ABSTRACT

Title of Document: INTEGRATED PEST MANAGEMENT OF FUSARIUM HEAD BLIGHT IN SOFT RED WINTER WHEAT.

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Fusarium head blight (FHB), caused by *Fusarium graminearum,* is a destructive disease of the soft red winter wheat grown in the Mid-Atlantic region. Management of FHB focuses primarily on foliar fungicides or cultivar resistance. The purpose of this research was to examine how type II resistance (resistance to spread of the pathogen) is affected by multiple infections along the spike. The combination of type II resistance and fungicide as a way to manage FHB was evaluated in both the greenhouse and field settings. Finally, the role of increased foliage density in an integrated pest management program that included fungicide and cultivar resistance was also evaluated. Multiple infections occurring along a single wheat spike can overwhelm the type II resistance present in some cultivars. The combination of type II resistance and fungicide was the best management practice for FHB than either alone. Foliage density did not improve FHB disease ratings.

INTEGRATED PEST MANAGEMENT OF FUSARIUM HEAD BLIGHT IN SOFT RED WINTER WHEAT

By

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Chapter 1: Introduction and Literature Review

Fusarium Head Blight (FHB) can be a highly destructive disease of small grains wherever those crops are grown. Fusarium head blight is caused by up to 17 different causal organisms, which include several related *Fusarium* species, such as *F. graminearum* Schwabe, *F. culmorum* (Wm. G. Sm.) Sacc. and *F. avenaceum* (Fr.:Fr.) Sacc. (Parry, 1995). In North America, however, *F. graminearum*, the anamorph of *Gibberella zeae* (Schwein.:Fr.) Petch, is the primary causal agent of the disease (McMullen, Jones and Callenberg, 1997). *F. graminearum* is a facultative parasite, which can colonize and overwinter on previously infected and senesced tissue, especially corn (*Zea mays* L.) stalks (Agrios, 2005). Wheat (*Triticum aestivum* L.) is susceptible to *F. graminearum* between flowering and the soft dough stage of kernel development. However, as early as the 1920's, studies have been conducted that have shown FHB is most severe when plants are inoculated during anthesis (Parry, 1995). Infected spikelets bleach prematurely and during periods of humid, warm weather, light pink spores can form on the glume and rachis. Under favorable environmental conditions, the disease can progress from the initial infected spikelet to bleach the entire wheat head (Schmale and Bergstrom, 2003). The optimum conditions for infection are during extended periods (36 to 72 hours) of high relative humidity and warm temperatures (20 to 30ºC) (Anderson, 1948). As *F. graminearum* colonizes the developing kernel, the seed becomes shrunken, shriveled and often has a chalky white or pink appearance (Schmale and Bergstrom, 2003).

Infected grain often contains mycotoxins produced by the fungus. *F. graminearum* produces a range of toxic chemicals, but the most common and abundant is deoxynivalenol (DON). DON, also called vomitoxin, has a low acute toxicity but it is so frequently produced by *F. graminearum* that it still poses a serious risk to humans and livestock that consume contaminated wheat seed over extended periods of time (Rotter *et al.*, 1996). DON, a tricothecene, is a significant inhibitor of eukaryotic protein biosynthesis. When contaminated grain is ingested it can cause dizziness, headaches, abdominal pain, nausea, fever, diarrhea and sleepiness in humans, as well as feed refusal and diarrhea in animals (Xu and Chen, 1993). The U.S. has set up a federal advisory limit of 1 ppm DON in grain that is used for human consumption, 10 ppm for cattle and chickens, and 5 ppm DON in grain intended for swine (Pestka, 1996). When delivered to the mill, contaminated grain is often downgraded or rejected outright, sometimes resulting in severe economic losses to the grower (Bai and Shaner, 1994).

In addition to seed quality reductions, FHB also causes yield losses. During an outbreak of scab in the Atlantic provinces of Canada in 1980, yield losses were reported to be between 30-70% (Martin and Johnston, 1982). Yield reduction often results from the light weight, shriveled seed characteristic of *Fusarium* infection lowering test weight, or if the grain is light enough, direct yield loss occurs when the grain is eliminated with chaff during harvest (Bai and Shaner, 1994). The mid-Atlantic region of the United States suffered from a severe FHB epidemic in 2003. Growers in 40 counties in Maryland, Virginia, and North Carolina were estimated to have lost over $13.6 million in 2003, compared to the 10-year average production

data for the area (Cowger and Sutton, 2005). Although other diseases and weather conditions, such as Stagonospora nodorum blotch (SNB) and delayed harvest, played a part in the yield and test weight losses, when Cowger and Sutton (2005) removed the effect of FHB, SNB and delayed harvests represented only a 10% loss from the 10-year average. In Maryland, yields were reduced by approximately 60% compared to the previous growing season and 70-100% of Eastern Shore growers were affected by FHB, compared to about 20% during a non-epidemic year (Cowger and Sutton, 2005). In addition to direct yield losses experienced by growers during the 2003 epidemic, extremely high levels of DON caused the major mills to stop sourcing wheat from the mid-Atlantic (Canty, 2004). For example, DON levels in Maryland research plots ranged anywhere from 2 to 12 ppm (Cowger and Sutton, 2005). The severe 2003 and more recently 2009 Mid-Atlantic epidemics have shown that more research is needed to determine the best methods of managing Fusarium Head Blight.

There has been a trend of increasingly, devastating FHB outbreaks in North America since the 1990's. McMullen et al. (1997) postulates that the primary contributing factors to FHB severity is high rainfall and high soil moisture levels, particularly in May and early June, when winter wheat is often flowering. Although controlling weather is beyond the scope of producers, McMullen et al. (1997) also cite several cultural practices which have contributed to an increase in FHB epidemics, namely an increase in conservation tillage, use of susceptible cultivars, and short rotations between susceptible crops. Corn is an important host of the FHB pathogen and *F. graminearum* often overwinters on infected corn debris. Teich and Hamilton (1985), during an observational study of wheat fields in Ontario, found

significantly higher blight and DON concentrations when corn was the previous crop compared to rotations where either soybean or barley were the previous crop. This observation was supported by Dill-Mackey and Jones (2000), who also found that disease incidence and severity were lowest in moldboard plowed plots, where the soil is turned over, compared with chisel plowed or no-till plots, which leaves a significant amount of residue on the soil surface. This clearly indicates that as the use of conservation tillage increases and wheat is planted after corn, the risk of an FHB epidemic also increases, making it even more important to understand the best management strategies for controlling FHB.

Fungicides have been used to attempt to manage FHB; however, the amount of disease reduction has not been consistent or adequate to recommend using fungicides alone (El-Allaf et al., 2002). Most researchers, many of which are spraying fungicides under more ideal conditions have better timing and more adequate spike coverage then producers, report less than 50 to 60% efficacy (Mesterhazy, 2003). Although fungicide efficacy is inadequate for complete control of FHB, some reduction in symptoms and DON content is possible. Until recently, tebuconazole was the most effective and available fungicide on the market (Homdork et al., 2000; Mesterhazy et al., 2003; Perry et al., 1995). Despite its reported efficacy, tebuconazole has only been shown to reduce FHB symptoms and DON accumulation by up to 50%, and only when applied with good coverage and at peak anthesis (McMullen et al., 1997; Schaafsma and Tamburic-Ilincic, 2005). These results demonstrate the potential of fungicides in an integrated management system; even

though they may not be consistent or efficacious enough to be used as the only technique for managing FHB.

Since fungicides have not yet proven effective for managing FHB, the most cost-effective control source may be in the use of resistant winter wheat cultivars. Resistance includes active mechanisms and passive ones. Active mechanisms for resistance to FHB have been categorized into five types (Mesterhazy, 1995). Type I is resistance against initial floral infection. Type II is resistance to the growth of fungal tissue through the wheat head after the initial infection has already occurred. Type III is resistance to kernel infection (glume infection has occurred but the pathogen is unable to penetrate the kernel). Type IV resistance is yield tolerance, and type V resistance is decomposition of toxins in the kernel. Although types I and II are relatively easy to test and recognize, types III, IV and V are highly correlated and much more difficult to breed into new wheat lines (Bai and Shaner, 1994). No completely resistant wheat source has been identified as of yet. Breeding for type I resistance alone provides minimal benefit since incomplete resistance can lead to the pathogen spreading throughout the rachis, causing complete blighting of the wheat head, after initial infection has occurred and given the appropriate environmental conditions. Because of this, type II resistance has been the focus of many breeding programs. "Sumai 3", a Chinese spring wheat cultivar, has been identified as the most effective source of type II resistance. "Sumai 3" is a spring wheat not adapted to the Mid-Atlantic region, where normally soft red winter wheat cultivars are grown. This cultivar is also low yielding and susceptible to other pathogens present in the region. Breeding programs have been attempting to transfer type II resistance from

"Sumai 3" and its progeny into soft red winter wheat lines, but type II scab resistant cultivars in regionally well-adapted lines are not numerous (Griffey et al., 2001).

Passive resistance mechanisms are morphological traits of the plant that contribute to a decrease in infection. Mesterhazy (1995) describes four types of passive resistance mechanisms which decrease either natural infection or disease severity: absence of awns, high spikelet density, escape or flowering in the boot stage and tallness. Dwarf genotypes have shown a higher natural infection rate than taller plants, perhaps due to a greater probability that rain splash from the soil surface which could carry ascospores or conidia to the susceptible wheat head (Mesterhazy, 1995). Another untested passive mechanism which could increase resistance is foliage density. Infected crop debris within the field is a significant source of inoculum, especially and most importantly, when conservation tillage is used and wheat is planted after corn. By increasing the seeding rate, a higher foliage density may be achieved, creating a barrier which could reduce the number of *F. graminearum* spores able to the reach susceptible wheat heads. Much like plant height, which creates a barrier of space, higher plant densities could decrease natural infection by creating a protective vegetative barrier. Although this would not affect any aerial spores from more distant sources, it could be part of an integrated system for decreasing FHB symptoms to acceptable levels.

There are three main objectives in this study. The first is to investigate the role of type II resistance in disease management. Namely, whether or not it is possible to overwhelm type II resistance when more than one infection occurs on the wheat head. Since the resistance of stopping the spread of fungal hyphae within the

rachis is not complete, there is a good chance that under highly favorable environmental conditions and severe disease pressure, this type of resistance may be overwhelmed. Secondly, I wish to determine whether the combination of a fungicide and a type II resistant cultivar reduces FHB incidence and severity, more than using either tool alone. The ability of the foliar fungicide tebuconazole to reduce disease incidence and severity has been documented, but it is unknown whether the disease reducing effects of this fungicide and a resistant cultivar can be stacked to reduce FHB to an economically acceptable level. Lastly, I would like to explore the role of foliage density in an IPM program along with a type II resistant cultivar and a fungicide application. By combining active and passive mechanisms of resistance as well as a fungicide, I hope to create a well-rounded and economically feasible IPM system, using a variety of disease reducing tools.

Chapter 2: The effect of multiple point inoculations on Fusarium Head Blight development in type II resistant cultivars in the greenhouse.

Introduction

The physical process by which type II resistant cultivars reduce the spread of *F. graminearum* through the wheat (*Triticum aestivum*) head is not fully understood. Pritsch et al (1999) have shown that 'Sumai 3', a type II resistant cultivar from China, accumulated thaumatin-like defense proteins, PR-4 and PR-5, earlier and in greater levels after infection than Wheaton, a susceptible cultivar. It might be possible to overwhelm the defense mechanisms of type II resistant cultivars when plants are subjected to highly favorable weather conditions for disease development or heavy inoculum loads.

A common practice for testing type II resistance is by point source inoculation in the greenhouse, a process by which a conidial suspension of *F. graminearum* is placed directly onto a wheat floret. Most plant breeders screening for FHB resistance use a single point source inoculation in order to see how far the disease progresses in the head. I hypothesize that if multiple inoculations were to occur, the resistance of type II cultivars would limit localized spread of the pathogen, but would be insufficient overall, and the wheat head would be overwhelmed with disease. Since resistance to Fusarium head blight is quantitative and can vary widely, I tested the

effect of increased point source inoculations on three soft red winter wheat cultivars with reported type II resistance.

Materials and Methods

A 4x3 factorial experiment in a randomized complete block design was conducted at the University of Maryland's Research Greenhouse Complex. Pioneer cultivars 25R54, 25R42, and 25R35, showing partial resistance from the 'Sumai 3' Chinese spring wheat source (Greg Marshall, Hi-Bred International, personal communication), were obtained from the Pioneer seed company (Pioneer Hi-Bred International, Johnston, IA). One susceptible cultivar, Jackson (Griffey et al, 2001)., was obtained commercially The four soft red winter wheat cultivars were germinated in 6-cell seed packs filled with soilless media and then vernalized for six weeks in a cold room below 10 ˚C (50 ˚F) in the winter of 2005. The cold room was equipped with fluorescent lights set for 12 hours of light per day. After vernalization, the plants were moved to the greenhouse where each plant was placed in a twelve-inch pot containing soil-less media and topsoil at a volumetric rate of three to one. Three pots per cultivar were placed randomly in each of the ten replications that were randomly arranged on the greenhouse bench. The main tiller of each plant was flagged with plastic tape to designate which head would be used for inoculation.

A carboxymethylcellulose (CMC) broth was prepared and seeded with a spore suspension of *Fusarium graminearum* prior to flowering to ensure it was ready whenever flowering occurred (Cappellini and Peterson, 1965). The CMC broth was incubated at room temperature on a table shaker at 200 rpm under continuous

supplemental fluorescent light to stimulate sporulation. The broth culture reached a carrying capacity of approximately 4 x 10^4 spores ml^{-1} six days after the initial seeding. Spores were counted with a haemocytometer under the microscope. When the plants reached flowering, a micropipette was used to inject either one or three of the flowering spikelets with 10 µl of the CMC spore suspension. The spore suspension was injected into the middle spikelet of each wheat head receiving one inoculation. Wheat heads subjected to three inoculations were also injected at the middle floret, as well as two florets above and below the initial injection. An uninoculated plant was used as a control. The inoculated and control plants were placed in a PVC pipe tent frame that was covered in landscape fabric (Figure 1). The tents were mist irrigated from above to create a humid chamber and ensure proper growth and development of *F. graminearum* within the wheat head. The experiment was repeated in 2006, substituting plain white muslin as the tent fabric to create a more humid environment. A wool mat was also added to the greenhouse bench, to help maintain humidity.

Visual disease severity measurements were taken 4, 8, 14 and 21 days after inoculation. These measurements consisted of a calculation of percentage of blighted spikelets per spike. Area under the disease progress curve was calculated using the trapezoidal method with units of percent disease severity and days (Campbell and Madden, 1990). After the last visual assessment, the plants were removed from the misting tents and were allowed to grow to maturity in the greenhouse. After senescence, the tagged spikes from each plant were cut, placed in labeled paper bags and taken back to the lab. The spikes were threshed by hand and the total number of

seed and seed weight was determined. The number of Fusarium damaged kernels (FDK) was visually determined for both the inoculated and control plants from the threshed seed. The percentage of FDK in a given grain sample is a way to determine the amount of seed infection and is indicative of the potential for mycotoxins contamination. Fusarium damaged kernels have a white, chalky appearance and are often shrunken or shriveled. Seed that is particularly shriveled and highly infected are generally termed tombstones. Any seed with visual symptoms or signs of *F. graminearum*, such as a pink, white or chalky discoloration as well as tombstones were counted as Fusarium damaged kernels.

All data was analyzed using the mixed procedure of the Statistical Analysis Systems software (SAS Institute, Cary, N.C.). Data from both years of the trial were combined for analysis with year being treated as a random factor. Year was significant when included in the statistical model, but no interactions occurred with the other factors so data was combined. Fischer's protected least significant difference at the 5% level was calculated for mean comparisons.

Results and Discussion

Significantly more disease developed with three inoculations than with one inoculation, and there was no disease development in uninoculated control spikes. The lack of disease in the controls caused a significant inoculation by cultivar interaction due to the large magnitude differences when spikes were inoculated. Since the interaction between the two factors was due solely to the control plants, the controls were removed from the final disease analysis. The ANOVA results of

disease severity (DS) at 4, 8, 14, and 21 days after inoculation, as well as the AUDPC of both data sets, with and without controls, are presented in Table 1.

It often takes 7 to 10 days for FHB symptoms and signs to appear in the field (Parry et al, 1995). Due to the optimum conditions for FHB development in the greenhouse, some symptoms were noticeable 4 days after inoculation. However, after 4 days, there were no major differences between cultivars inoculated at a single spikelet. There was a range DS but the average of treatments with one inoculation was 4.2% (Table 2). When the cultivars were inoculated at three points with a *F.* graminearum spore suspension the four cultivars were divided into two statistically different groups. 25R42 and 25R35 were equivalent with a lower DS of 7.7% and 7.6%. Jackson and 25R54 were also equivalent with a higher DS of 17.5% and 18.4%. After 8 and 14 days post inoculation, DS of all the cultivars increased, but the DS patterns observed at 4 days for the cultivars remained the same.

The final disease assessment occurred 21 days after inoculation. The final DS assessment is the most important because it is more closely correlated with yield loss and DON content than earlier assessments of FHB. Spikes with one point source inoculation showed varying degrees of disease severity ranging from 6% (25R42) to 61% (Jackson). All of the type II resistant cultivars with a single inoculation had a significantly lower disease severity than the susceptible control, Jackson, when inoculated at a single spikelet ($P < 0.0001$). However, there was a wide range of resistance between the three cultivars with 25R42 being the most resistant with a DS of 6%, 25R35 intermediate with a DS of 18%, and 25R54, the least resistant, with a DS of 42% (Table 2). Only the cultivars 25R42 and 25R35 had a significantly lower

DS, (16% and 47%, respectively), than Jackson, after spikes were inoculated at three points with the *F. graminearum* spore suspension. The DS of 25R54 was statistically equivalent to that of Jackson.

The area under the disease progress curve (AUDPC) is a useful way to analyze the amount of disease severity over time. The factors inoculation and cultivar both had highly significant differences ($P < 0.0001$). With one inoculation, 25R35 and 25R42 had equivalent AUDPC (Table 2). The AUDPC values of Jackson and 25R54 were higher than 25R35 and 25R42, but were equivalent to one another at one and three inoculations. The difference between 25R42 and 25R35 with three inoculations was statistically significant. The higher disease pressure of three inoculations increased the AUDPC of 25R42 and 25R35 to 236.9 and 486.3, respectively.

The average percent Fusarium damaged kernels (FDK), or tombstones, closely followed the disease severity trends. There was a highly significant inoculation by cultivar interaction due to magnitude differences in response to differing levels of inoculation among the cultivars. All three partially resistant cultivars had a significantly lower percent FDK than Jackson when inoculated at a single central spikelet along the wheat head. The resistant cultivars were not significantly different from one another for FDK, although 25R42 had the lowest and 25R54 had the highest percent FDK (Table 3).

Treatments with three inoculations resulted in higher FDK percentages for all cultivars with the exception of 25R42, which only showed a 2% increase in FDK when the number of point source inoculations increased. Unlike treatments with one

inoculation, the resistant cultivars were not similar in percent FDK. 25R35 and 25R54 had percent FDK values of 20% and 28%, respectively, but were not statistically different from one another. 25R42, in keeping with other presented data, was the most resistant to kernel infection and had the lowest FDK (Table 3). 25R54 had a numerically lower FDK value, but it was not significantly different from Jackson (Table 3). This was expected since the disease severity values were nearly identical, and disease severity and percent FDK are generally highly correlated.

Thousand kernel weight (TKW) is not as beneficial as a full grain yield analysis in determining the quality and marketability of a cultivar, but TKW is a direct measurement of one of the five components of yield, average seed weight (Slafer, Calderini and Miralles, 1996). The main effect factors of inoculation and cultivar both significantly affected TKW. There were no differences, however, between the cultivars when no inoculation occurred (Table 3). On average, one inoculation decreased TKW by 5.8 g and three inoculations decreased TKW by 12.4 g. The three cultivars with type II resistance and the susceptible cultivar, Jackson, also had equivalent TKW values when only one inoculation was administered, with an average of 27.9 g. When the cultivars received three inoculations, on the other hand, 25R42, the cultivar with the highest level of resistance had a significantly higher TKW than the susceptible and other type II resistant cultivars. In fact, increasing the number of point source inoculations did not lower TKW for 25R42. All other cultivars in this study lost, on average, 8.4 g/1000 kernels, when point source inoculations were increased from one to three (Table 3).

Since type II resistance is a quantitative trait, there is a wide range of level of resistance (Mesterhazy, 1995). This study shows that when multiple infections occur on the wheat head, much like they would in severe FHB epidemic years, it is possible that FHB may overwhelm certain partially type II resistant cultivars, increasing disease severity to levels similar to susceptible cultivars. This is of an important note considering many greenhouse tests of FHB resistance are done using only one point source inoculation per wheat spike. Homdork et al (2000) found that lower TKW, as well as a lower number of kernels per head, were the main sources of yield loss due to FHB. My data show that only cultivars with the highest level of type II resistance, such as 25R42, maintain TKW values when disease pressure is high. Although, type II resistance is clearly a beneficial component of FHB management, alone it may not be enough to properly protect producers from high levels of disease severity and yield losses.

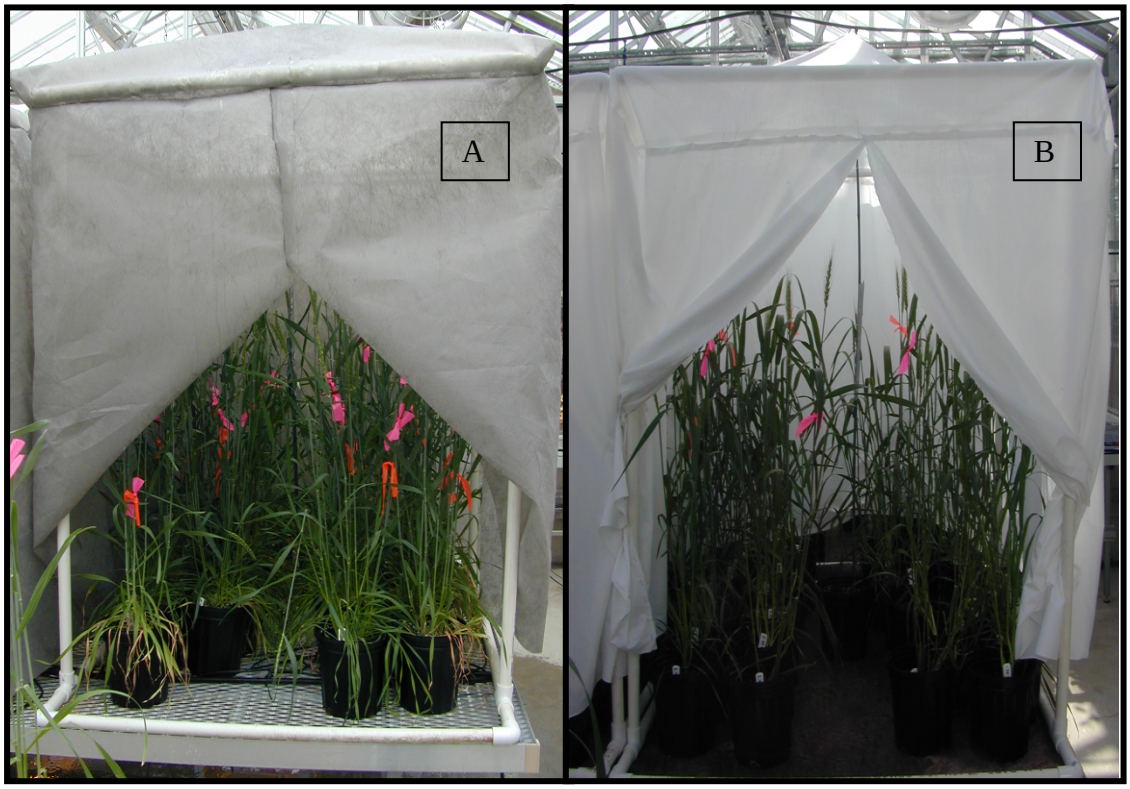

Figure 1. Humidity tents framed with PVC pipe used in the greenhouse trials in 2005 (A) with a cover of landscaping fabric and 2006 (B) with a cover of white cotton muslin and a wool blanket floor.

Table 1. ANOVA results of disease severity at 4, 8, 14, and 21 days after inoculation and area under the disease progress curve with and without uninoculated controls.

Controls included:

	Disease Severity				
	4 DAI	8 DAI	14 DAI	21 DAI	AUDPC
Effect	Pr > F	Pr > F	Pr > F	Pr > F	Pr > F
Inoculation	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Cultivar	0.0002	0.0041	<0.0001	<0.0001	<0.0001
Inoculation*Cultivar	0.0021	0.1311	<0.0001	<0.0001	<0.0001

Controls removed:

	Disease Severity				
	4 DAI	8 DAI	14 DAI	21 DAI	AUDPC
Effect	Pr > F	Pr > F	Pr > F	Pr > F	Pr > F
Inoculation	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Cultivar	0.0001	0.0032	<0.0001	<0.0001	<0.0001
Inoculation*Cultivar	0.0440	0.4943	0.0838	0.1641	0.0866

Table 2. The effect of multiple point inoculations on the disease severity and AUDPC of winter wheat cultivars differing in resistance to FHB over time.

		Disease Severity[c]				
Cultivar[a]	Inoculation[b]	4 DAI[d]	8 DAI	14 DAI	Final[e]	AUDPC[f]
25R42	1	3.9	4.8	6.0	6.3	92.9
	3	7.7	13.3	15.1	16.2	236.9
25R35	1	2.0	4.6	9.2	17.8	151.7
	3	7.6	14.8	35.4	47.0	486.3
25R54	1	5.0	8.2	24.3	42.4	357.4
	3	17.5	26.1	63.8	78.3	854.6
Jackson	1	6.0	12.3	40.5	61.3	550.3
	3	18.4	27.7	66.8	76.9	879.0
	$LSD_{0.05}$	3.0	5.3	8.5	9.3	98.7

[a]Soft red winter wheat cultivars with varying resistance to Fusarium head blight, numbered cultivars are all Pioneer brand.

[b]The number of *F. graminearum* point source inoculations along the wheat spike.

[c]Disease severity- percent infected spikes per spikelet.

[d]DAI- days after inoculation.

[e]Final disease assessment, 21 days after inoculation.

[f]Area under the disease progress curve

Table 3. The effect of multiple point inoculations on the FDK and TKW of cultivars varying in type II resistance to FHB.

Cultivar[a]	Inoculation[b]	FDK[c]	TKW[d]
25R42	0	0.0	35.6
	1	5.8	30.6
	3	8.5	30.5
25R35	0	0.0	30.0
	1	7.3	25.2
	3	20.3	19.3
25R54	0	0.0	34.6
	1	13.0	26.0
	3	27.7	17.3
Jackson	0	0.0	35.4
	1	25.8	29.7
	3	32.7	19.0
	$LSD_{0.05}$	5.4	5.6

[a]Soft red winter wheat cultivars with varying resistance to Fusarium head blight, numbered cultivars are all Pioneer brand.
[b]The number of *F. graminearum* point source inoculations along the wheat spike.
[c]The percent of visually Fusarium damaged kernels.
[d]Thousand kernel weights in grams.

Chapter 3: The effect of multiple point source inoculations, varying cultivar resistance and tebuconazole on the severity of Fusarium Head Blight in the greenhouse.

Introduction

Type II resistant cultivars are a promising solution to the problem of reducing Fusarium head blight (FHB) severity, but so far, few cultivars have been released commercially that show both a high level of resistance and agronomic properties sought after by farmers. As well as cultivar resistance, foliar fungicides are another tool available to producers. However, the use of fungicides for protection against FHB has been questioned since results have been inconsistent in different locations and in different years (McMullen et al., 1997). Tebuconazole is a fungal sterol inhibitor, which has been actively sought for use against FHB because it was the most effective and available product at the start of this research (Homdork et al., 2000; Mesterhazy et al., 2003; Perry et al., 1995). Many researchers have shown that tebuconazole can reduce FHB symptoms and DON accumulation when it is applied with good coverage at the very peak of anthesis, but this may not be enough to bring Fusarium head blight symptoms to economically feasible levels (McMullen et al., 1997; Schaafsma and Tamburic-Ilinicic, 2005). The main purpose of this study is to examine what effect the combination of tebuconazole and a type II resistant cultivar, under varying inoculum loads, has on reducing Fusarium head blight severity in a greenhouse environment.

Materials and Methods

Pioneer cultivar 25R42, a soft red winter wheat (*Triticum aestivum*) with type II resistance obtained from Pioneer Hi-Bred International, Inc (Johnston, IA), and Jackson, a susceptible control, were prepared as described for the greenhouse cultivar trial, but half of the plants in each of ten blocks were randomly selected to receive an application of tebuconazole, 1-(4-Chlorphenyl)-4,4-dimethyl-3-[1,2,4]triazol-1-ylmethyl-pentan-3-ol, at the beginning of anthesis. Tebuconazole, tradename Folicur 3.6F (Bayer CropScience, Research Triangle Park, NC), was applied to runoff at a concentration of 1.48 ml L^{-1}. The rate was based on US EPA label specifications for field use of 290 ml ha^{-1} of formulated product and a spray solution of 187 L ha^{-1}. A standard spray bottle was used to apply the fungicide to the wheat heads. One day after the fungicide application, sprayed and unsprayed plants received zero, one or three point source inoculations of 10 µl of a *F. graminearum* spore suspension as described previously. The inoculated plants were then placed in misting tents alongside uninoculated checks and visual and post-harvest observations and data collections were made as previously described.

The experiment was conducted at the Research Greenhouse Complex at the University of Maryland in 2005 and repeated in 2006. All data was analyzed using the mixed procedure of the Statistical Analysis Systems software (SAS Version 9.2). Data from both years was combined for analysis, and the year was used as a random factor for determining error. Year was significant when included in the statistical model, but no interactions occurred with the other factors so data was combined.

Fischer's protected least significant difference at the 5% level was calculated for mean comparisons.

Results and Discussion

The main objective of this research was to examine the effect of combining a type II resistant cultivar and a fungicide, both of which are partially effective against *Fusarium graminearum*, on the overall severity of FHB. All of the main effects: cultivar susceptibility, fungicide, and number of points of inoculation, had a highly significant effect on FHB severity ($P < 0.0001$). Significant interactions occurred between all possible factors when analyzing FHB disease severity, due to the inclusion of uninoculated control plants which did not develop disease symptoms. The uninoculated controls were removed from analysis since they did not contribute to an estimate of error. The ANOVA results for disease severity at 4, 8, 14 and 21 days after inoculation, as well as area under the disease progress curve of both data sets, with and without controls, are presented in Table 4.

FHB symptoms were visible as early as 4 days after inoculation when the first assessment occurred. Disease levels were relatively low at the time. No treatment had a DS higher than 20% (Table 5). All of the main effects, (cultivar, inoculation and fungicide), were significant. There were also several significant interactions that occurred only at this particular assessment. Both inoculation by cultivar and inoculation by fungicide were significant at this time. 25R42 had lower DS at both inoculation levels than Jackson. Tebuconazole reduced DS of Jackson at both inoculation levels and although there was a trend of reduced severity with the use of tebuconazole on 25R42, the result was not statistically significant ($P = 0.0986$).

The second disease assessment occurred eight days after inoculation. All of the main effects were significant but there was also a significant interaction between cultivar and fungicide. The significant interaction was due entirely to the different magnitudes of the effect that tebuconazole had on the FHB severity of the two cultivars. This interaction was also significant for every disease assessment that occurred after 8 days post inoculation. The disease severity on 25R42 treated with tebuconazole was not significantly different than Jackson treated with tebuconazole, at either inoculation level (Table 5). Although disease severity for all treatments increased when severity was assessed 14 days after inoculation, there was no change in rank of the cultivars or fungicide treatment.

The final disease assessment occurred 21 days after inoculation. The last assessment of disease is the most important since is the most likely to be correlated with yield losses and DON content. All of the main effects: cultivar, fungicide and inoculation, were highly significant ($P \leq 0.001$). The type II resistant cultivar, 25R42, showed no reduction in disease severity when a fungicide was applied at flowering ($P = 0.9477$). Whether 25R42 was sprayed or unsprayed, the plants had similar average disease severity of 5.4% with one inoculation and 16.1% with three inoculations (Table 5). Tebuconazole significantly reduced DS of Jackson at both levels of inoculation. The average reduction for Jackson at both levels of inoculation was 55%.

Significant interactions also occurred when analyzing the percent of Fusarium damaged kernels (FDK). But again, only the cultivar by fungicide interaction was significant after the uninoculated controls were removed from the analysis, because

an application of tebuconazole had very little influence on the percentage of FDK of 25R42. 25R42 had very low percentages of FDK in this study. FDK without a fungicide and with three inoculations of a *F. graminearum* spore suspension was 9.3%. Subjected to only one inoculation and no fungicide treatment, FDK of 25R42 was only 3.2%. Fungicide treatment of 25R42 slightly lowered the percentage of FDK to 6.6% with three inoculations and 2.4% with one inoculation. Although, these results were slightly lower than the unsprayed treatments, the differences were not statistically significant.

Greenhouse trials do not lend themselves to yield analysis; however, analyzing thousand kernel weights (TKW) does allow analysis of average seed weight, one of the five components of yield (Slafer, Calderini, and Miralles, 1996). The main factors of cultivar resistance, fungicide and the number of point inoculations were all significant for TKW (Table 6). All interactions between the main factors were also significant, including the 3-way interaction between inoculation, cultivar resistance and fungicide. Unfortunately, the number of significant interactions makes it difficult to generalize how the main factors affected TKW. Thousand kernel weights are presented in Table 6.

In this study, the combination of a type II resistant cultivar and a foliar fungicide did not reduce FHB disease severity, FDK or TKW. However, the failure of the fungicide treatment to reduce disease severity in 25R42 may be due to the inoculation method of introducing the pathogen directly to the flowers, and the lack of fungicide translocation to the ovaries. The level of type II resistance found in 25R42 is high enough that the spread of infection from the initial source did not

occur. As results from the previous trial demonstrated, as well as the results from numerous other researchers have shown, there are varying degrees of fungal inhibition from type II resistant cultivars (Bai, 1995 and Mesterhazy, 1999). In this trial, the disease severity of a FHB susceptible cultivar (Jackson) was reduced by approximately 55% with an application of tebuconazole at flowering. A reduction in disease severity of that magnitude is higher than most researchers report, but that may be due to the amount of control over timing and spike coverage that is available in the greenhouse as compared to field trials (Homdork et al., 2000). Tebuconazole reduced disease severity to the same level that type II resistance did in this trial. However, if tebuconazole is not translocated to the ovaries from the glumes and rachis, and since inoculum is directly introduced to the developing flowers within the glumes, the tebuconazole treatment could not prevent infection by *F. graminearum.* Although, the addition of a tebuconazole treatment did not reduce disease severity in a type II resistant cultivar in this study, the combination may still be the most beneficial for management of FHB in the field where infection may be prevented by a foliar fungicide.

Table 4. ANOVA results of disease severity at 4, 8, 14, and 21 days after inoculation and area under the disease progress curve with and without uninoculated controls.

Controls included:

	Disease Severity				
	4 DAI	8 DAI	14 DAI	21 DAI	AUDPC
Effect	Pr > F	Pr > F	Pr > F	Pr > F	Pr > F
Inoculation	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Cultivar	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Inoculation*Cultivar	<0.0001	0.0005	<0.0001	<0.0001	<0.0001
Fungicide	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Inoculation*Fungicide	0.0012	0.0017	<0.0001	<0.0001	<0.0001
Cultivar*Fungicide	0.0852	0.0006	<0.0001	<0.0001	<0.0001
Inoculation*Cultivar*Fungicide	0.4242	0.0393	<0.0001	<0.0001	<0.0001

Controls removed:

	Disease Severity				
	4 DAI	8 DAI	14 DAI	21 DAI	AUDPC
Effect	Pr > F	Pr > F	Pr > F	Pr > F	Pr > F
Inoculation	<0.0001	<0.0001	0.0002	0.0013	<0.0001
Cultivar	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Inoculation*Cultivar	0.0475	0.1905	0.6302	0.9917	0.4374
Fungicide	<0.0001	<.0001	<0.0001	<0.0001	<0.0001
Inoculation*Fungicide	0.0277	0.1349	0.4872	0.9232	0.328
Cultivar*Fungicide	0.0522	0.0005	<0.0001	<0.0001	<0.0001
Inoculation*Cultivar*Fungicide	0.8402	0.5687	0.8251	0.8571	0.7946

Table 5. The effect of multiple point inoculations and a foliar fungicide on the disease severity and AUDPC of winter wheat cultivars differing in resistance to FHB over time.

			Disease Severity[c]				
Cultivar[a]	Fungicide[b]	Inoculation[c]	4 DAI[d]	8 DAI	14 DAI	Final[e]	AUDPC[f]
25R42	Tebuconazole	1	2.3	3.9	4.7	5.3	72.6
		3	6.3	9.4	13.3	15.8	201.2
	None	1	2.7	4.0	4.4	5.4	72.6
		3	10.0	13.7	16.0	16.4	251.2
Jackson	Tebuconazole	1	4.8	6.1	7.4	19.4	157.2
		3	11.8	14.9	17.7	31.0	321.8
	None	1	8.0	19.8	54.0	79.4	744.4
		3	19.2	38.0	69.9	89.3	995.3
		$LSD_{0.05}$	2.1	4.8	6.1	6.5	68.8

[a]Soft red winter wheat cultivars with varying resistance to Fusarium head blight.

[b]The number of *F. graminearum* point source inoculations along the wheat spike.

[c]Disease severity - percent infected spikelets per total spikelets.

[d]DAI- days after inoculation.

[e]Final disease assessment, 21 days after inoculation.

[f]Area under the disease progress curve.

Table 6. The effect of point source inoculations and a spray application of a foliar fungicide on the thousand kernel weights (TKW) of two winter wheat cultivars.

Cultivar[a]	Inoculation[b]	Fungicide[c]	FDK[d]	TKW[e]
25R42	Zero	Sprayed	0.0	34.5
		Unsprayed	0.0	32.3
	One	Sprayed	2.4	31.9
		Unsprayed	3.2	31.9
	Three	Sprayed	6.6	31.4
		Unsprayed	9.3	32.3
Jackson	Zero	Sprayed	0.0	33.2
		Unsprayed	0.0	35.9
	One	Sprayed	7.5	37.1
		Unsprayed	30.4	16.7
	Three	Sprayed	11.3	26.7
		Unsprayed	41.4	16.7
		$LSD_{0.05}$	4.0	5.9

[a]Soft red winter wheat cultivars with varying resistance to Fusarium head blight. The susceptible Jackson and the partially resistant, Pioneer cultivar 25R42.

[b]The number of *F. graminearum* point source inoculations along the wheat spike.

[c]A foliar application of the fungicide tebuconazole at the time of flowering.

[e]FDK = Fusarium damaged kernels (%).

[e]TKW = thousand kernel weights (g).

Chapter 4: Effect of cultivar resistance and fungicide use on field management of Fusarium head blight in soft red winter wheat.

Introduction

Field testing is essential to understanding the true efficacy of pest management techniques. Unlike a greenhouse, field studies allow for testing the effect of combining a type II resistant cultivar with a fungicide on agronomic qualities, such as grain yield and grain quality after mechanical harvesting as it would be experienced by commercial producers. Type II resistance, or resistance to spread of Fusarium head blight (FHB) infection through the wheat (*Triticum aestivum*) head, is a promising management technique for disease control. However, resistant gene sources from unadapted germplasm may be linked to traits that are not suitable to the Mid-Atlantic climate or cropping systems. It is therefore essential to field test any cultivar that may be used in FHB management to ensure that the cultivar will be suitable for the Mid-Atlantic region. Tebuconazole, a fungicide which goes by the trade name Folicur and was one of the first registered for use in the U.S. market for FHB, has been inconsistent in controlling the disease particularly in the field. Some factors affecting the efficacy of tebuconazole, such as inadequate coverage of the wheat head at the time of spraying or difficulty of timing the application when flowering will occur.

Much like the previous greenhouse trial (Chapter 3), the purpose of this study was to examine the effectiveness of combining a type II resistant cultivar with a foliar fungicide in managing FHB. I hypothesized that the combination of a fungicide and a wheat cultivar with type II resistance would have the lowest amount of disease compared to a FHB susceptible cultivar or the same resistant cultivar not been treated with a fungicide.

Materials and Methods

A 2^3 factorial experiment in a randomized complete block design, with three replications, was planted on November 17, 2003 and November 2, 2004 at the Central Maryland Research and Education Center, Beltsville Facility, Beltsville, MD. The factors and their levels were: inoculated and uninoculated; a partially resistant and susceptible cultivar; and one application of tebuconazole at anthesis and unsprayed. The partially resistant soft red winter wheat cultivar, Pioneer cultivar 25R42, was obtained from Pioneer Hi-Bred International (Johnston, IA) and the FHB susceptible cultivar, Jackson, was obtained commercially. The plot size was 6 by 9 meters. The trial was seeded at a rate of 375 seeds m^{-2} following a corn (*Zea mays*) crop. The plots were inoculated with a spore suspension of *F. graminearum* at the beginning of wheat anthesis (10-15% of heads with exposed anthers) and then again four days later. After final dilution, *F. graminearum* spore concentrations were approximately $1x10^6$ ml^{-1} in 2004 and $6.7x10^5$ ml^{-1} in 2005. Spores were counted using a haemocytometer under the microscope. The trial procedures were modified in 2005, by the addition of an intermediate level of the inoculation factor. For 2005, the levels

of inoculation were uninoculated, a high level of inoculum and a low level of inoculum (created by diluting the original spore suspension 10 fold). Spore suspension preparation is described in the greenhouse cultivar trial materials and methods section (Chapter 2). The center rows (3 m) of inoculated plots were supplied the spore suspension through a 3 m spray boom equipped with flat fan 8001(Teejet Spraying Systems Co., Wheaton, IL) nozzles. The two person hand held sprayer was pressurized with CO_2 and operated at 27.5 Kpa pressure to deliver 187 L ha^{-1} spray solution. Tebuconazole, in the form of Folicur 3.6F (Bayer CropScience, Research Triangle Park, NC), was applied at a rate based on US EPA label specifications for field use of 290 ml ha^{-1}. The same boom and nozzles were used to spray tebuconazole, which was applied one day after the first inoculation. Misting irrigation was set up using a 4,500 L tank equipped with a pump to deliver the mist to plots via Micro-Bird II spinners, model SP24-340, (Rain Bird Corporation, Azusa, CA). The Micro-Bird spinners provided approximately 1.0 mm of water to the center of each plot every time the plots were irrigated. All plots were mist irrigated for 30 minutes in morning and 30 minutes in the evening one to two weeks prior to anthesis and for three weeks after anthesis, on days when no natural rain event had occurred. Misting irrigation was suspended for 24 hours after fungicide application to allow the chemical to penetrate the plant tissue. Weather data was obtained from a station located near the Beltsville, MD field site.

Disease incidence and severity were measured when the heads were still green, but FHB symptoms were clearly visible. At the soft dough stage of kernel development (Feekes' growth stage 11.2 (Large, 1954)), approximately three weeks

after inoculation, 30 heads were randomly sampled along a diagonal transect within the plot. The heads were bagged and placed on dry ice until they could be placed in a -18 °C freezer. Disease severity was measured as the percentage of infected spikelets spike^{-1}. Disease incidence was measured as the percentage of infected spikes per sample. A Massey Ferguson 8-XP (AGCO, Duluth, GA) plot combine was used to harvest the wheat plots. Yield, test weight and grain moisture were determined for each plot by a HarvestMaster data collections system (Juniper Systems, Logan, UT) installed on the combine. Average kernel weight, percent tombstones and DON were determined from seed samples gathered at harvest. DON content was determined by ELISA using a Veratox DON 5/5 (Neogen, Lexington, KY) test kit. Samples for the ELISA kit were prepared by obtaining a random 100 g sample from seed collected at harvest and grinding with a Mr. Coffee® coffee grinder so that 75% of the flour could pass through a 20 mesh sieve. The Veratox kit protocol was followed to obtain DON quantification of 0.5-5ppm. Ten g of the ground sample were shaken with 100 ml of distilled water for 3 minutes. The extract (10 to 20 ml) was then filtered through Whatman #1 filter paper. The filtered extract was then added to the enzyme linked wells along with the appropriate reagents supplied in the Veratox kit. A Bio-Rad Benchmark microplate reader (Bio-Rad Laboratories, Hercules, CA) was used to quantify the results of the ELISA test. Optical density data obtained with the microplate reader were interpreted by taking the natural log regression of the standards provided by Neogen and solving for the test samples.

All data was analyzed using the mixed procedure of the Statistical Analysis Systems software (SAS). Fisher's least significant differences were calculated at the

0.05 level for data when the ANOVA indicated significant treatment effects. Data from the two years of the study were not combined for analysis due to significant differences and interactions between the two years. The additional treatment of a low inoculum level (2005) was statistically not different than the uninoculated treatments and was left out of the final data analysis.

Results and Discussion

Disease incidence (DI) was visually determined from a sample of spikes randomly collected from each plot, was vastly higher in 2004 than in 2005 (Table 7). Conditions were so favorable for disease development in 2004 that all inoculated plots, regardless of fungicide treatment or cultivar resistance, had DI's of 98% to 100%. There were no significant effects for either fungicide or cultivar in 2004. However, preplanned comparisons of uninoculated treatments with and without an application of tebuconazole show that background DI, development of disease in the absence of inoculation, was reduced by the fungicide treatment by 14% ($P = 0.0493$) (Table 7). The resistant cultivar used in this study, 25R42, has primarily type II resistance to FHB, which is resistance to the spread of infection, and not resistance to initial infection. This could explain why cultivar resistance did not affect background DI in 2004. The differing results for the fungicide treatment between background DI and inoculated DI must lie in the use of artificial inoculation. The artificial inoculation used in this study was a concentrated spore suspension sprayed directly on the wheat heads at the time of flowering when the plants are most susceptible to FHB. The artificial inoculation was also sprayed onto the flowering wheat heads prior to the fungicide application. The timing of natural infection was most likely did

not occur in one day and probably occurred both before and after tebuconazole was applied. Timing and spore concentration differences between inoculation and natural infection are probably the reasons for the differences in between background DI and inoculated DI for the fungicide treatment.

Disease incidence was dramatically reduced in 2005 due to less favorable disease conditions. The DI was so low that uninoculated treatments (background DI) were not significantly different from zero (Table 7). Inoculated treatments on the other hand had a wide range of DI. This led to significant interactions of the main effects: inoculation by cultivar resistance, and inoculation by fungicide. As expected, treatments where Jackson did not receive a fungicide application at flowering had highest DI of 74.7% occurred. Unsprayed 25R42 treatments were significantly lower than Jackson, suggesting that 25R42 also has some amount of type I resistance, or resistance to initial infection, at least under low disease pressure. An application of tebuconazole at flowering significantly lowered DI by 22.0% and 14.7% for Jackson and 25R42, respectively (Table 7). The experimental design of the greenhouse trials (Chapters 2 and 3) was to inject a spore suspension directly into the spikelet. Fungicide had no affect on disease incidence due to this inoculation method, but when the spore suspension was sprayed over flowering wheat heads, instead of injected, tebuconazole was able to prevent some of the infection, lowering DI in the treatments that received a fungicide application near flowering.

Disease severity (DS), defined as the percent of infected spikelets spike^{-1}, is of particular importance when researching Fusarium head blight (FHB). Since there are no cultivars that have complete resistance to infection (type I resistance), breeding for

FHB has focused primarily on type II resistance. Cultivars with a high level of type II resistance, which is characterized by slower growth of *F. graminearum* through the wheat head, should have a lower level of DS, than cultivars without type II resistance. In 2004, the factors that most affected DS were inoculation and cultivar (P <0.0001). There was a significant inoculation by cultivar interaction in 2004, because inoculated Jackson had a much higher DS than inoculated 25R42. Although there was a trend towards DS reduction by an application of a foliar fungicide at flowering the results were not significant (P = 0.0701). When the susceptible cultivar, Jackson, was inoculated, DS ranged from 53.7%, when unsprayed, to 45.6%, with an application of tebuconazole at flowering (Table 7). Disease severity in inoculated 25R42 plots was 22.0% with a fungicide and 27.8% without a fungicide, respectively. In 2004, a fungicide application produced on average an 18% decrease in DS when the cultivars were inoculated with a spore suspension. When a no inoculation occurred, however, there was no difference between Jackson and 25R42 in DS (Table 7). Disease found in the uninoculated plots was a result of natural infection, most likely from local inoculum sources. Fungicide had no significant effect on DS in uninoculated plots.

In 2005, disease severity was much lower than in 2004 (Table 7). This was due to less suitable weather conditions in the spring prior to and during flowering and through the soft dough stage of kernel development, when the wheat head is susceptible to FHB infection (McMullen, 1997). In the mid-Atlantic region, those growth stages generally occur between May and June. During May and June of 2004, there was 36.1 mm more rain than in the same two months of 2005. Perhaps more importantly, there were also 11 more rain events in 2004 than in 2005. (Weather data

for 2004 and 2005 is presented in Table 12, Chapter 5). Optimum conditions for FHB disease development do not require heavy rainfalls, but rather extended periods of high humidity (Anderson, 1948), which more frequent rainfall could provide. The highest disease severity (DS) in 2005 was 3.2% in inoculated, no fungicide treatments of the susceptible cultivar, Jackson (Table 7). On the opposite end of the spectrum, uninoculated 25R42 had no disease, even where no fungicide was used in 2005. Inoculated 25R42 plots had DS levels of 1.7% and 1.6%, unsprayed and sprayed, respectively, significantly lower than Jackson for the same two fungicide treatments. Both inoculation and cultivar resistance had a significant effect on DS in 2005. Although there was a small trend of DS reduction when a fungicide was applied versus when it was not, those results were not significant ($P = 0.2784$).

Infection with FHB leads to direct yield losses due to low test weights and the loss of light weight seed during harvest and indirect losses due to the presence of mycotoxins in infected grain (Bai and Shaner, 1994). Deoxynivalenol (DON), or vomitoxin, is the most abundant toxin produced by *F. graminearum*. This toxin has a fairly low acute toxicity. However, the U.S. Food and Drug Administration has set advisory limits of 1 ppm for humans, 5 ppm for swine, and 10 ppm for chickens and cattle (Petska, 1996). In 2004, the only factor to cause significant effect on DON content was inoculation ($P < 0.0001$). Inoculation with a spore suspension of *F. graminearum*, supplied to the wheat heads at flowering increased DON content by 6.5 ppm compared with the background level of infection. Although I expected the partially resistant cultivar, 25R42, to have a lower toxin content, in this year both cultivars had equivalent DON content (Table 7). But in 2004, both cultivars also had

extremely high rates of DI, approximately 100%, which may be the reason there was not a cultivar effect on DON. There was also no effect of fungicide on DON, but fungicides have been shown to be inconsistent or less than adequate in reducing FHB in some years (El-Allaf et al., 2002). The DON of inoculated treatments was high, an average of 7.8 ppm for both cultivars. Grain with a DON content of 7.8 ppm would most likely be downgraded or rejected at the elevator or mill and be unsuitable for swine and human consumption. Due to the lack of significant effects in the 2004 study, it is difficult to assess the role of cultivar resistance and fungicide use on toxin levels in FHB infected grain.

The circumstances in the 2005 field season were much different, however. Inoculation was still a highly significant factor ($P < 0.0001$), but cultivar and fungicide were also significant in 2005, $P = 0.0001$ and $P = 0.0487$, respectively. An application of the foliar fungicide tebuconazole at flowering lowered the toxin levels of the grain by 2.6 ppm on averaged over both cultivars (Table 7). The reduction in DON caused by tebuconazole was higher in inoculated treatments than uninoculated treatments, but the interaction between the two factors was not significant. There was a significant interaction between inoculation and cultivar resistance, however. When inoculated, 25R42 was much lower in DON content than Jackson, a magnitude difference seen in other disease measurements as well. Tebuconazole lowered DON of inoculated Jackson by 7.1 ppm. Although 7.1 ppm is a significant reduction, DON content of 10.2 ppm that was present in the inoculated, fungicide treated plots was at a level which would be unacceptable for cattle or chicken feed products (Pestka,

1996). There was also a trend of a reduction in DON content by tebuconazole in 25R42, but the reduction was not significant (Table 7).

Grain moisture content did not vary widely during harvest in 2004, but there were significant differences due to inoculation and cultivar resistance, as well as a significant interaction between the two, but the main effect of fungicide was not significant. Although inoculation and cultivar were both significant overall, none of the main factors had an effect on the moisture of Jackson which had statistically equivalent moistures of 12.5% for all treatments (Table 8). Inoculated 25R42 treatments were 0.9% lower in moisture than uninoculated treatments. When the experiment was repeated in 2005, both cultivars had lower harvest moisture contents when inoculated. The average moisture across all treatments was higher by 0.9%, just slightly lower than the previous year of this study. In 2005, an application of tebuconazole increased grain harvest moisture in both cultivars by an average of 0.7%. It is clear that increased amount of disease and no fungicide application, lowers the average harvest moisture of soft red winter wheat.

Grain yield analysis is a crucial part of any production agricultural experiment. Although, our study was a not on the same scale as commercial production, both sample and plot sizes are adequate to allow discussion of differences between treatments. In 2004, there was a wide range in yield across treatments, 387 to 2909 kg ha^{-1} (Table 8). The main effects of fungicide, cultivar resistance, and inoculation were all highly significant. For both cultivars, an inoculation with a spore suspension of *F. graminearum* near flowering, lowered yield significantly. The yield of Jackson was lower in almost all treatments when compared with the equivalent

25R42 treatment, with the exception of the unsprayed, uninoculated treatment. The unsprayed, uninoculated treatments of Jackson and 25R42 had statistically equivalent yields of 2535 and 2385 kg ha^{-1}.

There were also several significant interactions of the main effects. There was a significant cultivar resistance by inoculation interaction, as well as a cultivar resistance by fungicide interaction. The latter interaction was mainly a result of the fungicide application having no significant effect on yield for Jackson, but the application of tebuconazole increased yield for 25R42 by approximately 496 kg ha^{-1}. The cultivar resistance by inoculation interaction resulted from large magnitude differences. Both cultivars were reduced in yield when inoculated with a spore suspension of *F. graminearum*, however, the yield for 25R42 was reduced much less dramatically than Jackson.

In 2005, when disease pressure was lower due to less favorable environmental conditions for disease development, yields were much higher, particularly for inoculated treatments (Table 8). There were no differences between any of the treatments involving the 25R42 cultivar. There is a significant trend for Jackson to have higher yields when neither cultivar was inoculated and therefore under little to no disease pressure. This suggests that Jackson has a slightly higher yield potential. In a year in which little disease development occurred, this research shows that using a resistant cultivar may have a lower yield potential than conventional, susceptible cultivars.

FHB can result in direct loss of kernels as well as lower TW due to the affect of FHB on grain fill. Although many diseased kernels are eliminated during

mechanical harvest, the ones that are not often lower test weight because they are small and light weight (Bai and Shaner, 1994). Analysis of TW from the 2004 field fungicide trial was difficult because several samples were unobtainable during harvest. Samples from inoculated Jackson plots were too low to be recorded. The missing data lead to several non-estimatable least square means and several interactions of the fixed effects could not be tested in the ANOVA. Specifically, the inoculation by cultivar interactions, as well as the inoculation by cultivar by fungicide interaction, could not be tested. Inoculation and cultivar were found to be significant factors affecting TW, however. Spraying 25R42 with a spore suspension of *F. graminearum* lowered TW by an average of 59.3 kg m^{-3}. The effects of inoculation on Jackson cannot be discussed due to the missing samples. Uninoculated 25R42 had test weights 41.2 kg m^{-3} higher than uninoculated Jackson. When the study was repeated in 2005, the only differences in TW were between cultivars. Due to low disease development, inoculation did not affect TW the way it did in 2004. Jackson had an average test weight of 651 kg m^{-3} where as 25R42 had a slightly higher average test weight of 706 kg m^{-3} (Table 8).

The decision to use a fungicide for FHB management should be dependent on several factors, such as the level of resistance of the cultivar, cultural practices like tillage and rotation that are used (Dill-Mackey and Jones, 2000; Teich and Hamilton, 1985), and the environmental conditions near flowering present in the current season. In this study the resistant cultivar, 25R42, had a fairly high level of resistance, as demonstrated by the lower disease ratings in most cases, but 25R42 is not commercially available and other type II cultivars may not have the same level of

resistance. The use of a fungicide at flowering may be even more effective when used in conjunction with less resistant cultivars than 25R42. In this study, an application of tebuconazole at flowering did not reduce DS, but did reduce DI in the second year. Unlike fungicide applications, a type II resistant cultivar consistently has lower DS even if environmental conditions are unfavorable for high levels of disease development. However, a reduction in DS of 1% to 2% during years when favorable conditions for disease development do not exist may not be of practical importance. This research indicates that years in which wheat is under lower disease pressure, due to environmental conditions or cultural practices such as tillage and rotation, a fungicide may not be needed if a highly resistant cultivar has been planted. On the other hand, a susceptible cultivar such as Jackson benefitted significantly from an application of a foliar fungicide at flowering. Although, previous greenhouse research suggested that an application of a fungicide at flowering on a FHB resistant cultivar may not be necessary for disease reduction, the results from this field trial suggest that even though disease severity was not significantly reduced by fungicide, there was a significant increase in yield. Overall, the results of this study indicate that the implementation of both a type II resistant cultivar and a foliar fungicide, such as tebuconazole, applied at flowering may be the best way to manage the affects of FHB, particularly in years when optimum environmental conditions for FHB development are present.

Table 7. Effect of inoculation with FHB and fungicide application at flowering on FHB disease incidence (DI), severity (DS), and deoxynivalenol (DON) accumulation on susceptible, Jackson, and a partially resistant, 25R42, soft red winter wheat cultivars in 2004 and 2005.

Year, cultivar[a]	Inoculation[b]	Fungicide[c]	DI (%)[d]	DS (%)[e]	DON (ppm)
2004					
25R42	Inoculated	Unsprayed	98.9	27.8	8.2
		Sprayed	100.0	22.0	8.3
	Uninoculated	Unsprayed	21.1	2.5	1.2
		Sprayed	11.9	1.0	1.0
Jackson	Inoculated	Unsprayed	99.7	53.7	5.8
		Sprayed	100.0	45.6	9.0
	Uninoculated	Unsprayed	24.5	3.3	1.7
		Sprayed	5.5	0.5	1.3
		$LSD_{0.05}$	10.1	4.9	1.7
2005					
25R42	Inoculated	Unsprayed	24.7	1.7	2.9
		Sprayed	10.0	1.6	1.6
	Uninoculated	Unsprayed	0.0	0.0	0.5
		Sprayed	0.0	0.0	0.0
Jackson	Inoculated	Unsprayed	74.7	3.2	17.3
		Sprayed	52.7	2.6	10.2
	Uninoculated	Unsprayed	5.5	0.9	1.5
		Sprayed	3.3	1.3	0.2
		$LSD_{0.05}$	5.4	0.4	2.5

[a]Year that study was performed and the winter wheat cultivar used.

[b]Inoculation with a *F. graminearum* spore suspension at the beginning of anthesis and again 4 days later.

[c]An application of the foliar fungicide tebuconazole at flowering.

[d]Percentage of infected spikes.

[e]Percentage of infected spikelets per spike.

Table 8. Yield, moisture, and test weight of a FHB susceptible and partially resistant cultivar under different levels of disease pressure and fungicide regime for 2004 and 2005.

Year, cultivar[a]	Inoculation[b]	Fungicide[c]	Yield[e] (kg ha^{-1})	Moisture (%)	TW (kg m^{-3})
2004					
25R42	Inoculated	Unsprayed	1139	12.3	630.6
		Sprayed	1586	12.9	629.3
	Uninoculated	Unsprayed	2394	13.2	682.1
		Sprayed	2909	13.8	696.3
Jackson	Inoculated	Unsprayed	387	12.5	.
		Sprayed	691	12.6	.
	Uninoculated	Unsprayed	2562	12.6	642.2
		Sprayed	2423	12.6	652.5
		$LSD_{0.05}$	163	0.4	15.4
2005					
25R42	Inoculated	Unsprayed	3283	13.5	698.8
		Sprayed	3433	14.3	710.4
	Uninoculated	Unsprayed	3078	14.3	695.0
		Sprayed	3038	14.9	716.9
Jackson	Inoculated	Unsprayed	2933	12.5	617.8
		Sprayed	3104	13.6	612.6
	Uninoculated	Unsprayed	3811	13.9	701.4
		Sprayed	3626	14.3	677.0
		$LSD_{0.05}$	364	0.4	41.2

[a]Year that study was harvested and the winter wheat cultivar used.

[b]Inoculation = a spore suspension of *F. graminearum* applied at flowering and 4 days later.

[c]An application of the foliar fungicide Folicur at a rate of 290 ml ha^{-1} during flowering.

[d]Yield corrected to 13.5% grain moisture.

Chapter 5: The effect of foliage density, cultivar resistance and fungicide on FHB disease management in the field.

Introduction

The objective of a fully integrated pest management field study is to establish if foliage density can reduce disease incidence, and if it can contribute to an integrated model that includes fungicide and a type II resistant winter wheat (*Triticum aestivum*) cultivar for the management of Fusarium head blight (FHB). The National Coalition on Integrated Pest Management (IPM) defines IPM as "a sustainable approach to managing pests by combining biological, physical, and chemical tools in a way that minimizes economic, health and environmental risks" (NCIPM, 1994). *F. graminearum* overwinters on in-field infected crop debris, such as corn. In the spring, perithecia that contain ascospores are produced. During humid conditions, ascospores are forcibly ejected from the perithecia through the plant canopy to the susceptible wheat heads (McMullen, 1997). An increase in foliage density may be able to block some of the ejected spores from reaching the wheat head. Although it is unlikely that foliage density as the only management technique would bring FHB down to acceptable levels, in combination with a partially resistant cultivar and fungicide applications, it may be a key management tool.

Materials and Methods

A 2^4 factorial experiment in a randomized complete block design with three replications was planted on November 4, 2003 and November 2, 2004 at the Central

Maryland Research and Education Center, Beltsville Facility in Beltsville, Md. The factors` and their levels were: inoculated and uninoculated; a seeding rate of 375 m^{-2} and 750 m^{-2}; unsprayed and one application of tebuconazole at the beginning of wheat anthesis; and a resistant and susceptible cultivar, Pioneer cultivar 25R42 (Pioneer Hi-Bred International, Johnston, IA) and Jackson (obtained commercially), respectively. The plot size was 10 by 7.6 m.

Inoculum consisted of *F. graminearum*-colonized corn kernels. The colonized corn kernels were produced by placing soaking corn kernels overnight in tap water and placing the soaked corn in 1-liter heat resistant jars with lids that were cut so that a thick filter paper disc could be inserted to maintain sterile but aerobic conditions. The jars were autoclaved at 121ºC for 20 minutes, allowed to cool, briefly, and then autoclaved for another 20 minutes. Under sterile conditions using a laminar flow bench, the jars of corn kernels were inoculated with a CMC broth culture of *F. graminearum*. Preparation details for a CMC broth culture can be found in the greenhouse cultivar materials and methods section. The inoculated corn kernels were incubated under fluorescent lights and shaken 2 to 3 times a week for approximately 4 weeks. After 4 weeks, the corn was spread on a lab bench to dry until it was time to be placed in the field. Approximately one month before flowering, 1.5 L of solid inoculum was placed in the center 1 m^2 area of each inoculated plot. All plots were mist irrigated for 30 minutes in morning and 30 minutes in the evening prior to anthesis and for three weeks after anthesis, if no natural rain event had recently occurred. Mist irrigation extended the dew period and was intended to create a more favorable environment for disease development.

Irrigation was set up using a 4500 L water tank with a pump and Micro-Bird II spinners, model SP24-340, from Rain Bird Corporation (Azusa, CA). The Micro-Bird spinners provided approximately 1.0 mm of water in thirty minutes every morning and evening.

Foliage density near the time of flowering was estimated by measuring leaf area index (LAI) using a LAI-2000 Plant Canopy Analyzer (LI-COR, Lincoln, NE) and canopy height. Disease incidence and severity were determined by sampling 20 green heads from each of 20 quadrants within the plot approximately 3 weeks after flowering and putting them on dry ice to preserve color and disease symptoms until they could be placed in a -18ºC freezer. The quadrants were approximately 2.5 by 1.5 m (8.25 x 5 feet) each. (Only six of the inner quadrants were used in data analysis.)

A Massey Ferguson 8-XP plot combine (AGCO, Duluth, GA) fitted with a Harvestmaster data collection system (Juniper Systems, Logan, UT) was used at harvest to collect yield, test weight and percent grain moisture for each plot on June 21, 2004 and July 5, 2005. Only the inner 3.4 by 7.6 m of each plot was harvested. The percent tombstones, average kernel weight and DON content were determined from seed samples taken during harvest. DON contamination was analyzed using Neogen's Veratox 5/5 ELISA test kit (Neogen, Lexington, KY). The methodology for preparing ELISA kit samples and analyzing data is described in the previous trial's material and methods section (Chapter 4).

All data was analyzed using the Mixed procedure of the Statistical Analysis Systems software (SAS). Data from the two years the trial was conducted were not combined due to significant differences and interactions that occurred within the

model. Fisher's least significant differences were calculated at the 0.05 level for mean separation when the ANOVA indicated significant treatment effects.

Results and Discussion

The main objective of this study was to analyze the combination of foliage density near flowering, fungicide, and cultivar resistance on the management of Fusarium head blight (FHB) in soft red winter wheat. Two levels of planting density were included in this study to create different foliage densities to test whether a higher amount of plant tissue could reduce the incidence of FHB infection by physically filtering spore dispersion from infield inoculum sources. Foliage density was calculated by dividing the leaf area index (LAI) by average plant height (Floyd and Anderson, 1987). Foliage density data, as well as LAI and height data, are presented in Table 9. Planting density was the only main effect to cause significant differences for LAI, height (2005, only) and foliage density. In 2004, there was also a significant interaction between planting population and cultivar resistance for foliage density. Increasing the seeding rate to 2X the normal rate only increased the foliage density of Jackson by 0.19 m^{-1} and 0.25 m^{-1}, in 2004 and 2005, respectively. But the same 2X rate increase for 25R42 increased its foliage density by 0.37 m^{-1} and 0.87 m^{-1}, in 2004 and 2005 (Table 9).

Figures 2 and 3 are graphical representations of the relationship between foliage density and disease incidence (DI). Only inoculated treatments are presented in the graphs, since they are the only ones that could have benefitted from an increase in foliage density. As the figures show there was not a consistent relationship

between foliage density and DI, in most cases. Sometimes the relationship between the two variables was positive, higher DI with increased foliage density, and sometimes it was a negative. Overall, it is obvious that there is no clearly defined relationship between foliage density and disease incidence when inoculum is coming from infield sources (Figures 2 and 3).

Disease incidence, the percent of infected spikes per sample, varied widely between treatments in 2004 from 9.0% to 52.3% (Table 10). The main effects of inoculation, cultivar, and fungicide were statistically significant ($P < 0.005$). Although there was a slight difference for DI between low and high planting density (PD) treatments, 30.3% and 27.0%, respectively, the effect on DI was not significant ($P = 0.2032$). On average, the fungicide tebuconazole decreased DI by 16.4%. The type II resistant cultivar, 25R42, had an average DI of 22.8%. Jackson, the susceptible cultivar, had a significantly higher average DI of 34.5%. The *F. graminearum* infested corn kernels placed in the center of the inoculated treatments increased DI by 8.1% on average. Overall, the treatment with the lowest DI, 9%, was low planting density, fungicide sprayed, uninoculated 25R42 plots. The highest average DI, 52.3%, was high planting density, no fungicide, inoculated plots of Jackson.

Disease incidence was significantly lower in 2005 compared to 2004; the result of a decrease in rainfall amount and the number of rainfall events. The same main effects were significant in 2005 as in 2004. (Weather data for May and June of 2004 and 2005 are presented in Table 12.) Inoculation, cultivar resistance, and fungicide all had a *P*-value of 0.0006 or less. Planting density was once again not

significant (P = 0.1702). There was a significant interaction between inoculation and cultivar in 2005 (P = 0.0018). This interaction was due to the large magnitude difference that inoculation had on DI between the two cultivars. Field inoculation with *F. graminearum* infested kernels prior to anthesis increased DI of 25R42 from 1.9% to 5.4%, whereas it increased DI of Jackson, the susceptible cultivar, from 7.1% to 20.2%. Since the range of DI was much smaller than in 2004, there was less differentiation between treatments. For instance, the lowest DI of 1.4% was shared by three separate uninoculated, 25R42 treatments. On average, fungicide reduced DI in 2005 by 5.5% across in 2005 (Table 10).

Disease severity (DS) is the average percent infected spikelets per spike. The main effects of inoculation, cultivar, and fungicide were all significant for DS in 2004 ($P \leq 0.0232$). Planting density was not significant with a P-value of 0.8526, but this was expected since the increased foliage density would only potentially decrease initially infection, not the secondary spread of the pathogen throughout the wheat head. Disease severity was relatively low, ranging from 0.8% to 11.7% (Table 10). Inoculation increased DS by only 1.8%, overall. Tebuconazole decreased DS by 3.3%. The treatment with the lowest DS, 0.8%, of all the treatments was 25R42 uninoculated, sprayed and at a low planting density. The treatment with the highest DS was Jackson at a low planting density, unsprayed and inoculated (Table 10).

Similar to the results for DI, DS was considerably lower in 2005 than in 2004. Disease severity ranged from 0.1% to 4.1% (Table 10). Inoculation and cultivar resistance to FHB were still significant, but fungicide use was not (P = 0.1966). On average, tebuconazole had a DS of 0.9% while DS of unsprayed plots was 1.3%.

Given the small range of DS seen in 2005, it is not surprising that the power of the statistical test was not high enough to detect a significant difference. There was a significant interaction between inoculation and cultivar due to the magnitude of change for inoculation of Jackson compared with that change for the resistant cultivar, 25R42. When Jackson was inoculated, DS increased 2%, but the DS of 25R42 only increased 0.2% when inoculated.

Deoxynivalenol (DON) content varied substantially in 2004 (Table 10). The lowest DON was 1.1 ppm, higher than the FDA guidelines for human consumption. The main factors of cultivar, fungicide and inoculation were statistically significant, but planting density was not. Artificial inoculation with infested corn kernels increased DON by 0.9 ppm on average. Tebuconazole reduced DON by 0.7 ppm. As was observed for other disease ratings, the partially resistant cultivar, 25R42, had a significantly lower average DON content, 1.6 ppm, than Jackson, the susceptible cultivar, 3.1 ppm (Table 10).

In 2005, the same main factors of inoculation, cultivar resistance, and fungicide were significant. However, there were also numerous significant interactions between the main effects: inoculation by cultivar, inoculation by fungicide, fungicide by cultivar, and inoculation by fungicide by cultivar. With the large number of significant interactions between the effects, it is difficult to generalize about the effects these factors had on DON content.

Moisture at harvest was relatively high for all treatments in 2004. Only the main factors of cultivar and fungicide had a significant effect on moisture levels. 25R42 had significantly higher average moisture of 16.4% compared with Jackson at

14.9% (Table 11). The grain moisture of Jackson may have been lower due to a greater number of scabby kernels in the harvested samples. A lower number of infected seed may also be the reason that tebuconazole increased average grain moisture by 0.8% (Table 11).

Overall, grain harvest moisture was lower during 2005 than 2004. The significant main effects in 2005 were inoculation, cultivar resistance, and fungicide. There were also numerous interactions between the main effects: inoculation by cultivar, inoculation by fungicide, and inoculation by fungicide by planting density. A 3-way interaction between the main effects makes generalizing how the main factors affected grain moisture difficult. Looking at individual treatments, however, the lowest grain moisture was unsprayed, inoculated, low density Jackson at 12.4% (Table 11). Sprayed, uninoculated, high planting density 25R42 treatments had the highest grain moisture at harvest of 14.0%.

Grain yield is the most important outcome with any disease management scenario for many commercial producers. During 2004 the main effect factors of inoculation, cultivar resistance and fungicide had all highly significant effects on yield ($P \leq 0.0001$). Planting density was not significant at a *P*-value of 0.1130. Inoculation with infested corn kernels prior to anthesis decreased yield of both cultivars an average of 357.8 kg ha^{-1} (Table 11). Tebuconazole applied near flowering increased yields for both cultivars by 313.9 kg ha^{-1}. In 2004, 25R42 had significantly higher yield of 3270 kg ha^{-1}, compared with Jackson, 2980 kg ha^{-1}. The difference in yield was due to the lower amount of FHB infection for 25R42 (Table 10). The treatment with the highest yield in 2004 was 25R42 at a low planting

density, sprayed with tebuconazole, and uninoculated (Table 11). It is not surprising that this treatment had the highest yield of all the treatments since it also had the lowest DS and DI in 2004 (Table 10). The treatment with the lowest yield was inoculated, unsprayed Jackson at a low planting density. And not surprising, this was also the treatment with the highest FHB DS and DI (Table 10).

The situation regarding yield was vastly different in 2005. Planting density was the only significant factor affecting yield ($P = 0.0194$). All other main effects and interactions were insignificant. Lower disease pressure in 2005 than in 2004 led to only minute differences in yield between the other factors. Increased planting populations, and subsequently increased foliage density, resulted in 470.8 kg ha^{-1} yield increase (Table 11).

FHB infection often lowers test weight, due to the inclusion of small, light weight, scabby seeds in the harvested grain (Bai and Shaner, 1994). The main effect cultivars of inoculation, fungicide and cultivar all significantly affected TW in 2004 ($P \leq 0.0005$). Tebuconazole increased TW by an average of 1.2 kg m^{-3} (Table 11). There was also a significant cultivar by inoculation interaction ($P = 0.0392$). Inoculation with scabby corn kernels lowered TW of Jackson by 1.5 kg m^{-3}, but only lowered TW of 25R42 by 0.4 kg m^{-3}. The interaction was due to the fact that inoculation of 25R42 treatments did not increase FHB disease severity as much as it did in Jackson treatments.

In 2005, the main effect factors of inoculation and cultivar resistance were still significantly affected TW, but fungicide did not. Inoculation lowered TW by 1.9 kg m^{-3} (Table 11.) Even though disease severity was lower in 2005, the TW of

25R42 was significantly higher than Jackson, 62.9 kg m^{-3} compared with 58.5 kg m^{-3}, respectively. There was also a significant fungicide by planting density interaction (*P* = 0.0435). The interaction occurred because there was no significant difference for TW between planting density levels when tebuconazole was sprayed, but the TW difference was significant when unsprayed. Like other measurements of yield, the treatment with the lowest TW was inoculated, unsprayed, low planting density Jackson.

Although we were able to successfully increase foliage density by increasing seed populations at planting, resulting in increased foliage density did not lower FHB incidence. Only in one year was planting density significant. A yield increase was seen in 2005 when planting density was high, but FHB disease pressure was low and the corresponding yield boost was not correlated with lower disease severity. It is possible that increased foliage density would be a beneficial part of an integrated pest management program if other cultivars were used such as cultivars with larger than average leaf size. For instance, in 2005, the foliage density of Jackson at flowering only increased by 0.19 m^{-1}, but for 25R42, it increased by 0.87 m^{-1}. Clearly cultivars differ in foliage density after increased seeding rates, and other cultivars may show an even greater density increase then 25R42, but more research would need to be done in order to determine which cultivars may maintain foliage density at flowering and what the economic cost or benefit may be for the producer.

The other components of our proposed integrated pest management system for FHB were more successful, however. 25R42, the cultivar with reported type II resistance also appeared to have some level of type I resistance, resistance to initial

infection. At all levels of fungicide, inoculation, and planting density, 25R42 had a lower or equivalent disease incidence than the FHB susceptible cultivar, Jackson. An application of tebuconazole reduced DI, DS and DON levels in most cases, (fungicide did not significantly affect DS in 2005). Tebuconazole also increased yield but only under the higher disease pressure scenario seen in 2004. Overall, 25R42 with an application of tebuconazole consistently had the lowest FHB disease ratings and the highest yields. Although increased foliage density does not seem to have a significant role in an IPM strategy at this time, the combination of a FHB partially type II resistant cultivar and a foliar fungicide, such as tebuconazole, is the most effective strategy at managing Fusarium head blight.

Table 9. The planting density, LAI, and height of two winter wheat cultivars with varying resistance to FHB when planted with low or high seeding rate.

Year, Cultivar	Planting Density	LAI[a]	Height (m)	Density (m^{-1})
2004				
Jackson	Low	2.30	0.71	3.26
	High	2.42	0.70	3.45
25R42	Low	2.31	0.71	3.27
	High	2.52	0.69	3.64
	$LSD_{0.05}$	0.13	N/A	0.21
2005				
Jackson	Low	2.62	0.65	3.97
	High	2.77	0.67	4.16
25R42	Low	2.29	0.63	3.61
	High	2.96	0.66	4.48
	$LSD_{0.05}$	0.31	0.02	0.37

[a]Leaf area index.

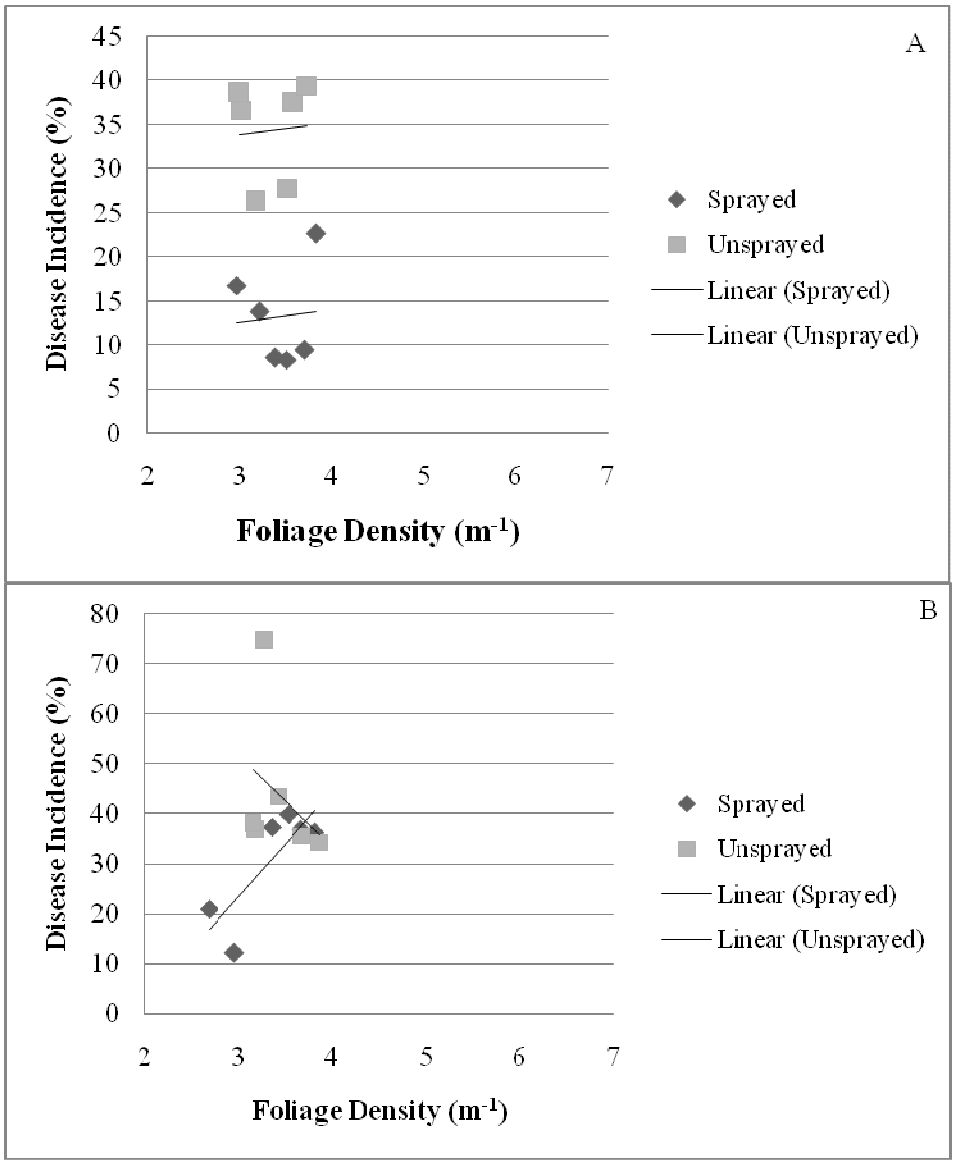

Figure 2. Foliage density by disease incidence of sprayed and unsprayed, inoculated treatments of 25R42 (A) and Jackson (B) in 2004.

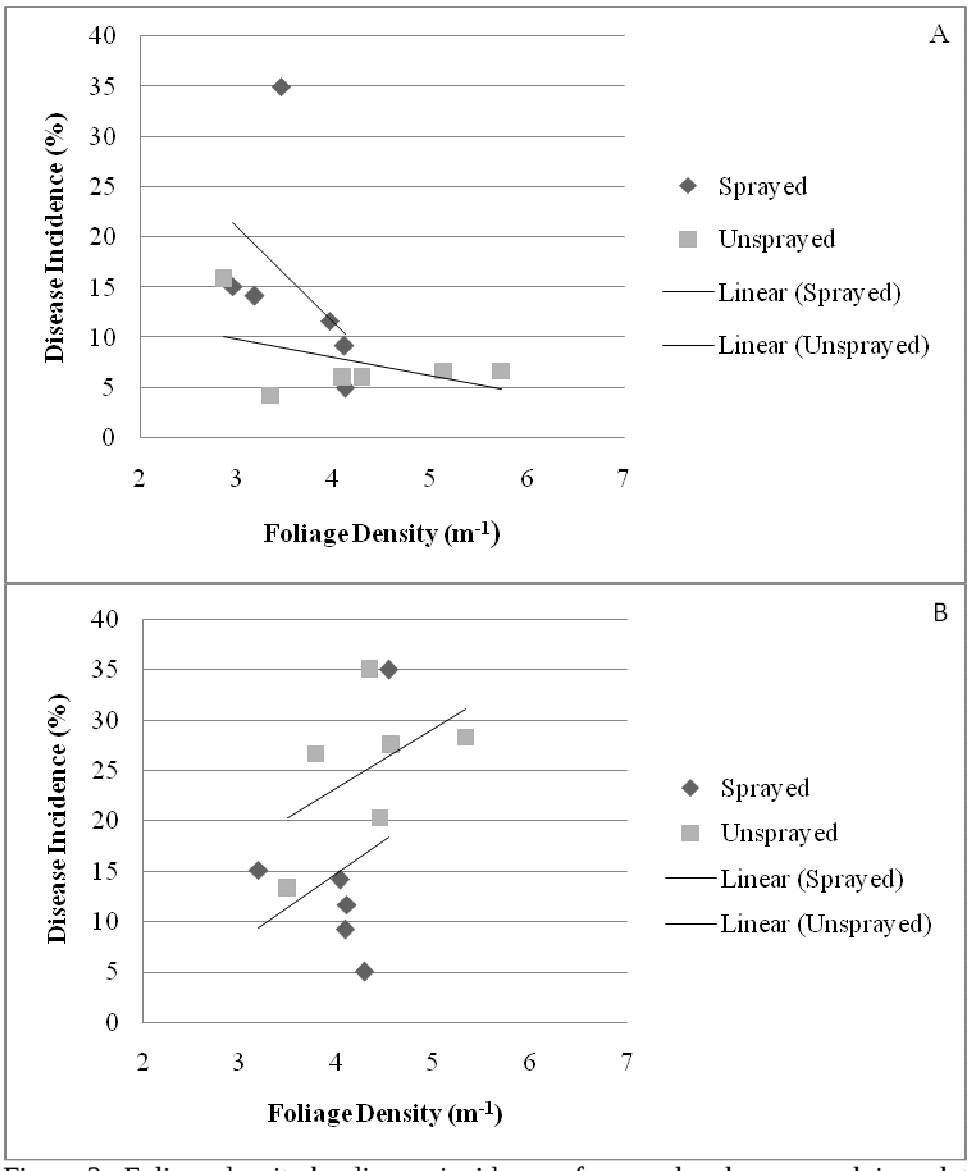

Figure 3. Foliage density by disease incidence of sprayed and unsprayed, inoculated treatments of 25R42 (A) and Jackson (B) in 2005.

Table 10. The effect of planting density, inoculation and the fungicide tebuconazole on FHB disease incidence (DI), severity (DS) and deoxynivalenol (DON) content.

Year, cultivar[a]	Inoculation[b]	PD[c]	Fungicide[d]	DI (%)	DS (%)	DON (ppm)
2004						
25R24	Inoculated	Low	Unsprayed	32.2	3.2	1.48
			Sprayed	16.9	1.7	1.22
		High	Unsprayed	36.4	4.7	1.53
			Sprayed	16.3	1.5	1.32
	Uninoculated	Low	Unsprayed	34.8	4.0	2.51
			Sprayed	9.0	0.8	1.20
		High	Unsprayed	24.1	2.5	2.64
			Sprayed	12.4	1.2	1.08
Jackson	Inoculated	Low	Unsprayed	52.3	11.7	2.46
			Sprayed	30.4	3.6	3.27
		High	Unsprayed	43.9	9.9	2.86
			Sprayed	33.0	5.1	1.29
	Uninoculated	Low	Unsprayed	34.0	4.8	4.47
			Sprayed	32.6	4.7	2.76
		High	Unsprayed	36.9	6.6	3.91
			Sprayed	12.6	1.9	4.03
			$LSD_{0.05}$	5.6	1.6	0.48
2005						
25R24	Inoculated	Low	Unsprayed	8.6	0.7	0.09
			Sprayed	3.0	0.3	0.38
		High	Unsprayed	6.4	0.5	0.54
			Sprayed	3.4	0.3	0.08
	Uninoculated	Low	Unsprayed	3.4	0.4	0.01
			Sprayed	1.4	0.2	0.08
		High	Unsprayed	1.4	0.1	0.09
			Sprayed	1.4	0.6	0.00
Jackson	Inoculated	Low	Unsprayed	22.9	2.4	3.40
			Sprayed	21.4	4.0	0.77
		High	Unsprayed	27.8	4.1	2.16
			Sprayed	8.8	0.7	0.33
	Uninoculated	Low	Unsprayed	11.8	1.7	0.22
			Sprayed	4.5	0.4	0.40
		High	Unsprayed	8.6	0.7	0.08
			Sprayed	3.4	0.5	0.04
			$LSD_{0.05}$	2.9	0.7	0.31

[a]Year that study was performed and the winter wheat cultivar used.

[b]Inoculation = 1.5 L of solid inoculum spread within one square meter in the center of the plot.

[c]PD = planting density. Low = seeding rate of 375 m^{-2}. High = seeding rate of 750 m^{-2}.

[d]An application of the foliar fungicide tebuconazole at a rate of 290 ml ha^{-1} during flowering.

Table 11. The effect of planting density, tebuconazole spray application, and inoculation of two cultivars differing in FHB resistance on yield, grain moisture and test weight (TW).

Year, cultivar[a]	Inoculation[b]	Fungicide[c]	PD[d]	Yield[e] (kg ha^{-1})	Moisture (%)	TW (kg m^{-3})
2004						
25R42	Inoculated	Unsprayed	Low	2857	15.7	54.7
			High	2916	15.4	54.3
		Sprayed	Low	3074	17.1	55.5
			High	3250	16.9	56.1
	Uninoculated	Unsprayed	Low	3237	16.0	54.7
			High	3283	15.9	55.4
		Sprayed	Low	3423	16.8	55.7
			High	3260	16.8	55.6
Jackson	Inoculated	Unsprayed	Low	2153	14.5	49.6
			High	2563	15.1	50.7
		Sprayed	Low	3017	15.5	51.7
			High	3051	14.2	51.3
	Uninoculated	Unsprayed	Low	3068	14.6	51.7
			High	3066	15.0	53.2
		Sprayed	Low	2956	15.3	52.4
			High	3325	15.3	53.2
			$LSD_{0.05}$	181	0.5	0.5
2005						
25R42	Inoculated	Unsprayed	Low	3739	13.1	64.9
			High	4298	13.4	61.6
		Sprayed	Low	2956	13.7	61.0
			High	3651	13.1	57.4
	Uninoculated	Unsprayed	Low	3934	13.4	65.2
			High	3634	13.5	63.4
		Sprayed	Low	4056	14.0	66.9
			High	4295	13.6	62.9
Jackson	Inoculated	Unsprayed	Low	3553	12.4	50.0
			High	4549	12.7	61.0
		Sprayed	Low	3548	13.0	56.0
			High	4528	13.7	65.3
	Uninoculated	Unsprayed	Low	4277	13.2	54.4
			High	4663	13.4	56.2
		Sprayed	Low	4057	13.6	61.5
			High	4295	13.6	62.3
			$LSD_{0.05}$	471	0.2	4.8

[a]Year that study was performed and the winter wheat cultivar used.
[b]Inoculation = 1.5 L of solid inoculum spread within one square meter in the center of the plot.
[c]An application of the foliar fungicide Folicur at a rate of 290 ml ha^{-1} during flowering.
[d]PD = planting density. Low = seeding rate of 375 m^{-2}. High = seeding rate of 750 m^{-2}.
[e]Corrected to 13.5% grain moisture.

Table 12. Weather data for the 2004 and 2005 field seasons when FHB development occurred.

	2004				2005			
	May		June		May		June	
Date	Air Temp °C	Rainfall (mm)	Air Temp °C	Rainfall (mm)	Air Temp °C	Rainfall (mm)	Air Temp °C	Rainfall (mm)
1	20.17	0.76	19.13	0.25	12.87	3.05	17.94	0.00
2	21.61	21.59	19.35	0.00	9.18	0.25	15.90	0.51
3	9.41	11.68	19.66	0.00	9.04	0.00	16.46	15.75
4	9.72	3.05	16.96	3.30	10.16	0.00	19.83	0.00
5	13.35	0.25	15.21	31.24	10.13	0.00	22.92	0.00
6	15.49	0.00	16.56	0.76	9.38	0.00	24.43	23.88
7	19.62	6.86	19.93	0.00	13.74	0.00	23.73	1.02
8	14.48	0.00	22.45	0.00	16.36	0.00	24.97	0.00
9	19.10	17.02	26.29	0.00	15.85	0.00	25.15	0.51
10	23.04	1.78	24.59	12.45	15.95	0.00	25.52	0.00
11	24.70	0.00	17.04	18.29	19.04	0.00	25.21	0.00
12	23.44	0.00	18.22	0.00	18.88	0.00	24.94	0.00
13	23.29	0.00	17.86	0.00	12.79	0.00	26.13	0.25
14	23.87	0.00	24.83	0.00	17.72	5.08	26.87	0.00
15	23.97	2.03	25.91	0.51	18.64	0.00	25.08	0.00
16	21.92	3.81	25.10	0.51	15.83	0.00	21.94	1.02
17	22.23	2.03	25.31	10.41	13.13	0.00	17.91	0.00
18	21.72	7.11	25.43	5.33	14.79	0.00	18.80	0.00
19	20.58	0.51	22.94	0.00	15.24	1.02	18.39	0.00
20	19.46	0.00	17.95	0.00	10.95	60.71	16.17	0.00
21	22.67	1.27	19.68	0.00	14.59	0.00	19.25	0.00
22	23.91	0.00	23.78	1.78	16.29	0.00	20.25	0.51
23	26.35	0.00	21.70	1.27	15.14	0.25	19.86	0.00
24	26.24	0.00	22.61	0.00	12.66	17.27	22.01	0.00
25	24.02	2.03	21.79	14.22	12.18	0.25	23.62	0.00
26	22.85	0.76	21.48	0.25	17.27	0.00	23.79	0.00
27	22.00	7.37	20.03	0.00	17.66	0.00	23.11	2.29
28	21.68	1.27	21.40	0.00	15.68	0.25	25.78	0.51
29	16.83	0.00	20.40	0.00	16.14	0.00	24.07	23.11
30	17.28	0.25	21.34	0.00	15.82	3.30	24.57	0.25
31	18.78	5.33	N/A	N/A	17.04	0.25	N/A	N/A
Monthly Averages:	20.44	3.12	21.16	3.35	14.52	2.96	22.15	2.32

Chapter 6: Conclusions

Fusarium head blight (FHB) can be a destructive disease of the soft red winter wheat (*Triticum aestivum*) grown in the Mid-Atlantic region. Due to the increasing number of FHB epidemics in recent years (McMullen, 1997, Canty, 2004, and Cowger and Sutton, 2005), much research has focused on the management of this potentially devastating disease. The research presented here focused on some of the management aspects for FHB.

There were three main objectives at the start of this research. The first objective was to determine if type II resistance, resistance to spread of the fungal pathogen throughout the wheat spike (Mesterhazy, 1995), could be overwhelmed when multiple infections occur along the spike. When optimum environmental conditions are present and inoculum is present, multiple infections would be common. I tested three partially resistant, type II soft red winter wheat cultivars and one FHB susceptible cultivar in the greenhouse with three levels of point inoculations: zero, one or three. When three inoculations were injected into the wheat spike of the type II resistant cultivars, two of the three cultivars continued to show some degree of resistance to FHB, but the third, Pioneer cultivar 25R54, had a disease severity equivalent to that of the susceptible cultivar, Jackson. It is clear that although not all resistance in type II cultivars can be overwhelmed by multiple infections, some can.

The second object of this research was to determine if FHB is best managed by the combination of a type II resistance and a foliar fungicide. Type II resistance is

a beneficial tool for managing FHB, but because some type II resistance can be overwhelmed by disease, I hypothesized that the combination of resistance and fungicide use would be the best way to control FHB. I tested this hypothesis both in the greenhouse and in a field setting. In the greenhouse, the combination of cultivar resistance and fungicide did not lower FHB disease severity. Disease severity was low in the resistant cultivar that I tested, and fungicide did not decrease the severity any further. This was most likely due to the experimental design of the experiment. In the field, however, the treatment with the lowest FHB disease ratings and the highest yield was usually a type II resistant cultivar that received an application of a foliar fungicide. This trend was particularly apparent when favorable environmental conditions were present for disease development.

Lastly, I wanted to study the role increased foliage density might play in an integrated management program for FHB that also included type II resistance and a foliar fungicide. It was hypothesized that an increase in foliage density might block or filter some of the spores of infield inoculum from reaching the susceptible wheat spike, thereby decreasing the disease incidence of FHB. An increase in foliage density was achieved by doubling the normal seeding rate of soft red winter wheat. However, the increase was small and did not correspond with a reduction in the incidence of FHB. More research is needed to determine if other cultivars, such as those with larger leaves, would maintain a higher foliage density than the two cultivars used in this study. At this time, however, it appears that foliage density does not have a role to play in the management of Fusarium head blight.

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