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RESEARCH

Quantitative Cephalosporium Stripe Disease Resistance Mapped in the Wheat Genome

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ABSTRACT

The soil-borne fungus *Cephalosporium gramineum* Nisikado and Ikata causes Cephalosporium stripe disease of winter wheat (*Triticum aestivum* L.), reducing yields significantly in severe cases. There is no known complete resistance to the disease and resistance mapping efforts in wheat have been few. This study was conducted to discover the genomic locations of disease resistance as a preliminary step in the process of molecular marker development. 'Finch' and 'Eltan' progeny were used for quantitative trait loci (QTL) mapping, and a diversity population of 459 individuals was compiled for the first-ever association mapping of resistance to this disease. These populations were genotyped respectively on emergent 9000 (9K) and 90,000 (90K) single nucleotide polymorphism marker platforms and were phenotyped for disease resistance in a 2-yr field study. Twelve resistance QTL were identified in Finch and Eltan, whereas 68 putative QTL were found in the diversity population. Though no QTL of major effect was found, the accumulation of favorable alleles improved resistance, confirming the quantitative nature of Cephalosporium stripe disease resistance. Further investigation will be needed to refine the loci reported here to develop molecular markers to help incorporate and accumulate favorable resistance alleles in new cultivars. Once developed, genetic resistance to Cephalosporium stripe disease will promote economically and environmentally sustainable winter wheat production on *C. gramineum*–infested soil.

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Abbreviations: CSD, Cephalosporium stripe disease; DP, diversity panel; F × E, Finch–Eltan cross; GWAS, genomewide association studies; h^2, heritability; LD, linkage disequilibrium; LOD, logarithm of the odds; PNW, Pacific Northwest; QTL, quantitative trait loci; RIL, recombinant inbred line; SNP, single nucleotide polymorphism; SSR, simple sequence repeat.

The soil-borne fungus *Cephalosporium gramineum* causes the systemic yield-reducing vascular wilt Cephalosporium stripe disease (CSD) of wheat (*Triticum aestivum* L.). First documented in Japan in 1932 (Nisikado et al., 1934), the disease has since been found in other wheat-producing areas of the world including the US Pacific Northwest (PNW), where the first U.S. case was identified in Washington State in 1955 (Bruehl, 1956). Infections of susceptible cultivars with virulent isolates in conducive environments can reduce yield by over 75% (Quincke et al., 2014; Mathre et al., 1977), making winter wheat production with susceptible varieties on infested soil economically unfavorable. Fortunately, the development of genetic resistance to the disease is a very promising avenue of control (Shefelbine and Bockus, 1989). The discovery of molecular genetic markers in the wheat genome associated with CSD resistance will accelerate the development and deployment of disease-resistant wheat varieties for use in an environmentally and economically sustainable integrated disease management package.

Though it is capable of infecting both spring and winter wheat, in standard field settings *C. gramineum* threatens only

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fall-planted varieties, infecting plants through root injuries, which are often caused by soil freeze–thaw cycles in winter and early spring (Raymond and Bockus, 1984). As the plant and fungus develop in spring, fungal hyphae and excretions invade lengthening tillers and begin to clog the stem and leaf vasculature. Symptoms of vascular restriction include plant stunting, chlorotic and necrotic leaf striping, and empty ears known as whiteheads; leaf striping reduces photosynthetic capacity, whereas impaired vasculature decreases water and nutrient uptake and translocation. The net effect is seen as reductions in the yield parameters of kernel size and quantity (Johnston and Mathre, 1972).

No fungicides are presently registered for *C. gramineum*, limiting control options to extended rotations with nonhost species, delayed seeding, destruction of infested straw by burning or plowing, liming of conducive acidic soils, and the use of resistant varieties (Smiley et al., 2009). These options, excepting resistant cultivars, carry negative economic or environmental costs. In contrast, consistent use of resistant varieties lessens disease severity by reducing *C. gramineum* reproduction and thereby cyclically diminishing inoculum volume over multiple growing seasons (Shefelbine and Bockus, 1989). The development and deployment, therefore, of adapted cultivars that are strongly resistant to *C. gramineum* will enhance wheat yield as well as the long-term suppression of CSD in the PNW and other regions where this disease is found. The rarity of the sexual stage of *C. gramineum*, limiting genetic recombination and the evolution of virulence, along with the slow spread of this soilborne pathogen, which hinders the dispersal of new races of unique virulence, enhances the potential for developing highly durable genetic resistance in winter wheat (Quincke et al., 2014; Anderson and Kohn, 1995).

Morton and Mathre (1980a) reported two independent mechanisms of winter wheat resistance to *C. gramineum*: (i) exclusion of the pathogen, resulting in reduced infection, and (ii) mitigation of systemic pathogen invasion and symptom severity once infection has occurred. Rahman et al. (2001) also demonstrated variety-dependent resistance to a toxin produced by *C. gramineum*, suggesting that toxin insensitivity may be an additional resistance mechanism. It is possible, however, that toxin resistance is classifiable within the second mechanism proposed by Morton and Mathre (1980a), which is broadly marked by a reduction in symptom severity. In any case, there is more than one mechanism of CSD resistance to be exploited in wheat, so improving multiple mechanisms within a single variety will provide the most effective means of combating the disease.

Morton and Mathre (1980b) reported significant differences between CSD-infected and noninfected wheat plants in relative water content and stomatal conductance, two physiological parameters that are impacted by *C. gramineum* vascular impairment. Ehrler et al. (1978) showed a strong correlation between water potential and the canopy temperature of wheat, suggesting that infrared measurements of canopy temperature could be used as a proxy to gauge the water stress of wheat plants. We therefore hypothesized that canopy temperature would indicate CSD infection severity.

Genetic mapping approaches such as quantitative trait loci (QTL) mapping and genomewide association studies (GWAS) (see Oraguzie et al., 2007) can be used to pinpoint the precise genomic regions associated with resistance to CSD in genetically variable winter wheat breeding populations. Further investigation of the associated genomic regions can then lead to the development of genetic markers that consistently segregate with disease resistance. The markers identified by genetic mapping can then be used to select for resistance alleles in the parents and progeny of wheat germplasm. In spite of the potential usefulness of such markers, only two known studies to date have analyzed segregating biparental populations of winter wheat to identify QTL conferring resistance to CSD (Quincke et al., 2011; Vazquez et al., 2015); otherwise, investigation of deployable sources of genetic resistance to this disease is severely limited (see also Quincke, 2009; Rahman et al., 2001).

In addition to the analysis of a new biparental population (via QTL mapping with >600 single nucleotide polymorphic [SNP] markers) with 'Finch' (Garland-Campbell et al., 2005) and 'Eltan' (Peterson et al., 1991) as parents, the present study also describes the original resistance screening of a diversity panel of winter wheat collected in the PNW and genetic mapping of resistance in this population via GWAS using 17,892 polymorphic SNP markers distributed genomewide. The purpose of this study was to expedite the discovery of selectable genetic polymorphisms associated with resistance to CSD in winter wheat to be applied in the breeding of resistant cultivars for sustainable wheat production on soil infested with *C. gramineum*.

MATERIALS AND METHODS

Mapping Populations

Two different winter wheat populations were used in this study to understand the genetics of resistance to CSD. A biparental population of 158 $F_{5:6}$ recombinant inbred lines (RILs) was developed by single-seed descent from a cross between the PNW-adapted soft white winter wheat cultivars Finch (PI 628640; Garland-Campbell et al., 2005) and Eltan (PI 536994; Peterson et al., 1991). These mutually adapted cultivars of the same market class were both released from the cooperative state–federal breeding program in Washington State and are therefore likely to be somewhat genetically similar, in spite of lacking immediate common ancestry. In its release publication, however, Finch was said to yield more than Eltan under severe CSD pressure and was touted as being significantly more resistant to the disease than the susceptible check cultivar 'Stephens' (Garland-Campbell et al., 2005; Kronstad et al., 1978); Eltan also received a relatively resistant disease index score in a regional variety testing project that included 43 breeding lines

and released varieties (Finch was not among them), indicating that Eltan, like Finch, is moderately resistant to CSD (Wetzel and Murray, 2014) and that both parents may have a number of quantitative resistance genes to offer. The observation of transgressive segregants in our screening, alongside our eventual mapping results, verified this to be the case. In addition to the biparental mapping population, a diversity panel (DP) comprising 459 advanced-generation soft white winter wheat breeding lines and released cultivars adapted to the PNW was compiled from regional breeding programs (Oregon State University, University of Idaho, USDA–ARS, Washington State University and private breeding companies) (Supplemental Table S1). Both Finch and Eltan, as well as two Finch–Eltan cross (F × E) RIL progeny, were included in the DP.

Phenotypic Evaluation

Three randomized replications of the F × E population and the unreplicated DP population were planted and rated in the 2012–2013 (2013) and 2013–2014 (2014) growing seasons near Pullman, WA. Both Finch and Eltan appeared 11 times within each of the three F × E replicated plantings; furthermore, the susceptible cultivar 'Bruehl' (PI 606764; Jones et al., 2001) was used as a check throughout the F × E and DP plantings, appearing 25 times as such in the DP and once as a constituent accession within that population. These repeated entries served to augment the experimental design and monitor for spatial variation within each planting. Each entry was planted as a single 1-m row of 4 g of seed in mid-September at a row spacing of 30 cm. The susceptible check cultivar Stephens was planted in border plots adjacent to the DP and F × E material. Since the 4-yr chickpea (*Cicer arietinum* L.), spring wheat, summer fallow, and winter wheat rotation scheme of the research plots would tend to minimize natural inoculum density, plantings were artificially inoculated with multiple *C. gramineum* isolates on infested oat (*Avena sativa* L.) kernels (Mathre and Johnston, 1975) broadcast in mid-October at a rate of 193 kg ha^{-1} in 2012 and 180 kg ha^{-1} in 2013 (Wetzel and Murray, 2014). The inoculant comprised the isolates Cg 84-20, 84-45, 84-47, 94-1, and 94-5 in 2012, and the isolates Cg 84-15, 95-4, and 10-15-150 in 2013 (from Dr. Murray's isolate collection).

To test the hypothesis that water potential, and thus CSD severity, is manifested in the canopy temperature of wheat, the canopy temperature of each individual accession row was measured with an infrared thermometer (Sixth Sense LT300, Total Temperature Instrumentation, Inc.) when most accessions were between booting and heading growth stages (Feekes 10 to 10.51) in 2013 and when most entries were between flowering and ripening (Feekes 10.51 to 11.2) in 2014 (Feekes, 1941). As this portion of the research was executed as a proof of concept, canopy temperature was taken at these two different growth stages to test which might better reflect the true CSD severity. A cumulative average temperature was recorded by the instrument while scanning the length of each row. Measurements were taken in early afternoon in calm, sunny conditions, facing away from the sun. The ambient air temperature was 24°C in 2013 and 25°C in 2014 when measurements were taken.

Wheat's physiological development, which corresponds closely with CSD development (Morton et al., 1980), was monitored both years by recording heading date, which trait also provided an internal standard by which to judge the effectiveness of the rating and genetic mapping of disease resistance. Heading date was recorded in ordinal days once 50% of the row had heads that fully extended from the flag leaf.

Disease severity was rated on a 0 to 4 scale analogous to that suggested by Bockus and Sim IV (1982), in which the flag leaf was assigned a value of 4, the second-to-flag leaf had a value of 3, and so on to the fifth leaf down from the flag leaf, which had a value of 0. An entry was given a score of 4 (susceptible) if striping symptoms appeared in the flag leaf. Note that although an entry may have received a score of 0, indicating a high level of resistance, no commercial winter wheat varieties have ever been found to be completely resistant to the disease (Mathre et al., 1977; Quincke et al., 2014). Likewise, we did not observe any symptomless entries among the >600 distinct genotypes tested. As it was impossible to evaluate each tiller of the individual plants of every entry, whole-number scores were assigned on a subjective level for each entry based on the general trend of severity within each row. This scoring method evaluated resistance to systemic spread of the disease through the plant vasculature and was recorded at three time points in both years to monitor developmental progress of both the disease and the expression of resistance, beginning when most accessions were heading and then twice more at approximately 1-wk intervals, the third rating taking place during grain filling (7, 17, and 24 June 2013, except the DP was not rated on 17 June; 6, 12, and 19 June 2014). These three ratings are henceforth referred to as the "first," "second," and "third" severity ratings.

Disease incidence, rated as the percentage of each row showing striping symptoms, was also recorded both years. This rating was designed to measure resistance in the form of the number of plants within each row infected by the disease and was recorded at the grain filling and ripening growth stage when the disease was the most visible but when plants had not yet senesced (25 June 2013 and 30 June 2014).

These severity and incidence rating scales were adopted to evaluate the two mechanisms of resistance proposed by Morton and Mathre (1980a) and reflected the rating methods previously deemed appropriate for screening large germplasm collections (Morton and Mathre, 1980b; Morton et al., 1980; Bockus and Sim, 1982). Blighted whiteheads, a common symptom of CSD, were not rated because they were observed inconsistently in our trials; additionally, rating the percentage of whiteheads would obscure differences between the two resistance mechanisms discussed above, as both incidence and severity would influence whitehead percentage. Also, as no disease-free controls were grown, we were unable to quantitatively evaluate the degree of stunting.

Statistical Analyses

The data were analyzed using SAS version 9.2 software (SAS Institute Inc.). Of the six ratings taken on the F × E population, though the histograms of residuals appeared to be near normal, none passed the Shapiro–Wilkes test for normally distributed residuals, and the first and third severity ratings further did not meet the assumption of constant variance. The first and third severity ratings were therefore square-root-transformed to improve the consistency of variance; otherwise, raw data values were used for statistical analyses, as the ANOVA procedure is robust to large datasets lacking perfectly normal residual distributions.

Spatial variation in the F × E ratings across replications within each year was corrected by adjusting trait values on the basis of the variability in Finch and Eltan throughout the planting. For this correction, an intercept and the least-square means were estimated using the LSMEANS statement and Tukey's adjustment for multiple comparisons in SAS PROC MIXED. Raw heading date, canopy temperature, second severity rating, and disease incidence data were subjected directly to least-square means adjustment, whereas the first and third severity ratings were square-root-transformed before adjustment.

Analysis of variance was executed in SAS PROC GLM to determine significant sources of variation in disease resistance contributed by F × E RILs, years, and replications nested within years and interactions between RILs and years. SAS PROC MIXED was used to estimate magnitude of genetic and phenotypic variances for the estimation of heritability (h^2), which was calculated for the F × E population across years as

$$h^2 = \frac{\sigma^2_G}{\sigma^2_G + \frac{1}{2}(\sigma^2_Y) + \frac{1}{2}(\sigma^2_{GxY}) + \frac{1}{6}(\sigma^2_{R(Y)}) + \frac{1}{6}(\sigma^2_{Resid})}$$

and within each year as

$$h^2 = \frac{\sigma^2_G}{\sigma^2_G + \frac{1}{3}(\sigma^2_R) + \frac{1}{3}(\sigma^2_{Resid})}$$

where G = RIL (i.e., genotype), Y = year, G×Y = RIL × year interaction, R(Y) = replication nested within year, R = replication, and Resid = residual.

Variance in DP disease resistances, canopy temperature, and heading date was analyzed as for the F × E population, except that, because of the lack of replication within years, heritability was calculated as

$$h^2 = \frac{\sigma^2_G}{\sigma^2_G + \frac{1}{2}(\sigma^2_Y) + \frac{1}{2}(\sigma^2_{Resid})}$$

where G = genotype, Y = year, and Resid = residual.

Genotyping and Analysis

DNA was extracted from seedlings of every individual in this study using the standardized methods and materials of the DNeasy 96 Plant Kit (Qiagen). All individuals in the DP were genotyped for 81,587 genomewide SNPs using the wheat-specific 90K iSelect beadchip assay (Wang et al., 2014). Parents and all RILs of the F × E population were genotyped for 8632 SNPs on the wheat-specific 9K iSelect beadchip assay (Cavanagh et al., 2013). In addition to the SNP markers, 156 simple sequence repeat (SSR) markers were assayed on the F × E parents, 49 of which were found to be polymorphic and were subsequently analyzed in the RIL population. Sequences of the available single sequence repeat (SSR) markers, along with their previously determined chromosomal locations, were obtained from GrainGenes (http://wheat.pw.usda.gov/GG3/, accessed 27 July 2015). Simple sequence repeat marker analyses were conducted using the polymerase chain reaction conditions described by Röder et al. (1998), except that primers were synthesized to include the M13-tail (Oetting et al., 1995). Appropriate fluorophores for the ABI 3130xl (Applied Biosystems) fragment detection system were included in the polymerase chain reaction mix.

The raw output of the 9K and 90K SNP tests was visualized and manipulated in GenomeStudio version 2011.1 software (Illumina); auto-called allelic clustering of F × E and DP individuals was visually reviewed for each polymorphic SNP, and clusters were manually reassigned if they were found to be incorrectly called. The resulting 1154 polymorphic F × E SNPs were then filtered to exclude markers for which >5% of the population was missing. Further filtering removed redundant markers and those that failed an $\alpha = 0.05$ χ^2 analysis of expected segregation between Finch and Eltan parental alleles. The remaining 618 SNPs, in addition to the 49 polymorphic SSRs, were assembled into 24 linkage groups in JoinMap version 4 (van Ooijen, 2006) via maximum likelihood mapping with Haldane's mapping function and the independence logarithm of the odds (LOD) parameter. Consensus positions of the SNPs in the 9K assay (Cavanagh et al., 2013) and of the SSRs (http://wheat.pw.usda.gov/GG3/, accessed 27 July 2015) were used to assign chromosome names and orientation to each linkage group, revealing that all but chromosomes 3 through 6 of the D subgenome were represented by the 24 linkage groups.

Diversity panel SNPs were similarly filtered to ensure marker quality and utility for mapping, except that the number of missing individuals per marker was restricted to <20%; minor allele frequency was limited to >5%. After filtering, 17,892 mapped SNPs remained, which, when assigned to their consensus positions (Wang et al., 2014), were found to represent all chromosomes in the wheat genome, although the D subgenome was generally covered with less marker density than the A and B subgenomes (only 9.6% of the markers were located on the D genome, rather than the 33% that would be expected if all genomes were equally covered).

Quantitative Trait Loci Mapping and Genomewide Association Studies

Quantitative trait loci were identified in the F × E population using WinQTL Cartographer version 2.5 software (Wang et al., 2007) running the composite interval mapping procedure (Zeng, 1994) under the following parameters: model, 6; forward and backward regression method; window size, 10.0 cM; 0.10 probability for into/out; walk speed, 2.0 cM. The LOD threshold was determined by 1000 permutations at $\alpha = 0.05$. Raw heading date, canopy temperature, second severity rating, incidence data, and square-root transformed first and third severity rating data of each replication from each year was analyzed separately as well as means-adjusted values for each trait within both years to evaluate the consistency of QTL identification across variable environments.

Genomewide association studies of the DP for all traits and experiments were executed in the GAPIT R package (Lipka et al., 2012) using the compressed mixed linear model. The compressed mixed linear model in GAPIT considers individuals as random effects, allowing the inclusion of marker-based relationships of individuals (kinship) as well as the structure of the entire population in the calculation of association probabilities for each marker; both parameters were calculated by GAPIT from the marker data that we supplied to the program for mapping. The VanRaden algorithm was used to derive kinship

from genotypes (VanRaden, 2008), and principal component analysis was used to calculate the population structure. This compressed mixed linear model method maximizes the statistical power of GWAS (Yu et al., 2006).

Associations were only reported at a nominal $P < 0.01$ and if they appeared in multiple ratings or years. (The more statistically conservative false discovery rate P values produced by GAPIT for each marker were too strict to be useful because of the high number of multiple comparisons involved in mapping with the thousands of SNP markers of the DP. That is, each additional marker analyzed inflates the false discovery rate; after a certain point when the number of markers analyzed rises into the thousands, depending on the nature of the trait under consideration, the false discovery rate can become unrealistically restrictive. The lowest marker false discovery rate P value observed was $P = 0.1$, and that for heading date.) These stringencies, combined with the powerful compressed mixed linear model method employed in GAPIT, were employed to reduce the possibility of reporting spurious associations. The high density of SNP markers in some genomic regions often led to the identification of many linked SNPs that were significantly associated with resistance. The marker within each of these continuous marker linkage groups with the most significant disease resistance association P value was therefore reported as the tagging SNP for that linked group of markers, and the R^2 and allelic effect were reported for that marker in the environment in which its most significant association P value was earned.

To verify if sequential loci were linked or not, chromosome-wise linkage disequilibrium (LD) was calculated between markers of unique consensus locations using GGT v2.0 software (van Berloo, 2008). Heat map visualization of LD intensity along the length of each chromosome helped to show which markers were likely to indicate the same locus. If LD grouping was not clear in the heat map, additional criteria were used to judge linkage: tagging markers were considered to be linked to one another if they were at a distance of <15 cM and if they appeared in similar, if not identical, ratings and environments. This multilevel decision method was implemented in cases of unclear LD patterns to overcome the unpredictability of wheat LD decay, which is locus specific and varies on the basis of preferential recombination frequency and can range from <1 to >40 cM (Somers et al., 2007). All tagging markers were still reported, but those suspected to be linked to the same locus were grouped together and annotated by a shared group number following the chromosomal number and letter designation (e.g., 1A.1).

RESULTS

Phenotypes and Statistical Analyses

Disease pressure was observed to be strong both years, judged by visibly severe striping and stunting symptoms in susceptible border plots of Stephens as well as visible striping across experimental rows. In both years, the first severity rating of the F × E RILs and the DP was typically lower than the subsequent second and third severity ratings, as disease severity evidently increased with time and plant development (Table 1). The range of F × E scores was also greater in the second and third severity ratings, demonstrating a more distinct separation of susceptible and resistant genotypes. Though the mean severity score of the DP also increased with time, the range was consistently 0 to 4 because of the wider genetic diversity in this population as opposed to that of the F × E RILs derived from moderately resistant parents. Least-square means-adjusted disease rating data from the F × E RILs exhibited roughly normal distributions within both years (Fig. 1), illustrating the quantitative nature of CSD resistance.

Both Finch and Eltan were moderately resistant and not statistically different from one another in the first ($P = 0.2375$) and second ($P = 0.6725$) severity ratings, though Finch demonstrated sometimes higher and often wider-ranging severity scores than Eltan, a result

Table 1. Mean and range of Cephalosporium stripe disease resistance ratings of Finch, Eltan, Finch × Eltan (F × E) recombinant inbred lines (RILs), and a diversity population (DP) of winter wheat recorded in Pullman, WA, in 2013 and 2014. First, second, and third represent severity ratings (0 = resistant, 4 = susceptible) taken at approximately 1-wk intervals from heading (first) to grain filling (third); disease incidence was rated at grain filling and ripening as the percentage of the row infected. Infrared canopy temperature was recorded between booting and heading stages in 2013 and between flowering and ripening in 2014.

	2013					2014				
	First	Second	Third	Incidence	Canopy temperature	First	Second	Third	Incidence	Canopy temperature
				%	°C				%	°C
Mean score										
Finch	1.5	2.9	2.4	38.8	24.8	1.6	1.8	2.6	31.5	29.6
Eltan	1.7	2.4	1.6	19.7	24.8	2.1	2.0	1.8	10.9	28.6
F × E RILs	1.7	2.8	2.0	32.0	24.8	1.9	2.3	2.7	31.8	29.2
DP	2.0	NA	2.4	46.0	25.9	2.0	2.5	2.6	43.3	28.8
Stephens†	4	NA	4	80.0	30.4	4	4	4	70.0	25.6
Range										
Finch	1–2	1–4	1–3	10–60	20.4–27.9	0–2	0–3	1–4	10–50	27.2–32.2
Eltan	0–2	2–3	1–3	0–40	21.8–28.8	1–3	1–3	1–3	0–30	26.3–31.3
F × E RILs	0–3	0–4	0–4	0–70	19.9–28.9	0–3	0–4	0–4	0–90	25.9–34.8
DP	0–4	NA	0–4	0–100	20.6–31.8	0–4	0–4	0–4	0–100	23.5–33.8

† Stephens, a well-known susceptible check, occurred as a single entry in the DP both years; therefore, the disease scores of Stephens given here are not mean values.

in contrast to the advertised and expected superiority of Finch for CSD resistance (Garland-Campbell et al., 2005), though we could not compare the yield of the two varieties as no yield data were collected. Both parents were nearly significantly different in the third severity rating (P = 0.0861) and disease incidence (P = 0.0565). In the mean and range of disease incidence, Finch rated consistently worse than Eltan.

Canopy temperature was not at all diagnostic of resistance nor significantly different between Finch and

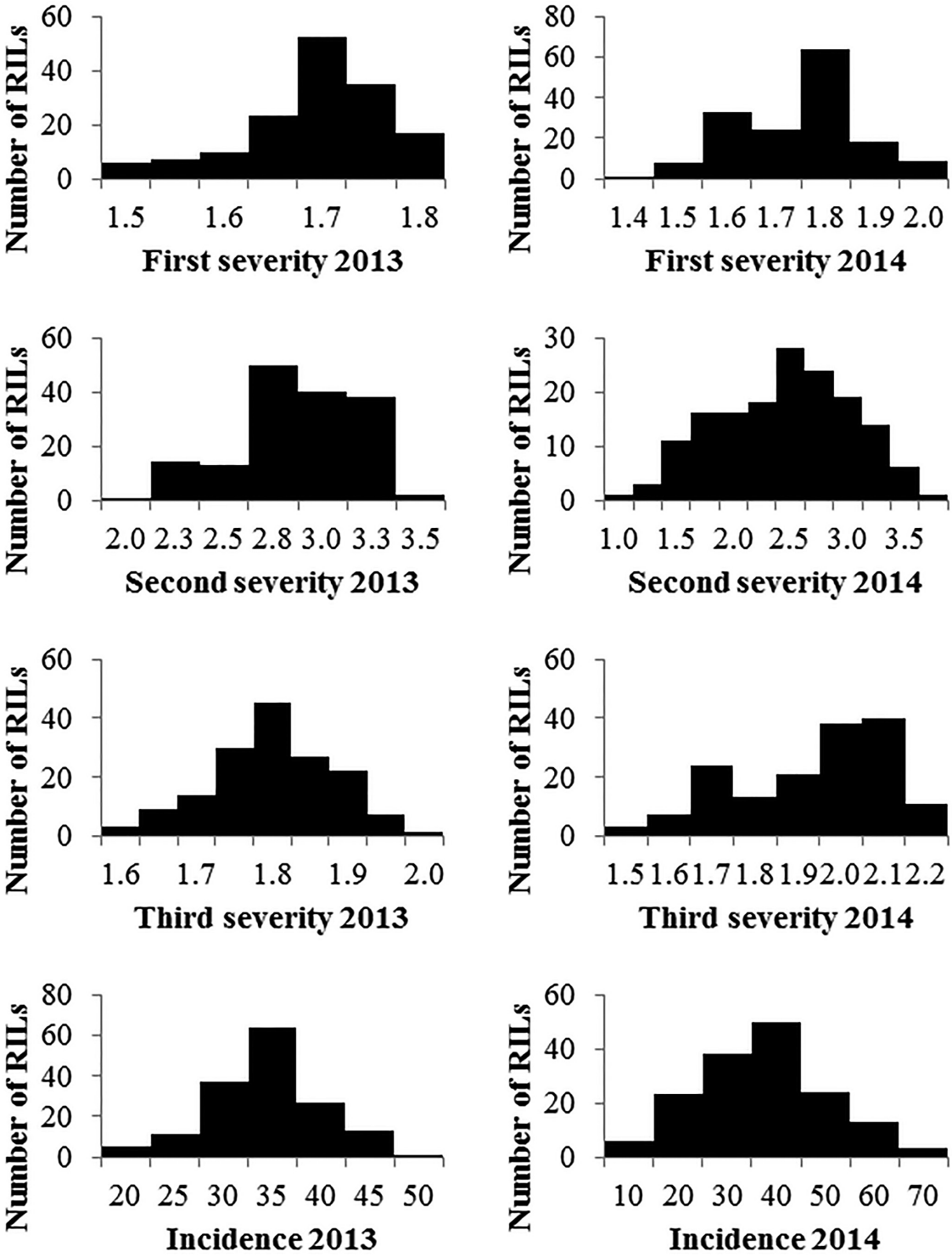

Fig. 1. Histograms demonstrating the quantitative nature of Cephalosporium stripe disease resistance in wheat as seen in the first, second, and third severity ratings and infection incidence for 2013 and 2014 least-square-means-adjusted data collected from the 157 Finch × Eltan (F × E) recombinant inbred lines in Pullman, WA. Severity ratings (lower = more resistant) were taken at approximately 1-wk intervals from heading (first) to grain filling (third); disease incidence was rated at grain filling and ripening as percentage of the row infected (lower = more resistant). First and third severity ratings were square-root-transformed before least-square-means adjustment. RILs, recombinant inbred lines.

Eltan (P = 0.4604), RILs (P = 0.0956), or DP members (P = 0.8318), whereas the random effects of year and replication had highly significant (P < 0.0001) impacts on variation in canopy temperature in all instances (Table 2). Finch and Eltan were not significantly different from one another in heading date (P = 0.1213), but there were highly significant differences in heading date between RILs and DP members (both P < 0.0001) (Table 2).

The ANOVA results and h^2 estimates for disease resistance traits, as well as heading date and canopy temperature traits, are presented for both populations in Table 2. Heading date, a highly heritable trait, acted as a standard to evaluate the effectiveness of disease resistance variance and h^2 estimation. The h^2 of heading date in our populations was 0.81 to 0.85, well within the range of heading date h^2 reported elsewhere (Kuchel et al., 2006). A higher level of environmental variance in 2013 than in 2014 is shown by the trend of lower h^2 in 2013 than in 2014 observed for all disease-related traits. The greater environmental variation in 2013 likewise depressed the disease resistance h^2 values that were calculated over both years (Table 2). Across all traits the DP consistently yielded lower h^2 values than the F × E population owing to lack of DP replication within years.

The ANOVA of least-square-means-adjusted RIL phenotypic data and DP phenotypic data revealed a significant genotype effect (P < 0.0001) on all disease trait values except canopy temperature (Table 2). The F × E and DP populations therefore contained significant genetic variation impacting the severity and incidence mechanisms of CSD resistance, warranting the genetic mapping of resistance in these populations.

Quantitative Trait Loci Mapping and Genomewide Association Studies

Multiple significant CSD resistance QTL were identified in the F × E population (Table 3). Only QTL from least-square means-adjusted data are reported, since these represent the genomic regions consistently identified across replications. Some QTL differed among the three severity ratings taken at progressive stages of disease development, suggesting that quantitative resistance to CSD may be progressively expressed throughout the course of plant and disease development. The QTL identified for heading date yielded the highest LOD scores of all traits analyzed (data not shown), indicating that heading date was less impacted by environmental variation than disease resistance traits; this observation is corroborated by heading date's high h^2 (Table 2). Another factor impacting the h^2 and mapping of heading date versus disease resistance may have been that heading date was more precisely and consistently rated than disease resistance because of the relative ease of accurately grading heading date as opposed to the more subjective disease-scoring methods employed in this study. On the other extreme, mapping of canopy temperature yielded few and inconsistent QTL (data not shown), none of which matched the QTL clearly associated with disease resistance as rated on severity and infection incidence scales, even though both of these resistance mechanisms would theoretically impact canopy temperature in the presence of infection, and in spite of the fact that canopy temperature was recorded as an entirely objective quantitative value.

Principal component analysis in GAPIT revealed three structural groups in the DP. Genomewide association studies of the DP revealed many loci associated with CSD resistance, some suspiciously close to one another and possibly within the same linkage block (Table 4;

Table 2. The ANOVA results for Cephalosporium stripe disease resistance ratings as well as heading date and canopy temperature of the bi-parental Finch × Eltan (F × E) recombinant inbred lines (RILs) and a diversity population (DP) of winter wheat evaluated in Pullman, WA, in 2013 and 2014. First, second, and third severity ratings were taken at approximately 1-wk intervals from heading (first) to grain filling (third);

		Trait P values and h^2†					
Population	Source of variation	First severity	Second severity	Third severity	Disease incidence‡	Heading date§	Canopy temperature¶
F × E	Replicate(year)	0.0384	0.3043	< 0.0001	< 0.0001	0.0002	< 0.0001
	Year	<0.0001	<0.0001	<0.0001	0.7810	0.0037	<0.0001
	RIL	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0956
	RIL × year	0.0090	0.0007	<0.0001	<0.0001	0.0329	0.7819
	h^2	0.62	0.53	0.53	0.66	0.85	0.01
DP	Year	0.6056	NA#	0.9644	0.0089	<0.0001	<0.0001
	Genotype	<0.0001	NA	<0.0001	<0.0001	<0.0001	0.8318
	h^2	0.39	NA	0.49	0.48	0.81	0.00

† h^2, heritability.

‡ Disease incidence was rated at grain filling and ripening as percentage of the row infected.

§ Heading date was recorded in ordinal days once 50% of the row had heads fully extended from the flag leaf.

¶ Infrared canopy temperature was recorded between booting and heading stages in 2013 and between flowering and ripening in 2014.

NA, not available; the second severity rating was not taken on the DP in 2013.

Table 3. Cephalosporium stripe disease resistance quantitative trait loci (QTL) in winter wheat and associated single nucleotide polymorphism (SNP) or simple sequence repeat (SSR) markers identified by quantitative trait loci (QTL) mapping in recombinant inbred lines from Finch and Eltan parents rated for disease resistance in Pullman, WA, in 2013 and 2014.

QTL	Trait (year)†	Closest marker	Peak position on linkage group	Linkage group position of closest marker (90K consensus position)	QTL boundaries; linkage group position	Nearest boundary SNP markers (90K consensus position)	LOD of QTL (LOD threshold‡)	Source of resistant allele	Allelic effect	R^2§
			cM¶							
QCsel.wak-1A	% (2013)	IWA3434	104.5	104.8 (105.7)	93.2–138.6	IWA1081–IWA475 (102.3–118.8)	5.67 (2.9)	Eltan	−0.20	0.11
	Third (2013)	IWA735	114.6	114.4 (113.2)	99.8–138.6		4.44 (2.9)	Eltan	−0.11	0.10
QCsel.wak-1B#	% (2013)	IWA4849	16.1	19.5 (70.1)	9.3–36.1	IWA1302–IWA7560 (64.9–74.4)	5.04 (2.9)	Eltan	−0.19	0.12
	Third (2013)	IWA4849	18.2	19.5 (70.1)	9.3–30.2		4.01 (2.9)	Eltan	−0.10	0.08
QCsfi.wak-2A	First (2014)	IWA2612	58.5	59.2 (116.6)	31.2–76.0	IWA3368–IWA2640 (103.6–116.2)	4.38 (3.1)	Finch	−0.15	0.12
QCsel.wak-2B	Second (2014)	IWA2946	9.3	9.3 (173.4)	0.0–12.0	IWA3315–IWA2094 (158.7–161.4)	4.29 (3.0)	Eltan	−0.17	0.08
	Third (2014)	IWA2946	9.3	9.3 (173.4)	0.0–12.0		4.46 (3.0)	Eltan	−0.23	0.10
QCsfi.wak-3B	Third (2013)	IWA2400	111.6	114.3 (75.2)	104.2–132.0	IWA4427–IWA4841 (74.3–76.6)	5.08 (2.9)	Finch	−0.10	0.13
	% (2013)	IWA2400	111.6	114.3 (75.2)	107.8–132.0		3.54 (2.9)	Finch	−0.17	0.08
	Third (2014)	IWA3402	114.6	115.0 (77.3)	107.8–132.0		3.94 (3.0)	Finch	−0.15	0.08
	First (2014)	IWA3402	114.8	115.0 (77.3)	104.8–132.0		3.45 (3.1)	Finch	−0.11	0.07
	Second (2013)	wmc471	117.5	117.3 (NA††)	107.8–132.0		5.58 (2.9)	Finch	−0.12	0.13
QCsel.wak-4A.1	First (2014)	IWA5897	107.5	107.6 (48.2)	83.1–122.7	IWA5363–IWA4023 (40.3–58.4)	3.65 (3.1)	Eltan	−0.11	0.07
	% (2014)	IWA6873	112.2	112.2 (48.5)	82.9–122.7		6.93 (3.0)	Eltan	−0.49	0.13
	Second (2014)	IWA6873	112.2	112.2 (48.5)	82.9–122.7		6.00 (3.0)	Eltan	−0.21	0.12
	Third (2014)	IWA2901	123.3	123.5 (49.0)	113.3–140.7		8.07 (3.0)	Eltan	−0.28	0.17
	Second (2013)	IWA6193	139.7	139.6 (54.5)	128.7–154.4		5.25 (2.9)	Eltan	−0.12	0.12
QCsfi.wak-4A.2	First (2013)	IWA2816	251.5	251.7 (102.0)	228.0–264.0	IWA2761–IWA4527 (91.2–108.7)	4.46 (2.9)	Finch	−0.08	0.10
QCsfi.wak-4B	% (2013)	gwm513	23.5	23.5 (NA)	19.5–33.2	IWA327–IWA3240 (62.2–69.9)	3.24 (2.9)	Finch	−0.15	0.06
	First (2013)	IWA8564	27.5	27.7 (64.0)	16.5–33.2		3.86 (2.9)	Finch	−0.08	0.09
QCsfi.wak-5A	Second (2014)	IWA4670	187.4	188.6 (56.5)	178.1–199.6	IWA6522–IWA7404 (56.5–62.7)	5.55 (3.0)	Finch	−0.21	0.12
	% (2014)	IWA4670	187.4	188.6 (56.5)	178.1–199.6		5.15 (3.0)	Finch	−0.41	0.10
QCsfi.wak-5B	% (2014)	IWA4103	101.4	99.2 (97.3)	82.8–109.9	IWA6894–IWA4057 (83.0–104.6)	3.82 (3.0)	Finch	−0.37	0.08
QCsel.wak-6B	Third (2014)	IWA1817	74.7	90.4 (91.1)	47.1–110.4	IWA5170–IWA5666 (72.3–95.8)	4.61 (3.0)	Eltan	−0.32	0.21
QCsel.wak-7A	% (2014)	IWA6124	57.7	59.8 (135.8)	40.6–97.0	IWA6124–IWA7933 (135.8–136.4)	3.47 (3.0)	Eltan	−0.42	0.10

† First, second, and third disease severity ratings were taken at approximately 1-wk intervals from heading (first) to grain filling (third); % signifies disease incidence, which was rated at grain filling and ripening as percentage of the row infected. Year is the year in which the reported QTL peak appeared in association with the noted trait.

‡ The logarithm of the odds (LOD) threshold was determined by 1000 permutations at $\alpha = 0.05$.

§ R^2 = proportion of variation in disease resistance score contributed by each QTL. The total R^2 of reported QTL for first (2013), 0.19; first (2014), 0.26; second (2013), 0.25; second (2014), 0.32; third (2013), 0.31; third (2014), 0.56; % (2013), 0.37; % (2014), 0.41.

¶ Reported genetic distances (cM) include both Finch × Eltan (F × E) specific recombination distances per linkage group as well as the 90K consensus map positions of indicated SNP markers; distinctions are indicated in the column headings.

The underlined QTL are similar in position to QTL found in the DP and may represent the same underlying gene.

†† NA, not applicable; SSR markers are not included in the 90K consensus map.

Table 4. Single nucleotide polymorphism (SNP) markers associated with Cephalosporium stripe disease resistance discovered by linkage disequilibrium mapping in a diversity population (DP) of winter wheat rated for disease resistance in Pullman, WA, in 2013 and 2014.

QTL†	Tagging marker‡	Position	Nominal *P* value	R^2§	Allelic effect¶	Years and ratings with significant marker–trait associations
		cM				
QCs.wak-1A.1	IWB73129	29.7	0.0039	0.02	–0.23	2013: third, %; 2014: second#
QCs.wak-1A.2	IWB35770	62.0	0.0010	0.02	–0.48	2013: %; 2014: %
	IWB5974	70.9	0.0006	0.03	–0.62	2013: first, third, %; 2014: first, %
QCs.wak-1A.3	IWB22117	139.7	0.0009	0.03	–0.16	2013: first, third, %; 2014: first
	IWB26254	148.7	<0.0001	0.04	–0.18	2013: first, third, %; 2014: %
	IWA1644	154.6	0.0004	0.03	–0.18	2013: first, third
QCs.wak-1B.1	IWB40971	31.0	0.0038	0.02	–0.99	2013: first, third, %; 2014: second, third
	IWB1655.1	43.9	0.0008	0.02	–0.73	2013: first, third; 2014: %
QCs.wak-1B.2	IWB58775	60.6	0.0029	0.02	–0.47	2013: first; 2014: third, %
QCs.wak-1B.3††	IWA188	70.9	0.0024	0.02	–0.39	2013: third; 2014: first, %
	IWA6917	74.4	0.0010	0.02	–0.90	2014: second, third, %
QCs.wak-1B.4	IWB11914	96.2	0.0016	0.02	–0.50	2014: second, %
QCs.wak-1B.5	IWB7459	117.8	0.0047	0.02	–0.26	2013: first, %
QCs.wak-1B.6	IWB23772	135.2	<0.0001	0.03	–0.71	2014: first, second, third, %
QCs.wak-1D.1	IWB21355	23.2	0.0040	0.02	–0.33	2013: third; 2014: second
QCs.wak-1D.2	IWA6675	65.1	0.0023	0.02	–0.51	2013: third; 2014: third
	IWA165	73.1	0.0012	0.02	–1.01	2014: third, %
QCs.wak-1D.4	IWA1559	164.7	0.0029	0.02	–0.32	2013: third; 2014: first
QCs.wak-2A.1	IWA6481.1	6.0	0.0070	0.01	–0.42	2014: second, %
QCs.wak-2A.2	IWB24342	26.0	0.0003	0.02	–1.14	2013: third; 2014: second, %
	IWA8091	47.2	0.0012	0.02	–0.67	2013: first, %; 2014: first, second, %
QCs.wak-2A.3	IWB9395	102.0	0.0023	0.02	–0.64	2013: first; 2014: first, third
QCs.wak-2A.4	IWB13459	149.9	0.0045	0.02	–0.22	2013: first, %; 2014: first %
QCs.wak-2B.1	IWB43910	20.9	<0.0001	0.03	–0.23	2013: third, %; 2014: %
	IWB26388	27.2	0.0007	0.03	–0.23	2013: third, %
QCs.wak-2B.2	IWB48659	109.5	0.0049	0.01	–0.42	2013: first, %; 2014: first, second, third
QCs.wak-2B.3	IWB25863.1	134.5	0.0013	0.02	–0.15	2013: first; 2014: %
QCs.wak-2D.1	IWB25308	6.8	0.0023	0.02	–0.17	2014: first, third, %
	IWB11455	11.2	0.0011	0.02	–0.35	2013: third; 2014: first, %
QCs.wak-2D.2	IWB8864	82.8	0.0011	0.02	–0.55	2013: third, %; 2014: first
QCs.wak-3A.1	IWB50475	15.1	0.0014	0.02	–0.75	2014: first, %
QCs.wak-3A.2	IWB53157	86.2	0.0008	0.02	–0.49	2013: first; 2014: first, second
QCs.wak-3A.3	IWB26072	145.6	0.0011	0.02	–0.30	2013: first, %
QCs.wak-3A.4	IWB12268	177.2	0.0031	0.02	–0.21	2013: first; 2014: first, second, third
	IWB67817	180.3	0.0029	0.02	–0.14	2013: first, %; 2014: first, second, third
QCs.wak-3B.1	IWB29578	9.0	0.0009	0.03	–0.30	2013: first, third, %; 2014: second, third
	IWB24979	14.1	0.0019	0.02	–0.41	2014: second, third, %
QCs.wak-3B.2	IWB2169	37.6	0.0003	0.03	–0.32	2014: third, %
	IWB24150	45.9	0.0047	0.01	–0.24	2014: third, %
	IWB25086	50.0	<0.0001	0.03	–1.14	2014: third, %
	IWB9716	62.6	0.0043	0.02	–0.24	2014: first, third
QCs.wak-3B.3	IWB12493	70.1	0.0002	0.03	–0.27	2013: %; 2014: second, third, %
QCs.wak-3B.4	IWB9095	99.4	0.0042	0.02	–0.42	2014: first, third
	IWB64607	107.2	0.0039	0.02	–0.20	2013: %; 2014: first, third
QCs.wak-3D.1	IWB42835	4.0	0.0029	0.02	–0.37	2013: first, third
QCs.wak-3D.2	IWB48173	119.4	0.0005	0.02	–0.22	2014: first, third
QCs.wak-4A.1	IWA3993	33.8	0.0018	0.02	–0.79	2014: third, %
QCs.wak-4A.2	IWB6937	49.0	0.0014	0.02	–0.76	2013: third, %
QCs.wak-4A.3	IWB36596	66.7	0.0005	0.02	–0.42	2014: second, third, %
QCs.wak-4A.4	IWB23723	79.3	0.0021	0.02	–0.27	2013: first, %
QCs.wak-4B.1	IWB35920	35.6	<0.0001	0.03	–0.96	2013: %; 2014: second, third, %

Cont'd.

Table 4. Continued.

QTL†	Tagging marker‡	Position	Nominal *P* value	R^2§	Allelic effect¶	Years and ratings with significant marker–trait associations
		cM				
QCs.wak-4B.2	IWB4673	64.0	0.0008	0.02	–0.22	2014: first, second, %
	IWB51658	68.5	0.0004	0.03	–0.19	2013: first, third, %; 2014: first
	IWB52994	71.5	0.0003	0.03	–0.18	2013: first, third, %; 2014: first, second
	IWB10847	75.6	<0.0001	0.04	–0.23	2013: first, third, %; 2014: first, second, third, %
	IWB71718	83.5	0.0057	0.02	–0.90	2013: first, third, %
QCs.wak-4D	IWB8050	74.0	0.0009	0.02	–0.47	2013: %; 2014: third, %
	IWA161	83.8	0.0020	0.02	–0.45	2013: third, %
QCs.wak-5A.1	IWB73735	49.7	0.0043	0.02	–0.18	2013: %; 2014: first
QCs.wak-5A.2	IWB6827	124.1	0.0010	0.02	–0.52	2013: first, third, %
QCs.wak-5B.1	IWB68150	11.2	0.0002	0.03	–0.30	2014: first, second, third
QCs.wak-5B.2	IWB7361	57.8	0.0035	0.02	–0.46	2014: first, %
QCs.wak-5B.3	IWB41910	89.5	0.0035	0.02	–0.45	2014: second, %
QCs.wak-5B.4	IWB36107	123.7	<0.0001	0.03	–0.49	2014: second, third, %
QCs.wak-5B.5	IWB28628	182.1	0.0012	0.02	–0.20	2013: first, third, %
QCs.wak-6A.1	IWB64084.1	4.7	0.0022	0.02	–1.00	2013: %; 2014: first
	IWB67412	13.5	0.0038	0.02	–0.31	2013: third; 2014: first, %
QCs.wak-6A.2	IWB73827	45.1	0.0016	0.02	–0.93	2013: third, %; 2014: second
	IWB61592	48.1	0.0007	0.02	–0.24	2014: second, third, %
	IWB55812	54.7	0.0063	0.01	–0.15	2013: third, %
QCs.wak-6A.3	IWA5930	63.0	0.0004	0.03	–1.04	2013: first, %
QCs.wak-6A.4	IWB30129	84.7	0.0083	0.01	–0.46	2014: first, third, %
QCs.wak-6A.5	IWB3566	95.9	0.0036	0.02	–0.34	2014: first, second, third
QCs.wak-6A.6	IWA7764	119.6	0.0005	0.03	–1.04	2013: third, %
	IWB65197	126.2	0.0013	0.02	–0.39	2013: third, %; 2014: second, %
	IWB997.1	140.9	0.0011	0.02	–0.59	2013: first, %; 2014: second
QCs.wak-6B.1	IWB12597	39.2	0.0030	0.02	–0.15	2013: %; 2014: first
QCs.wak-6B.2	IWB36146	66.6	0.0028	0.02	–0.37	2013: first, third; 2014: first, second, third, %
QCs.wak-6B.3	IWB30504	93.0	0.0003	0.03	–0.37	2013: first, third, %
QCs.wak-6B.4	IWB13533	119.0	0.0030	0.02	–0.64	2013: first, third, %; 2014: third, %
QCs.wak-6D.1	IWB64086	9.1	0.0010	0.02	–0.23	2013: third, %
QCs.wak-6D.2	IWB45050	24.8	0.0009	0.02	–0.42	2014: third, %
QCs.wak-6D.3	IWB10505	153.1	0.0053	0.02	–0.53	2013: first, third, %
QCs.wak-7A.1	IWB26501	46.0	0.0047	0.02	–1.08	2013: first, %
QCs.wak-7A.2	IWB61471	121.4	0.0005	0.03	–0.20	2013: first, %
QCs.wak-7B.1	IWB69202	10.1	0.0005	0.02	–0.45	2014: first, second, third
QCs.wak-7B.2	IWB7697	71.3	0.0040	0.02	–0.28	2014: second, third, %
	IWA1420	77.1	0.0009	0.02	–0.46	2013: first; 2014: second, third
QCs.wak-7B.3	IWA4750	133.6	0.0016	0.02	–0.21	2014: first, second, third, %
QCs.wak-7B.4	IWB10917	159.0	0.0041	0.02	–0.44	2013: third, %; 2014: first
QCs.wak-7B.5	IWB13363	171.1	0.0021	0.02	–0.14	2013: %; 2014: first, third, %
QCs.wak-7D.1	IWB72773	93.7	0.0016	0.02	–0.22	2014: second, third
QCs.wak-7D.2	IWB39510	148.9	0.0011	0.02	–0.15	2013: first, %; 2014: first
QCs.wak-7D.3	IWB74668	211.9	0.0047	0.02	–0.85	2013: %; 2014: first

† *QCs.wak-1A.1* indicates the first (in cM order) putative quantitative trait locus (QTL) located on chromosome 1A. Tagging markers, if more than one is listed within a single putative QTL, were originally selected from significantly associated marker groups identified across multiple ratings or years and after linkage disequilibrium, trait behavior, and cM distance analysis, were deemed to probably belong to the same larger QTL.

‡ Reported marker was, of all the significant markers in the original putative marker group, most strongly associated with resistance. R^2 and allelic effect values are for this marker in the environment and trait in which it earned its lowest *P* value.

§ R^2 = proportion of variation in the disease resistance score contributed by each marker in the year reported.

¶ Allelic effect is the relative magnitude of reduction in the disease severity or incidence score attributable to the favorable allele of the reported marker (more negative means more resistance).

Underlining indicates the year and rating in which the most significant SNP was found and from whence the *P* value, R^2, and allelic effect values are taken. First, second, and third represent the severity ratings taken at approximately 1-wk intervals from heading (first) to grain filling (third); % (disease incidence) was rated at grain filling and ripening as the percentage of the row infected.

†† Underlining of QTL indicates correspondence to QTL discovered in the F × E population on the basis of a comparison of the consensus map positions of the associated SNP markers.

Supplemental Table S1). Linkage disequilibrium analysis and visualization showed generally rapid LD decay (~1–5 cM), indicating that though they were in close proximity on the consensus map, in some cases significantly associated SNP were not obviously linked. (Note that the 90K consensus map is not population specific and therefore may not exactly represent the recombination frequency unique to the lines used in our research.) Low LD was expected because of the large number of individuals in the population and their mutual adaptation. Our LD results, however, were not always clear, so caution is advised when considering whether reported SNP are or are not linked; a closer look at the indicated loci separated by short recombination distances will be necessary to verify if they are signaling the same or distinct genes. A list of DP accessions, their disease scores, and their disease-associated marker alleles are provided in Supplemental Table S1. Note that most lines in the DP are not released cultivars and therefore do not have proper cultivar names and, furthermore, are exclusively held by the associated breeding program.

DISCUSSION

The indistinguishable difference in canopy temperatures among RILs, along with the highly significant differences between replications and years, indicates that the evaluation of canopy temperature as done in this study is an unsuitable method of gauging wheat resistance to CSD. Probably, the use of larger plots, more frequent scans, and infected versus clean controls would help moderate some of the environmental variation that reduced the utility of this measurement in our study. Further investigation into correlating CSD severity with canopy temperature is warranted and encouraged.

Previous QTL mapping studies of CSD resistance in winter wheat have identified from six to nine QTL in three different winter wheat biparental mapping populations representing six parental varieties (Quincke et al., 2011; Vazquez et al., 2015). In contrast, the biparental portion of our research revealed 12 QTL, and the GWAS of a linkage disequilibrium population revealed associations on every chromosome of wheat, except 5D, and many chromosomes carried more than one locus associated with CSD resistance. In no instance, however, was any single locus identified that controlled a majority of the variation in resistance, with most loci contributing only marginal effects (Table 3 and Table 4), though they were of cumulative value (Fig. 2). This quantity of diverse significant loci was surprising, although we did expect to uncover more QTL in the association mapping population than in the biparental population simply because there was more genetic diversity represented therein. Greater genome resolution was achieved in the DP because of the accumulation of meiotic recombination within the population and the high marker density characteristic of the 90K SNP marker platform. These numerous loci, in light of the range of disease scores seen throughout the study (e.g., Fig. 1 and Table 1), illustrate the quantitative nature of CSD resistance in winter wheat. Disease resistance on this quantitative level is more difficult to accumulate by breeding than simply inherited race-specific resistance to a specialized pathogen; therefore, selectable markers for components of CSD resistance would greatly improve breeding efficiency.

Bruehl (1983), the pathologist who first reported *C. gramineum* in the United States, reviewed the phenomenon

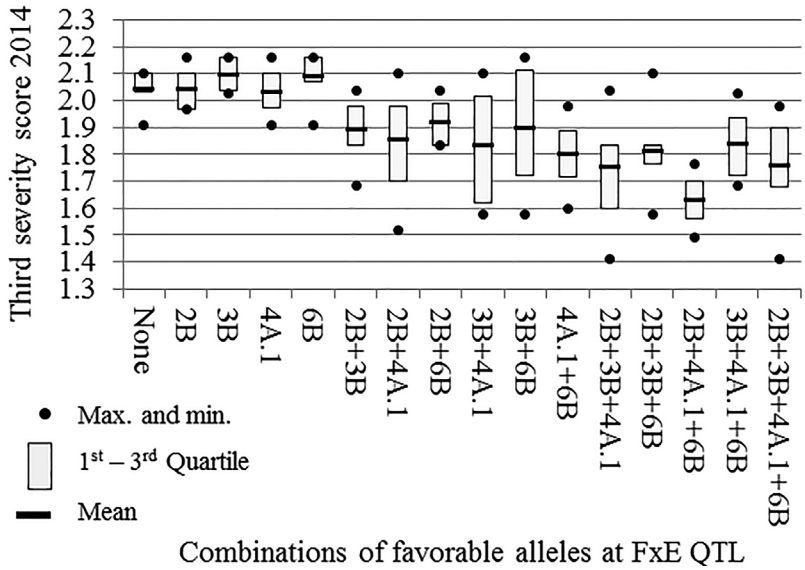

Fig. 2. Combinations of favorable alleles at the *QCsel.wak-2B* (2B), *QCsfi.wak-3B* (3B), *QCsel.wak-4A.1* (4A.1), and *QCsel.wak-6B* (6B) quantitative trait loci (see Table 3) in Finch × Eltan (F × E) wheat recombinant inbred lines and their cumulative effect on third severity score for Cephalosporium stripe disease in Pullman, WA, in 2014. The third severity rating, in which a low score indicates greater relative resistance, was taken at grain filling and was subsequently square-root-transformed and least-square-means-adjusted.

of quantitative resistance to soil-borne fungi. Considering diseases of sugarcane (*Saccharum officinarum* L.), maize (*Zea mays* L.), barley (*Hordeum vulgare* L.), and wheat, he concluded that the resistances that were most effective against their unspecialized fungal instigators often came from favorable variation in fundamental physiological processes impacting plant vigor, metabolism, phenology, and anatomical tissue quality (e.g., hypodermal thickness). These essential physiological processes are, understandably, controlled by many genes acting in complex networks in possibly synergistic interactions and, as a consequence, as Bruehl noted, are challenging to improve by breeding. Furthermore, the environment plays a larger role in these multigenic traits than in vertical resistance, as in any quantitative trait (Bernardo, 2010), lowering the heritability of resistance compared with a genetically simpler attribute such as heading date, as we also experienced (Table 2).

The following two examples illustrate how nonspecific genetic resistance to CSD may be manifested. Early planting increases CSD severity, because the greater root growth of an early-seeded wheat plant is more susceptible than the small root system of a late-sown plant to breakage by frost heaving over the winter; more root damage means more potential infection sites (Raymond and Bockus, 1984). Genes controlling the rate of root growth, root architecture, root tensile strength, et cetera, if allelically diverse in a population of winter wheat plants, could therefore understandably impact disease incidence differently from individual to individual even if all were planted at the same time in the same environment with the same exposure to *C. gramineum*. Evidence of similar consequence presented by Mathre and Johnston (1990) indicates that variation in anatomical structure in the crown transition zone between roots and shoots affects the rate of *C. gramineum*'s upward advance through the xylem and thus impacts ultimate disease severity. They concluded that if tortuous transition zone architecture is indeed a resistance mechanism in wheat, it is probably under polygenic control and would be difficult to transfer into new varieties. Root growth and vascular structure alone could account for the two mechanisms of tolerance observed by Morton and Mathre (1980b), where root growth correlated with infection incidence and vascular structure correlated with disease severity. However, both traits are, no doubt, controlled by multiple genes. In contrast, resistance to the toxin produced by *C. gramineum* might logically be assumed to follow a major gene model, as in other toxin pathogenesis systems (Lorang et al., 2012); however, genetic analysis of *C. gramineum* toxin sensitivity by Rahman et al. (2001) found even this trait to be quantitatively inherited in winter wheat. These three potential mechanisms of resistance fall under hypotheses no. 1 and no. 3 as described in a review of quantitative disease resistance by Poland et al. (2009). Only time will tell, however, which classes of genes are represented by the loci uncovered in this present study, as sequencing, cloning, and characterization efforts progress and coalesce for the wheat genome.

Of the 12 disease resistance QTL identified in the F × E population, resistance alleles for six of the loci came from Finch and six were contributed by Eltan (Table 3). The near equivalence in moderate resistance seen between these two parents—if anything, Eltan was slightly more resistant (Table 1)—was corroborated by the discovery of multiple loci with favorable alleles contributed by both parents. In general, the accumulation of favorable alleles improved the resistance score, as illustrated in Fig. 2; those lines carrying only one of the four QTL considered in the figure have a mean disease score in the susceptible range. Any appreciable genetic reduction of CSD will therefore require the deployment of more than one favorable QTL. There was still much observed variation in score, even with four favorable alleles, which can be explained by the quantitative nature of resistance, because the other eight loci controlling resistance in these RILs (and perhaps more loci at large) may have contributed positively or negatively to resistance and may also have been acting in synergistic or suppressive interactions not explicitly exposed by our analyses. Furthermore, there are nongenetic sources of variation in resistance (Table 2), which are only exacerbated when a subset of the population is considered, as shown in Fig. 2, where, for example, only six lines of the 158 individuals in the F × E population were found not to carry any positive alleles of the four QTL considered.

Comparison of the consensus-mapped positions of significant markers from the F × E population and the DP on the 90K consensus map (Wang et al., 2014) revealed some similarities between the loci found in our two populations (compare Table 3 and Table 4, in which putatively equivalent QTL are underlined). To facilitate this comparison, Table 3 includes the 90K consensus map position of the 9K markers that tag significant QTL in Finch and Eltan as well as the 90K positions of the approximate QTL boundaries. The rate of recombination between markers, which determines cM distance along a chromosome as well as which marker is most strongly associated with a trait of interest, differs between populations, making it difficult to exactly align QTL on the basis of cM distances of markers alone. However, it is probable that QTL found in the same general chromosomal vicinity between our two populations represent the same underlying resistance gene or genes. We expected to find some of the same QTL in both the F × E and DP populations because Finch and Eltan are members of the DP, along with a number of Finch- and Eltan-derived lines and close relations.

The F × E QTL *QCsel.wak-1B*, *QCsel.wak-4A.1*, *QCsfi.wak-4B*, *QCsfi.wak-5B*, and *QCsel.wak-6B* align well with the respective *QCs.wak-1B.3*, *QCs.wak-4A.2*, *QCs.wak-4B.2*, *QCs.wak-5B.3*, and *QCs.wak-6B.3* QTL from the DP,

indicating the same causative loci in both populations. This interpopulation corroboration serves to bolster the credibility of these matching QTL as true factors in disease resistance. Future work to develop markers for marker-assisted selection of CSD resistance in winter wheat breeding efforts or to further dissect the genetics of resistance should focus on these regions as well as attempt to pinpoint resistance within the proposed linked loci within the DP.

Together, the F × E QTL impacting disease severity (% in Table 3) together accounted for 37 and 41% of the phenotypic variation observed for this trait in 2013 and 2014, respectively. Likewise, QTL identified with the third severity rating controlled trait variation by 31% in 2013 and 56% in 2014, whereas cumulative QTL effects for first and second severity ratings tended to be less powerful. We gather from these results that the third severity and incidence ratings, both taken at later stages of plant and disease development, are the most indicative estimates of resistance and therefore uncovered the most impactful QTL. It is possible, however, that some QTL have time-sensitive roles and would only be identified early in disease development or may not be effective until later in the growing season, whereas others may have a more constitutive effect on resistance. The growth stage sensitivity and temperature sensitivity of genetic resistance to stripe rust (*Puccinia striiformis* Westend. f. sp. *tritici* Eriks.) in wheat has been duly exposed (Qayoum and Line, 1985), and many QTL for both seedling-stage and adult-plant resistance have been identified in that host–pathogen system (Rosewarne et al., 2013). In our experiments, the F × E QTL *QCsfi.wak-2A* and *QCsfi.wak-4A.2* were only found in association with the first severity rating. Chromosome 4A has never before been reported in previous QTL mapping studies, which have only relied on late-season phenotyping of blighted whiteheads (Quincke et al., 2011; Vazquez et al., 2015). In contrast, other QTL were indicated across all four ratings (*QCsfi.wak-3B* and *QCsel.wak-4A.1*), suggesting a more fundamental and constant resistance mechanism expressed at these loci. Some (*QCsel.wak-1A*, *QCsel.wak-1B*, *QCsfi.wak-5B*, *QCsel.wak-6B*, and *QCsel.wak-7A*) were only found in late-stage ratings, although the loci found in the DP corresponding to these 1B, 5B, and 6B F × E loci were also indicated in earlier ratings. Time course transcriptome profiling of diseased versus clean plants could help elucidate the genes involved in growth-stage-sensitive resistance.

In the interest of breeding winter wheat cultivars with resistance to CSD, the loci that are effective in the greatest number of environments and across all severity and incidence ratings should be targeted as the foundational sources of resistance and supplemented with other loci of minor effect. For instance, *QCsfi.wak-3B* and *QCsel.wak-4A.1* in the F × E population appeared across ratings and in both years. Also, the LOD scores were relatively strong at these QTL for some ratings, and *QCsel.wak-4A.1* alone contributed 17% of the variation in the third severity score in 2014, underlining the importance of this locus in CSD resistance. Recombinant inbred lines with combinations of QTL that included the Eltan allele of *QCsel.wak-4A.1* had lower mean and minimum scores than those with the Finch allele, especially when combined with the Finch allele of *QCsfi.wak-3B* (Fig. 2), illustrating the progress in disease resistance that can be made by selecting for and combining favorable alleles in breeding populations, a strategy that is made much more efficient with molecular marker technology. Stable QTL such as these, which appear in multiple years and throughout disease development, are the most promising candidates for cultivar improvement because of both their strong effect and their stability regardless of environmental variation.

We expected some QTL to differ year by year because of the random effect of yearly environment on expression of disease resistance and pathogen virulence. From the F × E RILs, *QCsel.wak-1A*, *QCsel.wak-1B*, *QCsfi.wak-4A.2*, and *QCsfi.wak-4B* were only observed in 2013, whereas *QCsfi.wak-2A*, *QCsel.wak-2B*, *QCsfi.wak-5A*, *QCsfi.wak-5B*, *QCsel.wak-6B*, and *QCsel.wak-7A* were only observed in 2014. (However, comparison of the F × E 1B, 4B, 5B, and 6B loci with their counterpart loci in the DP indicate that only the F × E 5B locus holds true to this year-specific rule in the DP.) We advise caution when considering QTL in the DP that only appeared in one of the two growing seasons evaluated; the lack of DP replication within years raises the probability that year-specific loci may have arisen from random stresses in the field that affected the visual rating of resistance in a certain pattern in one season but not the other and are thus not true indicators of resistance.

Different pathogen isolates were used for artificial inoculation in 2013 and 2014, which perhaps elicited differences in resistance reaction, thus highlighting isolate-specific chromosomal locations of resistance. Classical gene-for-gene pathogen resistance (Flor, 1956), based on the strong activity of one or a few major resistance genes that often confer near immunity, is typically race specific and could explain the yearly differences in QTL observed in this study. If this was our situation, however, we would expect to identify an isolate-specific locus controlling a large proportion of variation in resistance throughout all stages of disease development. Never was this found in our experience, as the R^2 values of all F × E CSD resistance QTL were between 6 and 21% (Table 3), and none was above 4% in the DP (Table 4), indicating a more quantitative, horizontal style of resistance for the QTL identified (although the 21% R^2 value, the highest observed in our study, was observed for *QCsel.wak-6B*, which only appeared in 2014). Another caveat is that we used isolate mixtures both years, which may have masked

race-specific reactions. A targeted study evaluating differential isolate responses in more than two environments would be needed to tease out the genetic basis for these observed year-specific and possibly race-specific differences in resistance reaction. Also, the extent of *C. gramineum*'s genetic diversity and race-dependent virulence, which would impinge on this question, is still unclear and is under investigation (Quincke et al., 2014).

In F × E QTL mapping and DP association mapping, the genomic regions indicated for the reduction of CSD incidence also reduced severity, except the F × E QTL *QCsfi.wak-5B* and *QCsel.wak-7A*, which were only indicated for incidence reduction, and the DP QTL *QCs.wak-1D.1*, *QCs.wak-1D.4*, *QCs.wak-2A.3*, *QCs.wak-3A.2*, *QCs.wak-3D.1*, *QCs.wak-3D.2*, *QCs.wak-5B.1*, *QCs.wak-6A.5*, *QCs.wak-7B.1*, and *QCs.wak-7D.1*, which were only indicated for severity reduction. (Again, comparison between the DP and F × E QTL suggests that *QCsfi.wak-5B* from F × E corresponds to *QCs.wak-5B.3* from the DP, which was identified in both the second severity rating as well as the incidence rating, eliminating this specific locus from the present consideration of resistance specificity.) We had originally hypothesized that the severity and incidence rating methods would be able to identify QTL that were unique to each of the two resistance mechanisms; by and large, however, the mapping results indicated that our visual subjective judgments of infection incidence may have been influenced by infection severity or vice versa, such that the same chromosomal regions were usually indicated by both scores. Nevertheless, this does not rule out the suggested explanation of pleiotropy in the control of both resistance traits.

A look at two previous reports on CSD resistance QTL also reveals some similarities, although precise correspondence is again lacking because of the differences in populations and marker platforms used; therefore, it is unclear how closely the chromosomal positions of our QTL match with those found in previous projects. Vazquez et al. (2015) used Marone et al.'s (2012) consensus map of the A and B wheat genomes to compare their QTL results with those of Quincke et al. (2011) and found five QTL in common. A comparison of 90K consensus map locations of the QTL from our study with Vazquez et al.'s groupings following Marone et al. (2012) revealed possible agreement between our *QCsfi.wak-5A* from F × E with a QTL on 5AL identified as *QCs.orp-5A.1* in Quincke et al. (2011) and as *QCsen.orz-5AL.2* by Vazquez et al. (2015). Likewise, *QCsns.orz-6BS* (Vazquez et al., 2015) and our *QCsel.wak-6B* (F × E) and *QCs.wak-6B.3* (DP) are in the same region; furthermore, *QCstb.orz-3BS* (Vazquez et al., 2015) suggests our *QCsfi.wak-3B* from F × E; *QCsen.orz-4BS* (Vazquez et al., 2015), *QCs.orp-4B* (Quincke et al., 2011), *QCsfi.wak-4B* (F × E), and *QCs.wak-4B.2* (DP) align well. Lastly, *QCsns.orz-C5BL* (Vazquez et al., 2015), *QCs.orp-5B* (Quincke et al., 2011), and our *QCsfi.wak-5B* (F × E) and *QCs.wak-5B.1* (DP) appear to match up. The appearance of these QTL in multiple experiments and populations indicates the commonality of some sources of genetic resistance in PNW germplasm and gives us additional confidence that we were able to find true sources of resistance in the experiments explained herein. Also, the new loci identified in both the F × E and DP populations underscore the untapped potential of genetic resistance to CSD accumulated in PNW germplasm, which we believe has had the longest exposure to the disease of all regions in the United States.

CONCLUSIONS

This study has again demonstrated that exploitable genetic variation in quantitative CSD resistance is available in PNW winter wheat breeding germplasm and has further contributed to the effort to localize that resistance in the wheat genome. The unique mapping populations, molecular marker platforms, and mapping methods used in this research have uncovered novel chromosomal regions of importance to the greater goal of improving winter wheat cultivars in resistance to CSD. On the basis of this work, further research should be pursued to fine-map resistance within the QTL identified herein and to develop consistently segregating DNA markers for use in the selection of genetically superior offspring from crosses to resistant adapted lines. Thus expedited, deployment of quantitative disease resistance in regionally adapted cultivars will improve the economic and environmental sustainability of winter wheat production on farms infested with *C. gramineum*.

Supplemental Material Available

Supplemental material is available with the online version of this article.

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