

LABORATORY STUDIES WITH PURIFIED ITURIN A, AND WITH  
*BACILLUS SPP.* GROWN IN COMPLEX AND DEFINED GROWTH  
MEDIA, TO ASCERTAIN THE IDENTITY AND ABILITY OF  
COMPOUNDS THAT INHIBIT *FUSARIUM GRAMINEARUM*

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**ABSTRACT**

Certain endospore-forming bacteria in the genus *Bacillus* are able to antagonize *Fusarium graminearum* in laboratory, greenhouse, and field-plot studies. We have worked with four strains of *Bacillus spp.* (likely related to *Bacillus amyloliquefaciens* and/or *Bacillus subtilis*) that have demonstrated ability to antagonize this wheat pathogen. Research has not yet entirely elucidated the mechanism of the antagonism, but it is probably due at least in part to bacterial antibiotics, such as cyclic lipopeptides in the iturin family. It is known from the literature that iturin production is enhanced in some growth media and suppressed in others. We have cultured these bacterial strains in potato dextrose broth (PDB), a complex medium containing glucose which may suppress iturin production to some degree. We have also cultured the *Bacillus spp.* in the defined broth medium of Besson et al. lacking glucose, containing mannitol, glutamic acid and inorganic salts. Bacterial cell numbers in this original formulation were lower than desirable for application of cells to wheat plants, so a modification of the original medium was used increasing the mannitol by 2.3 times, and increasing the glutamic acid by 2.1 times. After 10 days of growth in the modified broth medium with increased carbon and nitrogen sources, bacterial strain 1BA grew to over 10 times the optical density it achieved in the initial medium formulation. Plate count data also showed better growth of 1BA in the modified defined medium having elevated carbon and nitrogen, with plate counts of 10<sup>9</sup> CFU/ml or greater in the richer formulation, and plate counts that were orders of magnitude lower in the original growth medium formulation. Higher numbers of cells in the modified defined growth medium should allow better coverage of bacterial cells sprayed onto wheat surfaces when these bacteria are used in biocontrol trials. In addition, plate assays were done to see whether pure iturin would antagonize *F. graminearum*, and if the defined broth media in solidified form allowed the bacteria to antagonize the fungus. Purified iturin A was found to inhibit *F. graminearum* at a concentration of 40 µg/ml applied to a paper disk, challenging growth of the fungus on Potato Dextrose Agar. Analysis of extracts of broth cultures in defined media by absorption spectroscopy and HPLC indicated that iturin-like compounds were produced by all four *Bacillus* strains we have studied. Better understanding of the production of iturin and other compounds that might act in concert with iturin would allow better understanding and use of these and related bacteria as biocontrol agents to control FHB.

## CONTROL OF FUSARIUM HEAD BLIGHT WITH FUNGICIDES IN INDIANA, 2003

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### OBJECTIVES

To identify fungicides that are effective for control of Fusarium head blight.

### INTRODUCTION

Until wheat cultivars with a high and consistent degree of resistance to Fusarium head blight become available, fungicides may be a useful tool for maintaining yield and grain quality under conditions conducive for the disease. Fungicides currently registered for use on wheat in the U.S. are not highly effective against head blight nor for prevention of accumulation of deoxynivalenol (DON) in grain. This study was part of a cooperative effort to identify effective fungicides that will both reduce severity of head blight and accumulation of DON.

### MATERIALS AND METHODS

Fungicide trials were conducted at 2 locations in Indiana during 2003. At the Purdue Agronomy Center for Research and Education (ACRE) near West Lafayette, IN, wheat cultivar Patterson, treated with DividendExtreme and Reldan 4E seed treatment, was drilled at 7.5-in. row spacing on 26 Sep 02. At the Southeast Purdue Agricultural Center (SEPAC), near North Vernon, IN, wheat cultivar Patterson was drilled at 7.5-in. row spacing into disked corn stalks on 21 Oct 02. Before seeding at ACRE, 50 lbs /A of N was broadcast and incorporated. Plots were top-dressed on 22 Mar at 90 lb/A of N. Prior to planting at SEPAC, the field was fertilized with 524 lb/A of 7-17-38. Plots were top dressed with ammonium sulfate on 10 Mar at 90 lb/A of N. Harmony Extra at 0.5 fl oz/A was applied in early spring at both locations for weed control. Corn stalks were spread at ACRE between rows of wheat prior to heading to provide inoculum of *Gibberella zeae*. Plots at ACRE and SEPAC were 20 ft long and 5.5 ft wide. An intermittent mist-irrigation system was operated at ACRE in the plots from 13 May, when heads were in the boot stage, through the mid dough stage. Both experiments were randomized complete blocks with 4 replications. Rainfall at ACRE for the week prior to heading and 2 weeks past flowering was 1.7 in. Average high temperature for the same period was 69°F and average low temperature was 50°F. Rainfall at SEPAC was 3.1 in. during the same period, average high temperature was 72°F, and average low temperature was 54°F. Disease developed from natural inoculum. Fungicides were applied with a CO<sub>2</sub>-pressurized backpack sprayer and hand-held boom fitted with TJ 8001VS nozzles (12-in. spacing) that delivered 332 gpa at 40 psi. There were 2 nozzles at each drop, that directed the spray from the sides of the heads. Fungicides were applied at early boot stage (GS 45) on 7 May, head emergence (GS 59) on 10 May, beginning of anthesis (GS 61) on 15 May, and full anthesis (GS 63) on 22 May at ACRE. Fungicides were applied at SEPAC on 14 May at mid flowering (GS 63). Intensity of foliar disease was visually estimated on a whole-plot basis. Incidence of Fusarium head blight (FHB) was estimated by counting the blighted heads in 10 arbitrarily selected 1-ft lengths of row. We estimated severity of blight as the average percentage of spikelets

blighted on those heads that showed any blight. From the product of incidence and severity, we calculated an FHB index. Plots were harvested with a plot combine, on 14 Jul at ACRE and on 26 Jun at SEPAC. Grain was dried to 13 % moisture after which yield and test weight were measured. The frequency of *Fusarium*-damaged kernels (FDK) was determined by counting the number of such kernels in a sample of 100 kernels from each plot. We also determined the frequency of infection in kernels that were visually sound. We surface sterilized 25 healthy-looking kernels from each plot for 2 min in 5% bleach solution (0.2625 % sodium hypochlorite) and then rinsed them in sterile water for 1 min. These seeds were placed on Komada's medium, incubated at 25°C and examined for *F. graminearum* growth after 5 days. We ground 100 g of kernels from each plot and sent the coarse flour to Dr. Pat Hart at Michigan State University for DON analysis.

## RESULTS AND DISCUSSION

Weather conditions at ACRE were conducive to a moderate development of FHB. The supplemental mist irrigation promoted severe blight in this trial. All fungicide treatments, even those applied at the boot stage, reduced incidence (Table 1). Only the JAU6476 and V-10116 treatments applied during flowering reduced severity of blight. Most treatments reduced the number of FDK. Several treatments applied at flowering reduced DON level in grain. Three treatments increased yield significantly. Test weights were low in this trial and not improved by any treatment. Lodging was severe, and this, as much as head blight, contributed to low test weight. At SEPAC, all treatments reduced FHB incidence, but as at ACRE, JAU6476 and V-10116 were most effective (Table 2). By the time of the second assessment, incidence and severity had increased in all treatments, but several treatments were still superior to the unsprayed control.

Harvest was interrupted by rain at SEPAC, and some plots were never harvested. We were able to obtain samples from each treatment for analysis of FDK and DON. FDK values and DON were quite high at SEPAC, but 2 treatments reduced DON by more than 34%. Folicur did not reduce DON at either location.

**Table 1.** Control of Fusarium head blight on mist-irrigated wheat at the Purdue Agriculture Center for Research and Education (ACRE).

Product, rate per acre, growth stage at application	Leaf blotch <sup>1</sup>		FHB <sup>2</sup>		FHBX <sup>2</sup>	FDK <sup>3</sup>	DON <sup>5</sup>	Yield	Test weight
	20 Jun	FHBI <sup>2</sup>	FHBS <sup>2</sup>	FHBC <sup>4</sup>					
Folicur 3.6 F 4 fl oz + Induce 0.125% v/v, GS 59	27	32	47	15	10.3	35	1.7	63.6	45.2
Folicur 3.6 F 4 fl oz + Induce 0.125% v/v, GS 61	14	28	45	13	11.5	48	2.1	66.2	46.0
Folicur 3.6 F 4 fl oz + Induce 0.125% v/v, GS 63	22	35	42	15	7.8	53	1.7	65.9	46.9
JAU6476 480SC 5 fl oz + Induce 0.125% v/v, GS 61	8	22	20	5	4.8	21	0.8	73.5	49.3
JAU6476 480SC 5.7 fl oz + Induce 0.125% v/v, GS 59	6	24	29	7	5.3	35	1.1	72.1	49.6
JAU6476 480SC 5.7 fl oz + Induce 0.125% v/v, GS 61	15	25	39	10	10.5	41	1.6	73.1	45.8
JAU6476 480SC 5.7 fl oz + Induce 0.125% v/v, GS 67	29	25	37	10	6.8	34	1.3	64.1	47.2
JAU6476 480SC 3.6 fl oz + Folicur 3.6 F 4 fl oz + Induce 0.125% v/v, GS 61	6	21	21	6	7.8	28	1.1	69.2	46.4
V-10116 1.67 SC 6 fl oz + Induce 0.063% v/v, GS 61	7	30	34	10	8.8	30	0.9	63.7	47.0
V-10116 1.67 SC 8 fl oz + Induce 0.063% v/v, GS 63	37	29	40	12	6.3	29	0.8	59.3	45.4
Tilt 3.6 EC 4 fl oz, GS 45	35	36	41	15	8.5	38	2.5	61.5	43.6
Quadris 9.2 fl oz, GS 45	8	33	45	15	13.8	54	2.8	61.4	42.4
Stratego 250 EC 10 fl oz, GS 45	12	30	41	13	9.5	37	2.4	69.1	45.3
Folicur 3.6 F 4 fl oz, GS 45	22	31	45	14	13.8	46	2.7	68.1	44.9
JAU6476 480SC 5.7 fl oz + Induce 0.125% v/v, GS 45	17	36	45	16	8.5	46	2.3	64.8	44.4
Untreated	51	44	52	24	17.0	59	2.4	60.5	46.3
LSD (0.05)	15	13	2	2	4.3	20	0.6	9.3	3.5

<sup>1</sup> Leaf blotch was caused by both *Septoria tritici* and *Stagonospora nodorum*. Severity was rated as the percentage of flag leaf area affected.

<sup>2</sup> FHBI = FHB incidence, the percentage of heads showing blight symptoms. FHBS = FHB severity, the percentage of spikelets on affected heads that were blighted.

FHBX = FHB index, the product of incidence and severity expressed as percent.

<sup>3</sup> FDK = *Fusarium*-damaged kernels, determined by examining 25 kernels from each plot.

<sup>4</sup> FHBC = *Fusarium* contamination of apparently sound grain.

<sup>5</sup> DON (deoxynivalenol) content was determined by Dr. Pat Hart at Michigan State University.

**Table 2.** Control of Fusarium head blight on wheat at the Southeast Purdue Agricultural Center (SEPAC).

Product, rate per acre, growth stage at application	Leaf blotch <sup>1</sup>	FHBI <sup>2</sup> 4 Jun	FHBS <sup>2</sup> 4 Jun	FHBI <sup>2</sup> 13 Jun	FHBS <sup>2</sup> 13 Jun	FHBC <sup>4</sup> %	FDK <sup>3</sup> %	DON <sup>5</sup> ppm
Folicur 3.6 F 4 fl oz + Induce 0.125% v/v, GS 63	32	13	19	45	50	6	14 ab	14 cd
JAU6476 480SC 5 fl oz + Induce 0.125% v/v, GS 63	17	5	14	30	49	3	12 a	7 a
JAU6476 480SC 5.7 fl oz + Induce 0.125% v/v, GS 63	38	7	12	30	49	6	13 a	10 b
JAU6476 480SC 3.6 fl oz + Folicur 3.6 F 4 fl oz + Induce 0.125% v/v, GS 63	32	6	12	31	46	6	17 ab	11 bc
V-10116 1.67 SC 6 fl oz + Induce 0.063% v/v, GS 63	10	7	11	23	45	6	12 a	9 ab
V-10116 1.67 SC 8 fl oz + Induce 0.063% v/v, GS 63	15	5	10	25	44	8	14 ab	11 bcd
Untreated	60	20	17	57	58	11	20 b	13 cd
LSD (0.05)	23	4	4	21	13	6		

<sup>1</sup> Leaf blotch was caused by both *Septoria tritici* and *Stagonospora nodorum*. Severity was rated as the percentage of flag leaf area affected.

<sup>2</sup> FHBI = FHB incidence, the percentage of heads showing blight symptoms. FHBS = FHB severity, the percentage of spikelets on affected heads that were blighted.

<sup>3</sup> FDK = FHB index, the product of incidence and severity expressed as percent.

<sup>4</sup> FHBC = *Fusarium*-damaged kernels, determined by examining 25 kernels from each plot.

<sup>5</sup> DON (deoxynivalenol) content was determined by Dr. Pat Hart at Michigan State University.

POPULATION DYNAMICS OF THE FUSARIUM HEAD BLIGHT  
BIOCONTROL AGENT *CRYPTOCOCCUS NODAENSIS*  
OH182.9 ON WHEAT

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**ABSTRACT**

*Cryptococcus nodaensis* OH182.9 is a naturally occurring wheat anther colonist. Application of OH182.9 to wheat at anthesis reduced Fusarium head blight (FHB) severity by 56% and doubled 100-kernel weight in field trials. Confirmation of the ability of OH182.9 to survive on wheat heads prior to anthesis would support the possibility of applying OH182.9 earlier in crop development without sacrifice to efficacy. Biomass of OH182.9 was produced in liquid culture and applied to field and greenhouse grown wheat just prior to and during early anthesis. Populations of OH182.9 on extruded anthers were monitored for 10-12 d after application and on kernels harvested from treated heads. Significant populations of OH182.9 were recovered from all treated plants indicating that OH182.9 is able to survive in the absence of anthers. A significant increase in OH182.9 populations 4-8 d after application was observed suggesting that OH182.9 reproduced on the head. OH182.9 was recovered on kernels of treated wheat. Results of studies will be presented.

## 2003 UNIFORM FUNGICIDE PERFORMANCE TRIALS IN SOUTH DAKOTA

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### ABSTRACT

Fusarium head blight (FHB – scab) has been a serious concern for wheat producers in South Dakota for ten years. The objective of this study was to continue to evaluate the efficacy of various fungicides and fungicide combinations for the suppression of Fusarium head blight and other wheat diseases. Two hard red spring wheat cultivars, Oxen and Ingot, were planted at three South Dakota locations (Brookings, Groton, and South Shore/Watertown). Data were collected from two of three spring wheat study sites, Groton and Brookings, SD. Conditions were extremely dry at the third site and little disease developed. The winter wheat study site South Shore, was lost due to dry conditions at seeding. Trial treatments were all from the Uniform Fungicide Trial treatments list for the suppression of FHB and included an untreated check, Folicur at 4 fl oz/A, JAU6476 at 5.7 fl oz/A and 5.0 fl oz/A, JAU6476 at 3.6 fl oz/A + Folicur at 4 fl oz/A, and V-10116 at 6 or 8 fl oz/A. Trials were planted in a factorial randomized complete block design with six replications. Trial treatments were applied at anthesis. The following day, the crop was challenge inoculated with  $10^4$  macroconidia/ml of *Fusarium graminearum* 'Fg4'. The plots were misted for five minutes out of every twenty over a 14 days period. Twenty-one days following treatment, plots were evaluated for leaf diseases, FHB incidence, FHB head severity, and FHB field severity. Samples were collected for Fusarium damaged kernels (FDK), deoxynivalenol (DON), grain yield, test weight, and protein. At Brookings, all treatments significantly reduced leaf disease as measured by a whole plot, green leaf rating. When assessed by percent necrotic leaf tissue, only the low rate of JAU6476 was inferior. Similarly, leaf rust was significantly reduced by all treatments except JAU6476. While head severity of FHB was not reduced by any treatment, incidence and total disease were significantly reduced by all treatments. FDK was generally not affected. Yield and test weight were increased by all treatments. Similar trends resulted at Groton. All treatments significantly reduced leaf disease as measured by a whole plot, green leaf rating or percent necrotic leaf tissue. Leaf rust was only controlled by V-10116 at the high rate. While head severity of FHB was not reduced by any treatment, incidence and total disease were significantly reduced by all treatments.

## 2003 UNIFORM TRIALS FOR THE PERFORMANCE OF BIOLOGICAL CONTROL AGENTS IN THE SUPPRESSION OF FUSARIUM HEAD BLIGHT IN SOUTH DAKOTA

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### INTRODUCTION AND OBJECTIVES

Biological control agents (BCAs) have several advantages in the suppression of Fusarium head blight (FHB or scab). When organic crops are grown, fungicide options are not available and crops such as barley are susceptible over a long period of time following head emergence and before maturity. As such, biological control has a good fit for FHB management under those conditions.

The objectives to this study were to evaluate the efficacy of various BCAs relative to the standard fungicide comparisons for the suppression of Fusarium head blight on wheat and barley.

### MATERIALS AND METHODS

'Robust' barley was planted in a randomized complete block design with six replications and 'Oxen' hard red spring wheat were planted in a factorial randomized complete block design with four replications, both at Brookings, SD. Both spring wheat and barley were protected with isolates of *Bacillus subtilis*-type isolates SDSU-1BA and SDSU-1BC each of which were grown in either a potato dextrose broth or a defined medium intended to increase the production of iturin while reducing factors that might support growth of *Fusarium* on the plant surface; *Cryptococcus nodaensis* OH 182.9; *Bacillus*-type isolate TrigoCor 1448, and; *Lysobacter sp.* strains 'C3' and '7A4'. The BCAs were compared to a standard chemical treatment of Follicur (4 fl/oz/A) applied with Induce non-ionic surfactant (0.125%).

At initial anthesis of wheat or full head emergence of barley, the BCAs were applied to the heads as a spray of the growth medium + agent and allowed to dry. The following day, the plants were challenge inoculated with 10<sup>4</sup> macroconidia/ml of *Fusarium graminearum* 'Fg4'. The plots were also treated with Fg4 grown on corn grain inoculum.

Following challenge inoculation, a misting cycle was started for 5 minutes out of every 20 minutes, 24 hours a day for 14 days.

### RESULTS AND DISCUSSION

During the inoculation period, the environment was very hot and dry, but the moisture was retained on the heads between misting cycles. No misting was done before heading, so the corn grain inoculum had a minimal influence. No differences were detected among the FHB disease measurements in either study



(Table 1 and 2). Similarly yields were not impacted by the treatments, including the fungicide treatment. However, DON was significantly reduced by the addition of TrigoCor 1448 as compared to the untreated control. For some reason, Folicur resulted in a significantly higher DON content and OH 182.9, SDSU 1BA and 1BC from either growth media resulted in significantly lower DON content as compared to the Folicur treatment.

**Table 1.** Disease control and yield components on spring wheat at Brookings<sup>1</sup>.

Treatment	Fusarium Head Blight (FHB)			DON (ppm)	FDK (%)	Yield (bu/A)	Test Wt (lb/bu)	Protein (%)
	Incidence <sup>2</sup> (%)	Head Severity <sup>3</sup> (%)	Index <sup>4</sup> (%)					
Untreated	13.0	8.4	1.1	1.1	5	34.7	46.8	16.0
Folicur + NIS	14.0	8.9	1.2	1.5	5	34.6	47.3	15.9
7A4	15.0	9.1	1.4	1.4	4	34.3	46.3	16.1
C3	18.0	10.3	1.8	1.2	4.5	33.8	46.5	15.9
Trigo Cor 1448	21.3	9.2	2.1	0.5*	5.3	34.1	44.0	16.1
1 BA (defined)	19.0	9.6	1.9	1.1	4	34.0	45.1	16.1
1 BC (PDB)	12.5	8.6	1.0	1.0	4.5	34.6	47.4	15.9
1 BA (PDB)	12.5	7.3	0.9	1.0	4	35.8	46.0	16.0
1 BC (defined)	14.0	13.0	1.7	0.9	3.5	36.1	48.2	15.9
OH 182.9	11.0	8.4	0.9	1.0	4	34.9	46.0	16.0
LSD (P=0.05)	NS	NS	NS	0.4	NS	NS	NS	NS
CV	29.5	35.5	47.2	7.4	22.5	7.4	3.9	1.2

<sup>1</sup>Measurements of leaf disease were not significant.

<sup>2</sup>Incidence indicates the percentage of heads with any level of infection.

<sup>3</sup>Head severity indicates the percentage of disease on infected heads.

<sup>4</sup>Index indicates Incidence \* Severity.

**Table 2.** Disease control and yield components on barley at Brookings 2003<sup>1</sup>.

Treatment	Fusarium Head Blight (FHB)			DON <sup>1</sup> (ppm)	Yield (bu/A)	Test Wt (lb/bu)	Protein (%)
	Incidence <sup>1</sup> (%)	Head Severity <sup>2</sup> (%)	Index <sup>3</sup> (%)				
Untreated	69.5	9.5	6.5	3.0	85.6	40.0	12.2
Folicur + NIS	64.0	8.5	5.7	3.4	89.0	40.4	12.4
7A4	66.5	7.0	4.8	3.7	96.1	39.1	12.9
C3	64.0	11.3	7.5	2.9	88.2	40.1	12.3
Trigo Cor 1448	75.5	8.1	6.2	3.8	88.5	40.0	12.3
1 BA (defined)	71.5	8.9	6.6	2.8	89.3	40.2	12.7
1 BC (PDB)	67.0	10.2	6.9	2.9	90.7	39.1	12.5
1 BA (PDB)	64.0	8.0	5.1	3.3	88.2	40.7	12.7
1 BC (defined)	67.5	12.0	8.3	3.2	87.1	41.0	12.5
OH 182.9	62.5	7.0	4.5	2.9	87.9	40.1	13.0
1 BA	68.5	9.1	6.1	2.7	93.5	39.0	12.8
Untreated	67.0	9.0	6.0	4.1	86.7	39.5	12.3
Untreated	62.0	7.2	4.3	2.8	83.8	40.7	12.4
LSD (P=0.05)	NS	NS	NS	0.9	NS	NS	0.5
CV	14.8	33.0	40.7	21.1	6.5	3.6	27

<sup>1</sup>Measurements of leaf disease were not significant.

<sup>2</sup>Incidence indicates the percentage of heads with any level of infection.

<sup>3</sup>Head severity indicates the percentage of disease on infected heads.

<sup>4</sup>Index reflects Incidence \* Severity.

# GROUND SPRAY SYSTEMS AND SPRAY PARAMETER EVALUATION FOR CONTROL OF FUSARIUM HEAD BLIGHT ON A FIELD SCALE BASIS

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## OBJECTIVES

Evaluate field scale spray systems with differing application technologies for enhanced control of Fusarium head blight (FHB).

## INTRODUCTION

The production industry, growers and commercial pesticide applicators, often question the validity of research using small scale equipment. In the scientific community the validity of research methods receive constant review and revision. Reasons for using small scale equipment include a) parameter control, including reducing variance attributed to soil difference, b) the extensive time necessary to calibrate large spray booms, and c) the cost of the equipment necessary for conducting a research trial. At the request of the chemical and biological committee, and in response to the production industry, a trial was implemented at the Langdon Research Center to compare field scale spray systems and address the research from an interdisciplinary approach with collaboration between the agricultural engineering community and the plant pathology community.

## MATERIALS AND METHODS

A study was planned as a randomized complete block design (RCBD) arranged as a factorial replicated six times to determine differences between spraying systems with differing application technologies with emphasis on droplet size. Due to limitations among the spray systems and time constraints to equate droplet size parameters necessary for the factorial, the data was analyzed as a RCBD. A foundation seed production field, planted to Alsen, was selected for the suitability of plot layout to the spray systems, soil uniformity (Cavour-Cresbard loam), and the site's proximity to the mixing and other facilities of the station. Four replicates had previous crop small grains and two replicates were previous crop potatoes. The site was partitioned into 72 plots 35 x 60 feet to accommodate the field scale equipment. All units sprayed each individual replicate and did an end run around the trial to the front to align for the subsequent replicate and to minimize potential of drip contamination to other plots. About 20 ft from the front and backside of each plot was swathed and discarded from all disease assessment and harvest results. Harvest data was collected from the interior 20 feet of the spray area from each respective unit with a Hege small plot combine. Recommended, NDSU Extension Service, HRSW production practices for the northeast North Dakota were followed. A Fusarium spawn was hand broadcast on each individual plot three weeks prior to flowering at about 300 grams per plot. FHB was visually assessed by sampling 20 spikes per plot and counting the spikes per head and the infected kernels per spike 20 days after spray application. Data is reported as

incidence and (field) severity. Yield, test weight, and protein were determined on all plots and the percent deoxynivalenol (DON) was determined on three replicates after it was determined from the untreated plots that DON was present. Coverage parameters were assessed by including a Day Glo orange fluorescent dye mixed at 3% v/v with the spray solution. Twenty spikes were collected from the first replicate of each treatment, transported to North Dakota State University in Fargo, photographed under incandescent and UV light on both sides of the spike to determine the area covered by the spray solution from each spraying system. Prior to spraying three sampling stands, with water and oil sensitive paper at grain heading height (1" x 2.75" cards, Spraying Systems Co.), were oriented horizontal and vertical back to back east-west and north-south and spaced to collect spray pattern samples from each spray unit. DropletScan, a product of WRK, Inc. and Devore Systems Inc., was used to analyze the water sensitive cards and generate a report. The field scale sprayers, technical assistance, and operation were provided by AGCO Corporation (Chris Mohning) and Hardi Inc. (Richard Hundt). Technical assistance and operation of a plot scale sprayer previously provided by Spray-Air Technologies Ltd. was provided by Bob Dawes of Degelman Industries. A fourth unit utilized a Proptec system (Ledebuhr Industries Inc.-WWW.PROPTEC.COM) on one boom and a Spraying systems hydraulic flat fan nozzle system mounted on a double swivel with nozzles angled 30 degrees downward from horizontal and oriented forward and backward on the opposite boom. The unit was provided and operated by Dr. Gary Van Ee, Agricultural Engineer from Michigan State University. The five spray units each sprayed two treatments and were compared to two untreated checks for a total of 12 treatments. The technical representatives were encouraged to operate their respective system as close to optimum performance conditions for one spray treatment and readjust the equipment to change droplet size as much as possible within the limitations of each spraying system. Dye and water were provided for testing prior to the trial initiation. A CCD camera was available for visual assessment of the coverage. Sprayers and parameters included:

**1) AGCO's ESP (Energized Spray Process).** The spray solution is delivered through a hydraulic spray boom mounted on a Spray-Coupe. The spray solution is energized with a negative electric charge (40,000 volts) that is attracted to the positively charged wheat spike. The charge creates a high-intensity electrostatic field between the nozzle and the plant that increases spray velocity and attracts the solution to the plant. The ESP sprayed fungicide at 10 gpa through XR8003 nozzles operating at 60 psi and XR8002 nozzles operating at 110 psi. No adjuvant was added to the spray solution by recommendation of the company representative.

**2) Hardi Commander Plus 1200 80 ft Twin Force with Mustang 3500 Rate Controller.** The spray solution is delivered by hydraulic nozzles, ISO yellow #2 110° tips, which spray into an air stream that delivers the solution to the target. The air stream, generated by a centrifugal fan and dispersed through a bag type manifold, was angled forward 30 degrees. Spray solution was delivered at 15 gpa to both treatments, nozzles spaced 20 inches 22 to 24 inches above the canopy. One treatment was sprayed at 45 psi with fan speed operating at 1750 rpm which would deliver a droplet size of between 160-240 volume mean diameter (VMD). The second treatment sprayed at 100 psi with fan speed of 2300 rpm which would produce a VMD of 160-240 VMD. Greater pressure should produce the droplets in the lower end of the VMD range.

**3) Spray-air Technologies Ltd.** The spray solution was delivered by CO<sub>2</sub> pressurized system. The solution was dispersed through a metering orifice at 27 psi spaced 10 inches apart angled 15 degrees forward from vertical at 9 gpa. The droplet is formed by wind shear picking the drop off the end of an orifice in the center of the air stream. The speed of the wind stream determines the droplet size with greater wind speed (increased static pressure) producing smaller droplets. The air stream is generated by a centrifugal fan and dispersed by a manifold to individual orifices.

**4) Ledebuhr Industries Proptec.** This system produces an air stream generated by hydraulic driven axial fans spaced 48 inches apart. Blade pitch is adjustable. The spray solution is delivered by hydraulic pump through a metering orifice. The droplet is generated by a rotary atomizer spinning at approximately 5400 rpm. The Proptec atomizer was operated at approximately 2000 psi with a 4 gpm hydraulic flow rate delivering spray solution at 10.4 gpa. This system generates an extremely fine droplet (approximately 125 micron VMD) in a 40 to 50 mph air stream.

**5) Conventional F+B.** The control system utilizes hydraulic nozzles, XR8001, angle 30 degrees downward and oriented to deliver the spray solution forward and backward to spray both sides of the spike. The nozzles were operated at 40 psi delivering 10.4 gpa spray solution.

The spray solution consisted of Bayer's experimental fungicide JAU 6476, prothiaconazole. This fungicide was selected because of its linear disease reduction as rate increases. A rate of 2.85 oz/acre, half the recommended rate, was used to measure differences between the spraying systems and application technology parameters. Induce adjuvant was mixed with all solutions at 0.125% v/v except the ESP sprayer. A mixing error reduced the fungicide rate in both treatments of the Hardi system by approximately 20% to a rate of approximately 2.3 oz/acre. Data was analyzed with the general linear model (GLM) in SAS. Least significant differences (LSD) were used to compare means at the 5% probability level.

## RESULTS AND DISCUSSION

Spray application began at about 1:15 p.m. after the foliage had dried sufficiently to permit data collection. The final treatments were concluded by 6:00 p.m. Average wind speed ranged from 8.3 to 10.2 mph with occasional gusts exceeding 15 mph. Wind direction was WSW. Spray application commenced traveling from east to west minimizing drift between plots. Air temperatures ranged from 72 to 78° F and R.H. decreased from 65 to 55 % over the application period. FHB incidence was reduced by several of the treatments including both Spray-air and conventional treatments, the ESP with XR8003 nozzles with the coarse droplets, the Hardi with the fine droplets, and the Proptec angled at 45 degree angle compared to the untreated (Table 1). Field severity and leaf disease was reduced by all fungicide applications compared to the untreated. The recommended conventional system had smaller leaf disease levels than the other conventional treatment. The Spray-air (fine droplet), both Hardi treatments, and the Electrostatic with coarser droplets had smaller leaf disease levels compared to the conventional system 36 inches above target, and the Proptec system angled 45 degrees downward. Both Spray-air systems had increased yield over the untreated, the conventional 36 inches above the target, and the Proptec angled 70 degrees downward. No significant differences were measured in test weight or protein. Deoxynivalenol (DON) levels were reduced below 0.5 ppm by all fungicide applications.

Total spike coverage was reduced by about ¼ when the conventional sprayer was operated above recommended height and by ½ when the angle of the Proptec was increased from 45 to 75 degrees (Table 1). Spike coverage on the front side ranged from a high of 63% with the conventional unit at recommended height to 13% on the Proptec angled 70 degrees downward. The greatest backside coverage was also the conventional at recommended height at 25% and the smallest coverage on the conventional 36 inches above target (3%) and Proptec 70 degrees downward.

## CONCLUSION

This trial demonstrated the three major spray solution delivery systems, electrostatic, air stream, and hydraulic and further demonstrated three principal methods of spray atomization: hydraulic, wind sheer, and the rotary. The study indicates that operation and adjustment of each of the spray systems can affect one or more disease components and yield parameters. Water sensitive paper analysis indicated less but similar coverage compared to spike measurement (Table 2). The varying VMD indicates a wide range of choices between spraying systems and some variability when parameters of each individual spraying system changed. In most treatments a smaller droplet size was deposited on the back side of the deposition card compared to the front side indicating that a factor other than inertial impact contributed to deposition on the backside of the papers. Each of the spraying systems offers latitude to change application parameters as shown by GPA determinations from the conventional and Proptec systems (Table 2). Similar assessment can be made from the horizontal placed water sensitive paper which would affect leaf coverage and possibly leaf disease control (Table 3). Proper adjustment and operation is imperative to maximize the efficiency of all the systems. Coverage on the untreated indicates minimal intra plot spray drift.

One must conclude that all sprayers in this test successfully delivered the necessary fungicide dose to minimize disease infection. In the future, additional reductions or a range of fungicide rates may be necessary to measure sprayer differences. Measurement of the spray parameters indicates that further study to identify optimal adjustment factors to maximize spray coverage and fungicide efficacy should be undertaken. Evaluation on the appropriate fungicide rate for spray system and spray system parameter study should also be undertaken.

## ACKNOWLEDGEMENTS

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**Table 1.** FHB Incidence and field severity, leaf disease, yield, test weight, protein, and DON by spray systems, Langdon, 2003.

Spray System	Spray Parameters	Incidence		FHB		Leaf Disease	Yield	Test Weight	Protein	DON
		%	%	Field Severity	%					
Conv. F+B	10" above target	23.3	0.8	10.2	60.6	60.8	14.6	<0.5		
Conv. F+B	36" above target	22.5	0.8	15.9	60.0	60.4	14.4	<0.5		
Electrostatic	Twinjet XR8003 @ 60 psi	20.0	0.8	5.7	62.1	61.1	14.4	<0.5		
Electrostatic	Twinjet XR8002 @ 110 psi	36.7	1.4	10.4	60.4	60.5	14.8	<0.5		
Hardi Twin	45 psi 2200 rpm	39.2	1.6	7.0	61.5	60.7	15.2	<0.5		
Hardi Twin	95 psi 1800 rpm	23.3	0.8	8.3	62.5	60.6	14.2	<0.5		
Proptec	Angled 45° down	28.3	1.0	13.0	63.5	60.7	14.8	<0.5		
Proptec	Angled 70° down	32.5	1.2	9.1	59.1	60.5	14.7	<0.5		
Spray Air	Static Pressure 15	23.2	0.8	10.1	64.6	60.9	14.5	<0.5		
Spray Air	Static Pressure 25	29.2	0.9	6.7	64.0	60.9	15.1	<0.5		
Untreated		50.8	2.7	23.0	60.3	60.2	14.6	0.8		
Untreated		45.8	3.0	27.5	57.3	60.1	14.3	1.0		
LSD*		14.2	1.0	5.6	3.8	NS	NS	0.3		
% CV		39	64	40	5	1	6	28		

\* Significant at 0.05 probability level for mean comparisons

**Table 2.** Back, front, and total spike coverage and mean area, VMD, and GPA for front side and backside coverage placed vertically as measured on water sensitive paper by spray system, Langdon 2003.

Spray System	Spray Parameters*	Mean Spike Coverage**		WS Paper Front side Mean			WS Paper Backside Mean			
		Back	Front	Area	VMD	GPA	Area	VMD	GPA	
		%	%							
Conv. F+B	10" above target @10.4 gpa	24.91	63.18	44.04	38.7	577	7.9	18.2	481	6.0
Conv. F+B	36" above target @10.4 gpa	3.13	16.98	10.05	2.7	219	1.0	0.4	184	0.1
Electrostatic	Twinjet XR8003 @ 60 psi @ 10 gpa	12.62	28.02	20.32	15.3	478	5.2	6.7	472	2.6
Electrostatic	Twinjet XR8002 @ 110 psi @10 gpa	11.35	44.39	27.87	18.3	391	6.5	3.4	300	1.3
Hardi Twin	45 psi 2200 rpm @ 15 gpa	16.14	38.99	27.56	22.5	378	8.3	8.5	439	3.0
Hardi Twin	95 psi 1800 rpm @ 15 gpa	10.02	32.03	21.03	30.5	500	10.2	6.3	285	1.9
Proptec	Angled 45° down @ 10.4 gpa	7.36	21.52	14.44	22.4	270	7.6	2.5	136	0.7
Proptec	Angled 70° down @ 10.4 gpa	1.15	12.55	6.85	5.6	146	1.5	0.9	118	0.2
Spray Air	Static Pressure 15 @ 9 gpa	5.91	36.64	21.27	14.2	353	4.0	4.0	254	1.4
Spray Air	Static Pressure 25 @ 9 gpa	9.3	26.22	17.76	22.2	398	7.0	5.0	170	1.5
Untreated				0.07				0.03	87	.005
Untreated				0.25				0.01	121	.003

\*Increased water volumes result in increased coverage parameters when other parameters remain constant.

\*\*20 spike sample

**Table 3.** Mean area, VMD, and GPA for coverage as measured on water sensitive paper placed horizontally by spray system, Langdon 2003.

Spray System	Spray Parameters*	WS Paper* Horizontal Mean	
		Area	VMD GPA
Conv. F+B	10" above target @ 10.4 gpa	30.5	474 10.5
Conv. F+B	36" above target @ 10.4 gpa	19.3	354 7.5
Electrostatic	Twinjet XR8003 @ 60 psi @ 10 gpa	55.4	614 11.7
Electrostatic	Twinjet XR8002 @ 110 psi @ 10 gpa	46.7	562 11.1
Hardi Twin	45 psi 2200 rpm @ 15 gpa		
Hardi Twin	95 psi 1800 rpm @ 15 gpa	73.0	
Proptec	Angled 45° down @ 10.4 gpa	21.6	260 7.6
Proptec	Angled 70° down @ 10.4 gpa	3.8	155 1.0
Spray Air	Static Pressure 15 @ 9 gpa	32.4	448 9.7
Spray Air	Static Pressure 25 @ 9 gpa	37.5	398 11.7
Untreated		<0.1	64 <0.1
Untreated		<0.1	61 <0.1

\*Missing values due to coverage limitations of the water and oil sensitive paper.



## ANALYSIS OF 2003 UNIFORM WHEAT FUNGICIDE TRIALS ACROSS LOCATIONS AND WHEAT CLASSES

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### OBJECTIVE

The objective was to evaluate a common set of foliar fungicide treatments, across a range of environments, for effectiveness in managing Fusarium head blight (FHB) and deoxynivalenol (DON) accumulation in wheat.

### INTRODUCTION

FHB is a potentially devastating disease that can result in serious economic losses for wheat producers, millers and endusers of wheat products. In addition, grain contaminated with DON, a mycotoxin frequently associated with FHB, can cause health problems in both humans and livestock. Thus, identifying fungicides that significantly reduce FHB incidence and severity in the field, and DON accumulation in harvested grain, would have widespread benefits to growers and endusers of all market classes of wheat. The Uniform FHB Fungicide Trials were established as a means of evaluating fungicide treatments that may be useful in FHB management programs, nationwide.

### MATERIALS AND METHODS

Scientists from 11 states conducted 24 trials across a range of wheat classes (**Table 1**). Six fungicide treatments and a non-treated check were evaluated in each trial. All treatments were applied at early flowering (Feeke's stage 10.51) using a CO<sub>2</sub>-pressurized sprayer, equipped with Twinjet XR8001 nozzles mounted at a 60° angle backward and forward. Many of the trials were inoculated with *Fusarium graminearum* and mist-irrigated in order to promote sufficient disease pressure and allow for discrimination among treatments. Plot size, crop husbandry, spray volume and pressure, sprayer type, and number of replications varied by location. Consult individual state trial reports for details. For all trials, FHB incidence, severity, index (i.e., plot severity), and *Fusarium*-damaged kernels (FDK) were measured using a more or less standardized protocol. DON accumulation was measured by one of the two USWBSI-funded DON Testing Laboratories.

Data were grouped and statistically analyzed according to whether they involved spring or winter wheat. Locations were treated as replications in the analysis. In a few instances, more than one wheat class or variety were grown at the same location. These were treated as separate experiments for the purposes of this summary. The non-treated control was not included in analyses for percent control in order to determine if there were significant differences among fungicide treatments.

### RESULTS AND DISCUSSION

**Spring Wheat-** Data from eleven spring wheat trials are summarized in **Table 2**. FHB pressure was highly variable across locations. All treatments significantly lowered FHB incidence, severity, index and FDK compared to the non-treated control. Conversely, DON values were only reduced for the JAU6476 + Folicur combination treatment and both treatments of V-10116. Generally, Folicur applied at 4.0 fl oz/A was the least effective

fungicide treatment tested. However, no fungicide treatment consistently provided greater than 50% control of FHB or DON accumulation. Percent control values ranged from 32.1% to 66.5% across fungicide treatments and FHB parameters.

**Winter Wheat-** Data from nine winter wheat trials are summarized in **Table 3**. FHB pressure was heavy in most trials. All treatments significantly lowered FHB incidence, severity and index compared to the non-treated check. Conversely, no treatment resulted in significant control of FDK and only JAU6476 applied at 5.7 fl oz significantly lowered DON compared to the check. There were minimal performance differences among fungicide treatments for any parameter except DON accumulation, where significant differences were noted among treatments. In particular, Folicur at 4 fl oz provided significantly less DON control than other treatments, except for V-10116 at 8 fl oz. No fungicide treatment consistently provided greater than 40% control of FHB or DON accumulation. Percent control values ranged from 6.8% to 48.1% across fungicide treatments and FHB parameters.

**Spring and Winter Wheat Comparison-** When data were averaged by spring or winter growth habit, fungicide efficacy expressed as percent control, was significantly greater in spring wheat than in winter wheat (**Table 4**). The reason for this difference is unknown. It could be an artifact of the higher disease pressure in winter wheat trials, or it may be due to the longer grain-filling period associated with winter wheat crops. A longer grain-filling period would require fungicides to control FHB over a longer period of time. In any event, the possibility that fungicides are more effective in spring wheat should be monitored as it could play a significant role in fungicide label activities and/or the development of FHB management programs.

## SUMMARY

Six fungicide treatments and a non-treated check were evaluated in 24 trials across 11 states as part of the 2003 FHB Uniform Fungicide Trials. Generally, all fungicides reduced FHB and DON compared to the check, but only minimal differences were detected among fungicide treatments. No fungicide treatment was found to be “head and shoulders” above the rest, and no active ingredient, fungicide combination, or rate consistently provided greater than 50% control of FHB or DON accumulation. Fungicides were significantly more effective managing FHB and DON in spring wheat than they were in winter wheat.

**Table 1.** States, principal investigator, institution, wheat class evaluated, and number of tests conducted, 2003 Uniform Fungicide Trial.

State	PI	Institution	Wheat class*	No. trials
AR	Gene Milus	Univ. of Arkansas	SRRW	1
IN	Greg Shaner	Purdue Univ.	SRRW	2
MD	Arv Grybauskas	Univ. of Maryland	SRRW	1
MI	Pat Hart	Michigan State Univ.	SRRW	1
MO	Laura Sweets	Univ. of Missouri	SRRW	2
MN	Char Hollingsworth	Univ. of Minnesota	HRSW	1
ND	Marcia McMullen	North Dakota State Univ.	Durum	2
			HRSW	4
NY	Gary Bergstrom	Cornell Univ.	SWWW	1
OH	Pat Lipps	Ohio State Univ.	SRRW	1
SD	Martin Draper	South Dakota State Univ.	HRSW	6
			Durum	1
VA	Erik Stromberg	VPI and State Univ.	SRWW	1

\*SRWW = Soft red winter wheat

SWWW = Soft white winter wheat

HRSW = Hard red spring wheat

**Table 2.** FHB and DON results from eleven spring wheat trials, 2003 Uniform Fungicide Trials.

Treatment	% FHB Incidence		% FHB Severity		% FHB Index		% FDK		ppm DON	
	Mean	% Control	Mean	% Control	Mean	% Control	Mean	% Control	Mean	% Control
<b>Non-treated.....</b>	41.5a*	—	22.7a	--	9.2a	—	5.5a	—	4.3a	--
<b>Folicur 3.6F.....</b>	29.4b	32.1b	15.6b	35.3b	4.5b	49.1b	3.1b	33.0a	2.9ab	34.9a
4.0 fl oz + 0.125% induce										
<b>JAU6476 480SC.....</b>	23.9c	45.8a	11.1bc	54.4a	2.8c	68.3a	3.0b	40.0a	2.9ab	32.8a
5.7 fl oz 0.125% induce										
<b>JAU6476 480SC.....</b>	26.9bc	37.0ab	12.2bc	48.6a	3.2bc	62.8a	2.3b	47.2a	2.6ab	39.8a
5.0 fl oz + 0.125% induce										
<b>JAU6476 480SC.....</b>	23.7c	46.3a	10.4c	55.3a	2.8c	66.5a	2.2b	46.0a	2.3b	46.6bc
3.6 fl oz + Folicur 3.6F										
4.0 fl oz + 0.125% induce										
<b>V-10116 1.67SC.....</b>	27.8bc	38.1ab	10.9bc	50.0a	3.2bc	65.1a	2.6b	38.9a	2.0b	54.3c
6.0 fl oz + 0.125% induce										
<b>V-10116 1.67SC.....</b>	25.5bc	42.6a	10.7bc	53.2a	3.1bc	67.9a	2.5b	43.3a	2.1b	52.0bc
8.0 fl oz + 0.125% induce										
CV	19.3	21.5	29.5	24.2	62.0	15.9	52.4	29.9	43.3	34.4

\*Means followed by a common letter are not significantly different (P=0.05, Student-Newman-Keuls); except for DON ppm, statistics were performed on arcsine-transformed data.

**Table 3.** FHB and DON results from nine winter wheat trials, 2003 Uniform Fungicide Trial.

Treatment	% FHB Incidence		% FHB Severity		% FHB Index		% FDK		ppm DON	
	Mean	% Control	Mean	% Control	Mean	% Control	Mean	% Control	Mean	% Control
<b>Non-treated.....</b>	57.9a*	--	38.9a	--	22.8a	--	23.9a	--	4.4a	--
<b>Folicur 3.6F.....</b>	50.3b	20.4b	32.7b	17.2a	16.1b	33.4a	19.9a	18.9a	4.1a	6.8b
4.0 fl oz + 0.125% induce										
<b>JAU6476 480SC.....</b>	45.8b	29.1ab	31.3b	21.4a	13.2b	42.7a	14.5a	30.4a	2.5b	43.0a
5.7 fl oz + 0.125% induce										
<b>JAU6476 480SC.....</b>	45.7b	29.3ab	29.0b	21.9a	13.1b	43.5a	18.4a	33.4a	3.0b	31.8a
5.0 fl oz + 0.125% induce										
<b>JAU6476 480SC.....</b>	44.7b	30.7a	30.1b	24.8a	12.7b	45.4a	18.0a	27.4a	3.0b	31.8a
3.6 fl oz + Folicur 3.6F										
4.0 fl oz + 0.125% induce										
<b>V-10116 1.67SC.....</b>	48.1b	24.4ab	30.2b	24.6a	15.6b	36.8a	16.9a	26.4a	3.2ab	27.3a
6.0 fl oz + 0.125% induce										
<b>V-10116 1.67SC.....</b>	46.7b	25.4ab	30.4b	27.4a	12.7b	40.8a	15.4a	31.2a	3.7ab	15.9ab
8.0 fl oz + 0.125% induce										
CV	9.9	28.9	19.1	62.4	31.2	29.0	17.3	28.0	39.8	35.5

\*Means followed by a common letter are not significantly different ( $P=0.05$ , Student-Newman-Keuls); except for DON ppm, statistics were performed on arcsine-transformed data.

**Table 4.** Percent control of FHB and DON for combined fungicide treatments from eleven spring wheat and nine winter wheat trials, 2003 Uniform Fungicide Trials.

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<b>% CONTROL RELATIVE TO NON-TREATED CHECK</b>					
<b>Wheat Type</b>	<b>Incidence</b>	<b>Head Severity</b>	<b>Index</b>	<b>FDK</b>	<b>DON</b>
Spring Wheat	40.3a*	49.5a	63.3a	41.6a	37.9a
Winter Wheat	26.2b	22.9b	40.4b	28.0b	26.1b
Spring Advantage (%)	+14.1	+26.6	+22.9	+13.6	+5.9

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\*Means followed by a common letter are not significantly different

(P=0.05, Student-Newman-Keuls); statistics were performed on arcsine-transformed data.

## PERFORMANCE OF FOLICUR IN FUSARIUM HEAD BLIGHT UNIFORM FUNGICIDE TRIALS, 1998-2003

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### OBJECTIVE

The objective of this report was to summarize the performance of Folicur in FHB Uniform Fungicide Trials since 1998.

### INTRODUCTION

Folicur 3.6F (38.7% tebuconazole, manufactured by Bayer CropScience, Kansas City, MO) has been granted an emergency exemption (section 18) to manage Fusarium head blight (FHB) and deoxynivalenol (DON) in one or more states since 1999. In 2003, Michigan, Minnesota, Montana, North Dakota and South Dakota applied for, and were granted, an emergency exemption for this use. A significant FHB outbreak in several mid-Atlantic and Midwest states has resulted in additional states evaluating the possibility of applying for a Folicur section 18 in an attempt to manage future FHB/DON problems. This summary is a direct response to requests for information on how Folicur has performed in research plots since 1998. This report IS NOT a summary of all available data on the use of Folicur to manage FHB/DON; however, it is felt that a sufficient quantity of data has been summarized to accurately indicate the past and probable future effectiveness of Folicur for FHB/DON management.

### MATERIALS AND METHODS

Data used in this summary were gleaned from 1998-2003 Proceedings of the National Fusarium Head Blight Forum, and from various individual reports where Folicur was evaluated for FHB management. For each report, percent control of FHB variables was calculated as follows:

$$\% \text{ Control} = \frac{\text{non-treated mean} - \text{Folicur mean}}{\text{non-treated mean}} \times 100$$

Percent control for each variable in each test was classified into one of ten incremental ranges, and an overall percent control by Folicur was calculated.

### RESULTS AND DISCUSSION

Folicur applied at early flowering suppressed FHB symptoms and DON accumulation in wheat (**Table 1**). Overall, Folicur reduced FHB field symptoms (i.e., disease index) by about 40%. Lower levels of control (~30% control) were associated with the grain quality variables, Fusarium damaged kernels (FDK) and DON. When data were classified into incremental ranges of percent control (**Table 2**), it was clear that efficacy results were highly variable across tests and years. This variability may be due to overwhelming disease pressure in some trials, and possibly greater efficacy in spring wheat than winter wheat (see 2003 Uniform FHB Fungicide Trials report). Suppression of FHB and DON by Folicur is well below the industry standard for fungicide efficacy of 90+%.

Nonetheless, due to the lack of a more effective fungicide for FHB management, Folicur is considered by some individuals to be a valuable FHB/DON management tool.

**SUMMARY**

In research plots, Folicur suppressed, but did not control (by industry standards), FHB symptoms and DON accumulation in wheat when applied at early flowering.

**Table 1.** Percent control of Fusarium head blight and DON by Folicur compared to the non-treated check as gleaned from spring and winter wheat research reports, 1998 - 2003\*

**Average % control for Folicur relative to the non-treated check wheat**

<u>Incidence**</u>	<u>Severity</u>	<u>Index</u>	<u>FDK</u>	<u>DON</u>
19.7	22.5	39.4	26.7	27.4

\* Data summarized primarily from 1998-2003 Proceedings of National Fusarium Head Blight Forums.

\*\*Incidence = proportion of heads with any FHB symptoms

Severity = % florets diseased for heads showing FHB symptoms (excludes heads without FHB symptoms).

Index = FHB plot severity = incidence x severity

FDK = Fusarium damaged kernels

**Table 2.** Distribution of percent control by Folicur relative to the non-treated check for FHB variables among the tests summarized in this report.

**Number of individual test means in each range**

<u>Range of Control</u>	<u>Incidence</u>	<u>Severity</u>	<u>Index</u>	<u>FDK</u>	<u>DON</u>
0-10%	24	22	5	8	10
11-20%	9	8	4	10	2
21-30%	13	5	6	5	11
31-40%	12	16	5	0	9
41-50%	3	3	11	7	6
51-60%	4	2	7	4	5
61-70%	0	2	7	0	2
71-80%	1	0	2	1	0
81-90%	0	0	1	1	2
91-100%	0	0	0	0	0
<u>Number of Tests</u>	<u>66</u>	<u>58</u>	<u>48</u>	<u>36</u>	<u>47</u>

\*Data summarized primarily from 1998-2003 Proceedings of National Fusarium Head Blight Forums.

\*\*Incidence = proportion of heads with any FHB symptoms

Severity = % florets diseased for heads showing FHB symptoms (excludes heads without FHB symptoms).

Index = FHB plot severity = incidence x severity

FDK = Fusarium damaged kernels

DON = Deoxynivalenol

## PERFORMANCE EVALUATION OF AERIAL APPLIED FUNGICIDES

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### ABSTRACT

Fusarium head blight has caused considerable economic damage to small grain crops in the U.S. and Canada. This has caused producers to spray their fields with fungicide, which has produced mixed results over the years by both aerial and ground application. About 50% of the fungicide is being applied by air, and aerial applicators are looking for answers to improve application methods.

Two aerial application trials were completed in eastern North Dakota during the 2003 growing season. These trials were completed in producer fields near St. Thomas and Hunter, ND. Replicated spray applications were completed at the growth stage (Feekes 10.51) recommended for optimum fungicide efficacy. Plots were 100 ft. or more in width and either 850 or 1000 ft. in length. Four treatments were compared to an untreated check at both locations. The treatments included applying fungicide in one application and making two passes across a field in opposite directions at one half fungicide rate and at one half the amount of carrier. Both spray applications were conducted with large and small droplets. Multiple applications were theorized to improve the coverage to both sides of the grain head. Folicur fungicide was applied at 4 fl.oz./ac. in all treatments in both trials. A harvest sample was obtained by cutting one combine header width near the center of the plot over the length of the plot. Yield data was computed from plot weights recorded from a weigh wagon. A sub-sample was taken from the weigh wagon to test for deoxynivalenol (DON), test weight, and protein. No significant differences in FHB incidence, field severity, DON level, leaf disease, yield, test weight, and % protein were measured at St. Thomas. Significant differences in FHB incidence, yield and test weight were measured at the Hunter site. Coverage data was obtained by adding a food grade fluorescent dye to the spray tank and using an ultra-violet light to illuminate the dye on the grain heads. The spike coverage data did show a significant difference at Hunter but not at the St. Thomas site. Spray drop size information was obtained by using WRK DropletScan. This gives the VMD (volume median diameter) drop size, area of coverage and an estimate of the gallons per acre applied based upon the spray drops on water sensitive cards. In 2003, FHB infection was very low.



## UNIFORM FUNGICIDE TRIALS ON FHB OF WHEAT AND BARLEY IN MINNESOTA

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### OBJECTIVE

To provide a non-biased evaluation of experimental Fusarium head blight (FHB) chemical control products for hard red spring wheat and spring barley. Cooperatively, the multi-state uniform fungicide trial effort will indicate which fungicide compounds are most effective in reducing disease severity across diverse environments.

### INTRODUCTION

Fusarium head blight was originally described more than a century ago (Stack, 2000). Since that time the disease has been responsible for severe and repeated epidemics on small grain crops (Sutton, 1982; McMullen et al., 1997; Steffenson, 1998; Windels, 2000) that have resulted in billions of dollars in crop losses (McMullen et al., 1997; Wood, 2002). The disease is a constant threat to the economic stability of small grain growers in production areas with rain, humidity or heavy dews during critical fungal infection periods (McMullen, 1997).

Successful infection of FHB pathogens is largely dependent on weather conditions. Cultural disease management strategies (i.e.: crop rotation, tillage and field sanitation) have offered producers partial control. Likewise, moderate disease control has been achieved with fungicide application. Ongoing research of new, experimental fungicides is needed to further reduce small grain crop yield and quality losses from FHB.

### MATERIALS AND METHODS

Wheat and barley experiments were planted in randomized complete block designs with four replicates at the Northwest Research and Outreach Center in Crookston on 28 April 2003. Cultivars 'Oxen' hard red spring wheat and 'Robust' spring barley were seeded at 1.25 mil. live seed/acre and 1.375 mil. live seed/acre, respectively. Plots were inoculated with 250 g of *Fusarium graminearum* infested corn grain five weeks after planting. Night-cycle mist irrigation was initiated the day after inoculation and continued until plants began to senesce. Misting was discontinued temporarily during the growing season when weather events caused standing water at the testing site. Weeds were controlled as needed.

Treatments were applied to wheat nine weeks after planting [Feekes 10.51 growth stage (early flowering)] and to barley eight weeks after planting [Feekes 10.4 (early heading)]. Foliar applications were made with a CO<sub>2</sub> backpack type sprayer adjusted to 40 psi at 18-20 gpa with forward and backward facing nozzles. Disease severity responses were noted 22 and 25 days after treatment application from wheat and barley, respectively. Tests were harvested 15 weeks after planting.

Fusarium head blight severity was estimated based on the visual scale from Stack and McMullen (1995), while percent visually scabby kernels (VSK) was estimated using a set of standards provided by R. Jones

based on his recent publication (Jones and Mirocha, 1999). Percent leaf disease was estimated using James (1971). Grain sample deoxynivalenol (DON) levels were determined by the University of Minnesota Toxicology Lab in St. Paul utilizing the gas chromatography/mass spectrometry (GC/MS) method. ANOVAs were performed with SAS using PROC GLM. Fisher's protected least significant difference (LSD) mean comparisons were used to identify statistically different treatments.

## RESULTS AND DISCUSSION

**Hard Red Spring Wheat:** Weather conditions during anthesis were conducive for *F. graminearum* infection and disease progression. As expected, the nontreated control had the most severe disease response for all categories tested (Table 1). In seven of the nine categories noted, the JAU6476 + Folicur treatment significantly reduced FHB and leaf disease. Compared with other treatments, V-10116 6 fl. oz. had most reduced VSK and DON levels and most increased 1000-kernel weights. Those treatments controlling disease to a lesser extent were V-10116 8 fl. oz., JAU6476 5.0 fl. oz., and JAU6476 5.7 fl. oz. The least effective fungicide treatment at controlling FHB was Folicur 4 fl. oz.

**Spring Barley:** Severe weather conditions during Feekes growth stage 10 (booting) caused plants in all replicates to lodge. Plant lodging occurred approximately five days before treatments were applied. Therefore, data must be 'weighed' against the 'percent plot lodged' rating (Table 1) before assessing treatment effectiveness. Control plots had the least plants lodged (58.8%) which resulted in reduced disease severity compared with Folicur 4 fl. oz. treated plots (81% lodged). In four of nine parameters noted, the three JAU6476-containing treatments (JAU6476 5.0 fl. oz.; JAU6476 5.7 fl. oz.; and JAU6476 + Folicur) significantly reduced FHB and leaf disease. Compared with other treatments, V-10116 8 fl. oz. controlled FHB head severity, FHB field severity, and DON levels to the greatest extent in spite of its 80% lodging score.

## ACKNOWLEDGEMENTS

We would like to thank the U.S. Wheat and Barley Scab Initiative and the Northwest Research and Outreach Center for supporting these studies, as well as Bayer CropScience (Folicur and JAU6476) and Valent U.S.A. (V-10116) for providing fungicide materials.

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**Table 1.** FHB and leaf spot disease responses from ‘Oxen’ hard red spring wheat in Crookston, MN.

Treatment <sup>1</sup>	Fusarium Head Blight						Yield (bu/A)	Test Wt. (lb/bu)
	HS <sup>2</sup> (%)	I (%)	FS (%)	VSK (%)	DON (ppm)	LDS <sup>3</sup> (%)		
Nontreated control .....	5.9	98.5	35.7	17.5	15.0	3.4	52.0	53.8
Folicur 3.6F 4 fl oz .....	4.3	91.5	18.9	8.0	11.2	2.1	65.9	55.4
JAU6476 480SC 5.7 fl oz ...	3.6	73.0	13.9	10.0	12.9	2.4	64.4	55.5
JAU6476 480SC 5.0 fl oz ...	3.6	85.0	11.9	5.0	11.3	2.0	72.0	57.3
JAU6476 480SC 3.6 fl oz + Folicur 3.6F 4 fl oz .....	3.1	73.5	8.7	5.0	9.1	1.7	73.9	57.3
V-10116 1.67SC 6 fl oz .....	3.5	84.0	11.8	4.5	6.7	2.2	73.1	56.9
V-10116 1.67SC 8 fl oz .....	3.8	83.0	14.0	5.0	7.4	1.8	74.0	56.5
<i>LSD</i> <sub>0.05</sub>	0.3	16.5	10.1	4.8	3.2	0.3	8.4	1.2

<sup>1</sup>Each fungicide treatment included 0.125% Induce. Treatment abbreviations are HS: head severity; I: incidence; FS: field severity; VSK: visually scabby kernels; LDS: leaf disease severity.

<sup>2</sup>Square root transformation.

<sup>3</sup>Log transformation. Foliar diseases consisted of Stagonospora blotch (*Stagonospora nodorum*) and Tan spot (*Pyrenophora tritici-repentis*)

**Table 2.** FHB and leaf spot disease responses from ‘Robust’ spring barley in Crookston, MN.

Treatment <sup>1</sup>	% Plots Lodged <sup>2</sup>	Fusarium Head Blight					Plump Kernels	Yield (bu/ac)	Test Wt (lb/bu)
		HS <sup>3</sup> (%)	I (%)	FS (%)	DON (ppm)	LDS <sup>4</sup> (%)			
Nontreated control .....	58.8	4.8	100	24.7	36.2	2.7	73.2	88.4	41.6
Folicur 3.6F 4 fl oz .....	81.3	5.6	100	35.4	37.8	2.9	71.9	90.4	41.2
JAU6476 480SC 5.7 fl oz .....	86.3	4.6	100	24.1	25.5	2.7	76.6	96.1	42.6
JAU6476 480SC 5.0 fl oz .....	77.5	4.1	100	19.1	28.2	2.7	78.1	104.1	42.5
JAU6476 480SC 3.6 fl oz + Folicur 3.6F 4 fl oz .....	86.3	4.3	100	21.7	23.1	2.5	76.2	95.4	42.1
V-10116 1.67SC 6 fl oz .....	63.8	4.6	100	23.7	28.0	2.7	76.8	93.5	41.8
V-10116 1.67SC 8 fl oz .....	80.0	4.0	100	17.1	22.5	2.6	75.9	99.8	42.3
<i>LSD</i> <sub>0.05</sub>		0.4	NS	NS	8.0	0.4	NS	NS	NS

<sup>1</sup>Each fungicide treatment included 0.125% Induce. Treatment abbreviations are as follows: HS: head severity; I: incidence; FS: field severity; VSK: visually scabby kernels; LDS: leaf disease severity.

<sup>2</sup>Severe weather caused plants to lodge between 21-24 June, 2003; approximately five days before treatments were applied. Plot lodging assessments were taken six weeks after plants went down.

<sup>3</sup>Square root transformation.

<sup>4</sup>Log transformation. Foliar diseases consisted of Septoria speckled leaf blotch (*Septoria passerinii* and *Stagonospora avenae* f. sp. *triticea*), net blotch (*Pyrenophora teres*) and spot blotch (*Cochliobolus sativus*).

# DIFFERENTIAL RESPONSE OF BARLEY, HARD RED SPRING WHEAT, AND DURUM WHEAT TO MULTIPLE FHB INFECTIONS AND FUNGICIDE TREATMENTS

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## ABSTRACT

Wheat is most vulnerable to infection by *Fusarium graminearum* during anthesis, while spring barley becomes vulnerable to infection at early head emergence. If prolonged periods of favorable weather for infection occur after these growth stages, multiple infections may occur, resulting in lower yield and quality and higher deoxynivalenol (DON) than a single infection at optimum infection timing. Greenhouse studies in Fargo in 2001-2002 showed that with multiple infections, split applications of reduced rates of Folicur (tebuconazole) across multiple growth stages in spring wheat and durum did not significantly improve disease control over a single treatment of the full label rate applied at anthesis; ie. one optimum timing of fungicide may be sufficient in these two crops. However, in these trials, FHB field severities were only moderate for spring and durum wheat (untreated 13.3%, 34.1%, respectively), and very low in barley (untreated 2.8%). Additional tests were needed to determine response under more severe disease pressure.

In 2003, hard red spring and durum wheat, and spring barley were exposed to single or multiple (2 to 3) inoculations and fungicide applications in the greenhouse: prior to flowering (Feekes 10.3); early flowering (Feekes 10.51); or kernel watery ripe (Feekes 10.54). *F. graminearum* (10,000 spores/ml) was atomized onto grain heads at the test growth stage. For fungicide treatments, Folicur or AMS21619 (prothioconazole, Bayer experimental) was applied approximately four hours before inoculation, either at full rate or half rate, using a track sprayer equipped with XR8001 flat fan nozzles oriented forward/backward at 60° from the vertical. After inoculation and fungicide applications, plants were placed in a mist chamber for 48 hours and then returned to the greenhouse. Disease severity was evaluated at the soft dough stage.

FHB field severity in 2003 was greater (untreated up to 77%) than in the 2001-2002 tests. The higher level of disease provided greater separation of treatment effects. For hard red spring and durum wheat, a single full rate application of either fungicide at anthesis, in combination with three separate inoculations, provided similar FHB control to the two half rate applications of either fungicide in combination with two inoculations (58% vs 61% reduction). However, in barley, if three inoculations were applied, a single fungicide application applied at early full head emergence could not control the disease; disease levels were 26-38% higher than the untreated check exposed to two inoculations. Thus, multiple infection events in barley may have to be controlled with split applications of fungicide at multiple timings, whereas in spring wheat and durum, a single application of the full rate of fungicide may be adequate for FHB reduction.

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## ALTERNATIVES TO INCREASE AERIAL SPRAY DEPOSITS FOR FHB CONTROL

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### OBJECTIVE

Assess promising aerial application practices and methods that could increase spray deposits on wheat heads.

### INTRODUCTION

Fusarium head blight (FHB) has emerged as a major disease of wheat and barley in several major small grain production regions in the U.S. Cultural practices, resistant cultivars, and fungicides have made only limited impact on managing the disease (Parry et al. 1995). General observations from the literature show limited effectiveness and erratic results from fungicide applications to control FHB (Milus and Parsons 1994, Wilcoxson 1996, Shaner and Buechley 1999, and Gilbert and Tekauz 2000). This suggests that there are a number of factors that influence effectiveness of fungicidal applications that must be more thoroughly understood and coordinated before producers can rely on chemical control as an effective tool in FHB management. More detailed studies of these factors are needed to provide producers with options for effectively managing this disease of wheat and barley.

In the absence of robust disease control measures such as resistant cultivars and effective cultural or biological control practices, it is likely that some of the more conventional approaches could be optimized to help provide producers with technologies for FHB management or control. Spraying and dusting pest and disease control agents have been practiced in many crops for many years. Aerial application is an economical and effective means of applying materials to large acreages in a timely manner. Access and crop damage from other distribution means can also necessitate aerial application. Timeliness of application of chemical control materials is often a factor in effectiveness of the measures undertaken. Aerial application methods have been optimized and used more extensively in insect and weed control practices than in disease management. It is reasonable to expect that aerial application methods can be optimized for application of fungicides to wheat and barley heads for control of FHB. Numerous studies have been reported on optimization of aerial application practices for pest control in cotton, corn, and weeds and brush (Bouse et al. 1992, Carlton et al. 1995, Hoffmann et al. 1998, and Kirk et al. 1988, 1992, 1998 and 2001). Similar approaches were proposed to optimize aerial application technologies for improved deposition of disease control materials on wheat heads.

### MATERIALS AND METHODS

Based on experience with aerial application and consultation with FHB researchers, aerial application methodologies were selected to assess technologies that could offer improved spray deposits on wheat heads. These methodologies were implemented in three field studies in 2003. The aerial treatments were applied with an Air Tractor AT-402B (Figure 1) in 3-replication randomized blocks, with individual treatment plots of 3 to 5 acres. Aircraft hardware and spray performance variables were documented based on

ASAE Standards (2003), S327.2 FEB03 and S572 AUG99. These standards define Volume Median Diameter, ( $D_{v0.5}$ ), Droplet Spectra Classification (DSC), and other pertinent spray parameters. Weather parameters were monitored and recorded during all spray applications with a Gill 27005 UVW Anemometer, Young 43372VC Relative Humidity and Temperature Probe, and a Campbell 21-X data logger. Water-based spray mixes contained equal per-acre rates of the fluorescent tracer, Caracid Brilliant Flavine FFN, and a surfactant, Triton X-100, to monitor spray deposits and simulate a reasonable spray mix. Five ten-head wheat samples were taken at two locations in each treatment replication. Mylar plate samplers, soda straw samplers, and Water-Sensitive-Paper (WSP) samplers in two orientations were each placed in five positions across the center swath at two locations in each treatment replication (Figure 2). Spray mix samples, mylar plate samplers, and wheat head samples were analyzed spectrofluorometrically for tracer content and deposition. WSP samples were analyzed by computerized image analysis to characterize spray deposits. Deposit data were analyzed statistically to determine treatment effects.

***Spray rate and droplet size with conventional aerial hydraulic nozzles to optimize deposits on wheat heads*** – A range-finding study was conducted to determine the influence of spray rates of 2, 5, and 10 gpa and spray droplet sizes of 250 and 400  $\mu\text{m}$  volume median diameter ( $D_{v0.5}$ ) on spray deposits on wheat heads. These parameters describe the general range of normal aerial application practice. The 250  $\mu\text{m}$  droplet sizes were applied with CP-03 nozzles, DSC = F; and the 400  $\mu\text{m}$  droplet sizes were applied with disc orifice straight stream nozzles, DSC = M. Study protocols similar to those of Bouse et al. 1992 and Kirk et al. 1992 were used in the study.

***Spray rates with aerial rotary atomizers to optimize deposits on wheat heads*** – Previous research has shown that small droplets deposit more effectively on small targets and large droplets deposit more effectively on large targets (Kirk et al. 1992). A study with Curtis Dyna-Fog ASC rotary atomizers that produce a relatively narrow range of small droplets was conducted to exploit the small droplet – small target phenomenon. The rotary atomizers were operated at blade setting 6 for maximum no-load rpm of 9000 at 130 mph. The three treatments for the study were 20, 40, and 60 psi (flow-control orifice removed) for spray rates of approximately 3, 5, and 7 gpa, respectively, and DSC's of VF, F, and F, respectively. A study protocol similar to that used by Kirk et al. 1993 was used for the study.

***Multi-direction flight paths to increase deposits on both sides of wheat heads*** – Several FHB researchers indicated that fungicidal sprays need to be deposited on all sides of wheat or barley heads to maximize protection of the head. Limited small-plot research with ground sprays reported by Hart et al. 2001 indicate that sprays from both sides of the plots reduced the severity of FHB and reduced levels of deoxynivalenol. Four treatments: 5 gpa in 2 spray passes in opposite directions on same swath = 10 gpa, DSC = VF; 5 gpa, 1 pass, DSC = VF; 5 gpa, 1 pass, DSC = F; and 10 gpa 1 pass, DSC = F.



**Figure 1.** AT-402B applying treatments to wheat.



**Figure 2.** Artificial samplers for deposit analysis.

## RESULTS AND DISCUSSION

Aerial treatments, spray rates, and estimated spray droplet size spectrums are detailed in Table 1 along with measured deposits on wheat heads, mylar plates, and soda straws. Mylar plates were oriented horizontally at the top of the crop canopy and the soda straws were inclined at 45° and also placed at the top of the canopy, Figure 2. The primary objective of these studies was to assess various aerial technologies that would give highest spray deposits on wheat heads. Consequently, the brief discussion presented here will primarily highlight those treatments, along with data from artificial samplers that support observations of increased deposits on wheat heads. The highest observed deposits on wheat heads and artificial samplers were from rotary atomizers with a 5 gpa spray rate. The Fine and Very Fine sprays gave higher deposits than the Medium sprays; the lowest observed deposits on wheat heads were for the Medium DSC sprays, regardless of spray rate. The effects of spray rate on deposits on wheat heads are not particularly definitive, except that Medium sprays at 2 gpa resulted in lower deposits than the other treatments. Two aerial spray passes in opposite directions over the same swath did not give as much total deposit on wheat heads as the same amount of tracer applied in a single pass. A more detailed analysis of the spray droplet spectra as measured from wind tunnel studies and from deposits on water-sensitive paper samplers may provide a better understanding of reasons for the observed treatment differences in deposits on wheat heads. However, the more important issue is whether the aerial treatments that give higher spray deposits will also give improved control of FHB.

**Table 1.** Aerial spray deposit parameters for three field studies, Buffalo Ranch, Burleson County, TX, 2003

Treatment	Spray Rate, gal/acre	Droplet Size, $D_{V0.5}$ , $\mu\text{m}$ , (DSC)	<sup>[a]</sup> Deposits on Wheat Heads, $\mu\text{g}/\text{cm}^2$	<sup>[a]</sup> Deposits on Mylar Plates, $\mu\text{g}/\text{cm}^2$	<sup>[a]</sup> Deposits on Soda Straws, $\mu\text{g}/\text{cm}^2$
<b><i>Spray rate and droplet size with conventional aerial hydraulic nozzles</i></b>					
CP-03, 55°, 0.061 , 40 psi, 130 mph	2	249 (F)	0.25 bc	0.21 ef	0.12 de
CP-03, 90°, 0.125 , 20 psi, 130 mph	5	231 (F)	0.17 ef	0.23 def	0.16 e
CP-03, 90°, 0.171 , 40 psi, 130 mph	10	249 (F)	0.25 bc	0.30 bcd	0.26 cd
D-8 SS, 20°, 50 psi, 130 mph	2	415 (M)	0.14 fg	0.17 f	0.12 e
D-8 SS, 20°, 50 psi, 130 mph	5	415 (M)	0.17 ef	0.33 bc	0.18 de
D-8 SS, 20°, 50 psi, 130 mph	10	415 (M)	0.18 def	0.35 b	0.16 e
<b><i>Spray rates with aerial rotary atomizers</i></b>					
ASC, No Disc, 20 psi, 130 mph	3	220 (VF)	0.24 bcd	0.26 cde	0.27 cd
ASC, No Disc, 40 psi, 130 mph	5	240 (F)	0.36 a	0.47 a	0.42 a
ASC, No Disc, 60 psi, 130 mph	7	261 (F)	0.21 bcde	0.27 bcde	0.20 de
<b><i>Multi-direction flight paths</i></b>					
CP-03, 90°, 0.078 , 54 psi, 100 mph	5	304 (VF)	0.21 bcde	0.22 def	0.37 ab
CP-03, 90°, 0.078 , 54 psi, 100 mph	<sup>[b]</sup> 2@5 =	304 (VF)	0.10 g	0.16 f	0.31 bc
CP-03, 90°, 0.171 , 40 psi, 130 mph	10	249 (F)	0.27 b	0.26 cde	0.47 a
CP-03, 90°, 0.125 , 20 psi, 130 mph	5	231 (F)	0.24 bcd	0.20 ef	0.42 a

<sup>[a]</sup> Deposit measurements are amounts of the tracer dye deposited per unit of projected area on the respective samplers. Deposits in a single column followed by the same letter or same group of letters are not significantly different,  $\alpha = 0.05$ .

<sup>[b]</sup> Treatment composed of two 5 gpa spray passes over the same swaths in opposite directions for 10 gpa.

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## RESULTS OF THE UNIFORM FUSARIUM HEAD BLIGHT FUNGICIDE TEST ON WINTER WHEAT IN OHIO, 2003

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### OBJECTIVE

Evaluate a common set of fungicide treatments, as determined by the Chemical and Biological Control committee of the Wheat and Barley Scab Initiative, for efficacy against Fusarium head blight in Ohio. Additionally, to evaluate the effect of varying rates and application timing on efficacy of certain fungicides.

### INTRODUCTION

Fusarium head blight, caused by *Fusarium graminearum* and other *Fusarium spp.* occurs in most spring and winter wheat growing regions of the world. Although progress is being made in the development of resistant cultivars, most cultivars grown in the US are either susceptible or have a degree of susceptibility that permits yield loss and/or accumulation of DON in the grain. Wheat producers are interested in chemical control options as an additional tool for preventing yield losses. The Chemical and Biological Control Committee of the Wheat and Barley Scab Initiative establishes protocols for evaluating a set of fungicides each year. These materials are evaluated by researchers in a number of different states on several different classes of wheat in order to develop a data base for possible federal registration of the fungicide and to develop data on which recommendations for their use can be made. Ohio State University has been a cooperator in these evaluations since 1998 and this is a report of our results for 2003.

### MATERIALS AND METHODS

Seeds of Elkhart wheat, treated with Raxil-Thiram (tebuconazole 0.6% and thiram 20%, 3.5 fl. oz./cwt), were planted at the rate of 24 seeds/ft of row on 18 October, 2002 in Ravenna silt loam at the Ohio Agricultural Research and Development Center near Wooster, OH. Prior to planting, the field was mold-board plowed, then 75 lb/A of ammonium nitrate was broadcast over the field and incorporated with a disc. The experimental treatments were arranged in a randomized block design with five replicate blocks. Each experimental unit consisted of a 7-row plot, 15 ft long with 7 in. between rows. Additional nitrogen was applied on 27 Mar 03 as 240 lb of ammonium nitrate. Plots were inoculated by broadcasting *F. graminearum* colonized corn kernels (0.12 oz/sq ft) over the plot surface on 6 May. The plot was mist irrigated using NAAN 7110 series bridge with mist sprayer head 327122 fitted with nozzles having 0.35 in. openings that provided 10.2 GPH. Plots were misted each day from 1 wk prior to flowering to 2 wk after flowering (23 May to 12 Jun). The mist irrigation operated for 2.5 min out of each 10 min from 6:00 to 10:00 a.m. and from 8:00 to 10:00 p.m. each day. Fungicides were applied as sprays with an adjuvant (Induce, 0.125% v/v) in 33.4 gal water/A with a CO<sub>2</sub>-pressurized back pack sprayer with a constant boom pressure of 40 psi and 15 in. between twinjet XR8001 VS nozzles mounted at a 60 degree angle forward and backward. Sprays were applied on 27 May, 30 May or 2 June at heads one-fourth emerged, heads three-quarters emerged and flowering growth stages corresponding to Feekes' growth stage (GS) 10.2, 10.4 and 10.5.1, respectively. Plots were assessed for Fusarium head blight (FHB) on 20 June by determining the percentage of spikelets affected per head of 20 heads in each of five locations in each plot.

Severity was calculated as the average percentage of affected spikelets per head and incidence was calculated as the percentage of heads with disease. Plots were harvested on 30 Jul with a Hege 140 plot combine. Yield (bu/A) was determined from harvested grain adjusted to 13.5% moisture. Harvested grain was visually assessed for the percentage of Fusarium damaged kernels.

## RESULTS AND DISCUSSION

Daily mist irrigation favored disease development in spite of relatively cool temperatures during and 1 wk following anthesis (mean daily temperature 58.8° F) resulting in very high disease incidence (mean treatments ranged from 83% to 100%) (Table 1). Rain occurred on 19 of the 40 days between when disease assessments were made and plots were harvested. Frequent rain prevented harvest and kept the heads almost continuously wet during grain maturation. By harvest the grain was severely deteriorated resulting in very low yields (range 17.8 to 36.1 bu/A) and test weights (range 26.6 to 34.9 lb/bu) (Table 1).

Based on analysis of variance, the effect of treatment was significant for all disease assessments, percentage damaged kernels, yield and test weight. Results of the DON analysis of the grain is not yet available. All treatments, except Folicur 3.6 EC alone, had significantly lower FHB severity and FHB index, as well as significantly higher yield and test weight than the untreated control.

The impact of fungicide treatment on yield and test weight was difficult to determine due to the severe deterioration of the grain while in the field before harvest. Regardless, those treatments that had lower FHB severity levels and lower FHB index, generally also had statistically lower percentage of damaged kernels, and higher yield and test weight. Both the JUA6476 and V10116 materials appeared to be superior to Folicur in reducing the effects of Fusarium head blight in this experiment. Additionally, there did not appear to be a difference among the rates used for either material or among different application timings for JUA6476. Greater differences in percentage damaged kernels, yield and test weights may have occurred had protracted wet conditions not prevail prior to harvest. This was the first year we had seen this level of grain deterioration in over 20 years of conducting fungicide trials on wheat in Ohio.

**Table 1.** Efficacy of fungicides for control of Fusarium head blight of wheat in Ohio, 2003.

Treatment, rate/A	Application timing*	FHB Incidence (%)	FHB Severity (%)	FHB Index**	Damaged kernels (%)	Yield*** (bu/A)	Test weight (lb/bu)
Folicur 3.6 EC, 4.0 fl oz	GS10.5.1	100	36	36	50	21.4	28.5
JUA6476 480 SC, 5.7 fl oz	GS10.5.1	99	20	20	19	27.0	31.8
JUA6476 480 SC, 5.0 fl oz	GS10.5.1	95	26	25	56	28.0	31.2
JUA6476 480 SC, 3.6 fl oz + Folicur 3.6 EC, 4.0 fl oz	GS10.5.1	96	26	25	42	30.2	31.1
V10116 1.67 SC, 6.0 fl oz	GS10.5.1	94	23	22	32	31.1	33.0
V10116 1.67 SC, 8.0 fl oz	GS10.5.1	95	19	18	23	34.1	33.8
V10116 1.67SC, 4.0 fl oz	GS10.5.1	97	29	29	45	27.4	31.3
JUA 6476 480 SC 5.7 fl oz	GS10.2	100	20	20	25	32.9	34.9
JUA6476 480 SC 5.7 fl oz	GS10.4	95	20	20	21	36.1	33.9
JUA6476 480SC, 2.85 fl oz	GS10.4						
Then JUA6476 480 SC, 2.85 fl oz	GS10.5.1	94	21	21	41	29.0	32.0
JUA6476 480SC 3.56 fl oz + Folicur 43 SC 3.98 fl oz	GS10.5.1	83	20	17	28	31.5	33.4
Untreated		100	39	39	60	17.8	26.6
LSD ( $P=0.05$ )		13	10	10	29	5.1	4.2

\* Treatments applied at Feekes' growth stage (GS) 10.2 (June 27), 10.4 (June 30), or 10.5.1 (30 May)

\*\* Fusarium head blight (FHB) index = (FHB incidence\*FHB severity)/100.

\*\*\* Yield based on 13.5% moisture at 58 lb/bu.

## COMPARISON OF AERIAL APPLICATION WITH GROUND APPLICATION OF FOLICUR FUNGICIDE FOR THE CONTROL OF FUSARIUM HEAD BLIGHT (FHB) IN DURUM WHEAT

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### ABSTRACT

Fusarium head blight has caused severe economic losses in durum wheat in north central North Dakota in recent years. Decreases in yield and quality have resulted in millions of dollars of lost income. At this time, adapted durum cultivars are very susceptible to FHB. Cultivars with tolerance to FHB are not available, so efficient fungicide application is essential to control the disease.

Both aerial and ground applicators are interested in improving application, as acres protected by fungicides are increasing. Due to the importance of both ground and aerial application to North Dakota growers, further studies on the efficiency of both methods in applying fungicides to durum heads for FHB control was completed.

During the 2003 growing season, a trial was conducted comparing a ground sprayer and a spray plane in a commercial durum field north of Kenmare, N.D. A ground sprayer applying 18 GPA with twin jet nozzles was compared to an airplane applying 5 GPA with CP nozzles. The spray strips were 120 ft. wide by 1000 ft. long. All treatments, including the untreated check, had three replications. For each replicate, one pass was made with the ground sprayer applying a 96 ft. wide swath and the airplane made two passes with each pass being 45 ft. wide. Fungicide treatments included one application of 4 fl oz/acre of Folicur, both by ground and airplane, plus a split application of 2 fl oz/acre of Folicur by ground and air, applied 4 days apart. The full rate and first split application were made at Feekes 10.51, anthesis, while the second split application was made 4 days later at the end of flowering. FHB field severity was evaluated at soft dough stage. FHB did not develop in this field because of arid conditions following anthesis. Durum yields were determined by harvesting a 30-foot strip from each plot with a combine and weighing in a weigh wagon. A sub-sample was taken from the weigh wagon for deoxynivalenol (DON) analysis. All fungicide treatments increased yield by 2 to 4 bushels per acre compared to the untreated check. However, an ANOVA showed no significant difference in yield between the treatments or the untreated check. The DON levels for all treatments were below 0.5ppm.

## WHEAT UNIFORM FUNGICIDE TRIALS, ND, 2003

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### OBJECTIVE

To evaluate experimental fungicides for control of Fusarium head blight (scab) and leaf diseases in hard red spring and durum wheat in North Dakota.

### INTRODUCTION

Uniform fungicide trials have been established across grain classes and environments as part of the U.S. Wheat and Barley Scab Initiative (McMullen and Milus 2002). The purpose of these trials is to evaluate efficacy of fungicides in reducing Fusarium head blight severity (FHB), Fusarium damaged kernels (FDK), and deoxynivalenol (DON) levels. North Dakota continues to participate in these trials and tests fungicides at several locations across grain classes and cultivars.

### MATERIALS AND METHODS

A uniform set of six fungicide treatments were evaluated on hard red spring and durum wheat in ND in 2003 (Table 1). Fungicides tested included Folicur (tebuconazole), which had a Section 18 exemption for use on wheat in ND in 2003, JAU6476 (prothioconazole), an experimental fungicide from Bayer CropScience, and V-10116, an experimental product from Valent. Artificial inoculum in the form of inoculated grain was dispersed in plots at Fargo and Langdon, wheat straw was distributed at Carrington, and natural inoculum was the source of infections at Minot. Natural rainfall was augmented by mist irrigation at Fargo and Langdon and by some overhead irrigation at Carrington.

All treatments were applied at early flowering (Feekes 10.51) with a CO<sub>2</sub> backpack type sprayer, equipped with XR8001 nozzles mounted at a 60° angle forward and backward toward the grain heads. Water volume was 18-20 gpa applied at 40 psi. Disease ratings were taken at soft dough kernel stage. Plots were harvested with small plot combines. DON levels were determined by the NDSU Veterinary Toxicology Lab. Plots were in a Randomized Complete Block design and data were statistically analyzed across locations using ANOVA.

The uniform trial was done at four locations: Fargo in the southeast; Langdon in the northeast; Carrington in the central part of the state; and at Minot in the north central region. Each site represents different environment, soil type, and cropping practices. Fungicides were evaluated over two wheat classes and six cultivars: Fargo, Oxen hard red spring wheat; Carrington, Russ hard red spring wheat and Mountrail durum wheat; Langdon, Alsen hard red spring wheat, Grandin hard red spring wheat, and Lebsock durum; Minot, Mountrail durum.

## RESULTS AND DISCUSSION

All sites, except for the Minot location, had some level of FHB infection, from a low of 5.1% field severity in Alsen at Langdon, to a high of 10.2% in Oxen at Fargo. Because of the absence of FHB at Minot, the Minot data is not included in the summary Table 1. All fungicide treatments significantly reduced FHB field severity over the untreated check. The higher application rate of the JAU6476 product resulted in the lowest FHB field severity. All fungicide treatments also significantly reduced DON and FDK. All fungicide treatments significantly reduced the level of leaf disease from the untreated check. Yields were significantly increased by fungicide treatments, from 9.6 to 13.0 bushels, increases that are economic. Test weights also were significantly increased by fungicide treatments.

**Table 1.** Effect of fungicides on fungal leaf disease and FHB field severity, DON, FDK, yield and test wt., averaged across locations and wheat classes, North Dakota 2003.

Treatment and rate/acre <sup>1</sup>	FHB FS <sup>2</sup> %	DON <sup>3</sup> ppm	FKD <sup>4</sup> %	Leaf disease <sup>5</sup> % severity	Yield Bu/A	Test wt Lbs/bu
Untreated check	8.6 a	2.7 a	5.9 a	47.5 a	60.0 c	59.5 b
Folicur 3.6F 4.0 fl oz	3.7 b	1.5 b	3.1 b	14.3 b	70.0 b	60.9 a
JAU6476 480SC 5.7 fl oz	2.0 c	1.0 b	2.5 b	12.1 b	72.2 ab	61.4 a
JAU6476 480SC 5.0 fl oz	2.6 bc	0.9 b	2.4 b	11.0 b	69.9 b	61.4 a
JAU6476 480SC 3.6 fl oz + Folicur 3.6F 4.0 fl oz	2.7 bc	0.7 b	1.9 b	9.7 b	73.1 a	61.3 a
V-10116 1.67SC 6.0 fl oz	3.1 bc	1.2 b	3.2 b	19.2 b	69.7 b	60.9 a
V-10116 1.67SC 8.0 fl oz	2.5 bc	1.2 b	2.5 b	13.8 b	70.4 b	61.1 a

Numbers followed by different letters are significantly different at the 95% confidence level, using LSD analysis.

<sup>1</sup> All fungicide treatments had 0.125% Induce added; JAU6476 (= AMS21619) is an experimental fungicide from Bayer; V-10116 is an experimental fungicide from Valent

<sup>2</sup> FHB FS = Fusarium head blight field severity; field severity = incidence x head severity; ratings from all sites except Minot which did not have visible FHB

<sup>3</sup> DON (deoxynivalenol = vomitoxin) levels were only available from Carrington and Fargo at time of this report; All DON levels at Minot were <0.5 ppm and were not included in the summary

<sup>4</sup> FDK = Fusarium damaged kernels; data from Fargo and Langdon sites, only

<sup>5</sup> Leaf spot diseases primarily tan spot and Septoria leaf spot complex

All fungicides tested were effective in controlling FHB and leaf diseases and in improving yield and quality. Differences among fungicides generally were not significant, however. The high rate of JAU6476 did result in the lowest FHB field severity, while the combination treatment of JAU6476 and Folicur resulted in the lowest DON and significantly higher yield than some treatments.

## ACKNOWLEDGEMENT

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## REFERENCE

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## INFLUENCE OF METHODOLOGICAL AND TECHNOLOGICAL ASPECTS ON THE CONTROL OF FUSARIUM HEAD BLIGHT IN WHEAT

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### OBJECTIVES

The tests were made at full coverage of the heads by fungicides. In these tests based on latest experiments the effect of epidemic severity and cultivar resistance will be studied on the efficacy of fungicides. A way will be presented, how we can use the same experiment to demonstrate the efficacy of the same fungicides against leaf diseases. An attempt will be made to see, how the yield loss from these micro-pot test can be transferred to larger experimental plots. We would give further details on AMS 21619 and its combinations.

### INTRODUCTION

Among the fungicides used for the control of FHB until now the tebuconazole, metconazole and bromuconazole were identified with larger effect against the disease (Mesterházy 1996, 1997, 2001). However in our tests the tebuconazole containing fungicides with higher rate were the most effective, bromuconazole and metconazole were only of medium effect at lower rates applied in Hungary. Bromuconazole was only medium, but from metconazole (Caramba) also higher rates were tested. A part of the results was made public last year (Mesterházy and Bartók 2001, 2002). In the last years extensive investigations were made with the new Bayer experimental fungicide, signed as AMS 21619 in US or JAU 6476 in Europe, now the DON data are also available. Besides its efficacy the question was also what would be the best formulation and rating of the product. For this reason also leaf rust was rated, this was the most abundant, leaf spots and powdery mildew were only in traces registered.

### MATERIAL AND METHODS

Experimental design, spraying and inoculation technique and way of evaluation for traits and don were described by Mesterházy et al. 2002 and 2003. From 2001 48 hrs wet period by bagging was given as under dry conditions the 24 hrs coverage was not always suitable. 2001, 2002 and 2003 were very dry and hot, rain was only 30-50 % of the usual amount following inoculation. No additional mist irrigation was given. After harvesting the groups of heads, the ten heads for a group were separated and the not used heads were collected for the whole plot and threshed. Their mass was given to the plot yield harvested by combine. By this way comparable yield data were gained as from every plot 150 heads were separated for the Fusarium evaluation. Earlier results showed that efficacies for different traits can be different, therefore the mean efficacy characterizes better the fungicide than any of them separately. For this reason the mean efficacies (FHB, FDK, DON, and Yield loss) will be given. Active ingredients of the fungicides used for a L product: Folicur Solo: 250 g tebuconazole, Falcon 465 EC: 167 g tebuconazole, spiroxamine 250 g + triadimenole 43, Kolfugo Super carbendazime 200, Caramba SL metconazole 72 (1.2 l/ha) and 90 (1.5 l/ha), Juwel: Kresoxym-methyl 125 + epoxyconazole 125, Stratego: trifloxystrobin 125 + propiconazole 125EC, Sphera: trifloxystrobin 188 + cyproconazole 080 EC, Prosaro (AMS 21619 125 EC + HWG 125EC 1.0).

## RESULTS AND DISCUSSION

In 2001 the results (Table 1) the efficacies are very good, their mean is 91 % for the best fungicide Prosaro that is a composite of AMS 21619 and tebuconazole. This was necessary as AMS 21619 has lower efficacy against leaf rust. The results show that Prosaro is excellent also against leaf rust. Its general efficacy (91 %) is significantly better than that of the Folicur Solo, the best fungicide against FHB until now. Falcon 0.8, the most used fungicide in Hungary against FHB has 68 % mean efficacy. The newer fungicide are more effective, the 22 % efficacy difference to Prosaro 1.0 l/ha is very convincing. As cultivar resistance improves only slowly, the new fungicide will be important for the farms where fungicide application is a general practice. For longer time strobilurines were low rated against FHB. Their combinations with more effective active agents brought improvement also in this respect, like HEC combinations. The average efficacy for different traits is divergent, best is for FHB and yield loss, less for DON and FDK. The correlations between FHB traits are very close, all are significant at  $P = 0.1$  % indicating that improvement in FHB control is proportional with the response of other traits.

The means, however, overlap significant response differences. For this reason the data of the susceptible Zugoly are presented for FDK in Table 2. It is remarkable that three isolates have near the same FDK values, but the fungicide had significant differences in effect. At the 12551 isolates the FDK could be decreased to 1 % from about 60 %, but the picture at 44 F. graminearum isolate was different. Here Caramba and Falcon become ineffective, only the Prosaro variants, Folicur Solo and AMS 21619 could significantly reduce the FDK. This is important; because here we had a very severe epidemic situation and Prosaro 1.0 and AMS 21619 could decrease FDK lower than 20 %. This shows clearly that for the most sensitive cultivars at very heavy epidemic a really good solution has not been found. When we think on the recent devastating epidemics in US and Canada, the lesson is clear. And Zugoly is not the most susceptible cultivar ever seen. In a two years study (Mesterházy, unpublished) mean DON contamination for Zugoly was 45 ppm, but for the German Contra 247 ppm. At more resistant cultivars we do not have this problem in FHB control.

In 2002 we had similar results as ranks, but the efficacy become higher, the less effective fungicides had in this year higher values. We introduced first the Folicur Solo karbendazim mixture that contains the double amount of tebuconazole we had in the Falcon mixture. The result was very similar to Prosaro 1 l/ha, but somewhat weaker. The infection severity was lower than in 2001. The most distinct effect was measured at DON. In the plot yield data the total yield is included except the 150 heads used for FHB evaluation. Leaf rust was significant only at the Fusarium check and Kolfugo, the others gave excellent protection. As leaves were healthy the yield differences between fungicides are not only consequence of the control of leaf diseases. Folicur Solo caused 3 % yield increase; with limitation we can consider this the result of the disease control on the leaves. Caramba, Juwel and Folicur Solo+Kolfugo caused more than 10 % that significantly differs from the Folicur Solo yield. We think that this is a regulator effect that occurs at many triazole fungicides with the positive alterations in cytokinin metabolism. When this is consequent, it can increase the profitability of fungicide use in farms, even diseases occur at low severity.

From 2003 the DON data are not yet available; data are shown in Table 4. Again, the two Prosaro treatments are the best, the Folicur Solo + carbendazime mixture is not better, but in this year it is not always better than Folicur Solo alone. As Prosaro is better, it has no reason to test further this mixture. Until Prosaro does not come to market, the carbendazime mixtures might have some advantages.

## CONCLUSIONS

The AMS 21619 containing fungicides are superior to all other fungicide used to control FHB. Their efficacy is about 90 % or more, but in more humid years the efficacy will be possibly lower. It seems that the Fusarium tests allow the measuring of yield response of the wheat also for leaf diseases, independently from Fusarium. By this way the economics of testing fungicides can be increased. It seems also that several fungicides have significant regulator effect (yield increase) than can make the use of some fungicides more profitable when it is stable.

**Table 1.** Fungicide tests in wheat, summary for 2001, general means across cultivars and isolates (12 situations)

Treatment and rates/ha	FHB %	DON ppm	Yield loss %	FDK %	Mean FHB efficacy %	Leaf rust %	Eff. leaf rust %
Prosaro 1.0	0.60	1.66	3.9	5.61	90.81	3.00	95.95
AMS 21619 125 EC 0.8	0.92	1.88	3.5	6.59	88.68	32.52	56.10
Prosaro 0.8	0.99	2.29	1.1	7.44	86.96	2.56	96.55
Folicur Solo	1.14	4.09	4.8	9.08	80.99	1.74	97.65
HEC+AMS21619 1.0	1.43	3.17	9.5	9.93	81.95	10.96	85.20
Caramba 1.2	2.28	3.94	7.7	12.94	75.58	6.70	90.95
Falcon 0.8+Kolf. S.1.5	1.39	6.16	6.5	13.80	72.41	3.59	95.15
HEC+HWG 1.5	1.93	5.81	5.8	14.47	71.13	2.26	96.95
Falcon 0.8	2.91	5.55	12.3	14.91	68.34	5.37	92.75
Stratego 1.0	3.08	5.58	8.8	18.81	65.69	21.78	70.60
Kolfugo S 1.5	5.71	7.74	17.0	22.56	49.42	58.15	21.50
Fusarium check.	12.02	15.58	17.2	38.54	0.00	74.07	0.00
Mean	2.87	5.3	8.17	14.56		18.56	81.76
LSD 5 %	1.82	0.49	2.05	2.97	5.58	3.49	
Mean fung. efficacy %	83.08	72.07	83.07	67.89	75.63		81.76

### Correlations for Table 1

Traits	FHB %	DON ppm	Yield loss %
DON ppm	0.9529		
Yield loss %	0.8244	0.7933	
FDK %	0.9714	0.9793	0.8438



**Table 2.** Epidemic situations for the susceptible cultivar Zugoly, FDK values, 2001.

Fungicide	Zugoly				
	12551 FcA	12551FcB	44FgA	44FgB	Mean
Prosaro 1.0	0.56	1.11	0.00	14.44	4.03
Jau 0.8	0.00	2.56	0.33	8.44	2.83
Prosaro 0.8	1.33	1.44	0.22	34.44	9.36
Folicur Solo	0.50	2.00	0.67	24.44	6.90
Hec+Jau 1	4.44	10.22	0.22	21.11	9.00
Caramba 1.2	13.44	11.11	4.11	62.78	22.86
Falc 0.8+ Kolf 1.5	1.78	7.22	0.67	57.78	16.86
Hec 0.75 F250 069	5.11	17.78	1.78	62.22	21.72
Falcon 0.8	3.44	5.00	0.89	51.67	15.25
Sfera 1.0	17.00	18.33	2.67	60.00	24.50
Stratego	10.22	15.89	1.44	46.67	18.56
Kolfugo S. 1.5	38.89	25.56	4.44	69.44	34.58
Fus. check.	62.22	58.89	5.00	63.33	47.36
Mean	11.72	13.72	1.73	44.54	17.93
LSD 5 %					6.17

**Table 3.** Fungicides against FHB in wheat, 2002. General means across cultivars and isolates.

Fungicides and rates l/ha	FHB%		FDK	Yield loss	DON	Leaf rust %	Plot yield % to check	Mean - efficacy %
	FHB%	AUDPC	%	%	ppm	rust %		
Prosaro 1.0	0.40	4.51	0.77	3.85	0.79	0.22	106.01	91.74
Fol. Solo 1.0+Kolf. S 1.5	0.43	3.80	1.83	6.07	0.92	0.33	112.96	87.39
Prosaro 0.8	0.94	9.26	1.30	2.41	0.99	0.78	106.40	92.35
Folicur Solo 1.0	1.06	10.33	1.80	5.95	2.17	0.56	103.75	84.18
Caramba 1.5	1.70	17.74	1.53	4.92	1.61	0.44	109.41	86.50
Falcon 0.8	1.72	17.53	0.82	5.31	1.95	1.67	105.74	85.90
Juwel 1.0	2.97	32.22	1.86	5.56	1.92	0.22	111.13	83.37
Sfera 1.0	5.63	61.17	4.07	6.62	3.64	2.89	106.68	73.35
Kolfugo S 1.5	7.15	81.75	3.73	6.76	3.65	31.11	104.29	71.71
Fusarium check	23.88	294.27	25.79	18.10	10.87	52.22	100.00	0.00
Mean	4.17	48.42	3.95	5.96	2.59	8.22	96.94	68.77
LSD 5 %	1.12	12.13	1.41	3.44	0.87	3.91	4.12	5.98
Mean fungicide efficacy	89.7	91.0	92.3	70.8	81.9	91.8	-7.38	

	FHB%	AUDPC	FDK	Yd loss	DON
AUDPC	0.9996				
FDK	0.9795	0.9839			
Yield loss %	0.9620	0.9644	0.9719		
DON ppm	0.9924	0.9912	0.9742	0.9706	

All significant at P = 0.1 %

**Table 4.** Fungicide control of FHB in wheat, general means across.

Fungicide	cultivars and isolates, 2003		
	Traits		Yield loss
	FHB %	FDK %	%
Prosaro 1.0	1.75	2.42	6.41
Prosaro 0.8	2.31	2.68	8.10
F. Solo 1.0 +Kolf. 1.5	2.49	5.13	12.15
Falcon 460EC 0.8	2.82	5.71	14.17
Folicur Solo 1.0	3.05	5.18	11.80
Caramba 1.2	3.29	11.31	13.21
Caramba 1.5	4.49	7.50	12.55
Juwel 1.0	4.92	10.50	14.31
Tango Star 1.2	4.93	7.00	13.43
Kolfugo 1.5	15.81	20.77	22.78
Fusarium check	20.72	26.52	32.52
Mean	5.55	8.73	13.45
LSD 5 %	2.15	3.13	4.19

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## EFFICACY OF FUNGICIDES ON FHB OF SOFT RED WINTER WHEAT IN ARKANSAS, 2003

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### INTRODUCTION

Identifying fungicides that reduce incidence and severity of Fusarium head blight (FHB) in the field and levels of damage and mycotoxins in the grain could have widespread benefits to growers and users of all market classes of wheat in the event of FHB epidemics. This test in Arkansas is part of the Uniform Fungicide Trial that is coordinated by the Chemical and Biological Control Committee, and the objective is to hasten the integration of fungicides and biocontrols that are effective against FHB into cost-effective and environmentally-safe wheat disease management strategies.

### MATERIALS AND METHODS

The moderately susceptible wheat cultivar 'Agripro Patton' was planted at the University Farm at Fayetteville on 10 October 2002. Seed was treated with Dividend fungicide (1 fl oz / cwt) for loose smut and seedling diseases and Gaucho insecticide (3 fl oz / cwt) for aphids and barley yellow dwarf. Individual plots were 7 rows by 13 ft. Plots were fertilized with a total of 100 lb nitrogen as ammonium nitrate (75 lb applied on 4 March and 25 lb applied on 2 April). Ryegrass and broadleaf weeds were controlled with recommended herbicides. Infested corn kernel inoculum of *Fusarium graminearum* was applied to the plots on 4 and 14 April for a total of 6 kernels / sq ft. Fungicides were applied in a randomized complete block design with six replications on 28 April when 50% of the main stems had begun to flower. The mist system operated for eight 10-minute periods between midnight and 8:00 am for nine nights between 29 April and 13 May. On 27 May, 50 heads per plot were sampled randomly and evaluated for FHB incidence and head severity, and plot severity was calculated. Plots were harvested with a plot combine on 13 June, and grain was passed once through a seed cleaner before test weight and percentage of scabby grain were measured.

### RESULTS AND DISCUSSION

As measured by plot severity and incidence of infected heads, all treatments except V-10116 at 6 fl oz significantly reduced FHB (Table 1). Only three treatments significantly reduced infected head severity. There were no differences among treatments for percent scabby grain and test weight. Differences among treatments for yield were complicated by stripe rust and Septoria leaf blotch epidemics late in the season. Folicur and JAU6476 appeared to be more effective against these diseases than V-10116. Rainfall totaled 8.37 inches during April and May, but most of the rain came in late May after irrigation ceased. This high rainfall favored FHB and Septoria tritici blotch.

**Table 1.** Effects of fungicides on FHB variables, yield, and test weight of wheat in Arkansas.

<b>Product and rate per acre</b>	<b>Plot severity (%)</b>	<b>Incidence of infected heads</b>	<b>Infected head severity (%)</b>	<b>Scabby grain (%)</b>	<b>Yield (bu/ac)</b>	<b>Test wt. (lbs/bu)</b>
JAU6476 480SC 3.6 fl. oz. + Folicur 4 fl. oz. + 0.125% Induce	13.1a	0.74a	17.7a	32a	56.2a	47.9a
Folicur 3.6F 4 fl. oz. + 0.125% Induce	13.8a	0.82ab	16.7a	31a	50.4ab	47.3a
V-10116 (1.67 SC) 8 fl. oz. + 0.125% Induce	15.3ab	0.84b	18.2ab	35a	47.9bc	44.1a
JAU6476 480SC 5.7 fl. oz. + 0.125% Induce	15.4ab	0.76a	20.4abc	29a	51.8ab	46.8a
JAU6476 480SC 5.0 fl. oz. + 0.125% Induce	17.7b	0.83b	21.4bc	28a	51.9ab	48.4a
V-10116 (1.67 SC) 6 fl. oz. + 0.125% Induce	18.8bc	0.89bc	21.0bc	32a	49.0abc	46.1a
Non-treated check	22.3c	0.93c	24.0c	33a	42.0c	48.9a
CV (%)	19.4	8.5	16.2	14.2	13.3	10.3

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

## SPLIT APPLICATION OF FUNGICIDES FOR INCREASED CONTROL OF FHB AND DON ON BARLEY

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### ABSTRACT

Fusarium head blight (FHB) has reduced the quality of barley grown in the Midwest for the last decade due to fungus infected kernels, pinched grain and most importantly the presence of the toxin, deoxynivalenol (DON). Individual cultural and chemical control measures have reduced disease, but have been unsuccessful in getting the level of control necessary for the requirements of the malting barley industry. It is likely that the reduced control with fungicides is due to the long period during which the barley plant is susceptible to infection (flowering through to harvest) and the relatively short period of effectiveness of the fungicides used for control. Field experiments were to find a rate of multiple fungicide application that is both effective and economic. The fungicides tested were Folicur (Bayer CropScience), and JAU 6476 (Bayer CropScience experimental). Both 6-rowed Robust and 2-rowed Conlon were used as they are common barley cultivars sown in the Midwest and they differ in the amount of disease and DON they experience under the same environmental and inoculum conditions. In 2003 at Bottineau ND, fungicides were applied at the recommended rate either split between two applications at 50-75% headed and fully headed or in a single application at fully headed. Due to environmental conditions, FHB was too low to be assessed and only one plot had a positive DON reading. Flag leaf disease was 4.0% on Conlon and 6.0% on Robust. All timings of both fungicides on Robust and Conlon reduced flag leaf disease compared to the untreated control. Test weight in Robust was increased by both timings of both fungicides but yield was unaffected. In 2003 at Langdon ND, fungicides were applied at recommended and 2x recommended rates, either in one application or split between two applications at awns emerged and fully headed. Percentage of kernels infected by FHB was low, with Robust having 1.3% in the untreated and Conlon having 2.6%. In Robust, split application of the recommended rate or single application of 2x recommended rate of JAU 6476 reduced disease. In Conlon, the single application of the recommended rate and the double application of the recommended rate of Folicur reduced disease as did the single application of 2x recommended rate of JAU 6476. DON levels were significantly lower in untreated Conlon (0.95 ug/g) than Robust (2.28 ug/g). None of the treatments reduced DON in Conlon, but in Robust DON was reduced by a single application of 2x recommended rate of Folicur and both rates and timings of JAU 6476. Yield was unaffected by all treatments. The experiment at Bottineau demonstrates that the fungicides do not influence yield when there is very low levels of disease, but they may control leaf disease. At Langdon, disease was higher but still regarded as low, and under those conditions selected treatments reduced both disease and DON, but there was no significant advantage to using a split application of fungicide. These experiments need to be repeated under a range of environments and disease pressures.

USDA-ARS, OHIO STATE UNIVERSITY COOPERATIVE RESEARCH ON  
BIOLOGICALLY CONTROLLING FUSARIUM HEAD BLIGHT 1:  
*IN VITRO* AND FIELD TESTING OF THE EFFECT OF UV  
PROTECTANTS ON FHB ANTAGONISTS

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## OBJECTIVES

To 1) discover UV-protectants that enhance survival of FHB antagonist *Bacillus subtilis* OH 131.1 after UV exposure *in vitro* and 2) evaluate the influence of a lignin-based UV protectant on the efficacy of OH 131.1 and the yeast *Cryptococcus nodaensis* OH 182.9 in field tests.

## INTRODUCTION

In previous research, we have demonstrated the potential of several antagonists including *B. subtilis* OH 131.1 and *C. nodaensis* OH 182.9, to significantly reduce the severity of FHB in field environments when biomass was produced in laboratory and pilot-scale quantities in liquid culture (Schisler et al., 2002ab; Khan et al., 2003). We have developed an effective frozen biomass concentrate that can be commercially produced, and have produced a more commercially feasible, consumer friendly dried product that maintains CFU/ml but is less effective in reducing FHB, possibly due to the cryoprotectant in the dried product stimulating pathogen activity (Schisler et al., 2002b).

Formulation research, in terms of UV protectants (Behle et al., 1996; McGuire et al., 2001), may be crucial for the continued development of these biocontrol strains from bench discovery to biocontrol product. UV light is particularly devastating to the survival of vegetative microbial cells. A water soluble sodium salt of lignin (Westvaco PC1307, experimental product) and the optical brightener Blankophor BBH (Sigma, Detroit, MI) have demonstrated UV protectant activity (McGuire et al., 2001) but their effect on FHB antagonist survival and efficacy was unknown prior to these studies.

## MATERIALS AND METHODS

UV protectants (Westvaco PC1307 and Blankophor BBH) were tested, *in vitro*, for their ability to aid survival of dried cells of OH 131.1 when exposed to artificial sunlight supplied from a xenon light source (Suntest Atlas CPS solar simulator, Heraeus DSET Laboratories Inc., Phoenix, AZ). Cells of antagonist OH 131.1 were grown in flasks containing a semidefined liquid medium (SDCL), harvested from 24 h growth cultures, combined or not with UV protectants, added as microliter droplets of formulated cells to 96 well microtiter plates, air-dried for 1 h or not, and exposed or not to 6 h of UV light. Cell counts at the time of introduction to microtiter plates were approximately  $2 \times 10^8$  CFU/ml. Lignin concentrations were 0.2% and 0.3%, and BBH concentrations were 0.5%, 1.0% and 2.0%. Wells were rehydrated with weak

growth medium and the growth of surviving cells determined over time using a plate-reading spectrophotometer at 620 nm (Fig. 1). Predicted absorbance values (proportional to cell biomass concentration) were determined by weighted linear regression analysis of growth curves.

Because UV protectants enhanced OH 131.1 survival *in vitro*, OH 131.1 and yeast antagonist *C. nodaensis* OH 182.9 were tested with and without the lignin UV protectant in field trials at Peoria, IL and Wooster, OH. Inoculum of OH 131.1 and OH 182.9 was produced using SDCL medium with a carbon:nitrogen ratio of 11 and total carbon loading of 14 g carbon/liter (Schisler et al., 2002a). The soft red winter wheat cultivars Pioneer 2545 (susceptible) and Freedom (moderately resistant) were used at both locations. Biomass was harvested from Fernbach shake flasks and applied at the beginning of wheat flowering (Schisler et al., 2002a). Bacterial and yeast suspensions contained 50 % fully colonized broth ( $\sim 1 \times 10^8$  CFU/ml and  $\sim 5 \times 10^7$  CFU/ml, respectively) and were applied at a rate of 20 gal/acre. The fungicide Folicur 3.6F was applied at the recommended rate. Controls were untreated plants and plants treated with buffer/wetting agent only. Corn kernels colonized by *Gibberella zeae* (Schisler et al., 2002a) were scattered through plots ( $\sim 25$ -40 kernels/m<sup>2</sup>) two weeks prior to wheat flowering and mist irrigation provided periodically for approximately one week after treatment application. Heads were scored for disease incidence and severity 2 to 3 weeks after treatment using a 0-100% scale. Data for the deoxynivalenol content of grain and 100 kernel weight is being tabulated (ongoing). Randomized complete block designs were used in both trials ( $n=5$  at both locations). Analysis of variance, linear regression and Fisher's protected LSD or Student's t-test ( $p \leq 0.05$ ) (JMP 4.0 statistical software) were used as appropriate to separate treatment means in these studies.

## RESULTS AND DISCUSSION

*In vitro*, lignin and BBH enhanced the survival of dried cells of OH 131.1 vs. controls at all concentrations tested when cells were exposed to 6 h of UV as demonstrated by higher predicted absorbance (due to higher cell biomass) in wells during active cell growth 30 h after rehydration (Table 1). For 6 h UV treated wells, approximately 2 to 3 times as much cell biomass was present in UV protectant-amended wells as in unamended wells. The effect of UV protection was not necessarily improved as the concentration of protectants increased. UV protectants also enhanced OH 131.1 cell survival in wells that did not receive UV light, possibly due to reducing cell viability losses due to drying and rehydration stress. UV protectant amendment of fresh OH 131.1 cells generally had no influence on cell growth (data not shown).

Wheat treated with OH 131.1 or yeast OH 182.9 had decreased FHB severity and incidence vs. one or both controls in Peoria, IL and Wooster, OH field trials but the lignin UV protectant did not consistently influence antagonist efficacy (Table 2). Lignin (0.3%) amendment did not influence the efficacy of either antagonist in the Peoria, IL trial while lignin increased efficacy (OH 131.1 on Freedom), decreased efficacy (OH 131.1 and OH 182.9 on Pioneer 2545), or had no effect (OH 182.9 on Freedom) in the Wooster, OH trial. Repetition of these studies with washoff-resistant formulations of UV protectants and testing of additional UV protectants would clarify if these compounds can be used to enhance the efficacy of FHB antagonists in the field.

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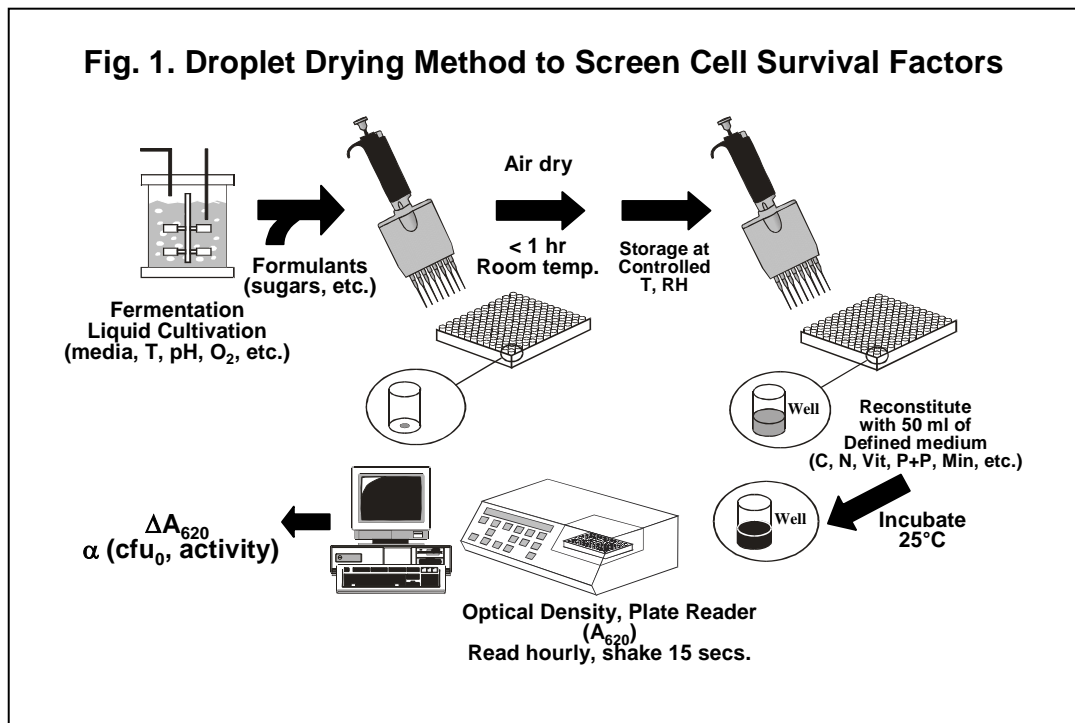
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**Table 1.** Influence of UV Protectants on Dried 24-h *Bacillus* sp. OH 131.1 Exposed to 0- or 6-h UV Prior to Rehydration of Cells.

Treatment	Predicted A <sub>620</sub> <sup>1</sup> 30 h after medium addition	
	No UV	6-h UV
OH 131.1 + 0.2% Lignin <sup>2</sup>	0.148 a	0.131 a
OH 131.1 + 0.3% Lignin	0.140 b	0.120 b
OH 131.1 Control	0.082 e	0.040 f
OH 131.1 + 0.5% Blankophor <sup>3</sup>	0.126 c	0.087 c
OH 131.1 + 1.0% Blankophor	0.110 d	0.081 d
OH 131.1 + 2.0% Blankophor	0.156 a	0.114 b

<sup>1</sup>Values followed by the same letter are not significantly different [Duncan's multiple range test (p<0.05)].

<sup>2</sup>Experimental product Westvaco PC 1307, water soluble lignin.

<sup>3</sup>Optical brightener.

**Table 2.** Field results in Peoria, IL and Wooster, OH biocontrol strains OH 131.1 and OH 182.9 with or without 0.3% Westvaco lignin PC 1307 formulation.

Treatment	Peoria, IL <sup>1</sup>			Wooster, OH <sup>1,2</sup>			
	Freedom	Pioneer 2545		Freedom		Pioneer 2545	
	DI <sup>3</sup>	DS	DI	DS	DI	DS	DI
Untreated Check	21.0#	4.0	32.0	25.8#	93.3	47.5	100
Buffer Tween Check	11.0*	3.2	29.3	20.6*	93.9	49.9	99.6
Folicur	12.0*	1.9*#	19.7*#	12.1*#	80.0*#	42.4*#	99.6
OH 182.9	12.7*	3.3	22.7*	17.4*#	92.8	40.9*#	98.9*
OH 182.9 + 0.3% Lignin	17.0*	2.8*	23.0*	16.3*#	89.4	44.3#	99.6
OH 131.1	10.7*	2.1*#	22.0*#	19.6*	95.6	42.5*#	100
OH 131.1 + 0.3% Lignin	9.0*	2.3*	22.0*	16.0*#	87.2#	48.6	100

<sup>1</sup>DS = % Disease severity, DI = % Disease incidence. Within a column, means followed by "\*" and "#" are significantly different from the untreated check and buffer/tween check, respectively (student's t-test, P ? 0.05).

<sup>2</sup>Extensive rain and wind caused wheat lodging and permitted excessive disease development at Wooster, OH.

<sup>3</sup>Disease severity values were very low (range = 0.9-1.9), and are therefore not reported.

# EVALUATION OF FUNGICIDES FOR THE CONTROL OF FUSARIUM HEAD BLIGHT AND LEAF DISEASES ON 'ELKHART' AND 'PIONEER VARIETY 2540' WINTER WHEAT IN MISSOURI

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## OBJECTIVES

To identify fungicides that are effective in minimizing the damage from Fusarium head blight and foliage diseases in winter wheat.

## INTRODUCTION

The severity of Fusarium head blight epidemics in the United States has caused enormous yield and quality losses in wheat and barley (McMullen, et al., 1997). The development of this disease is dependent on host genetics, a range of favorable environmental conditions, the prevalence of the causal fungus and the survival and spread of the cause fungus (Sutton, 1982). Control of this disease has been difficult because of the complex nature of the host/pathogen interaction. In addition to the development of varieties with resistance to Fusarium head blight, research focusing on fungicide treatments for the management of Fusarium head blight has been pursued.

In 1998, a Uniform Fungicide Trial was conducted across seven states (McMullen, 1998), which provided data on efficacy of five products or product combinations in reducing Fusarium head blight when applied at heading. This Uniform Fungicide Trial permitted evaluation of the performance of products across numerous states or sites, wheat classes and environments. Across the test sites that had substantial Fusarium head blight in 1998, an average of about fifty percent reduction in Fusarium head blight occurred, as well as a reduction in DON for most products, plus a substantial reduction in wheat leaf diseases. The Uniform Fungicide Trial has been continued since 1998 with additional test sites in more states and changes in products tested as new fungicides and biological control agents have become available. The Uniform Fungicide Trial continues to provide valuable information on efficacy and performance consistency of standard fungicides and new experimental fungicides. Missouri has participated in the Uniform Fungicide Trial since 1998 (Sweets, 2000). Results from the 2003 trial are presented in this report.

## MATERIALS AND METHODS

Six fungicide treatments and an untreated control were evaluated on 'Elkhart' and four fungicide treatments and an untreated control were evaluated on 'Pioneer variety 2540' soft red winter wheat at the Bradford Research Center, near Columbia, MO. 'Elkhart' and 'Pioneer variety 2540' were drilled directly into soybean stubble on 15 Oct 02. The soil type at the site was a Putnam silt loam. The planting rate was 100-lbs of seed/A. The experimental design for each variety was a randomized complete block with 6 replications. Individual plots were 4.5 ft (7 rows) by 30 ft in length. The entire plot area was fertilized with 30-lbs/A nitrogen preplant followed by 90-lbs/A nitrogen topdressed in the spring. Treatments were applied with a CO<sub>2</sub> backpack sprayer with nozzles directed towards the heads. Treatments were applied in 400 ml of water. Applications were made at Feeke's Growth Stage (FGS) 10.51 on 11 May 03. Plots were rated for foliage diseases on 24 May 03. Ratings were

done as estimates of the percentage of leaf area covered with Septoria leaf blotch or stripe rust on each of 10 flag leaves randomly collected from each plot. Fusarium head blight incidence and head severity measurements were taken 26 May 03. For harvest the plots were end trimmed and individual plot lengths measured. Plots were harvested on 30 June 03 with a Wintersteiger plot combine. Test weight and moisture were determined with a Dickey-John GAC 2000 Grain Analyzer. Samples were submitted to the Veterinary Diagnostic Services Department at North Dakota State University for DON analysis. Data was statistically analyzed using ANOVA.

## RESULTS AND DISCUSSION

Plants emerged well and early stands were uniform. The 2003 season was warm and dry early; cool and wet as the wheat was flowering and heading; and then cool and dry as the crop matured. Septoria leaf spot and leaf rust began to develop during flowering. When foliage disease ratings were made, both stripe rust and Septoria leaf blotch were evident across the trial so rating for these two diseases were made and are reported as percent total foliage disease. Fusarium head blight was widespread throughout the plot at the time Fusarium head blight incidence and severity ratings were made. At harvest most plots had noticeable amounts of shriveled, lightweight kernels or tombstone kernels.

There were no statistically significant differences in yield between the fungicide treatments and the untreated control on either 'Elkhart' or 'Pioneer variety 2540'. Total foliage disease ratings were significantly higher for the untreated control than the fungicide treatments on both 'Pioneer variety 2540' and 'Elkhart'. On 'Elkhart', JAU6476 at the 5.7 fl oz rate + Induce 0.125% v/v had lowest total foliage disease rating.

On 'Elkhart' there were no statistically significant differences between treatments for percent Fusarium head blight incidence, percent average head severity, percent field severity or percent scabby kernels but there was a significant difference between treatments for DON levels. The untreated control had the highest DON level, although it was only 1.10 ppm.

On 'Pioneer variety 2540' there were significant differences between treatments for percent Fusarium head blight incidence. The untreated control had the highest percent FHB incidence and the three JAU6476 480SC treatments had the lowest percent FHB incidence. There were also significant differences in DON levels between the treatments. Again the untreated control had the highest DON level (0.62 ppm) and the three JAU6476 480SC treatments had the lowest DON levels. There were no statistically significant differences between the untreated control and the four fungicide treatments for percent of percent average head severity, percent field severity or percent of scabby kernels on 'Pioneer variety 2540'.

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**Table 1.** 'Elkhart'

Treatment and Rate/A	Yield <sup>1</sup> bu/A	Test Wt. (lb/bu)	Foliage Disease		% Ave. Head Sev. <sup>4</sup>	% Field Sev. <sup>5</sup>	% Scab Kernels <sup>6</sup>	DON ppm
			Rating <sup>2</sup>	%FHB <sup>3</sup>				
Untreated control	55.4	59.0	15.23	42.00	19.33	7.94	20.8	1.10
Folicur 3.6F 4.0 fl oz + Induce 0.125% v/v	63.8	60.4	0.75	41.67	23.00	9.32	18.5	0.39
JAU6476 480SC 5.7 fl oz + Induce	60.6	60.5	0.57	41.00	19.83	8.04	16.2	0.21
JAU6476 480SC 5.0 fl oz + Induce	61.1	59.9	0.73	35.67	21.67	7.62	11.0	0.01
JAU6476 480SC 3.6 fl oz + Folicur 4.0 fl oz + Induce 0.125% v/v	70.1	60.3	0.70	40.67	24.33	9.61	13.6	0.26
V-10116 1.67SC 6.0 fl oz + Induce	55.8	59.2	0.62	41.33	19.85	8.56	21.0	0.09
V-10116 1.67SC 8.0 fl oz + Induce 0.125% v/v	57.1	59.7	0.74	40.67	25.67	10.48	19.3	0.01
LSD (P=0.05) <sup>7</sup>	N.S.	N.S.	1.38	N.S.	N.S.	N.S.	N.S.	0.32

<sup>1</sup> Yield based on 60-pound bushel weight adjusted to 13% moisture content.

<sup>2</sup> Total Foliage Disease Rating based on the average % of flag leaf showing symptoms of stripe rust and Septoria leaf blotch for 10 flag leaves.

<sup>3</sup> FHB or percent of Fusarium head blight incidence based on % of heads showing symptoms for 50 heads.

<sup>4</sup> % ave. head sev or percent of average head severity based on % of head showing FHB symptoms for 50 heads.

<sup>5</sup> % field sev or percent field severity calculated using the formula (%FHB x % ave. head sev.)/100.

<sup>6</sup> %scab kernels or percent scabby kernels based on % scabby kernels in a 200 kernel sample.

<sup>7</sup> Data was analyzed by ANOVA with means separated by LSD at P=0.05.

**Table 2.** 'Pioneer variety 2540'

Treatment and Rate/A	Yield <sup>1</sup> bu/A	Test Wt. (lb/bu)	Foliage Disease		% Ave. Head Sev. <sup>4</sup>	% Field Sev. <sup>5</sup>	% Scab Kernels <sup>6</sup>	DON ppm
			Rating <sup>2</sup>	%FHB <sup>3</sup>				
Untreated control	88.8	59.2	16.92	52.17	23.67	12.93	9.7	0.62
Folicur 3.6F 4.0 fl oz + Induce 0.125% v/v	94.5	59.5	0.80	46.67	22.83	10.57	11.3	0.34
JAU6476 480SC 5.7 fl oz + Induce 0.125% v/v	99.3	57.4	0.76	36.83	25.33	8.83	11.9	0.01
JAU6476 480SC 5.0 fl oz + Induce 0.125% v/v	97.9	59.1	0.72	41.00	19.00	7.48	12.2	0.11
JAU6476 480SC 3.6 fl oz + Folicur 4.0 fl oz + Induce 0.125% v/v	95.4	58.8	0.95	39.67	25.50	10.15	10.8	0.11
LSD (P=0.05) <sup>7</sup>	N.S.	N.S.	1.64	10.54	N.S.	N.S.	N.S.	0.29

<sup>1</sup> Yield based on 60-pound bushel weight adjusted to 13% moisture content.

<sup>2</sup> Total Foliage Disease Rating based on the average % of flag leaf showing symptoms of stripe rust and Septoria leaf blotch for 10 flag leaves.

<sup>3</sup> FHB or percent of Fusarium head blight incidence based on % of heads showing symptoms for 50 heads.

<sup>4</sup> % ave. head sev or percent of average head severity based on % of head showing FHB symptoms for 50 heads.

<sup>5</sup> % field sev or percent field severity calculated using the formula (%FHB x % ave. head sev.)/100.

<sup>6</sup> %scab kernels or percent scabby kernels based on % scabby kernels in a 200 kernel sample.

<sup>7</sup> Data was analyzed by ANOVA with means separated by LSD at P=0.05.

## CONTROL OF FUSARIUM HEAD BLIGHT VIA INDUCED RESISTANCE ELICITED BY *LYSOBACTER ENZYMOGENES* C3 - POTENTIALS AND LIMITATIONS

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### ABSTRACT

*Lysobacter enzymogenes* C3 is unique among biological control agents for Fusarium head blight (FHB) in that induced resistance is involved as a mechanism action. We investigated the spatial distribution of C3-elicited induced resistance by applying the bacterium or water to various parts of wheat plants and assessing the degree to which FHB developed in treated and non-treated spikelets following inoculation with pathogen conidial suspensions. When C3 was applied to flag leaves, there was no effect on FHB severity on heads of treated plants as compared to the water-treated control. When the agent was applied to spikelets on the lower half of flowering wheat heads, inhibition of FHB was observed in the lower half of treated heads but not in the non-treated upper portion. In another set of experiments, C3 was sprayed onto wheat heads and then the treated heads were challenged with pathogen conidia by floret injection. FHB severity in C3-treated heads was found to be lower than in heads treated with water. We conclude that resistance induced by C3 is not systemically expressed, but is localized to the spikelets on which the bacterium is deposited. As a consequence, biocontrol efficacy using C3 will depend in part on the effectiveness of application procedures in depositing the bacterium onto all spikelets. If C3 is well distributed across spikelets in a wheat head, induced resistance can reduce the incidence of infection in the head and inhibit pathogen spread from infected spikelets via the rachis. In studies on induced resistance in other host-pathogen systems, the effectiveness of induced resistance varied depending upon the host genotype to which the inducing agent was applied. Therefore, we also investigated whether or not C3 could be effective in reducing FHB development on different cultivars of spring wheat. In a series of greenhouse experiment in which C3 was applied to 11 cultivars varying in susceptibility to FHB, the bacterium reduced FHB severity, as compared to water-treated controls, in eight of the cultivars. Differential effectiveness of biocontrol by C3 was not associated with relative levels of resistance to FHB reported for the cultivars. These findings suggest that that host genotype may be another determining factor in biological control by C3 and that the interaction of each genotype with C3 must be assessed empirically.

## COOPERATIVE MULTISTATE FIELD TESTS OF BIOLOGICAL AGENTS FOR CONTROL OF FUSARIUM HEAD BLIGHT IN WHEAT AND BARLEY

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### OBJECTIVES

To evaluate biological control agents for efficacy in control of Fusarium head blight across a range of hosts (species, market class, and cultivars) and environmental conditions.

### INTRODUCTION

Microorganism strains are being investigated in several US laboratories as biological control agents against Fusarium head blight (FHB). Strains that have shown potential to reduce scab severity under field conditions include the yeast *Cryptococcus nodaensis* OH182.9 (Schisler et al., 2002), the gram-positive, spore-forming bacterium *Bacillus subtilis* TrigoCor 1448 (Hershman et al., 2002; da Luz et al., 2003), and the gram-negative bacterium *Lysobacter enzymogenes* C3 (Yuen and Jochum, 2002). Additional strains, such as *Bacillus* sp. 1BA (Baye et al., 2002; Luo, 2000), have shown promise in laboratory tests. Crucial to gauging the usefulness of a biocontrol agent is an assessment of its efficacy across the range of field conditions and host genotypes occurring in the different geographic areas in which FHB may be a problem. In 2003, experiments were conducted cooperatively among several biocontrol laboratories to evaluate the same agent strains in three states.

### MATERIALS AND METHODS

Six experiments were conducted, two each in Nebraska, Ohio and South Dakota. Nebraska experiments were located at Lincoln and Mead, separated by approx. 40 miles. Cultivar 2137, a FHB-susceptible hard red winter wheat, was planted at both locations. There were four and five replications per treatment at Lincoln and Mead, respectively. Ohio experiments were conducted in Wooster on soft red winter wheat cultivars Pioneer 2545 (susceptible) and Freedom (moderately resistant), with five and four replications per treatment, respectively. South Dakota experiments were conducted at Brookings in 'Oxen' hard red spring wheat and 'Robust' barley, both with four replications per treatment. Randomized complete block designs were used in all locations.

Bacterial strains C3, TrigoCor 1448, and 1BA were propagated in the laboratory at each location. The strains were grown in broth media and the resultant cultures were amended with the surfactant Induce (0.125%) prior to application. The yeast OH182.9 was supplied as fermentation biomass suspended in a

buffer amended with the surfactant Tween 80 (Schisler et al., 2002). Each experiment also included a treatment with tebuconazole (Folicur 3.6F, 4 fl oz./A) amended with Induce. Control plots in South Dakota were nontreated, whereas control plots in Nebraska were treated with water containing Induce; Ohio experiments involved both of these controls in addition to one treated with buffer/Tween solution. At anthesis, a single application of each treatment was made in 70 gal/A at Nebraska and Ohio and 20 gal/A in South Dakota. Pathogen inoculum was provided in the form of conidial suspensions (Nebraska and South Dakota) or pathogen-colonized corn kernels (Ohio and South Dakota). Mist irrigation was applied for over 1 week after treatment to promote FHB development. The incidence and severity of FHB was determined on over 40 heads per plot approx. 3 weeks after treatment. Seed yields, test weight and DON content were determined in Nebraska and South Dakota.

## RESULTS AND DISCUSSION

Biological control agents applied in Nebraska and South Dakota experiments did not affect FHB incidence or severity (Table 1). Treatment with Folicur reduced incidence in both Nebraska locations and decreased severity at Mead, but the fungicide had no effect in South Dakota. The biological control agents and Folicur did not affect DON content or plot yields, and only the fungicide treatment exhibited a positive effect on test weight in one Nebraska location (data not shown). Fusarium head blight severity levels were very low in Nebraska and South Dakota, and therefore, results from these experiments are not conclusive as to the efficacy of the biocontrol agents.

Efficacy was found for biological control treatments in Ohio, but the results varied depending upon the cultivar (Table 2). Very high incidence and severity levels recorded in the controls of susceptible Pioneer 2545 were unchanged by treatment with biological control agents or Folicur. In moderately resistant 'Freedom', FHB severity in plots treated with each of the biocontrol agents was lower than in the non-treated control and were not significantly different from plots treated with Folicur. Differences between the biocontrol treatments and treatment involving only adjuvants, however, were not statistically significant.

In this set of field experiments, no difference could be discerned for disease suppression among the biocontrol agents tested. Further research is needed to ascertain the effectiveness of the agents in different geographic areas and on barley and other wheat market types. Ohio results do lend support to the supposition that biological control agents will be most effective when integrated with host resistance.

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**Table 1.** Incidence (INC) and severity (SEV) of Fusarium head blight in Nebraska and South Dakota evaluations of biological control agents, 2003<sup>1</sup>.

Treatment	NE Lincoln		NE Mead		SD Wheat		SD Barley	
	%INC	%SEV	%INC	%SEV	%INC	%SEV	%INC	%SEV
Control <sup>2</sup>	41 ab	5	85 a	9 a	13	1	69	6
<i>Bacillus</i> sp. 1BA	52 a	7	81 a	8 a	19	2	72	7
<i>Bacillus subtilis</i> Trigocor 1448	51 a	6	74 a	7 a	21	2	76	6
<i>Cryptococcus nodaensis</i> OH182.9	54 a	8	84 a	8 a	11	1	62	4
<i>Lysobacter enzymogenes</i> C3	60 a	8	83 a	8 a	18	2	64	8
Folicur	32 b	3	44 b	4 b	14	1	64	6
<i>P</i>	0.008	0.051	0.001	0.006	0.053	0.125	0.795	0.664

<sup>1</sup>Means in a column with the same letter are not significantly different at P=0.05 according to Student-Newman-Keul's Multiple Range Test. Mean separations shown only when treatment effects in ANOVA were significant at the 95% level.

<sup>2</sup>Control plots in Nebraska were treated with water amended with Induce. Non-treated plots were the controls in South Dakota.

**Table 2.** Incidence (INC) and severity (SEV) of Fusarium head blight in Ohio, 2003, evaluations of biological control agents on two winter wheat cultivars<sup>1</sup>.

Treatment	Freedom		Pioneer 2545	
	% INC	% SEV	% INC	% SEV
Nontreated control	93	26 a	100	47
Induce	93	21 ab	>99	50
Buffer/Tween	94	21 ab	100	43
<i>Bacillus</i> sp. 1BA	88	18 bc	100	49
<i>Bacillus subtilis</i> Trigocor 1448	86	16 bc	100	47
<i>Cryptococcus nodaensis</i> OH182.9	93	18 bc	>99	42
<i>Lysobacter enzymogenes</i> C3	91	18 bc	100	49
Folicur	80	12 c	>99	42
<i>P</i>	0.136	<0.001	0.232	0.294

<sup>1</sup>Means in a column with the same letter are not significantly different at P=0.05 according to Student-Newman-Keul's Multiple Range Test. Mean separations shown only when treatment effects in ANOVA were significant at the 95% level.



# USDA-ARS, OHIO STATE UNIVERSITY COOPERATIVE RESEARCH ON BIOLOGICALLY CONTROLLING FUSARIUM HEAD BLIGHT 2: EFFECTS OF CARBON-TO-NITROGEN RATIO OF PRODUCTION MEDIA ON THE BIOCONTROL EFFICACY AND THE SURVIVAL OF *CRYPTOCOCCUS NODAENSIS* OH 182.9 AFTER FREEZE-DRYING

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## OBJECTIVES

To determine the effect of varying the carbon-to-nitrogen (C/N) ratio and carbon loading of production media and cultivation duration on the biocontrol efficacy of *C. nodaensis* OH 182.9 against Fusarium head blight and the viability of freeze-dried OH 182.9 cells over time.

## INTRODUCTION

Fusarium head blight (FHB), primarily incited by *Gibberella zeae*, is a devastating disease of wheat worldwide (McMullen *et al.*, 1997). The infection of wheat kernels by *G. zeae* reduces grain yield and quality in part due to the pathogen producing a vomitoxin deoxynivalenol (DON) in grain during infection. Extensive research has focused on the use of resistant wheat cultivars for control of FHB. However, no available cultivars are immune to FHB. Chemical management of FHB has proven inconsistent and is hindered by costs and concerns about post-harvest fungicide residues. Cultural practices such as crop rotation and a shift from reduced to conventional-tillage practices are not economically viable. *Cryptococcus nodaensis* OH 182.9 has been evaluated as an effective biocontrol agent for management of FHB (Khan *et al.*, 2001; Schisler, *et al.*, 2002). Development of a dried product of OH 182.9 is sought because of the potential advantages of ease of handling, favorable economics and acceptance by the users.

## MATERIALS AND METHODS

*C. nodaensis* OH182.9 cells were inoculated in 250-ml Erlenmeyer flasks containing 50 ml of semidefined complete liquid media (SDCL). Flasks were incubated for 48 and 72 h on a shaker at 250 rpm, 25°C. Two milliliters of samples were distributed in an autoclaved serum vial and then placed in a freeze drier with eutectic temperature – 8°C operating at – 45°C for 2 days. Vials were stored at room temperature and over time, cells were resuspended in 2 ml of phosphate buffer, diluted and plated on 1/5 Tryptic soy broth agar (TSBA/5). To determine the efficacy of fresh cells for controlling FHB, wheat heads (cultivar Norm) at anthesis were sprayed with 25% suspensions of fully colonized cultures of OH 182.9 in SDCL (approx.  $1 \times 10^8$  CFU/ml), followed by *G. zeae* Z-3639 ( $1 \times 10^4$  macroconidia/ml). Inoculated plants were placed in a humid chamber for 3 days before being transferred to greenhouse benches. Disease severity was recorded using a 0-100% scale 14 days after inoculation. Experiments were performed 2 or 3 times, and the treatments were arranged in a completely randomized design.

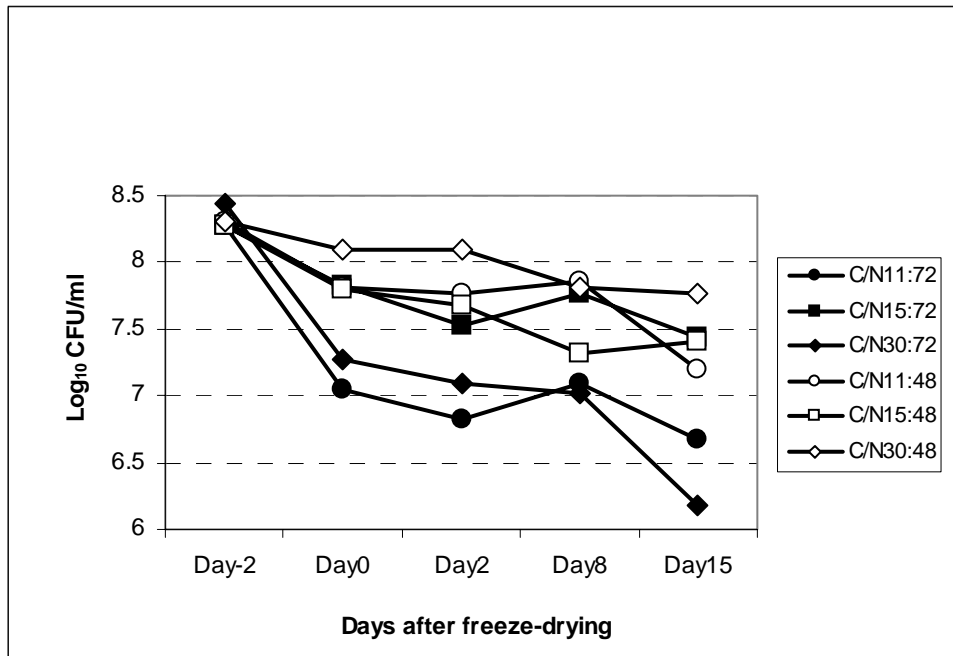
## RESULTS AND DISCUSSION

OH 182.9 was grown in SDCL with C/N ratios of 6.5, 9, 11, 15, 30 and the carbon loading of 14 g/L for 48 and 72 h. Total biomass production was similar for all combinations of cell age by SDCL medium C/N ratio. Survival of freeze-dried cells was greatest for cells grown in SDCL C/N 30 medium for 48 h (Fig. 1). Cells produced in C/N 6.5 medium exhibited the poorest survival (data not shown). In general, cells harvested after 48 h were more tolerant to freeze-drying than those cultured after 72 h; cells grown in higher C/N ratio SDCL survived better than those harvested from lower C/N media when being harvested at 48 h. OH 182.9 produced in C/N 9, 11 and 15 media for 48 h significantly reduced the FHB disease severity compared to the disease control (Table 1).

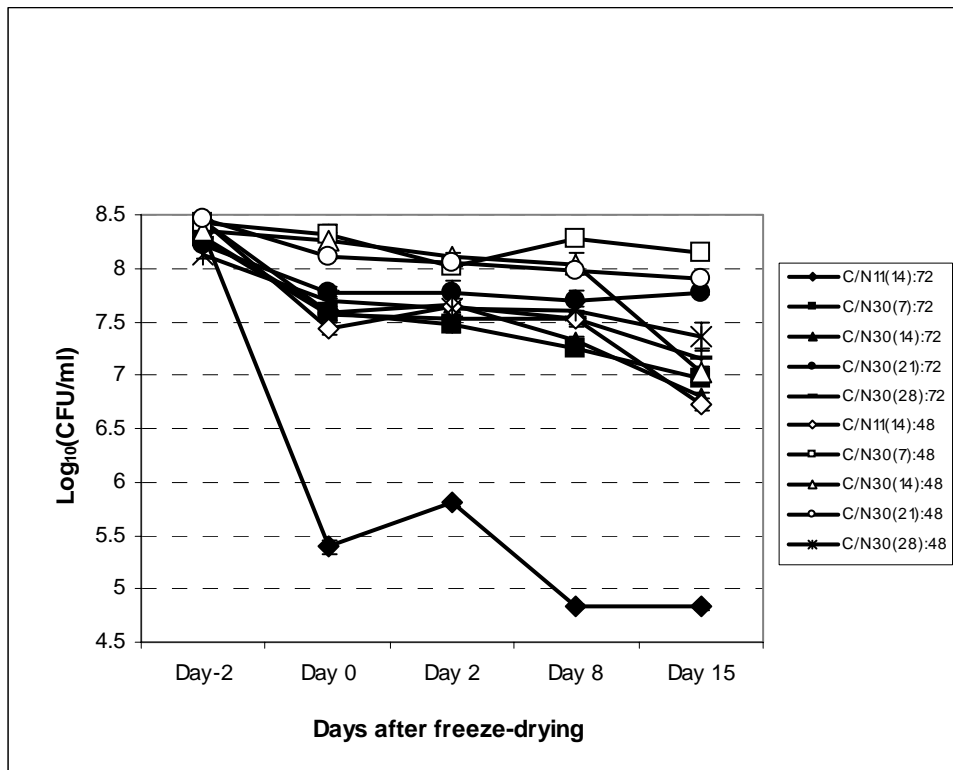
For the medium C/N 30, research on influence of carbon loading in production media on cell survival after freeze-drying and the efficacy of disease control was conducted. Total carbon loading of 7, 14, 21 and 28 g/L in SDCL C/N 30 medium was tested. In general, cells cultured for 48 h survived better than those harvested after 72 h. OH 182.9 cells from SDCL C/N 30 media with varied carbon loading maintained 6.5-8.5 log CFU/ml for 15 days after freeze-drying (Fig.2). Cells produced in C/N 30 media with 7, 14 and 21g/L carbon and harvested after 48 h had better survival than others, including the standard 48 h C/N 11 culture with 14g/L carbon. OH 182.9 grown in SDCL C/N 30 with carbon loading of 7 or 14 g/L was effective against FHB (Table 2). Our results indicate that C/N ratio, total carbon loading and the stage of microbial growth in production media greatly influence freeze-drying survival of the biomass and the biocontrol efficacy of OH 182.9.

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**Figure 1.** Effect of C/N ratio (11, 15, 30) and cell age (48, 72 h) on OH 182.9 cell survival after freeze-drying (14 g/L carbon).



**Figure 2.** Effect of carbon loading in SDCL and cell age (48, 72 h) on OH 182.9 cell survival after freeze-drying.

Table 1. Biocontrol efficacy of *C. nodaensis* OH 182.9 produced in SDCL C/N 11, 9 and 6.5 media with 14g/L carbon

Treatment <sup>x</sup>	Disease Severity (%) <sup>y</sup>			
	Trial 1	Trial 2	Trial 3	Pooled
C/N 11: 72 <sup>z</sup>	90 a	83 a	83 ab	85 a
C/N 9: 72	90 a	83 a	75 abc	83 ab
C/N 6.5: 72	90 a	77 a	79 abc	82 ab
C/N 11: 48	60 b	80 a	49 bc	63 bc
C/N 9: 48	74 ab	49 a	44 c	56 c
C/N 6.5: 48	90 a	76 a	83 ab	83 ab
Disease CK	86 a	90 a	90 a	89 a
Nontreated CK	0 c	0 b	0 d	0 d
LSD0.05	24	47	37	21

<sup>x</sup> 25 % full strength OH 182.9-colonized SDCL

<sup>y</sup> Different letters within a column indicate significant differences among means.

<sup>z</sup> C/N11: 72 indicates OH 182.9 was grown in SDCL C/N 30 medium for 72 h.

Table 2. Effect of carbon loading of 7 and 14 g/L in SDCL C/N 30 media and incubation time (48, 72 h) on biocontrol efficacy of *C. nodaensis* OH 182.9

Treatment <sup>x</sup>	Disease Severity (%) <sup>y</sup>			
	Trial 1	Trial 2	Trial 3	Pooled
C/N 11(14): 72	19 abc	44 abc	46 ab	36 abcd
C/N 30(7): 72 <sup>z</sup>	23 abc	57 ab	49 ab	43 abc
C/N 30(14): 72	48 a	54 ab	53 ab	52 ab
C/N 11(14): 48	7 bc	24 bc	19 bc	16 de
C/N 30(7): 48	24 abc	42 abc	26 bc	31 bcd
C/N 30(14): 48	14 abc	20 bc	28 bc	20 cde
Disease CK	37 ab	69 a	70 a	59 a
Nontreated CK	0 c	0 c	0 c	0 e
LSD 0.05	36	45	38	23

<sup>x</sup> 25 % full strength OH 182.9-colonized SDCL

<sup>y</sup> Different letters within a column indicate significant differences among means.

<sup>z</sup> C/N 30(7): 72 indicates OH 182.9 was grown in SDCL C/N 30 medium with carbon loading 7 g/L for 72h.