THE 1998 NATIONAL FUSARIUM HEAD BLIGHT FORUM

CHAPTER 1

EPIDEMIOLOGY AND DISEASE MANAGEMENT

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U.S. Wheat & Barley
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The 1998 National Fusarium Head Blight Forum

CHAPTER 1

Epidemiology and Disease Management

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Development of Fusarium Head Blight in Relation to Environment and Inoculum

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The willingness of Dr. Jeannie Gilbert, Agriculture and Agri-food Canada, Winnipeg, MB, to replicate this experiment in a second location is gratefully acknowledged. The assistance of James Jordahl and Samuel Markel in the conduct of this experiment is also appreciated. Dr. John Enz, NDSU climatologist, contributed environmental data for this report.

INTRODUCTION

A disease forecasting model for Fusarium head blight (FHB) could be developed from field observations of the three components of the disease triangle - pathogen, host and weather. *Gibberella zeae,* the main causal agent of FHB, overwinters on infested plant residue. Wheat is damaged most by infection during flowering and wet, warm weather favors infection. The objective of this research was to quantify disease progression as affected by environmental factors and inoculum. Repetition of this experiment in several more environments will permit a detailed analysis of disease onset and epidemic severity, which could be used in a system to advise if and when fungicide intervention is necessary.

MATERIALS AND METHODS

The experiment was conducted in 1998 on the North Dakota Agricultural Research Station, Fargo, ND. Wheat was grown in 1997 and the crop had 4% FHB severity at soft dough. Chisel and disk implements subsequently cultivated the soil, leaving the soil surface covered with wheat straw. The spring wheat cultivar Norm, susceptible to FHB, was sown in 1.4 by 15 m plots. Plots were planted on 22 April, 4 May and 19 May to increase the window of opportunity for data collection. In addition, the first planting date included plots that were covered with clear plastic to increase soil temperature and speed plant development. Planting date and plastic treatments were replicated once.

Temperature, relative humidity, precipitation, and solar radiation were measured on-site with an instrumented automatic datalogger (Campbell Scientific Inc. (CSI) model CR-10). The presence of moisture at the flag leaf height was averaged from measurements by six flat-plate resistance sensors (model 237, CSI). Environmental data were saved at half-hourly intervals and are reported here as 24-h summaries ending at 10:00 a.m., the approximate time of sampling. Supplemental environmental data were obtained from the North Dakota Agricultural Weather Network, which had a station 0.3 km from the experiment.

Data collection began once plants reached flowering. During late-morning hours on each day until mid-milk, 20 heads were collected at random and assessed for FHB incidence and severity. Heads were put into four 50 ml vials (five heads/vial) containing a floral preservative solution. Ten of the 20 heads were placed in a moist chamber for 24 h and then taken to a lab and placed under fluorescent lights on a 12-h light-dark cycle. The other ten heads were taken immediately to the same room. Temperature and relative humidity were monitored by a Vaisala HMP-35 probe on a CSI CR-10 datalogger and data were summarized hourly. Samples were again assessed for FHB incidence and severity after seven and 14 days.

An additional five heads were randomly sampled each morning for measurement of inoculum. Heads were placed into 50 ml sterile distilled water and shaken vigorously for 2 min, after which heads were discarded. Samples were frozen until processing. An aliquot of 0.5 ml was transferred to each of three plates of Komada's selective medium. Colonies of fungi were classified as to color and growth habit after seven days. Select colony types were transferred to PDA slants to obtain a pure culture type and then to carrot agar. Colonies which formed perithecia on carrot agar were classified as *G. zeae*. From this proportional information, the number of colony forming units (cfu) of *G. zeae* per head could be derived as an indicator of viable inoculum load. Inoculum estimates in this report are based preliminarily on speciation of about 70% of the culture type samples. Thus, only data associated with the earliest maturing plot will be included in this report. In addition to inoculum on the head, a Burkard volumetric spore sampler provided supplemental information about the presence of airborne inoculum from 10 June through 31 July.

Plots were rated for FHB incidence and severity four to six times (9, 13, 18, 21, 24 and 28 July) by assessing 30 heads at random. Ratings concluded when senescence masked the blight symptom in the middle of the soft dough stage.

RESULTS AND DISCUSSION

The three planting dates and plastic soil cover resulted in wheat plots that were in flower from 22 June until 10 July. Sampling of heads continued until 17 July (data not shown). The plastic cover accelerated flowering by about five days.

Ascospores of *G. zeae* were first detected in an air sample on 15 June, one week prior to flowering of the first plot. Ascospore maturity is probably related to growing season thermal units and moisture. Approximately 1600 degree days had accumulated by 15 June and about 200 degree days of that total occurred prior to planting. Total rainfall from 1 April to 15 June was 213 mm, with 6 mm falling on 15 June. Ascospores also were found on 17 June and daily from 19 to 29 June, with a peak on 24 June (day 175). Highest numbers were associated with the first night following rain. After 29 June, which corresponded to 2300 degree days, no ascospores were observed in air samples. However, *G. zeae* could be recovered from head samples in July (data not shown).

Heads were sampled for inoculum and disease level for 12 days beginning 22 June and ending on 3 July. *Gibberella zeae* could be detected on heads taken from the earliest plot when flowering commenced (Fig. 1D). Inoculum of *G. zeae* continued to be recovered from heads on every day but the last. The highest count on 25 June (day 176) correlated well with the peak airborne ascospore level during the previous 24 h. These results suggest that inoculation occurred on multiple occasions and the cumulative effect influenced final disease intensity. Inoculation prior to flowering may have played a role in disease progress but this must be confirmed. The rate of decrease in the absence of airborne ascospores during the last three days would suggest that inoculum remains viable on heads for about 48 h. These results finally suggest that the airborne ascospore concentration is a good indicator of disease potential.

The daily samples of wheat heads were kept in an environment that averaged 26 C and 42% relative humidity and the floral preservative keep the heads alive for two weeks. This environment did not seem to inhibit blight development; indeed, sporodochia could be observed on some severely damaged heads. Blight incidence was 30% on the heads removed from the field on 23 June (day 174) but severity was only 2% (Figs. 1E & 1F). (The 22 June sample was lost.) Incidence and severity varied from day to day but samples taken after flowering were representative of the final plot disease intensity ratings, which were 78% incidence and 32% severity. The addition of a wet period after sample collection generally increased FHB severity and incidence. Once again, the latter most samples had disease levels comparable to the final plot severity and incidence rating.

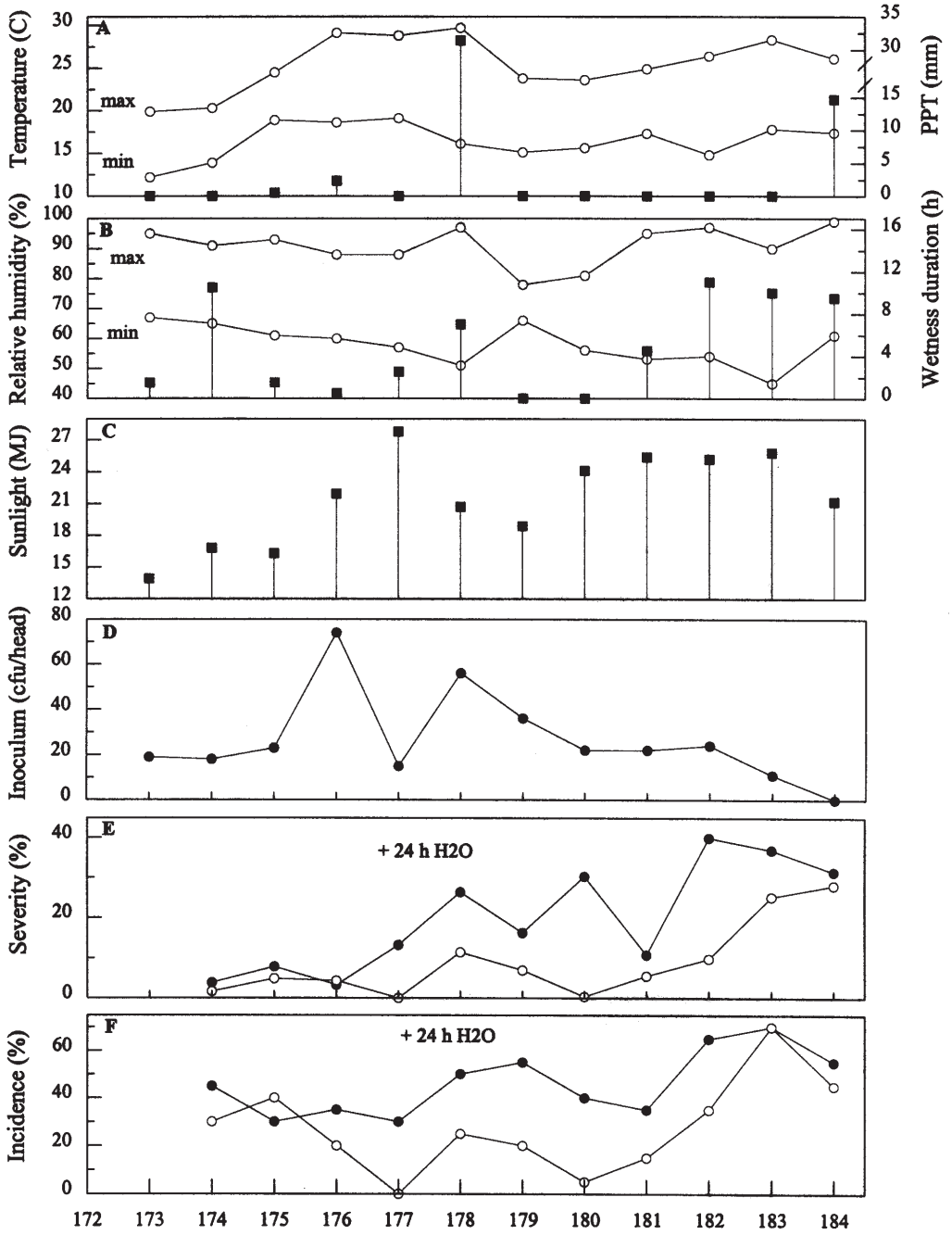
Precipitation and sunlight (Figs. 1A & 1C) were the environmental factors most highly related to inoculation (Fig. 1D). The highest measured concentration of cfu/head occurred on 25 June (day 176) after rains of 0.5 mm the previous morning and 2 mm the same morning. Then, inoculum levels dropped by 55 cfu/head during the sunniest day of the 12 measured (26 June or day 177). Finally, an increase of about 40 cfu/head on 27 June (day 178) could be associated with 31 mm of rain that had fallen between 6:00 and 11:00 p.m. the previous night.

Meanwhile, disease severity (Fig. 1E) seemed most closely related to wetness duration (Fig. 1B). The wetness period of 23 June (day 174) was associated with 2% severity; that of 27 June (day 178) with 10% severity; and, the period beginning on 1 July (day 182) with nearly 30% severity. Based on incidence data (Fig. 1F), the first two periods seemed conducive to an invasion of the fungus into other spikelets, while the last period affected the number of infected heads. Presumably, these infections were initiated at night when minimum temperatures were between 15 and 20 C (Fig. 1A).

Blight severity and incidence could not, however, be explained solely by environmental factors. Particularly intriguing is the sudden disappearance of airborne ascospores even though it rained on 2, 3, 5 and 7 July. This result may suggest that the supply of ascospores had become exhausted; however, this explanation must be confirmed.

FHB was also severe to moderately severe in plots where wheat flowered later in the season, with final levels of 9 to 24%. Therefore, analysis of those data may help explain the observations shown here. This experiment also should be repeated to confirm the associations observed in these preliminary data. Sampling of heads in the future should begin two days earlier in order to assess the role of preflowering inoculum in disease intensity. Finally, this experimental protocol appears promising as a method to define pathogen population dynamics and resulting epidemic development.

Fig. 1. Environmental conditions, level of *Gibberella zeae* on heads, and consequent disease intensity on samples (n=20) taken from a plot of wheat between flowering and early milk: (A) daily temperature minimums and maximums (lines) and rainfall (bars); (B) daily minimum and maximum relative humidity (lines) and hours of wetness (bars); (C) total solar radiation; (D) colony forming units of *G. zeae* recovered from head samples (n=5); (E) Fusarium head blight severity 14 days after collecting heads with (solid circles) and without (open circles) 24 h of additional moisture; (F) Fusarium head blight incidence 14 days after collecting heads with (solid circles) and without (open circles) 24 h of additional moisture.



Fusarium graminearum infection on wheat spikes: early events.

PRITSCH(1), W.R. Bushnell(2,3), D.A. Somers(1), G. Muehlbauer(1),

The early events in the infection process of wheat spikes by *Fusarium graminearum* Schw. were examined. Glumes from spray inoculated spikes (10 ^{6})conidia/ml) of Sumai (resistant) and Wheaton (susceptible) were sampled at 6,12,24,36,48,and 76 hours after inoculation and the abaxial surface examined using both scanning electron microscopy and light microscopy of calcofluor stained sections. Conidia germination occurred between 6-12 hours. Between 24-48 hours some subcuticular hyphal development was evident and other hyphae were found to penetrate stomata. By 48-76 hours after inoculation, hyphae were abundant on the glume surface, especially in areas occupied by stomatal rows and conidiophores were present. Also, hypahe were found within both epidermal and parenchyma cells. No major differences in the timing of these events were found between the two cultivars. Additionally, RNA was extracted from whole wheat spikes collected from the two cultivars at the same timepoints. RNA blot analysis was used to determine the expression pattern of several defense genes in the spikes during Fusarium infection. Preliminary results indicate that several defense genes were strongly induced by 48 hours after inoculation, including genes for chitinase, glucanase, peroxidase and thaumatin-like protein.

(This is a revised version of an abstract of a poster to be presented at the APS Meetings, Las Vegas, 1998).

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The effect of agronomic practice on the accumulation of deoxynivalenol (DON) in winter wheat fields in Ontario, 1996-97

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Objectives

The objectives of this study were to estimate the overall effect of machine harvesting on DON concentration; and to examine the effect of agronomic practice on the accumulation of DON in commercial fields of winter wheat during the epidemic of 1996 and the endemic of 1997 in Ontario.

Introduction

According to the Ontario Wheat Producers Marketing Board, well over $100 million CDN was lost in Ontario due to the Fusarium head blight [*Fusarium graminearum* (Schwabe)] epidemic in 1996. The epidemic afforded an opportunity to conduct a field scale survey of grain contamination across the province to test the incidental effect of agronomic practices. The survey was repeated in 1997 under endemic conditions. FHB in wheat scab can significantly reduce grain yield because infected kernels are often light and shriveled and often contain DON. DON is the most important mycotoxin produced by *F. graminerum*, and one of the best known *Fusarium* toxins found in wheat in Ontario (Miller et al. 1998). FHB may be affected by fungicides, host resistance, and management practice (Martin and Johnston 1982), but the effect of these factors on DON occurrence and accumulation is not clearly understood (Mills 1982). While tillage effects on FHB severity were inconclusive, and increased infection appeared to occur under minimum tillage (Miller et al 1998), tillage effects on DON accumulation were not measured.

Materials and methods

In 1996 and 1997, 86 and 72 paired-samples were taken from winter wheat fields across Ontario, respectively. Each field was sampled before machine harvest, followed by a second sample taken from the combine. Wheat producers were selected at random around predetermined points on a map until a representative geographical sample was obtained for each year across the counties of: Essex, Kent, Lambton, Elgin, Middlesex, Huron, Perth, Oxford, Waterloo, Brant, Grey, Wellington, and Halton. Each producer was asked to fill in a survey questionnaire related to each sample taken. Information collected for each field included: cropping history for three years, tillage program, fertility program, pesticide applications, variety, planting date, spacing and rate, and harvest date. Hand-harvested samples were taken a few days before machine harvest consisting of ca. ten primary heads picked at random at points about every 10 paces about 50 m into the field, and another 10 on the way out. The samples were air dried in a greenhouse, and then threshed with stationary plot thresher with every effort to recover all the infected and non-infected grain. Combine samples were taken while the grower was harvesting the field. As the grain was moved from the combine to the hopper, frequent samples of about 250 mL in volume were collected until about 5 kg of grain was accumulated. The grain was mixed thoroughly and a 2 kg sample was retained.

DON content was determined using CD-ELISA according to Sinha and Savard (1996), followed by a commercial kit of the same monoclonal antibody in 1997 (EZ-Quant DON Plate Kit, Beacon Analytical Systems, Inc., 4 Washington Avenue, Scarborough, ME 04074)

Results

All winter wheat samples collected in Ontario in 1996 contained DON, while in 1997 only 65 % of them were contaminated. Analysis of variance showed that effects of year, sampling method (hand versus combine harvested), and year·method on DON concentration was statistically significant (Table 1). In 1996, a *Fusarium* epidemic year, the effect of harvesting method on DON concentration was higher than in 1997, a *Fusarium* endemic year.

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There was a poor relationship between DON content in combine-harvested samples and those that were hand-harvested from the same field (R^2 of 0.43 and 0.33 for 1996 and 1997). Obviously many of the FDK's were expelled from the combine. The risk of confounded results are therefore high when using combine-harvested samples to test for agronomic effects on *Fusarium* disease as measured by DON content.

The most important factor affecting DON concentration in the survey samples was the variety of winter wheat, compared with other factors tested. None of the winter wheat varieties was free of DON (Table 2). DON concentration varied widely among varieties grown in Ontario in 1996 and 1997. In our survey, most growers in Southwestern Ontario grew the variety Harus. The varieties, Freedom and Fundulea were more resistant to accumulation of DON in the grain than other varieties tested, whereas AC RON and 2737W were more susceptible (Table 2). Regardless of the mechanism of resistance, planting susceptible varieties should be discouraged in areas with relatively high potential for scab infection, and DON contamination.

In both years DON concentration was lower in fields with conventional tillage, by comparison with minimum and no tillage (Fig 1 A). However, in recent years, various types of reduced tillage have become widely adapted in field-crop production in Ontario. The soil conservation and economic advantage of these tillage practice are firmly rooted and it is increasingly less likely that producers will clean plow crop debris as a disease management strategy.

In 1997, wheat fields fertilized with urea averaged lower DON contents than wheat fields fertilized with ammonium nitrate (Fig 1 B). This was not true for 1996 under epidemic conditions.

The use of starter fertilizer in the fall at planting appeared to have a slight effect on DON content at harvest in both years (Fig 1 C). Starter fertilizer could have increased the overall N in the head during the infection period, or it could have affected the flowering time (infection timing). The yield advantage of starter fertilizer applied at planting far outweigh the slight added risk of DON accumulation. We do not suggest changing this practice.

In 1996, later nitrogen application resulted in higher DON content, but earlier nitrogen application increased DON level in 1997 (Fig 1 D). Whereas the exact mechanism of the effect of N is still unclear, N balance in the plant certainly affects the risk of DON accumulation.

Assuming a corn-soybean-wheat rotation is the most common in Ontario, a simple pattern on DON accumulation occurred when the effect of crop from two years previous was examined. In both years, DON concentration was lowest in fields following soybeans by comparison with fields following corn or wheat in the second crop season, but the opposite relationship was noted for the crops three years previous (Fig 1 E,F). These data suggest that corn stubble is a more important reservoir for inoculum than is wheat stubble.

To reduce the risk of DON accumulation in wheat, our recommendation to wheat producers in Ontario based on the finding of this study are: to plant less susceptible cultivars, to avoid corn and wheat as a previous crop, to avoid growing wheat in soil that had corn in it within the last two crop years, use urea rather than ammonium nitrate as the N source, and practice a balanced N fertility program.

Table 1. Analysis of variance for the effect of harvesting method, and year on the accumulation of deoxynivalenol in commercial fields of winter wheat in Ontario.

Source	df	Mean squares	F-ratio	P
Year	1	2554.4	104.5	0.000
Harvest Method	1	304.6	12.5	0.000
Year . Method	1	215.3	8.8	0.003
Error	297	24.5		

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Fig 1: The effect of tillage practices (A), nitrogen sources (B), starter fertilizer (C), nitrogen application date (D), and previous crop (E,F) on DON (±SE) accumulation in hand-harvested winter wheat.

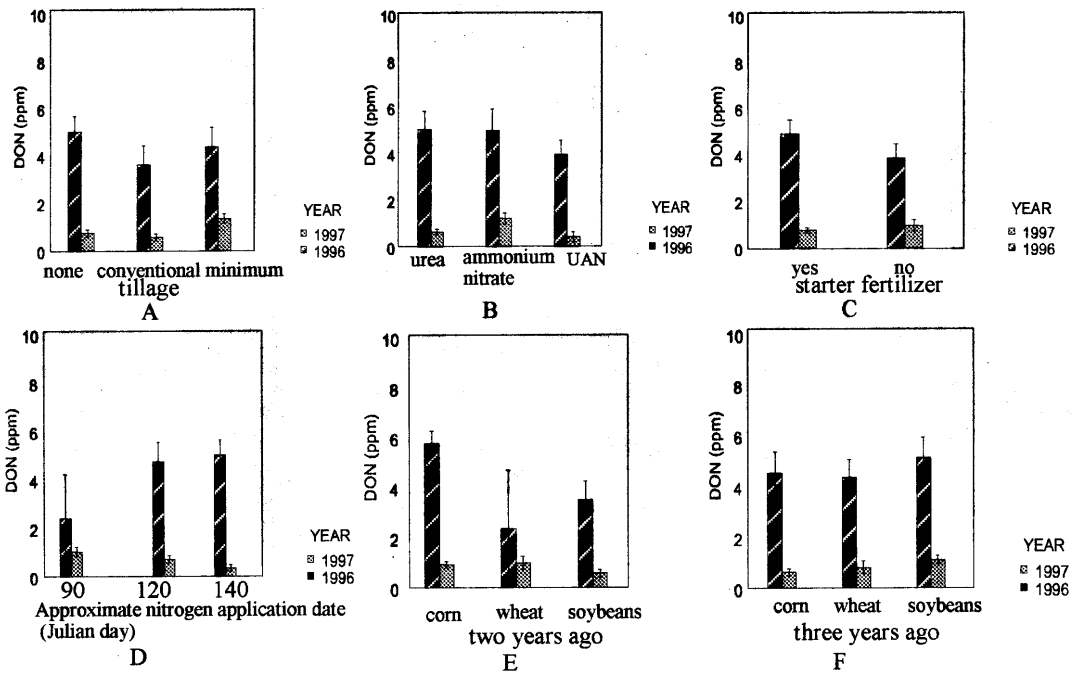

Table 2. The effect of variety on deoxynivalenol content (SE) in winter wheat in Ontario (1996-1997)

Variety	Hand-harvested (ppm)	Combine-harvested (ppm)	H/C	N	Hand-harvested (ppm)	Combine-harvested (ppm)	H/C	N
AC RON	10.2 (1.4)	5.0 (0.8)	2.1	18	1.7 (0.3)	0.9 (0.3)	1.9	7
HARUS	8.8 (1.3)	4.3 (0.7)	2.0	19	0.8 (0.2)	0.5 (0.1)	1.8	23
P 2510	6.5 (1.4)	4.8 (0.8)	1.4	6	1.3 (0.3)	0.6 (0.3)	2.0	6
FUNDULEA	4.9 (1.5)	2.2 (0.4)	2.2	11	0.4 (0.2)	0.1 (0.1)	6.6	9
FREEDOM	2.4 (0.6)	1.9 (0.8)	1.3	5	0.3 (0.1)	0.3 (0.2)	1.0	15
2737W	11.4 (4.4)	5.2 (1.7)	2.2	4	4.1	2.6	1.7	1
KARENA	3.4 (1.7)	4.9 (2.0)	0.7	6				

H/C - ratio of hand- over combine-harvested, N - number of fields sampled

Environmental parameters of ascospore discharge in *Gibberella zeae*

Frances Trail[1], David Gadoury[2] and Rachel Loranger[1].

Objectives

Head scab disease is initiated by infection of flowers from airborne ascospores. Our long-term objective is to understand the mechanisms that function to forcibly discharge the ascospores from the perithecia in *Gibberella zeae*.. Therefore, an understanding of the parameters that trigger discharge is important as a foundation in these studies. The study presented here includes an analysis of the effects of light and humidity on the release of ascospores.

Introduction

Gibberella zeae is thought to overwinter as hyphae in crop debris from the previous season. The following spring, perithecia develop on the debris and ascospores are forcibly discharged around the time of wheat flowering, serving as the primary inoculum source for the disease (Fernando et al., 1997). Several studies have been done exploring the effects of light, rain events, relative humidity, temperature and wind effects on ascospore discharge in the field. Tschanz et al. (1975) have studied the effects of relative humidity, and light/dark periodicity on ascospore discharge in the growth chamber. They suggested that discharge is initiated during decreases in humidity. In contrast, Paulitz (1996) studied the pattern of spore release in inoculated field plots over the course of 24 hours. He found that spore release peaked in correlation with a period of drying down during the day and a sharp rise in relative humidity in the evening. However, peak release occurred 2 to 4 days after a rainfall. He concluded that rainfall was needed for maturation of ascospores, but not release. Other workers have reported that ascospore release in the field is associated with high relative humidity or rainfall (Chen and Yuan, 1984; Reis, 1990). The requirement for free water and high relative humidity is difficult to assess in the field due to microclimates created by presence of vegetation, small changes in terrain, etc. In these studies, we assessed the effect of relative humidity of various values while maintaining constant light and temperature in the laboratory. In addition, we have used a wind tunnel to simulate constant rain, and temperature while varying light cycles to determine if light is important to discharge of ascospores. We have also explored the effect of light on the direction of ascospore discharge.

Materials and Methods

The role of light in spore release was tested using a wind tunnel and spore trap. The tunnel simulated a steady supply of rain, constant temperature (20°C), and air flow. Light (daylight-balanced artificial light) and dark cycles varied according to the experiment. The apparatus has been used in numerous studies exploring ascospore discharge in *Venturia inaequalis* and is described by Gadoury et al. (1994). Approximately 2 cm diameter circles were removed with a cork borer from a culture producing mature perithecia on carrot agar (Klittich and Leslie, 1988) and were mounted in the apparatus for each trial. Spores were collected at the end of the wind tunnel on cellophane tape mounted on a clock cylinder. The tape was removed at the end of each trial and mounted on slides. Spores deposited on the tape were counted under the microscope at intervals of 10 or 20 min depending on the periodicity of the clock cylinder. Two traps were run simultaneously with different light/ dark regimes for comparison. The experiment was repeated in four trials.

Relative humidity and free water requirements for ascospore discharge were also examined. These trials were set up in Mason canning jars using various glycerol concentrations to maintain constant relative humidities at 40%, 70%, 80%, 92% and 100% (Forney and Brandl, 1992). Agar blocks containing mature perithecia on their surfaces were mounted on small clay platforms and placed on the end of a slide. The agar blocks were placed on the vertical surface of the clay block and the block was oriented on the slide so that spores would be

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shot lengthwise down the slide. Slides were then placed above the surface of the various glycerol solutions and the jars were sealed with tape. After 24 hours in continuous light at 25°C, slides were removed and a line was drawn down the center of the spore deposit starting at the base of the clay (1mm mark) to the end of the slide. Spores deposited on the transect were counted, with the deposits on each mm recorded. Three trials were produced for each relative humidity. Analysis of variance was performed by the SAS System to determine the significance of variation between relative humidity tests.

Some fungi will shoot their spores in the direction of a light source. Phototropism of the asci was tested in glass chambers (1 cm in height, 7.5 cm length, 2.5 cm width) small enough to eliminate the effects of convection currents. Perithecia were mounted on clay bases as above and placed on coverslips in the chambers. The chambers were then placed in various orientations with respect to the light source. After 15 to 18 hours, the coverslips were removed and the orientation of the ascospore deposits were examined.

Results and Discussion

Ascospore release during simulated rain, at 20°C, was not affected by light in 3 of 4 trials. Figure 1 shows the pattern of spore release in the two wind tunnels (6.1 and 6.2) run simultaneously with different light regimes. Cumulative ascospore discharge was nearly identical in the two treatments, indicating that light does not affect spore discharge under these conditions. In one of the 4 trials, regression analysis indicated an effect of light on the rate of spore release during hours 3 and 4 of the test and an effect of darkness during hours 1 and 2. The rate of spore release in that trial was unsteady, and the effects were probably spurious. Other workers have observed the lack of a light effect in field studies (Paulitz, 1996). We have been able to confirm these results in vivo with simulated field conditions. Light has been implicated in the maturation of perithecia in the field. Indeed, perithecia production in the lab is dependent on light.

Our observations indicate a requirement for free water for significant ascospore discharge, although there is a low level of release at all relative humidities tested (Table 1). These test conditions may mimic the sharp increase in humidity thought to trigger ascospore discharge after relatively dry conditions (Paulitz, 1996). However, ascospore discharge did not occur for the first 12 hours of these trials, and the perithecia were removed from moist agar plates and placed directly in the humidity chambers. The dry conditions seen in the field occurred during the day, with increases in humidity triggering discharge at night, less than 12 hours later. Our results suggest a free water requirement for maximum ascospore discharge. The presence of free water under field conditions is difficult to determine due to formation of microclimates. Another possible explanation for these results is that ascospores continue to mature in the 100%RH trial and continue to discharge, whereas the dryer conditions prevent further ascospore maturation. Clarification of this possibility will require further study of the developmental aspects of ascospore formation. We are currently undertaking these studies.

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Fig. 1. Release of ascospores by G. zeae at 20°C. Treatment 6.1 was subjected to simulated rain and illuminated for 8.5 hr (430 min). Treatment 6.2 was subjected to simulated rain and darkness for 6.25 hr (375 min) and was then illuminated for 2.25 hr (195 min).

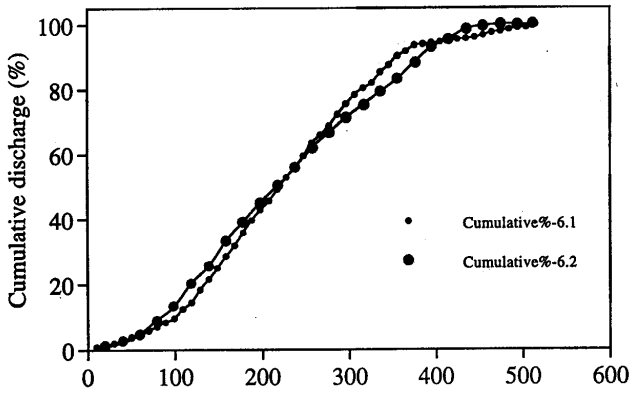

Table 1. Effect of relative humidity on discharge of ascospores.

Relative Humidity (%)	Mean number of spores [1]
40	7.2 ± 0.88 a
70	10.0 ± 1.22 a
80	17.3 ± 3.72 ab
92	25.1 ± 1.83 b
100	65.9 ± 7.41 c

1. Mean number of spores per mm of transect. Average of three trials. Data within columns that are followed by the same letter are not significantly different according to the 95% confidence limits. Analysis performed using Tukey's Studentized Range Test.

Variation in *Fusarium graminearum* Associated with Wheat Scab in North Carolina

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Introduction

Severe epidemics of wheat scab have occurred in the Midwestern United States during the past decade, but the disease has not been prevalent in North Carolina. However, growers in North Carolina are concerned about the possibility of a scab epidemic and have requested research on this disease and possible control measures. The purpose of this study was to collect isolates of *Fusarium* associated with wheat scab and compare these isolates for pathogenicity, *in-vitro* and *in-vivo* production of deoxynivalenol (DON) and zearalenone, and rate of growth of in culture. Typed cultures of *F. graminearum, F. avenaceum,* and *F. culmorum* were obtained and used as standards in each test. The information gathered in this study indicate a diverse population of *F. graminearum* exists in North Carolina and many isolates are both highly aggressive and produce high levels of DON.

Materials and Methods

Sixty-six isolates of *Fusarium sp.* were collected from wheat heads exhibiting symptoms of scab in the coastal, piedmont, and mountain regions of North Carolina. Single spore isolations were performed for each sample prior to any testing. Two isolates of *Fusarium graminearum* (R-6914) and (R-6925), an isolate of *F. culmorum* (R-6565), and an isolate of *F. avenaceum* (R-5314) were obtained from the Pennsylvania State University Fusarium Center and were included in all tests.

Rate of growth was determined by transferring a five mm plug to synthetic nutrient agar (SNA) for each isolate, and radial growth was measured at 3, 5, and 7 days. Treatments were arranged in randomized complete blocks and conducted under controlled light and temperature conditions.

Pathogenicity testing was performed using three soft red winter wheat cultivars: Cardinal (resistant), Wakefield (intermediate), and Caldwell (susceptible) in the greenhouse. A completely randomized design consisting of 70 isolates x 3 cultivars X 6 replications per cultivar was used, totaling 1260 pathogenicity tests. One ml of inoculum at a concentration of 1000 microcondia per ml was pipetted onto the top of the seed head and allowed to drain across the entire head. Plastic bags were misted with water and then placed over inoculated heads for five days. Controls used sterile water in place of inoculum. Symptoms of scab were recorded at ten and twenty days after inoculation.

Isolates for *in-vitro* toxin analysis were grown on rice media. Samples were divided into two groups, one incubated at 27C for 30 days; the other group were incubated 7 days at 27C, followed by 5 days at 4C, and followed by 18 days at 27C. Seed heads from pathogenicity tests were used to determine *in-vivo* DON levels. Seed heads inoculated with the five most aggressive and five least aggressive isolates were used for *in-vivo* toxin testing. DON levels were determined using methods described by Tacke and Casper (2). Zearalenone levels were determined using ELISA.

Results

All isolates collected in North Carolina were identified as *Fusarium graminearum* (1). Disease rating, rate of growth, *in-vitro* production of DON and zearalerone, and *in-vivo* production of DON were significantly different among collected isolates ($p > 0.0001$). Disease ratings differed significantly among the three cultivars tested ($p > 0.0001$). The resistant cultivar 'Cardinal' consistently showed less disease for each isolate than did the more susceptible cultivars. Levels of *in-vivo* DON were lower in the resistant 'Cardinal' cultivar than in the susceptible 'Caldwell' cultivar.

Several significant correlations between characters of different isolates were observed. Disease rating, the location where an isolate was collected, and the cultivar of wheat originally infected with the isolate were correlated. Rate of isolate growth was also correlated with the collection location and the cultivar of wheat originally infected with the isolate.

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In multiple regression analysis, *in-vivo* and *in-vitro* DON accounted for 55 percent of the variation in disease rating. Using cluster analysis, the isolates were separated into eight groups. The eight groups contained isolates from various regions of North Carolina and were not separated by the location where the isolate was collected.

Discussion

Aggressive isolates of *Fusarium graminearum* do exist in many regions of North Carolina and have the potential to cause extensive damage to wheat production. An isolates ability to produce DON appears to be highly linked to pathogenicity. The consistently lower disease ratings on the resistant 'Cardinal' cultivar suggest a lack of host specific virulence in the population.

The most aggressive isolate was collected in an area of very low wheat production. This may have occurred randomly, or possibly the pathogen population has favored highly aggressive strains due to a limited host reservoir. Infections of *F. graminearum* in maize may also maintain aggressive strains in the absence of wheat.

This study indicates the presence of a pathogenic fungal population. In most cases the host is susceptible, and in North Carolina, we frequently have rain and high humidity during flowering of wheat, yet a major outbreak of scab has not regularly occurred. It is possible the reservoir of inoculum is not as large in North Carolina as in the Midwestern United States. Levels of reduced tillage wheat and maize are currently low in North Carolina, so a potential source of primary inoculum is limited. Warm winters may also reduce levels of debris by increasing the rate of decomposition compared to the Midwest.

Literature Cited

1) Nelson, P.E., Toussoun, T.A. and Marasas, W.F.O. 1983. *Fusarium* Species - An Illustrated Manual for Identification. The Pennsylvania State University Press, University Park, Penn

2) Tacke, B.K. and Casper, H.H. 1996. Determination of deoxynivalenol in wheat, barley, and malt by column cleanup and gas chromatography with electron capture detection. Journal of AOAC International 79:472-475.