

**CHEMICAL, BIOLOGICAL
AND CULTURAL
CONTROL**

Chairperson: Martin Draper

CHARACTERISTICS, INCLUDING TOLERANCE TO ELEVATED
HEAT AND ELEVATED SALT CONCENTRATION,
OF A *BACILLUS* STRAIN USED AS A BIOCONTROL
AGENT TO CONTROL FUSARIUM HEAD BLIGHT

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ABSTRACT

Selected strains of *Bacillus* can be used as biocontrol agents (BCAs), to antagonize *Fusarium graminearum* which causes Fusarium Head Blight (FHB) of wheat and barley. The ability of the BCA strain 1BA to survive during storage at different temperatures was examined. Cells of strain 1BA grown for five days at 27°C in tryptic soy broth were stored at either 4°C, -20°C, or room temperature (27°C) for one month, then enumerated for viable cells. Best survival of cells was at 27°C (a 2 log decrease), with a 4-log decrease in cell numbers at -20°C and at 4°C. This was unexpected, since bacterial numbers usually remain more stable at refrigerator temperature rather than at room temperature. This has implications for using these BCAs for control of FHB, since due to unpredictable weather conditions and/or maturation of wheat or barley in the field, storage of BCAs before their use in the field is often required.

The production of endospores by strain 1BA at different temperatures was examined, using nutrient agar amended with manganese sulfate to help encourage spore production. Large numbers of spores were produced at selected incubation temperatures, ranging from 27°C to 50°C. Endospores survived pasteurization at 80°C for 10 minutes, indicating that endospores of this BCA could remain viable at elevated storage temperatures. Using plate assays, amylase but not chitinase activity was verified at all examined temperatures for 1BA.

To assay numbers of 1BA after spraying its cells onto wheat or barley heads in the field, a selective and/or differential growth medium is needed to either discourage growth of native wheat or barley microflora while allowing growth of this BCA. Temperature and salt stresses on 1BA were examined to develop such a medium. Strain 1BA grew on tryptic soy agar (TSA) and nutrient agar (NA) at various temperatures, ranging from 27°C to 50°C. 1BA also grew on TSA and NA amended with various NaCl concentrations, ranging from 2.5% NaCl to 10% NaCl. Strain 1BA also grew at elevated temperature and salt concentrations in the defined broth medium used for producing inoculum for field application. There were distinct colonial morphology changes in 1BA depending on temperature or NaCl concentration. The elevated temperature and NaCl concentrations that 1BA could withstand were used in preliminary plate counting of the microflora of wheat heads. Little or no growth of the native microflora was noted with these conditions, suggesting that we will be able to apply and count 1BA inoculum that has been sprayed onto wheat heads using a plate count methodology. The ability of these BCAs to grow at elevated temperature and salt concentration indicates these stresses can be used to select for these BCAs in plate count assays, and possibly to enrich for them on plant surfaces by spraying salt solutions on aerial plant parts.

2005 UNIFORM FUNGICIDE PERFORMANCE TRIALS
FOR THE SUPPRESSION OF FUSARIUM HEAD
BLIGHT IN SOUTH DAKOTA

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ABSTRACT

Fusarium head blight (FHB – scab) has been a serious concern for wheat and barley producers in South Dakota for ten years and a serious epidemic impacted the state's wheat and barley crop in 2005. 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, Briggs and Ingot, were planted at three South Dakota locations (Brookings, Groton, and South Shore/Watertown) and Robust barley was planted at Brookings. A winter wheat study site at South Shore/Watertown, was lost due to poor stand and heavy cheatgrass pressure. Only the spring wheat data is presented in this report. Trial treatments were from the Uniform Fungicide Trial treatments list for the suppression of FHB and included an untreated check, Folicur (tebuconazole) applied at 4.0 fl oz/A, Prosaro (a premix of prothioconazole and tebuconazole) applied at 6.5 fl oz/A, BAS 555 (metconazole) applied at 10 or 13.5 fl oz/A, and Punch (flusilazole) applied at 6 or 8 fl oz/A. All treatments included Induce, a non-ionic surfactant, applied at 0.125% v/v. Spring wheat trials were planted in a factorial randomized complete block design with six replications. Trial treatments were applied at anthesis (Feekes growth stage 10.51). Plots were inoculated by spreading *Fusarium graminearum* (isolate Fg4) inoculated corn (*Zea mays*) grain throughout the field and providing overhead mist irrigation applied for 3 min out of every 20 minutes from 5:00 pm until 9:00 am each day for two weeks following anthesis at the Brookings location only. Other sites had natural inoculum from corn stalk residue and natural moisture conditions. 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, and test weight. Under dryland conditions at South Shore/Watertown FHB was moderate, with 17.5% FHB field severity on the untreated. FHB incidence and severity were both reduced by all treatments except either rate of Punch. Prosaro and BAS 555 show a numeric improvement over tebuconazole alone. Folicur, Prosaro and BAS 555 provided the best yield and test weight response of the treatments. A similar response was noted at Groton, SD with BAS 555 generally providing the best response under those environmental conditions and slightly less FHB, 12.7% field severity in the untreated. At the irrigated Brookings location FHB was severe, 46% field severity on the untreated. Under these extreme conditions, no treatments reduced FHB incidence and only Prosaro and the BAS 555 treatments reduced FHB field severity and increased yield. Test weight was increased and FHB severity decreased by all treatments at Brookings except for the low rate of Punch.

2005 UNIFORM TRIALS FOR THE PERFORMANCE OF BIOLOGICAL CONTROL AGENTS IN THE SUPPRESSION OF FUSARIUM HEAD BLIGHT IN SOUTH DAKOTA

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ABSTRACT

Fusarium head blight (FHB – scab) has been a serious concern for wheat and barley producers in South Dakota for ten years and was very severe in parts of SD in 2005. 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. Ingot hard red spring wheat and Robust barley were planted at Brookings, South Dakota. Trial treatments included an untreated check; Folicur (tebuconazole) applied at 4.0 fl oz/A; C3R5 (*Lysobacter enzymogenes*) from University of Nebraska, Lincoln, NE, C3 + Folicur coapplied, 1BA (*Bacillus subtilis*) from South Dakota State University, Brookings, SD, 1BA + Folicur coapplied, TrigoCor 1448 (*Bacillus* sp.) from Cornell University, Ithaca, NY, and TrigoCor 1448 + Folicur coapplied. Treatments were grown on site according to specifications from their originating labs and applied at anthesis. Plots were inoculated by spreading *Fusarium graminearum* (isolate Fg4) inoculated corn (*Zea mays*) grain throughout the field at least ten days prior to flowering (wheat) or head emergence (barley) throughout the field and providing overhead mist irrigation applied for 3 min out of every 20 minutes from 5:00 pm until 9:00 am each day for two weeks following treatment. Twenty-one days following treatment, plots were evaluated for FHB incidence, FHB head severity, and FHB field severity. Plots were harvested for yield and test weight and samples were collected for Fusarium damaged kernels (FDK) and deoxynivalenol (DON). Substantial lodging occurred in the barley plots so yields of barley were highly variable.

Under the misted environment in 2005, FHB was severe at this location. FHB incidence was as high as 96% on spring wheat and near 100% on barley. FHB plot severity ranged from about 41-53% in spring wheat. Barley data is forthcoming. No significant improvements over the untreated were observed among the biological treatments for disease suppression or yield enhancement. Also, the Folicur treatment did not provide the level of disease suppression observed in other trials and in other years. The combined treatments of BCAs with Folicur also provided no greater response than any component individually. Test weight was impacted by two BCAs; C3 + Folicur and 1BA + Folicur improved test weight significantly over the untreated check, but not more than Folicur alone. However, the addition of the BCA allowed means separation from the untreated. Folicur alone, while numerically better than the untreated, did not significantly improve test weight unless the BCAs were present.

FLUID BED DRYING OF *CRYPTOCOCCUS NODAENSIS* OH 182.9; A BIOCONTROL AGENT OF FUSARIUM HEAD BLIGHT

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OBJECTIVE

To evaluate the feasibility of using fluid bed drying to prepare stable wettable granules of *Cryptococcus nodaensis* OH 182.9 for control of Fusarium head blight.

INTRODUCTION

Cryptococcus nodaensis (nomen nudem) OH 182.9 (NRRL Y-30216) has been shown to be effective in controlling Fusarium head blight (FHB) in greenhouse and field studies (Khan et al. 2004; Schisler et al. 2002). In an effort to transform *C. nodaensis* into a commercially viable FHB biological control option, studies in our laboratory have been performed to optimize its production and bioefficacy (Zhang et al. 2005a; Zhang et al. 2005b). Additional research is still needed to develop cost effective methods of drying and stabilizing *C. nodaensis* to produce a product with a suitable shelf-life. Optimization of the formulation and application parameters are critical steps in the product development process.

Wettable powders and granules have long been the ideal method of formulating microbial biological control agents. Successful wettable powders and granules offer ease of use, convenience for transportation, improved shelf-life and consumer acceptance. These dried products can be formed by a variety of methods such as, air drying, spray drying or fluid bed drying. Spray drying and fluid bed drying have long been used to produce active dry yeast for the food industry due to their reliability, low costs and high throughput (Grabowski et al. 1997; Luna-Solano et al. 2003; Luna-Solano et al. 2005). Fluid bed drying is gener-

ally considered the less stressful of the two for drying microbial cells.

MATERIAL AND METHODS

Biomass Production - *Cryptococcus nodaensis* (nomen nudem) OH 182.9 (NRRL Y-30216) was produced in a B Braun D-100 fermentor charged with 80 L of SDCL medium (Slininger et al., 1994). To initiate a production run, cells from a log-growth stage SDCL culture served as a 5% seed inoculum for the D-100 fermentor initially set at 25°C. Twenty-four hours after inoculation, the temperature was reduced to 15°C to cold shock the cells for 24 hrs prior to harvest. After completion of biomass production at approximately 48 h, colonized reactor broth was concentrated into a paste using a Sharples 12-V tubular bowl centrifuge. The cell paste was frozen at -80°C until use.

Granulation -Uniform spheres of *C. nodaensis* were produced by dropping droplets of 10% (w/w) aqueous solution into a rotating bed of perlite (Harborlite 1500 S, Harborlite Corp. Santa Barbara, CA) using a 20 ga needle and peristaltic pump. The rotating bed was a modified seed coater spinning at approximately 45 rpm. Excess free perlite was removed with sieving with 1 mm screen.

Fluid bed drying - was performed with a Niro-Aeromatic fluid bed dryer type STR-1 (Niro-Aeromatic Inc, Columbia, MD). Five hundred g batches of the wet *C. nodaensis* spheres were dried at 30°C and an air volume of 90 m³/h. Five replicate dryings were performed. The relative humidity of the ambient air was ~60% with no humidity controls in place. Samples were taken every 3 minutes of drying

and assayed for moisture content and viability. Moisture content ((wet-dry)/wet) of the samples was determined with a moisture analyzer (Mark I, Denver Instruments, Tempe, AZ).

Viability testing - Air-dried samples were resuspended in 50 ml of weak (0.03%) phosphate buffer, mixed in a Stomacher 80 (Seward Inc., England) with the normal setting for 60 s. Serial dilutions were made for each sample and plated on TSBA/5 media. Plates were incubated at 25°C for 2 days until colony counting.

RESULTS AND DISCUSSION

Fluid bed drying requires the product to be in granular form for the most cost-effective method of drying. To address this problem, a simple process for forming small (~3 mm) spheres of cell slurry was developed to provide a uniform product for fluid bed drying trials. The spheres had good flow behavior in the dryer with no problems of clumping and easily form a fluid bed. The process was developed after initial efforts to form extruded pellets proved unsuccessful. The method is applicable to other organisms, scalable and allows for easy incorporation of a number of adjuvants.

The results of fluid bed drying of *C. nodaensis* are presented in figure 1. Moisture content and viability of the cells were monitored over the course of drying. The results show *C. nodaensis* viability was well maintained until the moisture content decreased below 20%. Below 20% moisture content, a gradual loss of viability is observed until a final moisture content of 2-3%. The viability loss across this range of moisture is on the magnitude of one log. Additional experiments are needed to identify the optimum moisture content for storage. For comparison, the optimum moisture content for storage of baker's yeast is 7.0-8.5 % (Beker and Rapoport 1987).

The current work demonstrates fluid bed drying is a viable option for the drying of *C. nodaensis*. Additional optimization of the drying parameters, such as, temperature, humidity of incoming air, residence time, final moisture content and incorporation of stabilizing

adjuvants should enhance product shelf life and bioefficacy.

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DISCLAIMER

Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture. Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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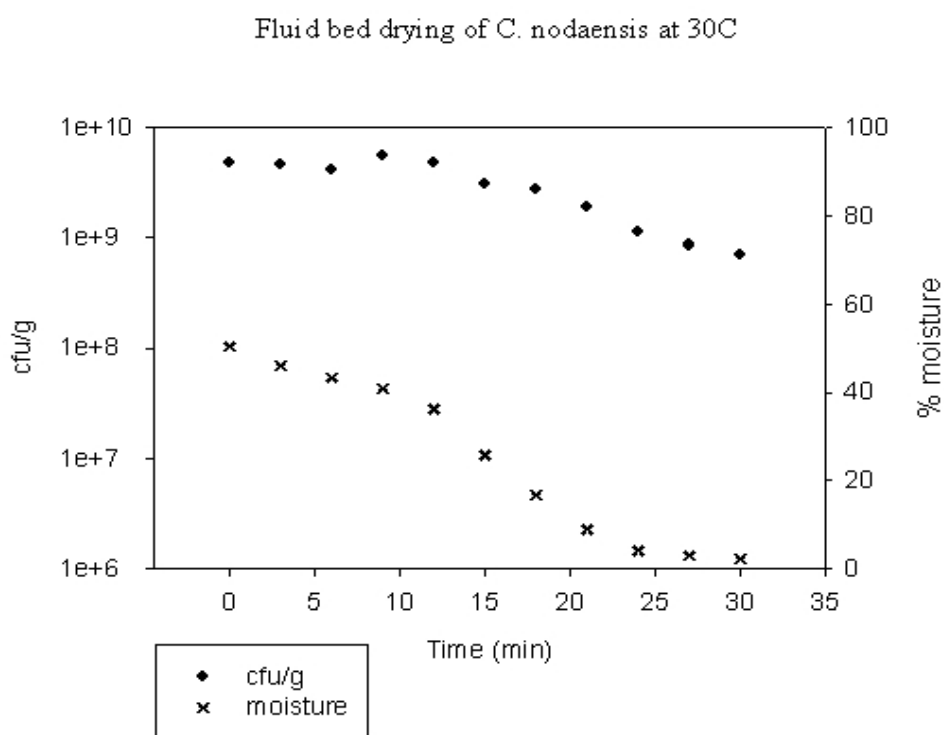


Figure 1. *C. nodaensis* viability and moisture content during fluid bed drying.

OSMOTIC SHOCK TOLERANCE AND MEMBRANE PROPERTIES
OF *CRYPTOCOCCUS NODAENSIS* OH 182.9; A BIOCONTROL
AGENT OF FUSARIUM HEAD BLIGHT

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ABSTRACT

Drying and stabilizing microbial biological control agents present a challenging problem. Drying and subsequent rehydration puts the microbe in high osmotic pressure gradients which can damage the cells. Understanding how cells respond to these pressures should lead to better methods for drying and rehydrating these cells. Our laboratory has previously shown *Cryptococcus nodaensis* OH 182.9 should be a suitable commercial biological control candidate for Fusarium head blight. Developing *C. nodaensis* into a commercially viable biocontrol agent requires knowledge of its environmental limitations. Our laboratory has previously shown *C. nodaensis* becomes more desiccation tolerant after cold shocking at 15°C for twenty-four hours. The current study evaluates the osmotic shock tolerance of *C. nodaensis* with and without cold shocking. In addition, the membrane transition temperature of the cells is determined through fluorescence anisotropy experiments. The results show cold shocking *C. nodaensis* results in improved osmotic shock tolerance and changes in the cell membrane.

EFFECT OF NOZZLES ON FUNGICIDE EFFICACY FOR CONTROL OF FUSARIUM HEAD BLIGHT ON BARLEY

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OBJECTIVES

The study objective was to determine if nozzles with differing drop formation technologies, differing drop sizes, and differing orientations affected the efficacy of fungicide for control of Fusarium head blight (FHB) on barley.

INTRODUCTION

Fungicide applications to small grains for control of FHB have often given results that are inconsistent and need to be improved. Preliminary results from ongoing studies at the Langdon Research Extension Center have shown spray deposition on the grain head can be improved with certain spray volumes and angle orientations. Barley has been the most difficult crop to show consistent control of FHB with fungicide application due to the extensive and tight structure of awns when heading is complete which the recommended time for fungicide application is. The awns provide a filter to minimize the amount of spores that contact and infect the developing grain kernels, but also provide a structure that makes it more difficult to deposit fungicide on the lemma and palea to protect against infection from FHB.

MATERIALS AND METHODS

A study was initiated using ground application equipment to compare several nozzles with different type and size drop formation. The nozzles evaluated were Spraying Systems Co.® (Spraying Systems, 2005) Flat Fan, XR TeeJet XR8002 and XR8003 oriented forward (F), Turbo Teejet TT11001 and TT11002 oriented forward + backward (F+B) and F, respectively, Air Induction AI110015 and AI11002 oriented

F, and AirJet 49880A (liquid orifice # 31) F+B at air pressure of 3.5, 5.0, and 8.5 psi. An untreated plot was included as a control. The classification for the nozzles were XR8002 (fine), XR8003 (medium), Turbo Teejet 11001 and 11002 (medium), Air Induction 110015 (coarse) and 11002 (very coarse), and AirJet 3.5 (very coarse), 5.0 (coarse) and 8.5 (fine) (Spraying Systems, 2005). The study was arranged as a randomized complete block with four replicates. The site was established to 'Tradition' barley on a Barnes/Svea soil on the Langdon Research Extension Center in May of 2005. Previously the site was fallowed. A Fusarium barley inoculum was hand spread 21 and 14 days prior to heading at 122 grams per plot. The most commonly used method to evaluate spray technology is the use of water and oil sensitive paper (WSP Spraying Systems Co.®, Wheaton, Illinois 60189). Water sensitive cards were placed on stands at grain head height in the center of two plots. The card data had not been compiled yet and will be presented in another manuscript at a later date. The tractor mounted sprayer traveled on the left side of the plots with a boom extending to the right of the tractor sprayed area measured 6 x 20 ft. The spray solution contained Bayer CropScience's Prosaro SC fungicide at 6.5 fl. oz. / Acre, Induce adjuvant at 0.125% v/v, and a dye (F D&C Blue #1) at 22 grams/acre. Additionally, barley heads were sampled after spray application from three replicates. Each sample consisted of 5 heads. The heads were shaken for 2 minutes to remove the dye in 80 ml of 95% ethanol and the absorbance determined by a Jenway spectrophotometer. A regression curve was established from a dilution of the original spray sample and the absorbance of the spectrophotometer is presented as dilution data for a comparison of head coverage among the nozzle treatments. The tractor traveled at 6 mph for the study and the spray volume was

10 GPA. North Dakota State University Extension recommended production practices for Northeast North Dakota were followed. A visual estimation was made from 20 samples per plot collected 20 days after fungicide application to estimate the incidence (number of spikes infected) and field severity (number of FHB infected kernels per head divided by total kernels per individual spike) of FHB in each plot. Each plot was harvested with a Hege plot combine and the grain sample cleaned and processed for yield, protein, and test weight determination and plump on barley. A sub sample was ground and analyzed for the toxin deoxynivalenol (DON) by North Dakota State University. 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

The North Dakota Agricultural Weather Network Weather Station recorded over 7 inches of precipitation in June. The total was more than double what is considered the 30 year normal. No additional misting was added to the trial. Disease levels were moderate to high considering the low levels of inoculum present in the area. Disease incidence was greater than 90% on all treatments but not different among treatments (Table 1). The greatest amount of dye on the head was with nozzles that produced fine or medium drops, dilution 4.3-Flat Fan XR8002, 4.9-Turbo Teejet 11001, and 4.3 AirJet 8.5 psi and smallest amount of coverage with coarse type drops. Yield, plump, and proteins were not different among treatments. Deoxynivalenol concentration was not different among fungicide treatments but coarse type drops trended toward less reduction in DON compared to the untreated. There were strong correlations between FHB

incidence and severity, test weight and incidence and severity, plump and test weight, and dilution on protein. Weaker correlations were determined between DON and plump and dilution and incidence and test weight (Table 2). F+B facing nozzles offer no measurable advantage when travel speeds were 6 mph. Data trends indicate that coarse and very coarse drops may reduce fungicide deposition on the grain head and result in increased DON. A study conducted on hard red spring wheat in 2004 (unpublished) showed increased fungicide levels on the heads with small medium drop size (XR8002) nozzles, compared to fine drop size (XR8001) nozzles and large medium drop size (XR8003) nozzles.

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Table 1. FHB Incidence and Field Severity, Yield, Test Weight, Plump, Protein, and Dilution by Nozzle Type, Size, and Orientation on Tradition Barley, Langdon 2005.

Nozzle Class ^y and Type	Size, Orient., or Air (Water) Pressure PSI	FHB		Field Severity %	Yield Bu/A	Test Weight Lb/Bu	Plump %	DON PPM	Protein %	Dilution 10 ⁻⁴
		Incidence %	Severity %							
Fine										
Flat Fan	XR8002 (35) F	96	13.3	98.9	47.8	93	1.1	11.7	4.3	
Air Jet	8.5 psi (21) F+B	98	9.2	99.5	47.6	95	1.2	11.8	4.3	
Medium										
Flat Fan	XR8003 (13) F	90	8.0	103.7	48.6	96	1.0	11.7	3.2	
Turbo Teejet	11001 (35) F+B	100	17.9	102.2	47.3	95	1.0	12.0	4.9	
Turbo Teejet	11002 (35) F	96	11.7	96.5	47.8	95	1.0	12.0	3.9	
Coarse										
Air Jet	5.0 psi (21) F+B	91	9.4	101.6	48.0	94	1.6	11.6	3.9	
Air Induction	110015 (60) F	96	10.9	101.2	47.8	95	1.0	11.7	3.5	
Very Coarse										
Air Induction	11002 (35) F	94	10.2	104.6	48.1	95	1.2	11.8	3.8	
Air Jet	3.5 psi (21) F+B	96	13.0	99.6	48.3	95	1.7	11.8	4.0	
Untreated		98	14.9	101.2	47.8	94	2.6	11.3	2.9	
LSD		NS	6.1 ^z	NS	0.8*	NS	0.8	NS	1.0	
% C.V.		7	36	7	1	1	40	6	11	

^y Spraying Systems Mobile Systems Products Catalog 49A. 2005. TeeJet Mid-Tech North 403 North Main Hartford, South Dakota 57033.

^z P=0.1

Travel Speed 6 MPH and application rate 10 GPA on all plots.

Table 2. Pearson Correlation of Dependent Variables in Nozzle Study, Langdon 2005.

Dependent Variable	FHB Incidence	Severity	Yield	Test Weight	Plump	DON	Protein	Dilution
Incidence	1.0	0.4746 0.0020	-0.2335 0.1470	-0.03217 0.043	0.0417 0.7985	-0.0251 0.8779	-0.0582 0.7213	0.3458 0.0613
Severity		1.0	-0.1473 0.3645	-0.3237 0.0416	-0.1720 0.2887	-0.0683 0.6754	0.1786 0.2702	0.2873 0.1237
Yield			1.0	0.2805 0.0800	0.2527 0.1156	0.0971 0.5510	0.0305 0.8517	0.0895 0.6381
Test Weight				1.0	0.3305 0.0372	0.0522 0.7489	0.0978 0.5483	-0.3483 0.0593
Plump					1.0	-0.3079 0.0533	0.0998 0.5401	0.1765 0.3508
DON						1.0	-0.1574 0.3322	0.3017 0.1051
Protein							1.0	-0.4004 0.0283
Dilution								1.0

Correlation Coefficient
 Prob > |r| under H0: Rho=0

THE EFFECT OF PREVIOUS CROP RESIDUE, CHAFF MANAGEMENT, AND TILLAGE SYSTEM ON FUSARIUM HEAD BLIGHT OF BARLEY

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ABSTRACT

The effects of durum or barley crop residue, chaff collection and removal from the field, and tillage system on Fusarium head blight (FHB) were examined in 2004 and 2005 at the Langdon Research Extension Center, Langdon North Dakota. Robust barley was planted to plots previously cropped durum or barley with the previous crops' chaff either dropped in the plot (typical of most producer management strategies) or collected and removed. A conventional tillage system, spring tooth cultivation both fall and spring before planting, was compared to rototill, both fall and spring to simulate moldboard plowing, and notill. The management systems evaluated typically leaves three distinct residue levels in the field. Barley as a previous crop left more residue in the field after planting. Collecting and removing chaff at harvest reduced residue levels the following spring but only slightly. Residue levels at planting were greater with notill than conventional or rototill. Yields were greater following durum and slightly greater when chaff was collected and removed. Yields were similar among tillage systems in 2004 when inoculum amounts were small but less with notill in 2005 when environment conditions provided greater amounts inoculum. Previous crop barley had greater plump than durum indicating advantage to rotation. Notill had less plump than the other systems. No differences were determined in FHB incidence, field severity, or deoxynivalenol levels regardless of treatment except for a small incidence difference one year. While it is likely that previous crop may contribute to FHB, this study indicates that localized residues may make minimal contribution to FHB when disease levels are moderate or high in the surrounding area.

AERIAL APPLICATION OF FUNGICIDE ON BARLEY

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ABSTRACT

Fusarium head blight (FHB) has been a major problem for cereal grain producers during the past decade. To combat this disease, growers have applied fungicide by both aerial and ground application. About 50% of the small grains acreage sprayed with fungicide in the Dakota-Minnesota region of the Great Plains are applied with spray planes. An aerial application study was conducted near Esmond to evaluate fungicide application for control of FHB on 'Drummond' barley. The study was designed as a randomized complete block with three replicates. The treatments included the fungicide Folicur (tebuconazole) applied with spray of 3 or 7 GPA applied with a fine and a medium size drop and a volume of 5 GPA applied with a medium size drop. The applications were made to heading barley (greater than 50% of main stem heads fully extended from the boot). The fungicide was applied with Induce adjuvant at 0.125% v/v and F D&C Blue #1 dye at 22 grams per acre. The dye is a food grade type. A common method to evaluate spray technology is the use of water and oil sensitive paper. Water sensitive cards, were placed at grain head height on stands. One card was placed horizontal. Other cards were placed vertical, back to back and oriented forward and backward and right and left on stands within the sprayed plots. Stain size was determined with WRK Droplet Scan system. Three 50 ft spray passes were made side by side (150 ft.) on each plot. All data was collected from the center of the plot. Additionally, 5 heads were collected at 5 points across the swath width and placed in Erlenmeyer flasks for determination of head coverage by washing the food dye with a 90% alcohol solution and recording absorbance with a spectrophotometer. Field counts were determined by a visual assessment of FHB and foliar disease at mid dough growth stage by assessing twenty heads in two locations per plot and determining the incidence of the disease and the severity of the individual head. The incidence x the severity of the 20 heads gave a field severity per plot. Foliar disease differences were determined by estimating the infected area on 5 leaves at two locations. Data were analyzed with the general linear model (GLM) in SAS. Least significant differences (LSD) were used to compare means at the 5% probability level. Differences in all measurement parameters were not significant due to almost 100% incidence in both the treated and untreated plots and high field severity. No differences in yield, test weight, plump, protein and DON were found. Drop size differences were also found to be insignificant due to large variations in coverage and drops deposited on the water sensitive cards were likely due to orientation of the plots and the variable wind speed recorded during spray application. Coverage on the head, although not significantly different trended to greater amounts with smaller spray volumes. This is due to the exceptional efficiency of small drops depositing on small collectors (awns).

ACKNOWLEDGEMENT

This information is based upon work supported by the US Department of Agriculture, under agreement No. 59-0790-3-079. This is a cooperative project with the US Wheat and Barley Scab Initiative. Thanks is provided to the barley grower, Louis Arnold of Esmond, ND for the use of his field to complete this study.

2005 FHB UNIFORM FUNGICIDE TRIAL ON HARD RED SPRING WHEAT IN MINNESOTA

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OBJECTIVE

Evaluate and compare *Fusarium* head blight (FHB) control efficacies of non-registered fungicide products when applied on hard red spring wheat in Minnesota.

INTRODUCTION

Fusarium head blight was originally described more than a century ago. Since that time the disease has caused severe and repeated epidemics on small grain crops resulting in billions of dollars in crop losses (McMullen et al., 1997; Wood, 2002). More specifically, Nganje et al. (2004) estimated the recent 1993-2001 FHB epidemics caused economic losses of greater than \$5.2 billion in Minnesota and North Dakota alone. The disease remains 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 plant infection by *Fusaria graminearum* is largely dependent on environmental conditions prior to, and during the time when the crops are in susceptible growth stages. Cultural disease management strategies (i.e.: crop rotation, tillage, and field sanitation) have resulted in partial disease suppression. Likewise, additional suppression has been achieved from the application of select fungicide products at Feekes 10.51 (early flowering growth stage). Ongoing research into disease control efficacies of experimental fungicides is needed to preserve yield and quality of small grains in regions most at risk for catastrophic crop losses.

MATERIALS AND METHODS

Hard red spring wheat cultivar 'Oxen' was planted 4 May 2005 into wheat residue at 1.25 million live seed acre⁻¹ in a randomized complete block design with four replicates. Each plot was inoculated with 112 kg ha⁻¹ of *F. graminearum* infested corn grain six weeks after planting. Early morning misting (10 minute intervals every 80 minutes between 4:00 am to 8 am) was initiated seven weeks after planting. When the weather moderated and soils were not saturated, the misting duration was increased. Misting was continued until approximately the hard dough growth stage (Feekes 11.2), but was discontinued temporarily during the growing season if weather events caused soil saturation. On 6 June, an application of tank mixed herbicides (MCPA, Harmony GT, and Puma) was made to control weeds.

Eight weeks after planting (1 July), fungicide treatments were applied to wheat at the early-flower growth stage. Treatment applications were made with a CO₂ backpack-type sprayer adjusted to 40 psi at 18-20 gpa with forward and backward positioned 'XR' Teejet flat fan 8001 VS nozzles. On 15 July, disease severities were recorded from flag leaves, and 50 spikes plot⁻¹ were collected and frozen until rated for FHB symptoms. The test was harvested 14 weeks after planting, on 9 August.

Fusarium head blight severities were estimated according to the visual scale published by Stack and McMullen (1995), while percent visually scabby kernels (VSK) was estimated using a set of grain standards based on Jones and Mirocha (1999). Percent leaf disease was estimated using James (1971). Grain 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

Two fungicide treatments resulted in the least amount of FHB incidence symptoms (Prosaro 6.5 fl oz/a and BAS555 13.5 fl oz/a) and were not significant from the duplicate Folicur 4 fl oz/a treatments ($P=0.01$) (Table 1). Two treatments (Punch 8 fl oz/a and Prosaro 6.5 fl oz/a) resulted in less FHB severity, but were not significant from either Folicur 4 fl oz/a treatment, BAS555 10 fl oz/a, or BAS555 13.5 fl oz/a ($P=0.1$). FHB index values ranged from a low of 3.4% to a high of 6%. The Prosaro 6.5 fl oz/a treatment resulted in the smallest FHB index value, but it was not significantly different from BAS555 13.5 fl oz/a, Folicur 4 fl oz/a, and Punch 8 fl oz/a ($P=0.001$). Visually scabby kernels varied from 5.5% to 10.3%. Numerically, the BAS555 (10 fl oz/a) treatment resulted in the most scabby kernels, but it was not significantly different than most other treatments ($P=0.03$). Application of Prosaro 6.5 fl oz/a and BAS555 13.5 fl oz/a resulted in significantly fewer scabby kernels compared with BAS555 10 fl oz/a. DON means were relatively similar across treatments, ranging from 2.2 ppm (Prosaro 6.5 fl oz/a) to 4.8 ppm (nontreated). The treatment resulting in the least amount of DON (Prosaro 6.5 fl oz/a) was not significantly different from BAS555 13.5 fl oz/a or Folicur 4 fl oz/a ($P<0.0001$). Fungicide treatments had increased kernel protein levels compared with the nontreated control. Folicur 4 fl oz/a, BAS555 13.5 fl oz/a, and Prosaro 6.5 fl oz/a treatment means were similar (15.3% to 15.4%) and were not statistically different from most of the other treatments ($P=0.0003$). Overall, Prosaro 6.5 fl oz/a, BAS555 13.5 fl oz/a, Punch 8 fl oz/a, and Folicur 4 fl oz/a performed well for test weight ($P=0.0003$) and yield ($P<0.0001$).

In general, the non-registered fungicides showed increased FHB control over the current industry stan-

dard treatment (Folicur 4 fl oz/a), but the difference was not significant. However, compared with the nontreated control, non-registered fungicides resulted in significantly better disease control (e.g.: FHB incidence, FHB index, DON levels, percent protein, leaf disease severity, and yield).

ACKNOWLEDGEMENTS

The authors would like to thank the U.S. Wheat and Barley Scab Initiative and the Northwest Research and Outreach Center for supporting this research; BASF Corp., Bayer CropScience, and DuPont Crop Protection for supplying fungicides; and the University of Minnesota Mycotoxin lab for providing DON results.

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Table 1. Fusarium head blight (FHB) and leaf spot disease responses from ‘Oxen’ hard red spring wheat in Crookston, Minnesota during 2005.

Treatment/Active Ingredient	Fusarium Head Blight									
	*DI ² (%)	**DS (%)	*DX (%)	*VSK (%)	*DON (ppm)	*Protein (%)	*LD ³ (%)	*Test Wt. (lb/bu)	*Yield (bu/A)	
1. Nontreated control.....	50.0a	12.3ab	6.1a	9.0abcd	4.8a	14.8c	2.5a	57.6c	57.1d	
2. Folicur ¹ 432SC 4.0 fl oz/a... • tebuconazole	36.0cd	10.3bc	3.7cd	6.5cd	3.0def	15.4a	0.3d	59.4a	64.6ab	
3. Folicur ¹ 432SC 4.0 fl oz/a... • tebuconazole	39.5bcd	11.5abc	4.5bc	7.0bcd	3.7bcd	15.3ab	1.2c	59.2ab	63.9b	
4. Prostaro ¹ 6.5 fl oz/a..... • tebuconazole & prothioconazole	33.0d	10.2c	3.4d	5.5d	2.2f	15.4a	1.9b	60.0a	67.3a	
5. BAS555 ¹ 01F 13.5 fl oz/a.... • metconazole	33.0d	10.3bc	3.5cd	6.0d	2.5ef	15.4a	0.2d	59.7a	65.7ab	
6. BAS555 ¹ 01F 10.0 fl oz/a.... • metconazole	45.5ab	11.7abc	5.3ab	10.3abc	4.6ab	15.2b	0.2d	59.4a	60.6c	
7. Punch 6.0 fl oz/a..... • flusilazole	43.5abc	12.7a	5.4ab	7.5abcd	4.3abc	15.2ab	0.3d	59.1ab	64.0b	
8. Punch 8.0 fl oz/a..... • flusilazole	45.5ab	9.8c	4.4bcd	6.5cd	3.4cde	15.3ab	0.2d	59.2ab	64.7ab	
CV	16	13	18	33	16	1	46	1	3	

¹Fungicide treatment included 0.125% Induce.

²Treatment abbreviations are DS, FHB severity; DI, FHB incidence; DX, FHB index; VSK, visually scabby kernels; DON, deoxynivalenol; LDS, leaf disease severity; Test wt., bushel test weight.

³Fungal foliar diseases consisted of Septoria/Stagonospora blotch complex (*Septoria tritici* and *Stagonospora nodorum*) and tan spot (*Pyrenophora tritici-repentis*). Data were logarithmically transformed.

NOTE: LSD significant at 0.10 probability level (***) and at 0.05 probability level (*).

2005 FHB UNIFORM FUNGICIDE TRIAL ON HARD
RED WINTER WHEAT IN MINNESOTA
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ABSTRACT

Hard red winter wheat cultivar 'Jerry' was planted on 29 Sept. 2004, into wheat residue at the Northwest Research and Outreach Center near Crookston as part of the uniform fungicide trials for Fusarium head blight control. The objectives of the trial were to evaluate and compare Fusarium head blight (FHB) control efficacies of non-registered fungicide products when applied to winter wheat in Minnesota. The test was arranged in a randomized complete block design with four replicates. Each plot was inoculated 10 June 2005, with 112 kg ha⁻¹ of *F. graminearum* infested corn grain. Fungicide treatments were applied on 20 June when plants were at the early flowering growth stage (Feekes 10.51). Fungicide treatments were applied using a CO₂ backpack-type sprayer adjusted to 40 psi at 18-20 gpa with forward and backward positioned 'XR' Teejet flat fan 8001 VS nozzles. Treatments consisted of: (1) nontreated control; (2) duplicate Folicur (tebuconazole) 4 fl oz acre⁻¹ treatments; (3) Prosaro (tebuconazole + prothioconazole) 6.5 fl oz acre⁻¹; (4) BAS555 (metconazole) 13.5 fl oz acre⁻¹; (5) BAS555 (metconazole) 10.0 fl oz acre⁻¹; (6) Punch (flusilazole) 6 fl oz acre⁻¹; and (7) Punch (flusilazole) 8 fl oz acre⁻¹. On 7 July, flag leaf disease severities were recorded. The same day 50 spikes plot⁻¹ were collected and frozen until rated for FHB symptoms. The test was harvested on 29 July. 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 of FHB incidence means ranged from 16.5% (Prosaro 6.5 fl oz) to 7.0% (BAS555 13.5 fl oz), FHB severity means ranged from 10.8% (Punch 8 fl oz) to 7.7% (BAS555 10 fl oz), and FHB indexes ranged from 1.7% (Punch 6 fl oz) to 0.7% (BAS555 13.5 fl oz). Treatment means were not significant for most test parameters (e.g.: FHB severity, FHB index, protein, test weight, thousand kernel weight and yield). FHB incidence, grain DON content, and leaf disease severity means were significant at $P=0.05$, $P=0.1$, and $P=0.05$ respectively. Test results will be published in the 2006 *Fungicide and Nematicide Tests*.

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2005 FHB UNIFORM FUNGICIDE TRIAL ON
SPRING BARLEY IN MINNESOTA
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ABSTRACT

A six-row spring barley cultivar 'Robust' was planted on 3 May 2005 into wheat residue at the Northwest Research and Outreach Center near Crookston as part of the uniform fungicide trials for Fusarium head blight control. The objectives of the trial were to evaluate and compare Fusarium head blight (FHB) control efficacies of non-registered fungicide products when applied to spring barley in Minnesota. The test was arranged in a randomized complete block design with four replicates. Each plot was inoculated six weeks after planting with 112 kg ha⁻¹ of *F. graminearum* infested corn grain and misted when weather was dry. Misting was discontinued during the hard dough development stage. Seven weeks after planting, fungicide treatments were applied to barley at Feekes 10.5 (early heading growth stage). Fungicide treatments were applied using a CO₂ backpack-type sprayer adjusted to 40 psi at 18-20 gpa with forward and backward positioned 'XR' Teejet flat fan 8001 VS nozzles. Treatments consisted of: (1) duplicate nontreated control treatments; (2) duplicate Folicur (tebuconazole) 4 fl oz acre⁻¹ treatments; (3) duplicate Tilt (propiconazole) 4 fl oz acre⁻¹ treatments; (4) Prosaro (tebuconazole + prothioconazole) 6.5 fl oz acre⁻¹; (5) BAS555 (metconazole) 13.5 fl oz acre⁻¹; (6) BAS555 (metconazole) 10.0 fl oz acre⁻¹; (7) Punch (flusilazole) 6 fl oz acre⁻¹; and (8) Punch (flusilazole) 8 fl oz acre⁻¹. On 8 July, flag leaf disease severities were recorded. The same day 50 spikes plot⁻¹ were collected and frozen until rated for FHB symptoms. The test was harvested 13 weeks after planting, on 1 August. 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 of FHB incidence means ranged from 56% (nontreated) to 32% (Punch 6 fl oz), FHB severity means ranged from 2.6% (Tilt 4 fl oz) to 2.0% (BAS555 10 fl oz), and FHB indexes ranged from 1.4% (nontreated) to 0.6 (Punch 6 fl oz). Treatment results were not significant for most test parameters (e.g.: DON content, FHB incidence, FHB severity, FHB index, leaf disease severity, protein, test weight, and yield). Barley plump and thousand kernel weight mean results were significant at $P=0.1$. Test results will be published in the *2006 Fungicide and Nematicide Tests*.

ACKNOWLEDGEMENTS

The authors would like to thank the U.S. Wheat and Barley Scab Initiative and the Northwest Research and Outreach Center for supporting this research; BASF Corp., Bayer CropScience, and DuPont Crop Protection for supplying fungicides; and the University of Minnesota Mycotoxin lab for providing DON results. This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0790-9-053. This is a cooperative project with the U.S. Wheat and Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

COOPERATIVE STUDY FOR IMPROVED MANAGEMENT OF FUSARIUM HEAD BLIGHT USING AERIAL APPLICATION OF FUNGICIDE

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OBJECTIVE

To evaluate the efficacy of aerial fungicide application with different spray volumes and droplet sizes for Fusarium head blight (FHB) management in hard red spring wheat.

INTRODUCTION

Fusarium head blight is difficult to manage in small grains if environmental conditions promote infection and disease development when crops are at susceptible growth stages. Extension plant pathologists, and others, suggest an integrated approach for managing the disease. Recommendations focus on crop rotation, residue management, cultivar resistance, and a timely application of fungicide (Watkins and Doupnik, 1996; McMullen and Stack, 1999; Hollingsworth, 2004; Stromberg and Thomason, 2005). During 1996, Wilcoxson published a comprehensive review of reported research that contributes to our body of knowledge regarding fungicide control efficacies. Progression of FHB disease symptoms after application of fungicides has been studied extensively. For more information on this topic, see the 'Chemical, Cultural and Biological Control' section of this publication for recent cooperative results from the Fusarium head blight uniform fungicide trial.

New fungicide chemistries offering increasingly effective control of FHB are limited, making it imperative that existing fungicides are applied in such a manner to achieve maximum disease control. Spreading a fungicide active ingredient uniformly onto glume tissues in

sufficient volumes to manage the disease is difficult. Non-target plant tissues (e.g.: awns, leaves) compete for droplets, reducing the amount of fungicide product available to protect susceptible plant tissues. Our objectives were to establish whether aerial fungicide application technologies could be modified in such a way as to increase fungicide deposition on plant tissues by adjusting droplet sizes and dilution volumes for increased disease control using fungicide.

MATERIALS AND METHODS

Treatment parameters. This cooperative research effort included three Red River Valley experimental locations situated within commercial fields of hard red spring wheat (locations north to south: St. Thomas, ND; Crookston, MN; Hunter, ND). A total of three wheat cultivars, susceptible to FHB, were tested ('Reeder' cv at St. Thomas, 'Polaris' cv at Crookston, and 'Briggs' cv at Hunter). All three fields were planted during late April and production inputs were managed by cooperating growers to achieve optimum yields. Three replications of treatments (five fungicide and one nontreated control) were arranged in a randomized complete block design at each test location. Treatments tested at each location included combinations of two fungicide dilutions (3.0 and 7 gpa) in two sizes of spray droplets (200 and 350 μm), as well as the industry standard treatment (5 gpa, 275 μm). Each plot area measured approximately 150 x 700-1,000 ft. to accommodate three 50 ft. wide fungicide application swaths from an aircraft. Folicur 3.6 F fungicide (e.g.: tebuconazole active ingredient) mixed with Induce adjuvant (0.125% v/v), and a blue food grade

type dye (22 g/a FD&C #1) were applied by a Cessna Ag Truck aircraft operated by Dakota Aviation of Grafton, ND. Different treatment spray volumes were attained by selecting CP-03 nozzles with two orifice sizes (0.125 and 0.171). One application of the fungicide mixture was applied at the labeled rate of 4 oz. acre⁻¹ during June or July (Hunter, 30 June; St. Thomas, 5 July; Crookston, 6 July) when the crop was at early flowering (Feekes growth stage 10.51).

Data collection. Droplet patterns and deposition data were determined from water sensitive cards and grain spikes. Coverage data will be reported elsewhere. Disease data as well as grain yield and quality parameters are reported here. FHB incidence, severity, and index ratings (incidence x severity/100) were recorded from 50-100 heads plot⁻¹ at soft dough stage of kernel development. Leaf disease severity ratings from 10-55 flag leaves plot⁻¹ also were collected at two test sites (Crookston and St. Thomas) during dough development (Feekes growth stage 11.2). At grain maturity, cooperating growers harvested one swath from each plot using commercial grain combines. Harvested grain was transferred into a weigh wagon. Yield (Bu/a) was calculated from swath area and grain weight. Grain sub-samples were collected to determine kernel moisture, protein, test weight, and deoxynivalenol (DON) concentration. A combined statistical analysis of data was conducted across test sites.

RESULTS AND DISCUSSION

Moderate FHB disease pressure occurred at two of three experiment locations (Hunter and St. Thomas, ND), while pressure was much less at Crookston (Table 1). All fungicide treatments resulted in less FHB incidence compared with the nontreated control ($P=0.002$). Severity of FHB was reduced at all test locations from two treatments (5 gpa, 275 μm ; 7 gpa, 200 μm), at two of three test locations from two treatments (3 gpa, 350 μm ; 7 gpa, 350 μm), and at one location from one treatment (3 gpa, 200 μm) compared with the nontreated control ($P=0.001$; Fig. 1). A significant location x treatment interaction occurred with FHB index data ($P=0.0002$). Compared with the nontreated control, all treatment combinations re-

duced FHB index at Hunter and St. Thomas, but not at Crookston. Flag leaf disease severities were reduced from all fungicide treatments at St. Thomas ($P=0.05$). Those treatments with fine droplet sizes (3 or 7 gpa with 200 μm) resulted in increased grain yield at all test locations over the nontreated control while treatments with larger droplet sizes (5 gpa, 275 μm ; 3 or 7 gpa with 350 μm) increased yield at the St. Thomas and Hunter, ND test sites ($P=0.0001$). Kernel test weight was increased at all test locations from one treatment (3 gpa, 200 μm); at two locations from two treatments (5 gpa, 275 μm ; 7 gpa, 350 μm), and at one location by two treatments (3 gpa, 350 μm ; 7 gpa, 200 μm) compared to the nontreated control ($P=0.004$). Only one treatment (3 gpa, 200 μm) at one location (Crookston) increased kernel test weight significantly compared with the industry standard treatment (5 gpa, 275 μm). Kernel protein and DON concentration results were not significant.

Overall, this research establishes that aerial application of fungicide on spring wheat, regardless of droplet size or dilution volume, was of benefit in locations with moderate FHB disease levels. The industry standard treatment (275 μm , 5 gpa) and the '200 μm , 7 gpa' treatment appear to offer a slightly greater and significant level of FHB control (Fig. 2), when averaged over all three locations.

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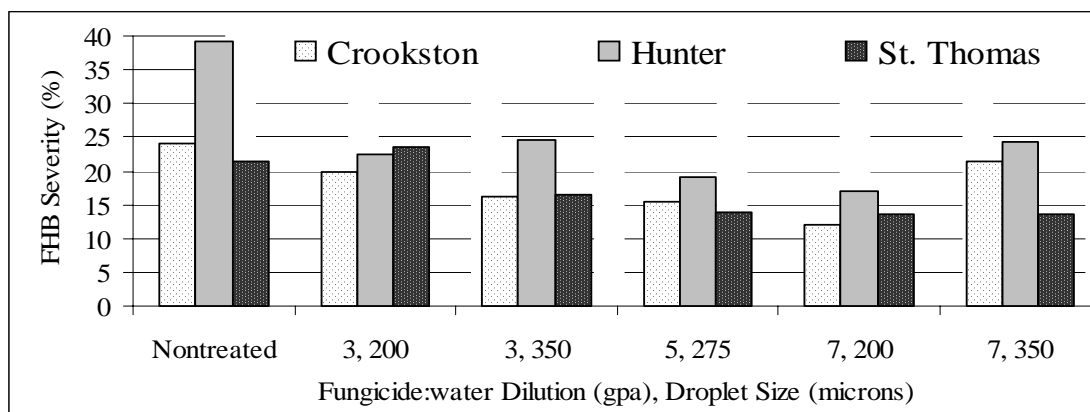


Fig 1. FHB severity of three spring wheat cultivars and locations using aerial application of fungicide with different treatment combinations of fungicide dilutions and droplet sizes.

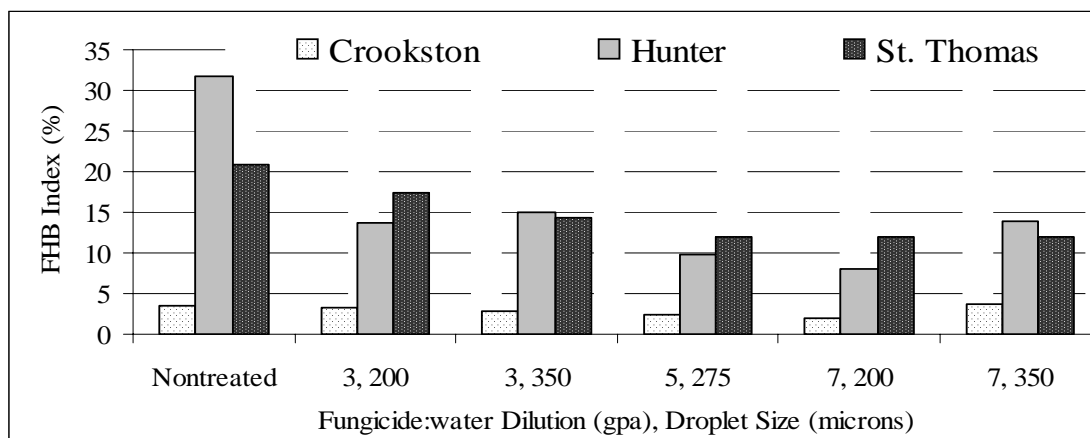


Fig. 2. FHB index values for three spring wheat cultivars and locations using aerial application of fungicide with different treatment combinations of fungicide dilutions and droplet sizes.

Table 1. FHB disease, crop yield, and grain quality parameters tested at three Red River Valley locations and from three spring wheat varieties during 2005.

Spray Volume (gpa)	Drop Size (µm)	Fusarium head blight			Yield (Bu/a)	Test		
		Incidence (%)	Severity (%)	Index (%)		Weight (lb/Bu)	DON (ppm)	Protein (%)
<u>Crookston 'Polaris'</u>								
3	200 µm	16.7	19.8	3.3	63.4	61.5	1.5	13.0
3	350 µm	16.7	16.2	2.8	60.6	60.9	1.3	12.5
7	200 µm	16.7	12.0	2.0	63.6	60.6	1.3	12.9
7	350 µm	16.0	21.4	3.7	61.3	60.8	1.3	12.5
5	275 µm	16.0	15.5	2.5	61.5	60.6	1.0	12.8
Untreated		16.0	24.0	3.4	59.3	60.4	1.2	12.9
<u>Hunter 'Briggs'</u>								
3	200 µm	59.2	22.6	13.8	60.6	61.0	3.1	15.0
3	350 µm	62.5	24.6	15.0	61.0	60.6	2.9	15.0
7	200 µm	47.5	17.0	8.1	56.2	60.5	3.2	15.4
7	350 µm	57.5	24.3	13.9	56.3	60.8	3.4	14.7
5	275 µm	52.5	19.2	9.8	59.4	60.8	2.8	15.5
Untreated		80.8	39.1	31.8	52.8	60.0	5.2	15.6
<u>St. Thomas 'Reeder'</u>								
3	200 µm	79.2	23.4	17.4	45.0	56.4	6.4	15.5
3	350 µm	77.5	16.4	14.3	45.2	56.5	7.2	15.5
7	200 µm	79.2	13.5	12.0	45.1	55.6	7.3	15.6
7	350 µm	76.7	13.7	12.0	45.1	57.0	4.9	15.5
5	275 µm	73.3	13.8	12.0	44.8	56.6	5.8	15.3
Untreated		89.2	21.4	20.9	38.9	54.8	11.8	15.6
Location		0.0001	0.0017	0.0001	0.0001	0.0001	0.0001	0.0001
Treatment		0.0024	0.0006	0.0001	0.0001	0.0040	0.1433	0.1599
Loc*Trt		0.0627	0.4165	0.0002	0.1422	0.5337	0.5952	0.5149
LSD		7.1	5.8	3.0	2.2	0.6	NS	NS
C.V.		14	31	30	4	1	60	3

EFFECTIVE APPLICATION OF FUNGICIDES ON WHEAT HEADS: WHAT'S THE BEST? D.C. Hooker^{1*}, H. Spieser² and A.W. Schaafsma¹

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ABSTRACT

Effective application of fungicides for protection against *Fusarium* head blight is a challenging goal for current application systems. For example, uniform coverage of a fungicide on wheat heads is critical. Spray coverage using a conventional sprayer with single-spaced nozzles on a boom has not been satisfactory. Timing of fungicide application is also critical. Unfortunately, the window of fungicide application in wheat is extremely narrow – just a few days – and the time often coincides with herbicide application in other crops. Therefore, both growers and custom applicators need methods to increase efficiency in chemical coverage within and among wheat heads, and to increase sprayer efficiency across land areas by increasing forward travel speeds – economically – using inexpensive nozzle configurations.

We've been investigating various sprayers and nozzle configurations since the late 1990s. In 2001, UV dye in various sprayers showed extremely variable coverage on wheat heads from various spray configurations. During each year between 2002, and 2005, water sensitive papers (Spraying Systems Co., Wheaton, IL) were used to evaluate spray coverage. These papers were transformed into cylinders to mimic wheat heads before spraying. After each spray treatment, the papers were unfolded, scanned, and analyzed for coverage using SigmaScan Pro Version 5.0 software. Labels on the papers were used to mark the position of the "wheat head" relative to the forward travel of the sprayer. Copper sulfate was used in the spray solution in all years, except 2001, to assess the amount of chemical applied on each "side" of the paper cylinders. The ground sprayer nozzle configurations included the use of Turbo TeeJet® nozzles in a forward-back configuration, TwinJet® nozzles, air induction nozzles, Turbo FloodJet® (single nozzles alternating forward and backward along the boom), FullJet nozzles, and the use of Twin Caps; except in 2005, all nozzle configurations on ground sprayers were compared at forward speeds of 10 and 19 kph (6 and 12 mph) and sprayed at the same water volumes. Ground sprayer configurations were compared with the airplane and helicopter in 2002. All of the nozzle configurations before 2005 were tested in optimal wind conditions (winds <5 kph) and boom heights above the wheat heads. In 2005, the best sprayer nozzle configurations from previous years were tested in sub-optimal spray conditions – wind conditions of approximately 15 kph, and with boom heights higher than the ideal, to mimic field conditions using wide spray booms. In 2005, we also assessed both coverage and fungicide efficacy of various nozzle configurations on field-scale strip plots.

Briefly, the backward-forward nozzle configuration and the FloodJet configuration produced the highest coverage and apparent distribution of chemical on the simulated wheat heads when compared to all other spray applicators. In these two sprayer configurations, a forward speed of 19 kph was equal in total coverage and uniformity of coverage compared to 10 kph at the same water volumes. All other spray nozzle configurations, however, had lower total coverage and higher variability when spraying at 19 kph compared to 10 kph. TwinJet nozzles at 10 kph produced half the coverage of the backward-forward nozzles, but coverage was relatively uniform compared to the Twin Cap and flat fan configurations. Although the spray coverage from the airplane and helicopter was relatively low (<3%), the amount of chemical that reached the "heads" was com

parable to most of the other ground applicator systems, but still less than the backward-forward and FloodJet configurations. Boom height and wind effects had a great impact on total coverage and uniformity of coverage. These data will be presented, along with a ranking of sprayer systems for effective application of fungicides for controlling Fusarium head blight.

EFFECTS OF INDUCED SYSTEMIC RESISTANCE-ACTIVATING AGENTS ON FUSARIUM HEAD BLIGHT

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OBJECTIVES

- I. To determine whether some of the known biocontrol agents for Fusarium head blight (FHB) can activate induced systemic resistance (ISR) in wheat against FHB.
- II. To determine the potential of using ISR-inducing strains of plant growth promoting rhizobacteria (PGPR) to control FHB.
- III. To evaluate PGPR strains and autoclaved fungal biomass (AFB) preparations for effectiveness in controlling FHB in the field.

INTRODUCTION

Chemicals and microorganisms have been reported to activate ISR in wheat and barley against a number of fungal diseases (Gorlach *et al.*, 1996; Steiner and Schonbeck, 1995). Theoretically, ISR could provide several advantages to controlling FHB: 1) protection using ISR-activating agents could be achieved without having to target flowering heads in spray treatments; and 2) application of the agents could be made prior to anthesis because ISR, once activated, can provide longer-term protection than conventional fungicides; and 3) ISR might provide control of other foliar pathogens as well, thus making application of ISR activators more economically practical (Van Loon *et al.*, 1998). The potential of any ISR activator to induce resistance against FHB, however, cannot be determined without direct testing. For example, benzothiadiazole, a commercially-available chemical activator, can induce ISR against powdery mildew in wheat (Stadnick and Buchenauer, 2000), but it did not provide protection against FHB (Yu and Meuhlbaauer, 2001). There are very few other reports

of materials being evaluated for induction of ISR against FHB. In one of them, autoclaved fungal biomass (AFB) prepared from several fungal isolates, reduced the severity of FHB in greenhouse experiments through induced resistance (Khan and Tisserat, 2004). In another, the bacterial biocontrol agent *Lysobacter enzymogenes* strain C3 did not induce systemic resistance in wheat against FHB when applied to flag leaves or roots, but presumably affected FHB partly through localized induced resistance (Yuen and Jochum, 2003). No other reported biocontrol agent for FHB has been investigated for induction of ISR in wheat. Numerous strains of PGPR are known to induce ISR (Kloepper *et al.* 2004; Ramamoorthy *et al.*, 2001; Van Loon *et al.*, 1998). While some were reported to induce resistance against fungal pathogens when applied to foliage of gramineaceous plants (Ekici-Kilic and Yuen, 2004; Ramamoorthy *et al.*, 2001), none have been tested for control of FHB.

MATERIALS AND METHODS

Biological control agents against FHB, strains of PGPR, and AFB preparation used in this study are listed in Table 1. Strain C3 of *L. enzymogenes* was included in all experiments for comparison as a presumed inducer of localized resistance. The PGPR strains were selected for this study because they induced resistance against a fungal pathogen when applied to tall fescue foliage in a previous study (Ekici-Kilic and Yuen, 2004). Strain C3 was cultured in chitin broth for 7 days, while all other bacterial strains were cultured on nutrient broth with yeast extract for 3 days. Whole broth cultures were used in treating plants in greenhouse and field experiments. AFB preparations were made from an isolate of *Aspergillus niger* (AFB1) and *Penicillium 2* (AFB2) by freeze-drying autoclaved mycelia and grinding to powder form. The

preparations were added to water at a rate of 400 mg/L for application in field experiments. All microbial and AFB treatment liquids were amended with the surfactant Induce (0.125%) prior to application to plants.

A greenhouse experiment was conducted to determine whether FHB biocontrol agents can induce ISR against FHB. Scab-susceptible spring wheat cultivar 'Bobwhite' was grown in 15 cm pots (6 plants per pot). Plants were treated with the biocontrol strains or water, as the control, by either spraying flag leaves 3 days prior to pathogen inoculation or spraying flowering heads 1 day prior to inoculation (6 pots per treatment). In the flag-leaf treatment, the heads were shielded from the spray by enclosing them in plastic bags during treatment. All plants were inoculated with the pathogen by spraying a conidial suspension of *Fusarium graminearum* (5×10^5 spores/ml) onto the heads at anthesis. Inoculated plants were placed in a mist chamber for 48 hours to stimulate infection and then transferred back to the greenhouse for scab development over a 14 day period. Scab severity (percent of spikelets on each inoculated head exhibiting scab symptoms) was assessed. Results from multiple heads per pot were averaged prior to performing analysis of variance.

A separate greenhouse experiment was conducted to evaluate PGPR strains for control of FHB. They were compared with *L. enzymogenes* C3 and a water control. Six pots with heads entering anthesis were sprayed with each treatment and inoculated 3 days later with the pathogen. All other methods were as described above.

Two field experiments were conducted, one in Lincoln, NE on winter wheat '2137', the other in Brookings, SD on spring wheat 'Russ'. The treatments in both experiments were *Pseudomonas fluorescens* WCS417, AFB1, AFB2, and *L. enzymogenes* C3. These were compared with tebuconazole (Folicur 3.6F; 295 ml/ha) and a water control. There were six replicate plots (1.2 m X 2.5 m) per treatment. All treatments were applied at 6.6 L/ha at early flowering (Feekes 10.5.1) using a CO₂-pressurized sprayer.

Both trials were inoculated with *F. graminearum* in the form of pathogen-infested corn kernels and utilized mist irrigation systems to stimulate infection. FHB incidence (% heads infected per plot), severity (% spikelets infected per diseased head), and index (plot severity) were determined from at least 40 heads per plot around 3 weeks after anthesis. Results from the two trials were pooled for analysis of variance.

RESULTS AND DISCUSSION

None of the FHB biocontrol agents (1BC, Trigocor 1448, and C3) provided protection against the disease when they were applied to flag leaves of wheat plants (Table 2). Therefore, we conclude that none of them can induce systemic resistance against FHB. The possibility that the biocontrol agents activated resistance locally when applied to wheat heads cannot be ruled out.

Among the three PGPR strains evaluated in the greenhouse, only *Pseudomonas fluorescens* WCS417 reduced FHB severity, but it was not as effective as *L. enzymogenes* C3 (Table 3). This supports findings from a previous study comparing the strains using a different pathogen-host system (Ekici-Kilic and Yuen, 2004); greater efficacy of C3 in that study was attributed to its ability to inhibit the pathogen through antibiosis and localized induced resistance.

In the two field experiments comparing the PGPR strain WCS417, AFB1, and AFB2 with C3 and tebuconazole, there were no significant treatment effects; none of treatments significantly inhibited FHB development compared to the control (Table 4). Thus, the identification of microbial agents that can induce a sufficiently high level of ISR to provide effect control of FHB in the field remains elusive. The number of agents evaluated for this trait in this study, however, is small. Further screening of strains among the large number reported to induce ISR is warranted. Performance of microbial agents in this field study may have been affected by such factors as application coverage and retention on foliage in response to environmental influences (e.g. rain). These also are problems that need to be addressed in subsequent research.

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Table 1. Biocontrol agent strains and activators of ISR used in this study

Strain/material	Organism	Source
FHB biocontrol agents		
1BC	<i>Bacillus sp.</i>	B. Bleakley, South Dakota State Univ.
TrigoCor1448	<i>Bacillus subtilis</i>	G. Bergstrom, Cornell University
C3	<i>Lysobacter enzymogenes</i>	G. Yuen, Univ. of Nebraska-Lincoln
PGPR strains		
INR7	<i>Bacillus pumilus</i>	J. Kloepper, Auburn University
89-B61	<i>Pseudomonas fluorescens</i>	J. Kloepper, Auburn University
WCS417	<i>Pseudomonas fluorescens</i>	L. Van Loon, Utrecht University
Autoclaved fungal biomass		
AFB1	<i>Aspergillus niger</i>	B. Tisserat, NCAUR, USDA-ARS
AFB2	<i>Penicillium 2</i>	B. Tisserat, NCAUR, USDA-ARS

Table 2. Results from greenhouse experiment evaluating FHB biocontrol agents for induction of systemic resistance in wheat to FHB.

Treatment	Severity (%)	
	Flag leaf treatment	Head treatment
Control (water)	49	93
<i>Bacillus sp.</i> 1BC	55	65
<i>Bacillus subtilis</i> TrigoCor1448	56	21
<i>Lysobacter enzymogenes</i> C3	63	33
	<i>P</i>	NS
	LSD _{0.05}	---
		0.002
		20

Table 3. Results from greenhouse experiment testing ISR activating strains of PGPR for control of FHB.

Treatment	Severity (%)
Control (water)	67
<i>Pseudomonas fluorescens</i> WCS417	45
<i>Pseudomonas fluorescens</i> 89B-61	56
<i>Bacillus pumilus</i> INR7	50
<i>Lysobacter enzymogenes</i> C3	26
	<i>P</i>
	LSD _{0.05}
	0.007
	19

Table 4. Results from 2005 field experiments evaluating bacterial strains and autoclaved fungal biomass (AFB) preparations for control of FHB. Values are means of two experiments.

Treatment	Severity (%)	Incidence (%)	Index (%)
Control (water)	16	79	27
<i>Lysobacter enzymogenes</i> C3	15	82	31
<i>Pseudomonas fluorescens</i> WCS417	13	87	27
AFB1	12	76	27
AFB2	16	81	27
Tebuconazole	11	76	24
	<i>P</i>	0.130	0.350
			0.095

**EFFECT OF ADJUVANTS ON EFFICACY OF FOLICUR
FUNGICIDE FOR FHB CONTROL**
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ABSTRACT

Currently, the triazole fungicide Folicur (tebuconazole) has special exemptions in some states within the United States (US) for use on wheat and barley to suppress *Fusarium* head blight (FHB). A standard adjuvant recommended for use with tebuconazole is Induce, a petroleum-based non-ionic surfactant. Various private companies in the US sell other non-ionic surfactants or have other adjuvants for sale that are silicone-based or are encapsulating products, and these companies also are experimenting with many new formulations of adjuvants. With so many products on the market, more information is needed about their efficacy with triazole fungicides such as Folicur. Our preliminary tests indicated few differences among adjuvants when combined with Folicur. We continued studies in the greenhouse with adjuvants in combination with Folicur on hard red spring wheat, durum wheat and barley and measured effects on reductions in *Fusarium* head blight severity index (% incidence x % head severity). All fungicide applications were applied once, at early flowering (Feekes 10.51) in spring wheat and durum wheat, and at early full head emergence (Feekes 10.5) in barley. Fungicide plus adjuvant applications were made using a track sprayer mounted with XR8001 flat fan nozzles oriented forward and backward at a 60° angle from vertical, delivering 18.3 gpa at 40 psi. Plants were inoculated with a mixture of three *F. graminearum* isolates, delivering 10,000 spores/ml, 20 ml/pot per spray event, with a DeVilbiss atomizer, 4 hrs after the fungicide was applied. Immediately following inoculation, plants were misted for 48 hours using a closed mist system at or near 100% RH at 23°C (+ or – 5°C).

Results showed that Interlock, an encapsulating adjuvant, in combination with an experimental adjuvant, both manufactured by Agriliance LLC, resulted in significant reduction of the FHB severity index as compared to Folicur without adjuvant, and had the lowest FHB rating of all adjuvants tested across three grain classes. In a separate study on durum and barley only, several experimental adjuvants from Wilbur Ellis Co. resulted in lower FHB severity ratings than Folicur plus Induce, but not significantly so, while other experimental adjuvants provided by Wilbur Ellis resulted in significantly higher FHB severity indices, indicating that appropriate adjuvant use can benefit or hinder efficacy of fungicides for FHB control.

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REAL WORLD RESULTS IN FHB MANAGEMENT

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ABSTRACT

In 1981, the Canadian Phytopathological Society held a symposium on the “Epidemiology and control of mycotoxigenic fusaria on cereal grains” as part of their 52nd annual meeting. In the Introduction to that Symposium, W. L. Seaman, from the Ottawa Agriculture Canada Research Station, described important steps for prevention of head molds: “development of resistant cultivars, rotation with nongramineous crops, effective cultivation of small grain and corn stubble, early planting, the use of high quality seed, prompt harvesting at maturity, and aerating and drying damp grain immediately after harvest”. J. C. Sutton, also a participant in that symposium, stated in his article on the epidemiology of wheat head blight, that “Disease forecasts and schemes to warn growers of the risks of mycotoxins... should be made... Given timely warnings, growers may find it possible to take actions to reduce the intensity and impact of disease”. This symposium in 1981 provided valuable information to scientists working with *Fusarium* diseases of cereals at that time, but it wasn’t until severe widespread epidemics in the 1990s in the US and Canada that an impetus for large regional research efforts to revisit these management guidelines occurred. And by 1998, a US national effort was organized, the US Wheat and Barley Scab Initiative, which funded multi-state and multi-grain class projects directed at finding resistant germplasm, developing resistant varieties, studying epidemiology and developing disease forecasting models, evaluating effects of modern cultural practices on disease severity, and finding new chemistries and biological agents for disease control. As a result of these efforts, today more tolerant wheat cultivars are now available to growers, disease forecasting tools are widely available, more information is available about crop rotation and tillage effects on the disease, and newer fungicide chemistries have shown improved reduction of FHB severity and DON levels. How have these research results been presented to growers, how have they been translated into use by growers, and how effective have they been under real world situations, including recent FHB epidemics in the US from 2003-2005? This presentation will focus on the above mentioned management strategies and how they performed in the real world in the US from 2003-2005, with emphasis on results in the northern plains in 2005.

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RESULTS OF THE UNIFORM FUNGICIDE TRIAL
ON BARLEY, NORTH DAKOTA, 2005
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ABSTRACT

As part of the national uniform scab fungicide trial, six fungicide treatments were compared for control of *Fusarium* head blight (FHB) in 'Robust' spring barley at the Fargo, ND Agriculture Experiment Station and in 'Tradition' spring barley at the Langdon Research Extension Center in northeast ND. The barley was planted on April 29th in Fargo and on May 9th in Langdon. Corn grain inoculated with *Fusarium graminearum* was spread evenly among plots in Fargo and naturally infected wheat seed was distributed among plots at Langdon. At Fargo, a misting system provided added water to the plots when the nighttime humidity dropped below 90%. Fungicides were applied on June 30th at Fargo and July 8th at Langdon, at early full head emergence (Feekes 10.5). Applications were with a backpack-type sprayer equipped with two XR8001 flat fan nozzles oriented toward the grain head at a 30 degree angle from the horizontal. The fungicides were applied at 18.5 gpa with 40 psi. Disease notes were taken at soft dough stage of development. The fungicide treatments included: Folicur (tebuconazole – A Bayer CropScience Section 18 compound) at 4 fl oz/A; Prosaro (19% prothioconazole + 19% tebuconazole - a Bayer CropScience experimental compound) at 6.5 fl oz/A; BAS555 (metconazole - a BASF experimental compound) at two rates, 13 fl oz/A and 10 fl oz/A; and Punch (flusilazole - a DuPont experimental compound) at two rates, 6 fl oz/A and 8 fl oz/A.

Fusarium head blight (FHB) field severity was very low at the Langdon location, only 0.6% in the untreated check, while at the Fargo location, it was 7.6%. Results indicated that all treatments significantly reduced FHB field severity at both locations. DON (deoxynivalenol) data was only available from Fargo at the time of this report. At Fargo, the Prosaro and BAS555 treatments significantly reduced DON over the untreated check. For yield, all treatments significantly increased yield at Fargo, but not at Langdon. The best yielding treatment at Fargo, Prosaro, increased yield by 29.5%.

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WHEAT UNIFORM FUNGICIDE TRIALS, ND, 2005

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OBJECTIVE

To evaluate experimental fungicides for control of Fusarium head blight (scab) 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). Results of these fungicide trials across states are reported (Hershman and Draper, 2004). 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 and in increasing yield and test weight. North Dakota continues to participate in these trials at several locations across the state.

MATERIALS AND METHODS

The uniform fungicide trial was established 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 on hard red spring wheat were evaluated on FHB susceptible hard red spring wheat cultivars 'Reeder' at Carrington and Fargo and on 'Grandin' at Langdon, and on a moderately resistant cultivar 'Glenn' at Langdon. For durum wheat, evaluations were done on susceptible cultivars 'Lebsock' at Carrington and Langdon, and on 'Mountrail' at Minot.

A uniform set of six fungicide treatments were evaluated (Table 1). Fungicides tested included Folicur (tebuconazole), which had a Section 18 exemption

for use on wheat in ND in 2005, Prosaro (equal parts prothioconazole and tebuconazole), an experimental fungicide from Bayer CropScience, BAS555 (metconazole), at two rates, an experimental product from BASF, and Punch (flusilazole) at two rates, an experimental product from DuPont. 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 by some overhead irrigation at Carrington.

All treatments were applied at early flowering (Feekes 10.51) with a CO₂ 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 using gas chromatography and electron capture. Plots were in a Randomized Complete Block design and data were statistically analyzed across locations using ANOVA with locations as replicates.

RESULTS AND DISCUSSION

FHB field severities varied across sites and wheat class. FHB field severity on untreated hard red spring wheat averaged as high as 36% at Carrington and as low as 5.6 to 7.3% at Langdon. Durum FHB field severities in untreated plots ranged from 3% at Minot to 27.7% at Carrington. Overall, hard red spring wheat trials were planted earlier than the durum trials and had much more severe FHB than the durum trials, because record high rainfalls occurred in June in North Dakota in 2005, favoring FHB in wheat crops that flowered in June, including the spring wheat trials. Analysis of treat-

ment differences in durum often were numerically different than the untreated, but not significantly so, when analyzed across sites. For durum, only the percent Fusarium damaged kernels (FDK) were significantly affected by fungicide treatments, with all fungicide treatments significantly lower than the untreated, and the Prosaro treatment having significantly lower FDK than the Punch fungicide treatments. Table 1 contains data only from the ND hard red spring wheat uniform fungicide trials.

For hard red spring wheat, all fungicide treatments significantly reduced the percentage of FHB incidence, head severity, field severity and FDK over the untreated check (Table 1). The experimental treatments of Prosaro and BAS555 at the high use rate had the lowest FHB field severity, but not significantly lower than other treatments. For the one site reporting DON levels, treatments with Prosaro and BAS555 resulted in DON levels significantly lower than other fungicide treatments. All treatments significantly improved yield and test weight.

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- McMullen, M. and Milus, E. 2002. History and accomplishments of the USWBSI uniform fungicide and biological control trials, 1998-2002. Page 96 in: Proc. 2002 national Fusarium Head Blight Forum, Erlanger, KY, Dec. 7-9, 2002. U.S. Wheat and Barley Scab Initiative, Michigan State Univ., East Lansing, MI.

Table 1. Effect of fungicides on Fusarium head blight (FHB) incidence, head severity, field severity, DON, Fusarium damaged kernels (FDK), yield and test wt., averaged across four hard red spring wheat trials at Carrington, Fargo and Langdon, ND, 2005

Treatment and rate/acre ¹	FHB I ² %	FHB HS ² %	FHB FS ² %	DON ³ ppm	FKD ⁴ %	Yield Bu/A	Test wt Lbs/bu
Untreated check	59.2	28.7	17.5	7.8	13.8	40.3	58.3
Folicur 3.6 EC 4.0 fl oz	41.1	19.6	9.0	5.8	7.7	49.7	59.1
Prosaro 421 SC 6.5 fl oz	35.0	17.6	7.4	4.0	5.9	53.7	59.9
BAS555 01 F 13.5 fl oz	38.4	17.5	7.8	4.1	7.0	51.4	59.5
BAS555 01 F 10.0 fl oz	42.4	21.2	9.2	4.6	5.6	50.4	59.5
Punch 6.0 fl oz	48.1	22.8	11.4	6.6	8.0	47.6	59.0
Punch 8.0 fl oz	44.0	19.3	10.1	6.0	8.1	47.3	59.1
LSD 0.05	11.0	5.9	4.6	1.1	2.8	2.3	0.7

¹ Folicur, Prosaro, and BAS555 treatments had 0.125% Induce added; Prosaro (19% prothioconazole + 19% tebuconazole) is an experimental fungicide from Bayer; BAS555 (metconazole) is an experimental fungicide from BASF; Punch (flusilazole) is an experimental product from DuPont

² FHB I = incidence; FHB HS = head severity; FS = Fusarium head blight field severity; field severity = incidence x head severity;

³ DON (deoxynivalenol = vomitoxin) levels were only available from the Fargo location at the time of this report;

⁴ FDK = Fusarium damaged kernels

**CONTROL OF FHB IN WHEAT BY IMPROVED
TECHNOLOGY AND FUNGICIDE CHOICE**
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ABSTRACT

The European Union set binding limit values for important *Fusarium* toxins in all cereals including maize and Sorghum. For DON the limit value will be 1,250 mg/kg or 1,25 ppm for wheat. The commodities over this value cannot be processed for food and feed above 1.75 mg/kg. For this reason the control of *Fusarium* toxins will be the most important task for the growers. As resistant cultivars are not yet on the market in the extent we would need them, the chemical control will have a highly important role to secure food and feed safety. The consequence is that the efficacy of the chemical control should be described by the most important parameter will be the toxin contamination and not FHB or FDK severity. As most cultivars are susceptible, and their change for resistant ones takes longer time, for short run the only possibility is to use better technologies and better fungicides. Tests were made with three cultivars with differing resistance and artificial inoculation was made 24-48 hrs after fungicide treatment at full head coverage with four *Fusarium* isolates with differing aggressiveness. By this way 12 epidemic situations can be modeled.

Efficacy of the fungicides at full coverage is 2-3 times higher and for the best fungicides reaches 90 % or more. With the same fungicides under field conditions the efficacies vary between 0 and 50 %, exceptionally higher. Therefore we need a technology that enables much better coverage. The first field tests with the new technology were made 2005 by the Turbo Flood Jet alternating nozzles suggested by Hooker and Schaafsma on 1 acre plots. The tests were made with 9 fungicides, but symptomless fields were registered only after Prosaro 1 L/ha and Falcon 0.8 L/ha. In the tests with full coverage shows very high efficacies, only

Prosaro was able to secure lower DON than 1.25 ppm for three isolates, in one case at very high control DON value (51.96 ppm) even Prosaro was not enough. The efficacy above 90 % may also be sometimes not enough. It is worth to mention that Folicur with 250 g tebuconazole/l could not control FHB in any situations effectively. We can say that Prosaro is the best fungicide now, but in extreme epidemic situations the DON contamination may exceed limit values. For the other is granted.

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Projects: GAK ALAP-00073/2004, Bayer AG, Germany

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Mesterhazy A., Bartok, T., Lamper, Cs. 2003. Influence of cultivar resistance, epidemic severity, and *Fusarium* species on the efficacy of fungicide control of *Fusarium* head blight in wheat and deoxynivalenol (DON) contamination of grain. Plant Disease, 87:1107-1115.

Table 1. Fungicides against FHB in wheat, DON in ppm, Szeged, ,means for three cultivars, 2004.

Treatment	Isolates				Mean
	L/ha	12377Fg	44Fg	12375Fc	
Prosaro 1.0	4.80	0.83	1.20	0.41	1.81
Input 1.0	4.15	1.09	2.42	1.06	2.18
F. solo 1.0	10.67	2.73	2.79	1.38	4.39
Falcon 0.8	13.62	1.84	2.13	1.87	4.87
Kolfugo 1.5	21.98	4.34	5.31	3.58	8.80
Fusarium contr.	51.96	35.59	22.72	18.02	32.07
Mean	17.86	7.73	6.10	4.39	9.02
LSD 5 %	6.74	6.74	6.74	6.74	3.37

NOVEL RESULTS ON FUNGICIDE APPLICATION AND CHOICE ON FHB IN WHEAT

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ABSTRACT

The European Union set binding limit values for important *Fusarium* toxin in all cereals including maize and Sorghum. For DON the limit value will be 1,250 mg/kg or 1,25 ppm for wheat. The commodities over this value cannot be processed for food and feed above 1.75 mg/kg. For this reason the control of *Fusarium* toxins will be the most important task for the growers. As resistant cultivars are not yet on the market in the extent we would need them, the chemical control will have a highly important role to secure food and feed safety. The consequence is that the efficacy of the chemical control should be described by the most important parameter will be the toxin contamination and not FHB or FDK severity. As most cultivars are susceptible, and their change for resistant ones takes longer time, for short run the only possibility is to use better technologies and better fungicides. Tests were made with three cultivars with differing resistance and artificial inoculation was made 24-48 hrs after fungicide treatment at full head coverage with four *Fusarium* isolates with differing aggressiveness. By this way 12 epidemic situations can be modeled.

Efficacy of the fungicides at full coverage is 2-3 times higher and for the best fungicides reaches 90 % or more. With the same fungicides under field conditions the efficacies vary between 0 and 50 %, exceptionally higher. Therefore we need a technology that enables much better coverage. The first field tests with the new technology were made 2005 by the Turbo Flood Jet alternating nozzles suggested by Hooker and Schaafsma on 1 acre plots. The tests were made with 9 fungicides, but symptomless fields were registered only after Prosaro 1 L/ha and Falcon 0.8 L/ha. In the tests with full coverage shows very high efficacies, only

Prosaro was able to secure lower DON than 1.25 ppm for three isolates, in one case at very high control DON value (51.96 ppm) even Prosaro was not enough. The efficacy above 90 % may also be sometimes not enough. It is worth to mention that Folicur with 250 g tebuconazole/l could not control FHB in any situations effectively. We can say that Prosaro is the best fungicide now, but in extreme epidemic situations the DON contamination may exceed limit values. For the other is granted.

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Table 1. Fungicides against FHB in wheat, DON in ppm, Szeged, ,means for three cultivars, 2004.

Treatment L/ha	Isolates				Mean
	12377Fg	44Fg	12375Fc	12551Fc	
Prosaro 1.0	4.80	0.83	1.20	0.41	1.81
Input 1.0	4.15	1.09	2.42	1.06	2.18
F. solo 1.0	10.67	2.73	2.79	1.38	4.39
Falcon 0.8	13.62	1.84	2.13	1.87	4.87
Kolfugo 1.5	21.98	4.34	5.31	3.58	8.80
Fusarium contr.	51.96	35.59	22.72	18.02	32.07
Mean	17.86	7.73	6.10	4.39	9.02
LSD 5 %	6.74	6.74	6.74	6.74	3.37

EFFECT OF FUNGICIDES ON FHB AND DON IN WHEAT - 2005 UNIFORM FUNGICIDE TRIALS

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OBJECTIVES

Evaluate foliar fungicides for effectiveness in managing Fusarium head blight (FHB) and deoxynivalenol (DON) accumulation in wheat across multiple trials representing different wheat classes and locations.

INTRODUCTION

FHB, caused predominantly by *Fusarium graminearum*, has had a great impact on every sector of the wheat and barley industries in North America. Wheat growers, millers, bakers, and consumers of wheat products all have been affected by this disease. This is largely due to the fact that in addition to yield losses associated with reduced kernel size and weight, reduced seed germination, and seedling blight, *F. graminearum* also produces a mycotoxin called deoxynivalenol (DON) (among other toxins) which may accumulate to unacceptable levels in harvested grain. DON levels above 2 ppm may render grain and their by-products unfit for commercialization and consumption. Efforts to minimize the impact of FHB and DON have been centered on the use of management strategies such as host resistance, crop rotation, tillage, and fungicide application. Through collaborative research involving scientists from multiple states, representing various wheat-growing regions, Uniform Fungicide Trials (UFT) have been used to evaluate fungicide effectiveness against FHB and DON. These trials follow standard protocols and have been conducted annually since 1998. The results of the 2005 UFT trials from 25 trials across 10 states are presented herein.

MATERIALS AND METHODS

Each trial consisted of six fungicide treatments and an untreated control in a randomized complete block design, with four replicate blocks (one trial had five replicate block). The treatments were:

- 1 - Non-treated control;
- 2 - Folicur 432SC 4.0 fl oz + 0.125% Induce;
- 3 - Prosaro 6.5 fl oz/a + 0.125% Induce;
- 4 - BAS555 01/F 13.5 fl oz/a + 0.125% Induce;
- 5 - BAS555 01/F 10 fl oz/a + 0.125% Induce;
- 6 - Punch 6 fl oz/a (no surfactant); and
- 7 - Punch 8 fl oz/a (no surfactant).

Treatments were applied at early flowering (Feeke's 10.51) using CO₂-pressurized sprayers, equipped with Twinjet XR8002 nozzles or paired XR8001 nozzles mounted at a 60° angle forward and backward.

Planting and crop production practices varied somewhat from trial to trial. See individual trial reports for details. Most plots were planted with a susceptible cultivar. To enhance disease development, plots were either planted into corn or wheat residue and/or artificially inoculated with *F. graminearum*-infested kernels. Many plots were mist-irrigated as a means of enhancing production of, and infection by fungal inocula. In each plot of each trial, percent FHB incidence (INC), diseased-head severity (SEV), index (IND; also known as plot severity), and *Fusarium*-damaged kernels (FDK) were measured as previously described (McMullen, et al., 1999). DON accumula-

tion was measured at one of the two USWBSI-funded DON Testing Laboratories.

For the purpose of data analysis, trials conducted at the same location, but using different cultivars, and trials conducted at different locations in the same state were considered separate studies. In the first part of the analysis, the data from all the trials with disease were pooled together. Trial was treated as a fixed effect, and a mixed effect model was fitted using PROC MIXED of SAS to determine the overall effects of trial, treatment, and the interaction between trial and treatment on the FHB and DON. The decision to treat trial as a fixed effect was based on the assumption that each location (or cultivar) was specifically chosen for the trial because of known characteristics, and researchers are interested in how these location- and cultivar-specific factors influence FHB and DON. In the second part of the analysis, each trial was analyzed separately to determine the most effective treatment within each trial. Linear contrasts were used to make pairwise comparisons between treatment means and means across groups of treatments. Studies with zero disease were not analyzed. A separate set of analyses was performed for each response variable IND, INC, SEV, and DON.

RESULTS AND DISCUSSION

FHB intensity varied from one trial to another. This was especially true when winter wheat trials were compared with spring wheat trials. In 2005, weather conditions in winter wheat areas were generally unfavorable for FHB development. Consequently, non-irrigated trials frequently had nominal disease development. Mean and maximum FHB index, across all replicates and treatments, ranged from 0 to 6.37 and 0 to 25.90%, respectively for winter wheat trials. For spring wheat trials, the corresponding values were 3.88 to 42.23% for mean index and 7.20 to 60.44% for maximum index (Table 1). In four of the 12 winter wheat trials, 0% index was observed in all treatments.

Based on the analysis of the pooled data, the treatment, trial, and the interaction between treatment and trial were significantly different from zero ($P < 0.05$).

When averaged across trials, all treatments significantly reduced FHB index relative to the check. When the data were grouped and analyzed according to wheat class, the effects of treatment and trial were significant for both winter and spring wheat trials; however, the interaction between treatment and trial was not significant ($P > 0.05$) among spring wheat trials. Within both groups of trials, all treatments significantly reduced FHB index relative to the check. In general, treatment 3 (Prosaro 6.5 fl oz/a + 0.125% Induce) was the most effective treatment, resulting in a greatest reduction in IND relative to the control. However, since there was a significant interaction between treatment and trial, the analysis was extended to determine the most effective treatment within each trial.

The mean level of disease and DON between each treatment and the untreated control was used to determine the most effective treatment within each trial. Similar results were observed for DON and for all measures of FHB intensity (IND, SEV, INC, and FDK), with the most effective treatment varying from trial to trial. Since IND is a direct function of INC and SEV (see Paul et al., 2005a,b), only the results for IND are summarized herein. The results for DON and FDK are presented in Tables 2 and 3. Treatment 3, application of Prosaro at a rate of 6.5 fl. oz per acre, resulted in the greatest reduction in IND relative to the untreated check in eight of the 21 trials analyzed (Table 1). In six of those trials, the difference in IND between Prosaro and the check was significantly different from zero ($P < 0.005$). A direct comparison between mean IND in Prosaro-treated plots and Folicur-treated plots showed that, although the absolute level of disease was generally lower in Prosaro-treated plots (in 15 of the 21 trials with disease), the difference between the two treatments was only significantly different from zero in two trials (Beltsville, MD and Fardo, ND). Treatments 4 (BAS555 01/F 13.5 fl oz/a + 0.125% Induce) and 5 (BAS555 01/F 10 fl oz/a + 0.125% Induce) were the most effective treatment in four trials each.

The percent control (Hershman and Milus, 2003) resulting from the most effective treatment was generally higher in trials with low levels of disease than in trials

with high levels of disease (overall mean and maximum disease IND in Table 1). This should be interpreted with caution since the ultimate effectiveness of a fungicide treatment should be based on results under high disease pressure. This can be done by jointly observing the percent control and the mean level of disease in the treated plots. In four of the seven trials with the highest levels of disease (mean IND across all treatments ranging from 10.95 and 42.23%), fungicide application significantly reduced IND when compared to the untreated check, with percent control between 21.42 and 59.36%. This is consistent with previously reported results (Hershman and Milus, 2003, Hershman and Draper, 2004). In the four trials with the highest levels of disease (Carrington 1 and 2 and Brookings 1 and 2), the mean index in plots treated with the most effective fungicides ranged from 16.25 to 27.72%.

The results for DON (Table 2) and FDK (Table 2) were very similar to those described for IND, with the treatment most effective at reducing DON and FDK varying among trials. The Prosaro treatment, treatment 3 was again the most effective. Based on the available data, fungicide treatment did result in a significant reduction in DON relative to the untreated check, with percent reduction being between 26.82 and 84.42%. Despite this reduction, however, DON levels in treated plots still exceeded critical thresholds in some trials. As was the case with IND, DON levels in Prosaro-treated plots was only significantly lower than DON levels in Folicur-treated plots in trials conducted at Beltsville, MD and Fardo, ND.

In summary, fungicide treatments did reduce FHB intensity and DON levels in harvested grain. The product and/or rate of application most effective at reduc-

ing FHB and DON varied from one trial to another; however, the application of Prosaro at a rate of 6.5 fl. oz per acre was the most effective treatment overall. The magnitude of FHB and DON reduction due to fungicide treatment depended on the level of disease. Under heavy disease pressure, relatively high levels of FHB and DON levels above threshold values may still occur in treated plots.

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Table 1. Fungicide effect on Fusarium head blight index.

Trial		Wheat Type	Most effective Treatment ^a				Disease index (%)	
State/PI	Location		Treat	IND (%)	% Control	<i>P</i> value	Mean	Max
IL/Adee	Monmouth	W	2,3,4,6	0.02	91.81	0.002	0.07	0.67
IL/Fakhoury	Carbondale	W	7	0.11	88.5	0.089	0.81	3.24
IN/Shaner	Lafayette	W	0.00	0.00
	Butlerville	W	0.00	0.00
LA/Padgett	Macon Ridge	W	4	0.37	82.22	0.029	1.56	5.26
MD/Grybauskas	Beltsville	W	3	1.62	89.64	<0.001	6.37	25.90
MI/Hart	East Lansing 1	W	0.00	0.00
	East Lansing 2	W	0.00	0.00
MN/Hollingsworth	Crookston 1	S	3	3.36	45.4	<0.001	4.55	7.20
	Crookston 2	W	4	0.66	47.6	0.089	1.17	2.62
MO/Sweets	Columbia 1	W	6	0.00	100	0.151	0.55	3.15
	Columbia 2	W	6	0.00	100	0.170	0.59	2.80
ND/McMullen	Fargo	S	3	2.97	85.92	<0.001	6.91	23.00
	Carrington 1	S	3	19.50	45.45	0.004	25.68	55.00
	Carrington 2	S/D	3	16.25	41.44	0.069	21.54	37.00
	Langdon 1	S/D	4	1.60	71.93	0.067	4.36	16.00
	Langdon 2	S	3	2.25	59.82	0.015	3.88	8.00
SD/Draper	Langdon 3	S	7	3.15	56.55	<0.001	4.81	7.90
	Brookings 1	S	3	35.83	21.42	0.122	42.23	60.44
	Brookings 2	S	2	27.72	45.26	<0.001	39.21	54.62
	Watertown 1	S	5	10.70	49.17	0.057	14.16	37.14
	Watertown 2	S	5	6.91	59.26	0.008	10.95	26.44
VA/Stromberg	Groton 1	S	5	7.80	59	0.042	12.01	39.94
	Groton 2	S	5	6.09	28.57	0.299	7.23	14.00
VA/Stromberg	Warsaw	W	2	2.68	64.89	0.001	4.54	8.40

^aTreat = the most effective treatment (s) within each trial based on the pair-wise difference between mean IND for each treatment and the check; IND (%) = mean index across plots receiving the most effective treatment; % control = percent control; *P* value = level of significance from *F* test of the difference between mean IND across plots receiving the most effective treatment and the untreated check. All tests of significance were done using arcsine-transformed IND.
 ... = Trials with no disease.

Table 2. Fungicide effect on DON.

Trial ^a		Wheat Type	Most effective Treatment ^b				DON (ppm)	
State/PI	Location		Treat	DON	% Reduction	<i>P</i> value	Mean	Max
MD/Grybauskas	Beltsville	W	3	2.40	84.42	<0.001	7.90	24.50
MN/Hollingsworth	Crookston 1	S	3	2.23	53.40	<0.001	3.50	5.70
	Crookston 2	W	4	0.73	26.82	0.329	1.01	1.80
ND/McMullen	Fargo	S	3	4.00	48.37	<0.001	5.55	8.50
	Carrington 1	S	7	4.85	51.74	0.041	6.33	19.10

^aDON data were not available for some trials or available but equally low (below 1 ppm) for all treatments.
^bTreat = the most effective treatment within each trial based on the pair-wise difference between mean DON for each treatment and the check; DON (ppm) = mean DON across plots receiving the most effective treatment; % reduction = percent reduction in DON; *P* value = level of significance from *F* test of the difference between mean DON across plots receiving the most effective treatment and the untreated check. All tests of significance were done using log-transformed.

Table 3. Fungicide effect on FDK.

Trial ^a		Wheat Type	Most effective Treatment ^b				FDK (%)	
State/PI	Location		Treat	FDK (%)	% Reduction	<i>P</i> value	Mean	Max
IL/Fakhoury	Carbondale	W	6	6.75	32.50	0.440	10.43	28.00
LA/Padgett	Macon Ridge	W	4	9.75	22.00	0.0269	12.78	19.00
MD/Grybauskas	Beltsville	W	3	13.20	55.10	0.004	22.91	52.00
MN/Hollingsworth	Crookston 1	S	3	5.50	38.89	0.051	7.32	15.00
ND/McMullen	Fargo	S	3	7.50	61.54	<0.001	12.61	20.00
	Carrington 1	S	3, 4	10.75	52.22	<0.001	12.86	25.00
	Carrington 2	S/D	3	4.00	77.14	<0.001	9.04	20.00
	Langdon 2	S	7	3.25	63.89	<0.001	5.30	10.50
	Langdon 3	S	3	0.75	31.25	<0.001	2.25	7.90
	SD/Draper	Brookings 1	S	4	32.5	13.33	0.025	36.79
Brookings 2		S	3	32.5	13.33	0.059	36.07	40.00
Watertown 1		S	5	2.50	50.00	<0.001	3.78	6.00
Watertown 2		S	2, 3	2.25	43.75	0.006	3.26	6.00
Groton 1		S	4	1.75	41.67	0.092	2.67	4.00
Groton 2		S	5	2.00	33.33	0.218	2.44	4.00

^a FDK data were not available for some trials or available but equally low for all treatments.

^bTreat = the most effective treatment (s) within each trial based on the pair-wise difference between mean FDK for each treatment and the check; FDK (%) = mean FDK across plots receiving the most effective treatment; % reduction = percent reduction in FDK; *P* value = level of significance from *F* test of the difference between mean FDK across plots receiving the most effective treatment and the untreated check. All tests of significance were done using arcsine-transformed FDK.

SPRAYER NOZZLE CONFIGURATIONS AND EFFECTS ON FUNGICIDE SPRAY DEPOSITION ON WHEAT HEADS

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ABSTRACT

Fusarium head blight (FHB) continues to cause significant yield and quality losses of wheat and barley in the US, and was very significant in portions of the Northern Plains during 2005. Fungicide application remains an accepted method for FHB control. Previous studies concerning fungicide application and control of FHB did not address detailed parameters of spray deposition on varying wheat head structures and the relationship of this coverage to FHB control. The objective of this trial was continue previous work to take steps in quantifying the parameters surrounding spray deposition on wheat heads and to identify methods whereby optimized fungicide application for efficacy can occur. Spring wheat (cvs Oxen and Ingot) were planted at the South Dakota State University Agronomy Farm and treated at anthesis (Feekes growth stage 10.51) by spraying in a single direction in nearly calm wind conditions with a tank mixture of Folicur (tebuconazole) at a rate of 4 fl oz/a (292.30 ml/ha) and Induce adjuvant (0.125% v/v) supplemented with a fluorescent orange water soluble dye (3% v/v). The mixture was applied using a wheeled cart to control sprayer height above the crop canopy, with a pressurized sprayer set at 40 psi (275.79 kPa) and an application rate of 18.6 gpa (173.97 l/ha). Nozzle configurations (treatments) included: 1) one flat fan nozzle pointing straight down (XR TeeJet 11002), 2) one flat fan nozzle angled 45 degrees forward (XR TeeJet 10002), 3) a twin-orifice flat fan nozzle (60 degrees between fans) (Twinjet TJ11002) and 4) a twin nozzle configuration (90 degrees between fans) (paired XR TeeJet 11001). Varieties and treatments were randomized in a 2 X 4 factorial design with four replications with varieties and nozzle types as factors. Plots were inoculated by spreading *Fusarium graminearum* (Fg4) inoculated corn (*Zea mays*) grain throughout the field and providing overhead mist irrigation on a 16 hr/8 hr on/off schedule (overnight mist) throughout anthesis. Wheat heads were evaluated for spray coverage, deposition pattern, FHB incidence, head severity, total FHB damage and location of diseased spikelets relative to direction of sprayer travel. Plot yield, test weight, and Fusarium damaged kernels (FDK) were also measured. Digital pictures of the incoming and outgoing side of the head were taken under UV light and spray coverage was analyzed digitally from the images. Spray coverage on the incoming side of heads was acceptable with all nozzles, while the side away from the application generally received much less product, regardless of nozzle configuration. No nozzle tested in this trial provided equal deposition on the incoming and outgoing sides of the head, although initial analysis shows that twin orifice configurations tend toward more uniform coverage on both sides of the head. Varietal and treatment differences were significant for yield, test weight and FDK. Initial data on FHB infection appears to show that there may be an effect of applied fungicide penetrating into the inner portions of the head, including the rachis, and thus limiting FHB infection to single spikelets and reducing FHB spread within the head. These data suggest that deep penetration of the spray droplets into the head may be as important into enhancing product efficacy and FHB control as total coverage of the head.

**FERMENTATION AND FORMULATION: CRUCIAL FOCUS AREAS FOR
EXPEDITING THE DEVELOPMENT OF BIOCONTROL PRODUCTS**
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ABSTRACT

Developing an effective, commercially successful biological control product is a complex, labor intensive undertaking. The process must begin with a carefully crafted microbial selection procedure, proceed by employing biomass production protocols that optimize product quantity and quality, and end with devising a product formulation that preserves shelf-life, aids product delivery and enhances bioactivity. Selection procedures that require prospective microbial biocontrol agents to possess both efficacy and amenability to production in liquid culture enhance the likelihood of selecting agents with improved commercial development potential. Scale-up of biomass production procedures must optimize product yield without compromise of product efficacy and amenability to stabilization and formulation. Considerations critical to designing successful formulations of microbial biomass are many fold and include designing production processes that enhance biomass amenability to formulation; an awareness of the mode of action of the microbial agent; durability of the life-stage to be formulated; the physical, chemical, and biological characteristics present on the application target; and the equipment used for field application. Solutions to these formulation considerations will not necessarily be compatible. Data from several systems for biologically controlling plant pests, with emphasis on the biological control of *Fusarium* head blight of wheat, will be used to demonstrate many of these concepts.

USDA-ARS AND THE OHIO STATE UNIVERSITY COOPERATIVE
RESEARCH: GREENHOUSE AND FIELD TESTS OF COMBINATIONS
OF CHOLINE METABOLIZING STRAINS AND ANTAGONIST
CRYPTOCOCCUS NODAENSIS OH 182.9 FOR
REDUCING FHB OF WHEAT

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INTRODUCTION

The primary causal agent of Fusarium head blight (FHB) in North America is *Fusarium graminearum* Schwabe Group 2 (Aoki and O'Donnell, 1999) (perfect state = *Gibberella zeae* (Schwein.) Petch). Considerable experimental evidence suggests that wheat anthers and specifically the compounds choline and betaine in anthers can enhance FHB disease development (Strange and Smith, 1978). Previously, we isolated 738 strains from wheat anthers, determined 16.5% were able to metabolize choline as a sole carbon source, and found that several strains reduced FHB disease parameters in greenhouse and field tests (Schisler et al, 2004). We have demonstrated the potential of several antagonists including *Cryptococcus nodaensis* nomen nudum OH 182.9 (NRRL Y-30216) 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., 2002; Khan et al., 2004). Combinations of biological control strains potentially increase the efficacy and consistency of biocontrol. The objective of this study was to determine if combinations of strain OH 182.9 and choline metabolizing strains (CMS) could be identified to enhance biocontrol compared to that obtained with individual strains.

MATERIALS AND METHODS

Antagonist combination tests (greenhouse)

Hard red spring wheat (cultivar Norm) was grown in plant growth chambers prior to conducting plant bioassays on greenhouse benches. Biomass of OH 182.9 and 3 CMS was produced by incubating flasks containing inoculated liquid medium (SDCL, Slininger et al. 1994) at 250 rpm and 25 C for 48 h. Conidial inoculum of *G. zeae* isolate Z-3639 was produced on clarified V8 juice agar under 12 h/day fluorescent light for 7 days at 24 C. At wheat anthesis, antagonist suspensions were individually misted onto approximately 14 wheat heads per treatment followed immediately by a mist application of a conidial suspension (5×10^5 conidia/ml). For treatments using single antagonist strains, fully colonized broths were diluted by one-quarter prior to use at log₁₀ (CFU/ml) of 7.73, 7.11, 9.63, and 9.56 for OH 182.9, AS 55.2, AS 64.4 and OH 221.3, respectively (Fig. 1). Treatments that combined antagonists were produced by mixing equivalent volumes of treatment suspensions of each individual strain. Heads treated with water followed by the conidial suspension of *G. zeae* isolate Z-3639 served as a "pathogen only" control. Plants were placed in humidity tents for 3 days, scored for disease severity after 16 days, and data analyzed using one-way ANOVA. The reported means are results from pooled replicate experiments.

Field testing of CMS

Field trials were conducted in Peoria, IL (insufficient disease development, data not shown) and in Wooster, OH in 2005 (Table 1). Biomass of antagonists was produced in Fernbach flasks using SDCL medium.

Soft red winter wheat cultivars Elkhart (susceptible) and Freedom (moderately resistant) were grown. Biomass of antagonists was applied at the beginning of wheat flowering at concentrations of \log_{10} (CFU/ml) of 7.18, 7.20, 9.36, and 9.35 for OH 182.9, AS 55.2, AS 64.4 and OH 221.3, respectively, and a rate of 80 gal/acre. The fungicide Folicur 3.6F was applied at the recommended AI rate as a chemical control and untreated plants served as an additional control. Corn kernels colonized by *G. zeae* were scattered through plots (~25-40 kernels/m²) two weeks prior to wheat flowering and mist irrigation was provided periodically for approximately two weeks after treatment application. Heads were scored for disease incidence and severity 26 days after treatment using a 0-100% scale. Randomized complete block designs were used in all field trials. Analysis of variance and Fisher's protected LSD ($P < 0.05$, Statistix 7 statistical software) were used to identify treatment means that were significantly different.

RESULTS AND DISCUSSION

All treatments that combined CMS with OH 182.9 reduced FHB disease severity with the exception of "OH 182.9 + OH 221.3" (Fig. 1) in greenhouse tests. Successful antagonist combination treatments did not reduce disease severity to a greater extent than treatment with OH 182.9 alone (Fig. 1).

Results varied in field testing depending on the wheat cultivar considered. The best disease control was obtained by combining the use of the resistant cultivar Freedom with antagonist combination treatments such as "OH 182.9 + AS 64.4" or OH 182.9 combined with all three CMS (Table 1). Folicur 3.6F and microbial treatments rarely reduced (and sometimes increased) FHB on the susceptible cultivar Elkhart (Table 1).

While enhanced efficacy of disease control was not demonstrated when OH 182.9 was combined with CMS, trends of enhanced consistency of biocontrol can only be determined with further data from field tests. Contrary to 2005 field results, 2003 field tests of individual CMS in Peoria, IL and Wooster, OH

demonstrated significant reduction in FHB disease parameters on cultivar Freedom (Schisler et al., 2004). Optimization of liquid culture growth conditions enhanced the biocontrol efficacy of OH 182.9 biomass (Zhang et al., 2005). Medium optimization may be similarly effective in improving the efficacy of CMS individually or in combination with OH 182.9.

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DISCLAIMER

Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture. Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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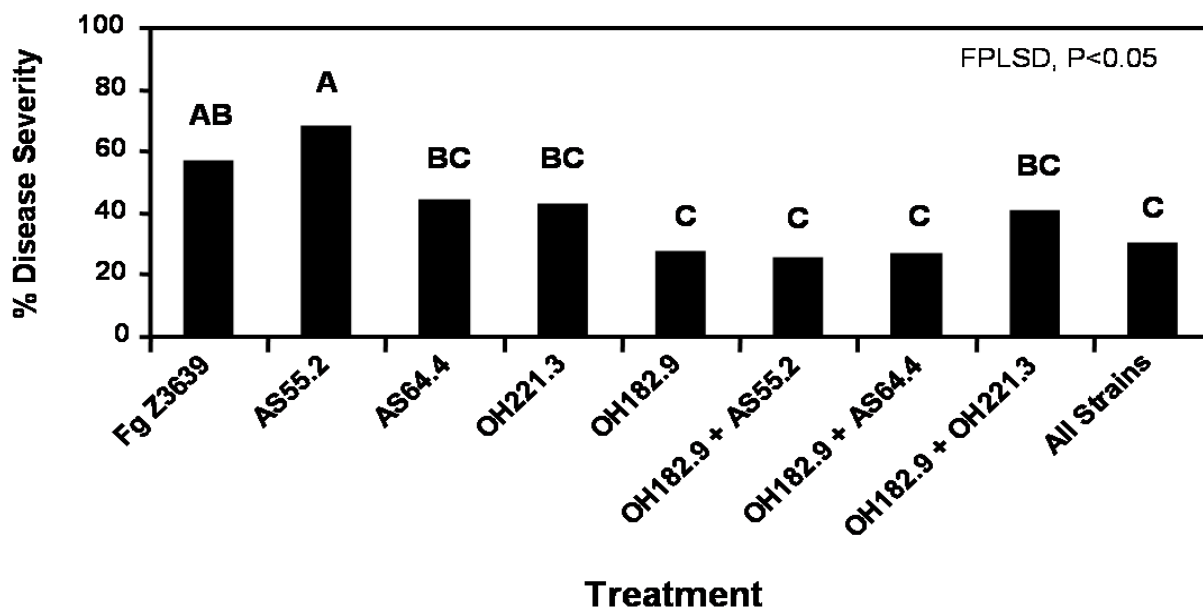


Figure 1. Influence of choline metabolizing strains AS 55.2, AS 64.4 and OH 221.3 alone or in combination with *C. nodaensis* OH 182.9 on FHB incited by *Fusarium graminearum* isolate Z-3639 in greenhouse tests.

Table 1. 2005 field trial results at Wooster, Ohio: influence of *Cryptococcus nodaensis* OH 182.9, choline metabolizing strains, Folicur 3.6F and combinations thereof on FHB disease parameters on two cultivars of winter wheat¹

Treatment	Wheat Cultivar			
	Freedom		Elkhart	
	% Disease Severity	% Incidence	% Disease Severity	% Incidence
Untreated control	4.8	29.3	20.8	57.0
Folicur 3.6F	2.8*	19.7*	20.3	57.0
OH182.9	3.3*	22.3*	22.1	57.0
AS55.2	3.7	26.7	29.1*	66.7*
AS64.4	4.6	28.0	25.8*	52.0
OH221.3	4.2	25.3	17.4	55.0
OH182.9 + AS 55.2	4.1	21.0*	22.3	55.7
OH182.9 + AS 64.4	2.9*	21.7*	16.6	45.3*
OH182.9 +OH 221.3	3.3*	21.7*	17.3	54.0
182.9 + 55.2 + 64.4 + 221.3	2.9*	18.3*	18.0	50.0
55.2 + 64.4 + 221.3	5.6	32.3	20.3	52.7
LSD _(0.05)	1.5	6.9	5.0	7.9

¹Within a column, values followed by an "*" are significantly different from the untreated control ($P \leq 0.05$).

EFFECT OF GLYPHOSATE ON THE *IN VITRO*
GROWTH OF FUNGAL ORGANISMS

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ABSTRACT

Glyphosate [N-(phosphonomethyl)-glycine] is a broad-spectrum, non-selective, post-emergence herbicide used to control weeds in agricultural production systems. The application of glyphosate prior to wheat planting has been reported to be associated with increased *Fusarium* head blight incidence in the wheat crop. Wheat frequently follows soybeans in crop rotations; therefore, it is important to determine the effect of glyphosate on fungal communities. The objectives of this study were to: 1) determine the effect of glyphosate on mycelial growth of *Fusarium graminearum* (causal organism for *Fusarium* head blight, or wheat scab) and six other common soil microorganisms, and 2) determine the effect of glyphosate on macroconidia production by *F. graminearum*. Three isolates of *F. graminearum* were tested in addition to six randomly selected isolates of common soil fungal organisms. Mycelial growth was measured daily on the isolates grown on potato dextrose agar (PDA) amended with different concentrations of glyphosate. Macroconidia production was evaluated by growing *F. graminearum* in carboxymethyl-cellulose (CMC) liquid media for five days and counting the number of macroconidia produced. Macroconidia production was greatly reduced at the recommended field rate of glyphosate. The mycelial growth of all seven species was reduced at all rates of glyphosate. At very low glyphosate concentrations the growth of the *Fusarium* spp. was inhibited less than the other four species. The application of glyphosate may alter the soil microflora and allow the less affected *Fusarium* spp. to fill vacant niches left in the soil community by other negatively affected microorganisms. An increased abundance of *Fusarium* spp. in soils could potentially result in a higher incidence and severity of diseases such as *Fusarium* head blight and sudden death syndrome of soybeans.

STANDARDIZED EVALUATION OF BIOLOGICAL AGENTS FOR THE CONTROL OF FUSARIUM HEAD BLIGHT: 2005 RESULTS

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OBJECTIVE

To evaluate, using standardized methodology, a set of treatments involving biological control agents alone and in combination with a fungicide for effectiveness in managing Fusarium head blight (FHB) in wheat across a range of environmental conditions.

INTRODUCTION

Biological control has been investigated for the control of FHB by laboratories in the United States, resulting in a number of agents with the potential for controlling FHB being identified. These agents, including *Cryptococcus nodaensis* OH 182.9 (Khan et al., 2004), *Bacillus* spp. strains Trigocor 1448 (Stockwell et al., 2001) and 1BA (Draper et al., 2001), and *Lysobacter enzymogenes* C3 (Yuen and Jochum, 2002), were effective when evaluated separately in field tests. Evidence also was obtained suggesting that biocontrol agent-fungicide combinations could provide higher levels of FHB control than biological or chemical methods alone (DaLuz et al., 2003; Yuen and Jochum, 2004). In 2005, standardized tests supported by the USWBSI were conducted over a wide range of environmental conditions and crop genotypes to compare two agents *Bacillus* sp. 1BA and *L. enzymogenes* C3 applied alone and in combination with the fungicide tebuconazole. The results of these efforts are reported here.

MATERIALS AND METHODS

Five trials were conducted across three states on a range of wheat market classes (Table 1). The trials

conducted on two cultivars in Missouri were separate experiments. In each trial, strain 1BA was provided by B. Bleakley and M. Draper, South Dakota State University and strain C3 was provided by G. Yuen, University of Nebraska-Lincoln as broth cultures. The pre-application population of each agent in the broth culture was determined by the local researcher using dilution plating. Each biocontrol agent was tested alone and in a tank mixture with tebuconazole (Folicur 3.6F; 4.0 fl oz/A; provided by Bayer Cropscience, Kansas City, MO). In addition, there was a non-treated control and a treatment with tebuconazole alone. All treatments were amended with the commercial surfactant Induce (0.125%) and applied at 20 gal. per acre. One application of each treatment was made at early flowering (Feekes 10.5.1) using a CO₂-pressurized sprayer (approximately 40 psi) equipped with flat-fan nozzles oriented forward and backward. The size and number of replicate plots varied among trials. Some of the trials were inoculated with *Fusarium graminearum* and utilized mist irrigation systems to stimulate infection. In all trials, FHB incidence (% heads infected per plot), severity (% spikelets infected per diseased head), and index (plot severity) were determined from at least 40 heads per plot around 3 weeks after anthesis. The incidence of Fusarium-damaged kernels (%FDK) was determined after harvest. Samples from each plot were sent to USWBSI-designated laboratories for analysis of deoxynivalenol (DON) content. Results from all trials first were analyzed separately using analysis of variance and then analyzed together, with each experiment being treated as a 'block'. Fisher's LSD test was used for means separation.

RESULTS

FHB pressure varied considerably among the trials, with incidence ranging from 8 to 94% and severity ranging from 5 to 91% in the controls (Table 2). Disease levels in MO experiments were too low to provide separation of treatments for any disease parameter. In the NE and SD experiments, significant treatment effects were found for some but not all disease parameters. Neither of the biological control agents or tebuconazole alone significantly reduced any disease parameter in more than one experiment. When data from all experiments were pooled, strain C3 and tebuconazole alone reduced scab incidence and index compared to control, but the level of reduction was low. Biocontrol agent-tebuconazole combinations were no better than tebuconazole alone, in individual experiments and across all experiments. No significant treatment effect was found for incidence of *Fusarium*-infected kernels or for DON content in any experiment (data not shown).

DISCUSSION

The identification of a biological control agent that can be effective across a range of environments and wheat genotypes remains a challenge. The finding in this study that tebuconazole, currently the most commonly applied fungicide for FHB in the US, did not provide consistent disease control or affect DON levels, is further confirmation as to how difficult it is to manage this disease. Combining biological control agents with fungicides theoretically should confer an advantage in the management of FHB by bringing diverse modes of action to play (Da Luz et al., 2003); this advantage, however, was not realized in this study.

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59-0790-1-079. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture. DON analysis was performed at the North Dakota State University Veterinary Diagnostic Laboratory, Fargo, ND.

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Table 1. 2005 biological control trial locations, wheat cultivars, and researchers.

State and location	Wheat market class and cultivar	PI and Institution
MO	Soft red winter wheat 'Elkhart'	L. Sweets, University of Missouri
MO	Soft red winter wheat 'Roane'	L. Sweets, University of Missouri
NE - Havelock	Hard red winter wheat '2137'	G. Yuen, University of Nebraska
NE - Mead	Hard red winter wheat '2137'	G. Yuen, University of Nebraska
SD	Hard red spring wheat 'Ingot'	B. Bleakley and M. Draper, South Dakota State University.

Table 2. Results across five uniform biocontrol trials on wheat, 2005

Treatment	MO 'Elkhart'	MO 'Roane'	NE Havelock	NE Mead	SD	Mean
SEVERITY (% spikelets infected)						
Control	6	5	20	31	91	31
Folicur	2	4	14	23	89	26
1BA	5	7	21	31	88	30
1BA + Folicur	3	2	19	21	85	26
C3	2	0	21	27	90	28
C3 + Folicur	4	5	16	17	95	27
P	NS	NS	0.006	NS	NS	NS
LSD _{0.05}	-	-	8	-	-	-
INCIDENCE (% heads infected)						
Control	8	8	94	71	57	47
Folicur	3	8	69	59	51	39
1BA	5	8	90	70	53	45
1BA + Folicur	5	3	90	58	48	41
C3	8	0	81	64	47	40
C3 + Folicur	10	8	88	55	50	42
P	NS	NS	NS	0.015	NS	0.05
LSD _{0.05}	-	-	-	13	-	6
INDEX (plot severity)						
Control	0.9	0.7	19	23	52	19
Folicur	0.2	0.7	10	14	46	14
1BA	0.5	0.7	19	22	47	18
1BA + Folicur	0.3	0.2	17	13	42	14
C3	0.7	0	17	17	42	15
C3 + Folicur	1	0.9	14	10	47	15
P	NS	NS	NS	0.006	NS	0.05
LSD _{0.05}	-	-	-	8	-	4

