USE OF A GFP STRAIN OF *FUSARIUM GRAMINEARUM* FOR HISTOLOGICAL INVESTIGATION OF INFECTED BARLEY

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ABSTRACT

We are investigating development of *Fusarium graminearum* on and in barley florets and other organs using a transformed strain of the fungus containing a constitutively expressed gene for green fluorescent protein (GFP). The GFP strain fluoresces green when viewed by fluorescence microscopy under blue or blue-violet incident light. The GFP strain is easier to see in inoculated living florets, leaf or coleoptile tissues (as viewed by epifluoresence microscopy) than are wild strains (viewed by conventional light microscopy). To remove surface mycelium which can obscure fungus development within underlying tissues, a 2 to 1 (v/v) solution of cellulose acetate in acetone is applied to the tissue surface, allowed to dry for a few minutes, then stripped away, leaving the tissue surface intact and free of mycelium. In initial experiments, inoculum in the form of mycelium growing on mung bean agar blocks (2x12x0.5 mm) was applied to cut ends of detached leaves, coleoptiles, the palea, and the lemma from Robust (susceptible) and Chevron (partially resistant) barley. After 24 hr, the block was removed. The fungus grew both into and on top of the inoculated tissues. By three days after inoculation, hyphae within tissues grew 2.9-3.1 mm in leaves, 1.2 - 1.5 mm in coleoptiles and paleas, but less than 0.2 mm in lemmas (as measured from the cut ends to the advancing hyphal front). In leaves, the distance was increased to 4.5 - 5.3 mm if the inoculum block was left in place 48 hr instead of 24 hr. Hyphae within tissues were subcuticular and intercellular. Plant cells in colonized leaves remained green 2-2.5 mm behind the advancing hyphae front. Tissues more than 2.5 mm from the front became chlorotic, losing the red fluorescence of chlorophyll. On the surface of the tissue, mycelium advanced about 0.3 mm ahead of the underlying subcuticular and intercellular hyphae. Hyphae appeared to grow into and out of leaf stomates. The results show that fungus development was limited in paleas, lemmas and coleoptiles compared to development in leaves, that exogenous nutrients can increase amount of colonization in leaves, and that development was the same in tissues of Robust and Chevron. Based on experience gained in these experiments, we will use the GFP strain to monitor pathways of infection from spot inoculations on exposed surfaces of the palea and lemma of intact barley heads, and to investigate the probable role of anthers in promoting head colonization. (This poster was presented at the 16th Annual Barley Researchers' Workshop, Idaho Falls, July 11-15, 1999. The abstract will be in the 1999 Barley Newsletter.)

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GENETIC ANALYSIS OF A *GIBBERELLA ZEAE* MUTANT WITH ALTERED MORPHOLOGY, REPRODUCTION, AND VIRULENCE

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ABSTRACT

Gibberella zeae (*Fusarium graminearum*) causes both wheat ear blight and maize ear rot, with yield reduction and mycotoxin contamination of the infected grain. During routine experiments to produce and regenerate protoplasts of *G. zeae* field strain GZ3639, we identified a mutant, designated *ear*$^-$ for ear rot, that was significantly reduced in ability to cause both wheat ear blight and maize ear rot. *Ear*$^-$ mutants produced normal macroconidia and were similar to the wild-type strain in growth and morphology on V8-juice agar medium, but were reduced in pigmentation on a range of other media. *Ear*$^-$ mutants were unable to produce perithecia, but were able to function as males in outcrosses with strain GZ3639. To conduct genetic analysis, strains were marked with different auxotrophic mutations, and heterozygous perithecia were identified by the presence of recombinant prototrophic progeny. Among random ascospores from heterozygous perithecia, 48 *ear*$^+$ and 26 *ear*$^-$ progeny were recovered, a slight deviation from the 1:1 segregation ratio expected for a single gene. Among these progeny, reduced pigmentation and female sterility showed perfect cosegregation with reduced virulence on wheat ears. These data suggest that a single genetic change may affect both morphogenesis and virulence of *G. zeae*. Future work will elucidate the genetic basis of this mutation and the function of the gene products in wheat ear blight and maize ear rot.

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ROLE OF ENVIRONMENT AND INOCULUM LEVEL IN WHEAT FUSARIUM HEAD BLIGHT DEVELOPMENT

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OBJECTIVES

Develop a forecasting system for wheat Fusarium head blight based on environment and pathogen propagule levels.

INTRODUCTION

Fusarium head blight (FHB) of wheat, caused by *Gibberella* zeae (Schw.) Petch., has proven to be a difficult disease to manage in many wheat producing regions of the world (Parry et al. 1995). In North America, severe epidemics of FHB have occurred in recent years resulting in large crop losses due to direct yield reduction and complications with mycotoxin contaminated grain (McMullen 1997). Management options for FHB include host resistance, crop rotation, tillage to destroy crop residues, and fungicide applications; however, research has shown that no one management option has been effective in controlling FHB (Parry et al. 1995, Bai and Shaner 1994). Successful disease management will require multiple strategies. The development of a reliable disease forecasting system would improve the ability of wheat producers to deal with the prognosis of disease and facilitate timely applications of chemical or biological control agents.

An early attempt to predict epidemics of FHB was based on observations of regional environmental variables correlated with severe epidemics (Atanasoff 1920). In 1965, Nakagawa et al. proposed a forecasting system for that utilized a regression equation for disease prediction based on environmental variables highly correlated with incidence of seed infection. Moschini and Fortugno (1996) proposed the use of linear regression equations developed from historical records of disease and environmental conditions in Argentina. More recently, Francl et al. (1999) reported on the importance of both inoculum level and environment as components of a FHB epidemic. Therefore, a thorough understanding of the factors contributing to the development of epidemics is needed to develop a disease forecasting system and identify potentially successful disease control options.

One approach to developing a disease forecasting system for FHB is to monitor and quantify the relationships between host, pathogen and environment. In order to define the role of these factors in the disease cycle of FHB and ascertain the effects of regional differences in environment and agricultural practices, a cooperative effort was initiated in 1999. Researchers in Ohio, North Dakota, Indiana, South Dakota, and Manitoba, Canada have completed replicated field trials in their respective locations. By presenting here the results from the Ohio and North Dakota locations, we demonstrate the type of information that such research can generate.

MATERIALS AND METHODS

Replicated plots of susceptible wheat cultivar, Hopewell, were planted near Wooster, Ohio, in the fall of 1998. Similar plots of the susceptible hard red spring wheat cultivar, Norm, were planted in the spring in Fargo, North Dakota. At

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both locations daily variation in inoculum level was monitored by arbitrarily selecting five wheat spikes each day from the replicated plots. Wheat spikes were placed into 50 ml of sterile deionized water, shaken to dislodge spores, and spikes then were discarded. A 0.5-ml aliquot was transferred to each of three plates of Komada's selective medium. Cultures were incubated for 10-14 days and Fusarium type colonies were transferred to potato dextrose agar and carrot agar to identify *Gibberella zeae*. Numbers of colony forming units per head were calculated as an indicator of viable inoculum. A Burkard volumetric spore sampler (sampling rate =16.5 L/min) located within the crop canopy (Ohio) or on an adjacent no-till area (ND) was used to provide supplemental information about the presence of airborne inoculum. Samples were collected every 24 h and observed microscopically for the presence of *G. zeae* ascospores and macroconidia. Disease severity and incidence of plants were evaluated by assessing 30 arbitrarily selected wheat spikes at the soft dough stage of growth (GS 83) (Zadoks et al. 1974).

Environmental variables, including ambient air temperature, relative humidity (RH), and precipitation, were measured in the wheat field with an automated datalogger (Campbell Scientific Inc.). The presence of moisture within the wheat canopy was measured with six replicate flat-plate resistance sensors. All wetness sensors were coated with an off-white latex paint and oriented south at the same height as the wheat spikes (80-90 cm).

RESULTS AND DISCUSSION

The environment in Wooster, Ohio, was favorable for wheat growth during the 1998-1999 growing season. Warm fall temperatures and a mild winter resulted in excellent winter survival of the crop. In the spring, favorable temperatures prevailed and precipitation events provided adequate moisture during the early stages of crop vegetative growth. As the crop progressed into the boot and heading growth stages (GS 45 to GS 59), dry conditions prevailed as precipitation events became less frequent (Figure 1). However, precipitation events and high RH were common during crop anthesis (GS 60 to GS 69). Four of the 7 days during crop anthesis corresponded with >11 h of wetness duration, >80% average RH, and measurable precipitation. Temperature during anthesis ranged from 10 to 20EC, and the average temperature was 15EC.

The daily average number of *G. zeae* colony forming units per wheat spike in Ohio ranged from 0 to 16 between heading emergence (GS 50) and early milk (GS 73) growth stages (Figure 1). The median number of colony forming units of *G. zeae* per day was 1 between head emergence and early milk stage of growth. During anthesis, the median daily number of colony forming units was 2. The Burkard sampler detected only low numbers of *G. zeae* spores; however, other fungi common to the air flora of Ohio were readily detected. The highest number of *G. zeae* spores collected was 1000 spores in a 24-h period (mean density 40 spores/m^3, which coincided to the watery ripe stage of growth (GS 71). Ascospores were the observed more frequently and in greater density than macroconidia. Final disease incidence and severity were less than 0.1% at Wooster, Ohio.

The environment in North Dakota was warm and frequent precipitation events provided adequate moisture for crop development. Three precipitation events occurred between boot (GS 45) and completion of heading (GS 60) stages of growth (Figure 2). Three of the 4 days during crop anthesis (GS 60 to GS 69) coincided with >9 h of wetness duration. RH during anthesis ranged from 75% to 87%, with an average of 80%. Ambient air temperature during crop anthesis ranged from 18 to 24EC, and the average temperature was 22EC.

Between the heading and early milk stages of growth, the daily average number of *G. zeae* colony forming units per wheat spike in North Dakota ranged from 0 to 44 (Figure 2). The median number of colony forming units of *G. zeae* per day was 4 during the same time period. While the crop was flowering, the median number of colony forming units per wheat spike was 7. The number and frequency of *G. zeae* spores collected in a Burkard spore sampler was greater than that collected in Wooster, Ohio. Although a low number of macroconidia were observed, ascospores were the major component of the *G. zeae* inoculum in Fargo. Final disease incidence at Fargo was 18%, and disease severity was 1.2%.

The median number of colony forming units per wheat spike observed at Fargo and Wooster were consistent with inoculum levels reported by Francl et al. (1999) for other non-epidemic years. Differences in disease level observed at the two locations may be the result of differences in inoculum level and timing of inoculum release events (Figures 1 & 2). At Wooster, dry conditions prior to anthesis may have resulted in delayed development of *G. zeae* perithecia, which would explain the low levels of inoculum detected during anthesis. Both the Ohio and North Dakota locations appeared to have wetness duration, RH and precipitation levels sufficient for infection by *G. zeae*. However, average ambient air temperature was 7EC lower at Wooster than in Fargo during crop anthesis, and may have also resulted in lower disease levels at Wooster. Based on this information we hypothesize that there are at least two critical periods of environmental conditions. The first critical environmental period occurs prior to head emergence and appears to be important for conidiogenesis, development of perithecia and ascospore maturation. The second critical period occurs while the crop is flowering and corresponds to inoculation and infection events. In the future, regional disease forecasts for FHB may be possible as more information from these and other wheat producing regions further defines the role of environment in the completion of FHB disease cycle events.

REFERENCES

Atanasoff, D. 1920. Fusarium-blight (Scab) of wheat and other cereals. J. Agric. Res. 20:1-32.

Bai, G. and Shaner, G. 1994. Scab of wheat: Prospects for control. Plant Dis. 78:760-766.

Francl, L., Shaner, G., Bergstrom, G., Gilbert, J. Pederson, W., Dill-Macky, R., Sweets, L., Corwin, B. Jin, Y., Gallenberg, R. and Wiersma, J. 1999. Daily inoculum levels of *Gibberella zeae* on wheat spikes. Plant Dis. 83:662-666.

McMullen, M., Jones, R. and Gallenburg, D. 1997. Scab of wheat and barley: a re-emerging disease of devastating impact. Plant Dis. 81:1340-1348.

Moschini, R. C. and Fortugno, C. 1996. Predicting wheat head blight incidence using models based on meteorological factors in Pergamino, Argentina. Eur. J. Plant Pathol. 102:211-218.

Nakagawa, M. Wanatabe, S., Gocho, H., and Nishio, K. 1965. Studies on the occurrence forecast of *Gibberella zeae* on wheat varieties caused by climatic factors. Bull. Tokai-Kinki, Agric. Expt. Sta. 15:55-67 (English summary and figure legends).

Parry, D. W., Jenkinson, P. and McLeod, L. 1995. *Fusarium* ear blight (scab) in small grain cereals-a review. Plant Pathol. 44:207-238.

Zadoks, J. C., Chang, T. T., and Konzak, C. F. 1974. A decimal code for the growth stage of cereals. Weed Res. 14:415-421.

Figure 1. The role of environment and *Gibberella zeae* inoculum level in the development of wheat Fusarium head blight was evaluated at Wooster, Ohio during the 1999 growing season. A bioassay was used to monitor the average daily number of colony forming units (CFU) per wheat spike in replicated field plots was used to monitor inoculum concentrations. Inoculum levels were combined with corresponding 24 h summaries of temperature, relative humidity, precipitation, and wetness duration.

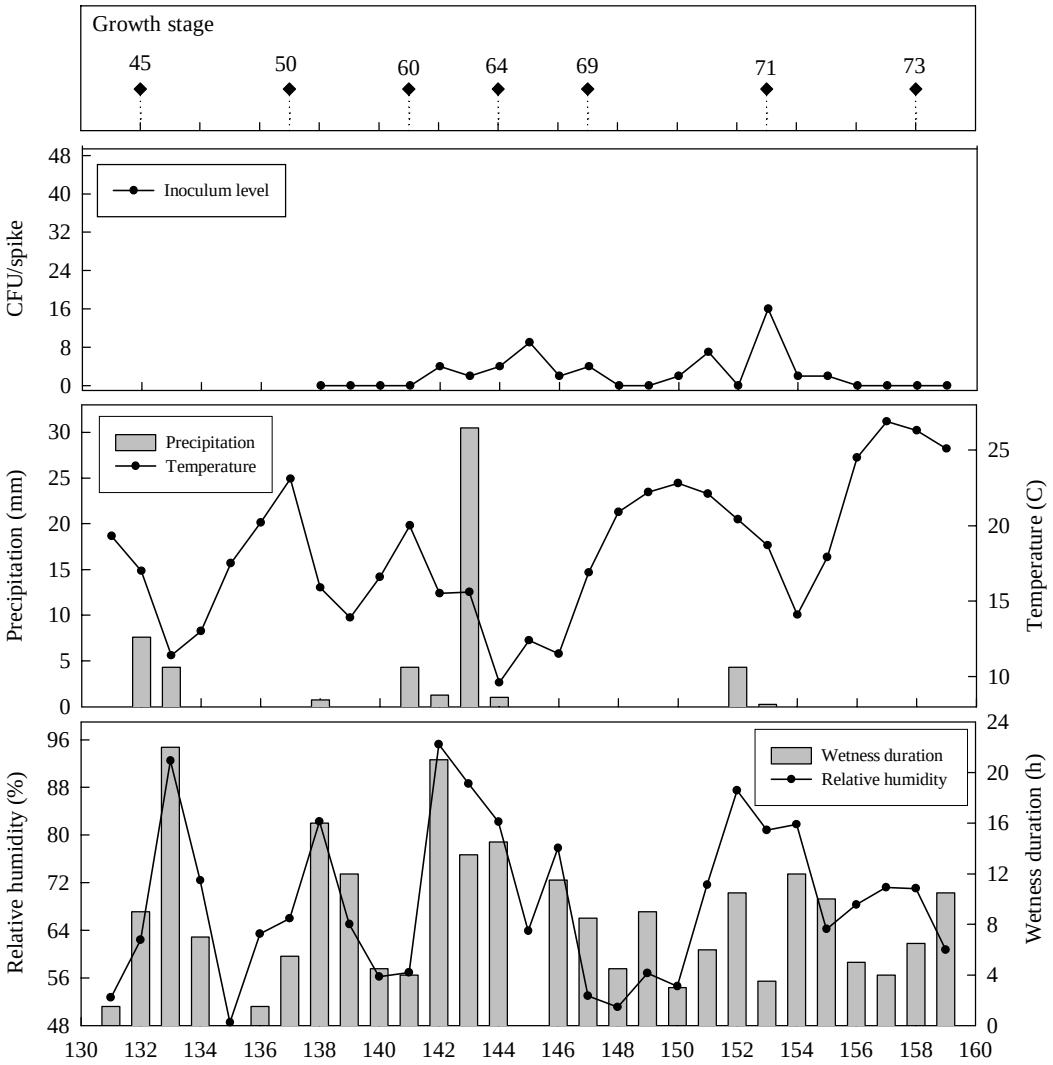
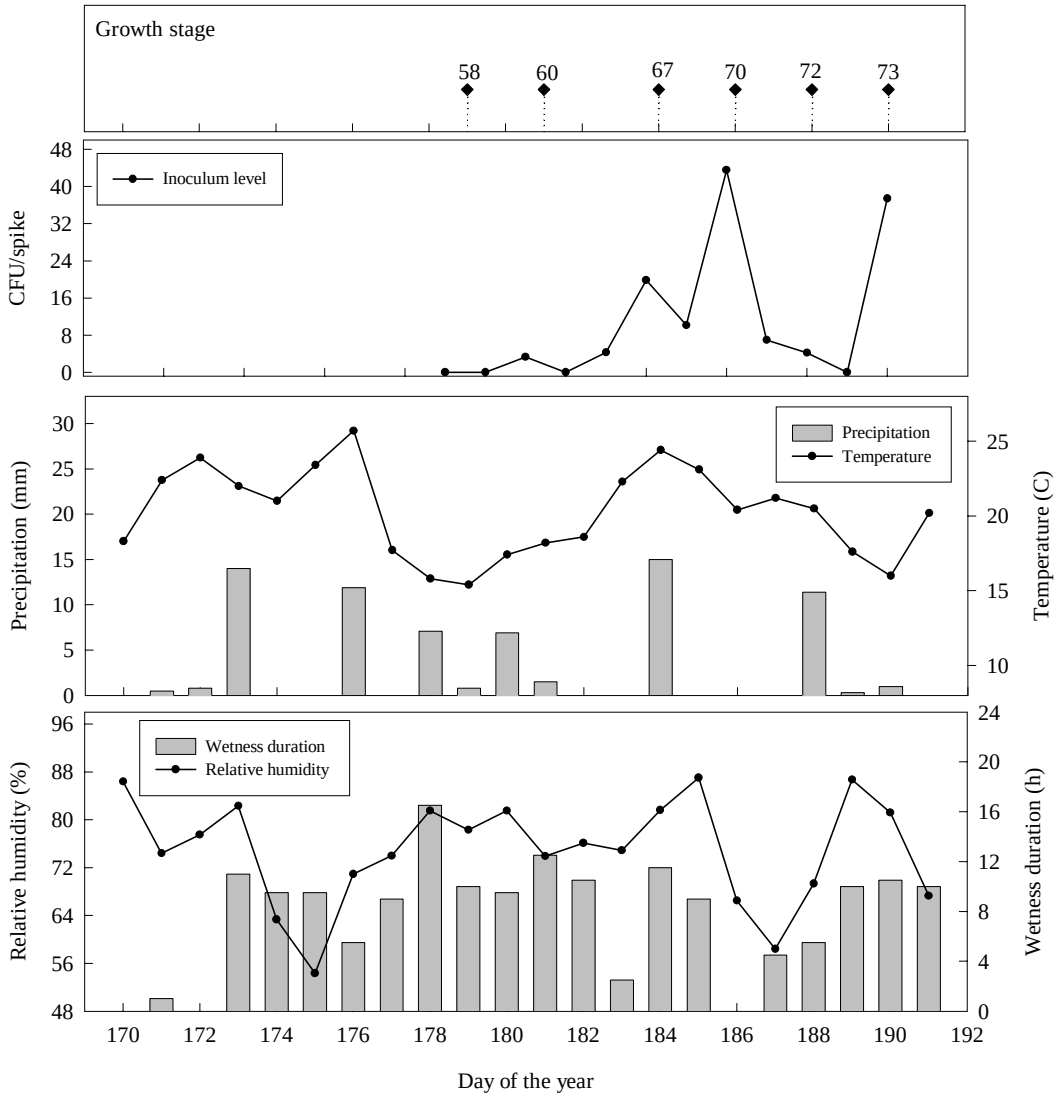


Figure 2. During the 1999 growing season in Fargo, North Dakota, the role of environmental conditions and *Gibberella zeae* inoculum levels in the development of wheat Fusarium head blight was evaluated. The daily average number of colony forming units (CFU) per wheat spike in replicated field plots was monitored with a bioassay. Inoculum levels were combined with 24 h summaries of temperature, relative humidity, precipitation, and wetness duration.



A WHEAT HEAD CULTURE SYSTEM TO SCREEN FOR PATHOGENICITY MUTANTS OF *GIBBERELLA ZEAE*

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ABSTRACT

An *in vitro* assay was developed to screen mutants of *Gibberella zeae* PH-1 for loss of pathogenicity. Wheat heads at anthesis were excised and placed in culture medium prior to inoculation with a suspension of macroconidia. The wheat heads were then incubated for the development of symptoms. The success of this method was evaluated by comparing the rate of infection in excised wheat heads of Norm (a susceptible variety of wheat) to that in intact wheat heads and by assessing the stability of resistance of Ning (a resistant line of wheat) in excised heads.

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USE OF REMOTE PILOTED VEHICLES IN AEROBIOLOGICAL STUDIES OF *GIBBERELLA ZEAE*

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OBJECTIVE

To determine whether viable ascospores of *Gibberella zeae* are dispersed in the planetary boundary layer of the lower atmosphere, and thus, exhibit the potential for regional dissemination.

INTRODUCTION

Gibberella zeae (Schw.) Petch (anamorph *Fusarium graminearum* Schwabe) is the causal pathogen of head blight of wheat and barley. Ascospores are forcibly discharged from perithecia, becoming airborne and infecting wheat spikes during flowering (Paulitz, 1996). Knowledge of the vertical profile of fungal spores in the air is important for a better understanding of the pathogen's distribution. Since the transition zone between the surface boundary layer (SBL) and the planetary boundary layer (PBL) is considered to be 2-2.5 times the crop canopy height in agricultural settings (Huschke, 1989) sampling the atmosphere between 0-152m is critical for understanding the initiation of long-ranged transport of this pathogen. Knowledge of the aerobiology of *G. zeae* will contribute to a better understanding of the regional epidemiology of Fusarium head blight (FHB).

MATERIALS AND METHODS

Two 2.4-m wingspan remote piloted vehicles (RPV's) were used to collect ascospores of *G. zeae* in the lower atmosphere. RPV's sampling devices were vertically mounted Petri dishes containing Komada's agar 1.75% as a collection surface and were outfitted with the Petri dish top as a door which can be remotely open when sampling periods were initiated. Each sampling device has the capacity to sample an average of $8m^3$ of air per minute. A total of 60 collection flights were performed during June 7- June 30, 1999 at Cornell's Musgrave Agronomy Farm near Aurora, NY. The landscape features wooded areas and small agricultural fields of corn, soybean, oat, wheat and fallow fields. Collections were made at approximately 15m aboveground and most circular flight patterns overlapped a plot of winter wheat in which we scattered autoclaved corn kernels infested with *G. zeae* (Gz014NY98) on May 14. Due to dry conditions at anthesis in early June, no FHB developed in the inoculated winter wheat.

RESULTS AND DISCUSSION

The use of RPV's is a feasible method for collecting viable ascospores of *G. zeae* from the lower atmosphere. Viable ascospores were collected each night, under a range of atmospheric conditions, and even following 7 days without a local rainfall event (Fig. 1). Therefore, airborne inoculum of *G. zeae* was present prior to and through the anthesis period of local winter wheat. The fact that FHB did not develop can be attributed to a lack of moisture favoring infection of wheat florets, rather than to

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a lack of inoculum. Peak colony counts of *G. zeae* were obtained during evenings with heavy cloud cover (Table 1) which increases the vertical mixing of air currents in the lower atmosphere.

REFERENCES

Huschke, R.E. 1989. Glossary of Meteorology. American Meteorological Society. 5th Ed. 638pp.

Paulitz, T.C. 1996. Diurnal release of ascospores of *Gibberella zeae* in inoculated wheat plots. Plant Dis. 80:674-678.

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Fig.1. Precipitation at Musgrave Agronomy Farm at Aurora (NY) during June, 1999.

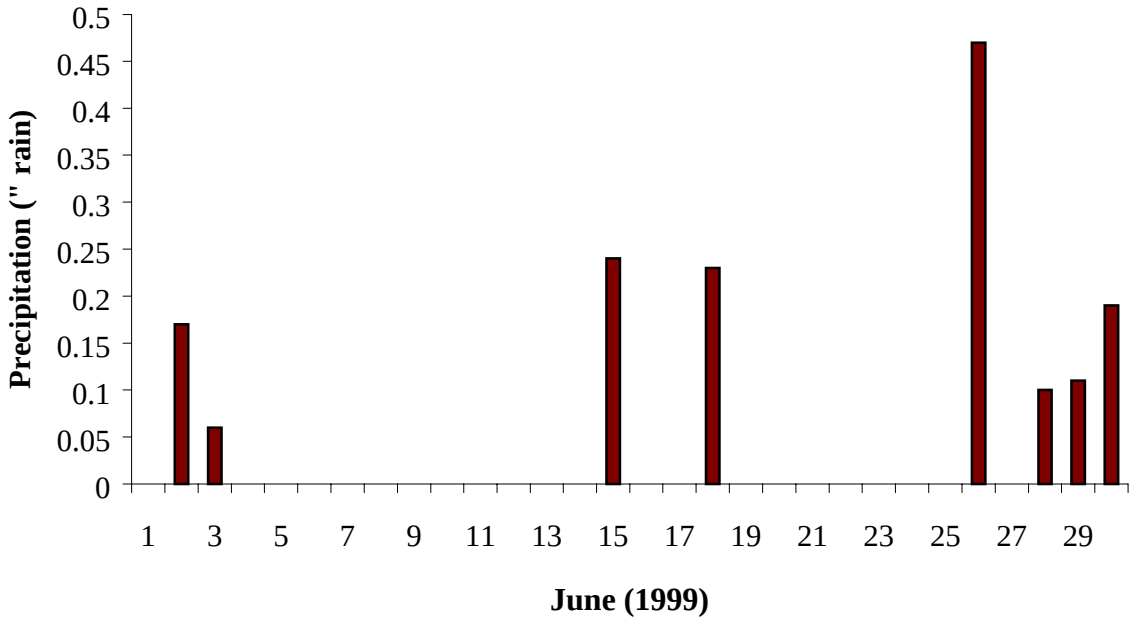

Table 1. Peak colony counts for *Gibberella zeae* collected using RPV s.

Date	Number of flights	Collection Time (PM)	Daily Temp (F)	Peak colony counts/15min	Flight conditions
June 7, 1999	3	6:30-8:15	93/67	10 @ 6:30	No cloud cover, no wind
June 8, 1999	6	6:30-9:30	95/66	27@ 9:30	No cloud cover, no wind
June 10, 1999	10	6:21-10:25	78/60	60 @ 9:15	Heavy cloud cover, very windy and cold
June 16, 1999	11	6:30-10:30	63/42	40 @ 8:40	Low cloud cover, no wind, cold
June 23, 1999	14	4:45-10:05	84/58	47 @ 4:45	Cloud cover, no wind, hot
June 25, 1999	5	4:40-5:45	90/67	23 @ 4:45	Hot, no wind, no cloud cover
June 30, 1999	11	4:50-9:45	78/49	87 @ 8:30*	Heavy cloud cover, no wind, cold

*flying 8m directly above inoculated wheat plot

SURVIVAL AND INOCULUM POTENTIAL OF FUSARIUM GRAMINEARUM IN WHEAT RESIDUES[1]

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OBJECTIVE

To determine the relationship between wheat residue decomposition and the survival and inoculum potential of *Fusarium graminearum*.

INTRODUCTION

Fusarium head blight (FHB) is economically important in wheat and barley worldwide (4,9). The disease has also become a major problem in the midwestern United States, producing devastating losses to growers in recent years (2,5). The principal pathogen associated with FHB in Minnesota is *Fusarium graminearum* Schwabe and its perfect stage *Gibberella zeae* (Schw.) Petch. (10).
F. graminearum survives saprophytically on residues of hosts like corn, wheat, barley and other cereals and some grasses (8). Management practices like crop rotation with non-host crops and plowing infected residues have been recommended to reduce primary inoculum of *F. graminearum* (1). The establishment of strategies for the management of *Fusarium* infected residue will require a thorough understanding of the viability and inoculum potential of *F.graminearum* in relation to residue decomposition.
A field experiment was established to examine the relationship between the decomposition of wheat residue and the survival and inoculum potential of *F. graminearum*. Data from this research may assist growers in establishing appropriate crop rotation periods and effective tillage systems in the management of *F.graminearum* infected residues.

MATERIALS AND METHODS

Stems of hard spring wheat residue (cv. Russ) in sections 24 cm long were placed in fiberglass mesh bags. Each bag contained 20 g of residue equivalent to 2857 kg/ha. Three hundred bags with inoculated residue (*F. graminearum* Group 2) were used for the determination of survival and inoculum potential of *F. graminearum.* Non-inoculated residue was used in a paired study of residue decomposition. Isolation from inoculated and non-inoculated nodes were made to determine the effect of the inoculation.

Field experiment

The residue was placed in the field in October 1997 at the Northwest Experiment Station, Crookston, MN.

Treatments:
1. Chisel plow, residue on soil surface
2. Chisel plow, residue buried at 7.5-10 cm depth
3. Chisel plow, residue buried at 15-20 cm depth
4. Moldboard plow, residue buried at 15-20 cm depth

Seventy-five samples in each treatment [15 residue bags (samples) in each strip, 5 replicates (strips)] were used to allow monthly samplings from April to October/November in 1998 and 1999. One set of samples from each strip was randomly collected during the sampling period. The residue was left undisturbed during the winter months when the ground was frozen.

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Determinations

The decomposition of the residue was determined by dry matter loss. Survival of *F. graminearum* was determined on stem pieces (30 per sample) 1.5 cm long including a single node. Pieces were surface disinfected, placed on pentachlorobenzene (PCNB) agar (6) and incubated at 20-22°C with 12 hr light per day for seven days. *'Fusarium'* colonies were counted and *F. graminearum* colonies were determined following transfer of 10 random colonies to carnation leaf agar (CLA) (3). CLA cultures were incubated at 20-22°C with 12 hr light per day for 10 days. Perithecia formation indicated *F. graminearum* Group 2 isolates. *Fusarium* colonies not forming perithecia were identified to species according to the descriptions of Nelson *et al*. (7).

Inoculum potential of *F. graminearum*: In April, May, July and September 1998, 24 stem/node pieces from each sample were surface disinfected and placed on sterile sand in culture boxes with vented lids. Nodes were incubated at 20-22°C with 12 hr light per day for 21 days and kept moistened. Ascospores were collected on silicone greased slides placed 2 cm above the nodes for 24 hours. The number of ascospores collected per slide and replicate were counted under the compound microscope. Due to reductions in the number of nodes recovered in November 1998 and subsequent samples, a weight of nodes was evaluated. Nodes with mature perithecia were placed in sterile distilled water (dilution 1:20) and a drop of Tween 20. Nodes were soaked for 10 hours to allow ascospore discharge and finally shaken for 10 minutes. Three aliquots (0.03 ml) were obtained from each treatment and used to determine the ascospore concentration.

Survival and inoculum potential data were subjected to ANOVA and treatments means were compared by Tukey test (P=0.05) using SAS (SAS Institute, 1985).

RESULTS AND DISCUSSION

F. graminearum was isolated from 96% and 97% of non-inoculated and inoculated nodes, respectively, suggesting that the inoculation had little impact on the colonization of wheat residue by *F. graminearum*.

Preliminary results (October 1997-May 1999) for the dry matter remaining and survival and inoculum potential *of F. graminearum* are presented in Tables 1, 2 and 3.

Buried residue decomposed substantially faster than residue at the soil surface (Table 1). No differences were evident in decomposition rate between the chisel plowed and moldboard plowed treatments buried at 15-20 cm. Approximately 20 and 50% of residue dry matter content remained in buried and surface residue treatments in May 1999, respectively.

Significant reductions in the colonization of residue by *F. graminearum* were observed once the residue had been in the field for at least eight months (Table 2) in those treatments where the residue had been buried. Even when a marked decreased in the survival of *F. graminearum* occurred in July 1998, the number of *Fusarium* spp. colonies isolated from residue samples tended to remain the same. This suggests that other species, probably with higher competitive ability, were colonizing the residue in preference to *F. graminearum*. Other *Fusarium* species recovered were: *F. sporotrichoides, F. equiseti, F. culmorum, F. semitectum, F. avenaceum, F. sambucinum, F. solani*.

Preliminary results of inoculum potential indicate that residue, from which *F. graminearum* can be isolated, support perithecial development and the production of mature ascospores (Table 3).

The reduction in the amount of residue colonization by *F. graminearum* is related to the decomposition of residues. Surface residues, which decomposed more slowly, seem to provide a host to *F. graminearum* for a longer period of time than buried residues. Residue from which *F. graminearum* can be isolated support perithecial development and ascospore production.

LITERATURE CITED

1. Bai, G. and Shaner, G. 1994. Scab of wheat: prospects for control. Plant Disease 78:760-766.

2. Dill-Macky, R. 1996. Fusarium Head Blight: Recent epidemics and research efforts in the Upper Midwest of the United States. Pages 1-6. *In*: Fusarium Head Scab: Global status and future prospects. H.J. Dublin; L. Gilchrist; J. Reeves and H. McNab eds. CIMMYT. Mexico.

3. Fischer, N.L.; Burgess, L.W.; Tousson, T.A. and Nelson, P.E. 1982. Carnation leaves as a substrate for preserving cultures of *Fusarium graminearum* species. Phytopathology 72:151-153.

4. Mathre, D.M. (ed.) 1997. Compendium of barley diseases. Second edition. The American Phytopathological Society. St Paul. 90 p.

5. McMullen, M.; Jones, R. and Gallemberg, D. 1997. Scab of wheat and barley: a re-emerging disease of devastating impact. Plant Disease 81:1340-1348.

6. Nash, S.M. and Snyder, W.C. 1962. Quantitative estimations by plate counts of propagules of the bean root rot *Fusarium* in field soils. Phytopathology 52:567-572.

7. Nelson, P.E.; Tousson, T.A. and Marasas, W.F.O. 1983. Fusarium species: an illustrated manual for identification. The Pennsylvania State University Press. University Park. 193p.

8. Sutton, J.C. 1982. Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum.* Can. J. Plant Pathol. 4:195-209.

9. Wiese, M.V. (ed.) 1987. Compendium of wheat diseases. Second edition. The American Phytopathological Society. St Paul. 112p.

10. Wilcoxson, R.D.; Kommedahl, T.; Osmon, E. A. and Windels, C.E. 1988. Occurrence of *Fusarium* species in scabby wheat from Minnesota and their pathogenicity to wheat. Phytopathology 78:586-589.

Table 1. Percent wheat residue dry matter, relative to initial weight at placement (October 1997), at sampling times between April 1998 and May 1997 in four tillage/residue placement depth treatments.

	Percent dry weight of wheat residue remaining at sampling										
	1997	1998								1999	
Treatments	Oct.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Apr.	May
Chisel plow surface	100[p]	94	86	85	76	72	61	55	50	53	43
Chisel plow 7.5-10 cm	100	86	75	62	46	39	22	19	18	21	21
Chisel plow 15-20 cm	100	88	77	66	51	41	28	19	22	22	22
Moldboard plow 15-20 cm	100	87	79	68	55	41	32	28	21	23	22

[p] values given are the means of five replicates

Table 2. Percent colonization of the nodes of wheat residue by F. graminearum in four tillage/residue placement depth treatments at time of residue placement (October 1997) and at sampling times between April 1998 and May 1999.

	Colonization of wheat residues by *F. graminearum* (%)										
	1997	1998								1999	
Treatments	Oct.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Apr.	May
Chisel plow surface	96[q]	93	96	96a[r]	69a	76a	80a	78a	81a	63a	67
Chisel plow 7.5-10 cm	96	81	91	93ab	57ab	61ab	60ab	58b	77ab	61ab	63
Chisel plow 15-20 cm	96	97	83	80b	46b	40b	56ab	57b	69ab	61ab	54
Moldboard plow 15-20 cm	96	81	90	87ab	47b	40b	50b	52b	51b	45b	50

[q] values given are the means of five replicates

[r] values followed by different letters are significantly different at *P=0.05*

Table 3. Inoculum potential of *F. graminearum*.

Sampling month	Chisel plow surface	Chisel plow 7.5-10 cm	Chisel plow 15-20 cm	Moldboard plow 15-20 cm
April 1998	55[s]	97	48	56
May 1998	50	72	27	33
July 1998	39	48	20	23
September 1998	49	58	31	27
November 1998	10[t]	10	16	12
April 1999	34	72	30	7
May 1999	104a[u]	40ab	21b	105a

[s] Ascospores per cm^2

[t] Thousands of ascospores per g of residue

[u] Means followed by the different letters are significantly

EPIDEMIOLOGY OF FUSARIUM HEAD BLIGHT OF WHEAT IN INDIANA, 1999

David Thomas, George Buechley and Gregory Shaner*

Scab, or Fusarium head blight, has been a difficult disease to control throughout the world because of the lack of effective disease management practices. Traditional disease control options (disease resistant cultivars, crop rotations, tillage to destroy residues, and fungicides) have not been used because of lack of availability, excessive cost, or negative impacts on soil conservation (McMullen et al. 1997). Evaluation of these various management options has shown that no one approach is effective in controlling Fusarium head blight. Bringing Fusarium head blight under control will require multiple disease management strategies (Parry et al. 1995, Bai and Shaner 1994), coupled with greater understanding of the epidemiology of the disease.

MATERIALS AND METHODS

The experiment was conducted in a long-term tillage-rotation experiment at the Purdue Agricultural Research Center (PARC) in Tippecanoe County (west central Indiana). Wheat plots adjacent to plots that had corn residue from the 1998 crop year were the site of spore sampling and assessment of disease incidence and severity.

A Burkard Cyclone sampler was placed in the wheat plot when plants were at the boot stage of development (GS 45). A continuous corn plot that received minimum tillage was located just west of this plot.

The spore sampler was operated from 19 May to 13 June. The Burkard drew in air at a rate of 16.5 liters per minute. The Eppendorf collection tube was replaced every 24 hours, between 10:00 and 11:00 am each morning. The contents of Eppendorf tube were processed directly after sampling, or placed in a freezer for processing later.

Spores were recovered from the Eppendorf tube by adding 1.5 ml of sterile water, shaking on a Vortex mixer for 1 minute and spreading 1 ml of the suspension evenly over a petri plate containing Komada's medium. The plate was placed under 24-hr UV light at 25°C. A 5ml sample of the water remaining in the Eppendorf was examined with a hemacytometer.

On 18 May, approximately 500 wheat heads between growth stages 48 and 50 were selected along the west edge of the long-term tillage plot. They were covered with waxed paper bags to shield them from airborne *Gibberella zeae* spores. Beginning on 20 May, 5 heads were uncovered and tagged each morning between 10:00 and 11:00 am. The heads were collected at the same time the following morning and 5 more heads were uncovered and tagged. The collected heads were then covered with plastic and taken directly to the lab for processing. This process was repeated until 11 June.

The 5 heads were shaken in 50 ml of sterile water for 1 minute. Five 1-ml portions of the washing were transferred to each of five plates of Komada's medium, which were placed under 24 hr UV light at 25°C.

For all platings, when colonies were clearly visible on the plates (approx. 0.5 cm in

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diameter), all *F. graminearum* colonies were counted.

Blighted heads and total heads in 20 arbitrarily-selected 61-cm lengths of row were counted on 17 June. Scab incidence was calculated as the percent of scabby heads in the 20 samples. On 22 June, 50 scabbed heads were taken for estimation of severity of infection. The spikelets on each head were counted, as well as blighted spikelets. These heads were threshed by hand, and total as well as scabbed kernels were counted.

RESULTS

Daily average temperatures varied dramatically at the beginning of the flowering season, ranging from 21.7°C on day 142 to 10.3°C on day 145 (Fig. 1). Average daily temperatures rose from then, reaching 13.9°C by the end of flowering and 27.8°C by the end of the sampling period. Precipitation occurred on only 6 days during this period, for a total of 87 mm; 38 mm fell during flowering (days 141-146).

The Burkard sampler collected spores on most days from 23 May through 11 June, when sampling ceased (Fig. 2). Although the highest number of spores collected occurred on the day with maximum rainfall during the sampling period, there was no consistent relation between rainfall amount and number of spores collected. The number of spores collected during the time wheat was flowering was quite low. The spore counts rose after anthesis (growth stage 70, day 147).

In addition to direct enumeration with a hemacytometer of spores of *Gibberella zeae* collected by the Burkard sampler, washings from the Eppendorf tube were plated on Komada's medium. Estimates of the number of CFUs/ml by this method were considerably lower than estimates from the hemacytometer counts (Fig. 3). There was a low correlation between the hemacytometer counts and the counts from Komada's medium (R=0.46). The CFU counts from the Komada's platings showed less relationship to precipitation than did the hemacytometer counts.

Air spora was also estimated by exposing heads for 24-hr periods and then washing them and plating the washing on Komada's medium. As with the Burkard samples, this method detected propagules of G. zeae on most days (Fig. 4). However, propagules were detected on only 2 days during anthesis, and this was toward the end of anthesis.

The mean incidence of scab calculated from the 20 61-cm field samples was 6.6% (st. dev.= 3.7%). On those heads that were blighted, mean severity was 29.9% (st. dev.= 23%). Mean incidence of scabby kernels from blighted heads was 8.2% (st. dev.= 11%).

DISCUSSION

Declining temperatures and only two, nonconsecutive rain events during anthesis evidently prevented severe development of head blight. Three consecutive days with rain occurred during the late milk stage of development, in conjunction with moderate temperatures. A large spore flight was detected on the third day of rain, but this evidently did not result in heavy infection. As found in a previous study (Francl et al. 1999), there was no clear association between precipitation and number of spores detected.

The spore number estimates derived from direct examination of the Eppendorf tube washing with a hemacytometer were more than two orders of magnitude greater than the estimates obtained by plating the washing on Komada's medium. There was also a low correlation between the methods of estimation. This discrepancy

between the two methods may arise in part from crowding of colonies on the Komada's plates. On the days where the hemacytometer counts revealed large numbers of spores, competition among colonies on the Komada's medium would probably have prevented all potential colonies from developing. Several platings resulted in the development of nearly 200 colonies, which covered the entire surface of the Komada's medium. A concentration of 200 CFUs/ml is probably the maximum for a reliable count on Komada's medium without further dilution of the Eppendorf tube washing. Dilution problems were evidently not the only reason for discrepancy between the two types of data, however. Given that competition may have restricted the number of colonies on Komada's medium, we regard the hemacytometer counts as a more reliable representation of the ambient spore concentrations.

Although the mean severity of blight on symptomatic heads was 30%, the incidence of scabby kernels from these heads was much lower. The likely explanation for this difference is that many of the scabby spikelets contained no kernels when dissected.

LITERATURE CITED

Bai, G. and Shaner, G. 1994. Scab of wheat: Prospects for control. Plant Dis. 78:760-766.

Francl, L., Shaner, G., Bergstrom, G., Gilbert, J. Pedersen, W., Dill-Macky, R., Sweets, L., Corwin, B. Jin, Y., Gallenberg, R. and Wiersma, J. 1999. Daily inoculum levels of *Gibberella zeae* on wheat spikes. Plant Dis. 83:662-666.

McMullen, M., Jones, R. and Gallenberg, D. 1997. Scab of wheat and barley: A re-emerging disease of devastating impact. Plant Dis. 81:1340-1348.

Parry, D. W., Jenkinson, P. and McLeod, L. 1995. *Fusarium* ear blight (scab) in small grain cereals-a review. Plant Pathol. 44:207-238.

Fig.1: Temperature and precipitation data from the beginning of anthesis to the end of sampling Day 140 is May 20.

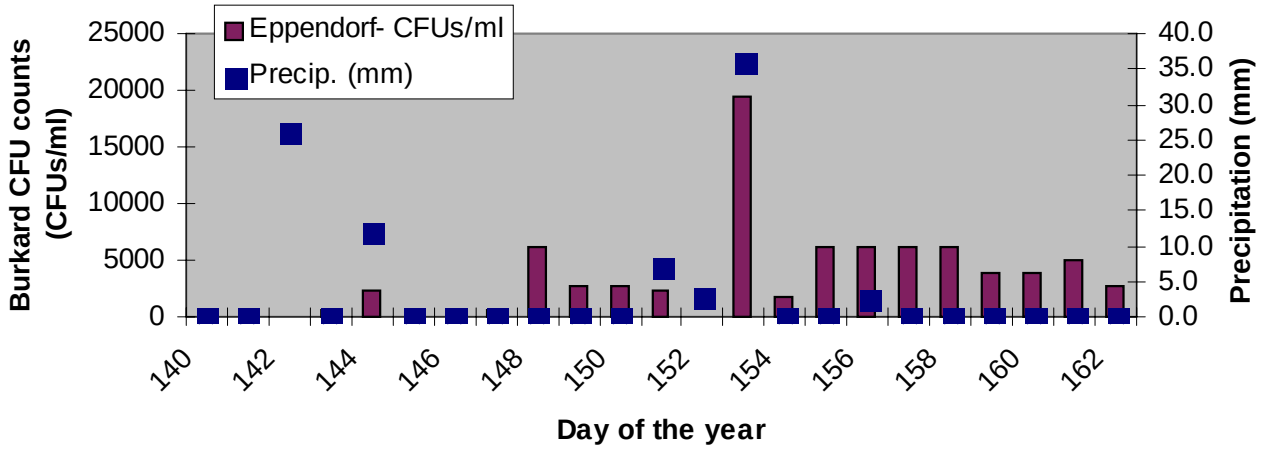


Fig.2: Hemacytometer counts of spores of *Gibberella zeae* from the Burkard sampler compared to precipitation data.

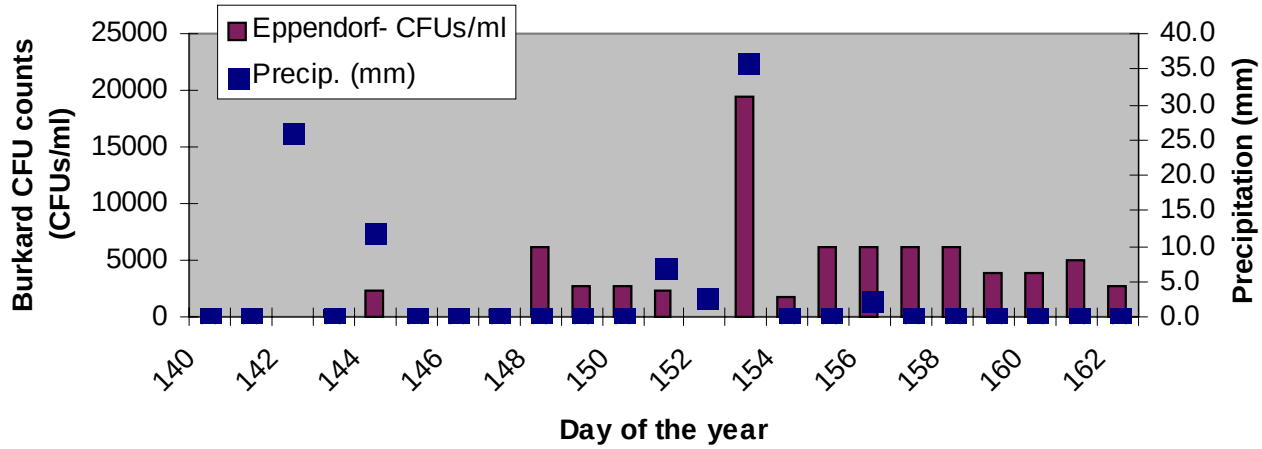


Fig.3: Concentrations of propagules of *Gibberella zeae* recovered from wheat heads exposed for 24 hours (calculated from Komada's plating) compared to precipitation.

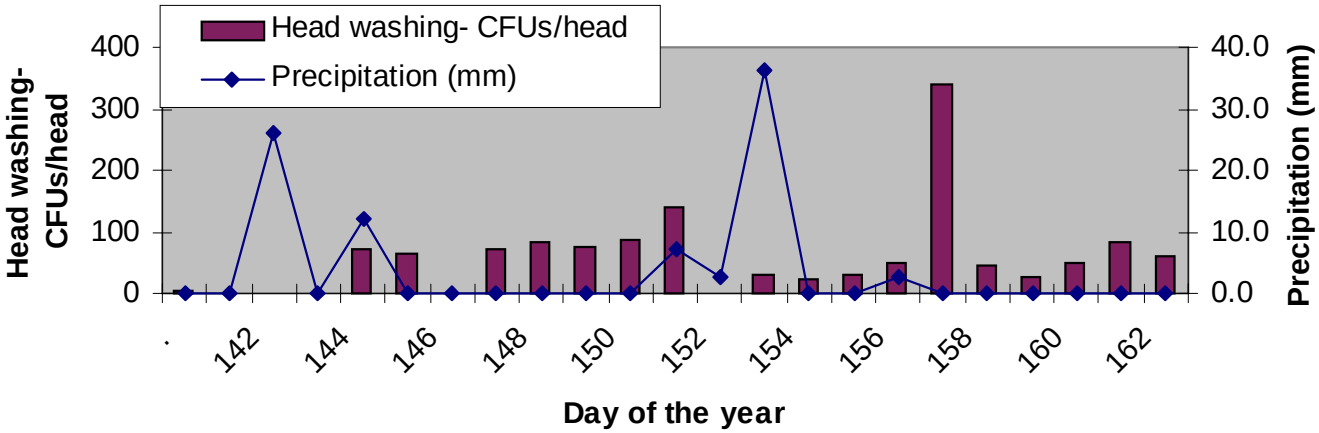
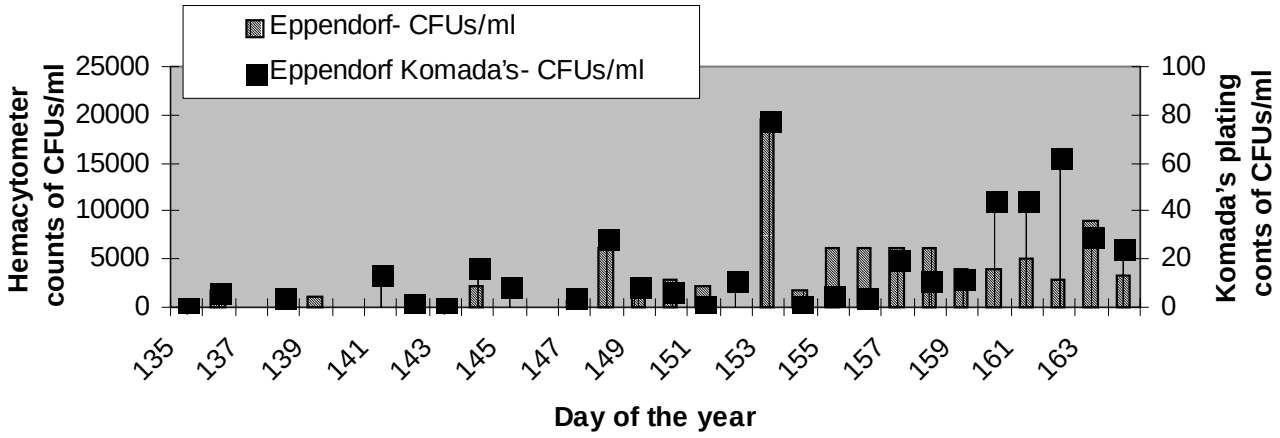


Fig.4: Comparison of counts of propagules of *Gibberella zeae* collected by a Burkard sampler using hemacytometer (bars) and by counting colonies on Komada's medium (squares).



PLANT RESIDUE IN THE CONTROL OF FUSARIUM HEAD BLIGHT

Robert L. Todd*, Robert Stack, Edward Deibert, and John Enz

ABSTRACT

Many approaches have been taken to control the disease Fusarium Head Blight, such as development of resistant-varieties, chemical control, crop rotation, and recently, attention to residue management. The fungus, *Fusarium graminearum*, causing Fusarium Head Blight persists and multiplies on infected crop residues of small grains and corn. It is the consensus of many investigators that *Fusarium* control via residue management may provide one means to control this disease. Treatments might include "plowing down" residue or use of nitrogen fertilization to enhance the decomposition process.

The research presented in this review is part of an ongoing investigation to establish the correlation between residue management and the survival of *Fusarium*. Residue decomposition and fusarium survival are quantified when wheat, barley and corn plant residues are placed on and below the soil surface. Cover crop and nitrogen (N) fertilizer treatments are included as well as monitoring parameters related to decomposition such as soil temperature and water, carbon to nitrogen ratio and lignin content of the residue. If *Fusarium graminearum* survival is related to residue decomposition, then residue management strategies which enhance displacement of *Fusarium* might be developed. Since residue decomposition is a microbial process, manipulation of the indigenous microorganisms might accelerate the loss of *Fusarium.*

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ASCOSPORE FORMATION AND DISCHARGE IN *GIBBERELLA ZEAE*

Frances Trail*, Corrie Andries and Haixin Xu

ABSTRACT

Gibberella zeae infects wheat and barley heads in flower by airborne ascospores that are forcibly ejected from perithecia produced on corn and wheat debris. We have been monitoring the timing of perithecium formation on crop debris in fields in southern Michigan since 1997. Since perithecia are ephemeral, the timing of formation may be critical to control of head scab. Results of our survey will be presented. We have completed a study of the development of perithecia in culture to coincide with our ongoing study of perithecium development on crop debris. The structure of the perithecium has implications on its ability to function effectively in ascospore dispersal. The cellular structure of the crop debris may also have important implications on perithecium formation.

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AFLP DIVERSITY OF *GIBBERELLA ZEAE*

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ABSTRACT

Fifty wheat heads with scab were sampled from a 0.25-m^2 quadrat in Kansas in 1993 and a similar quadrat was sampled in North Dakota in 1994. Isolations were made from the top, middle, and bottom of each head. For both quadrats, AFLP banding patterns showed that more than one AFLP haplotype colonized most heads. Several haplotypes appeared on more than one head, suggesting secondary colonization. AFLP haplotypes correlated very well with a previous vegetative compatibility group (VCG) diversity study on the same isolates. Allele frequencies in the two quadrats were similar. This suggests little genetic differentiation over large distances in the Great Plains region. Further studies with greater sample sizes are planned.

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