STEEP III PROGRESS REPORT - 2002

RESEARCH PROJECT TITLE: Improved Methods for Evaluating Resistance To Cephalosporium Stripe of Wheat

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FINAL REPORT

PROJECT OBJECTIVES:
1. Optimize procedures to produce fungal toxin(s) from *Cephalosporium gramineum*.
2. Purify and characterize biochemical variability of the toxin(s).
3. Determine reactions of modern Pacific Northwest cultivars to the dominant toxin form(s), determine inheritance of these reactions, and begin mass screening.
4. Identify and use molecular markers to determine the inheritance of Cephalosporium stripe resistance in a common wheat x synthetic wheat molecular mapping population.

KEY WORDS: Cephalosporium stripe, conservation tillage, fungal toxins, molecular mapping

STATEMENT OF PROBLEM: Cephalosporium stripe has become a limiting factor for many Pacific Northwest wheat growers in erosion-prone areas, especially when early planting and/or trashy fallow are practiced. Burning or plowing stubble and delayed seeding can provide substantial control of Cephalosporium stripe. However, these cultural control methods conflict strongly with attempts to control soil erosion. Though no soft white winter wheat cultivar shows complete resistance to Cephalosporium stripe, there is considerable variation in the degree of resistance among cultivars. Further, higher levels of resistance may be available in exotic germplasm. However, identifying resistance in breeding programs remains problematic. Expression of resistance is incomplete and environmentally dependent. In addition, the disease tends to be aggregated within fields, thus requiring large plots to make useful comparisons, which is not possible in early generations of cultivar development.

ZONE OF INTEREST: low and intermediate rainfall

ABSTRACT OF RESEARCH FINDINGS: Protocols were developed to improve the production of a toxic fraction produced by *Cephalosporium gramineum*, causal agent of Cephalosporium stripe. A rapid assay was developed that resulted in wilting by 72 hours after treatment of excised leaves with the toxic fraction. Comparison of laboratory with field data showed that sensitivity of 17 wheat genotypes to the toxic fraction was highly correlated with disease reaction in the field. It appears that toxin insensitivity may be an important mechanism of resistance to Cephalosporium stripe, but that other mechanisms are operative as well. We developed base-line data from the field regarding Cephalosporium resistance of advanced breeding lines with favorable agronomic and quality characteristics, and evaluation of potential resistance sources from outside of Oregon. We identified variation among advanced lines that will be useful in identifying appropriate cultivars for areas where Cephalosporium stripe is a problem. We identified several lines of French soft red winter wheat that have outstanding yield potential and a level of

resistance to Cephalosporium stripe greater than that of current PNW common soft white wheat cultivars. Populations were developed to study the inheritance of toxin insensitivity in genotypes adapted to Oregon growing conditions, and in resistance sources. Heritability of toxin insensitivity in a molecular mapping population was quantitatively inherited, but highly heritable. We also obtained field resistance data for 84 progeny of the mapping population for comparison with results of the lab-based toxin assay. Heritability of whitehead percentage was 88%. Regression analysis of DNA-based markers suggested the presence of determinants of resistance in chromosomes 2D, 4A, 6A, and 6D. These four regions accounted for 41% of the phenotypic variance. Results of the project will enable us to determine the potential for using the toxin assay to more rapidly screen for resistance to Cephalosporium stripe.

RESULTS AND INTERPRETATION:

Objectives 1 and 2. Toxin Production and Characterization - We were able to identify and isolate a toxic fraction from *Cephalosporium gramineum* that induces chlorosis and wilting of wheat leaves, and developed procedures to greatly increase the yield of the toxic fraction. Culture conditions and extraction of the toxic fraction were first done according to the methods of Kobayashi and Ui (1979. Physiol. Plant Pathol. 14:129-133). Extraction methodology was subsequently modified to further refine the toxic substance and increase its yield, as described in detail in Rahman et al. (2001. Phytopathology 91:702-707). To do so, the fungus was grown in broth culture for 35 days and filtered. With this modified method, the most time-consuming step of rotary evaporation of the culture filtrate was avoided.

Culture filtrate was directly extracted with ethyl acetate four times. Pooled ethyl acetate extracts were then subjected to rotary evaporation to isolate the crude toxin. This crude toxin was then solubilized in chloroform, dried, and dissolved in ethanol. The crude toxin was loaded onto a C18 reversed-phase Sep Pak cartridge, and then progressively eluted with mixtures of acetonitrile/water of varying concentration. Each fraction was subjected to rotary evaporation, dissolved in chloroform, dried under a hood overnight, and finally dissolved in ethanol. Initial assays indicated that the majority of the toxic activity was contained in the 75% acetonitrile/water fraction, which was subsequently used for the genotype evaluations described below.

Our revised procedures have greatly increased the amount of the toxic fraction that can be derived from the fungus. Further, we found that the toxic fraction can be frozen for future use, without loss of activity.

Objective 3. Genetics of Resistance and Screening - Our first step was to develop a reliable assay system for reaction of wheat genotypes to the toxic fraction. This toxic fraction from *C. gramineum* produces wilting symptoms within 72 hours in a detached leaf assay, in which excised leaves are placed in small glass vials containing the toxic fraction. Symptomology depends on concentration of the toxic fraction. We observed chlorosis of wheat leaves at low concentration (20ìl/ml), but this chlorosis did not reliably distinguish among wheat genotypes. At higher concentration (60ìl/ml), we obtained distinct wilt symptoms that consistently distinguished wheat genotypes, and this concentration was thus used in our assays. Leaves started showing wilting within 24 hour of exposure to the toxic fraction and sensitive genotypes were severely wilted within 72 hr of exposure, when data were recorded. Wilting was evaluated on a continuous scale of 1 to 5, with 1 indicating no change in leaf appearance and 5 indicating a fully wilted and dried or necrotic leaf.

Twenty wheat genotypes were initially evaluated, representing four taxonomic groups: 1) Common wheat included 9 soft white winter wheat cultivars and one soft white winter wheat breeding line that are adapted to the PNW region, and one hard red cultivar, Opata 85. Opata 85 is a parent of the International *Triticeae* Mapping Initiative (ITMI) mapping population. 2) Five winter club wheat cultivars adapted to the Pacific Northwest. 3) Three durum wheat genotypes, including two advanced winter durum lines from the Oregon State University wheat breeding program and one CIMMYT spring durum wheat cultivar (Altar 84) that is a "grandparent" of the ITMI mapping population (see below). 4) The synthetic hexaploid wheat M6, which is the second parent of the ITMI population. M6 was produced by crossing Altar 84 with an accession of *Aegilops tauschii*, followed by doubling chromosome number with colchicine to obtain a synthetic hexaploid. Two runs of three replications each were conducted with these 20 genotypes, and the run x treatment interaction was non-significant. Thus, the analysis of variance was combined over runs.

The common wheat genotypes were all highly sensitive to the toxic fraction (Table 1), with a wilting rating of 4 or greater. Fischer's protected LSD indicates that there is significant variation among genotypes within the common and club groups, but no overlap between these two groups. The three durum wheats showed highly similar reactions, and were intermediate between that of the common and the club groups. The synthetic wheat M6, which is a parent of the ITMI population, showed the highest level of resistance. Fortuitously, the other parent of the ITMI population (Opata 85) was the most susceptible genotype evaluated, thus providing an opportunity for studying inheritance of resistance to the toxic fraction among progeny of this cross (see below). Linear contrasts were highly significant (P ? 0.001) for the six pairwise combinations among the four germplasm groups. This association of germplasm groups with toxin insensitivity should be interpreted with caution, however, as only three durums and one synthetic wheat have so far been evaluated in this study.

After developing a repeatable assay, we studied the correlation between reaction of wheat genotypes to the toxic fraction and their disease expression in the field, by adding several treatments to another STEEP project ("On-farm Evaluation of Cephalosporium Stripe Severity and Yield for Wheat Cultivars and Cultivar Mixtures Grown in

Conservation Tillage Systems"). The experiments were arranged in a randomized complete block design with three replications at Condon and four replications at Dufur. All genotypes evaluated in the above laboratory/growth chamber experiment were included, except for the three spring genotypes Opata 85, Altar 84, and M6. Plots were planted into stubble mulch with a plot drill on 15 September 1999 at Condon and 16 September 1999 at Dufur.

There was more variation among genotypes within germplasm groups for the field data than for the toxin data (Table 1). Nonetheless, rankings of mean disease levels for the three germplasm groups tested in the field were the same as for the toxin assay, with the common wheats most susceptible, the club wheats most resistant, and durum wheats intermediate. Wilting symptoms measured in growth chambers were significantly ($P = 0.0001$) correlated with percent whiteheads estimated at each site (Figs. 1 and 2). In addition, a soft red winter wheat from France with very high yield potential in northcentral Oregon and a higher level of Cephalosporium stripe resistance than Madsen has shown reduced toxin sensitivity in preliminary studies. Some common wheats showed resistance in the field despite their sensitivity to the toxic fraction (Table 1). It is important to note, however, that we found no case of an insensitive genotype being susceptible in the field. These results suggest that toxin insensitivity may be an important mechanism of resistance to

Cephalosporium stripe, but that other mechanisms are operative as well.

We have also developed base-line data from the field regarding Cephalosporium resistance of advanced breeding lines with favorable agronomic and quality characteristics, and evaluation of potential resistance sources from outside of Oregon. Artificially inoculated plots at Pendleton in 2001 and 2002 provided whitehead data that were very repeatable between replicates. We identified variation among advanced lines that will be useful in identifying appropriate cultivars for areas where Cephalosporium stripe is a problem. We identified several lines of French soft red winter wheat that have outstanding yield potential and a level of resistance to Cephalosporium stripe substantially greater than that of Madsen. We are currently determining whether these lines also demonstrate insensitivity to the toxic fraction.

Populations are being developed to study the inheritance of toxin insensitivity in genotypes adapted to Oregon growing conditions. Three crosses of moderately resistant x susceptible cultivars have been made: Coda x Stephens, Madsen x Stephens, and Rossini (a French line with a high level of resistance) x Stephens. Progeny from these crosses are being increased through single seed descent in the greenhouse, and will be assayed with the toxic fraction and in field studies when sufficient seed of stable lines is available.

4. Mapping population - One-hundred and twelve recombinant inbred lines from the International *Triticae* Mapping Initiative (ITMI) population were evaluated for sensitivity to the toxic fraction. The detached leaf assay described earlier was used to obtain toxin sensitivity data from the individual mapping progeny. Frequency distribution of the wilting data (Fig. 3) suggests a continuous distribution skewed towards insensitivity. Seventeen of the progeny were numerically less sensitive than M6 and three were more sensitive than Opata 85, though none of these differences were significant at $P = 0.05$ based on either Duncan?s Multiple Range or Fisher?s Least Significant Difference Test. Analysis of variance indicated that differences among the progeny are highly significant (*P* ? 0.0001) with respect to toxin sensitivity. Heritability of sensitivity to the toxic fraction estimated on a genotype mean basis was 0.88, with a 90% confidence interval of 0.85 ? 0.91.

We planted and inoculated the mapping population at Pendleton in fall 2000 and 2001, in the hope that mild winters would allow survival and field evaluation of these spring genotypes. We were able to evaluate 84 mapping population progeny in a randomized complete block design with two replicates. Plots were scored for stand density, overall stand health, and percent whiteheads in spring. Heritability of toxin insensitivity was 88%. DNA-based marker genotypes and percent whitehead scores were subjected to regression analysis to identify marker-trait associations. Results suggested the presence of determinants of resistance in chromosomes 2D, 4A, 6A, and 6D. The proportion of the phenotypic variance accounted for by significant regions on chromosomes 2D, 4A, 6A, and 6D were 15, 12, 11, and 8%, respectively. These four regions accounted for 41% of the phenotypic variance. The region on chromosome 4A is particularly interesting since it has also been associated with resistance to chlorosis induction by *Pyrenophora tritici-repentis*, causal agent of tan spot of wheat.

Summary - The ultimate goal of the project is to produce wheat cultivars with resistance to Cephalosporium stripe combined with favorable quality, yield, and other important agronomic characteristics. Such cultivars will greatly increase ability of wheat growers to successfully implement conservation tillage practices in erosion-prone areas. In the short-term, we will identify and exploit useable levels of resistance in adapted germplasm. In the longer term, we hope to develop gene introgressions with very high levels of resistance from synthetic populations via molecular markers.

INTERACTION WITH OTHER SCIENTISTS CONDUCTING RELATED ACTIVITY:
Information regarding the biology and control of Cephalosporium stripe is exchanged with other pathologists in the Pacific Northwest, primarily Tim Murray (WSU) and Dick Smiley (OSU). In addition, contact is maintained with wheat breeding programs in Idaho, Oregon, and Washington to identify promising lines for evaluation, and to provide breeding programs with information regarding reactions of wheat cultivars and lines to Cephalosporium stripe.

PUBLICATIONS AND PRESENTATIONS IN 2002:
"Resistance to Cephalosporium Stripe and Strawbreaker Foot Rot in Wheat", Pendleton Station Field Day, June 2002.

"Resistance to Cephalosporium Stripe and Strawbreaker Foot Rot in Wheat", Moro Station Field Day, June 2002.

Table 1. Mean wilting reaction of 20 winter and spring wheat genotypes to a toxic fraction produced by *C. gramineum* in the laboratory and percentage of whiteheads expressed by 17 winter wheat genotypes in naturally infested fields at two locations.

		Whiteheads (%)			
		Condon[c]		Dufur[d]	
Variety	Wilting rating[a]	Original	Transformed	Original	Transformed
Common					
Opata 85[b]	4.94	-	-	-	-
Madsen	4.93	1.75	0.31	3.62	1.39
Macvicar	4.89	20.67	1.32	25.29	3.25
Malcolm	4.73	12.67	1.06	32.75	3.46
Stephens	4.68	14.17	1.13	46.75	3.83
Gene	4.52	8.67	0.96	33.00	3.50
Lambert	4.44	5.25	0.58	23.00	3.13
Rod	4.39	3.33	0.57	5.13	1.68
Weatherford	4.32	6.17	0.82	7.88	2.09
Hill 81	4.01	4.83	0.57	8.26	2.17
OR515	4.00	11.83	1.05	29.75	3.37
Mean	**4.53**	**8.93**	**0.84**	**21.54**	**2.79**
Durum					
OR948927	2.99	4.00	0.65	6.75	1.96
OR971897	2.89	2.17	0.42	9.50	2.29
Altar 84[b]	2.89	-	-	-	-

Mean	**2.92**	**3.09**	**0.54**	**8.13**	**2.13**
Club					
Temple	2.53	0.05	-0.25	1.44	0.61
Rohde	2.25	0.07	-0.25	1.13	0.49
Coda	2.09	0.62	0.05	1.38	0.55
Hyak	1.53	0.70	-0.04	0.35	-0.23
Tyee	1.47	0.68	0.03	0.05	-0.6
Mean	**1.97**	**0.34**	**-0.09**	**0.87**	**0.16**
Synthetic					
M6[b]	1.17	-	-	-	-
Mean	**1.17**	-	-	-	-
LSD(0.05)	**0.61**	**-**	**0.38**	**-**	**0.42**

[a]Continous scale with 1 = no effect of toxic fraction on leaf appearance and 5 = fully wilted and dried or necrotic leaf.

[b]Genotype could not be included in the field experiments because it is a spring type.

[c]Means of three replications, the transformed scale was $\log_{10}$(%whiteheads + 0.5).

[d]Means of four replications, the transformed scale was $\log_{e}$(%whiteheads + 0.5).

Fig. 1. Correlation of leaf wilting in response to a toxic fraction produced by *Cephalosporium gramineum* with percent whiteheads produced by 17 wheat genotypes in a naturally infested field in Condon, OR.

Fig. 2. Correlation of leaf wilting in response to a toxic fraction produced by *Cephalosporium gramineum* with percent whiteheads produced by 17 wheat genotypes in a naturally infested field in Dufur, OR.

Fig. 3. Frequency distribution of wilting for 112 recombinant inbred line progeny resulting from a cross between the sensitive parent Opata 85 and the insensitive parent M6 after exposure to a toxic fraction produced by *Cephalosporium gramineum*.