Proceedings of the 2016 National Fusarium Head Blight Forum

Hyatt Regency St. Louis at the Arch
St. Louis, Missouri, USA
December 4-6, 2016

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Proceedings compiled and edited by: S. Canty, K. Wolfe and D. Van Sanford

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Table of Contents

FHB MANAGEMENT

Integrated FHB Management of Spring Wheat in Idaho
S.S. Arcibal, C.A. Jackson, T.L. Shelman, L.L. Jones and J.M. Marshall .....Poster #1..... 3

2016 Field Plot Trials for Biological Control of Fusarium Head Blight on Winter Wheat in South Dakota using *Bacillus amyloliquefaciens* Strains
B.H. Bleakley, N.K.S. Murthy, P. Kulkarni, D.N. Yabwalo and E. Byamukama .....Poster #2.....7

Effects of Cultivar Resistance, Fungicide Application Timing, and Fungicide Chemical Class on FHB and DON in Winter Wheat
Carlos Bolanos-Carriel, Stephen N. Wegulo, Heather Hallen-Adams, P. Stephen Baenziger, Kent M. Eskridge and Deanna Funnell-Harris.....Poster #3..... 9

Effects of Fungicides, Time, and Grain Moisture Content on Postharvest Accumulation of DON in Winter Wheat
Carlos Bolanos-Carriel, Stephen N. Wegulo, Heather Hallen-Adams, P. Stephen Baenziger, Kent M. Eskridge, Deanna Funnell-Harris, Niki McMaster and David G. Schmale III .....Poster #4..... 11

Integrated Management of Fusarium Head Blight of Wheat in Central Alabama
Kira L. Bowen .....Poster #5..... 13

Integrated Management of Fusarium Head Blight of Wheat in South Alabama
Kira L. Bowen .....Poster #6..... 15

Effects of Fungicide and Fertility on Disease Development and Yield in Winter Wheat
M. Breunig, A.M. Byrne, M. Nagelkirk and M.I. Chilvers .....Poster #7..... 16

Fungicide Timing and Variety Resistance to Manage Fusarium Head Blight in Mid-Atlantic Winter Barley Crops
Christina Cowger and Consuelo Arellano .....Poster #8..... 17

Evaluation of Integrated Methods for Managing FHB and DON in Winter Wheat in New York in 2016
J.A. Cummings and G.C. Bergstrom 18

Evaluation of Fungicide Applications plus Cultivar Resistance to Reduce FHB and DON Infection of Barley in New England
Heather Darby and Erica Cummings..... 22

The Use of Integrated Management Strategies to Lower Fusarium Head Blight and Deoxynivalenol in Spring Barley
P.L. Gross, Venkata Chapara, J.Ransom, R. Brueggeman, Blaine Schatz, Audrey Kalil, Dimitri Fonseka, Chad Deplazes, Amanda Arens and A. Friskop .....Poster #10..... 27

Monitoring the Splash Dispersal of Spores of *Fusarium graminearum* from Wheat Plants using High Speed Video
Hope Gruszewski, Katrina Somers, Sunghwan Jung, Craig Powers, Regina Hanlon and David G. Schmale III ..... Poster #9 28
Variety and Fungicide Applications on FHB and DON in Delaware: A Collaborative Outreach Study
Nathan M. Kleczewski ..... Poster #11 29
Developing a Smart Phone App to Estimate *Fusarium* Damaged Kernels of Wheat Based on Computer Vision
Wenjing Ling, Pierce A. Paul and Laurence V. Madden ..... Poster #12 30
Influence of Rainfall Patterns on Deoxynivalenol Accumulation in Wheat after Fusarium Head Blight Symptom Development
Moraes, W.B., Lana, F.D., Schwarz, P.B., Madden, L.V., and Paul, P.A. ..... Poster #13 31
Effect of Cleaning and Fungicide Seed Treatment on Stand Establishment in Scabby Seed Lots in the Southern U.S.
Trey Price, Myra Purvis, Boyd Padgett, Steve Harrison, Erick Larson, Jenny Bibb, James Buck and John Youmans ..... Poster #14 33
Development of Fusarium Head Blight in Hard Red Winter Wheat during the 2016 Season in South Dakota
Dalitso Yabwalo, Richard Geppert, Shaukat Ali, Sunish Sehgal and Emmanuel Byamukama ..... Poster #15 34

FOOD SAFETY AND TOXICOLOGY

Development of Deoxynivalenol (DON) and DON-3-glucoside during Malting of *Fusarium* Infected Hard Red Spring Wheat
Zhao Jin, Bing Zhou, James Gillespie, John Barr, Thomas Gross, Robert Brueggeman and Paul Schwarz ..... Poster #16 37

GENE DISCOVERY AND ENGINEERING RESISTANCE

Development of a Fusarium Head Blight (FHB) Resistant Wheat via the Over-activations of Two Wheat Native Transgenes
Hussien Alameldin, Eric Olson, Elizabeth I. Brisco, Hayley West, Tahir Javaid, Julian Liber, Sohail Kamaran and Mariam Sticklen ..... Poster #17 41
Insight into the Mechanism of the *TRI6* RNA Enterference Ablating Deoxynivalenol Production in *Fusarium graminearum* with Patterns of siRNA Production
Thomas T. Baldwin and Phil Bregitzer ..... Poster #18 45
Host-induced Silencing of *Fusarium culmorum* Genes Protects Wheat from Infection
Wanxin Chen, Karolina Slominska, Christine Kastner, Daniela Nowara, Ely Oliveira-Garcia, Twan Rutten, Yusheng Zhao, Holger B. Deising, Jochen Kumlehn and Patrick Schweizer ..... Talk 46

FHB Resistance Genes - Genes with Multiple Benefits?
Doohan, F.M., Perochon, A., Walter, S., Kahla, A., and Gunupuru, L. ..... Talk..... 47
Transgenic Wheat Lines Upregulated for Genes in Lignin Biosynthesis as Potential Resistance Sources against Fusarium Head Blight
Deanna L. Funnell-Harris, Robert A. Graybosch, Scott E. Sattler, Stephen N. Wegulo and Thomas E. Clemente .....Poster #19..... 48
Exploring Fusarium Head Blight Disease Control by RNA Interference
Guixia Hao, Martha M. Vaughan and Susan McCormick .....Poster #21..... 49
Characterization of Small RNAs from *Fusarium*-inoculated Barley Spike Tissues
Yadong Huang, Lin Li, Kevin P Smith and Gary J Muehlbauer .....Poster #20..... 50
A Barley UDP-glucosyltransferase Provides Resistance to Nivalenol and Nivalenol-Producing *Fusarium graminearum*
Xin Li, Herbert Michlmayr, Wolfgang Schweiger, Alexandra Malachova, Sanghyun Shin, Yadong Huang, Yanhong Dong, Gerlinde Wiesenberger, Susan McCormick, Marc Lemmens, Philip Fruhman, Christian Hametner, Franz Berthiller, Gerhard Adam and Gary J. Muehlbauer .....Poster #22..... 51
Characterization of a Genus Specific Unidentified Open Reading Frame Found within the Mitochondrial Genome of *Fusarium*
Michael MacKillop, Haider Hamzah, Mauricio Diazgranados and John C. Kennell.....Poster #23..... 52
Antifungal Plant Defensins: Mechanisms of Action and Engineering Disease Resistant Crops
Dilip Shah, Jagdeep Kaur, Kaouar El-Mounadi, Tariq Islam, Siva Velivelli and John Fellers ..... Talk..... 53
Expression of Bean *PGIP2* under Control of the Barley *Lem1* Promoter Limits *Fusarium graminearum* Infection in Wheat
Silvio Tundo, Michela Janni, Ilaria Moscetti, Giulia Mandalà, Daniel Savatin, Ann Blechl, Francesco Favaron and Renato D'Ovidio .....Poster #24..... 54

PATHOGEN BIOLOGY AND GENETICS

Elucidating the Role of Silencing RNA *fgsiR34* in Fusarium Head Blight Pathogenesis in Wheat
Subha Daha, Aravind Galla, Bimal Paudel, Anjun Ma, Andrea Zavadil and Yang Yen .....Poster #26..... 57
Over-Expression of Translation Elongation Factor 1-alpha Modifies Pathogenic and Phenotypic Traits of a *Fusarium graminearum* Strain
Anas Eranthodi, Daria Ryabova, Ravinder K. Goyal and Nora A. Foroud .....Poster #27..... 58
Linking Host Community to *Fusarium graminearum* Distribution
Michael R. Fulcher and Gary C. Bergstrom .....Poster #28..... 59
Mapping of Fusarium Head Blight Resistance and Deoxynivalenol Accumulation in Kansas Wheat
Cristiano Lemes da Silva, Allan Fritz, Jesse Poland, Floyd Dowell and Kamaranga Peiris .....Poster #29..... 60

Identification and Characterization of *Fusarium graminearum* Pathogenesis Genes
Melissa M. Salazar, Frederic L. Kolb and Santiago X. Mideros .....Poster #30..... 61
Impact of Drought Stress on Wheat Root and Stem Base Infections (*Triticum aestivum* L.) with *Fusarium culmorum*
Sebastian Streit, Andreas von Tiedemann and Mark Winter .....Poster #31..... 62
Comparative Population Genomics of *Fusarium graminearum* Reveals Adaptive Divergence among Cereal Head Blight Pathogens
Todd J. Ward and Amy Kelly .....Talk..... 63
DON Modification in Naturally-Contaminated Wheat Samples using Microorganisms Isolated from the Environment
Nina Wilson, Dash Gantulga, Nicole McMaster, Ken Knott, Susan McCormick, Ryan Senger and David Schmale .....Poster #32..... 64
Deoxynivalenol (DON) and Nivalenol (NIV) Play a Role as Virulence Factors for Wheat Root and Stem Base Infection by *Fusarium culmorum* and *F. graminearum*
Mark Winter, Peter L. Samuels, Yanhong Dong and Ruth Dill-Macky .....Poster #33..... 65

VARIETY DEVELOPMENT AND HOST PLANT RESISTANCE

Tissue Culture Induced Variability: Critical Issues that Impact the Evaluation and use of Transgenic Parents
Phil Bregitzer.....Talk..... 69
Genomic Selection for FHB Resistance using the Uniform Scab Screening Nurseries
G. Brown-Guedira, J.M. Sarinelli, P. Tyagi, J.H. Lyerly, R. Acharya and J.P. Murphy .....Poster #34..... 75
Implementation of Genomic Selection for Resistance to Fusarium Head Blight into a Traditional Wheat Breeding Program
Neal Carpenter, Brian Ward, Subas Malla, Carl Griffey, Josh Fitzgerald, Niki McMaster and David Schmale III .....Poster #35..... 76
Association Mapping in a Panel of Minnesota Spring Wheat Breeding Lines Reveals QTL Maintained Over Decades of Phenotypic Selection
Emily J. Conley and James A. Anderson .....Poster #36..... 77
Evaluation of Germplasm Resistance to Fusarium Head Blight Disease
Sintayehu Daba, Rupesh Gaire and Mohsen Mohammadi .....Poster #37..... 78
Response of a Collection of Waxy (Reduced Amylose) Wheat Breeding Lines to *Fusarium graminearum*
Deanna L. Funnell-Harris, Robert A. Graybosch, Patrick M. O'Neill and Stephen Wegulo .....Poster #38..... 79
Simultaneous Mapping and Pyramiding Loci in Wheat Breeding Populations: Identity by Descent Mapping Approaches
Jose L. Gonzalez-Hernandez .....Talk..... 80

Evaluation of Southern Soft Red Winter Wheat Lines for Resistance to Fusarium Head Blight
Amanda L. Holder, R. Esten Mason and David E. Moon .....Poster #39..... 81
Epigenetic Control of FHB in Durum Wheat
Jitendra Kumar, Farhad Ghavami, Seyed M. Pirseyedi, Ajay Kumar, Steven Xu, Elias M. Elias, Ruth Dill-Macky and Shahryar Kianian .....Poster #40..... 82
The 2016 Uniform Southern Soft Red Winter Wheat Scab Nursery
J.P. Murphy, J.H. Lyerly, J.M. Sarinelli, P. Tyagi and G. Brown-Guedira.....Poster #41..... 83
Evaluating Methods of Updating Training Data in Long-Term Genomic Selection for Fusarium Head Blight Resistance in Barley
Jeffrey L. Neyhart, Tyler Tiede, Aaron J. Lorenz and Kevin P. Smith..... 86
Discovery of *Fusarium graminearum* Resistance in *Aegilops tauschii* Germplasm and Introgression into Wheat
Andrew T. Wiersma, Elizabeth I. Brisco, Linda K. Brown and Eric L. Olson..... Talk..... 91
Development of New Wheat Varieties Resistant to FHB through Microspore *in vitro* Selection Technology
Daria Ryabova, Harpinder S. Randhawa, Palak Kathiria, Fengying Jiang, Dean Spaner, Pierre Hucl, Robert Graf, François Eudes and Nora A. Foroud .....Poster #42..... 92
Trends in FHB Resistance in the Northern Uniform FHB Nursery
Clay Sneller, Mao Huang and Nelly Arguello.....Poster #43..... 93
A Meta-analysis of the Genetics of Fusarium Head Blight Resistance in Barley
Brian J. Steffenson, Matthew Haas and Ahmad Sallam ..... Talk..... 94
Development of High-throughput Diagnostic Markers for *Fhb1*, a Major Gene for FHB Resistance in Wheat
Zhenqi Su, Sujuan Jin, Amy Bernardo, Paul St. Amand and Guihua Bai.....Poster #44..... 95
Born, Bred and Brewed in New York
Daniel Sweeney and Mark Sorrells ..... Talk..... 96
Association Mapping for Fusarium Head Blight Resistance in Synthetic Hexaploid Wheat
A. Szabo-Hever, Q. Zhang, S. Zhong, T.L. Friesen, E.M. Elias, S.S. Xu and S. Chao .....Poster #45..... 97
Morphological and FHB Trait Variation in the Elite Eastern Mapping Panel
Lisa Tessmann, Anthony Clark and David Van Sanford.....Poster #46..... 98
Development of Fusarium Head Blight Resistance Germplasm in Highly Adapted Spring Wheat Background
Yaqoob Thurston, Jonathan T. Eckard, Karl D. Glover, James A. Anderson, Mohamed Mergoum, Shaukat Ali and Jose L. Gonzalez-Hernandez .....Poster #47..... 99
Development of Fusarium Head Blight Resistance Germplasm in Highly Adapted Winter Wheat Background
Yaqoob Thurston, Jonathan T. Eckard, Melanie Caffe, Shaukat Ali, Sunish K. Sehgal, Francois G. Marais and Jose L. Gonzalez-Hernandez .....Poster #48..... 100

Evaluation of Winter Barley Cultivar Eve for Quantitative Resistance to Fusarium Head Blight
Ullrich, J., S. Malla, C. Griffey, N. Carpenter, W. Brooks, D. Van Sanford, A. Clark, J.P. Murphy, R. Brueggeman, C. Cowger, N. McMaster, D. Schmale III, S. Chao and G. Brown-Guedira.....Poster #49..... 101

Effects of Elevated [CO_2] on the Defense Response of Wheat against *Fusarium graminearum* Infection
Martha M. Vaughan, Miroslava Cuperlovic-Culf, Guixia Hao, Karl Vermillion and Susan McCormick.....Poster #50..... 102

Genome-Wide Association Mapping of Fusarium Head Blight Resistance in Spring Wheat Lines Grown in Pacific Northwest and CIMMYT
Rui Wang, Jianli Chen, Junli Zhang, Weidong Zhao, Justin Wheeler, Natalie Klassen, James A. Anderson, Deven R. See and Yanhong Dong .....Poster #51..... 103

Growers' Needs and Industry Wants: A Retrospective of Two Decades in the Trenches in the Battle with FHB
Jochum J. Wiersma.....Talk.....104

Molecular Mapping of QTL for FHB Resistance Introgressed into Durum Wheat
Mingxia Zhao, Yueqiang Leng, Shiaoman Chao, Steven S. Xu and Shaobin Zhong .....Poster #52..... 105

OTHER PAPERS

GrainGenes: Supporting the Small Grains Community
Taner Z. Sen, Gerard R. Lazo, Yong Q. Gu, David L. Hane and Sarah G. Odell.....Poster #25..... 109

Expression of an *Arabidopsis* Non-specific Lipid Transfer Protein in Pichia Pastoris and Wheat
John E. McLaughlin, Dan Finn, Neerja Tyagi, Harold Trick, Susan McCormick and Nilgun E. Tumer .....Poster #53.....110

INDEX OF AUTHORS..... 111

FHB MANAGEMENT

INTEGRATED FHB MANAGEMENT OF SPRING WHEAT IN IDAHO

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OBJECTIVE

Evaluate the integrated effects of fungicide application and wheat resistance on Fusarium head blight (FHB) and deoxynivalenol (DON) in Idaho.

INTRODUCTION

FHB damage in spring wheat has substantially increased in southern Idaho over the last ten years. Many spring wheat fields in the area tested at 1-7 ppm DON even after appropriate fungicide treatments. Since the majority of the wheat varieties available to growers in the area are susceptible to FHB, it is crucial to develop integrated management strategies of FHB and DON specific to irrigated, high-desert production conditions in Idaho. Participation in the USWBSI FHB management coordinated project will not only provide a framework to educate local growers but also add a unique location to the national coordinated research program for meta-analysis.

MATERIALS AND METHODS

The coordinated study was conducted at the University of Idaho Aberdeen Research and Extension Center in Aberdeen, ID with four wheat varieties, Diva (moderately susceptible), IDO1202S (moderately susceptible) IDO851 (moderately resistant), and Klasic (susceptible) on 20 April 2016. Varieties were selected based on 2015 FHB screening data. The experimental design was compete randomized block with a split plot arrangement in 6 replications, with cultivars as main plots and treatments as sub-plots. Fungicide applications were at anthesis (Feekes growth stage 10.5.1) and anthesis + 4 days post-anthesis (A+4). Fungicide treatments were Prosaro® (6.5 fl. oz /A) at anthesis, Prosaro + Caramba® (6.5 + 14 fl. oz/A) at A+4, Caramba + Folicur® (14 + 4 fl. oz/A) at A+4 and Proline® + Folicur (5.7 + 4 fl. oz/A) at A+4. Fungicides were applied with a CO_2 sprayer using 8001 VS nozzles at a rate of 10 gallons per acre. Conidial suspensions (100,000 macroconidia/L) were sprayed a day following the anthesis fungicide application with a CO_2 backpack sprayer with Teejet 8003 VS nozzles at a ground speed of 1 second per foot at 40 psi. Severity (percent blighted spikelets per head) of 100 heads per plot was arbitrarily rated at soft dough (FGS 11.2) specifically 23-24 days after anthesis. FHB severity was used to calculate FHB incidence (incidence= number of blighted heads/100 sampled heads) and FHB index (FHB Index= Severity x Incidence / 100). Plots were harvested on 7 September using a Harvestmaster plot combine. *Fusarium*-damaged kernels (FDK) were assessed as a percentage of harvested kernels visibly affected by FHB out of the harvested grain from each plot. Data were analyzed using the generalized linear mixed model procedure (PROC GLIMMIX) in SAS (version 9.4). Subsamples were sent to Dr. Yanhong Dong of University of Minnesota for DON analysis and data will be provided on a later date.

RESULTS AND DISCUSSION

Significant differences in FHB ratings, yield and test weight were found among varieties (Table 1). IDO851 had the lowest FHB ratings and highest yield. Only FHB severity and test weight were significantly different between Diva

and IDO1202S. Klasic also had lower FHB than Diva and IDO1202S but had the highest FDK, and lowest yield and test weight among varieties. Klasic reached anthesis one week earlier than other varieties, which resulted to earlier and possibly lower FHB ratings.

Despite the moderately low disease pressure, fungicide applications significantly reduced FHB ratings and FDK as well as significantly increased yield and test weight compared to the untreated checks (Table 2). Inoculated and non-inoculated untreated checks significantly differ in test weight only. Although treatments with post-anthesis fungicide applications significantly reduced FHB severity and FDK, no significant differences in FHB index and yield were detected among fungicide treatments. The effectiveness of additional post-anthesis fungicide applications cannot be determined in this trial but may be effective in environments with highly conducive conditions.

Overall, FHB index ranged from 2 to 32 % (Table 3). Moderately susceptible varieties Diva and IDO1202S with fungicide treatments had significant FHB reduction but yields did not differ. However, test weights of fungicide-treated IDO1202S plots were significantly higher than the untreated checks. When treated with fungicides, the susceptible variety Klasic had significantly increased yield and test weight. Only Prosaro application at anthesis resulted to significantly higher yield and test weight of the moderately resistant ID0851. The 2016 growing season was very dry and under these conditions, split fungicide applications did not improve disease control compared to one application. Current recommendation of one fungicide application at anthesis remains the most cost effective method to reduce FHB under irrigation in southern Idaho.

ACKNOWLEDGEMENT AND DISCLAIMER

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Table 1. Main effect of varieties on FHB, yield, test weight and FDK at Aberdeen, ID in 2016.

Variety	Severity (%)	Incidence (%)	FHB Index	Yield (bu/A)	Test weight (g)	FDK (%)
Diva	16.4 a	27.4 a	6.1 a	133.3 ab	60.1 b	1.1 ab
IDO1202S	12.3 b	27.2 a	4.8 a	131.5 b	61.3 a	1.0 b
IDO851	2.4 c	9.3 b	0.3 b	144.4 a	59.7 b	0.4 c
Klasic	4.2 c	13.7 b	0.9 b	103.2 c	58.5 c	1.5 a
P=0.05	<.0001	0.0007	0.0013	0.0002	0.0005	0.003

Table 2. Main effect of fungicide on FHB, yield, test weight and FDK at Aberdeen, ID in 2016.

Treatment	Severity (%)	Incidence (%)	FHB Index	Yield (bu/A)	Test weight (g)	FDK (%)
Untreated check	16.5 a	29.2 a	6.8 a	117.9 b	58.5 c	1.8 a
Prosaro Anthesis	8.0 b	18.8 b	2.1 b	133.5 a	60.2 a	0.81 b
Prosaro + Caramba Anthesis + 4 days	2.1 c	8.4 d	0.2 b	132.7 a	60.6 a	0.45 c
Caramba + Folicur Anthesis + 4 days	3.2 c	12.9 cd	0.6 b	131.7 a	60.4 a	0.58 c
Proline + Folicur Anthesis + 4 days	7.0 b	16.0 bc	1.7 b	130.9 a	60.3 a	0.61 bc
Untreated non-inoculated check	16.2 a	31.0 a	6.7 a	121.8 b	59.2 b	1.6 a
P=0.05	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Table 3. Effect of variety and fungicide treatments on FHB, yield, test weight and FDK at Aberdeen, ID in 2016.

Variety	Treatment	Severity (%)		Incidence (%)		FHB Index		Yield (bu/A)		Test weight (g)		FDK (%)	
Diva	Untreated inoculated	31.6	a	43.3	a	14.2	a	128.2	f	59.3	def	1.9	a
	Prosaro	15.2	c	27.5	b	4.9	b	132.1	c-f	59.9	c-f	1.1	a
	Prosaro + Caramba	4.7	f-i	11.5	def	0.6	c	134.8	b-f	60.2	cde	0.6	a
	Caramba + Folicur	7.1	d-h	20.0	bcd	1.6	bc	135.8	b-f	60.6	bc	0.6	a
	Proline + Folicur	11.9	cd	21.8	bc	3.3	bc	137.8	b-f	60.4	c	0.9	a
	Untreated non-inoculated	28.2	ab	40.3	a	12.1	a	131.4	ef	60.1	c-f	1.7	a
IDO1202S	Untreated inoculated	23.9	b	44.5	a	11.2	a	120.9	h	59.8	c-f	1.9	a
	Prosaro	9.1	def	24.3	b	2.3	bc	130.4	ef	61.7	ab	0.8	a
	Prosaro + Caramba	1.9	i	9.8	ef	0.2	c	145.8	bcd	62.1	a	0.4	a
	Caramba + Folicur	3.5	ghi	14.3	c-e	0.6	c	132.0	c-f	62.0	a	0.6	a
	Proline + Folicur	10.9	cde	24.0	b	2.7	bc	133.6	c-f	62.0	a	0.4	a
	Untreated non-inoculated	24.2	b	46.3	a	12.0	a	126.0	f	60.1	c-f	1.7	a
IDO851	Untreated inoculated	3.8	f-i	11.0	def	0.5	c	135.7	b-f	59.1	ef	1.0	a
	Prosaro	2.6	ghi	10.8	def	0.4	c	160.6	a	60.3	cd	0.2	a
	Prosaro + Caramba	0.8	i	5.8	f	0.1	c	143.5	b-e	60.1	c-f	0.1	a
	Caramba + Folicur	0.9	i	7.3	f	0.1	c	148.6	b	59.9	c-f	0.1	a
	Proline + Folicur	2.4	ghi	8.8	ef	0.4	c	146.3	bc	59.8	c-f	0.0	a
	Untreated non-inoculated	4.0	f-i	12.5	c-e	0.5	c	131.7	def	59.0	f	0.8	a
Klasic	Untreated inoculated	6.6	d-i	18.0	b-e	1.3	bc	87.0	k	55.9	h	2.6	a
	Prosaro	5.3	e-i	12.8	c-e	1.0	c	110.9	hi	59.2	ef	1.2	a
	Prosaro + Caramba	1.1	i	6.8	f	0.1	c	106.6	hij	60.1	c-f	0.8	a
	Proline + Folicur	2.7	ghi	9.5	ef	0.3	c	106.1	ij	59.1	ef	1.2	a
	Caramba + Folicur	1.4	i	10.0	ef	0.1	c	110.6	hi	59.1	ef	1.1	a
	Untreated non-inoculated	8.4	d-g	25.0	b	2.5	bc	98.0	j	57.8	g	2.3	a
	$P = 0.05$	<.0001		<.0001		<.0001		0.0136		0.0006		0.2909	

2016 FIELD PLOT TRIALS FOR BIOLOGICAL CONTROL OF FUSARIUM HEAD BLIGHT ON WINTER WHEAT IN SOUTH DAKOTA USING *BACILLUS AMYLOLIQUEFACIENS* STRAINS

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ABSTRACT

Fusarium graminearium is mostly responsible for Fusarium head blight (FHB) in wheat and barley, which can cause significant economic losses. Yield losses can be controlled or reduced through using fungicides alone or in combination with biological control agents (BCAs). We have previously investigated the efficacy of *Bacillus amyloliquefaciens* strains 1BA and 1D3 in biological control of FHB on spring wheat cultivars. In the present study we assayed the ability of these bacteria to control FHB on winter wheat cultivars, to see if similar beneficial effects of BCA application would be observed. Field plot trials were conducted in 2016 in Brookings, South Dakota to analyze the efficacy of the *B. amyloliquefaciens* strains in biological control of FHB. Spray applications of *Bacillus* BCAs alone or in combination with Prosaro® (fungicide) and/ or Induce NIS (non-ionic surfactant) and/ or chitin were done on winter wheat cultivars Lyman and Redfield at Feekes 10.51.

Significant treatment differences (P=0.1) were observed for FHB Incidence, percentage disease, and test weight in the Lyman cultivar, compared to the untreated control; with the Prosaro alone (single application), and Prosaro plus BCAs/chitin treatments having the same percentage incidence (3.0%). Although not statistically significant, final yield (48.79 bu/ac), 1000 kernel weight (25.41), and protein percentage (14.88) were highest for the Prosaro plus BCAs/chitin treatment.

In the Redfield winter wheat treatments, significant treatment differences (P = 0.1) were observed for final yield, 1000 kernel weight, and percent *Fusarium* damaged kernels, compared to the untreated control. Although not statistically significant, the Prosaro plus BCAs/chitin treatment had the lowest percentage incidence (5.0%), and highest test weight (54.59 lb/bu). For both winter wheat cultivars, the results for DON are pending and not yet available.

These trials suggested that treatment with the *Bacillus* BCA strains in combination with Prosaro can help increase yield in some situations, and reduce some measures of FHB on these winter wheat cultivars. Further trials of these BCAs on winter wheat would help clarify the benefits of foliar BCA application in increasing yield and reducing FHB.

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EFFECTS OF CULTIVAR RESISTANCE, FUNGICIDE APPLICATION TIMING, AND FUNGICIDE CHEMICAL CLASS ON FHB AND DON IN WINTER WHEAT

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ABSTRACT

The frequency and severity of Fusarium head blight (FHB), caused by *Fusarium graminearum*, have increased in Nebraska during the last 10 years. Major epidemics occurred in 2007, 2008, and 2015, and varying levels of the disease have occurred from 2007 to 2016. *F. graminearum* produces the mycotoxin deoxynivalenol (DON). Losses to the grower include decreased yield and grain volume weight and discounts at the elevator if DON levels in grain exceed 2 ppm. Fungicide applications to control FHB are aimed at reducing disease intensity as well as DON. Because FHB infections occur on wheat heads mostly during flowering, optimal fungicide application is usually timed at anthesis. The narrow window (anthesis) of fungicide application presents challenges to the grower. Previous research has indicated that fungicides in the triazole class are more effective than those in the strobilurin class in suppressing FHB and DON. During 2015 and 2016, two field trials (dryland and irrigated) were conducted to accomplish the following objectives: 1) determine the effect of fungicide application timing at anthesis, as well as at 6 and 12 days post anthesis (dpa) on FHB and DON in a susceptible and a moderately resistant cultivar, and 2) compare the effects of Prosaro® (prothioconazole + tebuconazole, triazole) and Headline® (pyraclostrobin, strobilurin) on FHB and DON when applied at different timings in the cultivars Overley (susceptible) and Overland (moderately resistant). Data from the 2015 growing season, in which severe FHB and high DON levels occurred, have been presented (Bolanos-Carriel et al., 2015). In 2016, FHB and DON levels were very low. In the dryland trial, few differences were detected among treatments (cultivars and application timings). In Overley, FHB index was similar between fungicides (Prosaro and Headline) and application timings (range 14-33%; 38% in the unsprayed check) and the same was true for DON (range, 0.1-1 ppm; 1.4 ppm in the unsprayed check). In Overland, FHB index (range 2-13%; 14% in unsprayed the check) and DON (range 0.1-0.2 ppm; 0.1 ppm in the unsprayed check) were lower than in Overley. In the irrigated trial in Overley sprayed with Prosaro, FHB index was 28, 35, and 51% at anthesis, 6 dpa, and 12 dpa, respectively compared to 74% in the unsprayed check. The corresponding DON values were 2.9, 2.6, and 3.7 ppm, respectively compared to 4 ppm in the unsprayed check. In the same trial in Overley sprayed with Headline, FHB index was 23, 45, and 67% at anthesis, 6 dpa, and 12 dpa, respectively compared to 74% in the unsprayed check and the corresponding DON values were 4.1, 3.1, and 4 ppm, respectively compared to 4 ppm in the unsprayed check. In the irrigated trial in Overland, FHB index was similar between Prosaro and Headline (range 13 to 37% compared to 39% in the unsprayed check). Prosaro DON values were 0.6, 1.1, and 0.8 ppm, at anthesis, 6 dpa, and 12 dpa, respectively compared to 1.1 ppm in the unsprayed check and the corresponding Headline DON values were 0.6, 0.9, and 1.2 ppm, respectively compared to 1.1 ppm

in the unsprayed check. Results from 2015 (Bolanos-Carriel et al., 2015) and 2016 (this report) allow us to conclude that: 1) the window of fungicide application to control FHB and DON can be widened from anthesis to 6 days later without loss of efficacy in suppressing FHB and DON, and 2) moderate resistance coupled with fungicide application can significantly reduce DON in grain.

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EFFECTS OF FUNGICIDES, TIME, AND GRAIN MOISTURE CONTENT ON POSTHARVEST ACCUMULATION OF DON IN WINTER WHEAT

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ABSTRACT

Fusarium Head Blight (FHB), caused by *Fusarium graminearum*, is one of the most destructive diseases of wheat and other small grain cereals. *F. graminearum* produces the mycotoxin deoxynivalenol (DON). Losses to the grower are manifested as decreased yield and grain volume weight and discounts at the elevator if DON levels in grain exceed 2 ppm. DON toxicity has direct repercussions for human and animal health. During storage, the most critical factors for grain quality are moisture and temperature, parameters which are directly related to spoilage. The objective of this study was to determine the effect of time and grain moisture content on DON in stored winter wheat grain from field plots treated with the fungicides Headline® (pyraclostrobin, strobilurin) and Prosaro® (prothioconazole + tebuconazole, triazole) at anthesis. In 2015, plots of winter wheat cultivar Overland (moderately resistant to FHB), under rainfed environmental conditions, were treated with Prosaro (rate 6.5 fl oz/Acre (0.475 L/ha)) and Headline (rate 9 fl oz/Acre (0.658 L/ha)) at anthesis. After harvest, *Fusarium*-damaged kernels (FDK) were removed through a sorting process. Removal of FDK was necessary because during the growing season in 2015, conditions were highly favorable to disease development, leading to high levels of FHB and DON, which masked treatment differences in preliminary DON analysis. A mass of 300 g of clean grain from each of four replications per treatment was soaked, placed in a sterile micro-propagation container, and stored in a seed cooler under dark conditions at 50°F (10°C) and 40% relative humidity (RH). The conditions inside the containers were monitored using temperature and RH recorders. Moisture content was kept constant at 16% which corresponded to a water activity (Aw) value of 0.60 and at 20% which corresponded to an Aw value of 0.75. Sampling of each of the experimental units was made at 0, 30, 60, 90 and 120 days after soaking (DAS) and subsamples were subjected to DON analysis using Gas chromatography–mass spectrometry (GC-MS). Results showed that DON accumulation was higher in grain from plots treated with Headline (4.37 µg/g) than in grain from non-sprayed check plots (3.61 µg/g) (LSD, P = 0.05). Grain from plots treated with Prosaro, had the lowest DON (2.68 µg/g) which differed from the Headline and check treatments (LSD, P = 0.05). Following soaking, DON concentration declined during the first 30 days by an average (over the two moisture levels) of 24, 34, and 36% in the Headline, check, and Prosaro treatments, respectively. Then over the next 30 days, DON levels increased by an average of 23, 27, and 40% over the 30-day levels in the Headline, check, and Prosaro treatments, respectively. Thereafter, DON levels in all treatments stabilized with slight fluctuations. Averaged over time treatments, DON was slightly higher, similar,

and lower at 20% moisture compared to 16% moisture in the Headline, check, and Prosaro treatments, respectively. However, these differences were not significant at P = 0.05, indicating that grain moisture (16% or 20%) did not have an effect on DON during grain storage for 120 days. There was a consistent trend in DON levels over time in grain from plots treated with Headline, Prosaro, as well as in grain from the non-sprayed checks. The results clearly showed elevated DON (above the non-sprayed check) in the Headline treatment and reduced DON (below the non-sprayed check) in the Prosaro treatment. Overall, DON declined over time from 0 DAS to 120 DAS. However, DON in grain from plots treated with Headline was not different at 0 DAS versus 120 DAS (4.37 ppm vs 4.08 ppn; P = 0.1161) whereas significant differences were detected in DON at 0 DAS versus 120 DAS in grain from plots treated with Prosaro (2.68 ppm vs 2.24 ppm; P = 0.0142) as well as in grain from the non-sprayed checks (3.61 ppm vs 2.98 ppm; P=0.0005). The results from this study indicated that 1) Headline sprayed at anthesis elevated DON in wheat grain and therefore should not be used to control FHB and DON and 2) at a room temperature of 50^{o}F (10^{o}C) and 40% RH, DON decreased over time (120 days) in stored wheat grain from plots treated with Prosaro at anthesis and grain from non-sprayed plots, but not in grain from plots treated with Headline at anthesis.

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INTEGRATED MANAGEMENT OF FUSARIUM HEAD BLIGHT OF WHEAT IN CENTRAL ALABAMA

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ABSTRACT

Fusarium head blight (FHB), caused by *Fusarium graminearum*, is a disease of wheat that has sporadically but substantially impacted Alabama's wheat crop. Moderate epidemics occurred in 2015 and 2016 in the southern third of the state, and varying levels of the disease have occurred in other regions of the state in recent years. In addition to yield reduction, *F. graminearum* produces the mycotoxin deoxynivalenol (DON) which can reduce the price that growers receive for their grain and its value as livestock feed. Fungicide applications to control FHB must be carefully timed to protect the flower since infections occur during this growth stage. However, even with appropriately timed fungicide applications, disease can be severe when weather favors FHB. In addition, while no wheat cultivars have complete resistance to FHB, cultivars do differ in their susceptibility. It is important to understand how the use of differences in susceptibility plus fungicide application can impact FHB and subsequent DON levels. Thus, the objectives of this research were to: 1) evaluate the integrated effects of fungicide and soft red winter wheat cultivar differences FHB and DON in AL; and 2) evaluate late and multiple tebuconazole applications for control of FHB.

In December 2015, a field trial in central Alabama (32.500352, -85.891843) was planted to four wheat cultivars. The cultivars were Jamestown (moderately resistant, medium maturity), Pioneer 26R41 (moderately resistant, late maturity), AGS 2035 (moderately susceptible, medium maturity) and SS 8641 (moderately susceptible, medium maturity). Four blocks of a split-plot arrangement of treatments were included, with cultivars as main plots; sub-plots were fungicide and inoculation treatments. Treatments were: i) non-inoculated, non-treated; ii) inoculated, non-treated; iii) Prosaro® at FS10.51; iv) Prosaro at FS10.51 followed 3 to 5 days later with (fb) Caramba®; v) Caramba at FS10.51 fb Folicur®; and vi) Proline® at FS 10.51 fb Folicur; all treatments were inoculated. A mist irrigation system was placed in the plots and ran at hourly intervals for 10 minutes from 7 p.m. to 10 a.m. from 1 Apr to 12 May. Inoculation consisted of *F. graminearum* infested corn spread on plots at full flag leaf stage. Fungicides were applied on different dates based on cultivars reaching FS 10.51 (early flower), from 10 Apr to 1 May. Approximately 3 weeks after FS 10.51 for each cultivar, 20 heads were collected for determining FHB incidence and severity index. Plots were combined for yield calculations and samples were assayed for DON determination.

Cultivar did not have a significant effect on scab incidence or severity index; however, Jamestown had numerically lower values for both of these variables. SS 8641 and Pioneer 26R41 had higher ($P < 0.01$) DON content than AGS 2035, which also had higher ($P < 0.01$) DON content than Jamestown. Fungicide and inoculation significantly affected incidence and severity index, with both non-treated treatments having higher incidence than the Prosaro fb Caramba or the Proline fb Folicur treatments. The non-treated, inoculated treatment had a significantly ($P < 0.01$) higher FHB severity index than either Prosaro treatment or the Proline fb Folicur. The non-treated, inoculated treatment also had the highest ($P < 0.01$) DON content among treatments; lowest DON levels were found in the Prosaro fb

Caramba and Caramba fb Folicur treatments. Yields and test weights were improved with each of the fungicide treatments compared to the non-treated, inoculated treatment.

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INTEGRATED MANAGEMENT OF FUSARIUM HEAD BLIGHT OF WHEAT IN SOUTH ALABAMA

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ABSTRACT

Severe intensities of Fusarium head blight (FHB), caused by *Fusarium graminearum*, were noted in a wheat variety trial in southern Alabama (30^0 32' 5 N, -87^0 53' 7 W) in spring of 2015. In a nearby trial, with fungicide applications made at FS 10.5 (inflorescence complete), FHB intensities were noted at scores of 4 to 8 on a 0 to 10 scale where 10 = all heads and kernels affected by disease. These results demonstrated that even with fungicide applications aimed at protecting the flower, FHB can be problematic. Thus, in 2016, we sought to evaluate multiple fungicide applications and their effectiveness for managing FHB.

Two cultivars (Jamestown (moderately resistant) and AGS 2035 (moderately susceptible)) were planted in early December 2015 as main plots in a split plot arrangement with fungicide treatments as sub-plots. Four blocks of treatments were established. Fungicide treatments included applications made at FS10.51 or 4 days after FS10.51 (delayed); treatments also included two applications at both of these timings. Fungicides that were evaluated were Prosaro® (at FS10.51 and delayed), Prosaro (FS10.51) followed by Caramba® (delayed), Caramba (FS 10.51) followed by Folicur® (delayed), Proline® (FS10.51) followed by Folicur (delayed), Folicur at FS 6, and Folicur at FS6 followed by a delayed Folicur application, as well as a non-treated control treatment. Ten head samples were collected randomly from each plot for determination of Fusarium head blight (FHB) incidence and severity index. Plots were harvested at maturity and test weights determined. Samples were taken from combined samples and assayed for deoxynivalenol (DON) content. Data were subjected to generalized mixed model analysis followed by mean separation using Fisher's (protected) least significant difference (FLSD) with P=0.05.

No measured variables differed due to cultivar; however, Jamestown had a numerically lower FHB incidence, severity index and DON content, and higher yield than AGS 2035. All three fungicide programs that involved two applications during flower reduced FHB severity index ($P < 0.01$) compared to Folicur at FS6 and delayed Prosaro. Two treatments (Prosaro followed by Caramba, and Proline followed by Folicur) had lower FHB severity indices ($P < 0.01$) than the non-treated control. Yields were higher ($P < 0.01$) and DON was lower with the two (FS10.51 and delayed) application fungicide programs than other treatments.

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EFFECTS OF FUNGICIDE AND FERTILITY ON DISEASE DEVELOPMENT AND YIELD IN WINTER WHEAT

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ABSTRACT

Nitrogen fertilization in winter wheat is increasingly popular, as growers try to increase yields and profitability. However, nitrogen can also contribute to increased disease and lodging. Fungicide and growth regulator products may help mitigate these negative consequences. The objective of this study is to examine the interactions of fungicide, nitrogen, and growth regulators and their effects on disease and ultimately, yield. In 2015 and 2016, field trials were conducted in East Lansing, MI to evaluate leaf disease, Fusarium head blight (FHB), lodging, deoxynivalenol (DON) production, and yield in response to factorial combinations of the fungicides Stratego YLD® (Feekes 6) and Prosaro® (Feekes 10.5.1), and growth regulator Palisade (Feekes 6) under low (90 lbs N/A at green up) or high (additional 50 lbs N/A at Feekes 6) fertilization. In 2015, the highest yielding treatments were the low nitrogen treatments containing Prosaro, which were significantly higher than all of the high fertility treatments except those with both Stratego YLD and Prosaro. DON levels were greatest in the high fertility Feekes 6 application of Stratego YLD. Low nitrogen fertility treatments with Prosaro had the lowest DON values, and FHB disease index ratings followed the same trends. In 2016, there was very little FHB disease development due to dry conditions during flowering. No visible symptoms were apparent across plots, so no disease severity or incidence was measured. *Fusarium* damaged kernels (FDK) were assessed, but no significant differences were found. DON levels were extremely low across all treatments; all but two samples tested negative (below the detectable limit of 0.05 ppm). However, stripe rust (*Puccinia striiformis*) pressure was extremely high in 2016, which could explain the differences in yields compared to 2015. The highest yielding treatment was high nitrogen with both a Stratego YLD and Prosaro application, however this was not significantly different than the five treatments containing either one or both of Stratego YLD or Prosaro. This suggests the early Stratego YLD application can be important in protecting against reduced yield from stripe rust, but perhaps high nitrogen with Prosaro can also compensate for that loss of yield from stripe rust.

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FUNGICIDE TIMING AND VARIETY RESISTANCE TO MANAGE FUSARIUM HEAD BLIGHT IN MID-ATLANTIC WINTER BARLEY CROPS

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ABSTRACT

The mycotoxins resulting from barley Fusarium head blight (FHB) can render grain unsuitable for human or animal consumption, and there is a zero tolerance for deoxynivalenol (DON) in malting barley. At the same time, interest in barley production has increased in the mid-Atlantic U.S. because of the upsurge in craft malt and the demand for local grain. A three-year field experiment is underway to help mid-Atlantic barley producers select FHB-resistant varieties, judge the potential benefit from a fungicide, and choose the optimal timing for a fungicide application should FHB threaten. The split-plot experiment is taking place in a misted, inoculated nursery in Raleigh, NC, using main plots of three commonly planted six-row barley cultivars (Atlantic, Nomini, and Thoroughbred) and one two-row cultivar (Endeavor). Both Endeavor and Thoroughbred are used for malting. As sub-plots, three fungicide treatments are applied: Prosaro® at 100% spike emergence, Prosaro 6 days later, or no fungicide.

So far, two years of data have been obtained. FHB intensity was higher in 2015 than in 2016, with the mean levels of DON in the two years being 4.5 ppm and 1.5 ppm, respectively. There was no difference in DON for treatment*variety; i.e., the relative efficacy of fungicide timings did not depend on the variety to which they were applied. Spray efficacy in reducing DON did depend on year, however. In 2015, the higher-scab year, the two spray timings resulted in the same amount of DON (3.7 vs. 4.0 ppm for early and late, $P = 0.74$), and both produced less DON ($P \leq 0.0005$) than the unsprayed check (5.8 ppm). In 2016, the lower-scab year, neither spray timing was significantly less DON than the unsprayed check (1.6 vs 1.1 for early vs. late, and 1.9 for unsprayed; $P \geq 0.14$). The year*variety interaction for DON was significant ($P = 0.005$), although varieties maintained the same ranks by DON level in both years, with Endeavor consistently the lowest and Atlantic consistently the highest for DON. In 2015, the higher-scab year, Endeavor and Thoroughbred had the lowest DON, while Nomini was significantly higher and Atlantic was the highest, averaging across fungicide timings. In 2016, the lower-scab year, DON levels did not differ among varieties when averaged across fungicide treatments.

There was no significant effect of fungicide treatment on test weight in 2015 ($P = 0.44$). In 2016, however, the unsprayed treatment had slightly lower test weight than the mean of the sprayed treatments (47.4 vs. 48.5 lb/bu, respectively, $P = 0.01$).

So far, this experiment has shown that in a high-scab year, applying Prosaro at 100% spike emergence or 6 days later resulted in the same DON levels, and provided an approximately 34% reduction in DON relative to the unsprayed check when averaging across the two fungicide timings. Variety resistance also provided a significant reduction in DON in that year, with the average of the two MR varieties (Endeavor and Thoroughbred) providing a 68% DON reduction when compared to Atlantic (S) and a 56% DON reduction when compared to Nomini (MS). The experiment is being repeated for a third year.

EVALUATION OF INTEGRATED METHODS FOR MANAGING FHB AND DON IN WINTER WHEAT IN NEW YORK IN 2016

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OBJECTIVE

To evaluate the individual and interactive effects of moderately resistant cultivars and application timings and combinations of the fungicides Prosaro® and Caramba® on yield, Fusarium head blight (FHB), and deoxynivalenol (DON) on soft red winter wheat in New York.

INTRODUCTION

In response to the USWBSI goal to validate integrated management strategies for FHB and DON, the Disease Management RAC of USWBSI initiated a multi-state, multi-year, coordinated field study. In New York during 2016, we evaluated the disease and yield impact of cultivar susceptibility, inoculation with *Fusarium graminearum*, and treatment with Prosaro and Caramba fungicides alone or sequentially.

MATERIALS AND METHODS

The trial was conducted at the Musgrave Research Farm in Aurora, NY in a Lima silt loam soil planted with four soft red winter wheat varieties, 'Erie' (moderately susceptible to Fusarium head blight (FHB)), 'Otsego' (susceptible to FHB), 'Pioneer Brand 25R25' (moderately resistant to FHB), and 'Pioneer Brand 25R46' (moderately resistant to FHB), following soybean harvest on 6 Oct 2015. The experiment was set up as a completely randomized block design with a split-plot arrangement, with cultivar as the main plot and the treatments as subplots, randomized in six replicated blocks. Main plots were sown with wheat at 118.8 lb/A with a 10 ft wide commercial grain drill. Subplots were 20 x 10 ft including 15 rows with 7-in. row spacing. The plots were fertilized at planting (200 lb/A of 10-20-20) and topdressed on 10 Apr (120 lb/A of urea, providing an additional 55 lb/A of nitrogen). The first Prosaro (6.5 fl. oz./A) or Caramba (17 fl. oz./A) application was at anthesis (Feekes growth stage, FGS 10.51) on 31 May including the surfactant Induce at 0.125% V/V. After the fungicide had dried, plots were spray-inoculated with a conidial suspension of *F. graminearum* (40,000 conidia/ml) to augment the development of FHB. The second Caramba (17 fl. oz./A) application occurred seven days after anthesis on 7 Jun including the surfactant Induce at 0.125% V/V, and plants were inoculated with a conidial suspension of *F. graminearum* (40,000 conidia/ml) after the fungicide had dried. Fungicide and *F. graminearum* treatments were applied with a tractor-mounted sprayer with paired TJ-60 8003vs nozzles mounted at an angle (30° from horizontal) forward and backward, 20-in. apart, pressurized at 30 psi, and calibrated to deliver 20 gal/A. Incidence and severity (percent of symptomatic spikelets on symptomatic heads) of FHB in each plot were rated on 21 Jun and used to calculate FHB Index, where FHB index = (FHB severity * FHB incidence)/100. Foliar diseases were rated on 21 Jun as percent severity on flag leaves (average rating for whole plot). Grain was harvested from a 20 x 5 ft area in each subplot using an Almaco plot combine on 12 Jul. Grain moistures, plot yields, and test weights were recorded. Yields and test weights were adjusted to bu/A at 13.5% moisture. ***Fusarium*** damaged kernels (FDK) were evaluated post-harvest as a percentage of kernels visibly affected by FHB out of a 100 kernel subsample from each plot. Analysis of deoxynivalenol (DON)

content in grain was conducted in the mycotoxin analysis laboratory at the University of Minnesota, St. Paul, MN. Treatment means were calculated, subjected to analysis of variance, and separated by Tukey-Kramer HSD test ($P = 0.05$).

RESULTS AND DISCUSSION

The 2016 growing season was the driest in NY in more than 20 years. Severely dry conditions during the season were not conducive for the development of FHB, and incidence was < 1% throughout the trial despite inoculation with *F. graminearum*. In the virtual absence of FHB development, DON measurements were < 0.04 ppm in all plots, regardless of cultivar or treatment. The only significant difference observed in DON results occurred when the results of all treatments were combined and Otsego had significantly higher DON than all other varieties, though only at a very low mean of 0.02 ppm. Therefore, based on the extreme drought conditions of this trial, no strong conclusions or recommendations could be made for FHB and DON management regarding the cultivars or treatments evaluated.

However, there was measurable leaf and stripe rust observed throughout the trial. This was the first significant stripe rust epidemic observed in New York State. Significant responses among cultivars and treatments were observed for both leaf and stripe rust. When the results of all treatments were combined, Otsego had significantly higher leaf rust and Pioneer Brand 25R46 had significantly higher stripe rust than all other cultivars. However, when each of these cultivars were analyzed individually, all three fungicide treatments effectively eliminated both rusts. When the results of all cultivars were combined, all three fungicide treatments were equally effective at significantly reducing both leaf and stripe rusts as compared to non-fungicide treated plots. These results support our recommendations for NY growers to apply triazole fungicides for management of leaf and stripe rust during years of significant rust disease pressure. The results also indicate that although Pioneer Brand 25R46 has been shown in years past to be moderately resistant to FHB, it has notable susceptibility to stripe rust.

Also, despite the drought, we observed measurable Septoria and Stagonospora leaf blotches throughout the trial, though at fairly low levels ranging from 0.1 to 2.5% severity on the flag leaves. When the results of all treatments were combined, Pioneer Brand 25R25 had significantly higher leaf blotch than all other cultivars. When the results of all cultivars were combined, the non-fungicide treated plots had the highest levels of leaf blotches, though not significantly so in all cases. These results indicate that the triazole fungicide treatments evaluated under the conditions of this study may not provide effective control of leaf blotches even at fairly low levels of disease severity.

Surprisingly, the drought resulted in only slightly lower than average yields across the trial. When results of all the cultivars were combined or analyzed separately, none of the treatments had any significant effect on yield. This supports previous research and recommendations that fungicide applications in the absence of significant disease may not be cost effective. On the other hand, when the results of all the treatments were combined, Pioneer Brand 25R25 yielded significantly higher than all other varieties. Pioneer Brand 25R25 is a new release by Pioneer. In previous similar studies, we have found Pioneer Brand 25R46 to yield consistently higher than all other varieties. Perhaps the higher stripe rust susceptibility of Pioneer Brand 25R46 negatively impacted the yield of this variety, and the lack of stripe or leaf rust on Pioneer Brand 25R25 conveyed a yield advantage. These results indicate that cultivar selection for rust resistance may have a larger impact on yield than fungicide applications in the absence of significant FHB disease pressure.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported in part by the U.S. Department of Agriculture, through NIFA Hatch Project NYC153437, and

under Agreement No. 59-0206-4-006. The latter is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

Table 1. Main effect of treatment on leaf rust, stripe rust, leaf blotch, deoxynivalenol and grain yield at Aurora, NY in 2016.

Treatment	Leaf rust (%)	Stripe rust (%)	Leaf blotch (%)	DON (ppm)	Yield (Bu/A)
Non-sprayed, non-inoculated control	0.1 b	2.9 ab	0.9 ab	0.01	73.3
Inoculated FGS 10.51, and inoculated 7 days later	0.5 a	4.0 a	1.3 a	0.02	71.3
Prosaro SC (6.5 fl oz) and inoculated FGS 10.51, then inoculated 7 days later	0.0 b	0.0 b	0.8 ab	0.01	73.2
Caramba (17 fl oz) and inoculated FGS 10.51, then inoculated 7 days later	0.0 b	0.0 b	0.5 b	0.01	73.9
Prosaro SC (6.5 fl oz) and inoculated FGS 10.51, then Caramba (17fl oz) and inoculated 7 days later	0.0 b	0.0 b	0.8 ab	0.01	71.3
HSD (*P*=0.05)	0.30	2.99	0.66	NS	NS
CV (%)	339.1	290.7	105.9	100.0	9.9

Table 2. Main effect of cultivar on leaf rust, stripe rust, leaf blotch, deoxynivalenol and grain yield at Aurora, NY in 2016.

Cultivar	Leaf rust (%)	Stripe rust (%)	Leaf blotch (%)	DON (ppm)	Yield (bu/A
Erie	0.1 b	0.1 b	0.4 b	0.01 b	72.3 b
Otsego	0.3 a	0.6 b	0.5 b	0.02 a	68.9 b
Pioneer Brand 25R25	0.1 b	0.1 b	1.6 a	0.01 b	77.8 a
Pioneer Brand 25R46	0.0 b	4.7 a	0.9 b	0.01 b	71.3 b
HSD (*P*=0.05)	0.26	2.43	0.48	0.25	3.69
CV (%)	339.1	290.7	105.9	154.9	9.9

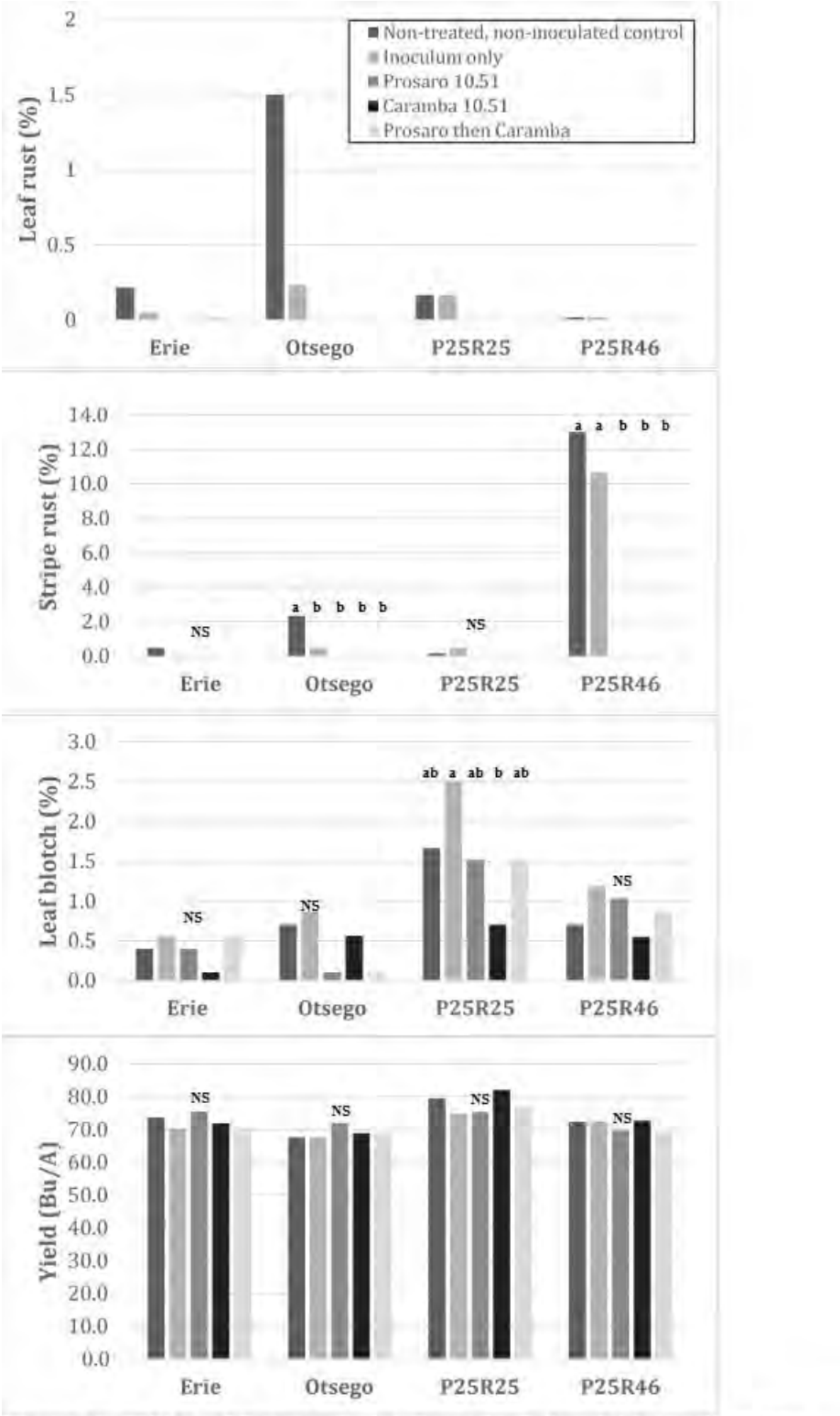

Figure 1. Effect of Prosaro® and Caramba® fungicide applications and *F. graminearum* inoculation on leaf rust, stripe rust, leaf blotches and yield of four winter wheat cultivars in Aurora, NY in 2016.

EVALUATION OF FUNGICIDE APPLICATIONS PLUS CULTIVAR RESISTANCE TO REDUCE FHB AND DON INFECTION OF BARLEY IN NEW ENGLAND

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OBJECTIVE

To evaluate the individual and interactive effects of moderately resistant cultivars and application timings of fungicides on barley yield and the integrated management of Fusarium head blight (FHB) and deoxynivalenol (DON) in Vermont.

INTRODUCTION

Public interest in sourcing local foods has extended into beverages. This had led to a rapid expansion of the northeast malting industry and has given farmers new markets. However these farmers are struggling to produce barley that is not infected with FHB and DON. Hence integrated management strategies are essential for reducing yield and quality losses from FHB. Most of these farmers have experienced significant crop loss from FHB and some farmers have already stopped growing barley. At present few farmers are specifically selecting varieties for resistance to FHB and even fewer are combining host resistance with fungicide applications. The use of a well-timed fungicide is an important management tool when suppressing FHB in barley production. In Vermont during 2016, we observed the disease and yield impact of cultivar susceptibility, inoculation with *Fusarium graminearum*, and treatment with fungicides at two timings.

MATERIALS AND METHODS

The trial was conducted at the Borderview Research Farm in Alburgh, VT in a Benson silt loam soil planted with two spring barley varieties, 'Robust' (susceptible to Fusarium head blight, FHB), 'Conlon' (moderately resistant to FHB) on 19 April 2016. The experiment was set up as a completely randomized block design with a split-plot arrangement, with cultivar as the main plot and the fungicide treatments as subplots, randomized in four replicated blocks. Fungicide treatments are shown in table 1. Main plots were sown with barley at 125 lb/A with a 5 ft wide Great Plains grain drill. Subplots were 5 x 20 ft including 7 rows with 7-in. row spacing. The first fungicide application was at heading (Feekes growth stage, FGS 10.1) on 17 June 2016 including the surfactant Induce at 0.125% V/V. After the fungicide had dried, plots were spray-inoculated with a conidial suspension of *F. graminearum* (40,000 conidia/ml) to augment the development of FHB. The second fungicide application occurred four days after heading on 21 June 2016 including the surfactant Induce at 0.125% V/V, and inoculated with a conidial suspension of *F. graminearum* (40,000 conidia/ml) after the fungicide had dried. Fungicide and *F. graminearum* treatments were applied with a CO_2 backpack sprayer with paired TJ-60 8003vs nozzles mounted at an angle (30° from horizontal) forward and backward, 20-in. apart, pressurized at 30 psi, and calibrated to deliver 20 gal/A. Incidence and severity (percent of symptomatic spikelets on symptomatic heads) of FHB in each plot were rated on 15 July and used to calculate FHB index, where FHB index = (FHB severity * FHB incidence)/100 (data not shown). Grain was harvested from a 5 x 20 ft area in each subplot using an Almaco plot combine on 27 July 2016. Grain moisture, plot yield, and test weight were recorded. Yield and test weight were adjusted to bu/A at 13.5% moisture. Analysis of deoxynivalenol (DON) content in grain was conducted at the University of Vermont Cereal Grain Testing Laboratory located in Burlington,

VT. Treatment means were calculated, subjected to analysis of variance, and separated by Fisher's protected LSD test (P = 0.05).

RESULTS AND DISCUSSION

Weather conditions in Vermont during the 2016 growing season can be characterized as having below average precipitation and above average temperatures. The incidence of *F. graminearum* even with inoculation was less than 1% in all treatments (data not shown).

The impact of supplemental inoculation with *F. graminearum* was determined by comparing the non-inoculated and inoculum only treatment. Overall, inoculation did not significantly impact DON concentrations or yield as compared with the non-inoculated plots (Table 2).

There was no significant cultivar by fungicide treatment interactions for DON or yield. This indicates that under low disease pressure the varieties responded similarly to the fungicide treatments (data not shown).

When results were combined across cultivars, the fungicide treatments did not significantly impact DON concentrations (Table 2). Barley yields did respond differently to the fungicide treatments (Table 2). The Prosaro® SC application at heading had significantly higher yields than all other treatments except the Prosaro SC applied 4 days after heading. A positive yield increase from Prosaro SC application has been shown in previous year's work as well.

Under low disease pressure, there were no significant differences detected in DON concentrations or yield among cultivars (Table 3).

Overall low disease pressure led to lack of treatment differences during the 2016 growing season. This underscores the necessity to conduct these types of experiments over numerous years and environments.

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This material is based upon work supported in part by the U.S. Department of Agriculture under agreement No. 59-0206-9-056. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

Table 1. Fungicide treatments, active ingredietns and rates applied.

Fungicide treatments	Company	Fungicide active ingredient	Application rates
Control			Water
Fusarium graminearum			40,000 spores/ml
Prosaro SC®	Bayer CropScience	Prothioconazole + tebuconazole	6.5 fl oz ac^{-1} + Induce at 0.125% V/V
Caramba®	BASF Ag Products	Metconazole	14 fl oz ac^{-1} + Induce at 0.125% V/V
Champ ION^{++}	NuFarm	Copper hydroxide	1.5 lbs ac^{-1}
Actinovate®	novozymes	Streptomyces lydicus WYEC	6 fl oz ac^{-1}
Sonata®	Bayer CropScience	Bacillus Pumllus strain 108	2 qt ac^{-1}

Table 2. Main effect treatment on deoxynivalenol (DON) contamination and grain yield at Alburgh, VT 2016.

Fungicide treatment	**DON**	**Yield**
	ppm	bu ac^{-1}
Non-sprayed, non-inoculated control	0.19	65.7
Inoculated FGS 10.1	0.28	61.4
Actinovate (6 fl oz) at heading	0.23	67.4
Actinovate (6 fl oz) 4 days after heading	0.29	67.3
Caramba (14 fl oz) at heading	0.20	73.1
Caramba (14 fl oz) 4 days after heading	0.35	68.0
ChampION (1.5 lbs) at heading	0.28	65.0
ChampION (1.5lbs) 4 days after heading	0.24	72.0
Prosaro SC (6.5 fl oz) at heading	0.19	82.5
Prosaro SC (6.5 fl oz) 4 days after heading	0.19	78.0
Sonota (2 qt) at heading	0.35	71.6
Sonota (2 qt) 4 days after heading	0.31	67.8
LSD (P=0.05)	NS	9.41

Table 3. Main effect of cultivar on deoxynivalenol (DON) contamination and grain yield at Alburgh, VT 2016.

Cultivar treatment	DON	Yield
	ppm	lbs ac^{-1}
Conlon	0.24	72.3
Robust	0.28	70.2
LSD (P=0.05)	NS	NS

THE USE OF INTEGRATED MANAGEMENT STRATEGIES TO LOWER FUSARIUM HEAD BLIGHT AND DEOXYNIVALENOL IN SPRING BARLEY

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ABSTRACT

Barley is an important crop in North Dakota and in most years Fusarium head blight (FHB) is the most devastating disease. Spring barley integrated FHB management trials were established at five locations in North Dakota: Fargo, Langdon, Carrington, Williston and Prosper. The trials evaluated the effect of fungicide treatment(s) and varietal resistance on the reduction of deoxynivalenol (DON) and protection of yield and test weight. Trials were designed in a randomized complete block with a split-plot arrangement with four replications at all locations. Barley varieties (at least two per location) differing in susceptibility to FHB served as whole plots. Fungicide treatments were the subplots and included prothioconazole+tebuconazole at heading, prothioconazole+tebuconazole at heading + metconazole 4-7 days later, metconazole at heading + tebuconazole 4-7 days later, prothioconazole at heading + tebuconazole 4 days later. Corn spawn served as the inoculum source at Langdon and Williston, ND. *Fusarium* spores were used at Fargo and *Fusarium* infected residue was used at Carrington and Prosper, ND. Inoculum was applied to all treatments except for the non-treated, non-inoculated check. The level of FHB severity was evaluated around the Feekes 11.2 growth stage (soft to mid dough) and DON, yield and test weight were obtained after harvest. Data were analyzed using Proc GLM and means were separated with LSD(P=0.05). Disease did not develop at Williston and Prosper and were excluded in the statistical analysis. Results indicated that, prothioconazole+tebuconazole at heading + metconazole 4-7 days later, had significantly lower DON levels when compared to the non-treated checks and the application of prothioconazole+tebuconazole at full head. Numerically, fungicides that were applied at two different times throughout the heading process had lower DON levels than a single application at full head. DON levels were lower in the resistant varieties when compared to the susceptible varieties. Both yield and test weight were significantly higher for treatments that included a fungicide application when compared to the non-treated inoculated check. Results from this study will help strengthen FHB fungicide recommendations for spring barley production.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No.59-0206-4-012. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

MONITORING THE SPLASH DISPERSAL OF SPORES OF *FUSARIUM GRAMINEARUM* FROM WHEAT PLANTS USING HIGH SPEED VIDEO

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ABSTRACT

Fusarium graminearum, causal agent of Fusarium Head Blight (FHB), produces macroconidia (asexual spores) and ascospores (sexual spores). Though both spore types may cause disease, they may be dispersed by different mechanisms; macroconidia may be dispersed by rainsplash, and ascospores may be forcibly discharged into the atmosphere from perithecia. Little is known about the dynamics of spore dispersal within and among wheat plants during rain events. We used high speed videos to study the dynamics of rain splash of macroconidia of *F.graminearum* on above-ground surfaces (leaves and spikes) of wheat plants grown under controlled environmental conditions. Different sizes of water droplets containing dye were released from different heights above healthy and infected wheat plants to simulate rainfall. The resulting splash events following impact with different surfaces of wheat plants were monitored with high speed photography, and the splashed droplets were captured on absorptive paper placed at the soil level surface around the treated plants. The splash traces were excised from the absorptive paper and plated on agar medium to confirm spores were associated with the observed dispersal patterns. Resulting knowledge could be used to inform FHB risk models, which may provide additional disease management information to growers of small grains in the U.S.

VARIETY AND FUNGICIDE APPLICATIONS ON FHB AND DON IN DELAWARE: A COLLABORATIVE OUTREACH STUDY

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ABSTRACT

Growers in Delaware and Maryland have been negatively impacted by Fusarium Head Blight (FHB) as recently as 2013. After the 2013 outbreak, it was determined that growers in the region required additional information, experience, and training with the use of moderately resistant (MR) wheat varieties and properly timed, FHB labelled fungicides for suppressing FHB and associated DON. In 2016, an on farm survey was conducted to show growers the value on integrating variety and fungicides for FHB management. A total of 21 fields were assessed 18-21 days after flower for rating visual FHB symptoms. Immediately prior to harvest, wheat heads were randomly collected from all wheat fields and processed for DON. Information on varieties was collected and each variety placed into a resistance category based on misted nursery results from the mid-Atlantic. MR varieties reduced visual symptoms by more than 91% and DON by more than 60% compared to susceptible varieties. Fungicide treatments reduced visual symptoms by 76% and DON by 72%. All MR varieties, even if untreated, had DON levels below 2ppm. DON levels in untreated fields or strips ranged from 2.0 ppm to 8.8 ppm. Growers participating in the studies indicated that the value of this project to them ranged from $30-80 /A and indicated that they will utilize USWBSI supported misted nurseries to help guide wheat variety selection.

DEVELOPING A SMART PHONE APP TO ESTIMATE *FUSARIUM* DAMAGED KERNELS OF WHEAT BASED ON COMPUTER VISION

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ABSTRACT

Fusarium head blight (FHB) is one of the most important diseases of wheat and barley. FHB affects grain yield and quality by reducing grain fill, leading to shriveled and lightweight kernels. Percent *Fusarium*-damaged kernels (FDK) is often estimated as one of the main ways of quantifying the effect of FHB on grain quality. This is commonly done by visually examining the grain and estimating percent damage based on grain color, size, and integrity. This is a time-consuming, inefficient, and often subjective process. In this study, we are developing a smartphone application to mimic the FDK quantification process using computer vision. Percent FDK is estimated by processing captured images and using FDK features such as whitish and reddish-pink discoloration to distinguish between healthy and scabby kernels. Kernels of soft red winter wheat cultivar Hopewell were spread out in a single layer on a black background and clear images were captured using a smartphone camera. Arithmetic algorithms, such as addition and subtraction, were implemented to clear the background of the image, and then edge detection was used to locate and separate each individual kernel from the background. Once the edges were detected, healthy and damaged kernels were counted by the program. To detect and count scabby kernels, histograms of FDK pixels were analyzed and HSV (Hue, Saturation and Value) and RGB (Red, Green and Blue) color spectra were used in combination to distinguish diseased from healthy kernels. The optimum threshold for distinguishing diseased from healthy kernels was determined through maximization of interclass variance. For each sample analyzed using the app, FDK was also visually estimated by counting the number of healthy and diseased kernels. Visual estimates were used as references (true values) against which the accuracy and precision of app estimates were compared based on concordance correlation coefficients. Preliminary results showed a strong concordance between true and app estimates of FDK, with 98.58% precision and 98.28% accuracy. The app successfully identified and distinguished between diseased and healthy kernels of Hopewell, with low false positive and false negative rates. However, when validated on a different set of cultivars, the results varied. It performed better on Cooper and Hopewell than on Malabar and Pioneer 25R47. Research continues to evaluate the app across cultivars and to determine the effects of light and image quality on it performance.

INFLUENCE OF RAINFALL PATTERNS ON DEOXYNIVALENOL ACCUMULATION IN WHEAT AFTER FUSARIUM HEAD BLIGHT SYMPTOM DEVELOPMENT

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ABSTRACT

Although there is often a positive correlation between visual symptoms of Fusarium head blight (FHB) and deoxynivalenol (DON) content in wheat, under certain conditions, DON levels may be higher than one would expect based on visual symptoms. A field experiment was conducted during the 2016 growing season to investigate the effects of intermittent rainfall patterns during the 22-day window between FHB visual symptom development and harvest on DON in wheat grains. The experimental design was a randomized complete block, with a split-plot arrangement of cultivar (moderately resistant and susceptible) as whole-plot, post-FHB symptom development simulated rainfall patterns (six levels) as sub-plot, and inoculum density (5×10^4, 1×10^5, and 2×10^5 spores.mL^{-1}) as sub-sub-plot. The six simulated rainfall treatments were initiated 24 days after visual symptoms developed and consisted of: 1) rainfall every day (Rain_1); 2) rainfall every other day (Rain_2); 3) two days of rainfall following by two days without rainfall (Rain_3); 4) three days of rainfall following by three days without rainfall (Rain_4); 5) four days of rainfall following by five days without rainfall (Rain_5); and 6) check (no supplemental rainfall). The combination of cultivar resistance and inoculation treatment resulted in a range of baseline FHB index levels under which the rainfall treatment effects were evaluated. Mean FHB index was 5.4, 11.4, and 12.8% for the moderately resistant cultivar, and 16.8, 23.2, 28.7% for the susceptible cultivar at low, medium, and high inoculum densities, respectively. As expected, plots with the highest levels of FHB index (high inoculum density) had the highest mean levels of DON, and plots of the moderately resistant cultivar had lower mean index and DON than plots of the susceptible cultivar, averaged across rainfall treatments. Plots that received simulated rain every day had higher mean DON levels than plots subjected to the other rainfall treatments, at all baseline index levels. Rain_3, Rain_4, Rain_5, and Rain_6 consistently resulted in higher mean DON levels than the check (no simulated rainfall) for the moderately resistant cultivar, and Rain_3 and Rain_5 resulted in higher mean DON than Rain_4 and Rain_6. However, Rain_3, Rain_4, Rain_5, and Rain_6 trended to have similar mean DON levels to that of the check for the susceptible at all baseline FHB index levels. Formal analyses will be conducted to quantify the effects of the aforementioned rainfall patterns on 1) FDK, after adjusting for baseline index, as a measure of their effects on grain colonization after visual symptom expression; 2) DON, after adjusting for FDK; and 3) DON-3-Glucoside, after adjusting for DON, as a measure of their effect on DON conjugation.

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EFFECT OF CLEANING AND FUNGICIDE SEED TREATMENT ON STAND ESTABLISHMENT IN SCABBY SEED LOTS IN THE SOUTHERN U.S.

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ABSTRACT

Six scabby wheat seed lots from the 2014-2015 season were either cleaned or not, and were treated with Raxil (0.1 oz/cwt), Stamina (0.8 oz/cwt), Vibrance (0.16 oz/cwt), or left non-treated. Seed were planted in five locations in Louisiana, Mississippi, and Georgia in randomized complete block designs with a total of four replicates per location. Plots were evaluated for stand, tillering, scab severity, and yield, and all four of these parameters varied with cultivar and location. Cleaning seed did not have a significant effect on any of these four parameters. The seed treatment, Stamina, significantly improved stand in two Louisiana locations, and no seed treatments improved stand in the Mississippi location. Fungicides did not have a significant effect on tillering, scab severity, or yield at any measured location. Based on these results, cleaning scabby seed lots may not be economically justifiable. Because of seed unavailability, this study was not repeatable, and more research is needed to confirm these results.

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This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-9-064. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

DEVELOPMENT OF FUSARIUM HEAD BLIGHT IN HARD RED WINTER WHEAT DURING THE 2016 SEASON IN SOUTH DAKOTA

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ABSTRACT

Fusarium head blight (FHB) caused by *Fusarium graminearum* is a major disease of wheat in South Dakota. Infected plants produce chalky shriveled kernels. In addition, F. *graminearum* produces the mycotoxin, deoxynevalenol (DON) that is harmful to livestock and humans. Infection of wheat by *F. graminearum* is promoted by rainfall and warm weather when wheat is at flowering stage. During the 2016 growing season, FHB development was monitored in three hard red winter wheat cultivars with varying resistance to FHB at two locations: South Dakota State University (SDSU) Volga Research Farm and SDSU Northeast Research Farm, South Shore, near Watertown. The cultivars were either treated with Fungicide or not treated. For treated plots, Prosaro® (6.5 fl oz/ac) was applied at flowering using a backpack CO_2 sprayer. A randomized complete block design with a split plot arrangement was used with cultivar as the main plot and fungicide or no fungicide as the subplot. Plots were assessed for FHB 21 days after fungicide application. At harvest, grain yield, thousand kernel weight, *Fusarium* damaged kernels and test weight were determined for each plot. At the Volga location, FHB index was very low (5%). The South Shore location had negligible FHB levels. No significant FHB index differences were observed between fungicide treated and non-treated at both locations and this is attributed to very low FHB pressure. Similarly, no significant grain yield increase was observed between fungicide treated and non-treated. However, grain yield was significantly different between cultivars at the South Shore location with Overland out yielding Wesley and Expedition. Low FHB pressure is attributed to very low rainfall and cooler temperatures when winter wheat was at flowering at both locations. The South Shore location had only 0.24″of rainfall throughout June and the average temperature was 70° F at this location. These results demonstrate the role of weather in the FHB development. There was no benefit of applying a fungicide for managing FHB in winter wheat at the two locations and generally in most of South Dakota for the 2016 winter wheat growing season. Use of resistant cultivars, monitoring conducive weather for FHB, and applying a triazole fungicide at flowering when moderate to high FHB risk is predicted, remain the most effective integrated management of FHB.

ACKNOWLEDGEMENT AND DISCLAIMER

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FOOD SAFETY AND TOXICOLOGY

DEVELOPMENT OF DEOXYNIVALENOL (DON) AND DON-3-GLUCOSIDE DURING MALTING OF *FUSARIUM* INFECTED HARD RED SPRING WHEAT

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ABSTRACT

The behavior Fusarium Head Blight (FHB) and fate of Deoxynivalenol (DON) during the malting and brewing of barley has been extensively investigated over the past 20 years. However, there is little to no information on wheat malt. This is of interest as the use of wheat by the brewing industry is growing as the result of the craft brewing segment, and also several successful wheat beers that are produced by the larger brewing companies. In addition, the growing craft malting industry makes extensive use of locally produced wheat, often from areas that are prone to FHB. The objective of this study was to assess the growth of *Fusarium* and the development of DON and DON-3-glucoside (DON-3-G) during the malting process. Twenty hard red spring (HRS) wheat samples from the 2015 crop in North Dakota were selected to provide a range in DON content (0.02-17.92 µg/g). The samples were micro-malted in duplicate following 2 and 6 months of storage at room temperature. DON was determined by GC-MS, DON-3-G by LC-MS and *Fusarium* DNA by real-time PCR. When malted shortly after harvest (2 months) levels of DON were observed to increase in all the samples (15) which had DON above the limit of quantitation (LOQ). The average increase was 560% over levels seen in the unmalted grain, but results varied from 113 to 1820 %. The increase in DON levels can be attributed to growth of *Fusarium* during malting, and DNA levels were observed to rise from an average of 1.4 pg/g on the wheat to 4.0 pg/g on malt. However, it also appears that a large portion of the DON was converted by the germinating wheat to DON-3-G. Consistent with other reports in the literature, levels of DON-3-G in the sound wheat were quite low, and were above the LOQ in less than half the samples. The ratio of DON-3-G/DON was approximately 20 mol% in wheat, but increased to 60 mol% in the malt. The samples were malted a second time following 6 months of storage. As expected there were no significant differences in the levels of DON for the most of ungerminated wheat samples when compared to the previous tests. However, increases were observed for 5 of higher DON samples. The amount of DON detected on malt after six months of storage was lower than that found on the unmalted wheat for approximately half the samples. This suggests some decrease in viable *Fusarium* following storage. Levels of DNA measured on the malt (average 1.76 pg/g) at 6 months were in fact generally lower than those detected with the previous malting (2 months). However, levels of DON-3-G produced during the second malting were not greatly different than those from the first, illustrating that storage likely does not influence the capacity of wheat grain to convert DON during malting.

ACKNOWLEDGEMENT AND DISCLAIMER

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GENE DISCOVERY AND ENGINEERING RESISTANCE

DEVELOPMENT OF A FUSARIUM HEAD BLIGHT (FHB) RESISTANT WHEAT VIA THE OVER-ACTIVATIONS OF TWO WHEAT NATIVE TRANSGENES

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OBJECTIVES

1. Search the GeneBank, and assemble constructs.
2. Transform wheat genome via biolistic gene-gun technology
3. Perform molecular analysis to confirm the transgenes integration and their real-time transcriptions.
4. Grow plants to maturity, and inoculate spikes using Ph1 isolates.
5. Measure and analyze disease spread severity.
6. Collect and analyze data.

INTRODUCTION

The wheat (*Triticum aestivum* L.) Fusarium head blight (FHB), mostly caused by *Fusarium graminearum* has resulted in $3 billion loss in North America. This pathogen not only reduces the crop yield, but also contains, deoxynivalenol (DON), a mycotoxin that is harmful to the human and animal health (Pierson et al., 2015).

Several reports confirm the resistance of Chinese wheat lines; Suami 3 and Wangshuibai to FHB. However, strategies to transfer the FHB resistance genes into commercial wheat genotypes via conventional breeding have not been successful (Buerstmayr et al., 2009) mostly because breeding of these two genotypes with commercial wheat lines are very difficult due to the complexity of the resistance to FHB pathogen trait (Xiao et al., 2013).

Although the *Fhb1* gene was identified for 17 years (Waldron et al., 1999), its map-based cloning very recently revealed the origin of the *Fhb1* gene in wheat genome (Rewat et al., 2016).

Xiao et al. (2013) inoculated FHB-resistant Wangshuibai and another FHB-susceptible wheat landrace, and performed a series of transcriptome-based discoveries of pathways and genes associated with the FHB resistance using high-throughput RNA sequencing. They reported that a few genes including the PR5, PR14, the ABC transporter and JA signaling pathway mediated by *Fhb1* were important in FHB resistance. They also confirmed the involvement of the coronatine insensitive 1-like (*coi1*) receptor in response to infection by the FHB pathogen, and reported that a chromosome deletion in the susceptible wheat landrace might play a role in FHB susceptibility in wheat.

Here, the authors studied the overexpression of genes encoding the wheat native protein *coi1* and *tlp1* in wheat genome for resistance to FHB.

MATERIALS AND METHODS

Construct assembly: The gene constructs (pCoi1-JS101 and pTLP-JS101; Figure 1) were developed. The *pHAtlp1* constitutes the wheat native *coi1* coding sequences regulated by the rice actin1 *(Act1)* promoter and the potato protease II terminator (*Pin3'*). The pTLP-JS101 constitutes the wheat native *tlp1* regulated by the *Act1* promoter and *Pin3'*. Both, pCoi1-JS101 and pTLP-JS101 constructs harbor the *bar* herbicide resistance gene cassette.

Plant Material and Genetic Transformation: Wheat cv. Bobwhite seeds were germinated in the greenhouse, plants were grown to maturity, and their immature embryos were isolated. Then, the sterilized immature embryos were bombarded with a 1:1 ratio of a combination of the *pCoi1* and *pTLP1* (Figure 1). The bombarded immature embryos were cultured in-vitro following Zhang et al. (2000). Putative transformants were transferred to a growth chamber and tested for herbicide resistance using a leaf painting assay with a 0.1 % aqueous Liberty™ solution containing 18.9 % glufosinate ammonium (Nguyen et al., 2013), and herbicide resistant plantlets were regenerated in a greenhouse to maturity.

Molecular Confirmation: Genomic DNA was extracted from herbicide resistant plantlets, as well as from their wild-type non-transgenic plants using the CTAB method (Xin and Chin, 2012), and polymerase chain reaction (PCR) was performed following the authors previous report (Nguyen et al., 2013) to confirm the integration of each of the two transgenes in wheat genome using specific primers.

qPCR**:** Total RNA was extracted from transgenic as well as the wild-type non-transgenic plants using Omega E.Z.N.A.® Plant RNA Kit, and cDNA strands were synthesized using GoScript™ Reverse Transcriptase (Promega catalogue no. A5003). Then the real-time (q) PCR was performed using Applied biosystems Fast SYBR® Green Master Mix.

Conidia Preparation Spike Inoculation and Data Collection: The *Fusarium graminearum* Ph-1 isolates were grown on Nash-Snyder media, and conidia were collected following the standard method, quantified using a hemocytometer, and diluted to a final concentration of 1.0x105 conidia/mL. Single central floret of each spike was inoculated by injecting 10µL of the *F. graminearum* suspension of ~ 1000 conidia into each floret, and the inoculated florets were marked for latter survivability data collections. Finally, disease rating was conducted at 7, 14 and 21 days after inoculation. Disease progression was recorded for each inoculated spike by counting the healthy spikelets in both directions (above and below) from the point of inoculation. The area under the disease progress curve (AUDPC) was calculated using Shaner and Finney's (1977) equation to describe the increase in plant susceptibility during the experiment.

RESULTS AND DISCUSSIONS

Herbicide resistant lines were identified, and PCR positive herbicide resistant plants showing the highest level of the two transgenes transcriptions were identified based on the qPCR data for both *Coi1* and *trlp1* genes in T1 plants.

The spikes of inoculated plants that showed a combination of highest transcripts of both *tlp1 and Coi1* showed an impressive level of resistance against the pathogen (Figure2), with disease severity ranging only from 1 to 15%, while the wild-type control plant spikes suffered at 95%. The AUDPC (Figure 3) showed a significant difference between the wild-type versus the transgenic lines (ANOVA $P<0.01$). Statistical analysis results show that the infection of wild-type non-transgenic line ($P=0.00$-0.018) was significantly higher than all six transgenic lines.

ACKNOWLEDGEMENTS

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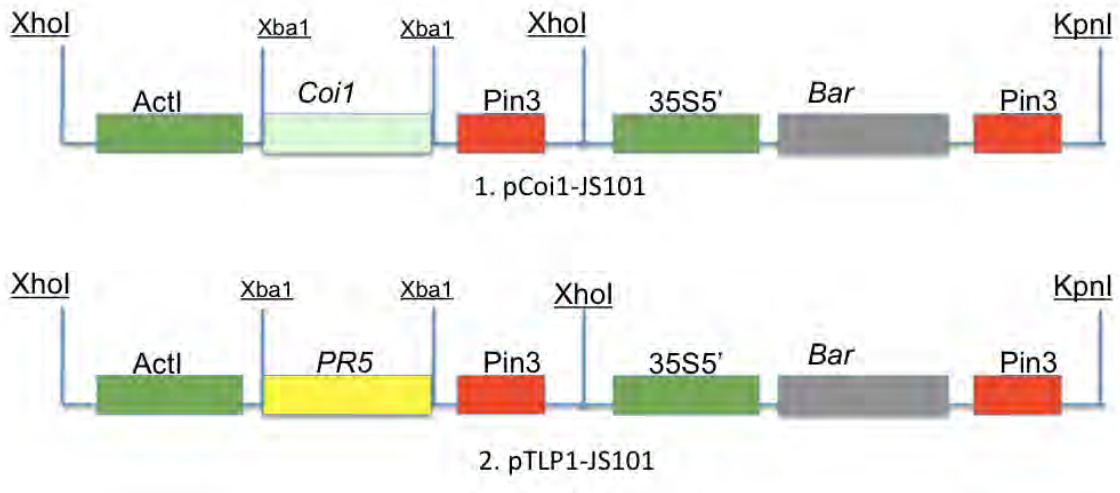

Figure 1. The pCoi1-JS101 constructs and pTLP1-JS101

Figure 2. Greenhouse level symptoms of *Fusarium graminearum* cell-free mycotoxin single spot microinjection of the wild-type non-transgenic spike (left) versus the first generation (T0) tlp1-coi1 real-time overexpressed spike (right) 21 days after inoculation. Note the site of inoculation (SOI) as single black spot on each spike.

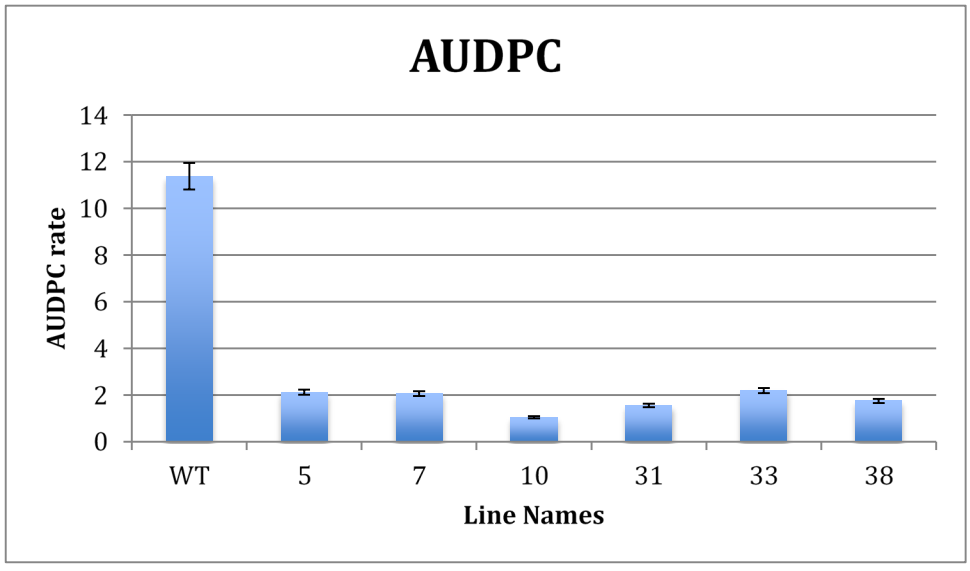

Figure 3. The area under disease progress curve (AUDPC) rates for the wild-type non-transgenic versus each of the six T1 (second generation) tlp1-Coi1 genetic lines.

INSIGHT INTO THE MECHANISM OF THE *TRI6* RNA ENTERFERENCE ABLATING DEOXYNIVALENOL PRODUCTION IN *FUSARIUM GRAMINEARUM* WITH PATTERNS OF SIRNA PRODUCTION

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ABSTRACT

Deoxynivalenol (DON) contamination of small grains caused by Fusarium head blight is a problem of economic and health importance that may be addressed by RNAi interference (RNAi)-based host-induced gene silencing (HIGS). RNAi in higher eukaryotes, including fungi, involves processing double stranded RNA (dsRNA) into small interfering RNA (siRNA) that silence genes based on homology. Six initial randomly inserted transgenic *F. graminearum* (strain PH1) mutants containing the RNAi vector pTRM-TRI6 were studied, containing a full length inverted repeat of *TRI6,* a transcription factor that positively regulates DON production. The mutant lines showed typical phenotypes of DON reduction, including reduced virulence on wheat, and reduced DON in barley infection and in non-host toxin inducing media. The sRNA populations of three mutant lines, but not PH1, had abundant siRNA species that mapped to *TRI6*, with 22 nt siRNA identified as the most abundant. The discontinuous and repeatable siRNA mapping, consistent peaks, and overrepresentation of siRNA with starting 5' uracil base demonstrated clear preferences for fungal dicer to produce specific siRNAs. Subsequent experiments, in which transformation with five shorter (200-250 nt) inverted repeats with homology to *TRI6* (each targeting a different region of *TRI6*), showed patterns of siRNA processing for each individual inverted repeat that were similar to that resulting from the processing of the corresponding section of the inverted repeat of full length *TRI6*. Dicer patterns for dsRNA processing have implications for design of efficient RNAi silencing vectors. Understanding the siRNA profiles that result from RNAi constructs is critical to optimizing RNAi applications, such as HIGS, that are designed to reduce pathogenicity and mycotoxin production in the field.

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HOST-INDUCED SILENCING OF *FUSARIUM CULMORUM* GENES PROTECTS WHEAT FROM INFECTION

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ABSTRACT

Plants producing antisense or double-stranded RNA molecules that target specific genes of eukaryotic pests or pathogens can become protected from their attack. This beneficial effect was also reported for plant-fungus interactions and is believed to reflect uptake of the RNAs by the fungus via a yet unknown mechanism, followed by target-gene silencing. Here we report that wheat plants pre-infected with barley stripe mosaic virus (BSMV) strains containing antisense sequences against target genes of the Fusarium head blight (FHB) fungus *F. culmorum* caused a reduction of corresponding transcript levels in the pathogen and reduced disease symptoms. Stable transgenic wheat plants carrying an RNAi hairpin construct against the β-1, 3-glucan synthase gene *FcGls1* of *F. culmorum* or a triple combination of Fc*Gls1* with two additional, pre-tested target genes also showed enhanced FHB resistance in leaf- and spike inoculation assays under greenhouse- and near-field conditions, respectively. Microscopic evaluation of *F. culmorum* development in plants transiently or stably expressing Fc*Gls1*-silencing constructs revealed aberrant, swollen fungal hyphae indicating severe hyphal cell wall defects. The results propose HIGS as a plant protection approach that may also be applicable to highly FHB-susceptible wheat genotypes. To better understand whether HIGS is a natural phenomenon, small RNAs from *F.graminearum* infected barley have been sequenced and functional analysis of potential HIGS targets in *F. culmorum* is in progress.

FHB RESISTANCE GENES - GENES WITH MULTIPLE BENEFITS?

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ABSTRACT

Fusarium head blight (FHB) is an economically important disease on both barley and wheat. One of the main foci of our team is to identify cereal genes that are of benefit to breeders in terms of marker-assisted selection for FHB resistance, and at the same time, we elucidate the signaling mechanisms involved in the host-pathogen interactions. This works compliments our research that aims to identify fungal endophytes that show potential for the enhancement of crop establishment and the inhibition of diseases. Having identified candidate genes we use a combination of gene silencing and gene overexpression studies to validate their role in resistance and use protein-protein interaction and other biochemical studies to determine their mode of action. Using this pipeline we have validated the role of specific genes in FHB resistance. For example, we used a functional genomics approach to identify genes up-regulated by the *Fusarium* mycotoxin deoxynivalenol (DON) in a population segregating for toxin resistance, this trait being a component of FHB resistance. This study delineated genes potentially involved in toxin resistance and further studies on an ABC transporter (*TaABCC3.1*), a novel orphan gene (*TaFROG*) and a cytochrome P450 (*TaCYP450*) validated their role as quantitative resistance genes. At the cellular level, the TaFROG protein interacts with and enhances the activity of the central stress regulator SnRK1. It also interacts with other cellular machinery including a novel transcription factor. Ongoing studies are determining the allelic diversity of candidate FHB resistance genes and their promoters. One emerging trend is that select FHB resistance genes affect wheat yield. In small-scale glasshouse trials we observed that either gene silencing and/or overexpression of select genes affected yield and future work will determine the effect of these genes on yield in larger scale yield trials.

TRANSGENIC WHEAT LINES UPREGULATED FOR GENES IN LIGNIN BIOSYNTHESIS AS POTENTIAL RESISTANCE SOURCES AGAINST FUSARIUM HEAD BLIGHT

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ABSTRACT

The goal of this research is to identify Fusarium head blight (FHB) resistance in transgenic wheat lines expressing genes involved in the monolignol biosynthetic pathway. Monolignols are the subunits of the lignin polymer, which are secreted into cell walls to provide structural support, and this pathway also is induced upon pathogen attack. It had been previously shown that increased resistance to *Fusarium* grain pathogens and elevated phenolic metabolites could result in sorghum lines with alterations in monolignol biosynthesis. For the present research, four sorghum genes, one a MYB transcription factor (*SbMyb60*) that acts as a positive regulator, and three for genes encoding enzymes in the sorghum monolignol pathway, caffeoyl_CoA 3-*O*-methyltransferase (*SbCCoAOMT*), 4-coumarate-coenzyme A ligase, (*Sb4CL*), and *p*-coumarate 3-hydroxylase (*SbC3H*), were cloned into expression constructs and individually transformed into wheat (spring CB037), using *Agrobacterium tumefacians*-mediated transformation. Previous research with sorghum overexpressing *SbMyb60* demonstrated that this transcription factor is associated with induction of lignin biosynthesis. Immunobolt analysis of protein extracts from transformed wheat lines showed that expression of *SbMyb60* resulted in increases of the endogenous phenylalanine ammonia lyase (PAL) and 4CL protein levels. Similar analysis of transformed wheat lines expressing *SbCCoAOMT* and *Sb4CL* resulted in detectable levels of the corresponding enzymes. *SbC3H* encodes for a membrane-associated cytochrome P-450, and is difficult to detect using immunoblot analysis; therefore, reverse transcriptase quantitative PCR was conducted to measure transcript levels of this gene in transformed wheat lines. Two elite events were identified for each of the four expression vectors. The eight transgenic lines, along with untransformed CB037 and resistant and susceptible checks, are being screened for Type I (to initial infection) and Type II (to spread after infection) resistance to FHB and accumulation of trichothecene mycotoxins in the grain. Metabolite analysis also will be conducted on inoculated and control plants to determine whether altered levels of one or more phenolic metabolites can be associated with increased resistance or tolerance to FHB. In this way, genes in the monolignol biosynthesis pathway or specific secondary metabolites may be identified as potential markers for breeding for FHB resistance in wheat.

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EXPLORING FUSARIUM HEAD BLIGHT DISEASE CONTROL BY RNA INTERFERENCE

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ABSTRACT

RNA interference (RNAi) technology provides a novel tool to study gene function and plant protection strategies. *Fusarium graminearum* is the causal agent of Fusarium head blight (FHB), which reduces crop yield and quality by producing trichothecene mycotoxins including 3-acetyl deoxynivalenol (3-ADON) and 15-acetyl deoxynivalenol (15-ADON). In this study, we designed and synthesized dsRNA targeting the transcription factor *tri6*, which is a key regulator of DON biosynthesis. Wheat heads were excised, point-inoculated with *F. graminearum*, and treated with a solution of *tri6*-dsRNA or a water control. FHB spread was scored after 8 days, and wheat heads were then collected to evaluate gene expression and DON production. Our results showed that *tri6*-dsRNA reduced disease spread and DON production in infected wheat heads in comparison to water treated controls. Furthermore, the expression of tri6 was significantly reduced in *tri6*-dsRNA treated wheat heads. Our study suggests that dsRNA application is a promising strategy for plant disease control. Further investigation will be focused on identifying the most effective dsRNA target and optimizing efficient delivery methods.

CHARACTERIZATION OF SMALL RNAS FROM *FUSARIUM*-INOCULATED BARLEY SPIKE TISSUES

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ABSTRACT

Fusarium species cause Fusarium head blight (FHB) disease in wheat and barley around the world. Transcriptomic analyses of barley-*Fusarium* interaction have revealed complex molecular mechanisms associated with disease resistance. Previously we analyzed the gene expression profiles of a near-isogenic line carrying a 2Hb8 QTL (2Hb8 R NIL) for FHB and its recurrent parent M69 using RNA-Seq. In this study, deep sequencing of small RNAs from infected and non-infected spike tissues of the same lines was performed. Small RNAs, including microRNAs (miRNAs), have regulatory functions in diverse biological processes such as development and response to environment. Twenty-four sRNA libraries were sequenced with the illumina platform and the average read counts were 12M per library. Mature miRNA reads were mapped to 36 barley miRNA families and 38 homologous miRNA families. Novel miRNAs were predicted using the miRDeep2 package. Overall, the total read count of miRNAs tended to decrease after infection in the susceptible genotype while the read count remained stable in the R NIL. At 96 hour after inoculation, the R NIL exhibited higher expression of miRNAs when compared to the susceptible recurrent parent. Targets of conserved barley miRNA families will be predicted using psTarget and the results will be presented.

A BARLEY UDP-GLUCOSYLTRANSFERASE PROVIDES RESISTANCE TO NIVALENOL AND NIVALENOL-PRODUCING *FUSARIUM GRAMINEARUM*

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ABSTRACT

Fusarium head blight (FHB) is a cereal disease that causes severe yield losses and mycotoxin contamination of small grains. The main causal pathogen, *Fusarium graminearum*, produces trichothecenes mycotoxins, such as deoxynivalenol (DON) or nivalenol (NIV). Nivalenol-producing *Fusarium* strains have been identified in North America and although not currently a major issue, could pose a potential future problem. A barley UDP-glucosyltransferase, *HvUGT13248*, was previously identified that efficiently detoxifies DON to the less toxic DON-3-*O*-β-D-glucoside and provides a high level of FHB resistance in transgenic wheat. Here we report that HvUGT13248 also converts nivalenol into nivalenol-3-*O*-β-D-glucoside, a much less toxic derivative. Interestingly, HvUGT13248 exhibits higher affinity and enzymatic activity for NIV than DON. Overexpression of *HvUGT13248* leads to increased nivalenol resistance in yeast and *Arabidopsis thaliana*. Also, transgenic wheat overexpressing *HvUGT13248* exhibit enhanced ability to detoxify NIV and high levels of type II resistance to a nivalenol-producing *Fusarium graminearum*. Taken together, our results demonstrate that *HvUGT13248* exhibits resistance to both DON and NIV.

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CHARACTERIZATION OF A GENUS SPECIFIC UNIDENTIFIED OPEN READING FRAME FOUND WITHIN THE MITOCHONDRIAL GENOME OF *FUSARIUM*

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ABSTRACT

Sequencing and annotation of the mitochondrial DNAs (mtDNAs) in species of the filamentous fungal genus *Fusarium* identified a highly variable region (HVR) located between the *rnl* and *nad2* genes. Prior characterization of this region identified the presence of a large unidentified open reading frame (LV-uORF) found in at least one strain of all species characterized to date. The predicted polypeptides of these LV-uORFs are variable in size, but in most cases are highly conserved within a species complex. Analysis of the HVR of 32 isolates from four species within the *Fusarium graminearum* Species Complex (FGSC) detected a highly conserved putative polypeptide of 1931 amino acids (ORF1931). The LV-uORF is actively transcribed, but the putative polypeptide (1931p) has yet to be detected. Current research aims to identify and localize 1931p within *F. graminearum* PH-1 mitochondria via immunoblotting and subcellular fractionation. Interestingly, isolates from the *Fusarium oxysporum* Species Complex (FOSC) containing the LV-uORF exhibit greater variability, ranging from 2200 to 2500 amino acids, with other isolates lacking the ORF entirely. As such, the FOSC is an ideal lineage for both phylogenetic and functional analysis to determine the function of this putative gene product. Here we examine phylogenetic evidence of the allelic diversity found within the LV-uORF of a single subspecies of the FOSC.

ANTIFUNGAL PLANT DEFENSINS: MECHANISMS OF ACTION AND ENGINEERING DISEASE RESISTANT CROPS

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ABSTRACT

Host defense peptides have evolved in plants to protect from the damaging effects of fungal pathogens. Defensins are sequence divergent cysteine-rich antifungal peptides of innate immunity expressed in all plants. They exhibit potent antifungal activity *in vitro* and therefore have potential for use in transgenic crops for fungal disease resistance. MtDef4 and MtDef5 are two sequence-divergent apoplast-localized defensins expressed in *Medicago truncatula*. MtDef4 is a monomeric defensin of 47 amino acids, whereas MtDef5 is a novel dimeric defensin containing two monomeric defensin peptides A & B joined by a 7-amino acid linker. Like all previously characterized monomeric plant defensins, MtDef4 inhibits the growth of filamentous fungi including *Fusarium graminearum* at micromolar concentrations. In contrast, dimeric MtDef5 inhibits the growth of these fungi at nanomolar concentrations. MtDef4 and MtDef5 rapidly permeabilize the plasma membrane of *F. graminearum* and translocate into the cytoplasm of this fungus. These defensins differ from each other in sequence, net charge and hydrophobicity. The mode-of-action studies have revealed that they exhibit different modes of antifungal action.

Transgenic wheat lines expressing apoplast-targeted MtDef4 exhibit strong resistance to an obligate biotroph *Puccinia triticina*, causal agent of an economically important leaf rust disease. Histopathological analysis suggested the presence of both pre- and posthaustorial resistance to leaf rust in these transgenic lines. MtDef4 did not, however, affect the root colonization of a beneficial arbuscular mycorrhizal fungus *Rhizophagus irregularis*. This study demonstrates that the expression of apoplast-targeted plant defensin MtDef4.2 can provide substantial resistance to an economically important leaf rust disease in transgenic wheat without negatively impacting its symbiotic relationship with the beneficial mycorrhizal fungus.

EXPRESSION OF BEAN *PGIP2* UNDER CONTROL OF THE BARLEY *LEM1* PROMOTER LIMITS *FUSARIUM GRAMINEARUM* INFECTION IN WHEAT

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ABSTRACT

Fusarium Head Blight (FHB) caused by *Fusarium graminearum* is one of the most destructive fungal diseases of wheat worldwide. The pathogen infects the spike at flowering time and causes severe yield losses, deterioration of grain quality, and accumulation of mycotoxins. Better understanding of the means of pathogen entry and colonization of floral tissue is crucial to providing effective protection against FHB. Polygalacturonase inhibiting proteins (PGIPs) are cell wall proteins that inhibit the activity of polygalacturonases (PGs), a class of pectin-depolymerizing enzymes secreted by microbial pathogens, including *Fusaria*. The constitutive expression of a bean PGIP (PvPGIP2) under control of the maize *Ubi1* promoter limits FHB symptoms and reduces mycotoxin accumulation in wheat grain [Janni et al. 2008 Molec. Plant Microb. Interact. 21:171]. To better understand which spike tissues play major roles in limiting *F. graminearum* infection, we explored the use of PvPGIP2 to defend specific spike tissues by expressing it under control of the barley *Lem1* promoter [Somleva and Blechl 2005 Cer. Res. Comm. 33:665]. We show here that the expression of PvPGIP2 in lemma, palea, rachis and anthers reduced FHB symptoms caused by *F. graminearum* compared to symptoms in infected nontransgenic plants. However, the expression of PvPGIP2 only in the endosperm under control of a HMW-glutenin gene promoter did not affect FHB symptom development, indicating that once the pathogen has reached the endosperm, inhibition of the pathogen's PG activity is not effective in preventing its further spread.

PATHOGEN BIOLOGY AND GENETICS

ELUCIDATING THE ROLE OF SILENCING RNA *FGSIR34* IN FUSARIUM HEAD BLIGHT PATHOGENESIS IN WHEAT

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ABSTRACT

Fusarium head bligt (FHB) is a devastating disease of small grains. Mycotoxin deoxynivalenol (DON) produced by *Fusarium graminearum* is believed to be an important virulence factor. Our research has led to the hypothesis that silencing RNA *fgsiR34* of *F. graminearum* may play a key role in regulating DON production. In our experiments to test this hypothesis, *Dicer-like 2* (*Dcl2*) gene, which control the biogenesis of *fgsiR34*, was knocked down, and *fgsiR34* was overexpressed. The mutants have been studied for their impacts on expression of the genes that control DON biosynthesis. The FHB pathogenicity of the mutants has also been studied. Here we report the preliminary results.

OVER-EXPRESSION OF TRANSLATION ELONGATION FACTOR 1-ALPHA MODIFIES PATHOGENIC AND PHENOTYPIC TRAITS OF A *FUSARIUM GRAMINEARUM* STRAIN

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ABSTRACT

Fusarium graminearum is the predominant causative agent in Fusarium head blight (FHB) of wheat and related cereals. We have generated a *F. graminearum* mutant (FgEF1a-OX) overexpressing a gene encoding for elongation factor 1-alpha (FgEF1a). Eukaryotic EF1a plays a vital role in protein synthesis, but has also been shown to involve in various other cellular activities including cytoskeletal organization, cell cycle and signalling. Compared to wild-type, the FgEF1a-OX mutant reduced the disease symptoms in susceptible wheat cultivar, Roblin by 77 and 61 % in spray and point inoculations, respectively. A reduction in visual symptoms was also observed in highly resistant cultivars, CM82036 and Tenacious. This apparent reduction in pathogenicity seems to be related to a loss of fitness in the FgEF1a-OX strain, which was observed through mycelial growth and spore germination assays. The germination of wild-type macroconidal spores was 60% at 6 h and increased to nearly 100% by 9 h incubation. In contrast, only 15 and 53 % of the FgEF1a-OX macroconidia were germinated at similar time points of incubation. The alteration in physiological levels of EF1a might have negatively impacted one or more aspects associated with cell biology/biochemistry. A report on EF1a overexpression in yeast leading to a similar loss in fitness due to interactions with actin suggests an altered cytoskeletal function in *F. graminearum*. Additional characterization of FgEF1a-OX is underway to identify changes in cell cycle and morphology to get in-sight into fitness loss associated with this strain.

LINKING HOST COMMUNITY TO *FUSARIUM GRAMINEARUM* DISTRIBUTION

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ABSTRACT

The host range of *Fusarium graminearum* includes many non-cultivated grass species. Communities of these hosts may serve as sources of inoculum and as reservoirs of pathogen genetic diversity. In New York, there is a greater acreage of naturalized grassland than of wheat, barley, and corn combined. With funding from the United States Wheat and Barley Scab Initiative, the relationship between *F. graminearum* and host community was investigated at a cereal-natural grassland interface. In 2015, preliminary samples of wheat heads and wild grass inflorescences were taken from three wheat fields. In 2016, naturally occurring wild grass debris, wheat spikes, and wild grass inflorescences were collected from five winter wheat fields and one national wildlife refuge. In 2015, 112 *F. graminearum* isolates were captured from inflorescences of winter wheat, smooth brome grass (*Bromus inermis*), and timothy grass (*Phleum pratense*). Field conditions in 2016 were not conducive to scab development in wheat or to the infection of wild grasses. However, debris gathered from 181 individual sampling sites across the six locations yielded 370 *F. graminearum* isolates. Incidence and distribution were analyzed with reference to grass community composition, and models were produced for pathogen presence and abundance at individual sampling points. The recovery rate of *F. graminearum* from plant debris sampled at the grassland was equal to or greater than that of material sampled at wheat field margins. The grassland had a higher host richness and density than the wheat field margins, and these factors had significant effects in logistic regressions of both *F. graminearum* presence and the number of isolates found at individual sampling sites. Spatial autocorrelation was not detected for *F. graminearum* presence or abundance. These results indicate the potential for non-cultivated grass species, both on and off farms, to support sizeable *F. graminearum* populations. Ongoing work is examining the pathogen population structure in these locations and the extent to which specific host assemblages or other ecological factors impact *F. graminearum* populations.

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MAPPING OF FUSARIUM HEAD BLIGHT RESISTANCE AND DEOXYNIVALENOL ACCUMULATION IN KANSAS WHEAT

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ABSTRACT

Fusarium head blight (FHB) is a wheat disease caused by *Fusarium graminearum* that significantly reduces grain yield and produces mycotoxins that contaminate wheat grains and flour. Deoxynivalenol (DON) is the most prevalent mycotoxin and its advisory limit in U.S. is 1 ppm for wheat products. Thus, the objective of this study was to map quantitative trait loci (QTL) associated with FHB resistance (type II, III, and IV), and estimate the effect of stacking multiple QTL within breeding lines. A doubled haploid (DH) population with 202 lines was developed from a cross between Everest and WB-Cedar, which are moderately resistant and moderately susceptible to FHB, respectively. The experiment was conducted in the field at Rocky Ford FHB during 2014/2015 and 2015/2016 growing seasons in a randomized complete block design with 3 replications. Evaluations of percentage of symptomatic spikelets (PSS) started 21 days after heading and repeated every 3 days for a total of 5 evaluations. After harvest, a sample of 100 grains from each plot was collected to measure DON accumulation and *Fusarium* damaged kernel (FDK) using a single kernel near-infrared spectroscopy instrument. DH lines and parents were genotyped using genotyping-by-sequencing (GBS). A pipeline on TASSEL 4 was used to call and filter SNP markers. The final linkage map consisted of 3,005 SNP and 165 DH-lines. Phenotypic traits were analyzed in SAS with PROC GLM. Composite interval mapping and multiple QTL mapping were performed in Rstudio using Haley–Knott regression. Three QTL for type II resistance were found on chromosomes 3BS, 6AL and 6BL explaining 30.5% of PSS. Another three QTL located on 1B, 5AL, and 5DS from Everest together explained 29.2% of DON accumulation. The QTL on 3BL and 5DL were significant in 2015 and 2016 growing seasons. FDK and DON data from the second year of experiment are currently being analyzed. DH-lines containing all QTL for each trait were significantly more resistant than DH-lines with none or only one QTL. Everest is an elite source of FHB resistance with multiple QTL. GBS sequences flanking significant QTL for both years of experimentation will be later converted into diagnostic markers to assist breeding for FHB resistance.

IDENTIFICATION AND CHARACTERIZATION OF *FUSARIUM GRAMINEARUM* PATHOGENESIS GENES

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ABSTRACT

Fungal pathogens overcome plant host defenses by producing pathogenesis compounds. *Fusarium graminearum*, the causal agent of Fusarium Head Blight (FHB) of wheat, produces trichothecene secondary metabolites such as deoxynivalenol (DON). Apart from DON, little is known about other pathogenesis compounds used by *F. graminearum* to cause disease on wheat. To identify fungal pathogenesis genes that are essential for *F. graminearum*, a paired strategy of isolates and transcriptome characterization was implemented. This should allow not only to capture biological samples containing the pathogenesis genes but also to determine their role in pathogenesis. A field pathogenomics strategy to identify common transcription signals in naturally infected wheat lines with various levels of resistance was used. In order to collect a variety of *F. graminearum* strains, research sites were established in the following Illinois locations: Urbana, Savoy, Brownstown, St. Jacob, and Carmi. Within each site, five wheat lines were planted in a random block design using University of Illinois soft red winter wheat improvement program plots. Wheat lines included the following: a resistant line (IL11-28222), a moderately resistant line (IL07-19334), a moderately susceptible line (IL10-19464), and two susceptible lines (Kaskaskia and Pioneer 25R47). Ten naturally infected heads were identified for each line, and two spikelets were collected. One spikelet was kept on ice for isolation of the fungus and the other was immediately submerged in RNAlater. Close to 200 *Fusarium* single spore isolates have been recovered from over 90% of the samples. Selected strains will be used in a greenhouse assay to characterize the levels of aggressiveness. RNA extraction of selected infected samples yielded high-quality RNA and will soon be submitted for sequencing. RNASeq analysis will then be conducted to compare the transcriptomes of resistant, moderately susceptible, and susceptible interactions and to identify pathogenesis genes that are required for infection on wheat.

ACKNOWLEDGMENT AND DISCLAIMER

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IMPACT OF DROUGHT STRESS ON WHEAT ROOT AND STEM BASE INFECTIONS *(TRITICUM AESTIVUM* L.*)* WITH *FUSARIUM CULMORUM*

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ABSTRACT

Fusarium culmorum is the predominant causal agent of *Fusarium* foot and crown rot (FCR) of wheat in arid and semi-arid growing regions world-wide. Drought stress is considered to play a crucial role during severe *F. culmorum* root and stem base infections of wheat. However, there are apparently no published studies documenting the impact of induced drought stress under controlled conditions. Our aim was to quantify the infestation of wheat root and stem bases with *F. culmorum* under drought stress and to document the water stress status in planta in order to show whether drought stress increases the colonization rate of *F. culmorum*. Pre-germinated wheat seedlings were dip-inoculated with a spore suspension of *F. culmorum* (800.000 spores/mL) and planted into potting soil. During stem elongation (Feekes 5), drought stress was induced in half of the plants by reducing the field capacity in the potting soil to 45%. In the well-watered treatment, we kept the field capacity at 75%. Plant water status was assessed by four different drought stress parameters: Relative leaf water content (RWC) (Feekes 11.1), leaf turgor (throughout the experiment), leaf surface temperature by thermal imaging (Feekes 11.1) and proline content (Feekes 11.1). Colonization of roots and stem bases with *F. culmorum* was determined by quantifying the fungal DNA with quantitative PCR (qPCR) at late milk ripeness (Feekes 11.1) and maturity (Feekes 11.4.) and rating the disease symptoms on the respective organs. All four water stress parameters assessed indicated the presence of drought stress in wheat plants in 45% field capacity. In both organs, the fungal DNA content was significantly higher under drought stress than under well-watered conditions (stem bases 17 times and roots 3 times higher; $P \leq 0.05$). However, fungal biomass in roots always exceeded the stem base levels, regardless if drought stress was present or not. Proline and DNA content in roots and stem bases were correlated ($r^2 = 0.47$). Based on these results we conclude that severe drought stress leads to higher colonization rates of *F. culmorum* in roots and stem bases of wheat.

This is the first study showing that water deficit in wheat plants followed by limited water supply significantly increases FCR severity in wheat caused by *F. culmorum*. Therefore, we conclude that FCR might be a threat to wheat production in areas with low precipitation with increasing importance.

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COMPARATIVE POPULATION GENOMICS OF *FUSARIUM GRAMINEARUM* REVEALS ADAPTIVE DIVERGENCE AMONG CEREAL HEAD BLIGHT PATHOGENS

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ABSTRACT

During the last decade, a combination of molecular surveillance and population genetic analyses have significantly altered our understanding of *Fusarium graminearum*, the major FHB pathogen in North America. In addition to the native NA1 population (largely 15ADON toxin type) and the invasive NA2 population (largely 3ADON toxin type), which has rapidly increased in frequency in some areas, isolates with a novel trichothecene toxin type (NX-2) were recently found to cause FHB in the northern U.S. and southern Canada. In this study, we sequenced the genomes of 60 *F. graminearum* isolates to understand how NX-2 isolates relate to the previously characterized NA1 and NA2 populations; and to identify potential adaptations that distinguish the various populations of *F. graminearum* responsible for FHB in the U.S. and Canada. Genome-wide patterns of SNP diversity revealed that most isolates with the NX-2 toxin type represent a novel genetic population (termed NA3), although genetic exchange among populations was documented. The three genetic populations were found to differ in gene content, with 122 genes showing population-specific patterns of gene conservation. An additional 16 loci, varying in size from 10-40 kb exhibited patterns of adaptive divergence between pathogen populations. Functional annotation of these population-differentiating genes and genomic regions indicated that *F. graminearum* populations in North America harbor unique sets of adaptations that contribute to differences in how these pathogens exploit the agricultural landscape.

DON MODIFICATION IN NATURALLY-ONTAMINATED WHEAT SAMPLES USING MICROORGANISMS ISOLATED FROM THE ENVIRONMENT

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ABSTRACT

The fungus *Fusarium graminearum* produces the toxic compound deoxynivalenol (DON) that contaminates wheat, barley, and maize. New strategies are needed to mitigate DON in the United States. Microbes were isolated from different soil types, and cultured in a mineral salt media using 100 ppm DON as the sole carbon source. We identified two mixed cultures, Soil 1 and Soil 2, which consistently modified DON. Nuclear magnetic resonance (NMR) was used to determine the structure of the culture byproducts of Soil 1 and Soil 2. Sequencing of the mixed cultures showed that Soil 1 contained mostly members of the genera *Acinetobacter* and *Enterobacter*, and Soil 2 contained mostly members of the genera *Pseudomonas* and *Comamonas*. Soil 1 and Soil 2 were incubated in two naturally contaminated wheat samples containing two different concentrations of DON (7 ppm and 41 ppm). Gas chromatography mass spectrometry (GC/MS) analysis showed nearly complete DON reduction in two samples (7 ppm DON) using the Soil 1 culture. GC/MS analysis of these two samples showed that DON was converted to another metabolite, 3-epi-DON. This research highlights the various ways DON can be modified under certain conditions and offers a platform to detoxify DON in naturally contaminated samples.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 52-0206-2-082. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S.Department of Agriculture.

DEOXYNIVALENOL (DON) AND NIVALENOL (NIV) PLAY A ROLE AS VIRULENCE FACTORS FOR WHEAT ROOT AND STEM BASE INFECTION BY *FUSARIUM CULMORUM* AND *F. GRAMINEARUM*

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ABSTRACT

Fusarium culmorum and *F. graminearum*, along with *F. pseudograminearum*, are the predominant causal agents of *Fusarium* foot and root rot in wheat. Populations of these two species vary broadly in their production of a variety of mycotoxins, including deoxynivalenol (DON) which is considered a virulence factor for wheat head and stem base infections. It is likely that DON is also a virulence factor in root infections incited by *F. culmorum* and *F. graminearum*, but there are apparently no published studies documenting infection under controlled conditions, or whether trichothecenes including DON are virulence factors in that process.

In this study, we screened a collection of 21 *F. culmorum* and six *F. graminearum* isolates for their ability to produce the mycotoxins DON, 3 acetyl-deoxynivalenol (3ADON), 15 acetyl-deoxynivalenol (15ADON), nivalenol (NIV), and zearelanone (ZEA) in rice culture. We selected two isolates of *F. culmorum* that demonstrated high and low production, respectively, of DON and 3ADON, and a third isolate for which ZEA was the only detected mycotoxin produced. Of the two *F. graminearum* isolates selected, one isolate only produced NIV and the other produced high levels of DON and 3ADON. Wheat plants were inoculated by planting pre-germinated seedlings (2 seedlings per pot) into potting soil enriched with *Fusarium*-colonized wheat straw (72 g straw per 10 kg soil). For each treatment, 16 plants were inoculated with the respective isolate. A non-inoculated treatment served as a control. For analysis, 4 plants were merged to one sample (n = 4). At the late milky ripe stage, root and above-ground biomass were determined. The roots and stem bases were excised and assessed visually for disease symptoms, and the DNA of *Fusarium* spp. and mycotoxin content were determined. Inoculation with the high DON/3ADON-producing isolate of *F. culmorum* resulted in the highest disease rating and a significant reduction in the biomass of roots and above-ground plant material. Additionally, levels of *Fusarium* DNA and DON in root and stem base tissues were significantly higher compared to the other isolates tested. *Fusarium* DNA and DON readings were significantly correlated ($r^2 = 0.67$ for roots, $r^2 = 0.92$ for stem bases). For *F. graminearum*, the NIV- and the DON/3ADON-producing isolates both caused higher disease symptoms on roots and stem bases, compared to the non-inoculated control, which was only significant for stem bases in plants inoculated with the NIV-producing isolate. Elevated DNA levels were seen in roots in both treatments and both isolates significantly reduced above-ground plant material compared to the control. Interestingly, only the DON/3ADON isolate caused a significant reduction of root biomass, and only the NIV-producing isolate colonized the stem base at significantly higher levels compared to the control plants, based on *Fusarium* DNA. NIV and DNA content were correlated in root and stem tissue ($r^2 = 0.66$ for roots, $r^2 = 0.59$ for stem bases). Based on these results it appears that the trichothecenes DON and NIV are virulence factors for root and stem base infection of wheat by *F. culmorum* and *F. graminearum*.

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VARIETY DEVELOPMENT AND HOST PLANT RESISTANCE

TISSUE CULTURE INDUCED VARIABILITY: CRITICAL ISSUES THAT IMPACT THE EVALUATION AND USE OF TRANSGENIC PARENTS

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OBJECTIVES

To 1) review the results of the performance of tissue-culture-derived non-transgenic and transgenic barley, and transgenic wheat, tested in multi-year, replicated tests in Idaho; and 2) discuss the impact of somaclonal variation on interpretation of results and on the use of transgenic plants as parents of potential new cultivars.

INTRODUCTION

Transformation of wheat and barley is dependent on the ability regenerate plants from cultured tissues that are amenable to biolistic- or *Agrobacterium*-mediated introduction of DNA. For both crops, tissue culture protocols have been worked out, particularly for Golden Promise barley and Bobwhite wheat, that enable a number of labs to routinely train scientists who successfully achieve transformation of these difficult-to-transform species. This was not always the case, and decades ago there was intense study of the process of regeneration (*e.g.*, embryogenesis or organogenesis) and the characteristics of regenerated plants (*e.g.*, abnormalities in phenotype and genotype, aka "somaclonal variation"). The accumulation of genetic variability was so widespread and striking that it was proposed as a method of generating varieties with unique characteristics, akin to other methods of mutagenesis (Larkin and Scowcroft, 1981). Despite the depth of the literature on somaclonal variation, most current reports of transgenic plants make no mention of its potential impact on the expression of engineered traits. Several sets of experimental lines were evaluated for agronomic performance and in various yield trials consisting of spaced plant, single row, or standard small plot yield trial formats of barley and wheat derived from tissue culture (both non-transgenic and transgenic). The results of these studies documented striking reductions in the agronomic performance and malting quality of almost all barley lines tested. In contrast, many wheat lines showed no or relatively modest reductions in performance and quality. For both crops, but especially barley, these results mean that somaclonal variation is a confounding factor in experiments that must be considered when evaluating plant performance, especially for traits in which incremental changes in performance are the intended result. In addition, breeding schemes—again, especially for barley—should take into account the near-certainty of heritable changes in performance in addition to that contributed by the transgene.

RESULTS AND DISCUSSION

Transformation induces variability in barley beyond that induced by tissue culture alone. Transformation protocols induce stress from chemical selection, osmotic changes, and/or *Agrobacterium* infection in addition to those imposed by the *in vitro* environment *per se*. Whether the transformation process would cause performance changes in addition to those caused by tissue culture was investigated in null-segregant (no transgene) Golden Promise barley plants derived from hemizygous, transgenic parents. They were tested as rows of spaced T_2 and T_4 plants at two Idaho locations in 1994 (two replicates) and 1996 (four replicates) (Bregitzer et al. 1998). In contrast to the tissue-culture-derived, non-transgenic plants

where no visual abnormalities were detected, these transgenic families contained clearly-mutant plants, including plants with extreme dwarfism (0 to 9%), a semi-prostrate growth habit (0 to 17%), and extremely late maturity (0 to 3%).

In contrast to the performance of tissue culture-derived, non-transgenic Golden Promise families, null-segregant families derived from transgenic, hemizygous plants performed remarkably—shockingly—poorly (Table 3, 4). Somaclonal variation is has been traced in many cases to epigenetic alterations (Kaeppler and Phillips, 1993), and may not be heritable. However, the observation of reduced performance in advanced generations meant that significant determinants of the observed performance must be heritable. Overall, these data suggested that most barley lines derived directly (via self-pollination) from regenerated transgenic plants would perform substantially worse than their non-transgenic parents.

Tissue culture alone induces significant variability in barley agronomic performance. A study (Bregitzer and Poulson 1995) was conducted to investigate agronomic performance of plants derived from 10–12-wk-old callus (Table 1). R_0 plants were advanced to R_2, space-planted in the greenhouse, and phenotypically normal plants (no abnormal plants were detected) were advanced and tested as R_4 and R_5 and tested in yield trials using a standard, small-plot format (randomized complete block design; four replicates at each of three locations over two years). None of the families were visually different from the controls. However, there was a clear trend towards reduced performance. The degree and frequency of observed alterations was affected by genotype (all Atlas 57-derived lines families were significantly reduced, but none derived from Steptoe were). Subsequently, malting quality was evaluated on grain derived from three of these cultivars (Bregitzer et al. 1995). Again, there was a trend towards reduced performance (Table 2), and the tissue culture-derived families presented malt profiles similar to that associated with stress (increased protein). Overall, the malting and agronomic data suggested that recovering barley lines from tissue culture that were equivalent in performance to their parent would be expected to be uncommon.

Thus, at least in barley, the recovery of performance would require introgression of transgenes into other backgrounds via one or more rounds of crossing. But—assuming that some performance loss was epigenetic in nature—what would be the expectations for heritability? If epigenetic alterations were involved, would they be stable? To answer this question eight lines derived from four transgenic events (containing either *PDR5* or *TRI101*) produced in the background Conlon (T_4 and T_5) and 35 lines derived from single backcrosses to one of the primary transgenic lines were tested in 2005 and 2006 in Aberdeen, ID, and Langdon, ND (Bregitzer et al. 2008). The backcross-derived lines included both transgenic and null-segregant lines. Each line was tested as a single row, with six (Aberdeen) and five (Langdon) replicates per line. Interestingly, the Conlon lines advanced by self-pollination were agronomically much better than the Golden Promise lines described above, showing again the potential for background genotype to influence the degree of somaclonal variation. The mean yield was 69% of non-transgenic Conlon (range 57 to 84%). The mean yield of the backcross-derived lines was 94% (range of 90 to 97%), and the performance of these lines was correlated with the relative performance of their respective transgenic parents. Thus, the amount of yield recovery was in line with expectations for the expectation that a single backcross would replace 75% of the donor (transgenic) parent genome with the wildtype Conlon genome. Therefore, regardless of the source (genetic or epigenetic), the determinants of reduced performance induced by tissue culture and transformation behaved as stable, heritable factors.

No differences were detected between null segregant and transgene-containing lines, suggesting that yield depression was a result solely of somaclonal variation. This, and the recovery of performance upon backcrossing, provided evidence that the

observed variation was not caused by transgene expression.

Malting quality was assessed also in these lines. The primary transgenic lines showed widespread reductions in malting quality, as seen before for the Golden Promise-derived lines, with substantial recovery of performance seen for the backcross derived lines (data not shown).

Transgenic wheat lines performed relatively better than transgenic barley lines. The performance of transgenic wheat was evaluated at one Idaho and two California locations in 2002 and 2003 (Bregitzer et al. 2006). The experimental format was a standard, small-plot yield trial format (randomized complete block design; four replicates at each location). Fifty-four independent transgenic wheat lines (each expressing a variant high-molecular-weight glutenin gene), and ten null segregant lines, were compared to the performance of the non-transgenic parent, Bobwhite. The performance of all of the null segregant lines, and of 33 of the 44 transgenic lines, was not significantly different from that of Bobwhite (data not shown). This suggested that in these wheat lines, expression of the transgenes was primarily responsible for significant reductions in performance, not somaclonal variation.

Since these wheat lines made it through tissue culture and transformation relatively unscathed, should the conclusion be that somaclonal variation can be discounted in wheat transgenics? Given that somaclonal variation has been documented in all plant species studied where it was searched for, it is unlikely that wheat is an exception. If it were, one would expect a population of wheat lines regenerated from transgenic wheat cultures to have mean performance equal to that of Bobwhite, and exhibit a normal distribution around the values for Bobwhite. Examining the performance of the null segregant lines shows this may not be true. Of the 30 data points for null segregants (10 lines x 3 locations; mean values over two years), only 10 were numerically higher for yield compared to the control, and their overall mean was 6200 kg/ha vs. 6301 for Bobwhite (Table 5). Perhaps these differences are indeed insignificant; nevertheless, the point to be made here is that the conservative assumption should be that the performance of transgenic lines developed by self-pollination may be compromised by somaclonal variation.

The effect of somaclonal variation on interpreting the results of genetic engineering experiments. It is obvious that various hybridization-based breeding approaches can remove determinants of somaclonal variation that are unlinked to the transgenic locus, and thus somaclonal variation is of no or negligible importance to the final product. Even in the absence of somaclonal variation, it is unlikely that the desired background would be Bobwhite, Golden Promise, or Conlon (the most commonly-transformed barley and wheat cultivars). The problem lies in interpreting the success of your initial experiments.

A transgenic alteration that eliminates susceptibility to FHB is of little value to a producer if it depresses yield or compromises end-use quality. Especially for qualitative traits, somaclonal variation makes interpretations of the potential agricultural utility difficult. Therefore, it is essential to develop proper controls that take somaclonal variation into account. The background parent that has not gone through the transformation process, a commonly-used control in transgenic studies, is nearly useless for comparing qualitative traits because the effects of the transgene and somaclonal variation are confounded. Null segregants, preferably more than one, derived from the same transformation event as the tested transgenic line, can be developed concurrently with the transgenic line and provide a superior control. Perhaps the best control, a near-isogenic line derived by backcrossing, is often impractical because of the time required to develop it before testing can begin.

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Table 1. Selected agronomic characteristics of lines derived from 10–12-wk-old callus, as measured in small-plot yield trials at three Idaho locations, 1992–1993.

Cultivar		Yield (# sig. dif. from control) (kg/ha)	Test weight ((kg/m^3)	Plump kernels (%)
Atlas 57	Control	5859	595	88.9
	6 R_2-derived families	4547–5128 (6)[a]	568–584 (6)	83.3–90.6 (3)
Golden Promise	Control	6165	618	58.8
	6 R_2-derived families	5612–6036 (2)	605–622 (2)	42.0–54.5 (3)
Klages	Control	5859	640	62.6
	4 R_2-derived families	4956–5379 (3)	609–649 (1)	45.4–61.2 (2)
Morex	Control	5364	632	69.3
	3 R_2-derived families	4929–5128 (0)	619–628 (2)	65.3–68.9 (1)
Piroline	Control	6063	667	78.4
	5 R_2-derived families	5208–5746 (2)	631–664 (3)	45.2–72.3 (3)
Steptoe	Control	6923	597	82.5
	6 R_2-derived families	6600–7036 (0)	588–601 (0)	80.4–83.5 (0)

[a]Number of families with means significantly different from the control for the specified trait.

Table 2. Selected malting quality characteristics of lines derived from 10–12-wk-old callus, as measured in small-plot yield trials at two Idaho locations, 1992–1993.

Cultivar		Barley protein (%)	Malt extract (%)	Soluble/total protein (%)	Diastatic power (°ASBC)	α-amylase (DU)
Klages	Control	12.7	77.0	36.2	109	39.5
	4 R_2-derived families	13.2–14.0 (2)[a]	76.0–77.9 (1)	34.1–40.0 (1)	104–118 (0)	36.1–38.0 (1)
Morex	Control	12.8	77.4	40.0	142	44.0
	3 R_2-derived families	13.6–14.0 (2)	76.2–77.6 (1)	39.9–40.6 (0)	167–192 (3)	39.4–40.5 (2)
Piroline	Control	12.2	76.7	34.6	115	34.4
	5 R_2-derived families	13.2–13.7 (3)	74.6–76.7 (2)	28.5–35.0 (2)	107–139 (1)	28.7–35.8 (2)

[a]Number of families with means significantly different from the control for the specified trait.

Table 3. Agronomic performances of transgenic barley grown at two locations in 1994.

Family[a]	# lines in family[b]	Height[c]	Yield[c]	100-seed-weight[c]
GP717B-2	1	88 (88–88)[d]	56 (56–56)	74 (74–74)
GP717B-4	5	98 (94–103)	85 (69–108)	84 (82–92)
GP717B-11	2	86 (84–88)	54 (50–58)	70 (68–73)
GP717B-14	2	73 (70–76)	16 (16–16)	57 (55–58)
GP717B-31	1	79 (79–79)	47 (47–47)	77 (77–77)
GP717B-32	5	94 (89–97)	66 (53–80)	79 (72–85)
GP717B-33	4	90 (86–93)	64 (57–73)	74 (72–75)
GP717B-59	1	87 (87–87)	64 (64–64)	81 (81–81)
GP717B-189	4	77 (69–87)	27 (16–41)	66 (58–75)
GP717B-197	5	82 (68–96)	49 (21–81)	72 (57–85)
GP724B-1	1	87 (87–87)	45 (45–45)	74 (74–74)
GP724B-4	4	87 (82–90)	60 (42–68)	93 (80–118)
GP724B-47	1	92 (92–92)	79 (79–79)	88 (88–88)
GP724B-96	4	80 (76–88)	50 (40–65)	75 (72–83)

[a]Each family represents an individual transformation event
[b]Each line derives from an individual regenerated plant
[c]Data are expressed as percentages of the non-transgenic GP control performance
[d]Data presented as: family mean (range of line means)

Table 4 Agronomic performances of Golden Promise and transgenic-derived null-segregant barley lines in 1996

Line	Traits			
	Heading date (d after Jan. 1)	Height (cm)	Yield per plant (g)	100-seed weight (g)
Golden Promise	193.9	49.6	30.2/1.2[a]	3.7/0.27
GP717B-14-8	196.2*	41.7*	5.2*/2.4*	2.3*/0.61
GP717B-14-12	198.3*	39.6*	4.4*/2.4*	2.3*/0.77*
GP717B-31-3	195.3	47.2*	15.9*/1.8*	3.2*/0.47
GP717B-32-6	195.2	46.3*	15.9*/1.8*	3.0*/0.50
GP717B-32-11	194.3	47.4*	18.2*/1.7*	3.0*/0.43
GP717B-33-3	196.7*	42.7*	12.1*/1.9*	2.8*/0.54
GP717B-33-3	193.6	46.2*	18.0*/1.6	3.4/0.26

* SigniÞcantly di□erent from G.P. as determined by Dunnett's multiple comparison procedure (P=0.05).
[a]Data presented as: trait mean/estimated deviation. Estimated deviation = range / trait mean, calculated on a per-plot basis.

Table 5. Agronomic performance of transgenic wheat lines, 2002 and 2003.

Location	Aberdeen, ID			Davis, CA		El Centro, CA	
Line	Yield (kg/ha)	Protein (%)	Test weight (kg/m^3)	Yield (kg/ha)	100-seed-weight (g)	Yield (kg/ha)	Test weight (kg/m^3)
Bobwhite	6486	12.5	792	2481	3.34	9936	746
Dx51Dy10-C null	6346	12.6	788	2547	3.25	11006	766
Hybrid-B null1	6023	13	790	2281	3.35	9133	763
Hybrid-B null2	5974	13.1	802	2333	3.1	9323	766
LongDx5-B null	6476	12.9	796	1780	3.2	10126	766
LongDx5-F null	6226	13	795	1927	3.19	10095	775
LongDx5-H null	6217	13	793	2064	3.41	9983	759
LongDx5-I null	5982	12.9	792	1905	3.34	10735	775
ShortDx5-C null	6113	13.8	792	2599	3.31	9771	766
ShortDx5-D null	6597	13	787	2399	3.09	11078	756
ShortDx5-H null	6082	13.9	792	1928	3.21	10960	788*

*, **, ***: significant at P=0.05, 0.01, and 0.001, respectively.

GENOMIC SELECTION FOR FHB RESISTANCE USING THE UNIFORM SCAB SCREENING NURSERIES

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ABSTRACT

Genomic selection (GS) involves use of genome-wide marker data in combination with phenotypic data to develop models for predicting performance of untested lines using only genotypic data. Wheat breeders in the eastern winter wheat region have collaborated to evaluate FHB resistance in adapted breeding lines across years and locations. Disease evaluation data from the 2011 through 2015 Uniform Southern Soft Red Winter Wheat Scab Nursery were analyzed using mixed model in SAS 9.3 to obtain BLUES for each genotype. Entries in the 2011 through 2016 USSRWWSN were genotyped using genotyping-by-sequencing and data were obtained for 15,013 markers distributed throughout the genome. In addition, KASP evaluations were performed for markers linked to FHB resistance QTL, the *Fhb1* locus, and the *Rht-B1* and *Rht-D1* loci. GS models were implemented with the R-package RR-BLUP and accuracy evaluated by correlation between Genomic Estimate Breeding Values (GEBVs) and BLUES for each line. The mean observed accuracies (r) from 100 cycles of five-fold cross validation were 0.46 for incidence, 0.66 for severity, 0.61 for Index, 0.59 for FDK, 0.59 for ISK and 0.53 for DON. Addition of markers for the *Rht1* and *Fhb1* loci as fixed effects in the model resulted in small increases in prediction accuracy. In particular, incidence accuracies increased with the addition of the *Rht-D1* marker (r = 0.50). DON accuracies were slightly increased with the addition of the *Fhb1* marker (r = 0.57). Based on GS models using the 2011-2015 nurseries as a training population, GEBVs were determined and reported for entries in the 2016 Uniform Southern Soft Red Winter Wheat Scab Nursery report. Results of genome-wide association mapping using this genotypic and phenotypic dataset will also be reported. Overall, our results suggest that GS for FHB resistance can be utilized to streamline variety selection and evaluation.

IMPLEMENTATION OF GENOMIC SELECTION FOR RESISTANCE TO FUSARIUM HEAD BLIGHT INTO A TRADITIONAL WHEAT BREEDING PROGRAM

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ABSTRACT

Genomic selection is a new method applied in plant breeding that uses high density genotyping associated with observed phenotypes to predict unobserved phenotypes via a marker-trait model. Our objective was to implement this method in a traditional soft winter wheat breeding program to increase overall genetic gains in developing wheat cultivars resistant to *Fusarium graminearum* (teleomorph: *Gibberella zea*). Phenotyping for Fusarium Head Blight (FHB) was conducted on a set of 417 lines from which a training population was derived. FHB incidence, severity, *Fusarium* damaged kernels (FDK), and deoxnivalenol levels were assessed on the lines grown in an inoculated and mist-irrigated scab nursery in Virginia. Genotyping was done using double digest rad-seq or often referred to as GBS using the enzymes *PstI* and *MseI*. SNPs were aligned using the International Wheat Genome Sequencing Consortium's whole genome assembly v0.4. Prior to imputation of missing genotypes, the genotypic dataset was filtered to remove SNPs with missing data frequencies >20%, heterozygous call frequencies >15%, and minor allele frequency < 5%. In addition, all unaligned SNPs were removed. Imputations were achieved using the R package *LinkImpute*. This package implements a nearest-neighbor algorithm using both the *k* nearest individuals and the *l* SNPs in highest LD with the specific missing SNP genotype that must be imputed. Genomic selection (GS) accuracies were assessed using best linear unbiased prediction (*rr-Blup*) using the *kin.blup* function. This function estimates genomic values in which performance of individuals are predicted based upon kinship to other lines in the training population. Average accuracies for Grain Yield, FHB Severity, Incidence, Index, and FDK were 60%, 38%, 47%, 45%, and 43%, respectively after 1000 permutation cycles for each trait. The R package *PopVar* was also used to predict parents to cross to generate high genetic variance for resistance to FHB.

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ASSOCIATION MAPPING IN A PANEL OF MINNESOTA SPRING WHEAT BREEDING LINES REVEALS QTL MAINTAINED OVER DECADES OF PHENOTYPIC SELECTION

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ABSTRACT

Developing varieties with improved resistance to Fusarium head blight (FHB) has been a major goal in the University of Minnesota's wheat improvement program since the scab epidemics of the 1990s. At that time, a number of diverse lines, particularly from Asian germplasm, were crossed into the program to introduce sources of genetic resistance. Early lines developed from resistance sources had poor agronomic and quality characteristics. In the intervening years, the focus has been on improving agronomic and quality traits while maintaining FHB resistance. We have selected for two major QTL, *Fhb1* (since 2001) and *Fhb5* (2005), using DNA markers and phenotype all F_5 and more advanced lines, about 3,500 per year, in controlled disease nurseries. To assess the number and locations of resistance QTL currently present in the UMN wheat breeding program, a panel of 383 F_7-derived lines in advanced yield testing were phenotyped for several FHB resistance-associated traits in a minimum of five environments between 2009 and 2013. The panel was genotyped at high density using the 90K Illumina Infinium iSelect Assay, resulting in 14,221 mapped SNP markers for association analysis. Association mapping revealed the presence of *Fhb1*, *Fhb2*, *Fhb5*, and over two dozen additional significant regions ($p<0.001$) across the genome, many corresponding to the locations of previously reported QTL. Analysis of the pedigrees confirms the presence of reported source lines in several cases. KASP markers based on significant Illumina 90K iSelect markers are being designed to facilitate the tracking of these QTL in the program. This study demonstrates the efficacy of phenotypic selection for long-term maintenance of favorable FHB resistance alleles.

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EVALUATION OF GERMPLASM RESISTANCE TO FUSARIUM HEAD BLIGHT DISEASE

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ABSTRACT

Fusarium Head blight (FHB) disease of wheat reduces yield and deteriorates quality. Breeding for FHB resistance requires identification of sources of resistance. This poster summarizes one year of FHB screening effort at Purdue University. The germplasm includes 80 advanced breeding lines developed at Purdue, 17 doubled haploid lines developed from the cross between Indiana wheats INW0412 and INW0411, 200 recombinant inbreeding lines from the cross between INW0412 and 992060G1-1-5, and lastly, 33 accessions introduced from the European FHB resistance breeding program in Austria. Plant materials were phenotyped in greenhouse and field conditions. Type II resistance for FHB was recorded following artificial infection. Germplasm showing type II FHB response of less than 20% were screened for further validation studies. Leveraging marker data for known loci indicated that a select number of lines carrying FHB resistance alleles also harbored important disease resistance genes such as stem rust resistance *Sr36* (effective against the race Ug99), height reducing loci (*Rht1* and *Rht2*), and *Bdv2/3* for barley yellow dwarf virus disease. After validation phenotyping, lines harboring multiple resistance alleles will be used in crossing schemes. Besides germplasm enhancement through phenotypic assessment, one future direction is to develop genome-wide markers to enable genomic prediction for FHB resistance and other traits of agronomic importance.

RESPONSE OF A COLLECTION OF *WAXY* (REDUCED AMYLOSE) WHEAT BREEDING LINES TO *FUSARIUM GRAMINEARUM*

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ABSTRACT

Loss of function mutations in the *Waxy* (*Wx*) gene encoding granule bound starch synthase I (GBSSI) that synthesizes amylose, results in starch granules containing mostly amylopectin. Wheat grain with this trait has increased functionality as an optimal substrate for production of modified food starches and for increased nutritional value in livestock and poultry feed. However, impaired GBSSI activity may alter grain structure and composition and, consequently, responses to pathogens. There are no published reports on response of *waxy* wheats to Fusarium head blight (FHB). A screen of colonization by *Fusarium graminearum* of *waxy* breeding lines and wild-type and *waxy* checks was conducted on grain grown at Mead, NE during 2013 and 2014. Grain was either surface disinfested before plating, or directly plated, onto medium semi-selective for *Fusarium* spp., indicating internal or both internal and superficial fungal infections, respectively. Fungi were identified using morphological characteristics. Chi-square analysis showed that internal and superficial total *Fusarium* infection rates (directly plated grain) were significantly higher among the *waxy* breeding lines and the waxy cultivar Mattern (55.1%) as compared with wild-type checks (45.6%) ($P < 0.01$). However, there were no significant differences in the proportion of these fungi that were *F. graminearum* in *waxy* (4.3%) *versus* wild-type (3.4%) grain ($P = 0.11$). Percent of internal infections (disinfested grain) of *waxy* (4.4%) and wild-type (3.9%) grains were not significantly different ($P = 0.45$). However, chi-square analyses indicated that the proportion of these fungi in *waxy* grain that were *F. graminearum* (4.7%) was significantly less than that of wild-type (17.5%) ($P = 0.03$). When grain was analyzed using GC-MS for four trichothecene mycotoxins, only deoxynivalenol (DON) was detected. In spite of internal and superficial levels of *F. graminearum* colonization, *waxy* breeding lines and Mattern combined had significantly higher levels of DON (0.58 ppm) than wild-type checks (0.52ppm) (SE= 0.02; $P = 0.03$). However, waxy breeding line NX12Y8213 (PI 677877) had mean rates of internal infection (0.00%) and DON levels (0.49 ± 0.05 ppm) which were the same as the FHB tolerant wild-type lines McGill and Freeman. The proportion of superficial and internal infection of NX12Y8213 by *F. graminearum* (1.7%) was not significantly different from that of the wild-type checks (3.4%) ($P = 0.47$). Therefore, NX12Y8213 is promising for breeding for *waxy* lines with tolerance to FHB.

SIMULTANEOUS MAPPING AND PYRAMIDING LOCI IN WHEAT BREEDING POPULATIONS: IDENTITY BY DESCENT MAPPING APPROACHES

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ABSTRACT

Pyramiding QTL from multiple sources for FHB resistance presents an opportunity to enhance the FHB resistance of elite wheat germplasm. Conventionally, pyramiding QTL using a marker assisted-selection approach would require preliminary mapping studies to identify the resistance QTL from each parental line and validation studies to assess the QTL effects in multiple genetic backgrounds. Mapping FHB resistance QTL directly in wheat breeding populations would eliminate the need for purpose built mapping populations, and thus accelerate marker-assisted pyramiding efforts. This presentation will discuss our recent studies showing how multiple QTL for FHB resistance can be mapped directly in early generation breeding populations by application of identical-by-descent (IBD)-based linkage mapping. We used IBD-based linkage analysis in spring and winter wheat segregating F_1 progeny derived from 43 and 28 four-way crosses respectively, among Fhb1 donor lines and multiple native sources of resistance including plant introductions, SDSU and UMN breeding lines in spring wheat; and Lyman, Overland, Ernie and Freedom in winter wheat. In the spring wheat experiment QTL for FHB resistance were identified on chromosomes 2A, 2B, 3B and 7B, explaining between 18 to 21% of the variance for FHB severity in different evaluations. The QTL detected on chromosome 2A appears to have a resistance allele conferred by MN99126 in the same region detected by QTL-meta analysis from Ning8026, Wangshuibai, Spark and Rubens. In the winter wheat experiment a total of 15 QTL for FHB resistance were mapped on chromosomes 1A, 1B, 2A, 3A, 3B, 4A, 4B, 4D, 5A, 6A, 6D and 7D, including known loci Fhb1, Fhb5, and Rht-B1. QTL conferring native resistance in the cultivars Lyman and Overland are mapped for the first time in this study, including a QTL on chromosome 1AS (Qfhb.sdsu-1A) explaining between 4.5 to 9.9% of the phenotypic variance in all evaluations. Marker haplotypes for these QTL regions can be used to conduct marker assisted selection and fixation of resistance alleles in subsequent generations of these breeding populations.

Subsequent efforts with double haploid derived from these breeding populations are validating the reported results.

EVALUATION OF SOUTHERN SOFT RED WINTER WHEAT LINES FOR RESISTANCE TO FUSARIUM HEAD BLIGHT

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ABSTRACT

Fusarium head blight (FHB) is a disease of small grains caused by the fungal pathogen *Fusarium graminearum*. FHB poses potential economic losses and health risks due to the accumulation of the mycotoxin deoxynivalenol (DON) on infected seed heads. The objectives of this study are; 1) evaluate soft red winter wheat (SRWW) lines for resistance to FHB in terms of resistance to initial inoculum (incidence); resistance to spread within the head (severity); resistance to DON accumulation; and resistance to *Fusarium* damaged kernels (FDK), 2) determine the frequency and effect of known FHB resistance genes and quantitative trait loci (QTL), and 3) Identify novel resistance loci using a genome wide association (GWAS) approach. In 2015-2016, 360 SRWW breeding lines were evaluated in inoculated misted FHB nurseries in Fayetteville and Newport, AR in a randomized complete block design. At both locations, lines were sown in two row plots, inoculated with *F. graminearum* infected corn (*Zea mays* L.) and overhead misted for a total of 480 and 520 minutes, for Fayetteville and Newport, respectively, throughout the months of April and May to provide optimal conditions for FHB infection. In addition to visual ratings and DON analysis, lines are currently being screened with molecular markers linked to known FHB resistance genes, including *Fhb1*, 3BSc from Massey and recently identified QTL for native resistance from Jamestown (1B, 6A) and Bess (2B, 3B). Future work will use markers generated through genotype by sequence to perform GWAS. The overall goal of this research is to produce marketable wheat cultivars with improved resistance to FHB using a combination of both traditional and molecular breeding methods.

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EPIGENETIC CONTROL OF FHB IN DURUM WHEAT

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ABSTRACT

Fusarium head blight (FHB) continues to be a serious problem for wheat production in the U.S. and elsewhere in the world. Economic losses associated with FHB occur due to low grain yield and contamination of grain with mycotoxins. *Fusarium graminearum* is the major causative agents of FHB in the U.S. Durum wheat (*Triticum turgidum* L. var. *durum*) is particularly susceptible to FHB and breeding for resistance has been impeded by low genetic variation. Thus, it is important to identify other means of enhancing resistance to FHB in durum wheat. DNA methylation and demethylation have been documented to be involved in immunity against the plant pathogens by regulating transcriptional and co-transcriptional immune-responsive genes.

We treated eight advanced durum breeding homozygous lines with 5-Methyl-azacytedine that removes CG methylation. The treated lines were selected for resistance at each generation and advanced to the M_4 generation, resulting in 32 selected lines that were further analyzed, along with the eight parental controls. All 40 lines were tested for FHB resistance under greenhouse and field conditions. Five of the 32 demethylated lines tested showed promise, having less than 30% disease severity as compared with a range of 50-100% for the parental lines and FHB susceptible lines included as checks. The proportion of *Fusarium*-damaged kernels (FDK) of the five lines identified ranged from 10 to 30%, whereas the parental and other treated lines showed values between 30 and 60%. The range of deoxynivalenol (DON) concentrations of grain harvested from the five lines was from 2.46 to 5.60 ppm, whereas the parental lines, checks and the remaining 27 treated lines had DON concentrations from 5.10 to 18.27 ppm. The FDK and DON analyses supported the findings of the disease development assessed in the field and the greenhouse. These five lines, together with their respective parental lines and some highly susceptible checks are being further analyzed to determine the specific epigenetic changes that are responsible for the enhanced resistance observed. We have advanced these lines by backcrossing them to the parental cultivars with the aim of testing the stability and inheritance of the resistance.

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THE 2016 UNIFORM SOUTHERN SOFT RED WINTER WHEAT SCAB NURSERY

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ABSTRACT

The Uniform Southern Soft Red Winter Wheat Scab Nursery provides breeders in the public and private sectors the opportunity for multi-environment, independent evaluations of FHB resistance in advanced generation breeding lines compared with the current most resistant check varieties 'Ernie', 'Bess' and 'Jamestown'. Valuable data are provided on resistance to other important fungal and viral diseases, milling and baking quality and agronomic characteristics. In addition, genotypic analyses identify alleles present at numerous important loci. For the first time we provide Genomic Estimated Breeding Values (GEBV) for nursery entries. These were estimated from a training population of nursery entries from 2011 to 2015. A combined mixed model analysis of the phenotypic data from 2011 to 2015 was performed using SAS 9.3 and BLUEs for each genotype were recorded. The number of SNP markers utilized was 70,081. The Genotypic Selection model utilized Ridge Regression BLUP through the R-package RR-BLUP to predict GEBVs for individuals in the 2016 nursery. GS model accuracy is evaluated by Pearson correlation between GEBVs and best linear unbiased estimate (BLUE) for the 2016 lines. Correlation varied between 0.55 for FHB Severity to 0.13 for FHB Index.

The 2015-16 nursery comprised 51 advanced generation breeding lines and four check cultivars, Ernie, Bess, Jamestown (partially resistant) and 'Coker 9835' (susceptible). Six U.S. public programs (Arkansas, Georgia, Louisiana, North Carolina, Virginia, and USDA-ARS), and two private companies (KWS and Limagrain) submitted entries. Data were returned from up to eight locations in the US and one in Hungary. In addition, three USDA-ARS laboratories conducted evaluations for Hessian fly resistance, milling and baking quality and marker genotypes.

Copies of the full report will be available at the 2016 National Fusarium Head Blight Forum and subsequently on line at the USWBSI web site: http://www.scabusa.org/.

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Table 1. Phenotypic means across locations, correlations between GEBV and phenotypic means and genotypic content of regions associated with FHB resistance.

	Cultivar/ Designation	FHB Incidence	RANK	FHB Severity	RANK	FHB Index	RANK	FDK	RANK	ISK	RANK	DON	RANK
1	ERNIE	49	40	25	33	14	29	32	38	31	32	3	10
2	COKER9835	84	55	56	55	49	55	56	54	57	55	7	52
3	BESS	31	6	17	12	7	5	15	3	19	1	2	1
4	JAMESTOWN	35	14	19	15	10	18	23	16	26	16	4	23
5	AR06024-7-2	33	8	13	3	8	8	20	10	25	14	2	1
6	ARS10-389	34	11	14	5	6	3	26	21	21	5	2	1
7	AR07010-7-1	30	4	19	15	9	15	25	20	24	10	5	35
8	AR07053-13-1	33	8	19	15	8	8	20	8	24	10	6	45
9	AR07078-7-4	44	28	27	36	17	38	24	17	33	36	6	45
10	AR07108-6-1	28	2	17	12	8	8	17	5	22	8	4	23
11	ARLA06146E-20-1	39	19	19	15	12	24	24	19	29	23	4	23
12	ARLA07084C-10-1	26	1	13	3	5	2	23	14	21	5	5	35
13	ARS11-2086	65	54	34	50	27	51	58	55	45	52	5	35
14	ARS12-201	59	51	31	47	24	50	50	50	40	47	5	35
15	ARS13-159	51	42	29	42	19	42	46	47	40	47	3	10
16	ARS13-215	50	41	28	41	19	42	27	26	32	34	15	55
17	ARS14W0539	32	7	16	8	6	3	34	40	29	23	5	35
18	ARS14W0623	40	23	24	30	10	18	47	48	32	34	11	54
19	ARS14W1012	47	33	27	36	19	42	40	46	40	47	4	23
20	ES14-0057	39	19	19	15	9	15	22	13	25	14	3	10
21	ES14-0528	58	50	21	22	15	32	30	34	30	27	3	10
22	ES14-1293	29	3	12	1	4	1	18	7	20	2	3	10
23	ES14-1350	54	46	29	42	21	47	48	49	42	50	2	1
24	GA08250-15ES14	35	14	16	8	8	8	12	1	21	5	3	10
25	GA08293-15ES3	46	31	29	42	19	42	35	42	34	39	5	35
26	GA09361-15ES38	47	33	31	47	19	42	27	27	34	39	6	45
27	GA091252-15ES35	52	44	25	33	15	32	34	41	34	39	6	45
28	GA08281-15ES1	46	31	24	30	14	29	29	31	30	27	4	23
29	GANC9337-15ES27	44	28	20	20	13	28	15	4	24	10	3	10
30	GA09343-15ES33	56	48	32	49	23	48	30	33	33	36	6	45
31	GANC 10014-15ES24	44	28	22	26	14	29	36	43	35	43	5	35
32	KWS 053	39	19	12	1	8	8	17	6	20	2	3	10
33	KWS 060	52	44	27	36	16	37	21	11	28	20	2	1
34	KWS 074	36	16	16	8	8	8	28	30	28	20	4	23
35	KWS 081	33	8	16	8	8	8	26	23	26	16	2	1
36	KWS 083	34	11	15	6	7	5	20	9	23	9	4	23
37	KWS 087	38	17	22	26	12	24	28	29	28	20	3	10
38	LA06146E-P4	38	17	21	22	12	24	30	35	30	27	5	35
39	LA08090C-9-2	34	11	24	30	12	24	32	36	31	32	6	45
40	LA08265C-50	40	23	27	36	15	32	30	32	30	27	4	23
41	LA09011UB-2	47	33	29	42	18	40	38	45	35	43	6	45
42	LA09225C-33	40	23	29	42	15	32	28	28	34	39	5	35
43	NC10435-11	51	42	27	36	18	40	22	12	30	27	3	10
44	NC12-22225	43	27	21	22	10	18	26	25	29	23	3	10
45	NC13-20076	30	4	15	6	7	5	15	2	20	2	2	1
46	NC13-22350	40	23	18	14	9	15	24	18	24	10	2	1
47	NC13-23449	56	48	34	50	27	51	37	44	38	46	4	23
48	VA12W-68	39	19	20	20	10	18	26	24	27	18	8	53
49	VA13W-38	48	39	21	22	11	23	26	22	27	18	2	1
50	VA09MAS6-122-7-1	47	33	23	28	17	38	23	15	29	23	4	23
51	VA08MAS1-188-6-4-1	47	33	23	28	15	32	32	37	33	36	3	10
52	VA13FHB-26	47	33	25	33	12	24	50	51	43	51	4	23
53	VA14FHB-14	62	52	39	53	30	53	55	53	48	54	3	10
54	VA14FHB-13	64	53	39	54	31	54	54	52	45	52	4	23
55	VA14FHB-28	55	47	35	52	23	48	33	39	36	45	5	35
	Mean	44		24		14		30		31		4	
	LSD (0.05)	37		28		26		34		23		4	
	CV%	43.0		59.6		90.1		56.4		37.3		50.1	
	Mean v GEBV Correlation	0.44		0.55		0.13		0.30		0.49		0.44	

Table 1. Continued

	Cultivar/ Designation	Heading Date	RANK	Plant Height	RANK	Flour Yield %	RANK	Softness Equivalent %	RANK	Hessian Fly Biotype L	Fhb1	Fhb Massey 3B	Fhb 5A	Fhb 2DL-Wuhan1/W14	Bess 2B	Bess 3B	Jamestown 1B	Jamestown 6A	NC-Neuse 1A	NC-Neuse 6A
1	ERNIE	123	12	33	12	63	50	52	42	0-19	no	yes	yes	no	no	no	no	no	yes	yes
2	COKER9835	126	27	32	3	66	30	60	4	0-17	no	no	no	no	no	no	no	no	no	no
3	BESS	127	33	35	31	65	41	54	24	0-19	no	no	no	no	yes	yes	yes	no	yes	no
4	JAMESTOWN	121	1	33	12	65	41	54	24	0-18	no	no	no	no	no	no	yes	yes	yes	no
5	AR06024-7-2	125	22	36	37	64	49	55	17	0-19	het	no	no	no	no	no	yes	no	yes	no
6	ARS10-389	121	1	35	31	71	1	39	53	0-16	no	no	no	no	no	no	no	no	no	no
7	AR07010-7-1	129	51	39	50	66	30	52	42	0-18	no	no	no	no	no	no	no	no	no	no
8	AR07053-13-1	128	39	39	50	66	30	54	24	0-13	no	no	no	no	no	no	no	no	no	no
9	AR07078-7-4	130	53	39	50	68	11	54	24	0-16	no	no	no	no	no	no	no	het	no	no
10	AR07108-6-1	128	39	40	53	67	21	55	17	0-18	no	no	no	no	no	yes	het	no	yes	no
11	ARLA06146E-20-1	126	27	40	53	65	41	56	10	0-14	no	no	no	no	no	no	yes	yes	yes	no
12	ARLA07084C-10-1	128	39	37	45	67	21	60	4	0-12	no	no	no	no	no	no	no	no	no	no
13	ARS11-2086	128	39	32	3	68	11	53	35	14-6	no	no	yes	no	no	no	yes?	no	yes	yes
14	ARS12-201	128	39	33	12	69	5	51	45	4-13	no	no	yes	no	no	no	yes	no	yes	yes
15	ARS13-159	127	33	38	48	68	11	61	1	0-18	no	yes	no	no	no	no	yes	no	no	no
16	ARS13-215	128	39	34	20	69	5	47	49	0-16	no	no	no	no	no	no	no	no	yes	no
17	ARS14W0539	131	54	32	3	.	.	.	.	0-20	no	yes	no	no	no	no	no	no	no	no
18	ARS14W0623	133	55	37	45	.	.	.	.	0-17	no	yes	no	no	no	no	no	no	no	no
19	ARS14W1012	127	33	33	12	66	30	48	47	0-13	no	no	no	no	no	no	no	no	no	yes
20	ES14-0057	129	51	36	37	65	41	58	7	0-18	no	no	no	no	no	no	het	no	yes	no
21	ES14-0528	124	16	36	37	70	2	54	24	0-19	no	yes	ND	no	no	no	yes	no	no	no
22	ES14-1293	128	39	40	53	67	21	54	24	18-0	no	no	no	no	no	no	no	no	yes	no
23	ES14-1350	126	27	36	37	66	30	47	49	0-19	no	yes	no	no	no	no	yes	no	yes	no
24	GA08250-15ES14	125	22	36	37	69	5	55	17	0-16	no	no	no	no	no	no	no	yes	yes	no
25	GA08293-15ES3	122	7	34	20	62	53	46	52	0-18	no	no	no	no	no	no	no	yes	no	no
26	GA09361-15ES38	125	22	34	20	70	2	52	42	0-20	no	no	yes	no	no	no	het	no	no	no
27	GA091252-15ES35	125	22	34	20	70	2	53	35	0-19	no	no	no	no	no	no	no	no	no	no
28	GA08281-15ES1	124	16	32	3	67	21	55	17	0-19	no	no	no	no	no	no	no	no	no	no
29	GANC9337-15ES27	122	7	33	12	66	30	56	10	0-18	no	no	no	no	no	no	yes	no	yes	no
30	GA09343-15ES33	121	1	32	3	68	11	56	10	0-20	no	no	no	no	no	no	no	no	no	no
31	GANC 10014-15ES24	128	39	33	12	63	50	54	24	0-15	no	no	yes	no	no	no	yes	no	no	yes
32	KWS 053	122	7	34	20	68	11	54	24	0-18	no	no	no	no	no	no	yes	no	yes	no
33	KWS 060	126	27	37	45	68	11	61	1	0-20	no	yes	no	no	no	no	no	no	yes	yes
34	KWS 074	126	27	34	20	65	41	61	1	0-23	no	no	no	no	no	no	no	no	het	no
35	KWS 081	127	33	38	48	67	21	60	4	0-16	no	no	no	no	yes	no	yes	no	no	no
36	KWS 083	128	39	35	31	63	50	55	17	0-19	no	no	no	no	no	no	no	no	yes	no
37	KWS 087	126	27	34	20	67	21	56	10	21-0	no	no	no	no	no	no	no	no	yes	no
38	LA06146E-P4	121	1	32	3	65	41	47	49	0-17	no	no	no	no	no	no	yes	yes	yes	no
39	LA08090C-9-2	128	39	35	31	67	21	48	47	0-20	no	no	no	no	no	no	no	no	no	no
40	LA08265C-50	124	16	35	31	68	11	54	24	0-17	no	no	no	no	no	no	yes	no	yes	no
41	LA09011UB-2	121	1	30	1	68	11	51	45	15-2	no	no	no	ND	no	no	no	no	yes	no
42	LA09225C-33	128	39	36	37	69	5	54	24	0-14	no	no	no	no	no	no	no	no	no	no
43	NC10435-11	123	12	34	20	68	11	53	35	13-1	no	no	no	no	no	no	yes	no	yes	yes
44	NC12-22225	128	39	33	12	65	41	53	35	17-5	Fhb1	yes	no	no	no	no	no	no	yes	no
45	NC13-20076	124	16	36	37	66	30	56	10	1-19	no	no	no	no	no	no	no	no	no	no
46	NC13-22350	127	33	34	20	66	30	56	10	13-4	Fhb1	yes?	Ning	no	no	no	no	no	no	yes
47	NC13-23449	127	33	36	37	69	5	56	10	0-16	no	no	no	no	no	no	yes	no	no	no
48	VA12W-68	123	12	32	3	66	30	53	35	21-0	no	yes?	no	no	ND	no	no	no	no	no
49	VA13W-38	122	7	32	3	67	21	53	35	0-18	no	no	no	no	no	no	yes	yes	no	no
50	VA09MAS6-122-7-1	124	16	31	2	68	11	57	9	0-20	no	no	no	no	no	no	no	no	no	no
51	VA08MAS1-188-6-4-1	124	16	32	3	66	30	53	35	0-19	het	no	yes	no	no	no	no	no	no	no
52	VA13FHB-26	125	22	35	31	65	41	55	17	0-19	no	no	het	no	no	no	no	no	no	no
53	VA14FHB-14	123	12	34	20	67	21	58	7	0-18	no	no	no	no	no	no	no	no	yes?	no
54	VA14FHB-13	122	7	33	12	66	30	55	17	0-17	no	no	no	no	no	no	no	no	yes	no
55	VA14FHB-28	121	1	34	20	69	5	54	24	0-20	no	no	no	no	no	no	no	no	no	no
	Mean	125		34		67		54		.										
	LSD (0.05)	4		4		.		.		.										
	CV%	1.7		5.6		.		.		.										
	Mean v GEBV Correlation	0.20		0.30		0.43		0.44												

EVALUATING METHODS OF UPDATING TRAINING DATA IN LONG-TERM GENOMIC SELECTION FOR FUSARIUM HEAD BLIGHT RESISTANCE IN BARLEY

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OBJECTIVE

To examine prediction accuracy and response to selection when updating the training population each cycle with the best predicted lines, the worst predicted lines, random lines, criterion-selected lines, or no lines.

INTRODUCTION

The improvement of populations in plant breeding through recurrent selection may benefit tremendously from genomic selection. Of particular worth are the high accuracies and shortened breeding cycles of genomic selection, which allow for greater genetic gains per unit time (Bernardo and Yu 2007; Heffner *et al.* 2009; Lorenz *et al.* 2011; 2012). Genomic selection has already been employed in established oat and barley breeding programs (Asoro *et al.* 2013; Sallam *et al.* 2015). The advantages of genomic selection depend on maintaining sufficient genetic gain over time. This requires accurate predictions, based on markers located throughout the genome, of the genotypic value of candidates within a selection population (Meuwissen *et al.* 2001). Training the statistical model necessitates reliable phenotypic data on a training population and sufficient marker data such that many or all quantitative trait loci (QTL) are in linkage disequilibrium (LD) with at least one marker (Lorenz *et al.* 2011). If such requirements are fulfilled, the training data will capture the effects of alleles at QTL. Selection can then act to increase the frequency of favorable alleles in a population and shift the mean of a population in a desirable direction.

Maintaining selection accuracy over time will likely require updating the training population with new genotypes and there are practical considerations in how a breeder selects lines to fulfill this need (Lorenz and Smith, 2015). All breeding programs will advance their "best" lines to the next stage of evaluation and this data could be used to update the training population. An important question is whether it is necessary to include other lines for phenotyping strictly for the purpose of building prediction models. If so, then then how to do this will be an important consideration when allocating resources for expensive traits like FHB resistance and DON concentration.

The objective of this study was to investigate various methods of updating a training population and their impact on genomewide recurrent selection. Using simulations, we envisioned a breeding program implementing genomewide recurrent selection for FHB resistance in barley. Six different training population update methods were compared, along with two scenarios of training population composition. Over 15 cycles of selection, we tracked prediction accuracy and response to selection.

METHODS AND MATERIALS

We simulated a barley breeding program selecting for FHB resistance using genomic selection and a one-year breeding cycle (Figure 1). To incorporate the observed LD structure in barley breeding populations into our simulations, we used empirical marker data from the University of Minnesota (UMN) and North Dakota State University (NDSU) breeding programs. Marker

genotypes from 768 six-row spring inbred lines at 3,072 bi-allelic SNP loci were obtained from the Triticeae Toolbox (T3) database (Close *et al.* 2009). Markers missing more than 10% data and lines missing more than 10% data were excluded. We set all heterzyogous genotype calls to missing and imputed missing genotypes using the mean genotype call across all samples, rounded to the nearest homozygote. This left a set of 764 breeding lines and 1,590 homozygous markers spanning 1,137 cM.

Genetic Model to Simulate QTL
Each iteration of the simulation was initiated by randomly selecting 100 SNP loci to become causal QTL. Genotypic values for QTL were drawn from a geometric series, as suggested by Lande and Thompson (1990). At the *k*th QTL, the value of the favorable homozygote was a^k, the value of the heterozygote was 0, and the value of the unfavorable homozygote was $-a^k$, where $a = (1-L)/(1+L)$. The value of the first allele of a QTL was randomly assigned to be favorable or unfavorable. The genotypic value of a given individual was calculated as the sum of the effects of QTL alleles carried by that individual. Phenotypic values were simulated by adding nongenetic effects to the genotypic values.

Phenotyping was assumed to take place in three environments with one replication. The variance of environmental effects and the variance of residual effects remained unchanged over cycles of selection, allowing the heritability to vary. The mean phenotypic value of each individual over the three environments was used in genomewide prediction.

Base Population and Cycle 1 of Genomic Selection
The base population (i.e. cycle 0 training population) consisted of genotypic and phenotypic data on the 764 breeding lines. Based on these simulated phenotypes, the top fifty (most resistant) UMN lines and the top fifty NDSU lines were intermated between breeding programs to generate the cycle 1 population. Specifically, fifty crosses were simulated, using each parent once, and twenty F_3-derived lines were generated per cross. Gametes were generated following Mendelian laws of segregation, with recombination events simulated according to the genetic map positions of all loci (Muñoz-Amatriaín *et al.* 2011) and assuming no cross-over interference or mutation. Population development resulted in a pool of 1,000 F_3 selection candidates.

The marker data for the training population and selection candidates comprised genotypes at all loci except the 100 QTL. This essentially simulated "genotyping" with complete accuracy. Monomorphic markers and those with a minor allele frequency less than 0.03 were removed prior to genomewide prediction. Marker effects were predicted using ridge-regression best linear unbiased prediction (RR-BLUP) according to the model $y = lu + Z_{TP}u + e$, where y was an Nx1 vector of the phenotypic means of N training population lines, *l* was a Nx1vector of ones, u was the grand mean, Z_{TP} was a Nxm incidence matrix of training population genotypes for m markers, u was a mx1 vector of marker effects, and e was a Nx1 vector of residuals. Elements of Z_{TP} were 1 if homozygous for the first allele, -1 if homozygous for the second allele, and 0 if heterozygous. Genotypic values of the F_3 selection candidates were predicted as $g=Z_{SC}u$, where g was a 1,000x1 vector of predicted genotypic values, Z_{SC} was a 1,000x *m* matrix of selection candidate genotypes, and u was a mx1 vector of predicted marker effects. Elements of Z_{SC} were the same as those in Z_{TP}.

Cycles 2 Through 15 of Genomic Selection
Subsequent cycles of the simulation consisted of three steps: 1) crossing and population development, 2) prediction and selection, and 3) training population updating. These are outlined in the diagram presented in Figure 1. Parents selected in the previous cycle were randomly intermated to form a pool of selection candidates. Again, fifty crosses were simulated and 1,000 F_3-derived selection candidates were generated. Prior to predictions, we removed monomorphic markers and those with a minor allele frequency less than 0.03 in both the pool of selection candidates and

in the training population. Since markers could become monomorphic due to selection or drift, the number of markers used for prediction decreased over breeding cycles. We predicted marker effects using the above linear model and phenotypic and genotypic data on the training population. These marker effects were then used to predict genotypic values of the 1,000 selection candidates, and those with the top 100 predicted genotypic values were designated as parents for the next cycle. A subset of all selection candidates were then designated as new additions to the training population according to one of the updating methods described below. We simulated phenotypes for these additions and merged the phenotypic and genotypic data to the pool of training population data.

Methods of updating the training population

Six different methods ("Top," "Bottom," "Random," "PEVmean," "CDmean," and "No Change") of updating the training population were explored in the simulations. Each method constituted an independent simulation experiment, and in each case 150 selection candidates from each cycle were chosen and added to the training population. For "Top" and "Bottom," selection candidates with the best ("Top") or worse ("Bottom") values were added to the training population. For "Random," a random sample of selection candidates were added to the training population, and for "No Change," the training population was not updated over breeding cycles. The other two methods were "PEVmean" and "CDmean" as described by Rincent et al. (2012). Using only genotypic data on all individuals, these algorithms aim to create a training population by optimally sampling individuals for which phenotypic data is available to predict the value of individuals for which no phenotypic data is available.

RESULTS AND DISCUSSION

Prediction accuracy (Figure 2) consistently decreased over cycles of selection for all methods of updating the training population and in both updating scenarios. Within and between scenarios, we observed differences among the update methods in the decay rate of prediction accuracy. A prominent observation was the precipitous decline in accuracy when not updating the training population (i.e. "No Change"). Early in breeding cycles, prediction accuracy for this method was similar to the remaining methods, but by cycle five had decayed beyond the remaining methods. As expected, identical trends were observed for "No Change" in both updating scenarios.

Among methods of actively updating the training population (i.e. excluding "No Change"), differences in prediction accuracy were observed in early cycles, but became increasingly similar in later cycles. The "Top" method resulted in a small, but noticeable accuracy advantage early on that persisted for several cycles. On the other hand, the "Bottom" method displayed a noticeable disadvantage that persisted for a similar length of time. The "Random," "PEVmean," and "CDmean" methods were highly comparable and yielded accuracies intermediate of the "Top" and "Bottom" methods. By cycle ten, the differences between active methods of updating were negligible. These patterns were observed in both the "Cumulative" and "Window" scenarios. Accuracy decay was slightly greater in the "Cumulative" scenario (Figure 2A) compared to the "Window" scenario (Figure 2B). By the fifteenth breeding cycle, the difference in these decay rates amounted to a difference in prediction accuracy of roughly 0.02 – 0.04.

In our simulation experiment of recurrent genomic selection, we confirmed the need to update the training population over breeding cycles. Among the tested methods of updating the training population, adding the lines predicted to have the greatest genotypic value (i.e. the "Top" method) is the most attractive. The desirability of this method stems not only from the resulting prediction accuracy and response to selection, but also from its simplicity and practicality. This means that a breeder can rely primarily on data from typical trials that include the best performing breeding lines to update training population data sets.

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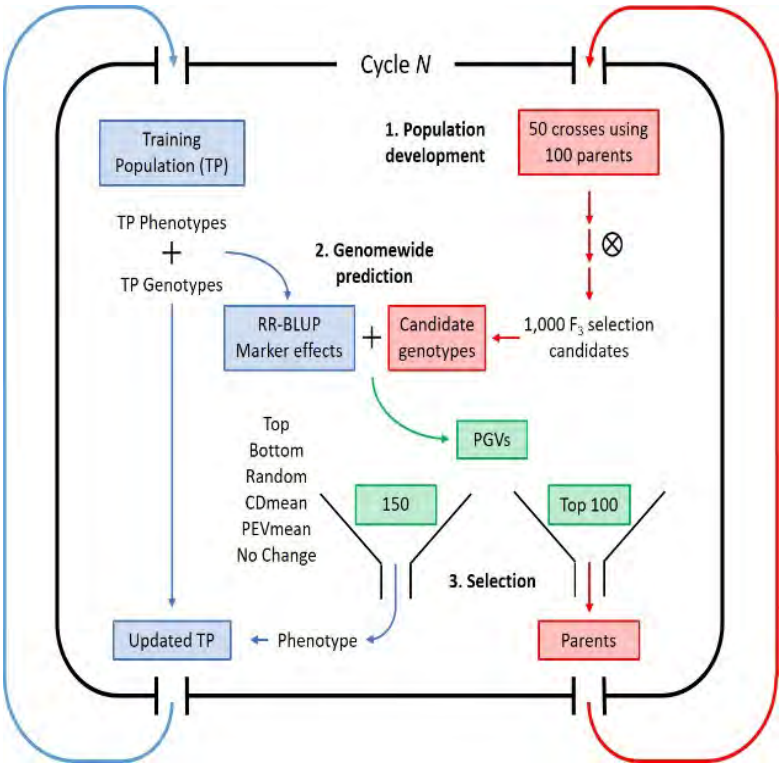

Figure 1. A single breeding cycle is broken down into two main streams. Blue indicates steps involving the training population, and red indicates steps involving crossing and population development. Green indicates the intermediate step of selection. 1) Fifty crosses are made using 100 randomly intermated parents from the previous cycle. Population development follows and 1,000 selection candidates are genotyped at the F3 stage. Marker effects are estimated using genotypic and phenotypic data from the training population (TP). 2) The predicted genotypic values of the selection candidates (PGVs) are used in decision-making. 3) The 100 best selection candidates are selected as

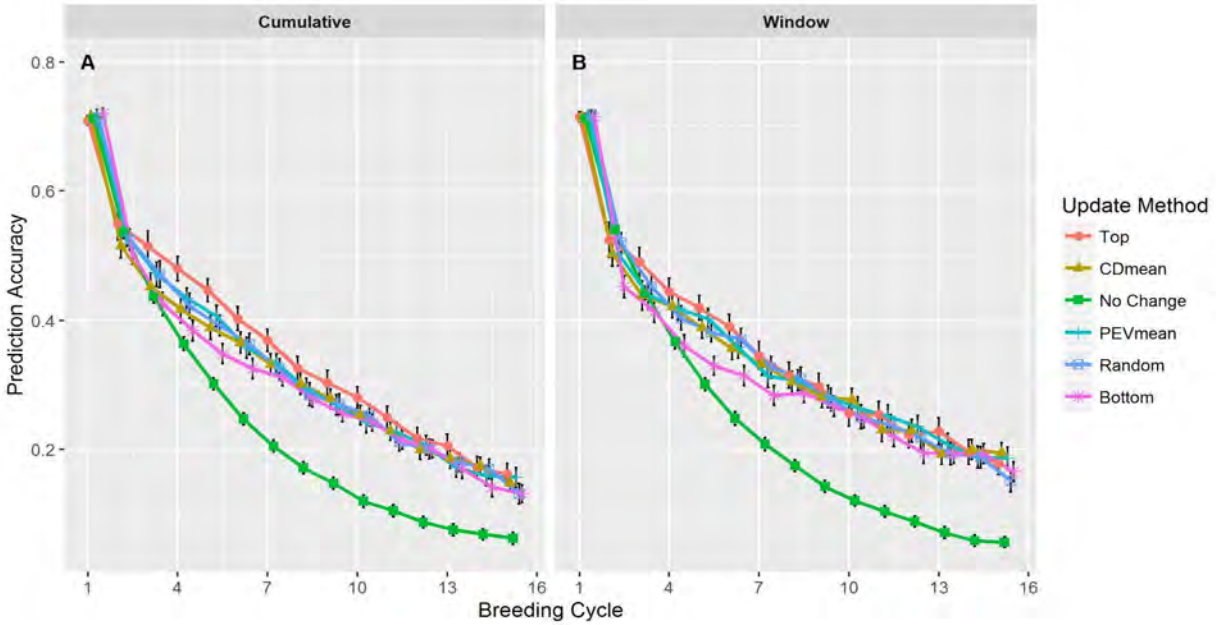

Figure 2. Prediction accuracy over breeding cycles of the simulation. Accuracy was measured as the correlation between the predicted and true genotypic values of the selection candidates. Line colors and point shapes delineate the different methods of updating the training population. Plots are separated into the "Cumulative" (A and C) and "Window" (B and D) updating scenarios. Average values are shown with 95% confidence intervals. To help reduce plot clutter, points for each update method are given a small, consistent jitter along the x-axis.

DISCOVERY OF *FUSARIUM GRAMINEARUM* RESISTANCE IN *AEGILOPS TAUSCHII* GERMPLASM AND INTROGRESSION INTO WHEAT

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ABSTRACT

Discovery of genetic resistance to Fusarium head blight (FHB), caused by *Fusarium graminearum*, is vital for the continued improvement of modern wheat. In this study, 109 accessions of the wheat D-genome progenitor species *Aegilops tauschii* Coss. were screened for FHB resistance. Greenhouse grown *Ae. tauschii* were infected with *F. graminearum* by single-floret inoculation (SFI) and disease severity was rated as the percentage of infected spikelets at 21 day post-inoculation. An apparent relationship was identified between the geographical origins of *Ae. tauschii* accessions and FHB resistance. Higher levels of FHB resistance were observed in accessions collected from regions bordering the Caspian Sea that receive higher levels of annual rainfall. In total, 12 resistant to moderately resistant *Ae. tauschii* accessions were identified. One accession, TA1662, with moderate resistance to FHB was crossed directly with the hexaploid wheat line KS05HW14 and backcrossed to restore typical D genome segregation. A population of 141 $BC_2F_{4:7}$ introgression lines (ILs) was planted in a replicated headrow nursery in Mason, MI, in 2015, and FHB incidence and severity were recorded. DNA was isolated from the ILs, genotyping-by-sequencing was performed, and linkage maps of introgressed loci were constructed. QTL analysis identified a QTL for FHB severity on the proximal portion of 7DL. Lines fixed for the *Ae. tauschii* allele at the 7DL QTL had lower average FHB severity than those fixed for the wheat allele. Then, using SFI under greenhouse conditions, three lines fixed for the *Ae. tauschii* allele at the 7DL QTL were compared to three lines fixed for the wheat allele. Those fixed for the *Ae. tauschii* allele had lower disease severity and fewer *Fusarium* damaged kernels (P-value < 0.05). The resistant germplasm identified and developed in this study will support long-term Fhb resistance breeding efforts.

DEVELOPMENT OF NEW WHEAT VARIETIES RESISTANT TO FHB THROUGH MICROSPORE *IN VITRO* SELECTION TECHNOLOGY

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ABSTRACT

Germplasm resistant to Fusarium Head Blight (FHB) is the most important and powerful tool to manage the disease. Based on natural selection, microspore culture offers a method to develop new germ lines with a varying degree of resistance to FHB. An *in vitro* selection in presence of *Fusarium* trichothecenes was used to develop homozygous doubled haploid lines of various genotypes showing resistance against FHB. Microspores from F_1 of 21 crosses of spring and winter wheat (*Triticum aestivum* L.) were subjected to selection against mixtures of *Fusarium* toxins (DON, 3-ADON, 15-ADON, NIV, T-2) in culture media. So far a total of 3232 doubled haploid lines were produced that have been incorporated into Canadian wheat breeding towards development of FHB resistant germplasm. The presence of trichothecenes in media had deleterious effects on the viability of microspores, formation of embryo like structures and regeneration rate of plants from embryo like structures. The response of different crosses was different to mycotoxins and as well as to media components and growth regulators. We found a novel epigenetic modifier Trichostatin A to be very efficient in stimulating embryogenesis and improving regeneration of plantlets. We were able to regenerate doubled haploids for crosses very recalcitrant to tissue culture. The doubled haploids lines produced through microspore culture technique will be screened for FHB resistance and DON accumulation in growth chamber followed by field trials in different regions of Canada.

TRENDS IN FHB RESISTANCE IN THE NORTHERN UNIFORM FHB NURSERY

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ABSTRACT

Wheat breeders in the Northeastern USA have been entering breeding lines in two uniform Fusarium Head Blight (FHB) nurseries (termed the PNUWWSN and NUWWSN) for many years. Each breeder has different criteria for entering lines into these tests, though most lines are elite lines and are candidates for release as new cultivars. Thus tracking trends over years in these trials is a way to assess changes in FHB resistance. The trials are conducted in multiple locations each year and data is collected on multiple FHB traits. The same checks have been used since 1998: Ernie [MR], Freedom [MR] and Pioneer 2545 [S]. In this study, we will assess the change in FHB trait values, the incidence of lines superior to the moderate resistant checks, and the incidence of QTL associated with FHB resistance over time.

A total of 1,212 lines from 18 breeding programs from 1998 to 2016 were evaluated. We determined the mean of lines first tested in a particular year and then regressed that mean on that year for each of seven FHB traits. Principal component analysis of the seven traits was conducted and the first PC score for each line was used as an integrative trait. Trends over time were clearer when using standardized data than when using simple Best Linear Unbiased Predictions (BLUPs). Overall years, there was a significant ($p < 0.05$) reduction in field severity (r^2=0.577), index (r^2=0.74), and ISK (r^2=0.29). There was considerable noise in the data from 1998 to 2002, and so we performed a second set of regressions using only data from 2003 to 2016. In these analyses, there was a significant reduction in severity (r^2=0.62), index (r^2=0.72), ISK (r^2=0.47), DON (r^2=0.43), and PC1 score (r^2=0.59). There has been a significant increase in the percentage of lines that have lower FHB trait values than the MR checks for all traits except for DON. In 1998 & 1999, just 19.8% of the lines were numerically lower than the MR checks for index. In 2015 & 2016, 68% of the lines had lower index values than the MR checks. On average 66% of the entries are better than the MR checks today, versus just 38% in 1998 & 1999. While no trend was noted for DON, in 2015 and 2016, 54.7% of the lines had lower DON than the MR checks and 33% had lower DON than Truman (the "R" check since 2004).

These analyses revealed a significant reduction in FHB trait values for lines entered in the USWBSI tests and especially since 2003. This is accompanied by a significant increase in lines with trait values that are lower than those of the MR checks.

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A META-ANALYSIS OF THE GENETICS OF FUSARIUM HEAD BLIGHT RESISTANCE IN BARLEY

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ABSTRACT

Fusarium head blight (FHB) devastated the once thriving malting barley industry in the Upper Midwest region of the USA after a series of epidemics starting in 1993. Due to the severe losses caused by these epidemics, breeding for resistance to FHB and the accumulation of mycotoxins produced by causal *Fusarium* pathogens became a priority for many barley improvement programs across North America. Extensive screening efforts of over 30,000 *Hordeum* accessions to FHB revealed very few sources of resistance. Early classical genetic studies revealed the quantitative nature of FHB resistance in barley. Subsequently, a number of molecular mapping studies were initiated to elucidate the number, chromosomal location and effect of resistance loci in these sources. To summarize this body of research, we conducted a meta-analysis of quantitative trait loci (QTL) reported for reduced FHB severity and DON accumulation, along with various agro-morphological traits thought to affect them, based on a single consensus map. This consensus map was constructed using marker data from eight mapping populations, plus two previously developed consensus maps based on simple sequence repeat and single nucleotide polymorphism markers. Consensus map construction was done using linear programming implemented in the LPmerge package of R. Marker order in the consensus map displayed high collinearity with other genetic maps for all chromosomes with an average correlation of 0.97. Sixty-seven and forty unique QTL were detected for low FHB severity and DON accumulation, respectively. These QTL were found across each of the seven barley chromosomes with most explaining just a small portion of the total phenotypic variation. Additionally, many of these QTL were not robust because they were detected in only one of several trials conducted at various locations over multiple years. Agro-morphological traits are thought to influence the level of FHB severity developing on barley. This aspect was investigated by considering these traits together with FHB severity and DON concentration on the consensus map. In chromosome 2H, several major effect genes such as *Ppd-H1* and *Eam6* for heading date, *Vrs1/vrs1* for two-rowed vs. six-rowed spike type and *Cly1*/*cly1* for chasmogamous vs. cleistogamous florets map to locations coincident for QTL controlling low FHB severity and DON. The same was true for the *Nud*/*nud* gene controlling the hulled vs. hulless character in chromosome 7H. These results suggest that some genes controlling agro-morphological traits may have a pleiotropic effect on FHB severity and the subsequent accumulation of mycotoxins. Although cultivars with moderate FHB resistance have been developed (e.g. Quest), the rate of progress has been relatively slow due to a lack of good resistance sources, the complex genetics underlying the trait, the variability associated with screening and selecting for FHB resistance in the field and the pleiotropic effect of various agro-morphological traits. Genomic selection offers a promising new approach for breeding for low FHB severity and DON accumulation in barley--whether the underlying selected loci represent true active resistance genes or the pleiotropic effect of an agro-morphological trait.

DEVELOPMENT OF HIGH-THROUGHPUT DIAGNOSTIC MARKERS FOR *FHB1*, A MAJOR GENE FOR FHB RESISTANCE IN WHEAT

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ABSTRACT

Fusarium Head Blight (FHB), mainly caused by *Fusarium graminearum*, is one of the most devastating wheat diseases worldwide. FHB not only significantly reduces grain yield but also affects grain quality due to *Fusarium* damaged kernels and mycotoxin contamination. Although FHB resistance is controlled by quantitative trait loci (QTLs), *Fhb1*, a QTL located on the short arm of chromosome 3B, shows a consistent major effect on reducing the disease spread within a spike in different genetic backgrounds. *Fhb1* has been widely used in wheat FHB resistance breeding programs worldwide. However, FHB resistance evaluation is laborious, time-consuming, and significantly influenced by the environments, which has significantly affected effective transfer of *Fhb1* into locally adapted wheat cultivars in breeding programs. Marker-assisted-selection (MAS) can increase the precision and efficiency of selection for a specific gene in breeding. Previously several markers tightly linked to *Fhb1* have been used for MAS, including the *Fhb1* flanking makers *Gwm533* and *Gwm493*, and tightly linked STS markers *STS256* and *UMN10*, and SNP markers *SNP8* and *SNP319*. However, none of them are functional markers, increased false positives reduces selection efficiency. Therefore, development of user-friendly and high-throughput diagnostic markers for *Fhb1* becomes critical for success in use of the gene in wheat breeding. More recently, we have cloned an *Fhb1* candidate by map-based cloning and found that lose-of-function of the gene confers FHB resistance. Two functional markers, *Fhb1-STS* and *Fhb1-KASP*, are developed based on the causal variation in the gene. *Fhb1-STS* is gel based marker that can be used in breeding programs with simple setup, whereas *Fhb1-KASP* is designed for medium throughput in these breeding programs that set up to run KASP markers. Both markers are codominance and feasible to genotyping segregating breeding populations, and are suitable for MAS to pyramid *Fhb1* with other resistance genes. Using thee markers, we screened a worldwide wheat collection and found that the *Fhb1* resistance allele is present only in some Chinese and Japanese accessions, not in the accessions from other areas. Among those with *Fhb1* resistance allele, many accessions including Sumai3, Ning7840, Huangcandou, Huangfangzhu, Baisanyuehuan, Wangshuibai and Nynbai have been reported to carry *Fhb1* in previous QTL mapping studies. Therefore, both *Fhb1-STS* and *Fhb1-KASP* can be used as diagnostic markers for *Fhb1* in wheat breeding programs.

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BORN, BRED AND BREWED IN NEW YORK

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ABSTRACT

The rapidly expanding craft brewing industry in New York has spurred interest in a self-sustaining local brewing economy from ground to glass. Barley production in New York is increasing with demand from craft malthouses but large scale production is still challenged by Fusarium head blight (FHB) and foliar pathogens. Variety testing over the past three years has not identified any cultivars with adequate FHB resistance and agronomics for New York. The Cornell Small Grains breeding program has begun a two-row spring malting barley breeding program to address these needs. High-throughput seed phenotyping and genomic selection are popular plant breeding buzzwords but their implementation in brand new breeding programs is can be challenging. We are using a single kernel near-infrared spectroscopy machine to phenotype large quantities of seed for malt quality traits and will be implementing multivariate genomic selection for disease traits, including FHB and deoxynivalenol, to rapidly advance superior breeding material to the evaluation stage.

ASSOCIATION MAPPING FOR FUSARIUM HEAD BLIGHT RESISTANCE IN SYNTHETIC HEXAPLOID WHEAT

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ABSTRACT

Synthetic hexaploid wheat (SHW) (×*Aegilotriticum* spp., 2n = 6x = 42, AABBDD) possess genetic diversity for resistance to several biotic and abiotic stresses. In order to investigate the prospects of transferring useful genes from wild and domesticated progenitors into hexaploid bread wheat (*Triticum aestivum* L.), we developed 150 SHW lines using durum wheat (*T. turgidum* L. var. *durum* Desf.) and other five tetraploid subspecies (*T. turgidum* spp. *carthlicum*, *dicoccum*, *polonicum*, *turgidum* and *turanicum*, 2n = 4x = 28, AABB) in crosses with *Aegilops tauschii* Coss. (2n = 2x = 14, DD). The goals of this project were to identify SHW lines carrying Fusarium head blight (FHB) resistance and to map putative novel FHB-resistant QTLs in the resistant SHW lines. In the evaluation experiments 150 SHW lines and their 73 tetraploid wheat parents have been tested in two greenhouse seasons and in the field nurseries at two locations (Fargo and Prosper, ND) for two years (2015 and 2016). The experiments were performed using a randomized complete block design (RCBD) with three replications. The common wheat cultivars 'Sumai 3' and 'Grandin' were used in all the experiments as resistant and susceptible checks, respectively. The statistical analyses of disease severity in the greenhouse and field nurseries showed a significant correlation among the experiments. According to the ANOVA and homogeneity tests, the FHB disease severity data from the two greenhouse seasons were pooled. For field experiments, the FHB data from the two locations were combined for each year. All the SHW lines and their tetraploid parents were genotyped using the Illumina wheat 9K-SNP array. When the mixed linear model (MLM) including both kinship and population structure was used for association mapping analysis, no significant associations were detected between marker data and disease severity. However, based on the general linear model (GLM) including population structure only, a number of marker loci showed significant association with disease severity both in the tetraploid and SHW lines. Several markers on chromosomes 1A, 2D, 3D, 6B and 7B of the SHW lines were verified in different environments including field and greenhouse seasons. By analyzing the FHB severity data, we found several resistant SHW lines having susceptible tetraploid parent, which supports the mapping results that the D genome has genomic regions associated with FHB resistance. These loci originated from *Ae. tauschii* may represent a source of novel resistance genes. Several SHW lines having resistant tetraploid parent showed as low FHB severity as the resistant check Sumai 3, indicating that they may be the useful base germplasm for improving wheat for FHB resistance in wheat breeding.

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MORPHOLOGICAL AND FHB TRAIT VARIATION IN THE ELITE EASTERN MAPPING PANEL

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ABSTRACT

Previous studies have suggested that morphological traits are related to FHB resistance. The objectives of this study were to evaluate morphological traits in the TCAP elite eastern mapping panel and use phenotypic and genotypic data to conduct a genome wide association study (GWAS). Two hundred sixty two wheat cultivars and breeding lines from the mapping panel were used in two experiments each conducted over two years (2015-2016) at Lexington, KY. In the first study, anther extrusion, plant height, spike length, spike density, number of florets, peduncle length and spike inclination were measured. Evaluation of FHB traits was carried out in an inoculated, irrigated nursery; heading date, plant height, disease incidence and severity, FHB rating, FHB index, *Fusarium* damaged kernels (FDK) and DON were measured. There were significant differences among the mapping panel entries for all traits evaluated. Significant genotype x year interaction for all morphological traits was observed; broad sense heritabilities ranged from 0.39 to 0.61. High heritabilities of all scab traits were recorded, though genotype x year interaction was significant. Correlations between morphological and scab traits varied by heading date. Eighteen of the panel entries had the R alleles at *Fhb1* though the average severity, FDK and DON was not lower in those lines than in the remainder of the entries. All FHB traits were significantly ($P < 0.05$) higher in lines with the height reducing alleles at the *Rht D-1* locus.

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DEVELOPMENT OF FUSARIUM HEAD BLIGHT RESISTANCE GERMPLASM IN HIGHLY ADAPTED SPRING WHEAT BACKGROUND

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ABSTRACT

Fusarium head blight (FHB or scab) caused by primarily by *Fusarium graminearum*, is one of the most devastating plant diseases to effect wheat (*Triticum* aestivum L.) and barley (*Hordeum vulgare* L.) worldwide. Despite the fact that FHB may decrease grain yield and quality, it may also lead to serious mycotoxin contamination in the infected grains, which is harmful to the health of human beings and livestock. Breeding for resistance to FHB in wheat is growing considering the scarce availability of varieties conveying adequate resistance to FHB. However, it has been demonstrated that pyramiding other resistance QTLs with *Fhb1* provides enhanced resistance to FHB. Therefore, our research here at SDSU was to screen for FHB severity using double haploid (DH) spring wheat lines derived from selected four-way crosses combining several sources of resistance, to validate *Fhb1* and putative QTLs (Xmc758, Gwm33, xbacr176, Xgm120, Xwmc317, Xwmc332, Xwmc522 and Xwmc296) that could minimize the threat of FHB including the reduction of mycotoxins, to the producers, processors, and consumers of wheat. A total of 225 spring wheat were initially screened in replicated field evaluation nurseries in 2014 and 2015 in three northern plains locations. Lines with low FHB severity were selected as putative resistant materials and were tested in for agronomical traits, in replicated trials, and fungicide application trials. We used molecular techniques to validate DH lines and their corresponding parents. In this study, we report on our finding that support recent discoveries of pyramiding different sources of FHB resistance with *Fhb1* as an opportunity to further enhance FHB resistance of adapted wheat germplasm.

DEVELOPMENT OF FUSARIUM HEAD BLIGHT RESISTANCE GERMPLASM IN HIGHLY ADAPTED WINTER WHEAT BACKGROUND

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ABSTRACT

Wheat is the third most important cereal in United States. However, production is severely constrained by many biotic stresses but the fungal pathogens *Fusarium graminearum* is the major causes of Fusarium head blight (FHB) which is a problematic disease for wheat and barley. FHB has seriously affected the production of wheat due to yield loss, low seed germination, and contamination of grain with mycotoxins. To date, no sources of resistance conferring complete resistance to FHB have been identified in wheat. We are using double haploid (DH) wheat lines derived from selected four-way crosses combining several sources of resistance to validate putative QTLs (Xmc758. Gwm33, xbacr176, Xgm120, Xwmc317, Xwmc332, Xwmc522 and Xwmc296) that could minimize the threat of FHB for the producers, processors, and consumers of wheat. This study attempted to develop and validate wheat lines that should display resistant characteristics to FHB given the materials genetic background. We report that over 50% of our lines had reduction to FHB which builds upon evidence accumulated from multiple studies in which pyramiding multiple sources and components of resistance with *Fhb1* serves to increase resistance to FHB.

EVALUATION OF WINTER BARLEY CULTIVAR EVE FOR QUANTITATIVE RESISTANCE TO FUSARIUM HEAD BLIGHT

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ABSTRACT

Fusarium head blight (FHB), caused by the pathogen *Fusarium graminearum* Schwabe, can result in severe yield and quality losses for barley (*Hordeum vulgare*) producers in the Mid-Atlantic region via kernel damage and production of mycotoxins. The demand for cultivars with enhanced resistance to prevalent diseases is essential to barley producers in order to meet the current and future market demands for winter barley in the production health foods, livestock feed, and malt products. The objectives of this study are to identify the FHB resistance QTL in the hulless winter barley cultivar Eve and to develop diagnostic markers for use in marker-assisted selection. Two mapping populations, comprised of recombinant inbred lines (RIL), were derived from crosses of 'Eve' to FHB susceptible parents (Eve/'Doyce' and Eve/VA07H-35WS) for use in mapping resistance to FHB. In 2015-2016 growing season, 180 RILs from each population were evaluated for FHB incidence and severity with the assistance from cooperators in KY and VA. In the 2014-2015 growing season both Eve RIL populations were evaluated in KY, VA, NC, and China for severity and incidence. Grain samples from both growing seasons were evaluated for *Fusarium* damaged kernels (FDK) and deoxynivalenol (DON) levels. In the Eve/Doyce (E/D) population a significant (P = <0.0001) correlations were observed between heading date and FHB incidence (r = -0.67376 and FHB severity (r = -0.61233) for only the 2016 Blacksburg, VA data. Eve populations were genotyped using a 9K SNP chip analysis. A putative QTL associated with higher FHB severity, FDK, and DON was identified in the E/D population with a logarithm of odds (LOD) of 5.57 and explaining 43.3% of the phenotypic variation. FHB resistant QTL identified in this population will be validated in the Eve/VA07H-35WS population and diagnostic markers will be identified for use in marker-assisted selection.

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EFFECTS OF ELEVATED [CO_2] ON THE DEFENSE RESPONSE OF WHEAT AGAINST *FUSARIUM GRAMINEARUM* INFECTION

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ABSTRACT

Fusarium head blight (FHB) is one of the world's most devastating wheat diseases, and results in significant yield loss and contamination of grain with harmful mycotoxins called trichothecenes. Despite emerging risks of increased mycotoxin contamination in food and feed associated with climate change, little is known about how rising [CO_2] will influence natural wheat resistance mechanisms against *Fusarium graminearum*, the primary etiological agent of FHB. In this study the defense response of wheat plants grown at ambient (400 ppm) [CO_2] and elevated (800 ppm) [CO_2] was evaluated and compared. The timing and magnitude of the phytohormone defense response was different at elevated [CO_2]. Additionally, pathogenesis-related (PR) and lipoxygenase (LOX) gene transcript levels and metabolite concentrations were altered. Our results suggest that elevated [CO_2] reconfigures the defense response of wheat leading to changes in susceptibility to FHB and mycotoxin contamination.

GENOME-WIDE ASSOCIATION MAPPING OF FUSARIUM HEAD BLIGHT RESISTANCE IN SPRING WHEAT LINES GROWN IN PACIFIC NORTHWEST AND CIMMYT

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ABSTRACT

Fusarium head blight is one of the destructive diseases of wheat in humid and semi-humid areas of the world. It has emerged in the Pacific Northwest (PNW) in recent years because of changing climate and rotation practice. The objectives of the present study were to characterize FHB resistance in spring wheat lines grown in PNW and CIMMYT and identify QTL associated with FHB resistance. A total of 170 spring wheat lines were evaluated in greenhouse and in field at Aberdeen, ID as well as at Saint Paul and Crookston, Minnesota in 2015 and 2016. Based on two years' data in greenhouse and field, 17 lines showing consistent resistance were selected as the starting resistance resources. These lines have no Sumai 3 or related backgrounds and can be used to develop FHB resistant cultivars for the PNW area. The 170 lines were genotyped using high-density Illumina 90K single nucleotide polymorphisms (SNPs) assay and ten other markers. A genome-wide association analysis was conducted with mixed model (Q+K). Consistent significant SNP associations with multiple traits (incidence, severity, FHB score, and deoxynivalenol concentration) were found on chromosome 2B, 4B, and 5B. The SNPs on chromosome 3B and the SSR marker *umn10* were not detected in any of the data sets, indicating the main FHB resistance loci in this panel does not include *Fhb1* locus. In summary, the resistance resources and associated SNP markers detected in this study can be used in the development of new FHB resistant cultivars in the PNW area.

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GROWERS' NEEDS AND INDUSTRY WANTS: A RETROSPECTIVE OF TWO DECADES IN THE TRENCHES IN THE BATTLE WITH FHB

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ABSTRACT

My position at the University of Minnesota was created in response to the historic FHB outbreaks of 1993 and 1994 in Minnesota and North Dakota. In the two decades since I took this appointment as extension specialist, I have been part of, and witnessed large changes in HRSW production practices. At first glance the public and private research communities have made gains in combating this opportunistic and ruthless pathogen. Albeit slower than producers and industry may have wanted or needed. Yet under this veneer of success lie some facts and statistics that suggest that a repeat of 1993 and 1994 epidemics is not out of the realm of possibilities, and that complacency has no place when it comes to scab management.

MOLECULAR MAPPING OF QTL FOR FHB RESISTANCE INTROGRESSED INTO DURUM WHEAT

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ABSTRACT

In the past years, great efforts have been devoted to introgress FHB resistance from tetraploid and hexaploid wheat accessions into adapted durum wheat cultivars. However, most of the quantitative trait loci (QTL) for FHB resistance existing in the introgression lines are not well characterized or validated. In this study, we aimed to identify and map QTLs for FHB resistance in durum line 10Ae564 and cultivar Joppa. 10Ae564 is a BC_1F_8 durum wheat line, which has FHB resistance derived from cross and backcross of the durum wheat cultivar Lebsock to PI 277012, a hexaploid wheat line carrying major FHB resistance QTL on chromosome 5A. Joppa is a newly released durum wheat cultivar with less FHB susceptibility than other durum wheat cultivars currently grown in North Dakota (ND), but no information is available on existence of QTL for FHB resistance in this cultivar. We developed a mapping population consisting of 205 recombinant inbred lines ($F_{2:7}$) from a cross between Joppa and 10Ae564. Genotyping was done with the wheat 90K-SNP chips and 6,323 polymorphic SNP markers were identified in the population. Excluding those co-segregated markers, 1,272 SNP makers were used to construct a genetic map, which consisted of 36 linkage groups with the total length of 472.14 cM. Phenotyping of the population for FHB reactions was also conducted in greenhouse for two seasons (2015GH and 2016GH), as well as in field FHB nurseries for three experiments (2015Fargo, 2015China and 2016Fargo). Meanwhile, grains of inoculated spikes collected from the 2015 greenhouse experiment (2015GH) and the 2015 Fargo field experiment (2015Fargo) were tested for DON content, referred to DON_2015GH and DON_2015Fargo, respectively. QTL analysis indicated that one QTL on chromosome 2A from Joppa and two QTL each on 5A and 7A from 10Ae564 were associated with FHB resistance. The 2A QTL was detected in the two greenhouse experiments (2015GH and 2016GH) and in two field experiments (2015Fargo and 2015China), explaining 15.4%, 17.3%, 8.8%, and 8.0 % of the phenotypic variation, respectively. The 7A QTL was detected only in the two greenhouse experiments (2015GH and 2016GH), explaining 10.4 and 12.6 % of the phenotypic variation, respectively. The QTL on 5A was detected in one greenhouse season (2015GH), one field experiment (2015Fargo), and the two DON tests (DON_2015GH, and DON_2015Fargo), which explained 20.0%, 17.9 %, 17.6, and 6.2% of phenotypic variation, respectively. The 2A QTL from Joppa was mapped to the QFhb.rwg-2A region identified in the ND durum cultivar Ben in a previous study. The 5A QTL was mapped to the same region where the major QTL Qfhb.rwg-5A.2 is located in PI 277102. However, the 7A QTL is located in a region where no FHB QTL have been reported and may represent a new QTL. The origin of the 7A QTL is not known, but it is probably from Lebsock, a parent in the pedigree of 10Ae564. This study further confirms that minor QTL exist in ND durum cultivars and combining major QTL from hexaploid wheat and native durum germplasm will be useful for improving durum FHB resistance.

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OTHER PAPERS

GRAINGENES: SUPPORTING THE SMALL GRAINS COMMUNITY

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ABSTRACT

Funded with hard funds by USDA, GrainGenes provides long-term data sustainability for small grains researchers and hosts a range of community newsletters, databases, and digital workspaces for wheat, barley, rye, and oats. GrainGenes is a gateway for integrated access to several types of peer-reviewed and curated genomic, genetic, and phenotypic data, along with QTLs and other experimental outcomes. The availability of reference genome assemblies of wheat and barley, along with their diversity data, is making a significant impact at GrainGenes and we are creating genome-centric views on our interface with rich links to data that is already housed at GrainGenes, curated over decades. We recently updated the GrainGenes Genome Browser with JBrowse, and are creating training videos for our users to smooth the learning curve for the new interface. GrainGenes will continue creating/implementing new tools and views for the small grains community, supporting them in their research, and providing them a long-term repository for their peer-reviewed experimental and computational data.

EXPRESSION OF AN *ARABIDOPSIS* NON-SPECIFIC LIPID TRANSFER PROTEIN IN PICHIA PASTORIS AND WHEAT

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ABSTRACT

Previously we found that overexpression of a non-specific lipid transfer protein (nsLTP), AtLTP4.4 (AT5G55450) in *Arabidopsis* and yeast protects against trichothecene-induced ROS stress. Protoplasts isolated from *Arabidopsis* expressing the nsLTP had basal ROS levels substantially lower compared to wild type (Col-0) protoplasts. Moreover, exposure to DON induced ROS generation in wild type protoplasts while protoplasts isolated from the AtLTP4.4-GFP transgenic line did not accumulate ROS. This ROS-protective mechanism of LTP protein likely accounts for observed protection against trichothecenes. To determine if this trichothecene protection mechanism extends to wheat, we have generated transgenic wheat lines expressing AtLTP4.4 and codon-optimized (for wheat) AtLTP4.4. We identified Bobwhite, Rollag, Forefront and RB07 lines that express high levels of nsLTP mRNA, but fail to produce detectable protein via Western analysis. Preliminary evidence suggested that the GFP-fusion may help stabilize the AtLTP4.4 protein and thus impact resistance to trichothecenes. We are currently analyzing transgenic wheat lines containing GFP fusions of AtLTP4.4 and a wheat nsLTP (TaLTP3/AY226580). In addition, to understand the mechanism of protection, we have produced both AtLTP4.4 and AtLTP4.4-GFP fusion proteins using the *Pichia pastoris* system for analysis of protein stability and efficacy. We generated X33 (Mut^+) and KM71H (Mut^s) transformants containing both the mature version of nsLTP and a GFP-tagged version of nsLTP. The results of the *P. pastoris* expression system and isolation of the protein will be presented.

INDEX OF AUTHORS

Acharya, R. - 75
Adam, Gerhard - 51
Alameldin, Hussien - 41
Ali, Shaukat - 34, 99, 100
Anderson, James A. - 77, 99, 103
Arcibal, S.S. - 3
Arellano, Consuelo - 17
Arens, Amanda - 27
Arguello, Nelly - 93
Baenziger, P. Stephen - 9, 11
Bai, Guihua - 95
Baldwin, Thomas - 45
Barr, John - 37
Bergstrom, Gary - 18, 59
Bernardo, Amy - 95
Berthiller, Franz - 51
Bibb, Jenny - 33
Bleakley, Bruce - 7
Blechl, Ann - 54
Bolanos-Carriel, Carlos - 9, 11
Bowen, Kira - 13, 15
Bregitzer, Phil - 45, 69
Breunig, Mikaela - 16
Brisco, Elizabeth - 41, 91
Brooks, W. - 101
Brown, Linda K. - 91
Brown-Guedira, Gina - 75, 83, 101
Brueggeman, R. - 27, 37, 101
Buck, James - 33
Byamukama, Emmanuel - 7, 34
Byrne, A.M. - 16
Caffe, Melanie - 100
Carpenter, Neal - 76, 101
Chao, Shiaoman - 97, 101, 105
Chapara, Venkata - 27
Chen, Jianli - 103
Chen, Wanxin - 46
Chilvers, Martin - 16
Clark, Anthony - 98, 101
Clemente, Thomas E. - 48
Conley, Emily - 77
Cowger, Christina - 17, 101
Cummings, Erica - 22
Cummings, Jaime - 18
Cuperlovic-Culf, Miroslava - 102
Daba, Sintayehu - 78
Dahal, Subha - 57
Darby, Heather - 22
Deising, Holger B. - 46
Deplazes, Chad - 27
Diazgranados, Mauricio - 52
Dill-Macky, Ruth - 65, 82
Dong, Yanhong - 51, 65, 103
Doohan, Fiona - 47
D'Ovidio, Renato - 54
Dowell, Floyd - 60
Eckard, Jonathan T. - 99, 100
Elias, Elias M. - 82, 97
El-Mounadi, Kaouar - 53
Eranthodi, Anas - 58
Eskridge, Kent, M. - 9, 11
Eudes, François - 92
Faroud, Nora - 58, 92
Favaron, Francesco - 54
Fellers, John - 53
Finn, Dan - 110
Fitzgerald, Josh - 76
Fonseka, Dimitri - 27
Friesen, T.L. - 97
Friskop, Andrew - 27
Fritz, Allan - 60
Fruhman, Philip - 51
Fulcher, Michael - 59
Funnell-Harris, Deanna - 9, 11, 48, 79
Gaire, Rupesh - 78
Galla, Aravind - 57
Gantulga, Dash - 64
Geppert, R. - 34
Ghavami, Farhad - 82
Gillespie, James - 37
Glover, Karl D. - 99
Gonzalez-Hernandez, Jose L. - 80, 99, 100
Goyal, Ravinder K. - 58
Gunupuru, L. - 47
Graf, Robert - 92
Graybosch, Robert A. - 48, 79
Griffey, Carl - 76, 101
Gross, Patrick - 27
Gross, Thomas - 37
Gruszewski, Hope - 28
Gu, Yong Q. - 109
Haas, M. - 94
Hallen-Adams, Heather - 9, 11
Hametner, Christian - 51
Hamzah, Haider - 52
Hane, David L. - 109
Hanlon, Regina - 28
Hao, Guixia - 49, 102
Harrison, Steve - 33
Holder, Amanda - 81
Huang, Mao - 93
Huang, Yadong - 50, 51
Hucl, Pierre - 92
Islam, Tariq - 53
Jackson, C.A. - 3
Janni, Michela - 54
Javaid, Tahir - 41
Jiang, Fengying - 92
Jin, Sujuan - 95
Jin, Zhao - 37
Jones, L.L. - 3
Jung, Sunghwan - 28
Kahla, A. - 47
Kalil, Audrey - 27
Kamaran, Sohail - 41
Kastner, Christine - 46
Kathiria, Palak - 92
Kaur, Jagdeep - 53
Kelly, Amy - 63
Kennell, John - 52
Kianian, Shahryar - 82
Klassen, Natalie - 103
Kleczewski, Nathan - 29
Knott, Ken - 64
Kolb, Frederic L. - 61
Kulkarni, P. - 7
Kumar, Ajay - 82
Kumar, Jitendra - 82
Kumlehn, Jochen - 46
Lana, F.D. - 31
Larson, Erick - 33
Lazo, Gerard R. - 109
Lemes da Silva, Cristiano - 60
Lemmens, Marc - 51
Leng, Yueqiang - 105
Li, Lin - 50
Li, Xin - 51
Liber, Julian - 41
Ling, Wenjing - 30
Lorenz, Aaron J. - 86
Lyerly, J.H. - 75, 83
Ma, Anjun - 57
MacKillop, Michael - 52
Madalà, Giulia - 54
Madden, Laurence V. - 30, 31
Malachova, Alexandra - 51
Malla, Subas - 76, 101
Marais, Francois G. - 100
Marshall, Juliet - 3
Mason, Eston R. - 81
McCormick, Susan - 49, 51, 64, 102, 110
McLaughlin, John - 110

McMaster, Nicole (Niki) - 11, 64, 76, 101
Mergoum, Mohamed - 99
Michlmayr, Herbert - 51
Mideros, Santiago - 61
Mohammedi, Mohsen - 78
Moon, David E. - 81
Moraes, Wanderson Bucker - 31
Moscetti, Ilaria - 54
Muehlbauer, Gary - 50, 51
Murphy, J. Paul - 75, 83, 101
Murthy, N.K.S. - 7
Nagelkirk, M. - 16
Neyhart, Jeffrey - 86
Nowara, Daniela - 46
Odell, Sarah G. - 109
Oliveira-Garcia, Ely - 46
Olson, Eric - 41, 91
O'Neill, Patrick M. - 79
Padgett, Boyd - 33
Paudel, Bimal - 57
Paul, Pierce Anderson - 30, 31
Peiris, Kamaranga - 60
Perochon, A. - 47
Pirseyedi, Seyed M. - 82
Poland, Jesse - 60
Powers, Craig - 28
Price, Trey - 33
Purvis, Myra - 33
Randhawa, Harpinder S. - 92
Ransom, J. - 27
Rutten, Twan - 46
Ryabova, Daria - 58, 92
Salazar, Melissa - 61
Sallam, A. - 94
Samuels, Peter L. - 65
Sarinelli, J.M. - 75, 83
Sattler, Scott E. - 48
Savatin, Daniel - 54
Schatz, Blaine - 27
Schmale III, David G. - 11, 28, 64, 76, 101
Schwarz, Paul B. - 31, 37
Schweiger, Wolfgang - 51
Schweizer, Patrick - 46
See, Deven R. - 103
Sehgal, S. - 34, 100
Sen, Taner - 109
Senger, Ryan - 64
Shah, Dilip - 53
Shelman, T.L. - 3
Shin, Sanghyun - 51
Slominska, Karolina - 46
Smith, Kevin P. - 50, 86
Sneller, Clay - 93
Somers, Katrina - 28
Sorrells, Mark - 96
Spaner, Dean - 92
St. Amand, Paul - 95
Steffenson, Brian - 94
Sticklen, Mariam - 41
Streit, Sebastian - 62
Su, Zhenqi - 95
Sweeney, Daniel - 96
Szabo-Hever, Agnes - 97
Tessman, Lisa - 98
Thurston, Yaqoob - 99, 100
Tiede, Tyler - 86
Tiedemann, Andreas von - 62
Trick, Harold - 110
Tumer, Nilgun - 110
Tundo, Silvio - 54
Tyagi, Neeri - 110
Tyagi, P. - 75, 83
Ullrich, Jordan - 101
Van Sanford, David - 98, 101
Vaughan, Martha - 49, 102
Velivelli, Siva - 53
Vermillion, Karl - 102
Walter, S. - 47
Wang, Rui - 103
Ward, Brian - 76
Ward, Todd - 63
Wegulo, Stephen - 9, 11, 48, 79
West, Hayley - 41
Wheeler, Justin - 103
Wiersma, Andrew T. - 91
Wiersma, Jochum - 104
Wiesenberger, Gerlinde - 51
Wilson, Nina - 64
Winter, Mark - 62, 65
Xu, Steven S. - 82, 97, 105
Yabwalo, Dalitso N. - 7, 34
Yen, Yang - 57
Youmans, John - 33
Zavadil, Andrea - 57
Zhang, Junli - 103
Zhang, Q. - 97
Zhao, Mingxia - 105
Zhao, Weidong - 103
Zhao, Yusheng - 46
Zhong, Shaobin - 97, 105
Zhou, Bing - 37

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