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ITEMS FROM THE UNITED STATES OF AMERICA

INDIANA

PURDUE UNIVERSITY
Departments of Agronomy, Botany and Plant Pathology, Entomology, and the USDA-ARS Crop Production and Pest Control Research Unit at Purdue University, West Lafayette, IN 47907, USA.

J.M. Anderson, S.E. Cambron, C. Crane, S.B. Goodwin, S. Scofield, B. Schemerhorn, R.H. Shukle and C.E. Williams (USDA–ARS); H.W. Ohm (Department of Agronomy); K. Wise (Department of Botany and Plant Pathology); and J. Stuart (Department of Entomology).

Wheat production.

According to the USDA National Agricultural Statistics Service, harvested wheat acreage in Indiana in 2009 totaled 450,000 acres. Wheat production was down from 560,000 acres in 2008. Total production was estimated at 30.1 million bushels, with an average yield of 67 bu/ac. Winter survival of wheat during the winter of 2008–09 was excellent. However, average temperatures from February to mid-June were below normal and soil moisture was higher than normal due to frequent rainfall, resulting in delayed growth and development of wheat and limited uptake of nitrogen, resulting in poor wheat growth in low and wet areas of fields. By mid-June, temperatures were higher and near normal, and there was mild soil moisture due to dry soil conditions, resulting in slightly reduced grain test weight.

Wheat disease summary.

Wheat diseases were generally at low levels throughout central and northern Indiana in 2009. Stagonospora leaf blotch and Septoria leaf blight were problematic in southern Indiana early, and a prolonged period of rainy and humid weather in early May contributed to significant Fusarium head blight (FHB) throughout southern Indiana. The resulting disease caused significant yield loss and reduction in grain quality due to the mycotoxin DON, especially on susceptible cultivars. Due to cool weather conditions, FHB developed late in the season in mid to northern Indiana, and was less severe than in the southern third of the state, although there was some grain yield loss. Leaf rust moved into Indiana late in the growing season, and stem rust was observed in southern Indiana, however, both diseases arrived too late in the growing

season to cause significant yield loss. Several viral diseases of wheat, including wheat streak mosaic virus, wheat spindle streak mosaic virus, soil-borne wheat mosaic virus, and barley yellow dwarf virus were confirmed in Indiana.

Performance of new cultivars.

Herb Ohm.

Cultivar INW0731 yielded well in Indiana and nearby regions. INW0731 has moderate resistance to yellow dwarf, moderate resistance to fusarium head blight from Freedom and Fundulea 201R, moderate resistance to yellow dwarf, leaf rust, resistance to powdery mildew, Stagonospora nodorum blotch, Septoria leaf blotch, soilborne wheat mosaic and wheat spindle streak mosaic viruses, and is susceptible to Hessian fly, stripe rust, and stem rust in Indiana. This cultivar, adapted to southern Indiana and surrounding regions, has survived winters very well in central and northern Indiana, but winters have been mild since 1996.

Cultivar INW0801, which has gene *Bdv3*, also performed well. INW0801 is well-suited to southern Indiana and adjacent areas because yellow dwarf is present many years, and its early maturity is suited to doublecropping, seeding soybeans no-till after wheat harvest.

Breeding/genetics: Combining multiple genes for resistance to foliar diseases, yellow dwarf, and Hessian fly in improved germ plasm and soft winter wheat cultivars adapted to Indiana.

Herb Ohm, Benjamin Campbell, Judy Lindell, Andy Linvill, Yanyan Liu, Dan McFatridge, Mahboobullah Nang, Brett Ochs, Kristen Rinehart, Wali Salari, Samantha Shoaf, Jin Sun, and Xiangye Xiao.

Fusarium head blight. *Bdv3* and *Qfhs.pur-7EL* were combined in coupling on 7DL; *Qfhs.pur-7EL* is more distal than *Bdv3*. *Bdv2* also was moved from 7DL to 7BL (both objectives were part of Ph.D. thesis research by K. Rinehart). We also combined *Qfhs.pur-7EL* and *Fhb1*, which is located on 3BS. The disease rarely spreads beyond the inoculated floret or spikelet in lines with these two strong FHB-resistance factors in our tests using point inoculation (inoculation of a single floret at flowering with 500 *F. graminearum* macrospores in 10 μl dH_2O and placing a plastic bag over inoculated spikes for 3 d). The disease severity averaged 0.75 diseased spikelets at 21 dai in greenhouse and field tests.

We completed the fourth and last phenotyping experiment to characterize a recombinant inbred population for type-II FHB resistance of a selection of the line Xing 117. Phenotyping in the four tests was carried out by undergraduate students Charlie Zila, Jill Recker, and Emily North; and visiting graduate student from Denmark, Stine Petersen; and Judy Lindell and Yanyan Liu, who also are screening the parent lines, bulks, and population with markers to map the resistance.

Stem and yellow rust. We have identified and obtained germ plasm lines that have resistance to stem rust race TTKS (Ug99) and yellow rust. We have developed recombinant inbred populations from crosses of the new resistant lines x susceptible lines. In collaboration with the USDA–ARS laboratory (Dr. Yue Jin) at St. Paul, MN (Ug99), and at Purdue University for resistance to our local isolates of the causal fungal pathogens, the populations are being phenotyped for resistance. We are mapping the resistance using the bulked-segregant analysis approach.

Marker-assisted seletion. We have significantly expanded MAS as an integral part of the breeding program to combine a large number of desired QTL/genes for various important plant traits. MAS is a necessary technology to genotype parent lines for various desired traits and to plan parental combinations for efficiently combining a large number of desired plant traits.

We released three soft winter wheat cultivars: **INW0801** (very early, moderate resistance to FHB and *Bdv3*), **INW0803** (early, short and stiff straw, excellent for high management), and **INW1021** (moderate resistance to FHB, yellow dwarf disease, WSSMV, SBMV, leaf, stripe and stem rusts (has *Lr37*, *Yr17*, and *Sr38*), powdery mildew, SNB, STB, susceptible to Hessian fly biotype L; is widely adapted, good soft wheat milling and baking qualities, has the *Bx70e* strong gluten allele, and the *Ppd* daylength insensitive allele).

Released germ plasm. Seed of the **91193/92201** RIL population (194 lines plus the two parent lines) was submitted to the UDSA–ARS GRIN, Aberdeen, ID.

Wheat management.

Kiersten Wise and George Buechley.

Fungicides for Fusarium head blight control. Research activities in 2009 focused on evaluating integrated management strategies for control of FHB. A trial conducted in west central Indiana tested the combined effects of a foliar fungicide application at Feekes 10.5.1, and cultivar susceptibility for improved FHB management. The fungicide Prosaro® was applied to experimental plots of six cultivars of varying susceptibility to FHB. Two susceptible cultivars, two moderately susceptible, and two resistant cultivars were included in the experiment. Unsprayed plots also were included.

Fungicide-treated plots had significantly (P = 0.05) lower FHB incidence, severity, FHB index, foliar disease severity, % FDK, and DON levels. Fungicide-treated plots also had significantly greater yields compared to untreated plots, however test weights were not significantly different. In comparisons between fungicide-treated and untreated plots of the same cultivar, fungicide-treated plots had lower disease levels and higher yields in all cultivars except one. Levels of FHB were generally low in 2009 at the research location, which may have contributed to why significantly reduced levels of FHB or DON were not observed in moderately resistant compared to susceptible cultivars. Additionally, yield and test weight results may have been confounded by BYDV infection in moderately resistant cultivars.

The results of this research project indicate that a well-timed fungicide application can significantly reduce the impact of FHB and DON in wheat cultivars and increase yields in most culariv ars. This information is of primary importance to growers and will be presented in extension programs and summarized in extension articles to aid growers in managing FHB and DON in wheat. Additional research is needed to more thoroughly investigate the interaction between fungicide and cultivar susceptibility under Indiana conditions.

Hessian fly: Interactions of wheat with virulent and avirulent Hf larvae.

Christie Williams, Jill Nemacheck, Kurt Saltzmann, Marcelo Giovanini, and Subhashree Subramanyam.

Wheat response to Hessian fly attack. A sequence encoding a new candidate type-1 lipid transfer protein from wheat, Hfr-LTP, was identified and its expression compared to a previously identified Hessian fly-responsive wheat LTP gene, *TaLTP3*. LTPs may be involved in maintaining the integrity of healthy cells. Although attack by a single virulent Hessian fly larva was sufficient to cause a detectable decrease in Hfr-LTP mRNA abundance, higher infestation levels led to near silencing of the gene with a 196-fold decrease in transcript abundance. Hfr-LTP transcript levels were not affected by other biotic factors or abiotic factors tested, so the response appears to be fairly specific to Hessian fly attack. Although TaLTP3 transcript abundance was confirmed to increase in resistant plants, a much larger effect was seen when quantified through eight days after egg hatch in susceptible plants. TaLTP3 mRNA abundance decreased markedly in susceptible plants, as was seen for Hfr-LTP. These decreases in wheat LTP transcript abundance in susceptible plants may contribute to degradation of epidermal cells at the larval feeding sites, resulting in nutrient delivery.

The potential role of reactive oxygen species (ROS) in defense of wheat and rice against Hessian fly larvae was examined. This study compared the rice non-host response to the wheat gene-for-gene response. A similar rapid and prolonged accumulation of H_2O_2 was detected in resistant wheat and rice plants at the attack site. Changes were detected in the abundance of 250 wheat transcripts and 320 rice transcripts from genes believed to be involved in generating ROS. Class-III peroxidase transcripts increased in abundance in both wheat gene-for-gene resistance and rice non-host interactions, whereas the levels of these transcripts decreased in susceptible wheat. In addition, elevated enzymatic activity of peroxidases was detected at the attack site in resistant wheat plants and non-host rice interactions. Thus, rice non-host resistance and wheat gene-for-gene resistance shared common elements in their defense against Hessian fly attack.

Williams Lab members. Subhashree Subramanyam is a Purdue University postdoctoral researcher, Kurt Saltzmann was a USDA–ARS postdoctoral researcher but now is an Assistant Professor at Purdue University, Jill Nemacheck is a

USDA–ARS research technician, Marcelo Giovanini was a joint student with Dr. Herbert Ohm who currently is a corn breeder for Monsanto in his home country of Brazil.

Ultrastructural changes in the midguts of Hessian fly larvae feeding on resistant wheat.

Richard H. Shukle, Christie E. Williams, and Subhashree Subramanyam.

The focus of this study was to compare ultrastructure in the midguts of Hessian fly larvae under different feeding regimens. Larvae were either fed on Hessian fly resistant or susceptible wheat, and each group was compared to starved larvae. Within three hours of larvae initiating feeding on resistant wheat midgut microvilli were disrupted, and after six hours midgut microvilli were absent. The disruption of midgut microvilli in larvae feeding on resistant wheat were similar to those reported for midgut microvilli of European corn borer larvae fed a diet containing the lectin wheat germ agglutinin. Results from the present ultrastructural study, coupled with previous studies documenting expression of genes encoding lectin and lectin-like proteins is rapidly up-regulated in resistant wheat to larval Hessian fly attack, are indications the midgut is a major target for toxic compounds elicited during the defense response of resistant wheat.

Development of a bioassay to evaluate the effects of toxic proteins on Hessian fly larvae. We have developed a bioassay to evaluate the effects of toxic proteins on Hessian fly larvae. Three lectins have been assayed to date and their effects on development and midgut ultrastructure of larvae documented. Additionally, we have obtained an expression clone for the 72-kDa toxic protein produced by the bacteria *Bacillus thuringiensis* subsp. *israelensis*, which is effective against mosquitoes and the close relatives of the Hessian fly the fungus gnats, and are currently expressing it for bioassay with Hessian fly larvae. Results impact development of transgenic resistance to compliment native resistance in wheat to Hessian fly.

Differential expression of genes encoding novel secreted salivary gland protein in the larval Hessian fly.

Richard H. Shukle and Alisha J. Johnson.

In collaboration with Dr. Ming-Shun Chen (USDA–ARS, Manhattan, KS) we are analyzing the salivary gland transcriptome of Hessian fly larvae using a custom Hessian fly Affymetrix array developed by Dr. Chen. These analyses are being conducted with three lab lines (vH9, vH13, and white), a field collection from Israel, and field collections from four states within the United State (Alabama, Georgia, Colorado, and Texas). Biotype GP is the reference 'wild-type' line. Initial results indicate significant differential expression in genes encoding novel secreted salivary gland proteins (SSGPs), which are hypothesized to be effectors in this insect/plant interaction. These results suggest each lab line and field collection evaluated has its own transcriptional signature with respect to genes encoding the SSGPs. Results impact knowledge of the interaction of Hessian fly larvae at the molecular level with wheat.

Multiplexed virus assays.

Mahua Deb and Joseph M. Anderson.

The addition of the high plains virus (HPV) and *Triticum* mosaic virus (TriMV) to a multiplex RT–PCR diagnostic assay previously developed for barley and cereal yellow dwarf viruses (CYDV), wheat spindle streak mosaic virus (WSSMV), wheat streak mosaic virus (WSMV), and soil-borne wheat mosaic virus (SBWMV).

A recent publication by Burrows et al. (2009, Plant Health Prog doi:10.1094/PHP-2009-0706-01-RS) demonstrated that TriMV and HPV as well as WSMV were the primary wheat viral pathogens in the Great Plains area. In response to this information, we have refined our wheat virus detection multi-plex PCR assay (Deb and Anderson 2008, J Virol Meth 148:17-24) to include TriMV and HPV. Like WSMV, these two viruses are vectored by the wheat curl mite and cause symptoms such as yellowing and stunting of plants that are similar to many other viruses attacking wheat. Because the disease phenotypes are similar, it makes a visual diagnosis quite difficult. ELISA is the standard diagnosis

method. Although a very effective detection method, it requires separate assays to identify which of these viruses are present. The multiplex PCR method we have developed uses a specific set of primers that detects the target viruses, TriMV and HPV, at 560 bp and 490 bp, respectively, in the presence of the other wheat and small grain viruses: B/CYDVs -PAV, -MAV, -SGV, -RPV, -RMV, WSSMV, SBWMV and WSMV at 295, 175, 237, 400, 365, 154, 219, and 193 bp, respectively. The different size virus-specific amplicons produced are readily visualized by agarose gel electrophoresis or capillary electrophoresis using a fluorescently tagged forward primer. All ten viruses can be amplified in a single reaction. Having the ability to detect all ten wheat viruses in a single test reduces the cost of the diagnostic assay and can readily identify mixed infections and also the presence of viruses. Therefore, this method reduces cost and leads to an improved diagnostic capacity.

Septoria tritici blotch.

Stephen Goodwin, Braham Dhillon, Yoon-E Choi, Jessica Cavaletto, and Ian Thompson.

Disease resistance. The Septoria tritici blotch resistance gene *Stb3* was mapped previously to chromosome 6DS by linkage to a single microsatellite locus. Additional markers on 6DS were tested to refine the map location and provide additional tools for marker-assisted selection. However, none of these markers was linked, indicating that the original location was incorrect. To find the correct location, more than 250 microsatellite primer pairs were tested by bulked-segregant analysis, but the level of polymorphism was quite low. Target region amplification polymorphism (TRAP) analysis identified a single linked marker that was located on chromosome 7A by analysis of nullisomic-tetrasomic stocks. Subsequent analyses of more than 50 SSR loci on chromosome 7A revealed that the correct location for *Stb3* is on the short arm. Locations of the SSR loci were confirmed by analysis of 7A deletion stocks. The linkage on 7AS was verified by analysis of two independent progeny sets. One SSR marker co-segregated with *Stb3* on all 97 doubled-haploid progeny so appears to be very tightly linked.

Work to backcross the resistance genes *Stb1–Stb8* into the highly susceptible, spring wheat background Taichung 29 are continuing. A progeny set of more than 700 lines is being developed for fine-scale mapping of the *Stb2* gene on chromosome 3BS. This work is aided by a stem inoculation technique that seems to give more reliable results compared to spray inoculation.

Work to develop isogenic lines of many of the *Stb* genes in the highly susceptible wheat background Taichung 29 is progressing. Many of the crosses are at the BC_3 or BC_4 stage. Ultimately, these lines can be used to analyze the effects of each *Stb* gene in a common susceptible background, and the progenies being developed can be used to validate previously published map locations.

Fungal genomics. Analysis of the repetitive content of the *M. graminicola* genome sequence identified a gene for methylation that is in multiple telomeric copies but occurs as a single copy in all other species analyzed. Further analysis revealed that the original copy in *M. graminicola* probably was duplicated and moved to a telomeric location, and then was amplified and spread to other chromosomes as part of the telomeric repeats. After becoming repetitive the gene seems to have become visible to the machinery for repeat-induced point mutation (RIP), a mechanism in fungi for inactivating transposable elements by introducing mutations that cause stop codons. The result of the RIP process was that all copies of the methylation gene appeared to be inactivated. To test this directly, DNA from several isolates of *M. graminicola* plus representatives of two close relatives, the barley pathogen *Septoria passerinii* and the banana pathogen *M. fijiensis*, was assayed for cytosine methylation. No methylation was detected in *M. graminicola*, but it appeared to be normal in the other two species, one of which (*M. fijiensis*) is known to have an unRIPed copy of the gene based on its genomic sequence. Therefore, *M. graminicola* is deficient in methylation, but with no obvious effect on phenotype.

Initial analysis of the genome sequence of *M. graminicola* is nearing completion. The genome sequence is finished, with 20 chromosomes from telomere to telomere and one telomere missing from the final chromosome. There are only two internal gaps. Eight of the chromosomes appear to be dispensable and could help give rise to the ability of the pathogen to adapt to new environments. Comparative genomics analyses with other sequenced relatives should soon provide an unprecedented understanding of the genetic content of these organisms.

Analysis of the function of genes in the *M. graminicola* genome is being pursued by developing knock-out mutants for particular genes of interest. So far, a number of new genes have been implicated in pathogenicity and that work will continue during the coming year.

Goodwin Lab members. Jessica Cavaletto and Dr. Ian Thompson are USDA–ARS Biological Science Research Technicians, Braham Dhillon is a Ph.D. student working on bioinformatics and genomics and Yoon-E Choi is a USDA postdoc who started during July of 2009.

Personnel.

Kristen Rinehart completed the PhD degree, August 2009, advisor Herb Ohm, and is in a corn breeding position with Pioneer stationed at Des Moines, IA. Yanyan Liu is a postdoctoral researcher since August 2009 with Herb Ohm.

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KANSAS

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Elevated Carbon Dioxide: Soil and Plant Water Relations.

M.B. Kirkham.

I have finished writing a book entitled ***Elevated Carbon Dioxide: Soil and Plant Water Relations***, now being considered for publication by Wiley-Blackwell. The book is developed from research that we in the Evapotranspiration Laboratory at Kansas State University did between 1984 and 1990 with field-grown sorghum, winter wheat, and rangeland plants under elevated carbon dioxide. Such experiments had not been done before in the semiarid Great Plains of the U.S. The rising levels of carbon dioxide in the atmosphere were of interest to the Department of Energy, which funded our work.

As the years have passed, the carbon dioxide levels in the atmosphere have increased, along with increasing interest concerning their effects. The carbon dioxide concentration in the atmosphere was first recorded by Charles D. Keeling (1928–2005) of the Scripps Institution of Oceanography, University of California, San Diego. He monitored it beginning in 1957 at Mauna Loa, Hawaii, and in Antarctica at the South Pole. In the 50-year period between 1958 and 2008, the carbon dioxide concentration in the atmosphere increased from 316 ppm to 385 ppm. Because no book documents soil- and plant-water relations under elevated carbon dioxide, I wrote this book to put the information in one source. It has been 26 years since we started our first experiments (1984–2010), so we can make some predictions, based on our early results, about how plants in the semiarid Great Plains of the U.S. are responding to elevated carbon dioxide, which has increased 55 ppm (from 330 ppm to 385 ppm) during this time.

Water and carbon dioxide are the two most important compounds affecting plant growth. In introductory botany textbooks, we have seen the familiar equation for photosynthesis, which shows carbon dioxide (CO_2) joining with water (H_2O), in the presence of light and chlorophyll, to form sugar ($C_6H_{12}O_6$) and oxygen (O_2), as follows:

$$6CO_2 + 6H_2O \xrightarrow[\text{chlorophyll}]{\text{light}} C_6H_{12}O_6 + 6O_2$$

Life on earth would not be possible without photosynthesis. We survive because of the oxygen produced by photosynthesis, as well as the food (sugars) produced by photosynthesis. Therefore, it is of critical importance to look at the water relations of plants under elevated carbon dioxide.

The book is technical and is based on information from peer-reviewed journal articles. I have written the book as if I were speaking to my graduate students and is organized as follows. I start with an introductory chapter