyespot resistance to differentiate among resistant and susceptible *A. longissima* accessions and genetic stocks. Madsen has been used in other studies (10,31,32) of eyespot resistance for the same purpose because it represents the most resistant cultivar in the PNW. In this study, we chose not to subdivide the disease reaction scale beyond resistant, intermediate, and susceptible because the purpose was to identify potentially useful accessions as a source of new eyespot resistance genes for wheat improvement programs and not to infer

the overall degree of effectiveness of eyespot resistance in *A. longissima* compared with Madsen. Furthermore, we did not have a statistical basis for subdividing the disease reaction scale beyond resistant, intermediate, and susceptible. Although several *A. longissima* accessions were statistically equal to Madsen, the overall effectiveness and usefulness of their resistance under field conditions compared with Madsen cannot be inferred from this comparison for two reasons. First, the comparison is based on a seedling test where the plant is exposed to the pathogen for only 8 weeks; further differentiation among resistant and susceptible plants may occur with age. Confirmation of the effectiveness of resistance should be based on field tests where the plant is exposed over a longer period of time and through all developmental stages. Second, and most important, *A. longissima* is a diploid species and any resistance genes it contains must be transferred to a comparable hexaploid wheat genotype for a direct comparison with Madsen. A subsequent study (46) has demonstrated that four quantitative trait loci (QTL) are responsible for eyespot resistance in *A. longissima*; whether and to what extent these QTL are effective in hexaploid wheat is the subject of current studies.

Table 2. (continued from preceding page)

Lines ^b	Description ^c	<i>O. yallundae</i>			<i>O. acuformis</i>		
		GUS	Rating	Reaction	GUS	Rating	Reaction
TA6501	DS 1S ¹ (1A)	1.6*	3.2*	S	1.4*	2.5*	S
TA3711	Selkirk DA ?S ¹	1.6*	3.6*	S	1.4*	2.3	S
TA7515	DtA 1S ¹ /S	1.6*	2.8	S	1.5*	3.5*	S
TA6541	DtS 6S ¹ /S (6B)	1.6*	3.0*	S	1.5*	2.8*	S
TA3714	Selkirk DA ?S ¹	1.6*	2.4	S	1.6*	2.0	S
TA3636	DA ?S ¹	1.6*	2.8	S	1.6*	2.8*	S
TA6548	dDtS 3S ¹ (3D)	1.6*	2.8	S	1.6*	1.8	S
TA6521	DtS 1S ¹ /L (1A)	1.6*	3.0*	S	1.6*	3.3*	S
TA3574	DA 2S ¹	1.6*	3.2*	S	1.6*	3.0*	S
TA6525	DtS 1S ¹ /L (1D)	1.7*	3.1*	S	1.1	1.8	R
TA6604	dDtS 2S ¹ (2D)	1.7*	3.3*	S	1.1	1.5	R
TA6546	DS 2S ¹ (2D)	1.7*	2.5	S	1.2	1.8	R
TA6515	DS 5S ¹ (5D)	1.7*	2.9*	S	1.2	1.7	R
TA6538	DtS 4S ¹ /S (4D)	1.7*	3.2*	S	1.2	1.8	R
TA3465	DS 4S ¹ (4B)	1.7*	2.9*	S	1.3	2.0	R
TA7546	DA 4S ¹	1.7*	3.0*	S	1.3	2.2	R
TA6608	dDtS 5S ¹ (5D)	1.7*	3.2*	S	1.3	2.2	R
TA7516	DtA 1S ¹ /L	1.7*	3.3*	S	1.3	2.0	R
TA7527	DtA 7S ¹ /S	1.7*	2.7	S	1.4*	2.7*	S
TA6605	dDtS 3S ¹ (3D)	1.7*	2.8	S	1.4*	2.2	S
TA7517	DtA 2S ¹ /S	1.7*	3.0*	S	1.4*	2.3	S
TA6544	DtS 7S ¹ /L (7D)	1.7*	3.1*	S	1.4*	2.2	S
TA6509	DS 3S ¹ (3D)	1.7*	3.2*	S	1.4*	1.8	S
TA6611	dDtS 7S ¹ (7D)	1.7*	3.3*	S	1.4*	2.8*	S
TA7545	DA 3S ¹	1.7*	3.4*	S	1.4*	2.7*	S
TA3573	DA 1S ¹	1.7*	2.6	S	1.5*	2.5*	S
TA3715	Selkirk DA ?S ¹	1.7*	2.6	S	1.5*	3.0*	S
TA7518	DtA 2S ¹ /L	1.7*	2.8	S	1.5*	3.2*	S
TA6606	dDtS 4S ¹ (4D)	1.7*	2.8	S	1.5*	2.0	S
TA7522	DtA 4S ¹ /L	1.7*	3.1*	S	1.5*	2.8*	S
TA6530	DtS 2S ¹ /L (2D)	1.7*	2.5	S	1.6*	2.2	S
TA6507	DS 3S ¹ (3A)	1.7*	2.8	S	1.6*	2.7*	S
TA7523	DtA 5S ¹ /S	1.7*	3.2*	S	1.6*	2.3	S
TA6533	DtS 3S ¹ /S (3B)	1.8*	3.0*	S	1.2	1.8	R
TA7593	DA ?S ¹	1.8*	2.7	S	1.5*	2.3	S
TA6640	DS 5S ¹ (5D)	1.8*	3.0*	S	1.5*	2.5*	S
TA7521	DtA 4S ¹ /S	1.8*	3.2*	S	1.5*	2.8*	S
TA7519	DtA 3S ¹ /S	1.8*	2.9*	S	1.6*	2.3	S
TA6531	DtS 3S ¹ /S (3A)	1.8*	2.9*	S	1.6*	3.5*	S
TA6522	DtS 1S ¹ /S (1A)	1.8*	3.0*	S	1.7*	3.2*	S
TA6547	dDtS 1S ¹ (1D)	1.9*	3.5*	S	1.2	1.8	R
TA3575	DA 3S ¹	1.9*	3.1*	S	1.4*	2.0	S
TA6526	DtS 2S ¹ /S (2A)	1.9*	3.3*	S	1.5*	2.7*	S
TA1910	Accession	1.4	2.5	R	1.0	1.7	R
'Madsen'	Control (R)	1.0	0.9	R	1.0	0.8	R
'Hill 81'	Control (S)	1.6*	3.3*	S	1.4*	2.2	S
CS	Control (S)	1.7*	3.2*	S	1.3	2.5*	I
'Opata'	Control	1.6*	3.2*	S	...	...	...
'Selkirk'	Control	1.6*	2.9*	S	1.4*	3.2*	S
MSD _{0.05}	...	0.52	1.88	...	0.34	1.6	...

However, the accessions and genetic stocks classified as resistant to either *O. yallundae* or *O. acuformis* and statistically equal to Madsen are potentially useful to breeding programs.

The *A. longissima* addition or substitution lines resistant to *O. yallundae* possessed chromosomes 1S¹, 2S¹, 5S¹, and 7S¹ and the 4S¹/7S¹ translocation. Most lines resistant to *O. acuformis* had chromosomes 1S¹, 2S¹, 4S¹, or 5S¹, and lines resistant to both *O. yallundae* and *O. acuformis* possessed chromosomes 1S¹, 2S¹, 5S¹, and 7S¹. These results demonstrate that the genetic control of eyespot resistance is located in multiple regions of the *A. longissima* genome. The long arms of chromosomes 1S¹, 5S¹, and 7S¹ contained genes conferring resistance to both *O. yallundae* and *O. acuformis*, which indicates that resistance to both pathogens may be controlled by the same genes or genes at the same loci on chromosomes 1S¹, 5S¹, and 7S¹. QTL conferring resistance to *O. yallundae* were found on the long arms of chromosomes 1S¹, 5S¹, and 7S¹ and the short arm of 3S¹ (46). The finding of resistance on the long arms of 1S¹, 5S¹, and 7S¹ is consistent between this study and the QTL mapping study by Sheng et al. (46). However, resistance on the short arm of 3S¹ was only found for *O. acuformis* in this study.

Genetic diversity for eyespot resistance was also observed in the *A. longissima* addition or substitution lines; lines with the same alien chromosome composition differed in reaction to eyespot, which may indicate that different genes for eyespot resistance are present. Polymorphism among these lines should be useful in mapping genes for eyespot resistance.

Uslu et al. (49) found effective resistance for *O. yallundae* on chromosome 4V and for *O. acuformis* on 5V even though resistance to both eyespot pathogens in *D. villosum* was controlled by genes on chromosomes 1V, 2V, and 3V. Law et al. (30) also found resistance to eyespot on Cappelle Desprez chromosomes 1A, 2B, 5D, and 7A. In our study, resistance to *O. yallundae* was found on *A. longissima* chromosomes 1S¹, 2S¹, 5S¹, and 7S¹ and two 4S¹/7S¹ translocation lines. Because no lines containing 4S¹ were resistant to *O. yallundae*, it is possible that resistance is on the 7S¹ segment in the translocation lines. If so, only *A. longissima* chromosomes 1S¹, 2S¹, 5S¹, and 7S¹ carried resistance to *O. yallundae*. Resistance to *O. acuformis* was found on all *A. longissima* chromosomes, with greater resistance from chromosomes 1S¹, 2S¹, 4S¹, and 5S¹.

A. longissima chromosome 4S¹ only showed resistance to *O. acuformis*. This finding is unexpected because resistance to *O. yallundae* has been found on homologous chromosome group 4 in other studies (31,32,40,49). Even though two 4S¹/7S¹ translocation lines were resistant to *O. yallundae*, evidence for resistance to *O. yallundae* on 4S¹ was not found in the genetic stocks tested in this study. Evidence for resistance on homologous chromosome group 5 has been reported in other studies. Muranty et al. (37) found eyespot resistance on Cappelle Desprez chromosome 5A. Recently,

Burt et al. (4) reported that a QTL on chromosome 5AL of Cappelle Desprez conferred effective resistance to both *O. acuformis* and *O. yallundae*. Eyespot resistance gene *Pch1* was located on 7DL of VPM-1 based on linkage with an isozyme marker (*EP-D1b*) (25,35). The major genetic control of eyespot resistance in Cappelle Desprez was mapped to 7AL by an RFLP linkage map (16). de la Peña et al. (16) suggested that *Pch1* and *Pch2* were homoeoloci, which is a conclusion supported by Chapman et al. (9) due to the similar positions of microsatellite markers linked to *Pch1* and *Pch2*.

A. speltoides is considered the B genome donor for bread wheat (43,44). However, Feldman (19) observed that more wheat B genome chromosomes paired with those of *A. longissima* than *A. speltoides*. Feldman (19) also found that the S¹ genome of *A. longissima* paired primarily with the B genome of *T. aestivum* but relatively less with the A and D genomes. Kota et al. (29) reported that the heterohomologous 6B¹ (= 6S¹) chromosome of *A. longissima* substituted for chromosome 6B of *T. aestivum*. Naranjo (41) showed that *A. longissima* chromosomes 1S¹, 2S¹, 3S¹, 5S¹, and 6S¹ paired with wheat chromosome groups 1, 2, 3, 5, and 6, respectively. Zhang et al. (56) constructed a comparative genetic map of the *A. longissima* genome and found that colinearity was conserved between 1S¹, 2S¹, 3S¹, 5S¹, and 6S¹ and wheat chromosomes 1D, 2D, 3D, 5D, and 6D, respectively. Mello-Sampayo (36) also demonstrated that *A. longissima* could actively promote homologous pairing in crosses with *T. aestivum*. All of the above studies imply the crossability of *A. longissima* with wheat and the possibility of transferring useful genes into adapted wheat cultivars to improve wheat production.

Based on these results, *A. longissima* is a new source of eyespot resistance, multiple resistance genes to *O. yallundae* and *O. acuformis* are present in the S¹ genome, and the genetic control of resistance to these pathogens differs in some lines. Based on consideration of both GUS scores and visual ratings along with the standard deviation of each line, seven resistant lines (PI 542196, PI 604112, PI 604116, PI 604119, PI 604136, PI 604137, and PI 604140) and three susceptible lines (PI 330480, PI 604111, and PI 604117) with the same responses to both species are potentially useful parents for further genetic studies. It remains to be seen whether and to what extent the resistance observed in these studies is expressed under field conditions in a hexaploid wheat background.

The chromosome-specific resistance to *O. yallundae* and *O. acuformis* identified in *A. longissima* addition or substitution lines provides important information for future studies of eyespot resistance. The resistant genetic stocks identified in this study should be useful for transferring resistance genes into adapted wheat cultivars. TA3710, TA3716, and TA3717, which are DA lines in a Selkirk background, were resistant to *O. yallundae* and TA3710 is resistant to *O. acuformis*. Identifying the alien chromosomes in these lines should be useful in locating the genes for eyespot resistance. The introgression of these new sources of resistance into wheat has potential to broaden the genetic base of eyespot resistance improve its effectiveness.

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Literature Cited

- Allan, R. E., Peterson, C. J., Rubenthaler, G. L., Line, R. F., and Robert, D. E. 1989. Registration of 'Madsen' wheat. Crop Sci. 29:1575-1576.
- Anikster, Y., Manisterski, J., Long, D. L., and Leonard, K. J. 2005. Resistance to leaf rust, stripe rust, and stem rust in *Aegilops* spp. in Israel. Plant Dis. 89:303-308.
- Bock, C. H., Wan, A. M., and Fitt, B. D. L. 2009. Development of *Oculimacula yallundae* and *O. acuformis* (eyespot) on stems of winter wheat in relation to thermal time in the UK. Plant Pathol. 58:12-22.
- Burt, C., Hollins, T. W., and Nicholson, P. 2011. Identification of a QTL conferring seedling and adult plant resistance to eyespot on chromosome

Fig. 2. Distribution of *Oculimacula yallundae* and *O. acuformis* resistance among chromosomes of *Aegilops longissima*. Black bars are number of *Aegilops longissima* addition or substitution lines containing that chromosome in a line resistant to *O. yallundae* and white bars are number of lines resistant to *O. acuformis*. Data for *O. yallundae* is the mean of 12 plants per line in two experiments and *O. acuformis* is the mean of 6 plants in one experiment.

- 5A of Cappelle Desprez. *Theor. Appl. Genet.* 122:119-128.
5. Burt, C., Hollins, T. W., Powell, N., and Nicholson, P. 2010. Differential seedling resistance to the eyespot pathogens, *Oculimacula yallundae* and *Oculimacula acufiformis*, conferred by *Pch2* in wheat and among accessions of *Triticum monococcum*. *Plant Pathol.* 59:819-828.
6. Cadle, M. M., Murray, T. D., and Jones, S. S. 1997. Identification of resistance to *Pseudocercospora herpotrichoides* in *Triticum monococcum*. *Plant Dis.* 81:1181-1186.
7. Cenci, A., D'Ovidio, R., Tanzarella, O. A., Ceoloni, C., and Porceddu, E. 1999. Identification of molecular markers linked to *Pm13*, an *Aegilops longissima* gene conferring resistance to powdery mildew in wheat. *Theor. Appl. Genet.* 98:448-454.
8. Ceoloni, C., Del signore, G., Ercoli, L., and Donini, P. 1992. Locating the alien chromatin segment in common wheat-*Aegilops longissima* mildew resistant transfers. *Hereditas* 116:239-245.
9. Chapman, N. H., Burt, C., Dong, H., and Nicholson, P. 2008. The development of PCR-based markers for the selection of eyespot resistance genes *Pch1* and *Pch2*. *Theor. Appl. Genet.* 117:425-433.
10. Cox, C. M., Murray, T. D., and Jones, S. S. 2002. Perennial wheat germ plasm lines resistant to eyespot, Cephalosporium stripe, and wheat streak mosaic. *Plant Dis.* 86:1043-1048.
11. Crous, P. W., Groenewald, J. Z. (Ewald), and Gams, W. 2003. Eyespot of cereals revisited: ITS phylogeny reveals new species relationships. *Eur. J. Plant Pathol.* 109:841-850.
12. Cunningham, P. C. 1981. Occurrence, role and pathogenic traits of a distinct pathotype of *Pseudocercospora herpotrichoides*. *Trans. Br. Mycol. Soc.* 76:3-15.
13. Daniels, A., Lucas, J. A., and Peberdy, J. F. 1991. Morphology and ultra-structure of W and R pathotypes of *Pseudocercospora herpotrichoides* on wheat seedlings. *Mycol. Res.* 95:385-397.
14. de la Peña, R. C., and Murray, T. D. 1994. Identifying wheat genotypes resistant to eyespot disease with a β -glucuronidase-transformed strain of *Pseudocercospora herpotrichoides*. *Phytopathology* 84:972-977.
15. de la Peña, R. C., Murray, T. D., and Jones, S. S. 1996. Linkage relations among eyespot resistance gene *Pch2*, endopeptidase *Ep-A1b*, and RFLP marker *Xpsr121* on chromosome 7A of wheat. *Plant Breed.* 115:273-275.
16. de la Peña R. C., Murray, T. D., and Jones, S. S. 1997. Identification of an RFLP interval containing *Pch2* on chromosome 7AL of wheat. *Genome* 40:249-252.
17. Doussinault, G., Delibes, A., Sanchez-Monge, R., and Garcia-Olmedo, F. 1983. Transfer of a dominant gene for resistance to eyespot disease from a wild grass to hexaploid wheat. *Nature* 303:698-700.
18. Ecker, R., Cahaner, A., and Dinor, A. 1990. The inheritance of resistance to Septoria glume blotch III. The wild wheat species *Aegilops longissima*. *Plant Breed.* 104:224-230.
19. Feldman, M. 1978. New evidence on the origin of the B genome of wheat. Pages 120-138 in: *Proc. 5th Int. Wheat Genet. Symp.* New Delhi, India.
20. Figliuolo, G., Jones, S. S., Murray, T. D., and Zeuli, P. L. S. 1998. Characterization of tetraploid wheat germplasm for resistance to *Pseudocercospora herpotrichoides*, cause of eyespot disease. *Genet. Res. Crop Evol.* 45:47-56.
21. Friebe, B., Tuleen, N., Jiang, J., and Gill, B. S. 1993. Standard karyotype of *Triticum longissimum* and its cytogenetic relationship with *T. aestivum*. *Genome* 36:731-742.
22. Gomez, K. A., and Gomez, A. A. 1984. Pages 319-322 in: *Statistical Procedures for Agricultural Research*. Wiley, New York.
23. Goulds, A., and Fitt, B. D. L. 1991. The development of eyespot on stems of winter wheat and winter barley in crops inoculated with W-type or R-type isolates of *Pseudocercospora herpotrichoides*. *J. Plant Dis. Prot.* 98:490-502.
24. Hollins, T. W., Lockley, K. D., Blackman, J. A., Scott, P. R., and Bingham, J. 1988. Field performance of Rendezvous, a wheat cultivar with resistance to eyespot (*Pseudocercospora herpotrichoides*) derived from *Aegilops ventricosa*. *Plant Pathol.* 37:251-260.
25. Jahier, J., Doussinault, G., Dosba, F., and Bourgeois, F. 1979. Monosomic analysis of resistance to eyespot in the variety 'Roazon'. Pages 437-440 in: *Proc. 5th Int. Wheat Genet. Symp.* New Delhi, India.
26. Jiang, J., Friebe, B., and Gill, B. S. 1994. Recent advances in alien gene transfer in wheat. *Euphytica* 73:199-212.
27. Johnson, R. 1992. Past, present and future opportunities in breeding for disease resistance, with examples from wheat. *Euphytica* 63:3-22.
28. Jones, S. S., Murray, T. D., and Allan, R. E. 1995. Use of alien genes for the development of disease resistance in wheat. *Annu. Rev. Phytopathol.* 33:429-443.
29. Kota, R. S., McGuire, P. E., and Dvorak, J. 1986. Latent nonstructural differentiation among homologous chromosomes at the diploid level: Chromosome 6B' of *Aegilops longissima*. *Genetics* 114:579-592.
30. Law, C. N., Scott, P. R., Worland, A. J., and Hollins, T. W. 1976. The inheritance of resistance to eyespot (*Cercospora herpotrichoides*) in wheat. *Genet. Res.* 25:73-79.
31. Li, H. J., Arterburn, M., Jones, S. S., and Murray, T. D. 2004. A new source of resistance to *Tapesia yallundae* associated with a homoeologous group 4 chromosome in *Thinopyrum ponticum*. *Phytopathology* 94:932-937.
32. Li, H. J., Arterburn, M., Jones, S. S., and Murray, T. D. 2005. Resistance to eyespot of wheat, caused by *Tapesia yallundae*, derived from *Thinopyrum intermedium* homoeologous group 4 chromosome. *Theor. Appl. Genet.* 111:932-940.
33. Li, H. J., Conner, R. L., and Murray, T. D. 2008. Resistance to soil-borne diseases of wheat: Contributions from the wheatgrasses *Thinopyrum intermedium* and *Th. ponticum*. *Can. J. Plant Sci.* 88:195-205.
34. Lucas, J. A., Dyer, P. S., and Murray, T. D. 2000. Pathogenicity, host specificity, and population biology of *Tapesia* spp., causal agents of eyespot disease in cereals. *Adv. Bot. Res.* 33:226-258.
35. McMillin, D. E., Allan, R. E., and Roberts, D. E. 1986. Association of an isozyme locus and strawbreaker foot rot resistance derived from *Aegilops ventricosa* in wheat. *Theor. Appl. Genet.* 72:743-747.
36. Mello-Sampayo, T. 1971. Promotion of homoeologous pairing in the hybrids of *Triticum aestivum* $\times$ *Aegilops longissima*. *Genet. Iberica* 23:1-9.
37. Muranty, H., Jahier, J., Tanguy, A. M., Worland, A. J., and Law, C. 2002. Inheritance of resistance of wheat to eyespot at the adult stage. *Plant Breed.* 121:536-538.
38. Murray, T. D. 1996. Resistance to benzimidazole fungicides in the cereal eyespot pathogen, *Pseudocercospora herpotrichoides*, in the Pacific Northwest 1984-1990. *Plant Dis.* 80:19-23.
39. Murray, T. D. 2010. Eyespot (Strawbreaker foot rot). Pages 32-34 in: *Compendium of Wheat Diseases and Insects*, Third Edition. W. W. Bockus, R. L. Bowden, R. M. Hunger, T. D. Murray, R. W. Smiley, and W. Morrill, eds. American Phytopathological Society, St. Paul, MN.
40. Murray, T. D., de la Peña, R. C., Yildirim, A., and Jones, S. S. 1994. A new source of resistance to *Pseudocercospora herpotrichoides*, cause of eyespot disease of wheat, located on chromosome 4V of *Dasyphyrum villosum*. *Plant Breed.* 113:281-286.
41. Naranjo, T. 1995. Chromosome structure of *Triticum longissimum* relative to wheat. *Theor. Appl. Genet.* 91:105-109.
42. Poupard, P., Grare, S., Cavelier, N., and Lind, V. 1994. Development of *Pseudocercospora herpotrichoides* (Fron) Deighton var. *herpotrichoides* and var. *acufiformis* on wheat plants measured by ELISA. *J. Phytopathol.* 140:301-311.
43. Riley, R., Unrau, J., and Chapman, V. 1958. Evidence on the origin of the B genome of wheat. *J. Hered.* 49:91-98.
44. Sarkar, P., and Stebbins, G. L. 1956. Morphological evidence concerning the origin of the B genome in wheat. *Am. J. Bot.* 43:297-304.
45. Sears, E. R. 1983. The transfer to wheat of interstitial segments of alien chromosomes. Pages 5-12 in: *Proc. 6th Int. Wheat Genet. Symp.* Kyoto, Japan.
46. Sheng, H., See, D. R., and Murray, T. D. 2012. Mapping QTL for resistance to eyespot of wheat in *Aegilops longissima*. *Theor. Appl. Genet.* 125:355-366.
47. Strausbaugh, C. A., and Murray, T. D. 1989. Inheritance of resistance to *Pseudocercospora herpotrichoides* in three cultivars of winter wheat. *Phytopathology* 79:1048-1053.
48. Thiele, A., Schumann, E., Peil, A., and Weber, W. E. 2002. Eyespot resistance in wheat $\times$ *Aegilops kotschy* backcross lines. *Plant Breed.* 121:29-35.
49. Uslu, E., Miller, T. E., Rezanor, N. H., and Nicholson, P. 1998. Resistance of *Dasyphyrum villosum* to the cereal eyespot pathogens, *Tapesia yallundae* and *Tapesia acufiformis*. *Euphytica* 103:203-209.
50. Van Slageren, M. W. 1994. Pages 1-7 in: *Wild Wheats: A Monograph of Aegilops L. and Amblyopyrum (Jaub. & Spach) Eig (Poaceae)*. Wageningen Agricultural University, Wageningen, The Netherlands.
51. Worland, A. J., Law, C. N., Hollins, T. W., Koebner, R. M. D., and Giura, A. 1988. Location of a gene for resistance to eyespot (*Pseudocercospora herpotrichoides*) on chromosome 7D of bread wheat. *Plant Breed.* 101:43-51.
52. Yildirim, A., Jones, S. S., and Murray, T. D. 1998. Mapping a gene conferring resistance to *Pseudocercospora herpotrichoides* on chromosome 4V of *Dasyphyrum villosum* in a wheat background. *Genome* 41:1-6.
53. Yildirim, A., Jones, S. S., Murray, T. D., Cox, T. S., and Line, R. F. 1995. Resistance to stripe rust and eyespot diseases of wheat in *Triticum tauschii*. *Plant Dis.* 79:1230-1236.
54. Yildirim, A., Jones, S. S., Murray, T. D., and Line, R. F. 2000. Evaluation of *Dasyphyrum villosum* populations for resistance to cereal eyespot and stripe rust pathogens. *Plant Dis.* 84:40-44.
55. Zadoks, J. C., Chang, T. T., and Konzak, C. F. 1974. A decimal code for the growth stages of cereals. *Weed Res.* 14:415-421.
56. Zhang, H., Reader, S. M., Liu, X., Jia, J. Z., Gale, M. D., and Devos, M. K. 2001. Comparative genetic analysis of the *Aegilops longissima* and *A. sharonensis* genomes with common wheat. *Theor. Appl. Genet.* 103:518-525.