USDA-ARS, OHIO STATE UNIVERSITY COOPERATIVE RESEARCH ON BIOLOGICALLY CONTROLLING FUSARIUM HEAD BLIGHT: 3. FIELD TESTING OF ANTAGONISTS

M.J. Boehm[1*], N.I. Khan[1], and D.A. Schisler[2]

OBJECTIVE

Determine if microbial strains effective in reducing Fusarium head blight severity in greenhouse bioassays are effective in reducing FHB in field trials on soft red winter wheat cultivars which vary in susceptibility to FHB.

INTRODUCTION

Over the last two years, research conducted at the NCAUR in Peoria, IL, in conjunction with The Ohio State University (Khan et al, 1998a; Khan et al., 1998b; Khan et al., 1999a; Khan et al., 1999b; Schisler et al., 1999a; Schisler et al., 1999b), has culminated in the isolation, screening and identification of several putative biological control agents for suppression of FHB. This update provides a summary of the results obtained in our 1999 field evaluation trials conducted on soft red winter wheat cultivars in Ohio and Illinois. Results of the 1998 field tests on the soft red winter wheat cultivar 'Pioneer 2545' were presented previously (Khan et al., 1998b; Schisler et al., 1999a).

MATERIALS AND METHODS

Isolation, screening and selection of microbial antagonists

As described previously (Khan et al., 1998a; Khan et al., 1998b; Schisler et al., 1999a Schisler et al., 1999b), approximately 740 strains of microorganisms were isolated from wheat anthers collected from fields in OH and IL and screened for their ability to either use choline as a sole carbon source or for *in vitro* inhibition of *Fusarium graminearum*. Strains capable of using choline or inhibiting *F. graminearum* were subsequently screened for their ability to suppress FHB in a series of greenhouse assays (Khan et al., 1998b). Strains capable of significantly reducing the severity or incidence of FHB were further evaluated for their ability to suppress various isolates of *F. graminearum* (Khan et al., 1999a; Khan et al., 1999b). Seven strains, four yeasts and three *Bacillus* sp., were selected for field testing on either or both durum and soft red winter wheat. The ability of several of these isolates at suppressing FHB on durum wheat is presented in another report at this symposium (Schisler et al., 1999b).

1999 Peoria, IL, and Wooster, OH, field trials of FHB antagonists on soft red winter wheat cultivars 'Pioneer 2545' and 'Freedom'

Three *Bacillus* sp. and three yeast isolates (Table 1) were screened on soft red winter wheat cultivars "Pioneer 2545" and "Freedom." These cultivars were selected because of their widespread use throughout the Midwest and in an attempt to acquire preliminary data regarding the integration of biocontrol and genetic resistance for managing FHB. Antagonists were produced in liquid culture in Fernbach flasks as described previously (Schisler et al., 1999b) and applied at the beginning of flowering in aqueous suspensions. Bacterial suspensions contained either 10% or 50% liquid culture or ~$2x10^8$ or $1x10^9$ cfu/ml, respectively. Yeast suspensions contained either 10% or 50% liquid culture or

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~$1x10^7$ or $5x10^7$ cfu/ml, respectively. Pathogen inoculum consisted of ascospores released from *F. graminearum* Z3639 colonized corn kernels scattered throughout the plots (~25-40 kernels/ m^2) 2 wk prior to wheat flowering. Non antagonist/buffer treated plants served as controls. Mist irrigation was applied regularly to promote FHB disease development. Plots were scored for disease severity and incidence and harvested to determine 100 kernel weights. Randomized complete block designs were used in both trials (*n*=4 in Peoria; *n*=6 in Wooster).

RESULTS AND DISCUSSION

In the field trial at Peoria, IL, five of the six antagonists tested reduced FHB disease on wheat cultivar 'Pioneer 2545' at one or both of the concentrations assayed (Table 2) despite poor environmental conditions for disease development across the Midwest. On cultivar 'Freedom', all six antagonists reduced FHB disease at one or both of the concentrations tested. At the Wooster, OH, field site, five of the six antagonists reduced FHB disease at one or both of the concentrations assayed (Table 3) on cultivar 'Pioneer 2545'. Yeast antagonist OH 71.4 reduced disease severity by nearly 56%. Antagonists OH 131.1, OH 181.1 and OH 182.9 reduced disease severity and disease incidence at both of the antagonist cell concentrations tested (Table 3). No appreciable amount of disease was observed on the 'Freedom' plots in Wooster.

The potential for these microbial antagonists to contribute to the management of FHB was demonstrated in these replicated field trials. Future research focusing on the: 1) development of liquid culture fermentation methodologies aimed at enhancing biocontrol agent efficacy; 2) determination of the genes, regulatory mechanisms or other cellular processes responsible for biocontrol agent efficacy; and, 3) continued evaluation of biocontrol agent efficacy under field conditions at various sites, on various hosts, and in tandem with other management practices, may greatly enhance our ability to manage FHB.

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Table 1. Microbial antagonists effective in reducing the severity of Fusarium head blight in greenhouse bioassays and selected for evaluation in the 1999 field tests.

Antagonist Strain	Accession Number	Type of Organism
AS 43.3	NRRL B-30210	*Bacillus* sp.
AS 43.4	NRRL B-30211	*Bacillus* sp.
OH 71.4	NRRL Y-30213	Yeast
OH 131.1	NRRL B-30212	*Bacillus* sp.
OH 181.1	NRRL Y-30215	Yeast
OH 182.9	NRRL Y-30216	Yeast

Table 3. Influence of two antagonist cell concentrations on Fusarium head blight development on soft red winter wheat cultivar Pioneer 2545" in a 1999 field trial at Wooster, Ohio[a].

Cultivar Pioneer 2545"				
	10% Antagonist		50% Antagonist	
Treatment	Disease[b] Severity (%)	Disease Incidence (%)	Disease Severity (%)	Disease Incidence (%)
F. graminearum	11.0	34.4	11.0	34.4
AS 43.3	9.8	31.4	10.3	33.9
AS 43.4	8.5	33.6	10.7	38.1
OH 71.4	4.8*	23.1*	9.6	30.6
OH 131.1	6.5*	25.3*	5.1*	22.0*
OH 181.1	6.7*	27.8*	6.3*	25.3*
OH 182.9	4.6*	23.1*	7.1*	27.0*

[a] Wheat heads were sprayed to run-off with an antagonist cell suspension. Naturally occurring inoculum of *F. graminearum* was supplemented with ascospores released from *F. graminearum* Z3639 colonized corn kernels that had been spread across the test plot (≈20 colonized kernels/m^2).

[b] Within a column means followed by an asterisk are significantly different from the *F. graminearum* control ($P \leq 0.05$).

Table 2. Influence of two antagonist cell concentrations on Fusarium head blight development on soft red winter wheat cultivars Pioneer 2545 and Freedom in a 1999 field trial at Peoria, Illinois[a].

	Cultivar Pioneer 2545"						Cultivar Freedom					
	10% Antagonist			50% Antagonist			10% Antagonist			50% Antagonist		
Treatment	Disease[b] Severity (%)	Disease Incidence (%)	100 kernel wt. (g)	Disease Severity (%)	Disease Incidence (%)	100 kernel wt. (g)	Disease Severity (%)	Disease Incidence (%)	100 kernel wt. (g)	Disease Severity (%)	Disease Incidence (%)	100 kernel wt. (g)
F. graminearum	2.0	11.2	3.3	2.0	11.2	3.3	1.0	8.3	3.1	1.0	8.3	3.1
AS 43.3	1.0	6.2*	3.3	2.6	16.7	3.2*	0.4	3.8*	3.2*	0.2*	3.3*	3.1
AS 43.4	0.4*	5.4*	3.3	2..2	12.5	3.3	0.4	4.6	3.1	0.3*	4.2*	3.2
OH 71.4	1.1	8.8	3.4	0.6*	6.2*	3.2*	0.7	5.4	3.3*	0.2*	2.9*	3.2
OH 131.1	1.9	10.4	3.2*	0.7*	4.6*	3.3	0.7	6.7	3.2	1.6	6.2	3.3*
OH 181.1	1.6	9.2	3.4	2.4	9.2	3.1*	0.2*	2.9*	3.2	0.5	3.8*	3.3*
OH 182.9	0.9*	5.4*	3.4	1.6	6.7*	3.4	0.3*	3.3*	3.2*	0.6	3.8*	3.0

[a] Wheat heads were sprayed to run-off with an antagonistic cell suspension. Naturally occurring inoculum of *F. graminearum* was supplemented with ascospores released from *F. graminearum* Z3639 colonized corn kernels that had been spread across the test plot (~20 colonized kernels/m^2).

[b] Within a column, means followed by an asterisk are significantly different from the *F. graminearum* control ($P \leq 0.05$).

OH 131.1	1.9	10.4	3.2*	0.7*	4.6*	3.3	0.7	6.7	3.2	1.6	6.2	3.3*
OH 181.1	1.6	9.2	3.4	2.4	9.2	3.1*	0.2*	2.9*	3.2	0.5	3.8*	3.3*
OH 182.9	0.9*	5.4*	3.4	1.6	6.7*	3.4	0.3*	3.3*	3.2*	0.6	3.8*	3.0

A NOVEL METHOD TO IDENTIFY *FUSARIUM* SPECIES THAT ATTACH TO ROOTS OF WHEAT AND BARLEY SEEDLINGS

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OBJECTIVES

To examine roots of seedlings grown from FHB-contaminated seed for infection by *Fusarium* spp.

INTRODUCTION

Seed dressings effectively control *Fusarium* species permitting infested seeds to emerge and thrive. However, it is not known if *Fusarium* added to the soil on infested seed is able to infect root systems of the emerging plants, or if the fungicide contains the fungus at the seed surface. Roots were examined to determine *Fusarium* species and levels of infection among 3 varieties each of spring wheat and barley treated with 2 seed dressings.

MATERIALS AND METHODS

Germination and level of infection were determined on 2 replicates of 100 seeds of cv.s Glenlea (extra strong), AC Morse (durum) and AC Vista (Canada Prairie Spring) wheats, fungicide-treated, or left as an untreated control. The three barley varieties were Stander, Robust and Foster. Experimental design was a 4 replicate split plot with variety as the main plot and treatments as the subplots. Plots were 0.9 X 3 m consisting of 4 rows each 30 cm apart. At GS 15-20 roots were washed thoroughly in water and frozen for later examination. Each root was surface sterilized in 0.1% NaOCl for 1 min and allowed to dry under a laminar flow hood before being placed on PDA agar plates and covered with a thin layer of Komada's medium (specific for *Fusarium* species). Plates were examined for colonies of *Fusarium* sp. and transferred to fresh PDA plates for identification.

RESULTS

Germination of pre-treated seed ranged from 73% to 91%, and for infection with *Fusarium* spp. 16% to 47% (Table 1). Foster barley germinated at 91%, despite high (31%) *Fusarium* infection. However, there were no significant treatment or variety differences in *Fusarium* spp. and number of isolations from roots. Nine *Fusarium* species were identified from the roots (Table 2). The predominant species isolated were *F. equiseti* (43.8% of isolations), *F. graminearum* (17.9%), and *F. sambucinum* (13.3%).

Table 1. Mean percent germination and level of infection in *Fusarium*-infected spring wheat and barley cultivars.

	Germination (%)	Infection with *Fusarium* sp. (%)
Glenlea	76	31
AC Morse	76	32
AC Vista	73	47
Stander	87	20
Foster	91	31
Robust	87	16

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Table 2. Percent isolations of *Fusarium* species from roots of spring wheat seedlings.

Species	Isolations (%)
F. equiseti	43.8
F. graminearum	17.9
F. sambucinum	13.3
F. sporotrichioides	8.0
F. solani	5.7
F. poae	3.4
F. culmorum	3.0
F. oxysporum	3.0
F. avenaceum	1.9

CONCLUSIONS

Seed treatment did not reduce *Fusarium* levels on roots compared to untreated controls. A successful method for isolating *Fusarium* species from whole roots was developed.

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SPRAYER MODIFICATIONS FOR ENHANCED CONTROL OF FUSARIUM HEAD BLIGHT WITH FUNGICIDES

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INTRODUCTION AND OBJECTIVES

Fusarium head blight (FHB) has been difficult to control with fungicides; due in part, to the difficult target a grain spike presents. Deposition and retention of fungicide on the grain spike is hindered by the vertical orientation of the spike, along with the interference of awns, and the waxy, glabrous surface of the spike. Conventional application methods intended primarily for foliar targets have shown to be ineffective in delivering fungicide to the grain spike. This study was initiated to identify spray application parameters of currently available equipment that are most effective in improving fungicide deposition on the spike. The primary objective of the study was to correlate the amount of fungicide delivered to the spike with FHB control. The control of fungal leaf spot on the flag leaf was also measured.

METHODS

Two types of spraying systems (CO_2 pressurized and air assist), several nozzle orientations, and two water volumes were evaluated for delivery of fungicide to the grain spike. Applications were made to Grandin hard red spring wheat (*Triticum aestivum*) at the early flowering period. A three percent v/v solution of orange Day Glo dye was included in the spray for detection of spray captured by the spike. Selected grain spikes were detached and examined under ultra violet light for fluorescence, and a digital camera system measured the percent of grain spike covered by the dye. FHB incidence, FHB head severity, FHB field severity, and flag leaf disease were evaluated at Zadok's 85. A grain-spawn inoculated with *Fusarium graminearum* was evenly spread over the plot area two weeks prior to flowering to enhance FHB infection. Plots were harvested with a plot combine, and yield and test weights were determined. Treatments were randomized in a complete block design, and data were analyzed using GLM, and Pearson's correlation coefficient.

RESULTS

All applications of Folicur significantly reduced flag leaf necrosis and field severity over the check (Table1). Spike coverage increased by changing the nozzle orientation of Spray Air from vertical to 45º forward, and by changing the vertical orientation of the Standard sprayer nozzles to two XR11001 nozzles per opening, one angled forward, and one backward 30 degrees from horizontal (F+B). The XR11001 nozzles orientated F+B significantly improved control of field severity over straight down nozzle orientation treatments at 9 gallons per acre water volume. The XR11001 orientated F+B provided the lowest FHB incidence, FHB head severity, FHB field severity, yield, and test weight of all treatments. FHB parameters negatively correlated to amount of dye deposited on head (Table 2).

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Table 1. Comparison of Sprayers and Spray Nozzle Configurations for FHB Control

					FHB			Flag Leaf Necrosis				
Sprayer	Nozzle	Nozzle Orient.	gpa	Press-ure	Incid-ence	Head Severity	Field Severity	Leaf Rust	Leaf Spot[a]	Yield	Twt	Head Cover-age
				psi	%	%	%	%	%	bu/A	lbs/bu	%
Check	NA	NA	0	0	53.4	10.8	5.6	4.6	29.7	56.9	58.6	0.2
Spray Air	0.0424 Orifice	St Down	9	10	37.5	7.5	3.1	0	2.2	63.3	57.6	4.2
Spray Air	0.0424 Orifice	45" F	9	10	27.5	6.7	1.9	0	2.1	65.1	58.7	27.0
Standard	XR110 01	St Down	9	40	40.0	8.4	3.4	0.6	4.4	64.9	58.3	1.5
Standard	XR110 01	F+B	9	40	25.0	4.1	1.1	0	2.0	65.9	58.7	29.5
Spray Air	0.0424 Orifice	45" F	3	10	25.0	6.9	1.9	<0.1	2.8	64.5	57.7	10.6
LSD p>0.05					17.9	4.1	1.9	1.4	10.7	NS	NS	11.4
C.V. %					35	39	48	108	99	12	2	66

a. Leaf spots are a mix of tan spot and *Septoria nodorum* and *Septoria tritici*

Table 2. Pearson Correlation Coefficients between Coverage, Disease and Yield Parameters

	FHB Incidence	FBH Head Sev.	FHB Field Severity	Leaf Spot	Leaf Rust	Coverage	Yield	Test Weight
Incidence	1.0	0.49	0.87	0.78	0.61	-0.49	-0.69	-0.25
Severity	0.49	1.0	0.83	0.44	0.46	-0.28	-0.46	-0.08
Field Severity	0.87	0.83	1.0	0.80	0.68	-0.42	-0.63	-0.14
Leaf Spot	0.78	0.44	0.80	1.0	0.80	-0.35	-0.54	-0.04
Leaf Rust	0.61	0.46	0.68	0.80	1.0	-0.33	-0.42	<0.01
Coverage	-0.49	-0.28	-0.42	-0.35	-0.33	1.0	0.26	0.18
Yield	-0.69	-0.46	-0.63	-0.54	-0.42	0.26	1.0	0.54
Test Weight	-0.25	-0.08	-0.14	-0.04	<0.01	0.18	0.54	1.0

FUNGICIDE EFFICACY TRIALS AT MICHIGAN STATE UNIVERSITY

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Fungicides were evaluated for their potential to reduce the incidence and severity of Fusarium Head Blight (FHB) in winter wheat, and concomitantly for a reduction in levels of deoxynivalenol (DON). Three projects associated with the use of fungicides in disease management were conducted: 1) evaluate efficacy of different fungicides as part of a regional Uniform Fungicide Trial; 2) the effect of different application methods on FHB; 3) the effect of fungicide application timing on FHB. Winter wheat varieties used in these experiments were planted in October 5th, 1998. Fertilizer and herbicides were applied as per Michigan State University recommendations. Corn inoculum infested with *Gibberella zeae* was spread over the fields on May 4th and again on May 14th. Low volume overhead irrigation was turned on May 13th. Irrigation was on for 15 minutes and off for ninety minutes, 24 hours/day from May 13th to June 14th. The irrigation was turned off for 24 hrs the days fungicides were applied. In projects 1 and 2 the variety Harus was used, and in project 3 the varieties Frankenmuth, Pioneer 2510, and Freedom were used. Perithecia were first observed on the corn inoculum on May 17th, and mature ascospores were first observed on May 28th. Fungicides in project 1 and 2 were applied to Harus at Feekes growth stage 10.5 on June 3rd. For the timing experiment, Project 3, Folicur (4 oz/acre + 0.06% induce) was applied on Pioneer 2510 and Freedom on 28 May (GS 10.1) and 1 June (GS 10.5). Frankenmuth was treated only at GS 10.5 on June 1st. Fungicides were applied with a CO_2 backpack sprayer and flat fan nozzles in projects 1 and 3. For project 2, Folicur was applied at GS 10.5 on the variety Harus using either a Proptech sprayer, or boom sprayer with flat fan nozzles directed at sixty degree angle above the horizontal. Folicur was applied at two rates, 2.5 or 4.0 oz per acre + 0.06% induce. All treatments in each project were replicated three times. Each plot was rated three times for disease incidence and severity using the scoring system developed by Stack and McMullen. Mature grain was harvested, milled and analyzed for DON by ELISA (Hart et al., 1998).

RESULTS

Project 1: evaluate efficacy of different fungicides as part of a regional Uniform Fungicide Trial.

Differences in yield, FHB incidence and severity, and DON were not significantly different between treatments including the untreated controls (Table 1). The Folicur treatment had the lowest amount of disease and DON, and highest yield. The previous two years levels of DON in Quadris treatments were often significantly higher than the untreated controls but not in 1999. Maintaining irrigation for two weeks after flowering may have provided an environment highly favorable for FHB to develop, more so than under natural conditions, and may have minimized differences that would normally be larger.

Project 2: the effect of different application methods on FHB.

All treatments had significantly lower levels of

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DON (P=0.05) than the untreated controls, but were not signicantly different from each other.(Table 2). Adjusting the angle of the flat fan nozzles appears to be an effective method of application (as is recommended by North Dakota extension). The apparent efficacy of lower rates is interesting, but should be validated over years and environments prior to being made a recommendation. Project two used the same variety and conditions as project one, but fungicides were applied more like a commercial operation because the plot size was 21 x 84 feet compared to 10 x 15 feet for project one. The significant differences in DON levels could have resulted from a more efficient application of fungicides compared to CO_2 back pack sprayer applications used in project one .

Project 3: the effect of fungicide application timing on FHB.

Concentrations of DON were significantly lower across all three varieties sprayed with Folicur at GS 10.5 (Table 3). DON concentrations were significantly lower in Freedom sprayed at GS 10.1, but not in Pioneer 2510. Frankenmuth was not sprayed at GS 10.1. These results are in line with the work of others also showing overall better efficacy in spring wheat and barley when fungicides were applied at GS.10.5 compared to GS. 10.1.

SUMMARY

Fungicides may be an effective management tool for FHB in winter wheat. Timing of application, and possibly the application method could influence the efficacy of fungicide treatments. Although differences between fungicides were not significant, the trend for Folicur to reduce FHB and DON below all other fungicide treatments is in line with previous years (Hart et al., 1998; McMullen, 1998).

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Table 1: Results from 1999 FHB Uniform Fungicide Trial

		Yield	DON	Disease Rating 15-June	
Treatment	**rate**	**(bu/a)**	**(ppm)**	**Incidence**	**Severity**
BASF 500 00F	15.3 fl oz/a	52.7	9.3	48%	3.5
Benlate / Manzate	0.5 lb/a , 1 lb/a	48.7	9.6	63%	4.8
Folicur	4 fl oz/a	55.2	6.0	58%	3.0
Penncozeb	1 lb/a	51.2	10.9	62%	4.7
Quadris (9.6 oz/a)	9.6 fl oz/a	50.0	7.7	42%	3.7
Quadris (12.3 oz/a)	12.3 fl oz/a	50.0	7.8	42%	3.8
Stratego (10oz/a)	10 fl oz/a	54.8	8.2	42%	3.7
Stratego (14oz/a)	14 fl oz/a	53.2	9.7	47%	3.5
Untreated	-	50.6	8.7	73%	4.8
		NS	**NS**	**NS**	**NS**

Table 2: Results from 1999 Sprayer Comparison Fungicide Trial

		DON	Disease Rating 29-June	
sprayer	**rate (oz/a)**	**(ppm)**	**Incidence**	**Severity**
Proptech	4.0	2.4*****	43.5	4.8
Proptech	2.5	6.5*****	40.4	5.1
Boom	4.0	5.9*****	35.2	4.6
Boom	2.5	3.4*****	42.9	5.6
Untreated	-	13.4	45.4	5.8

***** significantly different from the untreated control (p=0.05)

Table 3: Results from 1999 FHB Folicur Application Timing Fungicide Trial

	application	Yield	DON	Disease Rating 15-June	
variety	**stage**	**(bu/a)**	**(ppm)**	**Incidence**	**Severity**
Freedom	10.1	41.8	9.8*****	50%	3.3
Freedom	10.5	45.4	9.2*****	40%	2.3
Freedom	untreated	38.0	13.3	82%	3.8
Pioneer 2510	10.1	37.3	16.9	90%	4.0
Pioneer 2510	10.5	40.7	11.9*****	67%	4.0
Pioneer 2510	untreated	31.2	18.6	88%	4.5
Frankenmuth	10.5	37.3	7.8*****	22%	3.7
Frankenmuth	untreated	23.1	11.8	26%	5.0

***** significantly different from the untreated control (p=0.05)

USDA-ARS, OHIO STATE UNIVERSITY COOPERATIVE RESEARCH ON BIOLOGICALLY CONTROLLING FUSARIUM HEAD BLIGHT: 2. INFLUENCE OF PATHOGEN STRAIN, INOCULUM SPRAY SEQUENCE AND INOCULUM SPRAY TIME

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OBJECTIVES

To test the ability of microbial antagonists to reduce the severity of scab of wheat when coinoculated with any of three different isolates of *Gibberella zeae* and when sprayed at different times before or after the arrival of pathogen inoculum on wheat heads.

INTRODUCTION

Fusarium head blight (FHB), also known as wheat head scab, causes extensive damage to wheat throughout the world. In North America, the primary causal agent of FHB is *Gibberella zeae* (anamorph= *Fusarium graminearum*) which can produce potent toxins during colonization of grain including the estrogenic toxin zearalenone (F-2) (Vesonder and Hesseltine, 1980) and the trichothecene deoxynivalenol (vomitoxin) (Proctor et al., 1995) which can inhibit amino acid incorporation and protein production in plant tissues (Casale and Hart, 1988). Because wheat cultivars with a high degree of resistance are not currently available and the use of chemical control measures is complicated by concerns regarding potential residues and cost, biological control has been suggested as a promising method for reducing FHB (Khan et al., 1998).

In a separate report (Schisler et al., 1999), a screening method for selecting microbial antagonists that suppress FHB has been described as have biocontrol studies on durum wheat. In this study we explore the efficacy of our antagonists in reducing FHB incited by several isolates of *G. zeae* obtained from different geographical locations. We also studied how the sequence and timing of antagonist and pathogen inoculum arrival on the infection court influences the level of biological control observed. The field testing of these antagonists is reported elsewhere in this proceedings (Boehm et al., 1999).

MATERIALS AND METHODS

Greenhouse plant bioassay of biocontrol: point inoculation

Hard red spring wheat cultivar Norm was used in these experiments. Ten µl of a suspension containing conidia (10^5 conidia/ml), biocontrol agent (yeast at approximately $2x10^7$ cfu/ml; bacteria at approximately $5x10^8$ cfu/ml) and 0.04% Tween 80 in weak PO_4 buffer was used to inoculate the middle floret of a centrally located spikelet on a head. Heads inoculated only with conidia of *G. zeae* in the Tween 80-buffer suspension were the control. Three isolates of *G. zeae* (Z3639, DOAM, and Fg-9-96) were tested in a completely randomized experimental design with 16 heads per treatment. After inoculation, plants were kept in a plastic humidity chamber for 72 h and then transferred to greenhouse benches. Disease severity was visually estimated (Stack and McMullen, 1995) 10 days (data not shown) and 16 days after inoculation. One-hundred kernel weight and the percentage of healthy kernels (data not shown) were also assessed. The experiment was

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repeated at least once. The data on disease severity was normalized using arcsine transformation before statistical analysis using PC SAS (version 6.12).

Greenhouse plant bioassay of biocontrol: spray inoculation

A spray inoculation method was used to mimic the arrival of inoculum at the infection court in the field. Inoculum of *G. zeae* isolate Z3639 was sprayed on wheat heads immediately before or after and 4 h before or after spraying heads with microbial antagonists (formulation of pathogen and antagonist inoculum as described above). Heads sprayed only with conidia of *G. zeae* were the control. All other procedures were as described above.

RESULTS AND DISCUSSION

In the assays against *G. zeae* isolate Z3639, six antagonists, including four choline-utilizing strains, reduced disease as indicated by increased 100 kernel weights of microbially treated wheat heads ($P<0.05$, Table 1). Three antagonists decreased disease severity. Bacterial strain AS 43.3, AS 43.4, and yeast strain OH 182.9 reduced disease severity by >77%, 93%, and 56%, respectively. Treatments with antagonist strains AS 43.3, AS 43.4, and OH 182.9 increased the 100 kernel weight by >140%, 144 %, and 100 %, respectively ($P<0.05$, Table 1). In bioassays against isolate DOAM of *G. zeae***,** only bacterial strains AS 43.3 and AS 43.4 reduced disease as measured by reduction in disease severity, and disease incidence. Five antagonists increased 100 kernel weight (Table 1)**.** Conversely in bioassays using isolate Fg-9-96 of *G. zeae*, all antagonists except OH 72.4 increased 100 kernel weights and five of seven antagonists reduced disease severity ($P<0.05$, Table 1). Overall, bacterial strains AS 43.3 and AS 43.4 consistently reduced FHB disease regardless of the isolate of *G. zeae* used in the bioassays (Table 1). The other antagonist strains tended to be effective against one or two but not all three isolates of *G. zeae*. Further tests using a broader array of *G. zeae* isolates will provide an indication of how widely efficacious individual antagonist strains have the potential to be.

In spray inoculation experiments, all antagonists significantly reduced disease severity, regardless of the sequence, timing, and concentration of inoculum application ($P<0.05$, Table 2), though some antagonists did not increase 100 kernel weight when applied 4 h after inoculum of *G. zeae* (Table 3). These results suggest that biological control of FHB in the field may become less effective if cells of antagonists are significantly delayed in arriving at the infection court compared to inoculum of *G. zeae*.

Further studies on formulation technologies including identifying compounds that support growth of antagonist strains without enhancing the growth of *G. zeae* may offer a method of improving both the breadth and level of efficacy of FHB antagonists. Studies on optimizing liquid media to maximize biomass production and efficacy while utilizing cost effective nutrient sources, and field testing of antagonists showing promise in the greenhouse assays at different geographical locations are planned.

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Table 1. Influence of microbial antagonists on FHB incited by three isolates of *G. zeae* on the hard red spring wheat cultivar Norm [a]

	G. zeae Isolate								
	Z 3639			DOAM			Fg-9-96		
Treatment	Disease Severity (%)	Disease Incidence (%)	100 Kernel Weight (g)	Disease Severity (%)	Disease Incidence (%)	100 Kernel Weight (g)	Disease Severity (%)	Disease Incidence (%)	100 Kernel Weight (g)
G. zeae	90	95	1.5	76	91	1.8	54	66	3.2
AS 43.3	20 *[b]	63 *	3.6 *	17 *	41 *	3.7 *	3 *	3 *	4.0 *
AS 43.4	6 *	46 *	3.9 *	14 *	31 *	3.6 *	11 *	12 *	3.8 *
OH 71.4	78	82	1.9 *	75	87	2.0 *	3 *	12 *	4.0 *
OH 72.4	82	89	1.8	73	84	2.0 *	51	56	2.8 *
OH 131.1	79	89	2.1 *	75	87	1.9	26 *	34 *	3.8 *
OH 181.1	82	89	1.9 *	88	91	1.7 *	44 *	50	4.0 *
OH 182.9	39 *	72 *	3.0 *	69	84	2.0 *	51	65	3.5 *

[a]The middle floret of a central spikelet of a wheat head was co-inoculated with 10 l off a 25% suspension of antagonist liquid culture (10^7-10^8 cfu/ml) and *G. zeae* conidia (1 x 10^5 conidia/ml).
[b]Within a column, means followed by an * are significantly different from *G. zeae* control (P=0.05).

Table 2. Percent FHB disease severity when varying the time and sequence of pathogen and antagonist inoculum application to wheat heads

	% Disease Severity			
	Pathogen Inoculum Applied:			
Treatment	4 h Before Antagonist	Immediately Before Antagonist	Immediately After Antagonist	4 h After Antagonist
G. zeae Z3639	59	86	81	85
AS 43.3 (10%)[a]	13 *[b]	2 *	3 *	21 *
AS 43.3 (50%)	5 *	1 *	0 *	15 *
AS 43.4 (10%)	42 *	3 *	3 *	30 *
AS 43.4 (50%)	19 *	33 *	0 *	18 *
OH 71.4 (10%)	26 *	24 *	18 *	49 *
OH 71.4 (50%)	28 *	51 *	37 *	63 *
OH 182.9 (10%)	43 *	64 *	60 *	45 *
OH 182.9 (50%)	43 *	49 *	60 *	58 *

[a]Antagonists were applied at concentrations of 10% or 50% of a fully colonized complete liquid medium.
[b]Within a column, means followed by an * are significantly different from *G. zeae* control. Means were separated by Fisher protected LSD at P=0.05.

Table 3. One hundred kernel weights when varying the time and sequence of pathogen and antagonist inoculum application to wheat heads

	100 Kernel Weight (g)			
	Pathogen Inoculum Applied:			
Treatment	4 h Before Antagonist	Immediately Before Antagonist	Immediately After Antagonist	4 h After Antagonist
G. zeae Z3639	1.8	1.6	1.7	1.4
AS 43.3 (10%)[a]	2.4 *[b]	3.4 *	3.4 *	2.6 *
AS 43.3 (50%)	2.6 *	3.6 *	3.1 *	2.7 *
AS 43.4 (10%)	2.5 *	3.3 *	3.2 *	2.2 *
AS 43.4 (50%)	1.7	3.0 *	3.1 *	2.6 *
OH 71.4 (10%)	1.9	2.3 *	2.8 *	2.4 *
OH 71.4 (50%)	2.4 *	2.6 *	2.5 *	2.0 *
OH 182.9 (10%)	1.9	2.3 *	2.3 *	2.7 *
OH 182.9 (50%)	1.8	2.2 *	2.3 *	2.0 *

[a]Antagonists were applied at concentrations of 10% or 50% of a fully colonized complete liquid medium.
[b]Within a column, means followed by an * are significantly different from *G. zeae* control. Means were separated by Fisher protected LSD at P=0.05.

BIOLOGICAL CONTROL OF FUSARIUM HEAD BLIGHT (FHB) OF WHEAT BY *BACILLUS* STRAINS

Yongmei Luo and Bruce Bleakley*

ABSTRACT

Fusarium Head Blight (FHB) or scab, caused by some pathovars of *Fusarium graminearum* (teleomorph of sexual stage *Gibberella zeae*), is a fungal disease of wheat which can cause serious crop-yield reduction. Biocontrol agents that antagonize the pathogen could be useful in management strategies for controlling FHB. Four *Bacillus* stains designated 1BA, 1BC, 1BE and 1D3 were isolated from South Dakota wheat foliage as potential biocontrol agents of scab. Both whole cells and cell-free, concentrated ethyl-acetate extracts of the broth supernatants of each bacterial stain inhibited growth of the pathogen in plate assays. The compounds found in cell-free ethyl acetate extracts of bacterial culture supernatants were separated by thin layer chromatography (TLC) in a solvent system of 1-butanol:acetic acid:H_2O (3:1:1) and detected by UV light. Each strain had 11-12 individual spots that were separated on TLC plates. Extracts of 1BE and 1BC were used in disease nursery research in the summer of 1998. The results of MIXED t test statistical analysis showed significant reduction of scab symptoms on heads of spring wheat that received cell-free extracts of the *Bacillus* strains. Application of strain 1BE extract resulted in 25.88% disease reduction and application of strain 1BC extract resulted in 20.95% of disease reduction. The results showed that cell-free extracts of these *Bacillus* strains helped protect wheat against scab in a disease nursery, and that some biocontrol strains can be differentiated from one another based on TLC analysis of cell-free culture supernatant extracts of *Bacillus* strains. One or more of these *Bacillus* strains may prove useful in management schemes for controlling Fusarium Head Blight.

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CHEMICAL & BIOLOGICAL CONTROL OF FUSARIUM HEAD BLIGHT 1999 PROJECTS AND PROGRESS

Marcia McMullen[1*] and Gary Bergstrom[2]

OBJECTIVES

The control of Fusarium head blight (FHB = scab) has been difficult, because of the complex nature of the host/pathogen/environment interaction. While host resistance is the most promising and effective long-term management solution, wheat and barley farmers have needed some immediate solutions for keeping this disease from causing severe economic loss. In addition, optional tools for management even in the presence of host resistance may be necessary under severe epidemics or for crops or regions that don't have high levels of resistance available. The goals of the Chemical and Biological Control area of the National Wheat and Barley Scab Initiative have been: to evaluate fungicides and biological antagonists that will be effective, safe, easy to apply, and economic; to devise and test application methods that will enhance efficacy and economics; and to test the consistency of performance of fungicides, other antagonists, and methods across multiple environments. This discussion will highlight the 1999 research supported in this area through the U.S. Wheat and Barley Scab Initiative.

UNIFORM FUNGICIDE TRIAL

A set of core fungicide treatments was evaluated for efficacy against FHB across seven states (IN, MI, MN, MO, ND, OH, SD) in 1998 (McMullen, 1998). The data on efficacy of five products or product mixes in reducing FHB when applied at heading indicated an average of about 50% reduction in FHB severity occurred with some of the products. In 1999, additional fungicide treatments and locations were included in the Uniform Fungicide Trial. Nine fungicide treatments were compared to the untreated check in 14 states (AR, IL, IN, KY, MD, MI, MN, MO, NY, NC, ND, OH, SD, and VA). A summary report, with all collaborators and treatments listed, is provided elsewhere in these Proceedings - ***1999 Uniform Fungicide Trials to Identify Products Effective Against Fusarium Head Blight in Wheat,*** by M. McMullen, G. Milus, and L. Prom. Collaborators in these 14 states provided fungicide testing on three classes of wheat across very different environments, some of which had low to high levels of FHB in 1999, while others had drought conditions. The summary report indicates that, averaged over the environments that had FHB, eight of the nine fungicide treatments, applied once at flowering, significantly reduced FHB. DON levels were reduced on the average by 45% with the best treatment (Folicur at 6 fl oz/acre applied at flowering). Folicur fungicide had a Section 18 emergency exemption for use in many states in 1999, while two other promising treatments in these trials, BAS 500 and Stratego, are not yet registered for wheat. Greg Shaner, Purdue University, will provide some additional information on ***Scab and Fungicide testing in the Corn Belt***, during the 1999 Forum's session on the review of the Chemical and Biological Control Area.

Individual cooperators have also provided information on their fungicide results in several formats: manuscripts in these Scab Forum Proceedings; in Progress Reports provided on-

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line at http://www.scabusa.org/; in the *Fungicide and Nematicide Tests* publication; or at professional meetings. An example included in the 1999 Scab Initiative's Forum Proceedings is a report titled ***Fungicide Efficacy Trials at Michigan State University*** by P. Hart, J. Froedtert, R. Ward, L. Siler, G. VanEE, and R. Ledebuhr, all of Michigan State University. A. Grybauskas provided his 1999 results from Maryland in a poster titled ***Timing of Fungicide Applications for Fusarium Head Blight Management of Winter Wheat***, published in *Phytopathology* 89:S30. Among reports submitted to *Fungicide and Nematicide Tests* volume 55 are ***Efficacy of Fungicides for Control of Fusarium Head Blight on Wheat in Arkansas***, *1999* by L.K. Prom, E.A. Milus, and C. Weight, and ***Evaluation of Fungicides for Control of Fusarium Head Blight and Leaf Diseases on Winter Wheat at Columbia, MO., 1999*** by L.E. Sweets. The core set of fungicides also was tested against FHB on barley in Minnesota and North Dakota. The results of these tests are provided elsewhere in these Scab Forum Proceedings, under the title ***1999 Uniform Fungicide Trials for FHB Control in Barley***, by M. McMullen, R. Jones, J. Pederson, S. Halley, and J.Lukach.

BIOCONTROL STUDIES

Two sets of studies for evaluating biocontrol agents were supported by the National Scab Initiative in 1999. A cooperative project among G. Bergstrom and C. Stockwell, Cornell University, Ithaca, NY, and W. da Luz, EMBRAPA-TRIGO, Passo Fundo, Brazil, is described in a separate report provided in these Proceedings, titled ***Selection of Microbial Antagonists for Biological Control of Fusarium Head Blight of Wheat***. This team has isolated, preserved and characterized approximately 120 candidate biocontrol organisms from 70 different sources, and have developed bioassays to evaluate these isolates as to the most promising for efficacy against *Fusarium graminearum* and for tolerance to environmental stresses. This project is emphasizing organisms that are likely to be robust under harsh field conditions. This research project is evaluating the top candidate biocontrol organisms as protectant sprays during anthesis, as treatment of scabby wheat seed, and as treatment of infested maize debris.

Another group working on biocontrol includes M. Boehm and N. Khan at Ohio State University, Columbus, OH, and D. Schisler, USDA-ARS, Peoria, IL. They have provided results of their 1999 research in several poster presentations here at the Forum, titled ***Optimization and Field Testing of Biocontrol Agents Active Against Fusarium Head Blight***, and the team also will provide an oral summary during the presentation on the Chemical and Biological Control Area. This group has developed selective screening methods for identifying microbial strains with an enhanced likelihood of reducing the severity of FHB. They screened wheat anther colonists for their ability to metabolize choline and betaine, two *Fusarium graminearum* growth stimulating compounds found in anthers. They tested six strains of selected microorganisms in greenhouse bioassays and field tests in Illinois and Ohio, and plan to continue field testing in other environments. They also are looking at methods to enhance bioactivity of these microorganisms.

APPLICATION TECHNOLOGIES

Two groups in the United States have devoted considerable time and resources to evaluating methods of application of fungicides to improve FHB control. The grain spike is the site of infection, but the grain spike is a difficult target. Deposition and retention of fungicide on the grain spike is hindered by the vertical orientation of the spike, along with the interference of awns, and the waxy or glabrous surface of the glumes. Conventional application methods intended

primarily for foliar targets have shown to be ineffective in delivering fungicide to the grain spike. Generally, only a small percentage of the spray volume remains on the grain spike. Studies at North Dakota State University have been established to identify spray application parameters that are most effective in improving fungicide deposition on the spike. Angled, rather than vertically oriented, sprays have shown great improvement in deposition and disease control, and some air assist spray systems have also shown great promise. A report on some of these results in North Dakota is given within these Proceedings and as a poster titled ***Sprayer Modifications for Enhanced Control of Fusarium Head Blight with Fungicides***, by S. Halley, J., Pederson, M. McMullen, and J. Lukach.

At Michigan State University, Gary Van Ee and coworkers also have looked at spray application techniques, and compared the deposition characteristics of four different types of application equipment using a fluorescent dye tracer on the heads of wheat. They tested flat fan nozzles set at an angle from the horizontal and tested a modified version of the Proptec sprayer, an air assist type sprayer. Both methods provided average vomitoxin levels below 5 ppm while the untreated control exceeded 13 ppm. A summary of their results is available in the Initiative's Progress Reports and as part of a report included in this year's Forum Proceedings, titled ***Fungicide Efficacy Trials at Michigan State University***, by P. Hart, J, Froedtert, R. Ward, L. Siler, G. Van Ee, and R. Ledebuhr.

Overall, substantial progress has been made in the past year with identifying fungicides that are most efficacious in reducing FHB and vomitoxin, identifying biological agents that have good activity against *F. graminearum*, and in identifying application techniques that improve performance of these products. Additional research is needed in evaluating newer fungicide chemistries and rates of use across environments and grain classes, in evaluating biological agents in the field and in increasing their survivability and establishment, and in transferring application techniques to available, affordable, and practical equipment for producers.

1999 UNIFORM FUNGICIDE TRIALS TO IDENTIFY PRODUCTS EFFECTIVE AGAINST FUSARIUM HEAD BLIGHT IN WHEAT

Marcia McMullen[1*], Gene Milus[2] and Louis Prom[2]

INTRODUCTION

Wheat growers are very interested in finding effective fungicides that will substantially control Fusarium head blight (FHB) and be safe and economical to use. The severity of the FHB epidemics in the US in 1993, 1996, and 1997 led to interest in a cooperative project to evaluate a core set of fungicide treatments across wheat classes and environments. During these epidemics, few fungicides had federal registration for heading application to wheat. In 1998, a uniform fungicide trial was conducted across seven states (ND, MN, SD, OH, IN, KY, MO), with five fungicides or fungicide mixes (Benlate + mancozeb, Folicur, Tilt, Quadris, Quadris + Benlate) evaluated for reducing FHB when applied to wheat at the flowering stage. In 1998, only Benlate and various mancozebs had full registration; Folicur received Special Emergency Exemptions (Section 18) for use in many states; Tilt had 24C state labels for heading application in other states; and Quadris became registered after the growing season. In 1998, only three of the seven states had substantial levels of FHB in which to evaluate efficacy against FHB; across these three states (ND, SD, MN), FHB was reduced by an average of about 50% with the best treatments (McMullen et al., 1997). More information was needed on these and additional products potentially close to registration, and additional sites with potential for FHB were needed for effective evaluation of product efficacy across environments.

METHODS

Plant Pathologists in 14 states participated in the 1999 uniform fungicide trial. These states represented hard red spring wheat, hard red winter wheat, soft red winter wheat, and soft white winter wheat production areas. The states and the principal cooperators were:

Arkansas	*Gene Milus, Univ. of Arkansas, Fayetteville*
Illinois	*Wayne Pedersen, Univ. of Illinois, Urbana*
Indiana	*Greg Shaner, Purdue Univ., West Lafayette*
Kentucky	*Don Hershman, Univ. of Kentucky, Princeton*
Maryland	*Arvydas Grybauskas, Univ. of Maryland, College Park*
Michigan	*Pat Hart, Michigan State Univ., East Lansing*
Minnesota	*Roger Jones, Univ. of Minnesota, St. Paul*
Missouri	*Laura Sweets, Univ. of Missouri, Columbia*
New York	*Gary Bergstrom, Cornell Univ., Ithaca*
North Carolina	*Steven Leath, North Carolina State Univ., Raleigh*
North Dakota	*Marcia McMullen, North Dakota State Univ., Fargo*
Ohio	*Pat Lipps, Ohio State Univ., Wooster*
South Dakota	*Marty Draper, South Dakota State Univ., Brookings*
Virginia	*Erik Stromberg, Virginia Tech, Blacksburg*

The treatment list in 1999 included a nontreated check and nine fungicide treatments, including several new experimental products, various timings of application of two products, and two rates of one product (Table 1). Two of the products tested were strobilurin chemistries (BAS 500, Quadris) one was a triazole fungicide (Folicur), two treatments tested Stratego [a combination product of a triazole (Tilt) and an experimental strobilurin], and two treatments contained the protectant mancozeb fungicide (Penncozeb, Benlate + mancozeb). At some, but not all locations, artificial inoculum of *Fusarium graminearum* was applied as infected corn or corn and barley grain, distributed evenly over the plot area 10 to 14 days before flowering. At some sites, water was added to the plot sites prior to flowering and during flowering via a

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misting system or irrigation system. Fungicides were applied at the flowering stage of development, unless otherwise indicated. Fungicides were generally applied with a CO_2 pressurized hand held sprayer, delivering from 18-25 gpa. Nozzle configurations on sprayers varied among states, as did plot size. Disease parameters measured at soft dough stage of development included FHB incidence (% of heads showing symptoms), FHB head severity (% of head area infected), FHB index (= field severity = incidence x head severity), deoxynivalenol (DON) content in ppm, Fusarium damaged kernels (FDK), and % flag leaf disease, as well.

Table 1. Uniform fungicide treatments evaluated in 1999 for control of Fusarium head blight

Treatment	Products Manufacturer	Rate of product/acre	Adjuvant, if applied	Timing of application [a]
Control	-----	-----	-----	-----
Folicur	Bayer	6 fl oz	0.06 % v/v Induce	Feekes 10.51
Benlate + Manzate	DuPont + Griffin	0.5 lb + 1 lb	0.25% v/v CS7	Feekes 10.51
Penncozeb	AtoChem	1 lb + 1 lb		Feekes 10.3 + 10.51
BAS 500	BASF	15.3 fl oz	1 % v/v Agridex	Feekes 10.3
BAS 500	BASF	15.3 fl oz	1 % v/v Agridex	Feekes 10.51
Stratego	Novartis	10 fl oz		Feekes 10.51
Stratego	Novartis	14 fl oz		Feekes 10.51
Quadris	Zeneca	12.3 fl oz		Feekes 10.51
Quadris	Zeneca	9.2 fl oz		Feekes 10.51

[a] Feekes growth stage 10.3 = 50% heading; Feekes growth stage 10.51 = early flowering

RESULTS

Several of the 14 states participating in the 1999 trial had severe to moderate drought during the growing season, and little or no FHB developed. These states included New York, North Carolina, Ohio, Virginia, and Indiana. The fungicide trial in Arkansas had the greatest level of FHB, a test site on spring wheat in Watertown, SD had moderate levels of FHB, while other states or sites had FHB index levels below five when averaged across all treatments. Some states, such as Michigan, Illinois, Minnesota, Missouri, and Maryland had FHB in 1999, but the index level or field severity in untreated plots averaged below five percent. To examine how treatments performed across locations, and determine variability across locations, Gene Milus and Louis Prom did the following analyses for FHB index (field severity), Fusarium damaged kernels (FDK), and DON levels. Statistical analyses were done using mean FHB data from various locations that reported on all ten treatments of the uniform list or had FDK and DON data. Yields were not included in these analyses because they may reflect leaf disease control as well as FHB control. Individual reports from each state are provided in the state's progress reports provided to the U.S. Wheat and Barley Scab Initiative and listed on the Internet at http://www.scabusa.org/.

FHB Index (Field Severity)

The FHB index values were significantly different among locations and treatments (Table 2). The highest severities were at Fayetteville, AR, and the lowest average FHB index in this analysis was at Princeton, KY. The Fayetteville, AR location had an FHB index that was about the same as the sum of the other nine locations. When data was re-analyzed without Fayetteville data, treatments and locations were still significantly different. Variation in FHB index across sites indicates the problems associated with achieving enough infection and a uniformity of infection across locations. Factors affecting FHB infection include amount of inoculum available or applied, variety tested, amount of water applied, and natural environmental conditions.

All treatments significantly reduced the FHB index over the untreated control, when averaged across all 10 locations (Table 2), but few treatments varied significantly from each other. Penncozeb and Quadris appeared to be the least effective in reducing FHB, while Stratego at 14 fl oz, BAS 500 applied at flowering, and Folicur were the most effective.

FDK levels

Five trials in these studies had useable data for Fusarium damaged kernels (FDK) and locations were significantly different, but treatments were not (Table 3). Of the five trials, the Fayetteville, AR study had significantly more FDK than the other sites, while the Watertown, SD study on winter wheat had the least. Data were analyzed with (5 sites) and without (4 sites) the Fayetteville location.

DON levels

At the time of submitting this report, only eight locations in the uniform trials had useable or available data for DON; there were significant differences among the locations, but not among the treatments (Table 4). When Fayetteville data were excluded (7 sites), treatments were significantly different at $P = 0.08$. The nontreated control was intermediate among the treatments, and no treatment had significant reduction in DON over the control. Treatments of Penncozeb, Quadris, BAS 500 at heading or Stratego at the low rate averaged above or equal to the control in DON levels.

CONCLUSIONS

FHB levels varied considerably across testing sites in 1999. Additional sites with the presence of FHB at moderate levels and with all treatments included would have added to the data and provided more information about product efficacy. The best treatments in these composite analyses were the Folicur treatment and the BAS 500 applied at flowering, reducing the FHB index by about 50%, DON levels by about 22%. Additional improvements are needed to achieve substantial control of FHB with fungicides. Possibilities for improved control include application technologies that deposit more fungicide on the heads and untested experimental fungicides.

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Table 2. FHB Index (incidence x head severity) across locations and across treatments

Location[a]	Mean FHB Index across treatments	t grouping LSD (P=0.05)	Treatment	Mean FHB Index across locations	t grouping LSD (P=0.05)
Fayetteville, AR	25.1	A	Control	7.9	A
Watertown, SD	7.2	B	Penncozeb	6.2	B
Carrington, ND	4.2	C	Quadris 9.2 fl oz	5.9	CB
Langdon, ND	3.6	DC	Quadris 12.3 fl oz	5.3	CBD
Groton, SD	2.8	DCE	Stratego 10 fl oz	4.8	CBD
Fargo, ND	2.5	DFE	BAS 500 at 50% heading	4.8	CBD
Watertown, SD	2.4	DFE	Benlate + mancozeb	4.8	CBD
Keysburg, KY	1.4	FE	Stratego 14 fl oz	4.5	CD
Columbia, MO	1.2	F	BAS 500 at flowering	4.2	D
Princeton, KY	1.1	F	Folicur	4.0	D
LSD (P=0.05)	1.4		LSD (P=0.05)	1.4	

[a] Treatments applied to soft red winter wheat, hard red winter wheat, or hard red spring wheat

Table 3. Fusarium damaged kernel (FDK) levels across locations and treatments.

Location	Mean FDK 5 sites	t grouping 5 sites	Mean FDK 4 sites	t grouping 4 sites
Fayetteville, AR	61.6	A		
Langdon, ND	8.0	B	8.0	A
Watertown, SD	7.0	CB	7.0	A
Groton, SD	4.1	CD	4.1	B
Watertown, SD[a]	1.6	D	1.6	C
LSD (P=0.05)	3.3		1.1	

[a] hard red winter wheat

Treatments	Mean FDK 5 sites	Mean FDK 4 sites
Quadris 9.2 fl oz	23.0	7.3
Quadris 12.3 fl oz	19.6	6.3
BAS 500 at heading	18.5	5.6
Stratego 10 fl oz	16.9	4.9
Control	16.8	6.0
Stratego 14 fl oz	16.4	4.9
BAS 500 at flowering	15.3	3.9
Penncozeb	14.2	5.4
Folicur	14.1	4.6
Benlate + Mancozeb	14.0	4.1
LSD	NS	NS

Table 4. DON levels across eight locations and across fungicide treatments.

Location	Mean DON (ppm)	t grouping
Fayetteville, AR	14.8	A
Langdon, ND	10.8	B
E. Lansing, MI	8.6	C
Groton, SD	7.9	C
Columbia, MO	0.7	D
Princeton, KY	0.6	D
Urbana, IL	0.5	D
Keysburg, KY	0.5	D
LSD (P=0.05)	1.2	

Treatment	Mean DON (ppm)
Penncozeb	7.8
Quadris 9.2 fl oz	6.2
Quadris 12.3 fl oz	6.1
Stratego 10 fl oz	5.6
Control	5.6
BAS 500 at heading	5.6
Stratego 14 fl oz	5.1
Benlate + Mancozeb	5.0
BAS 500 at flowering	4.9
Folicur	4.3
LSD	NS

1999 UNIFORM FUNGICIDE TRIALS FOR FHB CONTROL IN BARLEY

Marcia McMullen[1*], Roger Jones[2], Jeremy Pedersen[1], Scott Halley[1], and John Lukach[3]

INTRODUCTION AND OBJECTIVES

Barley growers are very interested in finding effective fungicides that will substantially control Fusarium head blight (FHB) and reduce the deoxynivalenol (DON) content in the harvested grain. The severity of recent FHB epidemics in the US led to a cooperative project to evaluate a core set of fungicide treatments on barley.

METHODS

In 1999, fungicide tests on barley were done at two locations in North Dakota and one in Minnesota. A set of 10 treatments plus an untreated check were evaluated at Fargo and at Langdon, ND on Stander barley (Table 1), while six of these treatments were evaluated at Crookston, MN on MnBrite barley. MnBrite is a newly released variety from the Univ. of Minnesota that has more tolerance to FHB than Stander. Treatments were applied with CO2 backpack sprayers at 18-20 gpa at 40 psi during heading. Grain spawn inoculated with *Fusarium graminearum* was distributed evenly among plots at the Langdon and Fargo sites approximately 10 days before heading. The Crookston site was not inoculated. Plots at each site were in a randomized complete block design with four replicates per treatment. Disease ratings were taken at soft dough stage of kernel development. Disease parameters included FHB incidence (% of heads showing symptoms), FHB head severity (% head area infected), FHB index (= field severity = incidence x head severity), deoxynivalenol (DON) content in ppm, and % flag leaf disease.

RESULTS

FHB Index (Field Severity)

FHB levels were generally low across all three sites in 1999, with the untreated control having an FHB index of 1.3-1.5 at Fargo and Crookston, and 6.4 at Langdon. The mean FHB index values ranged from 0.8-3.2 when averaged across locations (Table 1). At Fargo, all treatments gave significantly lower FHB values than the untreated check, but treatments were not significantly different from each other. At Crookston, the differences among all treatments were non-significant. At the Langdon location, all treatments resulted in a significantly lower FHB index value than the untreated, and the BAS 500 treatment at heading, the Stratego at 14 fl oz, and Quadris at the 12.5 fl oz rate gave significantly lower FHB severity ratings than some of the other treatments. The mean values across locations (Table 1) indicate that the Benlate + mancozeb treatment, the Penncozeb treatment, and the BAS 500 treatment applied prior to full head emergence resulted in the highest FHB field severity ratings.

Deoxynivalenol (DON Levels)

At the time of submitting this report, only the Crookston location had DON analysis completed. At Crookston, all treatments significantly reduced DON levels over the untreated control, but all levels of DON were low, under 1 ppm. Only the Benlate + mancozeb treatment significantly reduced the DON levels over the other fungicide treatments.

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Leaf Disease

Leaf disease levels also were low across the sites, with flag leaf necrosis due to net blotch infection in untreated checks at 1.3% at Fargo, 1.8% at Crookston, and 38% at Langdon.
At Langdon, where net blotch was most severe, all treatments significantly reduced this disease over the untreated check, but the Benlate + mancozeb treatment and the Penncozeb treatment resulted in greater net blotch than other treatments.

Table 1. Fungicide treatments evaluated in 1999 on barley for control of Fusarium head blight (FHB) and effect on FHB field severity

Treatment	Product s Manufacturer	Rate of product/acre	Adjuvant, if applied	Timing of application [a]	Mean FHB Field Severity across locations
Control	-----	-----	-----	-----	3.1
Folicur	Bayer	6 fl oz	0.06 % v/v Induce	Feekes 10.51	1.6
Benlate + Manzate	DuPont + Griffin	0.5 lb + 1 lb	0.25% v/v CS7	Feekes 10.51	3.2
Penncozeb	AtoChem	1 lb + 1 lb		Feekes 10.3 + 10.51	2.2
BAS 500	BASF	15.3 fl oz	1 % v/v Agridex	Feekes 10.3	2.1
BAS 500	BASF	15.3 fl oz	1 % v/v Agridex	Feekes 10.51	0.8
Stratego	Novartis	10 fl oz		Feekes 10.51	1.8
Stratego	Novartis	14 fl oz		Feekes 10.51	1.0
Quadris	Zeneca	12.3 fl oz		Feekes 10.51	1.0
Quadris	Zeneca	9.2 fl oz		Feekes 10.51	1.1
Folicur	Bayer	4 fl oz	0.06% v/v Induce	Feekes 10.51	1.0

[a] Feekes growth stage 10.3 = 50% heading; Feekes growth stage 10.51 = head completely emerged

"SCES": AN OBJECTIVE FUNGICIDE COVERAGE EVALUATION SYSTEM FOR CONTROL OF FUSARIUM HEAD BLIGHT

S. Panigrahi[1*], H. Gu[1], V. Hofman[1], M. McMullen[2], and S. Halley[2]

INTRODUCTION

Effective chemical spray coverage on wheat or barley heads is critical for scab disease control and high crop yield. Effective chemical application is also always desirable for protection of the environment, and it will reduce input costs and increase wheat production profits. Furthermore, by thoroughly understanding spray coverage on wheat heads, we can learn how chemicals work on wheat heads. Variations produced by different combinations of spray parameters can be understood and thus the best spray configuration can be recommended.

In order to process spray images acquired, appropriate software needed to be developed for processing and analyzing images. Image processing techniques have been implemented in some spray coverage analysis applications. The most important step in analyzing these spray images is to segment the images correctly. That is to correctly separate the spray droplets from background in the images. One of the techniques is to use histogram-based thresholding techniques. Many algorithms have been potential to accomplish this task, for example, the modified Otsu's algorithm (Panigrahi et al., 1995), the edge-based algorithm (Kohler, 1979), multi-modal algorithm (Beveridge et al., 1989), moment-preserving algorithm (Tsai, 1985), entropy method (Kapur, 1985) and Shapiro's automatic thresholding algorithm (Shapiro, 1992). All these algorithms were implemented in our study.

OBJECTIVES

The objectives of this paper were to evaluate and compare the performances of different thresholding algorithms for quantifying spray coverage on wheat/barley head.

MATERIALS AND METHODS

Image Acquisition Methods

Wheat heads were sprayed using a mixture of chemicals and Dayglow Blaze Orange (EPX-15 from Day-Glo Color Inc.) at 1.0 % volumetric concentration. Surfactant Latron CS-7 (.05%) was also used along with the Dayglow Blaze Orange dye tracer to provide better-contrasted images. The sprayed wheat heads were put in a light chamber under Ultra Violet light (long wavelength). Dye-covered images of the wheat heads were taken with a high-resolution low light CCD camera (VI470, Optronics) with high integration time (two seconds). Two optical filters were used with cut off and cut on at 700 nm and 520 nm respectively. These filters filtered out unwanted backgrounds while provide best spectral ranges for the dye tracer to pass through. The images taken with the CCD camera were digitized and captured with a Coreco (TCI-SE) frame grabber. The same wheat head images were also acquired under visible lighting. The spray coverage was calculated by dividing the image area of sprayed chemical by image area of wheat head.

Algorithms Used

Figure 1 shows the flow chart of the image processing/analyzing software used for processing the sprayed images of wheat heads. These procedures were applied to both the regular as well as the dye-covered wheat head images.

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Algorithm 1: Modified Otsu's Algorithm (Panigrahi et al., 1995)

At first, Otsu's algorithm (Otsu, 1979) was used to calculate the threshold 'th'. The Otsu's method is based on discriminate analysis. The following criterion was used to determine the threshold,

(1) th = max $\{\sigma_B^2(th)\}$, for 0 <= th <= L and
$\sigma_B^2 = [\mu_T w(th) - \mu(th)]^2 / \{w(th)[1-w(th)]\}$

where w(th) and μ(th) are the zeroth and the first order cumulative moments of the histogram up to 'th' level and is defined by:

(2) $$w(th) = \sum_{i=0}^{th} p_i \ , \ \mu(th) = \sum_{i=0}^{th} ip_i$$

μ_T, the total mean level of the image, is given by:

(3) $$\mu_T = \sum_{i=0}^{L-1} ip_i$$

where

(4) $p_i = n_i/N$, $p_i > 0$ and $\sum_{i=0}^{L-1} p_i = 1$

and L represents the total number of gray level (256), n_i represents the number of pixels at any gray level 'i' and N represents the total number of pixels in the image. After 'th' was found, statistical procedures were implemented to to find a more accurate threshold

Algorithm 2: Shapiro's Automatic Threshold Algorithm (Shapiro, 1992)

Shapiro (1992) developed an adaptive method to find an optimum threshold. This method utilizes local thresholding techniques with subimages. The method is independent of object/background area ratio and insensitive to noises. Let us define a subimage R with size *m* x *m* pixels.

1) For each R, calculate g_{min}, g_{max}, g_{mid} where g_{min} is the minimum pixel value in subimage R, g_{max} is the maximum pixel value in the subimage R and g_{mid} is the average of the above two,
2) For each R, find g_1 and g_2 where g_1 is the histogram mean in the range of g_{min} to g_{mid} while g_2 is the histogram mean in the range of g_{mid}+1 to g_{max} , and find standard deviation, s of histogram of the whole subimage.
3) For each threshold candidate 'th' from 0 to 255, calculate weight function for each subimage with following formula,

(5) $$W(R,th) = \begin{cases} \exp\{-\dfrac{4.5(th - g_{mid}^{\ 2})}{(g_{mid} - g_1)^2}\}, \\ \quad if\ th \in [g_1, g_{mid}]; \\ \exp\{-\dfrac{4.5(th - g_{mid}^{\ 2})}{(g_{mid} + 1 - g_2)^2}\}, \\ \quad if\ th \in [g_{mid} + 1, g_2]; \\ 0, \textit{elsewhere} \end{cases}$$

4) for each threshold candidate 'th', find 'dt' where 'dt' is calculated using

(6) $$dt = \sum_{for\ all\ R} \sigma(R) \times W(R,th),$$

and find 'jt' with jt = dt / n where n is the number of subimages with non zero weight functions.
5) For all threshold candidates 'th', finds the one that maximizes 'jt' and that becomes the optimum threshold.

Algorithm 3: Edge-based algorithm (Kohler, 1979)

Kohler (1979) has proposed an algorithm that will select a threshold automatically so as to maximize the global average contrast of edges. Such method performs multiple passes over the image, creating histograms of contrast per threshold-value, from which the maximum is chosen. For each pass of the algorithm, two stages are established. In the first stage, each edge in the image is examined and

two histograms are build, one for total-contrast per threshold-value and the other edge-count per threshold-value. In the second stage, a third histogram of average-contrast per threshold-value is computed by dividing the total-contrast histogram by the edge-count histogram. Threshold is chosen based on an user defined minimum contrast criterion.

Algorithm 4: Entropy Method (Kapur, 1985)

Kapur (1985) has introduced a method that will find optimal threshold value that will maximize the information content of both the object and background. For each threshold value, the object and background distributions are derived from the original gray level distribution of the image and the two respective entropy values are calculated. Their sum is then used to represent the information content of both the object and background mentioned above. The optimal threshold is chosen as the gray level at which the sum of the two entropy values is maximum.

Algorithm 5: Moment Method (Tsai, 1985)

In this method, the threshold values are computed deterministically in such a way that the moments of an image to be thresholded are preserved in the output (binary) image. The i-th moment is calculated as

(7) $$m_i = \frac{1}{n}\sum_{g=0}^{l-1} g^i h(g), \quad i = 1,2,3$$

where n is the total number of pixels in the image. The threshold value t is obtained from the gray level histogram of the image by choosing t as the p_0-tile, where p_0 is given by

(8) $$p_0 = \frac{z - m_1}{(c_1^2 - 4c_0)^{1/2}}$$

and

(9) $$c_0 = \frac{m_1 m_3 - m_2^2}{m_2 - m_1^2}$$

(10) $$c_1 = \frac{m_1 m_2 - m_3}{m_2 - m_1^2}$$

(11) $$z = \tfrac{1}{2}\{(c_1^2 - 4c_0)^{1/2} - c_1\}$$

Algorithm 6: Multi-Modal Method (Beveridge et al., 1989)

Beveridge et al. (1989) has developed a region segmentation algorithm called multi-modal method developed from the Localized Histogram Segmentation algorithm. There are two phases to the histogram segmentation process.

In the first phase, the image is partitioned into a set of rectangular local subimages called sectors. The histogram (frequency distribution) of pixel values from the input image is then computed for each of these sectors. Within the histogram of each sector "significant" clusters are identified by a means of a peak-valley analysis.

The second phase of the process involves the peak addition in which "ambiguous" peaks in the histogram can be verified according to their presence or absence in adjacent sectors. The intensity value of each of the selected peaks is then used as the output label for all pixels in the input sector that map to the corresponding peak. The domain of this mapping includes all intensity values lying between the valleys on either side of the peak.

RESULTS AND DISCUSSIONS

All these algorithms were implemented using a image processing software called Aphelion. Several methods were available in the software package except the modified Otsu's method and Shapiro's Automatic Method. These two methods were then developed using Microsoft C/C++ and introduced in Aphelion environment as user defined operators.

Wheat heads with high, medium and low spray coverage were evaluated as well as their images

under regular lighting. Ten images of each category were chosen randomly to evaluate the performances of these algorithms. The images were binarized according to thresholds selected by different methods. The same images were also binarized according to a threshold selected by manual thresholding. The images thresholded manually served as a standard for other methods to be compared against.

The histograms of all segmented images were calculated. The object pixels in the manually segmented image were compared with those in the images segmented by each algorithm. Table 1-4 lists the results of the comparisons.

For high, medium and low coverage spray images of wheat heads, the average pixel difference is the least for the edge-based method. They are at 312, 165 and 73 pixels respectively. Since these spray images are sizes of 640 by 480, which contain 307200 total number of pixels, therefore, differences in the magnitude of hundreds or even lower portion of thousands are very small. The edge-based method was the best for these images followed by entropy method, modified Otsu's method, moment method, multi-modal method and Shapiro's method respectively. However, Shapiro's method seems to work well on low coverage wheat heads with average pixel difference of only 335 pixels, which ranked right behind edge-based method.

For wheat head images under visible lighting, the modified Otsu's method performs the best with an average pixel difference of 2394 pixels. The edge-based method failed miserably in this case. The other methods did not perform any better with average pixel differences of tens of thousands of pixels.

Figures 2(a), (b) show sprayed images of wheat head and its corresponding segmented image. Similarly, figures 3(a), (b) show an image of wheat head under visible lighting and its corresponding segmented image.

SUMMARY AND CONCLUSIONS

Image processing technique were developed and evaluated to process and analyze images of sprayed wheat head. The evaluation of six automatic thresholding methods worked reasonably well for most of the images with the edge method performs the best for sprayed images and the modified Otsu's method performs the best for images under regular lighting. Some of these methods were not exactly right for several low quality images. But our findings are satisfactory. Currently, we are extending the same study to 1000 images to obtain consistency and accuracy of such findings. Thought it might not be possible to find a thresholding method that would provide exact 100% segmentation accuracy for all types of images, we postulate that based on our current research, an optimum technique will be developed.

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Figure 1. Flow Chart Image Processing Software

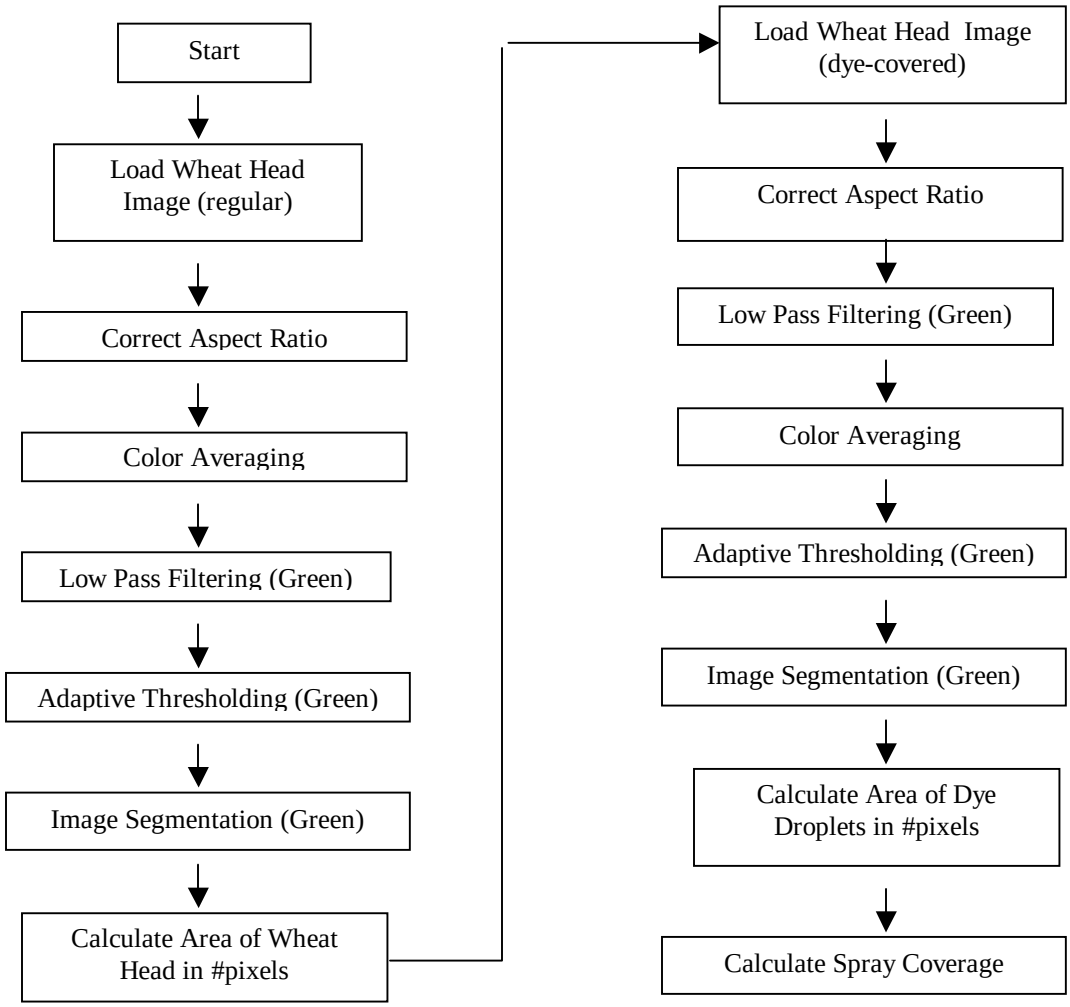

Table 1. Threshold Comparisons (Object Pixel Differences) of High Spray Coverage Wheat Heads

Image No.	Edge-Based Method	Moment Method	Entropy Method	Multi-modal Method	Modified Otsu's Method	Shapiro's Method
1	373	9866	4452	44739	4807	277582
2	0	20198	5244	477	6921	272988
3	336	9527	4359	42770	3677	273108
4	571	14064	7146	3011	8100	2196
5	0	18439	7450	1148	8479	2561
6	692	10322	4796	437	7180	2073
7	270	11089	7986	2337	6420	1685
8	706	9088	4984	97072	7990	2310
9	0	53263	11290	523	5944	1538
10	173	7233	4803	87562	9109	1660
Avg.	**312.1**	**16308.9**	**6251**	**28007.6**	**6862.7**	**83770.1**

Table 2. Threshold Comparisons (Object Pixel Differences) of Medium Spray Coverage Wheat Heads

Image No.	Edge-Based Method	Moment Method	Entropy Method	Multi-modal Method	Modified Otsu's Method	
1	76	9628	4760	49200	Not Available	
2	0	6240	2222	47907	Not Available	
3	170	9644	2330	41441	Not Available	
4	0	6138	2375	42084	Not Available	
5	245	15277	2893	435	Not Available	
6	99	12510	3128	2122	3351	
7	242	7973	2273	46764	3265	
8	219	10074	3099	46627	4396	
9	438	8984	4742	575	4635	
Avg.	**165.44444**	**9607.556**	**3091.333**	**30795**	**3911.75**	**2**

Table 3. Threshold Comparisons (Object Pixel Differences) of Low Spray Coverage Wheat Heads

Image No.	Edge-Based Method	Moment Method	Entropy Method	Multi-modal Method	Modified Otsu's Method	Shapiro's Method
1	0	11570	2886	2949	3462	704
2	166	10452	1926	101572	2374	355
3	92	10416	2189	89022	2659	434
4	0	17101	3898	87446	3539	688
5	106	463	896	101838	1557	84
6	19	7734	1462	86665	1865	283
7	116	7302	1521	87021	1825	300
8	50	5535	1197	102201	1782	274
9	146	690	848	76929	1636	166
10	36	318	420	549	1855	64
Avg.	**73.1**	**7158.1**	**1724.3**	**73619.2**	**2255.4**	**335.2**

Table 4. Threshold Comparisons (Object Pixel Differences) of Wheat Heads Under Visible Lighting

Image No.	Edge-Based Method	Moment Method	Entropy Method	Multi-modal Method	Modified Otsu's Method	
1	4733	18546	5876	3059	604	
2	15571	38990	1438	42265	9551	
3	648	14662	213638	910	167	
4	3364	15789	207814	63963	1099	
5	2359	17727	4951	3027	387	
6	253885	17370	4999	2073	750	
7	243293	14362	209125	1167	801	
8	1779	14605	217450	509	35	
9	15540	36048	1238	15540	8156	
Avg.	**60130.222**	**20899.89**	**96281**	**14723.6667**	**2394.444444**	**2**

Figure 2(a) -- A typical sprayed image of wheat head

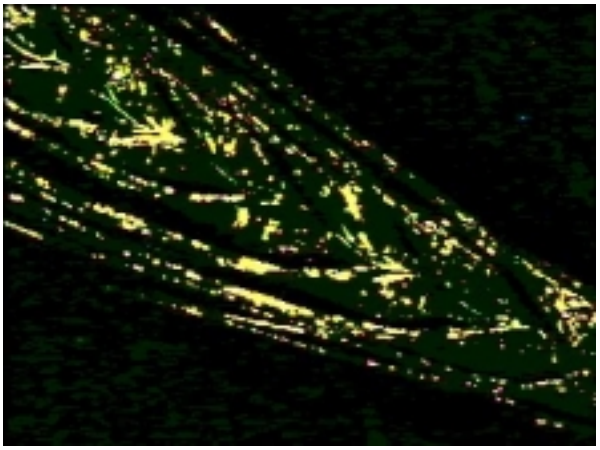

Figure 2(b) -- Segmented sprayed image using edge-based method

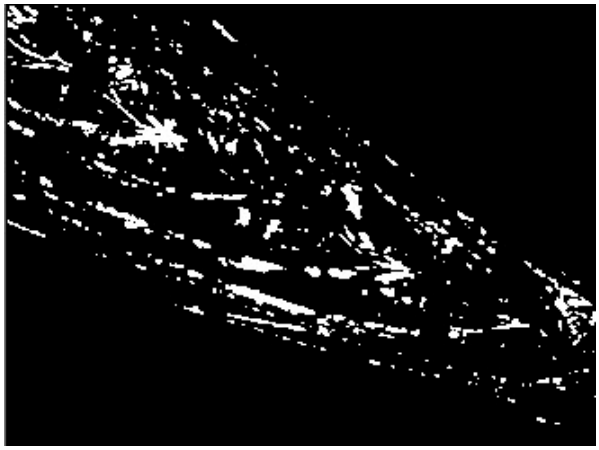

Figure 3(a) -- A typical image of wheat head under visible lighting

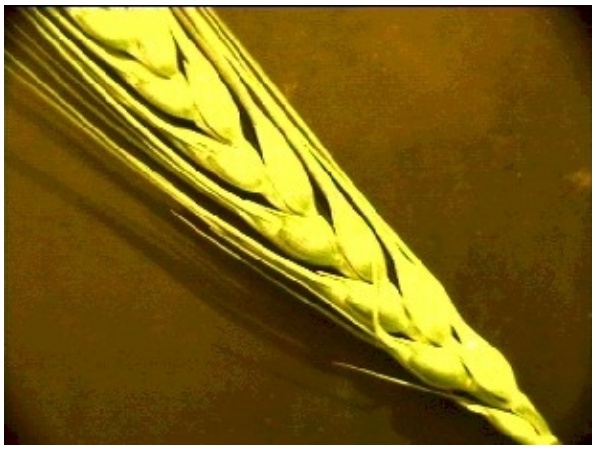

Figure 3(b) Segmented wheat image using modified Otsu s method

USDA-ARS, OHIO STATE UNIVERSITY COOPERATIVE RESEARCH ON BIOLOGICALLY CONTROLLING FUSARIUM HEAD BLIGHT: 1. ANTAGONIST SELECTION AND TESTING ON DURUM WHEAT

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OBJECTIVE

Determine if microbial strains that are effective in reducing Fusarium head blight disease severity on hard red spring and soft red winter wheats are effective in reducing the disease in greenhouse and field tests on durum wheats.

INTRODUCTION

Fusarium head blight (FHB), also known as wheat scab, is a devastating disease of wheat and barley that is primarily incited by the fungus *Gibberella zeae* (anamorph= *Fusarium graminearum*). FHB epidemics cause extensive damage to wheat and barley in humid and semihumid wheat growing areas of the world (McMullen et al., 1997). In durum wheats, the pathogen-produced toxin deoxynivalenol (DON) is retained in semolina at approximately 50%, and *G. zeae* has a strong adverse effect on pasta color when *G. zeae* damaged kernels make up as little as 2% of a lot (Dexter et al. 1997).

High levels of *G. zeae* inoculum are generally found on susceptible wheat heads in fields that experience FHB epidemics (Francl et al., 1999). The use of biotic agents to reduce the severity of FHB holds considerable promise since their application to wheat heads can be timed to be coincident with the most susceptible stages in wheat development: anthesis to early milk (Fernando et al., 1997). Mechanisms of biological control activity that have been identified in a variety of pathosystems include preemptive colonization of infection sites, induced systemic disease resistance, mycoparasitism, nutrient competition and antibiotic production. A traditional method for selecting putative biological control agents is an *in vitro* Petri plate bioassay designed to identify those strains capable of producing antibiotics that inhibit mycelial growth of a pathogen. Unfortunately, the results of Petri plate antagonism assays regularly do not correlate with the biocontrol efficacy of the same strains tested against the pathogen on plants (Reddy et al., 1993).

In research conducted at the NCAUR in Peoria, IL, in cooperation with The Ohio State University (Boehm et al., 1999; Khan et al, 1998; Khan et al., 1999) a selective microbial screening method (Schisler and Slininger, 1997) that does not rely on the traditional Petri plate antagonism assay was developed. *Gibberella zeae* primarily infects the heads of wheat plants from the time of flowering until the soft dough stage of head development (Fernando et al., 1997). Strange and Smith (1978) observed that choline and betaine, compounds present in anthers and wheat heads, stimulated germ tube elongation of conidia of *G. zeae*. We postulated that some of the microorganisms present on wheat anthers may be effective in biologically controlling FHB. Furthermore, since choline and betaine provide a growth stimulus to the pathogen, we surmised that screening anther colonists for their ability to metabolize these growth stimulating compounds (choline and betaine) could provide a method for significantly narrowing the search for antagonists of *G. zeae*.

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Over 700 strains of microorganisms were isolated from anthers collected from wheat plants across Illinois and Ohio. Approximately 55 of more than 700 strains of microorganisms assayed utilized choline as a sole carbon source. Of the seven microbial strains that significantly reduced FHB disease in repeated greenhouse trials, five of the strains were able to utilize choline as a sole carbon source while the remaining two were not. Selecting from a collection of microbial strains those that utilize choline, therefore, was an effective method for rapidly narrowing the search for microbial strains that reduce FHB in wheat. Four effective biocontrol strains (Table 1) were chosen for use in multiple greenhouse tests and a field test of the efficacy of these strains to reduce FHB on durum wheat.

MATERIALS AND METHODS

Greenhouse testing of FHB antagonists on durum cultivars "Renville" and "Ben."

Seedlings were grown in pasteurized potting mix in a growth chamber for 8 weeks prior to transfer to greenhouse benches. Inoculum of the four microbial antagonists (Table 1) used in experiments was grown in a complete liquid medium in Erlenmeyer flasks. Conidia of *G. zeae* isolate Z 3639 were produced on CV-8 agar. At the onset of wheat head flowering, a centrally located spikelet of a wheat head was inoculated with an aqueous suspension containing antagonist cells and broth, 1 x 10^5 conidia/ml of *G. zeae*, 0.04% Tween 80, and a weak phosphate buffer. Antagonists' colony forming units were approximately 2 x10^7 cfu/ml for yeast antagonists and 5 x 10^8 cfu/ml for bacterial antagonists. Controls consisted of "*G. zeae* only" and "tween-buffer only" treatments. Wheat plants were incubated at high relative humidity for 72 h and were scored for disease severity and incidence 16 days after inoculation. There were at least four heads per replication and four replications per treatment. Treatments were distributed in a completely randomized design, and data from experiments that were conducted at least twice were combined. Differences between treatments were determined using analysis of variance (ANOVA) and means separated from controls using Fisher's protected LSD test.

1999 Peoria, IL, field trial of FHB antagonists on durum cultivars "Renville" and "Ben."

Inoculum of antagonists generally was produced as described above except in larger volumes in Fernbach flasks. Due to abnormally hot field conditions from boot through crop maturation (high temperatures ranging from 30-36 C), inoculum of *F. graminearum* was provided as conidia produced as described earlier and as ascospores released from *F. graminearum* Z 3639 colonized corn kernels scattered throughout the plot (?approx 25 kernels/ m^2) 2 weeks prior to wheat flowering. Antagonists and conidia were applied at flowering in aqueous suspensions. Mist irrigation was applied regularly and heavily to promote FHB disease development. Plots were scored for disease severity and incidence.

RESULTS AND DISCUSSION

In greenhouse tests, all four antagonists significantly reduced disease severity on cultivar Renville compared to the positive control (Z3639), and three of the four reduced disease incidence (Table 2). Bacterial antagonist AS 43.3 decreased disease severity by >90% and disease incidence by >75%. Two of the four biocontrol treatments increased, and two of the four decreased the percentage of kernels that were scored as visually faultless though this subjective rating was more severe than the quantitative rating of 100 kernel weights where three of four biocontrol agents significantly increased this factor. Three of four antagonist treatments reduced FHB disease severity on cultivar Ben in greenhouse tests, and two of four reduced FHB disease incidence (Table 3). Because plants were harvested somewhat prematurely in one of the two experiments with Ben, the percent visually faultless kernels and 100 kernel weight data is difficult to interpret.

Though hot dry weather caused an extremely unfavorable season for growing durum wheat and for inciting scab disease, some significant treatment effects with field grown Renville were observed (Table 4). Bacterium AS 43.3 and yeast OH 182.9 significantly reduced disease severity. OH 182.9 also reduced disease incidence. For the Ben portion of the field trial, high levels of variability precluded statistically separating any of the means obtained (data not shown).

With these results, the considerable potential for applying these microbes to reduce the severity of FHB on durum wheat has now been demonstrated. Further studies on durum wheat, including planned cooperative field studies at locations where durum wheats are traditionally grown, should help clarify what role these biocontrol agents could play in the integrated management of FHB.

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prematurely in one of the two experiments with Ben, the percent visually faultless kernels and 100 kernel weight data is difficult to interpret.

Table 1. Microbial antagonists used to reduce the severity of FHB on durum wheat

Antagonist Strain	Accession Number	Type of Microorganism
AS 43.3	NRRL B-30210	Gram positive bacterium
AS 43.4	NRRL B-30211	Gram positive bacterium
OH 71.4	NRRL Y-30213	Yeast
OH 182.9	NRRL Y-30216	Yeast

Table 2. Influence of microbial antagonists on FHB in greenhouse tests on durum cultivar Renville

Treatment	Percent Disease Severity[a]	Percent Incidence	Percent Visually Faultless Kernels[b]	100 Kernel Weight (g)
G. zeae Z3639	50	96	25	1.9
Buffer	0 *	0 *	90 *	2.8 *
AS 43.3	3 *	21 *	46 *	2.3 *
AS 43.4	17 *	62 *	53 *	2.4 *
OH 71.4	36 *	83	11 *	1.9
OH 182.9	27 *	79 *	18 *	2.1 *

[a]Means in a column followed by an * are significantly different from the *G. zeae* Z3639 control (P=0.05). Means were separated using Fisher s protected LSD.
[b]Perfectly formed kernels with no discoloration or sunken areas were scored as faultless.

Table 3. Influence of microbial antagonists on FHB in greenhouse tests on durum cultivar Ben

Treatment	Percent Disease Severity[a]	Percent Incidence	Percent Visually Faultless Kernels[b]	100 Kernel Weight (g)
G. zeae Z3639	46	97	37	3.2
Buffer	0 *	0 *	69 *	3.6 *
AS 43.3	10 *	41 *	68 *	3.4 *
AS 43.4	9 *	44 *	69 *	3.2
OH 71.4	26 *	87	54 *	3.0 *
OH 182.9	43	87	30 *	2.7 *

[a]Means in a column followed by an * are significantly different from the *G. zeae* Z3639 control (P=0.05). Means were separated using Fisher s protected LSD.
[b]Perfectly formed kernels with no discoloration or sunken areas were scored as faultless.

Table 4. 1999 Peoria field results: microbial antagonists against FHB on durum wheat cultivar Renville.

Treatment	Percent Disease Severity[a]	Percent Incidence
G. zeae Z3639	1.9	18.8
AS 43.3	1.3 *	14.8
AS 43.4	1.9	18.4
OH 71.4	1.9	16.7
OH 182.9	1.3 *	11.8 *

[a]Means in a column with an * are significantly different from the *G. zeae* Z3639 control (P=0.05). Means were separated using Fisher s protected LSD.

SELECTION OF MICROBIAL ANTAGONISTS FOR BIOLOGICAL CONTROL OF FUSARIUM HEAD BLIGHT OF WHEAT

C.A. Stockwell[1*], G.C. Bergstrom[1] and W.C. da Luz[2]

OBJECTIVES

To select microbial antagonists effective in controlling *Fusarium graminearum* when applied to cereal spikes, seed, or crop residue, and to evaluate bio-compatible residue treatments for their ability to interfere with perithecial development or ascospore release.

INTRODUCTION

US wheat and barley producers, grain elevators, brewing, malting, and milling industries have suffered severe economic losses in recent years due to Fusarium head blight (FHB) caused by *Fusarium graminearum* (teleomorph=*Gibberella zeae*) (McMullen et al., 1997). Biological control is looked to as an additional strategy in an integrative approach to FHB management in cereals. FHB may be amenable to biocontrol for two reasons: 1) the disease cycle is monocyclic and 2) the length of time during which the plant can be infected is quite short, from flowering to kernel soft dough stage.

Screening microoganisms to control wheat scab was initiated in Brazil a decade ago (Luz, 1988). Treatment with individual bioprotectants significantly diminished the severity of the disease under field conditions, raising the yield of wheat between 7 and 31 % when compared to untreated plants (Perondi et al. 1996). In laboratory assays, *Paenibacillus macerans, Pseudomonas putida* and *Sporobolomyces roseus* reduced the *in vitro* growth of *F. graminearum* up to 95 - 100 % (Stockwell et al., 1997). In greenhouse trials, flowering spikes co-inoculated with *Paenibacilus macerans* and *F. graminearum* yielded seeds with one tenth the mycotoxin DON (=vomitoxin) concentration (ppm by HPLC analysis) as that found in the seeds from plants inoculated with the pathogen alone (Stockwell, et al., unpublished).

A project was initiated in May 1999 to select microbial isolates with potential for biological control of FHB when applied to cereal spikes, seed or infected crop residues. We are using the following protocols for selection and evaluation of candidate microbial isolates.

MATERIALS AND METHODS

Initial selection and evaluation of isolates

Several bioassays have been adopted in order to efficiently evaluate the large number of isolates and choose the most promising organisms and bio-compatible treatments for inclusion in greenhouse and field trials.

The funnel-method test (Luz, 1990), which compares the effect of the diffusate of individual test organisms on the radial growth of *F. graminearum*, is used to evaluate the isolates for antibiosis. Bioassays which have been employed to identify organisms tolerant to environmental stresses include: NaCl-nutrient medium for osmotic stress tolerance, an assay for UV tolerance and heat tolerance for selection of spore-formers.

Evaluations as protectant sprays during anthesis

Candidate biocontrol organisms are evaluated for their efficacy as protectants by co-inoculating them

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with the pathogen onto the spikes of glasshouse-grown wheat plants during anthesis. The most promising of the newly acquired isolates will be evaluated in this way as well as re-confirming the results of previously-tested biocontrol agents. In the 2000 field season, *Paenibacillus macerans* and possibly several other outstanding biocontrol candidates will be included as treatments alongside chemical treatments in the Uniform Fungicide Trial at Aurora, NY. Sufficient primary inoculum is assured by the distribution of artificially-infested maize kernels throught the experimental plot. This material produces mature perithecia by the time the wheat reaches anthesis.

Evaluations as treatment of scabby wheat seed

The isolates will be evaluated on scabby seed for their ability to improve emergence and seedling vigor using both *in vitro* and glasshouse assays. The laboratory assay uses 24-well culture plates to evaluate the efficacy of candidate biocontrol agents, bio-compatible treatments and combinations of treatments. In the glasshouse assay, treated scabby seed are planted in sterile soil mix and rated for percent germination and seedling height.

Evaluations as treatment of infested maize debris

An *in vitro* ascospore discharge inhibition assay is used to identify organisms and other bio-compatible treatments of maize debris which will reduce perithecial formation and ascospore discharge. Several treatments are being evaluated during autumn/winter 1999-2000 for their ability to interrupt the production of primary inoculum on artificially-infested maize stalk pieces exposed to ambient environmental conditions.

RESULTS AND DISCUSSION

We have isolated, preserved and characterized approximately 120 candidate biocontrol organisms, from 70 different sources including wheat, maize, and over-wintered maize debris, wild grasses and sedges on non-agricultural land, and air-borne samples. In addition, one of the authors, Dr. Wilmar Luz of EMBRAPA-TRIGO, Brazil provided us with 14 promising microorganisms which, when used as seed treatments in field trials in Brazil, were shown to reduce losses due to several soil-borne fungal diseases of wheat and maize.

In the 1999 field season, the very promising candidate biocontrol agent, *Paenibacillus macerans*, was included as a treatment alongside chemical treatments in the Uniform Fungicide Trial at Aurora, NY. Unfortunately, the lack of precipitation at anthesis resulted in negligible infection of all treatments. This biocontrol treatment will be included again in the 2000 field trials.

One of the key issues in the development of efficient biocontrol agents is *adaptability*. Organisms which give good control in *in vitro* assays may be ineffective or unreliable under field conditions. For this reason the selection of organisms which are likely to be robust under harsh field conditions is emphasized in this project. Spore-forming bacteria are desirable as biocontrol agents because of their tolerance to environmental stresses and their stability in commercial formulations. Several candidate biocontrol agents already have been identified which exhibit both antibiosis and tolerance to several environmental stresses. We are optimistic that we will find among our collection one or more isolates that will significantly control *Fusarium graminearum*, alone or in combination with other treatments when applied to cereal spikes, seed or infected crop residues.

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