IDENTIFICATION (BASED ON MEMBRANE FATTY ACID METHYL ESTER ANALYSIS AND PARTIAL SEQUENCING OF 16S RIBOSOMAL RNA) OF BACTERIAL STRAINS USED IN THE BIOLOGICAL CONTROL OF FUSARIUM HEAD BLIGHT

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ABSTRACT

Our laboratory has been working for the last several years with bacterial strains (designated as 1B-A, 1B-C, 1B-E, and 1D-3) isolated from South Dakota wheat foliage and residue which are able to antagonize *Fusarium graminearum* in laboratory plate assays and in field plot trials. Although we have known for many years that the bacterial strains are endospore formers that are able to grow aerobically, likely being members of the genus *Bacillus*, the exact identity of the strains has remained problematic. Systematics of the genus *Bacillus* have undergone great changes since modern methods for bacterial identification, such as analysis of membrane fatty acid methyl esters (FAME analysis) and analysis of small subunit 16S ribosomal RNA sequences have become available. Analysis of FAME patterns of the four strains done about six years ago suggested that bacterial strain 1D-3 was almost certainly *Bacillus amyloliquefaciens*, whereas the other three strains were not extremely similar to any bacteria in the FAME database, but were related to a degree to *Bacillus atrophaeus* (formerly *B. subtilis* variety *niger*). This year another laboratory did FAME analyses on the four strains. Results strongly indicated that strains 1B-A and 1D-3 were *Bacillus lentimorbus*, and that strains 1B-E and 1B-C were *Bacillus subtilis*. In addition, the first 500 base pairs of the 16S rRNA gene of each strain were sequenced and compared to known sequences of ribosomal RNA genes, and alignment profiles and phylogenetic trees were derived from the sequences. Usually knowing the first 500 base pairs of the 16S rRNA gene is sufficient to determine the identity of most bacterial strains. All four strains (1B-A, 1B-C, 1B-E, and 1D-3) had identical sequences in the first 500 base pairs of their 16S RNA genes, and all were most closely related to *Bacillus amyloliquefaciens* and had less but significant relatedness to *Bacillus atrophaeus*. This and other studies have found that FAME analysis will not necessarily agree with the results of 16S rRNA sequencing. Extent and completeness of the database used in each taxonomic analysis is extremely important when attempting to identify a bacterial strain, and in some cases (such as this study) further standard physiologic tests will be needed to help make a confident identification of the bacterial strains. Complete 16S RNA sequences of the strains would be very valuable in helping to determine whether the strains truly belong to known bacterial species, or are one or more new species in the genus *Bacillus*. In addition, detailed physiologic tests will be conducted to help further evaluate the relatedness of these bacterial strains to known bacterial species. Thorough understanding of the enzymatic activities of these strains will help optimize their formulation and application as biological control agents used to control FHB in the field.

BIODIVERSITY OF MICROBIAL ANTAGONISTS TO *GIBBERELLA ZEAE* IN BRAZIL

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OBJECTIVES

To determine the diversity of microbial antagonistic agents to *Gibberella zeae.*

INTRODUCTION

Knowledge of biodiversity of living organisms is mainly important to determine their potential functions. Fusarium head blight (FHB), induced by *Gibberella zeae* (anamorph = *Fusarium graminearum* Schw.) is a prevalent wheat disease in Brazil, causing crop production losses varying from 10% (Luz, 1984); to 54 % (Picinini & Fernandes, 1994). Due to the difficulty in controlling the disease by chemical treatment, crop rotation and varietal resistance, biological control agents are beeing evaluated as an additional tactics for integrated management of FHB. To have an idea of potential bioprotectants, biodiversity of promining isolates need to be established.

MATERIALS AND METHODS

Thousands of microorganisms were screened in vitro and in vivo against *G. zeae.* Biodiversity of most effective microbial strains was established by systematic determination using physiological, biochemical and morphological features as well as systems such as Biolog, GC-Fame (analysis of fat acids) and comparison of the sequences of the small subunits of the RNA 16S (for bacterial strains), comparing 500 base pairs.

RESULTS AND DISCUSSION

The diversity of microorganisms that shows potential for managing FHB in Brazil comprises 15 different species as presented in Table 1. Searching different isolates of these microorganisms with different degree of control efficiency may be of valuable interest to investigators in other countries as well as in Brazil. This microbiota may have an important impact in inproving FHB control either alone or as an additional measure to supplement chemical, cultural and resistance control methods.

Table 1. Biodiversity of microorganisms for biocontrol of Fusarium head blight Wheat in Brazil[1]

Bacillus licheniformans
Bacillus lentimorbus
Baccillus megaterium
Bacillus pumilus
Bacillus subtilis
Clavibacter michiganense insiduosum
Enterobacter cloacae
Klebsiella planticola
Kluyvera cryocrescens,
Paenibacillus macerans
Pantoea agglomerans
Pseudomonas putida
Pseudomonas fluorescens
Sporobolomyces roseus
Rhodotorula sp.

[1] Identifications of bacterial isolates by Microbe Inotech Laboratories, Inc. Saint Louis, Mo, USA, based on GC-FAME, Biolog, and/or 16Sr RNA sequence. Bacterial colony morphology, physiological tests, and morphological indentification of fungi by Plant Pathology Laboratory, Embrapa Trigo, Passo Fundo, RS, Brazil.

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GREENHOUSE SCREENING OF BIOLOGICAL CONTROL AGENTS FOR SUPPRESSION OF FUSARIUM HEAD BLIGHT

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ABSTRACT

In order to better understand the potential application of biological control agents for the suppression of Fusarium head blight (FHB), several bacterial isolates were selected that had shown evidence of biological activity against *Fusarium graminearum* in culture. These agents were selected and screened in two consecutive greenhouse trials in 2001. Oxen hard red spring wheat was planted in four replications of twenty plants each in a greenhouse ground bed for the first trial and in cone-tainers for the second trial. Treatments included untreated/uninoculated (negative control), untreated/inoculated (positive control), a chemical control standard (Folicur at 4 fl oz/A), and four biological control agents (SDSU-1BA, SDSU-1BC, TrigoCor 1448, and Trigo Cor 9790). Ten heads per treatment were exposed to the biocontrol agents and the agent was allowed to dry. The heads were then challenged with a 10^6 CFU/ml of *Fusarium graminearum* conidia about 12 hours later. The plants were incubated in a humid chamber, with mist applied for ten minutes each hour to maintain nearly 100% relative humidity for a period of two weeks. Three weeks after inoculation, the treated heads were evaluated for FHB. In the first study, FHB damage was fairly high, about 20% incidence and 37% head severity in the untreated/inoculated check. Only Folicur significantly reduced FHB incidence, head severity, and disease index (incidence X severity) in this trial. In the second study, a lower density of challenge inoculum (10^4 CFU/ml of *Fusarium graminearum* conidia) was used to avoid excessively high disease severity. Incidence and severity of FHB were reduced from levels observed in the first study, about 4% incidence and 28% head severity in the untreated/inoculated check. However, no treatments significantly reduced any measurement of FHB in this trial. Additionally, a background level of FHB escaped to the negative control, making it more difficult to differentiate treatments. However, numeric differences were observed that indicate there may be cause for further study. Two biological treatments, SDSU-1BA and TrigoCor 1448 reduced disease index by 73% and 75% respectively. While this may seem unimportant due to the lack of statistical validity, the important point is that the Folicur treatment only reduced disease by 85% in the same study. A similar relationship was noted in field studies with SDSU-1BA under low disease pressure in 2000. As such, further study will be conducted on these and other potential biological control agents.

EFFECT OF FOLIAR FUNGICIDES AND BIOCONTROL AGENTS ON FUSARIUM HEAD BLIGHT DEVELOPMENT AND CONTROL IN OHIO

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OBJECTIVES

i) to evaluate the effect of three foliar fungicides (Folicur, AMS21619, and BAS505) and two biocontrol agents (TrigoCor 1448, and USDA/Peoria-OH182.9) against Fusarium head blight,

ii) to determine the relationships between the disease, DON and yield, and iii) to document the effect of these materials on scab development.

INTRODUCTION

Fusarium head blight (FHB) or scab, caused by *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae*) is a major disease in many wheat and barely production regions of North America and throughout the world (Bai and Shaner 1994; Parry et al.1995; McMullen et al. 1997). This disease has been difficult to control. Although recent advances in host resistance are beginning to improve disease management in some wheat production regions, many wheat and barley producers have few management options. Commonly used methods of disease management including tillage and crop rotations, have not been effective in eliminating wide spread disease epidemics (McMullen et al. 1997). Controlling Fusarium head blight will require multiple disease management strategies, coupled with greater understanding of the epidemiology of the disease (Bai and Shaner, 1994; Parry, et al., 1995; Shaner and Buechley, 2000).

Effective fungicides could provide growers with management options when susceptible cultivars are grown, and may help protect yield and grain quality of cultivars with partial resistance under conditions favorable for disease. Although a few fungicides have shown some efficacy against scab, their results have been inconsistent over locations and years (Parry, et al., 1995; McMullen et al. 1997; Shaner and Buechley, 1999; Gilbert and Tekauz, 2000). Treatment with some fungicides reduced DON contamination of grain, but others caused an increase in DON levels (Shaner and Buechley, 1997, 1999 and 2000; Gilbert and Tekauz, 2000).

MATERIALS AND METHODS

Seeds of wheat cultivar Elkhart treated with Raxil-Thiram, were planted using 24 seeds/ft of row on 11 Oct., 2000 in Ravenna silt loam soil at the Ohio Agricultural Research and Development Center, Wooster. For each treatment, there were three replicate plots. Each plot was 15-ft long, and consisted of 7-rows with 7 in. between rows. Plots were inoculated by

broadcasting colonized corn kernels (0.12 oz/sq ft) over the plot surface on May 14. Plots were misted each day from one week prior to flowering to two week after flowering, using NAAN 7110 series bridge with mist sprayer head 327122 fitted with nozzles having 0.35 in. opening. Biological agents and fungicides were applied as sprays in 26.2 gal. water/A with a CO- pressurized back pack sprayer with a constant boom pressure of 40 psi and 15 in. between twinjet XR8001 nozzles mounted at a 60 degree angle forward and backward. Sprays were applied at flowering (30 May). Disease assessments were made twice a week (June 11 - June 26) for both incidence and severity in one ft. of row at 15 locations in each plot. Plots were harvested on 17 of July. Yield (bu/A) was determined from harvested grain adjusted to 13.5% moisture, and grain was analyzed for DON content.

RESULTS AND CONCLUSIONS

Disease development varied greatly among the different fungicide and biological control treatments tested (Fig. 1). Based on the coefficient of determination (R), evaluation of the residual plots, the standard error of estimates (SE) and mean square errors (MSE), the Gompertz model was appropriate for describing the disease incidence and severity data sets (R ranged from 83 to 93%). The various treatments had a significant effect on disease development. Rates of disease increase for the various treatments and the control ranged from 0.138 to 0.229 per day based on disease incidence, and from 0.093 to 0.172 per day for disease severity (Table 1). Area under the disease progress curve based on disease incidence (AUDPCI) ranged from 418.0 to 804.2 and from 125.1 to 307.5 when based on disease severity (AUDPCS) (Table 1). Final disease incidence for the various treatments ranged from 55.0 to 89.6% and final disease severity ranged from 23.9 to 57.9% (Table 2).

Plots treated with Folicur at 6.0 fl oz/A, AMS21619 or BAS 505 had significantly lower rates of disease increase and AUDPCI values than the untreated control (Table 1). Only plots treated with AMS21619 and BAS 505 fungicides had both significantly lower rates of disease progress and AUDPCS values than the untreated control plots (Table 1). Additionally, only plots treated with AMS21619 and BAS 505 had significantly lower final disease incidence and severity than the untreated control (Table 2).

Plots treated with AMS21619, BAS 505 and the BAS 505 plus Folicur had significantly higher yield than the untreated plots. However, of the plots treated with these fungicides only grain from the AMS21619 and the BAS 505 treated plots had significantly lower DON levels than grain from the untreated control plots. Although the biocontrol agent USDA OH182.9 did not have a significant effect on reducing disease development, grain harvested from plots treated with this biocontrol agent had significantly lower DON than grain from the untreated control plots.

There were positive correlations between DON and final disease severity, AUDPCI, AUDPCS with correlation coefficient (r) of 0.79, 0.68, and 0.69 respectively. On the other hand, there were negative correlations between yield and final disease severity, AUDPCI, and AUDPCS with correlation coefficient (r) of 0.63, 0.54, and 0.55 respectively.

In conclusion, the treatments exhibited different effect on Fusarium head blight development and control. Treatments AMS21619 and BAS505 had low maximum disease, low epidemic

rates, and small AUDPCI and AUDPCS values that were significantly different from the control. On the other hand, treatments TrigoCor 1448 and USDA/OH182.9 had high maximum disease, fast epidemic rates, and large AUDPCI and AUDPCS values that were not significantly different from untreated control. These results indicate the AMS21619 and BAS505 fungicides have greater potential for management of Fusarium head blight than the other treatments tested.

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Table 1. Fit of models, epidemic rates, and area under disease progress curve for Fusarium head blight incidence (AUDPCI) and severity (AUDPCS) for fungicides and biocontrol agents tested in Ohio, in 2001.

Treatment and rate/A	Incidence			Severity		
	Model Fits	Rate	AUDPCI	Model Fits	Rate	AUDPCS
Control	Gompertz	0.212	759.3	Gompertz	0.159	291.9
Folicur 3.6 EC 4.0 fl oz+ Induce (0.125%, v/v)	Gompertz	0.194	634.1	Gompertz	0.141	235.9
Folicur 3.6 EC 6.0 fl oz+ Induce (0.125%, v/v)	Gompertz	0.158*	592.1*	Gompertz	0.119*	192.6
AMS21619 480SC 5.7 fl oz +Induce (0.125%,v/v)	Gompertz	0.138*	418.0*	Gompertz	0.093*	125.1*
BAS 505 50G 3.1 oz	Gompertz	0.143*	469.2*	Gompertz	0.117*	159.4*
BAS 505 50G 3.1 oz Folicur (3.6 EC 2.0 fl oz)	Gompertz	0.166	647.9	Gompertz	0.148	254.7
TrigoCor 1448	Gompertz	0.231	798.4	Gompertz	0.169	315.7
USDA/ OH182.9	Gompertz	0.229	804.2	Gompertz	0.172	307.5

*Indicates means significantly different ($p \geq 0.05$) from untreated control based on LSD.

Table 2. Mean final disease of Fusarium head blight, yield, and DON content of grain for fungicides and biocontrol agents tested in Ohio in 2001.

Treatment and rate/A	Mean Final Disease		Yield (bu/A)	DON (ppm)
	Incidence (%)	Severity (%)		
Control	82.5	50.9	62.3	16.0
Folicur 3.6 EC 4.0 fl oz+ Induce (0.125%, v/v)	75.8	41.5	66.6	12.0
Foliur 3.6 EC 6.0 fl oz+ Induce (0.125%, v/v)	69.7	35.3	66.8	14.6
AMS21619 480SC 5.7 fl oz +Induce (0.125%,v/v)	55.0*	23.9*	74.0*	7.2*
BAS 505 50G 3.1 oz	60.1*	28.6*	77.1*	8.4*
BAS 505 50G 3.1 oz Folicur 3.6 EC 2.0 fl oz	73.0	41.6	70.2*	14.9
TrigoCor 1448	89.6	57.9	56.0	24.0*
USDA/OH182.9	87.5	51.8	62.0	10.7*

* Indicates means significantly different ($p \geq 0.05$) from untreated control.

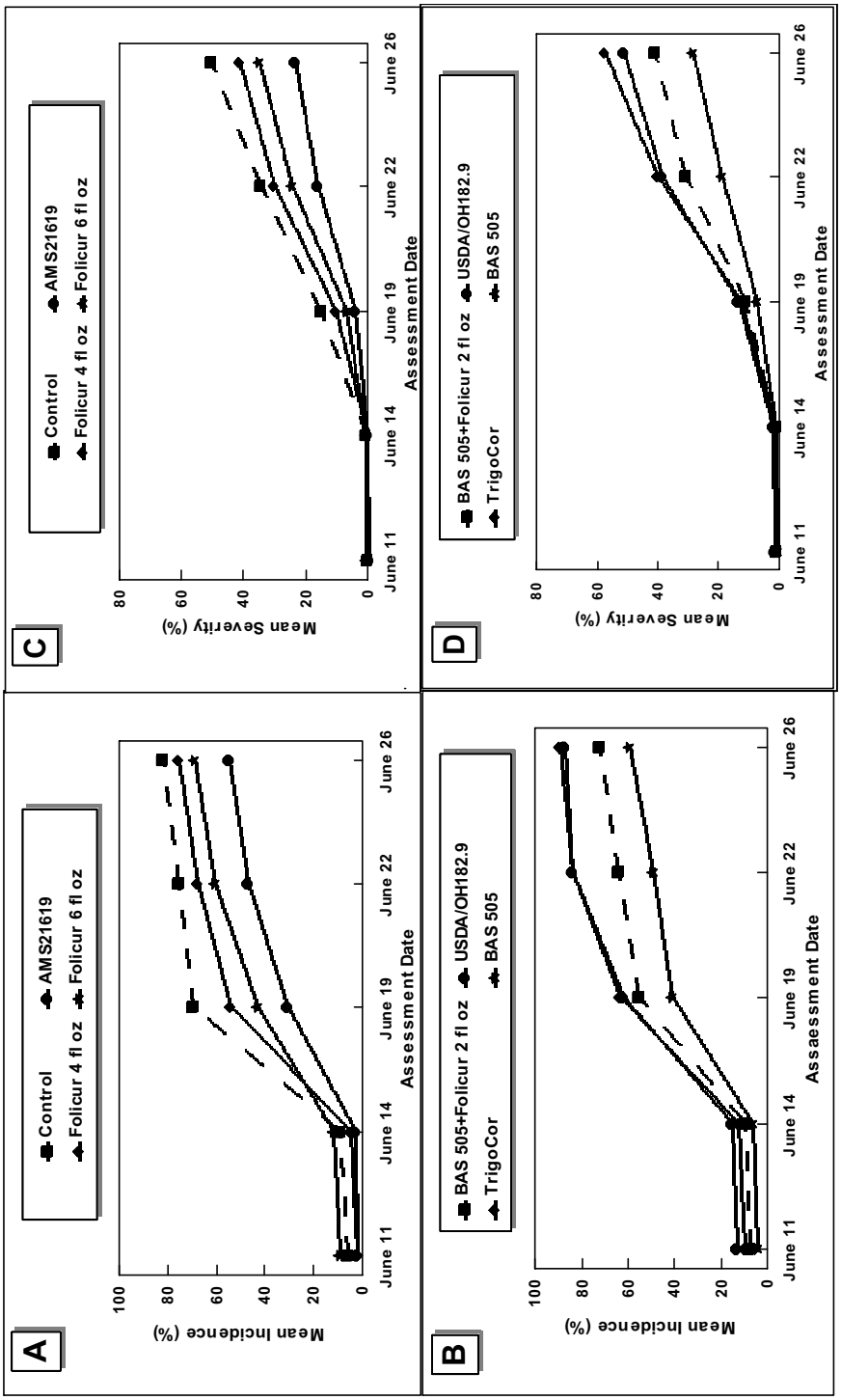

Fig. 1 Disease progress curves for Fusarium head blight incidence (A, B) and severity (C, D) for fungicides and biocontrol agents tested in Ohio in 2001.

UNIFORM FUNGICIDE TRIAL COLLABORATIVE STUDY 2001– MICHIGAN STATE UNIVERSITY

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OBJECTIVE

Evaluate efficacy of fungicides on reducing incidence, severity and deoxynivalenol (DON) on Fusarium head blight (FHB) in winter wheat.

INTRODUCTION

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, vomitoxin). This project was part of an ongoing collaborative study among states participating in the FHB Initiative (Milus and McMullen, 2000; McMullen et al, 1999; and Hart, et al 1999).

METHODS AND MATERIALS

Winter wheat varieties, Frankenmuth, Harus, and Freedom were planted in October 2000. Fertilizer and herbicides were applied as per Michigan State University recommendations. Corn inoculum infested with *Gibberella zeae* was applied on May 17th and again on June 4th. Low volume overhead irrigation was started on May 24th. Irrigation was on for 15 minutes and off for ninety minutes, 24 hours/day until June 16th. The irrigation was turned off for 24 hrs the days fungicides were applied. Fungicides were applied at Feekes growth stage 10.5 to Harus and Freedon on May 31st, and to Frankenmuth on June 7th. Fungicides were applied with a CO_2 backpack sprayer and flat fan nozzles directed at a sixty-degree angle above the horizontal. Each treatment was replicated four times, and replicated plot was 15 by 30 feet. Plots were rated on June 27th. Because the disease incidence was always 100 percent, the disease severity was used to calculate differences among treatments. Normally the incidence is multiplied by the severity to get a disease index value (DI). Incidence is the number of heads in a plot with symptoms, and severity is the percent of spikelets infected. Mature grain was harvested, weighed, milled and analyzed for DON by ELISA (Hart, et al, 1998).

Treatments:

1. Untreated
2. Folicur 4 fl oz + 0.125% v/v Induce
3. AMS 21619 at 5.7 fl oz/A + 0.125% v/v Induce
4. BAS 505 0.2 lbs ai/acre + 0.125% Induce
5. BAS 505 0.1 lb ai/acre + Folicur 2 fl oz + 0.125% v/v Induce

6. Cornell biological agent
7. USDA/Peoria biological agent

A second project evaluating a prototype sprayer was held at the Michigan Bean and Beet Farm. Folicur was applied at GS 10.5 (June 8th) on the variety Harus using either a conventional boom sprayer using 25 gal of water/acre with flat fan nozzles directed at a sixty-degree angle above the horizontal; or the prototype sprayer using 5 gal of water/acre. Treatment 2 above, 4 oz of folicur + 0.125% Induce, was the only fungicide applied. Each plot was 75 x 525 feet, and the center 30 feet x 525 was harvested on July 16th. The treatment were; 1) wheat was sprayed from two sides with the prototype to ensure complete coverage of the head with fungicide; 2) wheat was sprayed on only one side with the prototype sprayer resulting in incomplete coverage; 3) Conventional flat fan sprayer with nozzles aimed downward; and 4) Untreated controls. There was only one replication per treatment. Twenty-five grain probes per treatment were collected directly from the combine at harvest. Each probe sample was analyzed was analyzed separately for DON (Hart, et al, 1998). The plots were not rated for yield or disease severity.

RESULTS AND DISCUSSION

Uniform Fungicide Trial. FHB developed late in 2001, toward the end of flowering (Figure 1). Frankenmuth headed and flowered 7-10 days later than Harus or Freedom.

Heading and flowering were occurred later compared to previous years, and the flowering was longer, probably due to the cool temperature during flowering (Figure 1). FHB incidence was one hundred percent in all the plots, and severity was moderate (Table 1). Several treatments significantly reduced the severity of FHB, but did not significantly affect yield or DON. The rain and temperature data suggest that favorable infection periods probably occurred only toward the end of flowering, and may account for the limited affect of fungicide treatments on yield and DON (Figure 1; Hart, et al 1984).

Saginaw Trial. Treatments were not evaluated for FHB incidence, severity or yield. DON levels in the different treatments wer:

Treatment	DON (PPM)
1	0.3
2	0.9
3	0.9
4	0.9

Although these results are preliminary and not replicated, they do suggest that thorough coverage of the wheat head is essential to reduce DON, and new technologies using very low spray volumes may compete very will with conventional sprayers. The conventional sprayer used here would not have provided coverage for both sides of the wheat head.

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Table 1. Comparison of fungicide efficacy on FHB incidence, severity, yield, and DON in Uniform Fungicide Trials at Michigan State University.

Treatment #	Incidence	Mean Severity	Mean Yield	DON (ppm)
1	100	31.1a	70.3a	1.2a
2	100	22.5ab	74.8a	1.2a
3	100	19.7b	72.4a	0.8a
4	100	21.3b	72.7a	1.1a
5	100	20.6b	72.3a	1.2a
6	100	29.0ab	65.0a	1.3a
7	100	26.7ab	70.0a	1.2a

Individual Varieties

Frankenmuth

Treatment #	Incidence	Mean Severity	Mean Yield	
1	100	20.3ab	60.7a	0.8a
2	100	18.3ab	61.6a	0.5b
3	100	18.8ab	51.7a	0.5b
4	100	12.0a	51.7a	0.6a
5	100	14.5a	52.9a	0.7a
6	100	19.3ab	48.0a	0.9a
7	100	27.5b	50.9a	0.7a

Freedom

Treatment #	Incidence	Mean Severity	Mean Yield	
1	100	45.0a	71.2a	1.2a
2	100	30.0b	84.4a	1.2a
3	100	22.0b	81.4a	0.6b
4	100	33.5ab	84.7a	1.0a
5	100	30.0b	78.2a	0.9a
6	100	34.8ab	70.5a	1.1a
7	100	31.5ab	62.1a	1.1a

Harus

Treatment #	Incidence	Mean Severity	Mean Severity	
1	100	28.0a	79.6a	1.7a
2	100	19.3b	83.2a	1.8a
3	100	18.3b	88.1a	1.3a
4	100	18.3b	87.8a	1.5a
5	100	17.3b	87.4a	1.9a
6	100	33.0a	77.9a	2.0a
7	100	21.0b	79.6a	1.7a

Overall Variety Comparison

Variety	Mean Yield	Mean Severity	DON
Frankenmuth	53.9a	18.6a	0.7a
Freedom	78.9b	32.4b	1.0b
Harus	83.2b	22.1b	1.7c

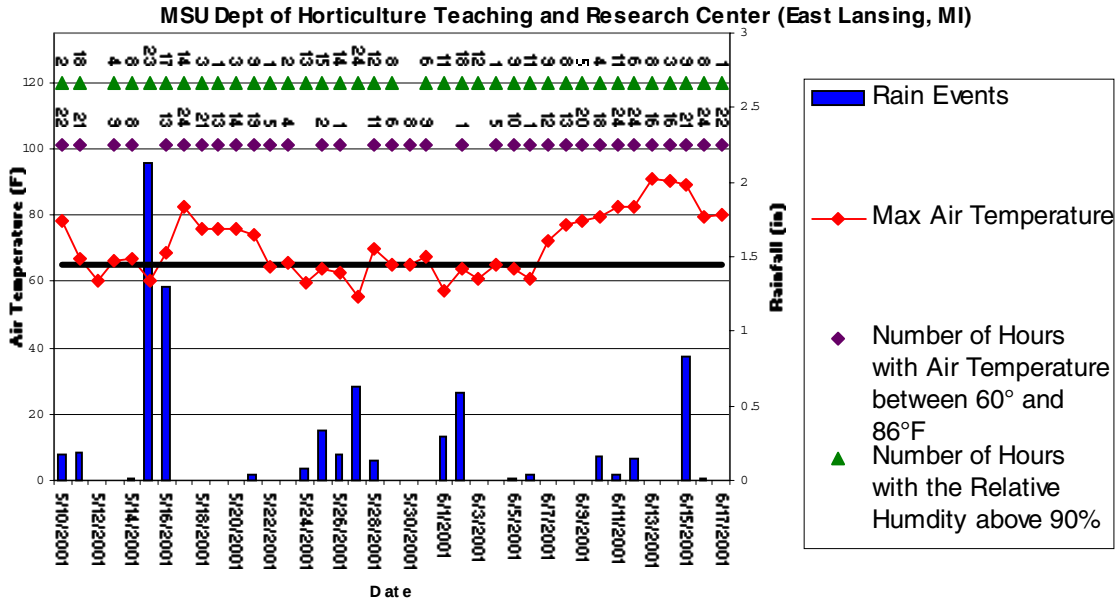

Figure 1. Rain fall and temperature patterns at the MSU Horticulture Farm between May 11th and June 17th, 2001. Flowering occurred between May 25th and June 15th. Temperature above 60°F coinciding with three or more days of rain and flowering may be favorable for infection of wheat by *Gibberella zeae*.

MANAGEMENT OF FUSARIUM HEAD BLIGHT IN WHEAT USING SELECTED BIOLOGICAL CONTROL AGENTS AND FOLIAR FUNGICIDES, 2001

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OBJECTIVES

To evaluate selected foliar fungicides and biological control agents for potential use in soft red winter wheat Fusarium head blight management programs in Kentucky. Also, to generate data as a cooperator in the 2001National Fusarium head blight Uniform Fungicide and Biocontrol Test.

INTRODUCTION

Fusarium head blight (FHB) of wheat and barley is a significant disease concern in all wheat and barley producing regions of the United States. Statewide, epidemics in Kentucky are rare, but each year some fields are severely damaged by FHB. Currently, the only options available for the management of FHB are the use of cultural practices that encourage escape from disease. These include the use of multiple planting dates and varieties representing different flowering dates and periods. Moderate resistance is also available in several different wheat varieties, but severe FBH will occur under conditions that favor FHB. Preliminary studies conducted in various states indicate that foliar fungicides (Milus and McMullen, 2000) and biological control agents (BCA's) (Schisler et al, 2000) may be capable of providing safe, effective and economical management of FHB. Nonetheless, specific and consistent data are lacking in regards to which products and rates are most suitable for use in FHB management programs. The National FHB Uniform Fungicide and Biocontrol Test program was established as a means of addressing this deficiency in data. This test involves cooperators at various test locations across the county, the use of a standard set of promising treatments, and a reasonably standardized testing protocol. Each state, including the one in Kentucky during 2001, also evaluate unique treatments of interest locally.

MATERIALS AND METHODS

The test site was established at the University of Kentucky Research and Education Center in Princeton, KY. The core set of treatments evaluated was determined by collective agreement of the scientists involved in the National FHB Uniform Fungicide and Biocontrol Test. Specific local treatments were also evaluated. Treatments included a variety of foliar fungicides and two BCA's. The test site was planted in a conventionally-tilled seed bed on October 18, 2000 and maintained according to standard crop husbandry practices for soft red winter wheat production in west Kentucky (Bitzer and Herbek, 1997). The wheat variety planted was 'Clark'; maize was the previous crop grown in the test site.

Plots were inoculated on April 1, 2001 with sterilized, cracked corn infested with a mixture of several highly pathogenic isolates of *Fusarium graminearum*, the primary causal agent of FHB. Test plots were mist-irrigated according to a strict regime in order to encourage the causal fungus to produce infectious spores and infect the test plots. Between inoculation and the onset of flowering, plots were mist-irrigated for two hours daily, between 7pm and 9 pm. Following the onset of flowering, plots were mist-irrigated twice daily from 5-7am and 7-9pm. Fungicides were applied to plots on May 7, 2001 when the crop was in the early flowering. Treatments were applied using a CO^2-propelled plot sprayer delivering at 40 PSI in 18-20 GPA. The spray boom was equipped with twinjet XR8001 nozzles oriented at a 60 degree angle forward and backward. FHB incidence, severity, and field severity data were obtained by collecting and visually rating 100 heads from each test plot. Plots were harvested with a small plot combine and grain yield and test weight where calculated. Deoxynivalenol (DON) levels were determined at the Michigan State University Don Testing Laboratory. Tests for standard germination, percent dead seed, and percent seed infected by Fusaria were conducted at Dr. TeKrony's seed technology laboratory in Lexington, KY. Percent visually scabby kernels (VSK) was determined by segregating healthy from scabby kernels for two sets of 100-seed samples for each treatment replication.

RESULTS AND DISCUSSION

Overall conditions of the test favored moderate crop yield and significant, but not excessive, FHB pressure. At the first rating date (late milk wheat stage), all treatments except Folicur alone had significantly lower disease incidence than the non-treated check (Table 1). Disease severity and field severity, however, were similar among treatments. By the soft dough stage eight days later, the following treatments had significantly lower disease incidence ratings compared to check plots: Folicur 4.0 fl oz/A, AMS 12619 5.7 fl oz/A, BAS 505 0.2 lb a.i./A, and Tilt 4 fl oz/A plus Quadris 4.11 fl oz/A. Of these treatments, none had significantly lower severity ratings and only the treatments involving AMS 12619 and BAS 505 alone had significantly lower field severity ratings. The only significant yield difference was with the AMS 12619 treatment. In contrast, test weight values were significantly higher than the check for treatments involving Folicur alone, AMS 12619, BAS (0.1 lb. a.i.) + Folicur (2 fl oz) and BAS 500 alone. None of the treatments resulted in significantly reduced percent Fusaria as determined by culturing fungi from surface-sterilized seed (Table 2); lack of significance appeared to be the result of significant variability between treatment replications treatments. Regarding percent VSK, only AMS 12619 and BAS 505 alone resulted in values significantly below the check. AMS 12619, BAS 505 + Folicur, BAS 505 alone, and the Cornell BCA (TrigoCor 1448) each significantly reduced ppm of DON compared to the check. Standard germination of harvested seed was significantly greater than the check for treatments involving AMS 12619 and BAS 505 alone. Number of dead seed was statistically similar between all treatments.

No fungicide or BCA reduced FHB severity at either rating date. This is consistent with previous studies (McMullen et al, 1999). In our study, AMS 12619 (5.7 fl. oz) + induce (0.125% v/v) was the only treatment that resulted in a significant yield advantage when compared with the check. Foliar and other head diseases were not a factor in this test, so this yield result was apparently directly related to partial control of FBH. Several foliar fungicide treatments, including AMS 12619, suppressed FHB to a moderate extent, reduced

DON levels in grain and minimized test weight losses when compared with the check. No treatment provided excellent control of FHB. Seed quality, as indicated by standard germination and percent VSK, was maintained at higher levels in treatments involving AMS 12619 and BAS 500 alone. Other treatments, including both BCA's, had no positive effect on any of the seed quality parameters measured. Overall, the treatment involving AMS 12619 was the most consistent and effective performer across all parameters measured. In contrast, Folicur performed very poorly in this study. Specifically, there was only a slight reduction in FHB incidence (at the early but not late rating date, Table 1) and a higher test weight when compared with the check; other measurements were statistically similar to the check. This is an interesting finding considering that Folicur is usually among the most efficacious fungicides for managing FHB (E. Milus, *personal communication*; McMullin et al, 1999). The two BCA's studied where ineffective across all data sets. The one exception was a significant reduction in DON for the Cornell University BCA, TrigoCor 1448. Similarly, three treatments involving Tilt performed very poorly in the test, with the exception of a significant reduction in FHB incidence when Tilt (4.0 fl oz/A) was mixed with Quadris (4.11 fl oz/A).

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Table 1. Effect of various fungicides and BCA's on FHB, yield and test weight.

	Disease Ratings*							
	May 22			May 30				Tst Wt
Treatment and rate/A	**Inc**	**Sev**	**Fld Sev**	**Inc**	**Sev**	**Fld Sev**	**Bu/A****	
Non-treated	19.2	12.9	2.4	43.3	43.4	19.0	61.3	55.6
Folicur: 4.0 fl oz + Induce 0.125% v/v	14.3	10.4	1.5	37.1	45.6	16.9	61.6	56.4
AMS 12619 5.7 fl oz + Induce 0.125% v/v	7.3	9.3	0.7	30.3	41.3	12.4	67.7	57.2
BAS 505: 0.1 lb a.i.+ Folicur: 2.0 fl oz + Induce 0.125% v/v	7.5	15.3	0.8	37.4	52.5	19.6	63.7	56.6
BAS 505 0.2 lb a.i. + Induce 0.125% v/v	6.1	24.4	1.3	24.8	48.5	10.8	66.1	57.2
Cornell BCA (TrigoCor 1448)	8.8	13.9	1.1	38.8	52.1	20.1	60.4	55.9
USDA BCA (OH 182.9)	11.9	14.3	1.7	39.5	55.5	22.0	63.2	55.7
Tilt 4 fl oz + Induce 0.125% v/v	9.1	11.3	1.1	42.4	57.8	24.4	60.9	55.5
Tilt 4 fl oz + Quadris 3.42 fl oz + Induce 0.125% v/v	12.8	9.7	1.3	43.6	61.5	26.2	60.4	55.7
Tilt 4 fl oz + Quadris 4.11 fl oz + Induce 0.125% v/v	8.5	18.1	1.5	31.6	59.9	16.8	65.1	56.2
LSD P=0.05	**6.1**	**NS**	**NS**	**5.9**	**6.5**	**5.3**	**6.1**	**0.7**

*Inc = Incidence; Sev = Severity; Fld. Sev = Field Severity. All ratings are based on 100 heads collected and rated at late milk (May 22) and soft dough (May 30) stages. ** Based on 13% moisture and 60lb/bu test weight.

Table 2. Effect of various fungicides and BCA's on FHB on various seed quality parameters.

Treatment and rate/A	% Fusaria	VSK*	DON (ppm)	Std** Germ	No. Dead Seed
Non-treated	38.0	25.8	5.7	75.2	18.0
Folicur: 4.0 fl oz + Induce 0.125% v/v	39.5	23.8	4.6	76.4	17.3
AMS 12619 5.7 fl oz + Induce 0.125% v/v	24.8	14.8	1.7	84.1	10.4
BAS 505: 0.1 lb a.i.+ Folicur: 2.0 fl oz + Induce 0.125% v/v	33.2	20.0	3.8	77.0	15.6
BAS 505 0.2 lb a.i. + Induce 0.125% v/v	26.4	14.4	3.4	82.5	12.7
Cornell BCA	42.0	24.8	3.5	80.3	14.8
USDA BCA	34.4	23.6	4.7	76.1	16.4
Tilt 4 fl oz Induce 0.125% v/v	33.2	26.8	5.7	77.0	16.3
Tilt 4 fl oz + Quadris 3.42 fl oz + Induce 0.125% v/v	41.2	27.2	6.1	73.5	18.9
Tilt 4 fl oz + Quadris 4.11 fl oz + Induce 0.125% v/v	28.8	21.0	5.5	74.4	18.7
LSD P=0.05	**NS**	**7.9**	**1.8**	**6.2**	**NS**

* Visually Scabby Kernels: 100 seed per plot were examined twice for scabby kernels and the average was used.
** Percent of seed germinated.

POTENTIAL FOR BIOLOGICAL CONTROL OF FUSARIUM HEAD BLIGHT BY *LYSOBACTER SP*. STRAIN C3

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ABSTRACT

Control of Fusarium head blight (FHB) remains a challenge for wheat and barley producers. Host resistance and fungicides provide only partial control of infection and are not very effective in reducing production of deoxynivalenol in seed. Biological control is being explored as another strategy for disease management. *Lysobacter sp.* strain C3 (previously reported as *Stenotrophomonas maltophilia*) is a bacterial agent that is active through chitinolysis and induced resistance. In previous field studies it exhibited efficacy against a number of turfgrass diseases and rust in common bean. C3 also was effective in greenhouse tests in controlling spot blotch (*Bipolaris sorokiniana*) and leaf rust (*Puccinia triticina*) on wheat. Application of chitin broth cultures of C3 provided the highest level of disease control; the culture fluid contained high levels of lytic enzymes, which aided in pathogen suppression, and supplied nutrients for colonization of the bacterium on the plant surface. The culture fluid also was found to elicit induced resistance in turfgrasses. Our objectives in this study were to evaluate, under greenhouse conditions, the potential for using C3 to control FHB and to determine the parameters for application of C3 in future field trials. C3 cell suspensions and whole C3 chitin broth cultures (7 days-old) were applied to 'Bobwhite' wheat heads at anthesis. Plants were then held overnight in 90-100 % relative humidity, inoculated with a sprayed suspension of *Fusarium graminearum* conidia, and then held in high humidity for another 48 hours. C3 cells suspended in distilled water exhibited little control of FHB. Treatments with C3 chitin broth cultures, on the other hand, effectively reduced the severity of FHB and were consistent between experiments. When applied to wheat heads 1 day prior to *Fusarium graminearum* inoculation, C3 treatments reduced the percent of infected spikelets to less than 10 %, whereas the controls typically exhibited greater than 50% infected spikelets. A 1:125 dilution of the whole chitin broth culture was as effective as a full strength application. FHB was controlled with a ½ strength broth culture of C3 even when the treatment was applied 7 days prior to pathogen inoculation. The results suggest there is potential for using C3 chitin broth cultures to control FHB in the field. Experiments are currently underway to test whether FHB control by C3 is due to antagonism or induced resistance. Future experiments will involve testing C3 broth cultures for efficacy in the field with spring and winter wheat cultivars.

FURTHER STUDIES ON THE EFFECTS OF TIMING OF APPLICATION AND OF ADJUVANTS ON FUNGICIDE CONTROL OF FHB

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ABSTRACT

Application techniques that will improve fungicidal control of Fusarium head blight in spring wheat, durum wheat and barley are needed. Early flowering (Feekes growth stage 10.51) has been defined by our research group as the optimum timing for a single fungicide application in spring wheat and durum. However, questions have arisen on whether multiple or split applications of fungicide, from early head emergence through kernel watery ripe stage, would provide better control than this single application. A greenhouse experiment was designed to test efficacy of single applications of the full label rate (4 fl oz) vs multiple applications of split rates of Folicur fungicide to durum wheat. Application timings tested were: 50% head emergence (Feekes 10.3); full head emergence prior to flowering (Feekes 10.5); early flowering (Feekes 10.51); and/or kernel watery ripe stage (Feekes 10.54). FHB disease was achieved by atomizing spores (5000/ml) of *Fusarium graminearum* onto the durum heads at Feekes 10.51. Results indicated that the greatest reduction of FHB severity was with a 2 fl oz/acre rate of Folicur applied at Feekes 10.5 followed by a second 2 fl oz application at Feekes 10.51. The second greatest reduction in FHB occurred with a split application of 1 fl oz at Feekes 10.3, followed by 2 fl oz at Feekes 10.51, and then 1 fl oz at Feekes 10.54. The smallest reduction of FHB occurred when 4 fl oz of Folicur were applied once at Feekes 10.54. In addition to studying timing of application to improve fungicide efficacy, use of various adjuvants has been examined. Field experiments in 2000 showed that some adjuvants used in conjunction with Folicur or Tilt fungicide for FHB control resulted in greater reduction in FHB than others. Further adjuvant tests in the greenhouse in 2001 showed that the addition of some experimental humectant adjuvants resulted in slightly improved control of FHB in spring wheat than did Induce or Silwet adjuvants when added to Folicur fungicide, while just the opposite results occurred when the same adjuvants were added to Tilt fungicide.

UNIFORM BARLEY FUNGICIDE TRIALS IN NORTH DAKOTA, 2001

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ABSTRACT

A uniform set of five fungicide treatments was evaluated on Robust barley in ND in 2001 for control of Fusarium head blight (FHB) and fungal leaf diseases. Treatments were tested in replicated plots at Fargo and Langdon. Artificial inoculum in the form of inoculated grain was dispersed in plots at both locations. Natural rainfall was augmented by mist irrigation at Langdon, but not at Fargo. All treatments were applied in 15-20 gpa at early full head emergence (Feekes 10.5) with a CO_2 backpack type sprayer equipped with XR8001 nozzles mounted at a 60 degree angle forward and backward toward the grain head. Treatments included Folicur (tebuconazole) fungicide, AMS 21619 (an experimental from Bayer, Corp.), BAS 505 (an experimental from BASF), a combination of BAS 505 + Folicur), and Caramba (metconazole; not registered in the US). Disease ratings were taken at soft dough stage of kernel development. Plots were harvested with small plot combines. Plots were in a RCB design with four replicates, and data were statistically analyzed across locations using ANOVA. Disease development at both locations was relatively low compared to recent years, with FHB field severity in the untreated plots averaging 6.7% at Fargo and 8.9% at Langdon. All fungicide treatments significantly reduced FHB incidence, head severity, and field severity from the untreated check, but differences among fungicide treatments were not significant. The AMS fungicide gave the greatest reduction (70.5%) in FHB field severity. DON values were less than 0.5 ppm at Fargo for all treatments, and were not yet available from Langdon at the time of this report. Leaf diseases, primarily net blotch and *Septoria passerinii*, were reduced by all fungicide treatments, but not significantly. Yield was increased by 1-6.5 bu/acre by fungicide treatments, but differences were not statistically significant. Test weights were increased by 0.6-2.7 lb/bu, but differences were not statistically significant.

ND UNIFORM WHEAT FUNGICIDE AND BIOCONTROL TRIALS, 2001

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OBJECTIVE

To evaluate fungicides and biological agents for control of Fusarium head blight (scab) and leaf diseases in spring wheat and durum wheat.

INTRODUCTION

North Dakota wheat producers continue to be very interested in having effective fungicides or biological agents that will substantially reduce Fusarium head blight (FHB) severity and DON vomitoxin) levels, will reduce leaf diseases,and will increase yields. Although a recent North Dakota release of a resistant hard red spring wheat cultivar (Alsen) may reduce the need for fungicides in those spring wheat fields, acreages of susceptible spring wheat cultivars are still planted in North Dakota and durum cultivars grown are still quite susceptible to the disease. In recent years, data from the Uniform fungicide trials in North Dakota and other states have shown that, of the registered or near-registration, available fungicides, Folicur (tebuconazole) has more consistently provided better FHB control and gave greater reduction of DON than other fungicides or biologicals tested (Jones 2000, McMullen, et al. 1999, Milus and McMullen 2000). This information has been used to help obtain section 18 emergency exemptions for use of Folicur in North Dakota in recent years. Although Folicur has been consistent over several years and locations, some experimental fungicides and biologicals may provide even better control, be more cost-effective, and/or more environmentally safe. Uniform fungicide trials across North Dakota, a state with a consistent recent history of this disease, provide additional information on the performance of various products against FHB.

MATERIALS AND METHODS

A uniform set of four fungicide treatments and a biological agent were evaluated on wheat in ND in 2001 for control of Fusarium head blight (FHB) and fungal leaf diseases. Treatments were tested across five locations and across three spring wheat and two durum wheat cultivars: Oxen hard red spring wheat at Fargo; Grandin hard red spring wheat at Langdon and at Minot; Monroe durum at Garrison; Russ hard red spring wheat at Carrington; and Munich durum at Carrington. Artificial inoculum in the form of inoculated grain was dispersed in plots at Fargo and Langdon, while infection was solely from natural sources at Minot, Garrison and Carrington. Natural rainfall was augmented by mist irrigation at Fargo and Langdon, and by overhead irrigation at Carrington. All treatments were applied at early flowering (Feekes 10.51) with a CO_2 backpack type sprayer equipped with XR8001 nozzles mounted at a 60 degree angle forward and backward, in 15-20 gpa. Treatments were applied either in the early morning hours, prior to 9 am, or in the late afternoon or early evening

hours. Treatments included Folicur (tebuconazole) fungicide, AMS 21619 (an experimental from Bayer, Inc.), BAS 505 (experimental from BASF), a combination of BAS 505 + Folicur), and a biological agent OH182.9 (developed by the USDA in Peoria, in conjunction with Ohio State University). An additional biological agent developed by Cornell University, was tested at the Fargo location, but data is not presented here.

Disease ratings were taken at soft dough stage of kernel development. Plots were harvested with small plot combines. Plots were in a RCB design with four replicates, and data were statistically analyzed across locations using ANOVA.

RESULTS AND DISCUSSION

Disease levels varied substantially among locations; untreated durum plots at Carrington had the highest FHB field severity (42.1%), while spring wheat plots at Fargo had the lowest (5.7% untreated). Continuous rainfall and high humidities occurred at the Minot, Garrison, Carrington, and Langdon locations, beginning July 11, coinciding with flowering periods of the crops. At Fargo, the wheat crop flowered the first week of July during a hot, dry spell, and measurable rainfall in July did not begin until July 17.

All fungicide treatments significantly reduced FHB. DON levels were reduced, but not significantly, by all fungicide treatments, with the AMS product resulting in the lowest DON. All fungicide treatments also significantly reduced % flag leaf disease, predominately tan spot and Septoria/Stagnosopora leaf spots. All fungicide treatments significantly increased yield over the untreated check, and three fungicide treatments significantly improved test weight. The AMS product and the BAS 505 product look promising for further evaluation. The biological agent did not significantly improve disease control, yield or test weight. In some locations, the biological agent was applied in the early morning hours, instead of in the evening, and UV radiation may have inhibited some activity of the organism.

Results of fungicide and biocontrol tests on spring wheat and durum across ND locations, 2001

Treatment*	% FHB incidence	% FHB head sev.	% FHB Field Sev.	DON ppm****	% flag leaf disease***	Yield bu/A	TWT. lbs/bu
Untreated	64.9	17.9	13.0	5.1	58.9	43.2	55.6
Folicur 4 fl oz	42.8	9.6	4.1	3.4	32.1	54.2	57.1
AMS 21619 5.7 fl oz	42.9	8.4	3.6	1.4	20.0	56.1	58.7
BAS 505 6.4 fl oz	44.2	9.0	3.9	2.8	34.0	53.9	58.5
BAS 505 3.2 fl oz + Folicur 4 fl oz	41.4	8.6	3.5	2.7	29.9	55.6	57.9
USDA biological OH182.9	56.3	15.4	10.1	5.8	54.1	44.7	55.8
LSD P = 0.05	13.2	7.2	8.8	NS	18.3	8.0	1.7

*All fungicide treatments had 0.125% Induce added;

** DON (vomitoxin) levels were not available from Langdon at the time of report

*** Flag leaf disease primarily tan spot and Septoria/ Stagonospora leaf spots

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FUNGICIDE CONTROL OF FUSARIUM HEAD BLIGHT IN WHEAT

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OBJECTIVES

To understand betted the fungicide effect by the analysis of the influence of cultivar resistance and isolate aggressiveness and longevity of fungicide effect.

INTRODUCTION

As the majority of cultivars is susceptible to FHB, in epidemic situations the fungicides may help to lessen the damages and toxin contamination. However, their use is often insufficient and the causes would be important to know better (Anon. 1993, Caron 1990, McMullen et al. 1997, Mauler-Machnik and Zahn 1994, Mauler–Machnik and Suty 1997). Wilcoxson (1996) agrees and lists several reasons for this from the less effective fungicides, methodical problems and application deficiencies. He represents the view that a fungicide treatment is effective when the visual grain infection (FDK) will be lower than 5 %. As agronomy gives only moderate results, especially under very favorable epidemic conditions the last hope can be the use of fungicides.

MATERIALS AND METHODS

In the methodology we introduced the artificial inoculation of group of heads known from resistance tests (Mesterházy 1995, Mesterházy et al. 1999) that allowed testing the fungicide activity in our system on two or three cultivars differing in resistance and four isolates (two *F. graminearum* and two F*. culmorum*) with differing aggressiveness (Mesterházy and Bartók 1996, 1997). By this way 8-12 epidemic situations could be analyzed at the same time. As a mean product of these situations the results are more convincing and give a more accurate picture about the antifusarium ability of the fungicides. Each treatment contained 3 plot replicates per cultivar; in each 5 m^2 plot: the four isolates in three replicates was made and in each plot three control head of groups were bagged without inoculation. Besides the fungicide treatment a Fusarium check was also included without fungicide application. As control fungicide the Kolfugo Super (carbendazime 20 % a. i.) was used. The same was true for the longevity test.

Fungicide spraying was made at full flowering at rates suggested by the fungicide producers. One day thereafter the inoculation was performed. The sprayed head groups were covered for 24 hours with polyethylene bags, to secure 100 % rel. humidity for infection. Head symptoms were evaluated 10, 14, 18, 22 and 26 days after inoculation. After ripening the groups of heads were separately harvested, 10 heads of each group were randomly separated and threshed at low wind not to loose light infected scabby grains. Yield was measured; visual grain infection for scabby grains was estimated as percentages. In 1998 also the mass ratio of infected grains was measured and beside this the small grain ratio was also given.

In another test the durability of fungicide effect was studied, of the tests the 1999 and 2000 trial will be shown. Here 5, 10 and 15 days as well as 7, 14 and 21 days after spraying additional inoculations were made on separate plots. For controlling leaf disease the durability is easy to measure, we should observe only when the increase of the rusts or powdery mildew starts again to grow after treatment. For the Fusarium effect such correct data are not available in the literature only observations occur. The question is of practical importance as the necessity of a second treatment can be decided only by such data. Here the problem is whether the fungicides sprayed at flowering can combat late rainy period favoring FHB infection.

RESULTS AND DISCUSSION

Table 1 shows the results of the 1998 trial as means across cultivars and isolates. It is important that the decrease of kernel infection, yield loss or toxin contamination correlates very closely with the decrease of FHB data. This means that a fungicide treatment is effective not only against the FHF symptoms, but similarly also to toxin contamination. It is remarkable also that the fungicides are similarly effective to both *Fusarium* spp. meaning that there is no danger to have selectivity of the fungicides to individual Fusarium species. There is a significant difference between fungicides. The best were the tebuconazole containing ones, however they differed significantly according to their tebuconazole content. The best was the combination between Folicur Top and carbendazime mixture that seems to be equivalent or better to Folicur Solo with 250 g a. i. / ha.. Amistar was very poor and it increased toxin contamination in the susceptible cultivar by 20 % related to *Fusarium* check. This effect of Amistar was not found at more resistant cultivars. In Kolfugo we found this DON increase first since ten years. The ratio of small size FDKs and total amount of infected kernels shows that 34-40 % of the total infected mass belongs to the small size group. This means that by screening only this part can be separated, the major proportion remains in the staple with its toxin contamination. This agrees well with the literature data about the 30 % effectiveness of this procedure and gives its reason.

In Table 2 we present the 1999 mean results. In the mixture the Folicur BT was replaced by Falcon 0.8 l/ha. It seems that this combination is more powerful than the Folicur BT was. The conclusions are the same we gained in 1998.

On more resistant cultivars the infection severity with the best fungicides could be decreased dawn to several percent, on susceptible genotypes, however, 20 % infection usually remained. This is in comparison with 80 % infection severity of the check is considerable, but not enough to grow a well marketable wheat. The efficacy was different among isolates, but a clear tendency was not observed at lower or higher aggressiveness. The efficacy differed also according to traits like FHB %, yield, kernel infection or deoxynivalenol content. They were lowered very parallel according to fungicide efficacy, the correlation coefficients were above 0.90 (P = 0.001) between traits indicating as much the disease severity decreased by the given fungicide, the improvement was similar also in other traits. In our tests the efficacies are significantly higher (80 % at the best entries) than published in relevant literature. It is due to the fact that we aimed a full protection of the head on its whole surface. Therefore these results show the maximal efficacy that can be achieved controlling FHB. As practical efficacy is 20-30 % lower, the application of a fungicide with 50 % efficacy

may raise problems. We could confirm data that Azoystrobin increased toxin contamination. New result that this refers on susceptible and not more resistant cultivars.

In our tests the efficacies are significantly higher (80 % or more at the best entries) than published in relevant literature. Therefore these results show the maximal efficacy that can be achieved controlling FHB. As practical efficacy is 20-30 % lower, the application of a fungicide with 50 % efficacy in our tests may raise problems. We could confirm data that Azoystrobin increased toxin contamination. It is new that this refers on susceptible and not more resistant cultivars.

The durability data show that two weeks after spraying all fungicides kept their protective effects, interestingly the Falcon 0,6 l/ha showed improvement later in 1999 (Table 3). In 2000 the two weeks data show similar results on the 14th day inoculation, but on the 21st day Kolfugo does not give effective protection. For the others the protective effects lasted. (Table 4).

Effective control of FHB is possible now for the cultivars that are not highly sensitive to FHB. Preventive treatment is suggested at flowering; the use of twin nozzles is important to cover correctly the heads from every side to utilize the antifungal capacity of the fungicides. The efficacy of fungicides depends besides others on cultivar resistance, isolate aggressiveness and weather conditions. The efficacy of the best fungicides exceeded 70-80 %, but differs according to the parameter (FHB %, FDK %, yield loss, DON contamination) measured. Therefore a mean efficacy is suggested to describe more correctly the fungicide effect. There was a very close correlation between decrease of toxin contamination and FHB reduction, above r=0,90 meaning that as far the FHB symptoms can be decreased, the decrease of DON content will be proportional.

Table 1. Summary of the fungicide tests against FHB in wheat, 1998.

Fungicide and rate l/ha	Traits						Small/
	Grain inf.[2]	FHB %	Yield loss %	Grain inf.[1]	FDK %	DON[3] ppm	Total %[4]
Folicur Top 1.0+Kolf. 1.5	7.45	7.88	16.00	21.69	15.53	4.19	34.33
Folicur Solo 1.0	6.74	8.13	20.87	18.78	19.73	3.79	35.90
Falcon 0.8	9.92	9.85	20.57	27.51	28.08	6.24	36.05
Falcon 1.0	8.60	11.63	18.43	24.55	25.86	5.72	35.05
Folicur Top 1.0	10.73	12.41	21.26	27.82	30.81	5.94	38.56
Juwel 1.0	9.74	13.44	30.45	27.06	28.83	5.86	36.01
Duett 1.0	8.00	13.90	25.38	26.52	25.91	not tested	30.18
Falcon 0.6	13.43	15.92	28.24	37.74	35.70	not tested	35.59
Kolfugo Super	14.93	21.72	38.37	36.58	41.92	10.42	40.80
Amistar 1.0	17.48	24.75	37.60	42.94	46.39	10.98	40.70
Fus.check	23.95	41.55	50.36	53.98	58.56	11.79	44.37
Mean	11.91	16.47	27.96	31.38	32.48	7.21	37.05
LSD 5 %	1.95	0.71	2.89	2.90	3.33	2.07	

Correlations between traits

	Grain inf.2	FHB %	Yield loss %	Grain inf.[1]	FDK %
FHB %	0.9678				
Yield loss %	0.9247	0.9491			
Grain inf.[2]	0.9861	0.9514	0.9155		
Grain inf. %[1]	0.9788	0.9522	0.9398	0.9752	
DON ppm[4]	0.9460	0.9080	0.9186	0.9565	0.9650

All correlations are significant at P = 0.1 %.

[1] Mass ratio of all infected grains , [2] Mass ratio of small size infected grains

[3]Correlations with DON n = 9, the others n = 11, [4]Ratio of small FDKs to total mass of FDKs

Table 2. Fungicides against Fusarium head blight of wheat. General means for 1999.

Fungicide and rate l/ha	Yield loss %	Kernel inf. %	FHB %	DON ppm
Falc.0.8+Kolf.1	12.19	14.36	23.43	10.22
Fol. Solo 1	13.42	22.37	27.14	12.85
Falcon 0.8	14.99	20.47	28.61	13.29
Juwel 1,0	23.75	32.69	38.97	20.84
Kolfugo1.5	25.13	31.31	39.93	14.04
Fus. check	41.97	58.79	56.11	36.23
Mean	14.93	19.67	24.15	17.91
LSD 5 %	0.91	3.21	2.93	3.94

Table 3. Fungicide durable effect on FHB in wheat, relative grain infection data (%)to the Fusarium check, 1999.

Inoculation: days after fungicide application	Fungicides				
	Fus.check	Falcon 0.6	Kolfugo 1.5	Caramba 1.0	Falcon 0.8
1	100.00	79.98	68.81	53.75	46.15
5	100.00	66.19	57.67	42.69	27.62
10	100.00	55.54	48.94	44.26	44.63
15	100.00	37.66	57.24	41.28	48.51
Mean	100.00	59.84	58.17	45.50	41.73

Table 4. Longevity of fungicide effect against FHB in wheat, Summary, 2000
Kernel infection. Related data to FHB control.

Inoculation: days after	Fungicides					
fungicide application	Fus. check	Kolfugo 1.5	Caramba 1.2	Falcon 0.8	Falcon 1	Mean
1	100.00	44.79	8.91	15.42	11.15	20.07
7	100.00	43.54	80.03	22.62	18.97	41.29
14	100.00	37.62	7.23	28.94	27.49	25.32
21	100.00	92.59	18.52	27.78	18.52	39.35
Mean	100.00	54.63	28.67	23.69	19.03	31.51

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ANALYSIS OF THE 2001 UNIFORM WHEAT FUNGICIDE AND BIOCONTROL TRIALS ACROSS LOCATIONS

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INTRODUCTION

Identifying fungicides and biocontrols that reduce incidence and severity of Fusarium head blight (FHB) in the field and levels of damage and mycotoxins in the grain could have wide spread benefits to growers and users of all market classes of wheat in the event of FHB epidemics. The overall objective of the Chemical and Biological Control Committee is to hasten the integration of fungicides and biocontrols that are effective against FHB into cost-effective and environmentally-safe wheat disease management strategies. The current objective is to identify the most efficacious treatments. Uniform trials across the range of wheat market classes and environments prone to FHB epidemics is believed to be the best means for evaluating the efficacy of treatments. This analysis will consider only variables that are directly related to FHB because other variables, such as yield, are likely to be affected by diseases other than FHB.

METHODS

Plant pathologists in 13 states (Table 1) participated in the 2001 wheat uniform fungicide and biocontrol trials. These states represented hard red spring wheat, hard red winter wheat, soft red winter wheat, soft white winter wheat, and durum wheat production areas. The seven uniform treatments for 2001 (Table 2) included Folicur that has received several Section 18 registrations for FHB management, two experimental fungicides (BAS 505 and AMS21619), and two biological agents (TrigoCor 1448, a bacterium, and OH 182.9, a yeast). The biological agents were developed in part through USWBSI funding.

All treatments were applied at flowering stage using a CO2-powered sprayer equipped with twinjet XR8001 nozzles mounted at a 60 degree angle forward and backward. Details such as plot size, volume per acre, CO2 pressure, and number of replications varied slightly among the locations but were not considered to significantly affect the results. Inoculation and/or some form of overhead misting were used at most locations to promote head blight development, and these practices likely increased the incidence and severity of head blight. Disease variables included in this analysis were field severity (= FHB index = incidence x head severity) measured at soft dough stage and deoxynivalenol (DON) content in the grain and percentage of Fusarium-damaged kernels (FDK) measured after harvest. Cooperators analyzed results of their individual locations and provided treatment means to the authors for analysis across locations. The experimental design was a randomized complete block using the various locations as blocks. Analyses of FHB variables using all available data

were followed by analyses of the variables in high, moderate, and low categories of the variables in order to increase the probability of finding real differences among the treatments.

RESULTS

Seventeen locations across 11 of the participating states reported some FHB data (Table 3). As expected, there were significant differences among locations for each of the FHB variables. Averaged across all of the locations with data for the FHB variables, there were significant differences among treatments for field severity, but not for FDK or DON (Table 4). Compared to the non-treated check, all treatments except TrigoCor 1448 significantly reduced field severity and the fungicides reduced field severity by about 50%. Although the difference was not statistically significant, AMS21619 reduced FDK by about 50%.

Analyzed across locations with similar levels of field severity (Table 5), all treatments significantly reduced field severity at locations with low levels of disease, and all fungicides significantly reduced field severity at locations with moderate levels of disease. The best treatments reduced field severity by about 50%. At locations with high levels of disease, none of the treatments were significantly different from the non-treated check, but AMS21619 did reduce field severity by more than 50%.

Analyzed across locations with similar levels of FDK (Table 6), there were no significant differences among treatments at high, moderate or low levels of FDK. However, the best treatments in each of the three analyses did reduce the level of FDK by about 50% or more.

Analyzed across locations with similar levels of DON (Table 7), there were no significant differences among treatments at high-moderate or low levels of DON. Compared to the non-treated check, none of the treatments reduced DON by 50% or more.

CONCLUSIONS

All of the tested treatments had some efficacy against at least some of the FHB variables in some of the analyses. In general, fungicides were more efficacious than biological agents, and the most efficacious treatments reduced the values for field severity, FDK, and DON by about 50% compared to the non-treated check. Frequently, these differences were not statistically significant at $P = 0.05$ because of variability in the data. Perhaps it would be appropriate to use a less stringent significance level in future analyses.

Table 1. States and principal cooperators in the uniform wheat fungicide and biocontrol trials.

Arkansas	Gene Milus, University of Arkansas, Fayetteville
Indiana	Greg Shaner, Purdue University, West Lafayette
Iowa	Gary Munkvold, Iowa State University, Ames
Kentucky	Don Hershman, University of Kentucky, Princeton
Maryland	Arvydas Grybauskas, University of Maryland, College Park
Michigan	Pat Hart, Michigan State University, East Lansing
Minnesota	Hala Toubia-Rahme, University of Minnesota, Crookston
Missouri	Laura Sweets, University of Missouri, Columbia
New York	Gary Bergstrom, Cornell University, Ithaca
North Dakota	Marcia McMullen, North Dakota State University, Fargo
Ohio	Pat Lipps, Ohio State University, Wooster
South Dakota	Marty Draper, South Dakota State University, Brookings
Virginia	Erik Stromberg, Virginia Technical, Blacksburg

Table 2. Treatment, rate, and adjuvant used in the uniform trials in 2001.

#	Treatment	Rate of product / A	Adjuvant
1	Nontreated		
2	Folicur 3.6 F	4 fl oz	0.125% Induce
3	AMS12619 480SC	5.7 fl oz	0.125% Induce
4	BAS505 50DF	6.4 oz	0.125% Induce
5	BAS505 50DF + Folicur 3.6F	3.2 oz + 2 fl oz	0.125% Induce
6	TrigoCor 1448	varied among locations	
7	OH 182.9	varied among locations	

Table 3. The means for field severity, Fusarium-damaged kernels (FDK), and deoxynivalenol (DON) across all seven treatments at locations that reported some level of FHB in the 2001 uniform trials.

Location	Location	Field severity[1]	FDK[1]	DON[1]
(#)	(state and city or variety)	(%)	(%)	(ppm)
1	Ohio	33.0a	33.2b	13.8b
2	Michigan (Freedom)	32.4a		1.0c
3	Michigan (Harus)	22.1b		1.7c
4	Minnesota	19.2b	10.5cd	2.0c
5	Michigan (Frankenmuth)	18.6bc		0.7c
6	Kentucky	17.3bc	21.0c	3.9c
7	North Dakota (Carrington)	17.2bc		
8	North Dakota (Langdon durum)	16.7bc		
9	Arkansas	12.8cd	52.8a	29.2a
10	Missouri	10.1de		1.6c
11	Virginia	5.1ef		
12	North Dakota (Fargo)	3.8f	2.8d	1.2c
13	North Dakota (Langdon)	3.2f		
14	North Dakota (Minot)	2.7f		
15	New York	1.6f	6.2d	
16	Iowa	1.6f		
17	Indiana			0.8c

[1]Values within a column followed by the same letter are not significantly different by a LSD test at P=0.05

Table 4. Treatment means for field severity, Fusarium-damaged kernels (FDK), and deoxynivalenol (DON) level averaged across all of the locations in Table 3.

Treatment	Field severity[1]	FDK[1]	DON[1]
	(%)	(%)	(ppm)
Non-treated	20.0a	29.4a	6.2a
TrigoCor 1448	18.0ab	24.4a	5.6a
OH 182.9	15.9bc	23.1a	4.4a
Folicur	12.1cd	21.1a	5.3a
BAS505 + Folicur	11.0d	18.6a	6.7a
BAS505	10.9d	17.0a	6.2a
AMS21619	9.2d	13.9a	4.6a

[1]Values within a column followed by the same letter are not significantly different by a LSD test at P=0.05

Table 5. Treatment means for field severity averaged across locations with high, moderate, or low levels of field severity.

Treatment	High severity[1,2]	Moderate severity[1,3]	Low severity[1,4]
	(%)	(%)	(%)
Non-treated	43.5a	25.2a	5.1a
TrigoCor 1448	43.3a	19.9ab	2.5bc
OH 182.9	38.4a	20.1ab	3.5b
Folicur	30.7a	14.8bc	2.3c
BAS505 + Folicur	30.1a	12.7c	2.4c
BAS505	25.4a	13.8c	2.4c
AMS21619	17.6a	12.0c	2.6bc

[1]Values within a column followed by the same letter are not significantly different by a LSD test at P=0.05. [2]Locations (by number from Table 3) in this analysis are 1 and 2.
[3]Locations (by number from Table 3) in this analysis are 3, 4, 5, 6, 7, 8, 9, and 10.
[4]Locations (by number from Table 3) in this analysis are 11, 12, 13, 14, 15, and 16.

Table 6. Treatment means for Fusarium-damaged kernels (FDK) averaged across locations with high, moderate, or low levels of Fusarium-damaged kernels.

Treatment	High FDK[1,2]	Moderate FDK[1,3]	FDK severity[1,4]
	(%)	(%)	(%)
Non-treated	60.5a	21.0a	6.7a
TrigoCor 1448	50.5a	16.7a	6.1a
OH 182.9	45.5a	18.7a	5.2a
Folicur	44.2a	15.7a	3.6a
BAS505 + Folicur	41.4a	11.2a	3.2a
BAS505	33.0a	13.8a	4.3a
AMS21619	25.9a	13.4a	2.5a

[1]Values within a column followed by the same letter are not significantly different by a LSD test at P=0.05. [2]Locations (by number from Table 3) in this analysis are 1 and 9.
[3]Locations (by number from Table 3) in this analysis are 4 and 6.
[4]Locations (by number from Table 3) in this analysis are 12 and 15.

Table 7. Treatment means for deoxynivalenol (DON) level averaged across locations with high-moderate or low levels of deoxynivalenol.

Treatment	High-moderate DON[1,2]	Low DON[1,3]
	(%)	(%)
Non-treated	23.1a	2.3a
TrigoCor 1448	21.5a	1.9a
OH 182.9	15.2a	2.0a
Folicur	20.0a	2.1a
BAS505 + Folicur	27.7a	1.8a
BAS505	25.1a	1.8a
AMS21619	18.0a	1.6a

[1]Values within a column followed by the same letter are not significantly different by a LSD test at P=0.05. [2]Locations (by number from Table 3) in this analysis are 1 and 9.
[3]Locations (by number from Table 3) in this analysis are 2, 3, 4, 6, 10, and 12.

EFFICACY OF FUNGICIDES AND BIOCONTROLS AGAINST FUSARIUM HEAD BLIGHT IN ARKANSAS, 2001

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OBJECTIVE

To identify fungicides and biocontrol agents that are effective against Fusarium head blight.

MATERIALS AND METHODS

Seeds of Hazen soft red winter wheat, treated with Gaucho (3 fl oz / cwt) and Dividend (1 fl oz / cwt), were planted at the rate of 114 lb/A on 19 October 2000 at University Farm in Fayetteville. Each plot was 7 rows (4.1 ft) by 15 ft and trimmed to 12 ft before harvest. Plots were fertilized with 40 lb N/A on 23 February and 6 March. Colonized corn kernel inoculum was spread in the field on 18, 20, and 25 April for a total of 14 kernels / sq ft. The TrigoCor1448 bacterium was grown in shake culture of nutrient broth for 10 days. The OH 182.9 yeast suspension was prepared from a frozen paste according to directions. At early flowering stage, treatments were applied in the late afternoon (to promote the establishment of the biological agents) on 27 April at 20 gal/A and 40 psi using a CO2-powered backpack sprayer equipped with three sets of twinjet XR8001 nozzles mounted at a 60 degree angle forward and backward. The design was a randomized complete block with six replications. To promote ascospore formation in the corn kernel inoculum and head infection, the plot was misted for eight 11-minute periods between midnight and 8:00 am on 23 nights between 18 April and 18 May. Fifty heads per plot were collected at soft dough stage on 25 May to determine the incidence and severity of head blight. Plots were harvested with a plot combine on 11 June. Yields were adjusted to 13% moisture and test weights were determined after passing the grain once through an air-blast seed cleaner. A 50-g sample of grain from each plot was evaluated visually for the percentage of scabby grain and then sent to Michigan State University for DON analysis.

RESULTS AND DISCUSSION

Fusarium head blight developed later than normal, probably because ascospores were not released from the inoculum until after flowering. Septoria tritici blotch was the only other disease prevalent in the plots, but it developed late in the season and likely did not affect results. Plots treated with OH 182.9 had the lowest levels of scabby seed and DON (Table 1). Plots treated with TrigoCor 1448 also had a low level of DON. The high efficacy of the biocontrol agents relative to the fungicides may have been due to 1) treatments were applied in the late afternoon to help the biocontrol agents establish, 2) frequent mist cycles may have allowed the populations of the biocontrol agents to increase before head blight infection occurred, and 3) disease developed late after fungicide activity likely dissipated. Plots treated with BAS505 had significantly higher levels of DON than the non-treated checks.

Table 1. Results of the uniform fungicide and biocontrol trial at Fayetteville, AR, in 2001.

Trt No.	Treatment and rate per acre	Yield (bu/A)	Test Wt. (lbs/bu)	Plot severity (%)	Head severity (%)	Incidence (%)	Scabby Seed (%)	DON (ppm)
1	Non-treated #1	87.1	54.5	12.7	16.5	75	59.2	29.5
2	Folicur 3.6F 4 fl oz + 0.125% Induce	87.2	55.2	13.7	18.1	74	55.0	27.9
3	AMS21619 480SC 5.7 fl oz. + 0.125% Induce	92.2	55.9	12.1	17.9	65	47.5	28.8
4	BAS505 50DF 6.4 oz + 0.125% Induce	88.5	54.3	12.3	17.5	69	59.2	41.8
5	BAS505 50DF 3.2 oz + Folicur 3.6F 2 fl oz + 0.125% Induce	84.4	53.9	13.3	17.5	76	65.0	40.5
6	TrigoCor 1448 $1.7x10^{14}$ cfu	87.2	54.8	14.1	18.6	75	49.2	19.0
7	OH 182.9 $2.4x10^{14}$ cfu	91.1	56.5	11.7	17.7	64	34.2	16.9
8	AMS21619 480SC 3.6 fl oz. + Folicur 3.6F 4 fl oz. + 0.06% Induce	88.2	56.1	11.8	17.0	69	46.7	22.5
9	AMS21619 480SC 5.7 fl oz. + 1% crop oil concentrate	88.1	55.3	12.7	18.2	69	55.8	33.5
10	Non-treated #2	81.6	55.0	14.6	18.6	78	50.0	27.4
	Prob > F	0.32	0.017	0.71	0.99	0.002	< .0001	0.0001
	LSD (P=0.05)	NS	1.5	NS	NS	7	11.0	10.1
	CV (%)	7.4	2.3	22.9	19.2	8.90	17.9	29.7

EFFICACY OF FUNGICIDES IN CONTROLLING FUSARIUM HEAD BLIGHT ON BARLEY GENOTYPES WITH PARTIAL RESISTANCE

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INTRODUCTION

Fusarium head blight (FHB), incited primarily by *Fusarium graminearum*, adversely affected the quality of barley grown in eastern North Dakota and northwestern Minnesota the last nine years. Quality of harvested grain was reduced because of blighted kernels and the presence of deoxynivalenol (DON), a mycotoxin produced by the pathogen. Non-detectable, or low levels of DON are needed for malting barley because DON has been found to carry through malting and brewing into finished beer (Schwarz et al., 1995). Anheuser-Busch, Inc., the largest brewer in the U.S., will not purchase malt produced from barley with DON levels greater than 0.5 ppm.

Research to test the efficacy of fungicides in reducing FHB and DON levels in barley has been conducted using cultivars susceptible to FHB. Pederson and McMullen (1999) found that the fungicides Folicur, Tilt, Benlate, Mancozeb, and Quadris significantly reduced FHB severity and DON content of barley. However, the fungicides were not successful in reducing DON content to a level that would be acceptable to maltsters and brewers. The most successful fungicide treatment reduced DON content of barley to 17.2 ppm.

In a preliminary study, Horsley et al (2000) evaluated the efficacy of Folicur in controlling FHB on barley genotypes with different levels of resistance. They concluded that Folicur did not significantly reduce FHB levels in any of the 14 genotypes included in the study.

OBJECTIVES

The objective of this study is to further investigate the integrated use of fungicides and resistant or moderately resistant barley genotypes to reduce FHB severity and accumulation of DON to levels acceptable to the malting and brewing industry.

MATERIALS AND METHODS

Fourteen barley genotypes with different levels of FHB resistance were grown at sites near Osnabrock, Langdon, and Fargo, North Dakota during the 2000 and 2001 growing seasons. Treatments were assigned to 35 ft2 experimental units using a randomized complete block design with a split-plot arrangement and three replicates at each location. Whole plots included; no fungicide, 4 fl oz Folicur/acre, and in the 2001 growing season only, 5.7 fl oz/acre of an experimental triazol from Bayerâ. Subplots were genotypes. Evaluated genotypes were either resistant to FHB (Chevron, Svanhals, and Kaoto Nijo 2), moderately resistant to FHB (MNBrite, F101-78, F103-61, F103-52, and F102-61), or susceptible to FHB (Foster,

Stander, Conlon, Logan, Drummond, and Legacy). Experimental units were not inoculated with *F. graminearum.*

Fungicides were applied at Feeke's growth stage 10.3 using a CO2-pressurized handheld boom sprayer operating at 40 psi, and calibrated to deliver 24 gallons of solution acre-1. Fusarium head blight severity was assessed at Feeke's growth stage 11.2 by determining the ratio of infected kernels to total kernels on 10 spikes per row. Disease severity was expressed as percent FHB severity. At maturity, each experimental unit was harvested with a small-plot combine. Grain samples were dried and cleaned prior to yield determination. Grain samples from each experimental unit were submitted to Dr. Paul Schwarz's laboratory in the Department of Cereal Science, North Dakota State University for DON analysis. To date, DON data for the 2001 Osnabrock and Fargo locations are not available.

Data from individual locations were analyzed separately using analysis of variance (ANOVA) and error mean squares from each location were tested for homogeneity of variance. Combined ANOVA's were done using data from locations in which error mean squares were homogeneous. Means were separated using an F-protected LSD (P=0.05). In the combined analyses, fungicide and genotypes were considered fixed effects and environment a random effect.

RESULTS AND DISCUSSION

Environmental conditions at Langdon and Osnabrock were more conducive for development of FHB than conditions at Fargo. Mean FHB severity was 4.3% at Langdon, 3.7% at Osnabrock, and 0.8% at Fargo. Fusarium head blight severity was not significantly reduced by Folicur in any of the genotypes (data not presented). Deoxynivalenol content was not significantly reduced by Folicur in any of the evaluated genotypes (Table 1); however, there was a trend for slightly lower DON resulting from Folicur application. Reductions of DON to levels acceptable for the malting and brewing industry (<0.6 ppm) occurred only in genotypes with resistance or partial resistance.

Genotypes sprayed with Folicur generally had greater yield than unsprayed genotypes (Table 2). Much of the yield improvements may be due to reductions of foliar disease in genotypes sprayed with Folicur. Foliar disease severity data were collected at Langdon and Osnabrock. (Data not presented). The predominant foliar disease at each location was septoria leaf blotch, incited by Septoria spp. Significant yield increases were mainly observed for the cultivars developed and released from upper Midwest barley breeding programs (i.e.Legacy, Conlon, Drummond, Foster, Logan, MNBrite, and Stander.) This suggests that factors other that foliar diseases were limiting yield in the other genotypes.

In the barley-growing region in the upper Midwest U.S., it costs growers about $14/acre for Folicur and its application. For this cost to be recovered, a yield increase of at least 10.8 bushels/acre is needed based on a farmgate-selling price of $1.30/bushel for feed barley. Based on the yield increases observed in this study, the cost of Folicur and its application was recovered only when applied to the adapted cultivars Legacy, Conlon, Foster, Logan, MNBrite, and Stander (Table 3). If DON content could be reduced to levels required by the

malting and brewing industry (<0.6 ppm) additional net profit returns could be realized due to a $1.00/bushel premium for malting barley.

Preliminary results indicate that the efficacy of the experimental triazol was slightly better than Folicur in reducing FHB and DON (data not presented). Deoxynivalenol data were available from only one location at the time of preparing this report. The study including the experimental triazol will be continued in the next growing so that more definite conclusions can be made.

CONCLUSIONS

Folicur application did not significantly reduce FHB severity or DON level in resistant, moderately resistant, or susceptible genotypes.

Genotypes sprayed with Folicur generally had greater yield

Yield gains due to control of foliar diseases tended to be sufficient to cover the cost of Folicur and its application on cultivars developed and released by upper Midwest barley breeding programs.

Further research is needed to determine if a fungicide with greater efficacy than Folicur for FHB control can be used with moderately resistant genotypes to reduce DON

Table 1. Effect of Folicur and genotype on DON content of barley.

	Environment							
	2000 Fargo		2000 Langdon		2000 Osnabrock		2001 Langdon	
Genotype	Folicur	No Folicur	Folicur	No Folicur	Folicur	No Folicur	Folicur	No Folicur
	ppm							
Chevron	0.1	0.1	0.5	1.5	0.7	0.6	0.2	0.9
Svanhals	0.0	0.0	0.5	0.6	0.4	0.1	1.2	0.6
Kaota Nijo 2	0.1	0.1	1.0	1.9	1.9	2.4	0.3	0.2
F101-78	0.3	0.2	0.8	2.0	0.8	1.2	0.5	0.4
F102-61	0.3	0.4	0.3	0.7	0.6	0.7	1.5	1.5
F103-52	0.3	0.5	0.3	2.2	1.1	0.8	0.6	0.9
F103-61	0.4	0.3	1.4	2.2	0.9	1.7	0.4	0.4
MnBrite	0.3	0.4	1.6	2.9	1.1	1.4	0.6	0.7
Legacy	0.2	0.4	1.2	2.0	1.0	1.5	1.4	0.9
Drummond	0.6	0.7	2.1	3.8	1.3	1.6	0.6	0.5
Foster	0.4	0.5	1.6	2.4	2.9	2.6	0.8	0.6
Stander	0.7	0.6	1.8	2.5	2.3	2.2	1.4	1.7
Logan	0.2	0.1	1.3	1.9	1.2	1.6	0.4	0.2
Conlon	0.1	0.2	1.1	1.2	1.6	1.1	0.2	0.3
LSD(0.05)	ns							

Table 2. Effect of Folicur and genotype on yield of barley.

	Environment									
	2000 Fargo		2000 Langdon		2000 Osnabrock		2001 Fargo		2001Osnabrock	
Genotype	Folicur	No Folicur	Folicur	No Folicur	Folicur	No Folicur	Folicur	No Folicur	Folicur	No Folicur
	bu/acre									
Chevron	49.4	50.1	36.7	33.8	75.9	68.4	35.2	33.2	47.2	44.7
Svanhals	42.3	37.8	32.3	30.4	71.3	56.9	32.9	37.7	39.2	35.1
Kaota Nijo 2	52.7	48.1	77.6	73.5	76.9	62.8	40.9	43.0	57.0	46.4
F101-78	48.6	47.5	76.1	72.6	63.6	63.7	32.2	36.1	46.5	55.7
F102-61	47.6	45.9	65.9	60.6	69.8	71.4	23.0	32.6	52.3	45.9
F103-52	39.2	33.1	46.8	42.2	67.2	63.5	23.1	23.8	34.8	39.1
F103-61	51.0	47.7	81.1	75.5	74.6	68.0	31.5	40.6	62.9	56.6
MnBrite	56.8	46.3	102.5	81.6	91.6	90.9	42.5	40.8	75.2	66.1
Legacy	57.7	57.0	107.4	91.5	109.3	94.1	38.7	36.4	61.7	55.9
Drummond	61.8	55.4	100.3	90.7	90.4	79.1	45.1	48.0	63.4	52.6
Foster	64.1	60.9	115.4	79.6	107.6	84.5	43.9	55.4	70.2	61.9
Stander	68.5	59.9	111.9	96.2	91.9	83.4	45.7	47.6	64.0	64.7
Logan	59.3	50.3	109.8	77.4	100.1	85.7	48.5	45.9	70.4	50.2
Conlon	53.4	45.0	85.6	68.3	86.4	75.1	46.7	49.3	61.4	54.8
LSD(0.05)	9.7									

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USDA-ARS, OHIO STATE UNIVERSITY COOPERATIVE RESEARCH ON BIOLOGICALLY CONTROLLING FUSARIUM HEAD BLIGHT: PILOT-PLANT-SCALE PRODUCTION AND PROCESSING OF BIOMASS OF YEAST ANTAGONISTS

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OBJECTIVES

To select one of two superior yeast antagonists (Cryptococcus sp. OH 181.1 and C. nodaensis OH 182.9) for use in the 2001 Uniform Wheat Fungicide and Biocontrol Trial (UWFBT) based on antagonist amenability to liquid culture production in shake flasks and 30 L fermentors. Additionally, to evaluate the suitability of two methods of freezing antagonist biomass in order to maximize viable cell counts until the time of application.

INTRODUCTION

Research on developing strategies and microorganisms for biologically controlling Fusarium head blight (FHB) was initiated in 1997 at the NCAUR in Peoria, IL, in conjunction with The Ohio State University. Several biological control agents remain under consideration for commercial development (Schisler et al. 2000; Khan et al., 2001). A critical step in the transition from conducting laboratory experiments on biological control agents to producing a commercially available biocontrol product is devising economically feasible procedures for large-scale, liquid culture production of biomass of the biological agent. Antagonist strains considered for commercial development must also be able survive cell preservation techniques and maintain high viable cell counts over time.

MATERIALS AND METHODS

Growth of *Cryptococcus sp.* OH 181.1 and *C. nodaensis* OH 182.9 in liquid culture

In preliminary medium optimization experiments, a semidefined complete liquid medium (SDCL; Slininger et al., 1994) supported excellent growth of both yeast antagonists. A liter of this medium contains approximately 15 g and 1.2 g of total carbon and nitrogen, respectively. Glucose serves as a carbon source while Casamino acids provide carbon and nitrogen. In standard laboratory use, the glucose and amino acid portions of the medium are sterilized separately (A+B). A version of SDCL medium where all ingredients are autoclaved together (AB) has an enhanced commercial potential due to requiring less costly production parameters. However, the influence of heat-induced condensation products in the AB form of SDCL on microbial growth was unknown. In shake flask experiments, 125 ml flasks were

charged with 50 ml of the AB or A+B version of SDCL and inoculated with 18-24 h precultures of yeast antagonists to an optical density (ODA620) of 0.1. Cultures were incubated at 25 C and 250 rpm for 72 h in a shaker incubator. Colony forming units (CFU) per ml were determined at 48 h and 72h.

Yeast strains OH 181.1 and OH 182.9 were also produced in a B Braun D-30 fermentor charged with 20 L of either SDCL AB or SDCL A+B medium. To initiate a production run, 24 h old cells grown in the same medium as used in the production run served as a 5% seed inoculum. Reactor medium pH, temperature, dissolved O2, antifoam, agitation rate were monitored and/or maintained to insure near identical production runs. Colonized broths were sampled and plated on 1/5 strength Tryptic soy broth agar (TSBA/5) for CFU/ml after 48 h.

Processing and freezing of biomass of *Cryptococcus sp*. OH 181.1 and *C. nodaensis* OH 182.9

Cells of the yeast antagonists were produced in a 30 L fermentor as described above. After completion of biomass production at approximately 48h, cells in the broth were concentrated into a paste using a Sharples 12-V tubular bowl centrifuge. The paste was split into two parts and resuspended using either buffer or spent broth and frozen at -18 C. Samples of the frozen biomass were gradually thawed and plated on TSBA/5 every seven days for a total of 70 days to determine CFU/ml. Log10 CFU/ml data obtained over the coure of the experiment were analyzed using linear regression.

RESULTS AND DISCUSSION

In both shake flask and fermentor experiments, CFU/ml production by OH 181.1 or OH 182.9 was not deleteriously affected by autoclaving all components of the SDCL medium together (SDCL AB)(Table 1) demonstrating the utility of a form of the medium that would be most advantageous for commercial use. Antagonist OH 182.9 actually tended to produce more CFU/ml in the AB than the A+B version of SDCL in the shake flask and fermentor experiments (Table 1). Antagonist OH 182.9 showed a trend of producing more CFU/ml than did OH 181.1 in every comparison of like medium and production vessel (Table 1). A linear relationship described CFU/ml over time for frozen OH 182.9 cells resuspended in buffer ($P<0.001$) or in spent broth ($P<0.001$)(Fig. 1). Biomass viability of OH 182.9 decayed more rapidly for cells that were resuspended in spent broth before freezing than for cells resuspended in buffer (Fig. 1). Nearly identical results were obtained for OH 181.1 (data not shown).

C. nodaensis OH 182.9 was chosen over *Cryptococcus sp.* OH 181.1 for use in the 2001 UWFBT due to OH 182.9 obtaining higher maximum CFU/ml and obtaining CFUmax in less time than OH 181.1. The efficacy of the frozen biomass of both antagonists in reducing FHB severity was similar in greenhouse trials (data not shown). As a result of these and other studies, cells of OH 182.9 were produced in SDCL AB medium in 20 L and 80 L quantities, harvested after 48 h, concentrated by centrifugation, resuspended in buffer, frozen, and sent frozen to participants in the 2001 UWFBT. Selected results of using antagonist *C. nodaensis* OH 182.9 in the 2001 UWFBT are presented by Milus and coauthors (2001) in this volume.

A portion of our future research on enhancing the commercial development potential of OH 182.9 will concentrate on identifying cryoprotectant compounds that further enhance the survival and shelf-life of frozen biomass of the antagonist as well as determining the feasibility of alternative biomass processing procedures such as air, fluidized bed or spray drying.

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Table 1. Comparison of cell production by antagonists *Cryptococcus* spp. OH 181.1 and OH 182.9 in shake flasks and 30L fermentors charged with different liquid media

	Maximum Log_{10} (CFU/ml)	
Antagonist/Medium[1,2]	Shake Flask[3]	Fermentor
OH 181.1/A+B	8.59	8.63
OH 181.1//AB	8.59	8.63
OH 182.9/A+B	8.71	8.72
OH 182.9/AB	8.85	9.19

[1] OH 181.1 is a *Cryptococcus* sp. with NRRL accession number Y-30215. OH 182.9 is a strain of *C. nodaensis* with NRRL accession number Y-30216.
[2] Medium "A+B" is a semidefined complete medium (Slininger et al., 1994) where the glucose and amino acid portions of the medium are sterilized separately while in medium "AB" all media ingredients are autoclaved together.
[3] CFU/ml values are the maximum obtained and occurred between 48 and 72 hours after inoculation of liquid cultures.

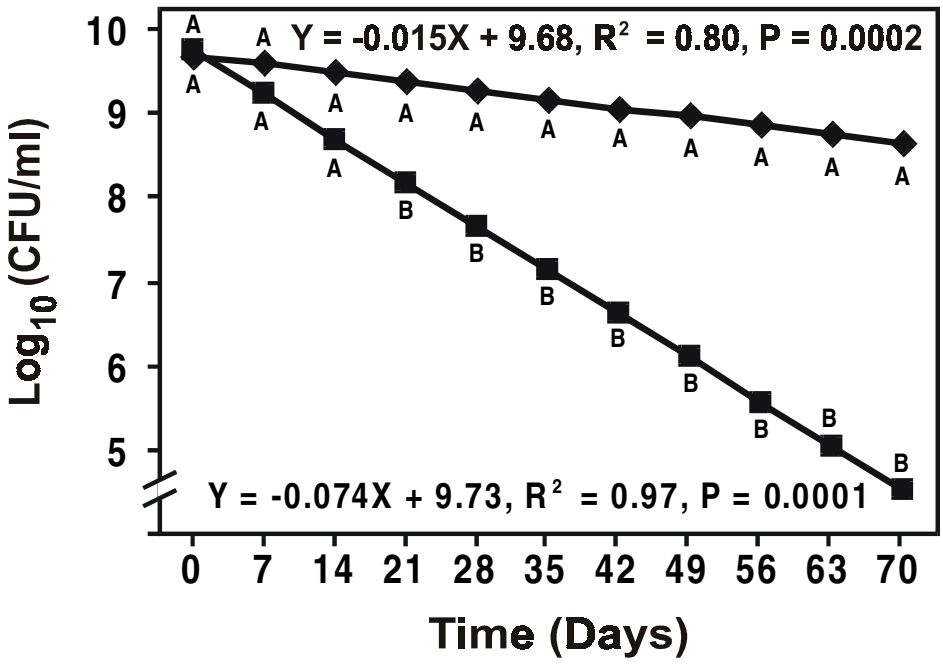

Figure 1. Survival of 30-liter fermentor-produced biomass of Fusarium head blight antagonist *Cryptococcus nodaensis* OH 182.9 resuspended in spent broth or weak PO4 buffer and stored at -20 C. -♦- biomass resuspended in buffer. -■- biomass resuspended in spent broth. Data points at same time that do not have identical letters are significantly different (P=0.05)

BIOLOGICAL CONTROL OF FUSARIUM HEAD BLIGHT WITH *BACILLUS SUBTILIS* TRIGOCOR 1448: 2001 FIELD RESULTS

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OBJECTIVES

To quantify the ability of the bioprotectant TrigoCor 1448, applied to flowering spikes, to control Fusarium head blight (FHB) and to reduce deoxynivalenol (DON) contamination of the harvested grain.

INTRODUCTION

Efforts are being made to provide safe, affordable and efficacious biological protectants for the integrated management of FHB of wheat and barley (Schisler, et al. 2000; Luz, W.C. da 2000). The reduction of DON contamination of the harvested grain to acceptable levels remains of critical importance in the management of this disease. In previous exploratory trials the *Bacillus subtilis* isolate, TrigoCor 1448, has shown promise in field and laboratory tests (Stockwell, et al., 1997; Stockwell, et al, 2000). Repeated field trials have been done to demonstrate the efficacy of this bioprotectant when applied to the spikes during flowering in a variety of environments, under varying levels of disease pressure and over several years. Preliminary comparisons can also be made between TrigoCor 1448 and other bioprotectants based on the data generated under field conditions.

MATERIALS AND METHODS

Uniform Fungicide/Bioprotectant Field Trial - Musgrave Farm, Aurora, NY

Twelve treatments were included in the uniform fungicide/bioprotectant trial conducted at Aurora, NY. Treatments were replicated 4 times and arranged in a randomized block design. In addition to TrigoCor 1448 and the USDA/Peoria Yeast which were included as core treatments tested at all locations, this trial included the commercial *Bacillus subtilis* bioprotectant product, Serenade (AgraQuest; Davis, CA) and, the resistance elicitor, Messenger (Eden Biosciences; Bothell, WA). Commercial products were applied at labeled rates. In this same trial, TrigoCor 1448 and similarly, TrigoCor 4712 were combined with tebuconazole (4 fl oz Folicur) to determine if the combination would give enhanced FHB control over either bioprotectant or fungicide alone. Bacteria were grown for 5 days in nutrient broth with yeast extract, NBYE, (2-4 X 108 cfu/ml) and applied undiluted as whole broth. Test weight, yield, % Fusarium damaged kernels (fdk), % seed infection (on SNAWS selective medium) and

DON were determined from the harvested grain. Seed from each plot were sent to Michigan State University for DON analysis.

Bioprotectant Trial - McGowan Field, Ithaca, NY

Seven treatments were included in the biocontrol trial conducted at Ithaca, NY on "Caledonia" soft white winter wheat. Treatments were replicated 5 times and arranged in a randomized block design.

Messenger (Eden Bioscience Corp., Bothell, WA) was applied on May 4 (Feekes 3.5) and again on May 23 (Feekes 9.5). The bioprotectants TrigoCor 1448 and TrigoCor 4712 were grown with constant agitation in nutrient broth for 5 days and were applied as diluted whole broth. The TrigoCor 1448-Reconstituted was prepared from frozen cells of 5 day-old cultures grown in nutrient broth that were re-suspended in sterile distilled water to the original volume of the broth. The treatments were visually rated for the incidence of Fusarium head blight and for severity. Standard data set was taken from the harvested grain.

National 2001 Uniform Fungicide/Bioprotectant Trials

A core set of treatments including TrigoCor 1448 were tested at 14 sites in 13 states. A culture of the bioprotectant was sent along with instructions and dry ingredients to make sufficient NBYE for field application. Undiluted broth of 3 to 5 day old cultures were applied to wheat or barley spikes during anthesis.

RESULTS AND DISCUSSION

Uniform Fungicide/Bioprotectant Field Trial - Musgrave Farm, Aurora, NY

FHB incidence was shown to be significantly different between treatments. This reflects both the elevated incidence of the Serenade treatment and a substantial decrease in incidence by Folicur (Fig. 1). There was no significant difference between treatments for all other responses including DON contamination of the harvested grain. However, some trends may be discerned for this data. TrigoCor 1448 reduced DON content by 0.6 ppm from the non-treated control (Fig. 2). When Folicur (4 fl oz) was combined with TrigoCor 1448, FHB incidence was reduced by 27% and the DON contamination was reduced by 1.6 ppm compared to non-treated wheat. Similarly, when Folicur was combined with TrigoCor 4712, FHB incidence was reduced by 16% and the DON contamination was reduced also by 1.6 ppm . In comparison, Serenade raised DON levels by 1.0 ppm while the USDA/Peoria yeast lowered DON by 1.1 ppm.

Bioprotectant Trial - McGowan Field, Ithaca, NY

Although FHB incidence was shown to be significantly different between treatments, this primarily reflects the elevated incidence of the Serenade and Messenger treatments rather than a substantial decrease in incidence by any treatment. Although not significantly different from the non-treated check, plants treated with TrigoCor 1448 whole broth had the lowest level of DON contamination in the harvested grain, followed closely by theTrigoCor

1448-Reconstituted (washed cells). This represents a 1.08 and 0.96 ppm reduction in DON, respectively. In a year of low scab incidence (over-all incidence of 5.7% and DON contamination of 4.4 ppm), all treatments including Folicur, generally one of the best synthetic fungicides for scab control, had little measurable effect.

National 2001 Uniform Fungicide/Biological Trials

At all but the Ohio site, the incidence of FHB was reduced by treatment with TrigoCor 1448 when compared to the non-treated control (Fig. 3). Reduction of DON to market acceptable levels remains the most critical challenge for integrated management of FHB. Of 10 field experiments where non-treated grain was contaminated with greater than 0.5 pmm DON, nine showed a decrease in DON or an increase of less than 0.5 ppm in response to TrigoCor 1448 application (Fig. 4). Again, only the Ohio site produced a result where TrigoCor 1448 increased significantly DON as well as FHB. By contrast, under severe epidemics at Arkansas and Kentucky, TrigoCor 1448 reduced DON by 33% and 39%, respectively. We have no explanation for the contradictory results from Ohio.

Conclusions - The modest success of the bioprotectant TrigoCor 1448 in reducing FHB and DON in most, but not all, locations suggests that bioprotectants may be a useful component of integrated management of FHB. The combination of the TrigoCor1448 with the fungicide Folicur gave the most promising results in New York tests and suggests one of the thrusts of future research, the combination of bioprotectants with fungicides. While the results are not spectacular, the consistent reduction in FHB incidence and DON contamination, across many test gives us encouragement to look for ways to increase the efficacy of TrigoCor 1448 and other bioprotectants. There is also a need to elucidate the conditions under which bioprotectants reduce DON levels.

Acknowledgements - We wish to thank all of the regional collaborators in the 2001 Uniform Fungicide Trial who included TrigoCor 1448 as a core treatment and provided us with results: Gene Milus/AR, Greg Shaner/ IN, Gary Munkvold/IA, Don Hershman/KY, Arv Grybaukas/MD, Pat Hart/MI, Hala Toubia-Rahme/MN, Laura Sweets/MO, Pat Lipps/OH, Marcia McMullen/ND, Marty Draper/SD and Erik Stromberg/VI.

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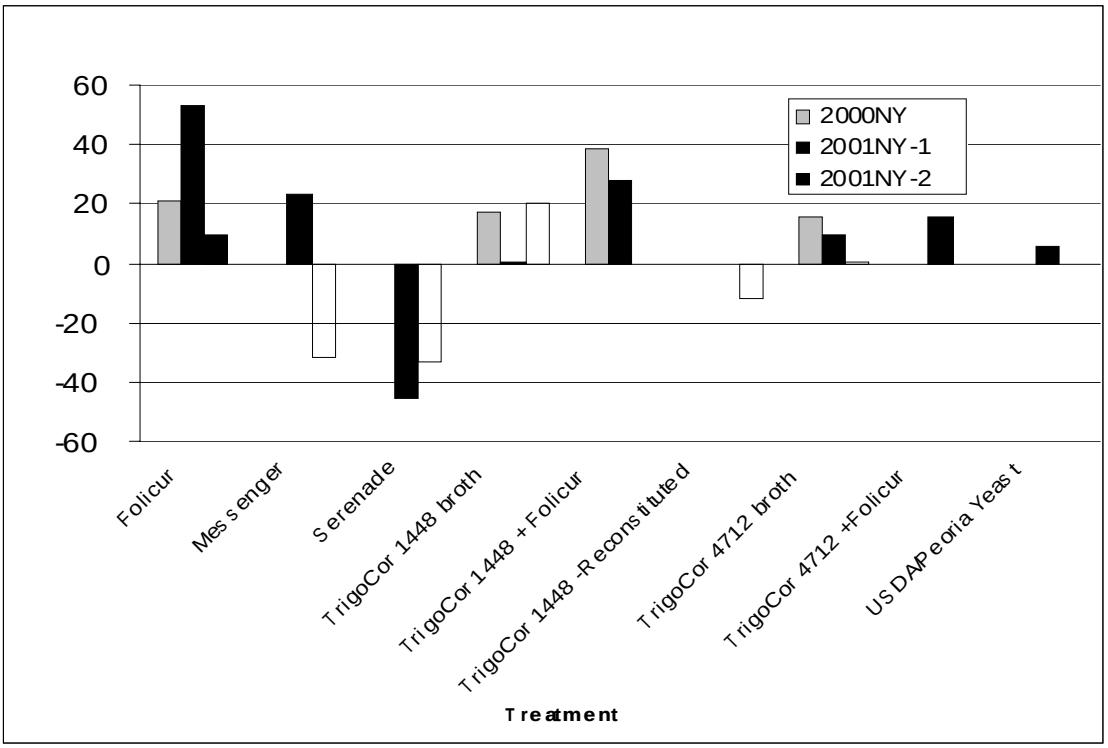

Figure 1. Control of Fusarium head blight in NY. Bars represent the % change in the incidence or severity of FHB for each treatment with biological or chemical control (Folicur) in trials located in NY in 2000 and 2001.

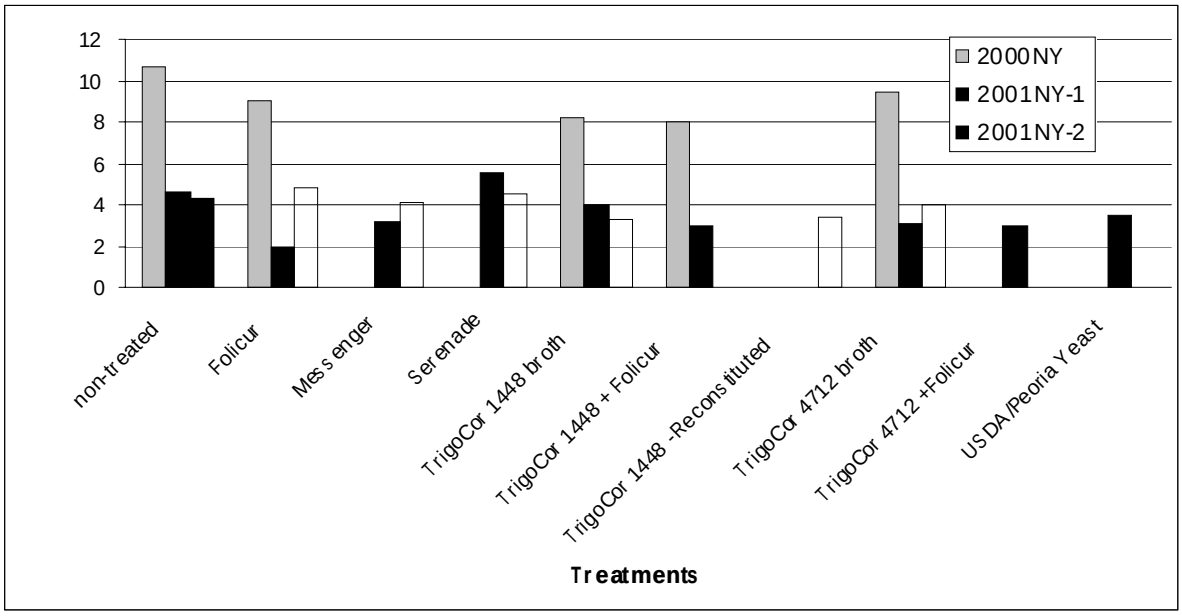

Figure 2. Effect of treatments to control Fusarium head blight on DON contamination of harvested grain (ppm) of 'Caledonia' winter wheat in NY in 2000 and 2001.

Figure 3. Control of Fusarium head blight with TrigoCor 1448. Bars represent the % change in the incidence or severity of FHB at each of the locations of the Uniform fungicide/biological trial. Trials in which disease incidence was negligible in the non-treated control have not been included in this figure.

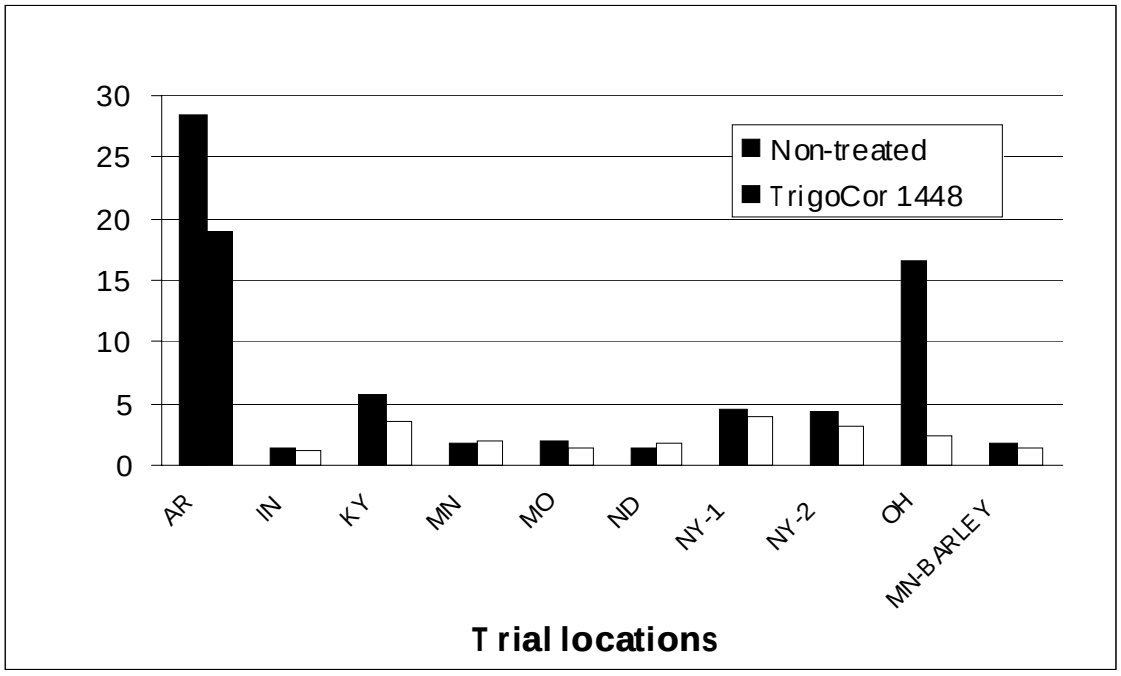

Figure 4. Effect of treatment of wheat spikes with TrigoCor 1448 on DON contamination of harvested grain (ppm) of wheat and barley at ten trial locations in eight states in 2001.

EFFICACY OF FOLIAR FUNGICIDES AND BIOLOGICAL CONTROL AGENTS FOR THE CONTROL OF FUSARIUM HEAD BLIGHT IN SPRING WHEAT

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ABSTRACT

The effect of four fungicides and two biological control agents on leaf disease severities, Fusarium head blight, grain quality parameters and the production of deoxynivalenol (DON) in the susceptible hard red spring wheat cultivar "Ivan" was investigated in a field trial in Minnesota in 2001. This study was done in collaboration with other researchers in several states that participate in the uniform fungicide trial. The objective of this cooperative study is to assess the performance of these products over a wide range of environments.

The treatments included Folicur (4 fl oz/acre), AMS 21619 (5.7 fl oz/acre), BAS 505 (0.4 lb/acre), BAS 505 + Folicur (0.2 lb + 2 fl oz/acre), Cornell biological agent (TrigoCor 1448, an antagonistic bacterium), and USDA/Peoria biological agent (*Cryptococcus nodaensis* OH 182.9, an antagonistic yeast). These treatments were applied at early flowering. The trials were planted on May 14, 2001. The plots were arranged in a randomized complete block design with four replications. Artificial inoculation of *Fusarium graminearum*, in the form of infected corn kernels were added to the plots on June 25, 2001. Treatments were applied at 40 psi in 20 gpa; using hand-boom sprayers equipped with XR8001 flat fan nozzles angled forward/backward at 30° from horizontal. Fusarium head blight incidence and severity and leaf disease severities was assessed at soft dough stage of kernel development. Plots were harvested for yield and quality measurements, and DON concentrations were determined. Fusarium damaged kernel percentages was determined on the harvested samples. Data collected were subjected to analysis of variance using SAS (Statistical Analysis System). After a significant F test (P = 0.05), treatment means were separated using a least significant difference test at P = 0.05. All treatments significantly reduced leaf diseases that were primarily Septoria and Stagonospora leaf blotches, and FHB severity compared to the untreated control. Three treatments (AMS 21619, BAS 505, and OH 182.9) reduced FHB incidence significantly. All treatments except OH 182.9 reduced significantly the percentage of scabby kernels. Two treatments (BAS 505 and BAS 505 + Folicur) resulted in significantly higher yield compared to the untreated control. Test weight and deoxynivalenol levels were not significantly affected by the treatments compared to the untreated control.

CONTROL WHEAT SCAB WITH IMPROVED FUNGICIDE APPLICATION TECHNOLOGY - 2001

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OBJECTIVE

Develop and field test a low-volume, air-assisted, small droplet prototype spraying system specifically applicable for spraying wheat and grasses.

INTRODUCTION

A project evaluating an MSU prototype sprayer was held at the Michigan Bean and Beet Farm; Saginaw, MI. The MSU sprayer was a low-volume, air-assisted, small-droplet, tower sprayer that was "skid" mounted into the bed of a 4 x 4 pick-up truck. The spray plume moved horizontal to the ground and sprayed a 75 foot wide swath at 4 mph. Folicur was applied at GS 10.5 (June 8th) on the variety Harus using either a conventional boom sprayer using 25 gal of water/acre with flat fan nozzles straight down; or the MSU sprayer using 5 gal of water/acre. Four oz of Folicur + 0.125% Induce, was the only fungicide applied. Each plot was 75 x 525 feet, and the center 30 feet x 525 was harvested on July 16th. The treatments were:

1) Wheat was sprayed from two sides with the prototype to ensure complete coverage of the head with fungicide;
2) Wheat was sprayed on only one side with the prototype sprayer resulting in incom plete coverage;
3) Conventional flat fan sprayer with nozzles aimed downward;
4) Untreated controls.

There was only one replication per treatment. Twenty-five grain probes per treatment were collected directly from the combine at harvest. Each probe sample was analyzed was analyzed separately for DON (Hart, et al, 1998). The plots were not rated for yield or disease severity.

RESULTS AND DISCUSSION

Treatments were not evaluated for FHB incidence, severity or yield. DON levels in the different treatments were:

Treatment	DON (PPM)	Standard Deviation
1	0.3	0.10
2	0.9	0.21
3	0.9	1.17
4	0.9	0.25

Although these results are preliminary and not replicated, they do suggest that thorough coverage of the wheat head is essential to reduce DON, and new technologies using very low spray volumes may compete very will with conventional sprayers.

The oral presentation will include a "five minute" video that illustrates the application technologies used.

TIME (min:sec)	TOPIC
0:00	Original “field testing” of the truck-mounted sprayer in a grass field.
1:50	Operating the truck-mounted sprayer in a wheat field at the Michigan Bean and Beet Farm.
2:30	Using an alternate “air-assisted” spraying technology (®PROPTEC) in wheat. (Note: this sprayer was used for a 2001 study of fungicide application to sugar beets in a nearby field. Originally, we had intended to include it in this study.)
3:05	Self-propelled, Hagie sprayer with a “50 foot” wide PROPTEC boom spraying asparagus.
4:00	Spraying Christmas trees with the truck-mounted wheat sprayer.
5:00	End