**U.S. Wheat and Barley Scab Initiative**
**Annual Progress Report**
**September 18, 2000**

## Cover Page

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| **Year:** | **FY2000** |
| **Grant Number:** | |
| **Grant Title:** | **Fusarium Head Blight Research** |
| **Amount Granted:** | **$44,800.00** |

## Project

| Program Area | Objective | Requested Amount |
|---|---|---|
| Epidemiology | To investigate pathways of head invasion by Fusarium graminearum. | $44,800.00 |
| | **Requested Total** | $44,800.00 |

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Principal Investigator Date

(Form – PR1)

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Project 1: To investigate pathways of head invasion by *Fusarium graminearum*

1. What major problem or issue resolved and how are you resolving it?

Fusarium head blight emerged in the 1990s in the U.S. as an extremely destructive disease of wheat and barley. Efforts to develop resistant varieties have been only partially successful. Furthermore, the initial stages of infection in heads of wheat and barley are poorly understood. Accordingly, we are using microscope techniques to investigate how the head blight fungus initiates infection in the florets of developing wheat and barley heads. Understanding infection pathways will favorably impact virtually all aspects of Fusarium head blight research, including screening of breeding lines for resistance, disease management in farmers' fields, and use of genetic transformation (biotechnology) to develop disease resistance.

2. Provide a comparison of the actual accomplishments with the objectives established.

Toward the principal objective of understanding pathways of invasion and pathogenesis by the Fusarium head blight fungus, we observed the fungus microscopically in living tissues of whole leaves. Progressive changes in invaded leaves were documented, confirming that the fungus first grows in between plant cells without killing them, then induces yellowing (loss of chlorophyll) and, one to two days later, enters and grows within cells as the cells begin to collapse and die. The fungus advanced most rapidly between cells bordering vascular bundles. To supplement microscopy of whole leaf tissues, methods were developed to embed invaded tissue in resin and section them for viewing microscopically. These methods were used in preliminary investigation of barley florets (the tissues invaded by the head blight fungus), confirming that thick-walled cells armor the floret surface against fungal entry. Methods were developed to inoculate selected microscopic spots on the floret surface to elucidate potential invasion pathways through stomates or in the narrow gap between the palea and lemma (the two petal-like structures enclosing the florets).

3. What were the reasons established objectives were not met?

Autofluorescence of plant tissues unexpectedly reduced our ability to see the invading fungus in advanced stages of disease development. The autofluorescence obscured the view of the fluorescent fungal mutant (GFP) used to help see the fungus using fluorescence microscopy. The GFP strain is clearly visible in early stages of infection but not in advanced stages. Consequently, we developed methods for sectioning and staining invaded tissue for viewing by conventional microscopy.

4. What were the most significant accomplishments this past year?

First, we confirmed that the Fusarium head blight fungus grows initially between cells without killing them (in a biotrophic relationship with the plant tissue). This indicates that antifungal substances produced by genetically engineered plants or fungicides applied to plants will be most effective if they are targeted to intercellular spaces within the plant. Second, we gained an improved understanding of the complex anatomy of barley florets and developed improved histological methods to follow infection processes. This provides the foundation

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needed for investigation of potential avenues of fungus invasion.
Publications

Bushnell, W.R., R.W. Skadsen, T.N. Goff, and T.M. Hohn. 1999. Use of a GFP strain of *Fusarium graminearum* for histological investigation of infected barley. Abstracts, National Fusarium Headblight Forum. Sioux Falls, S.D. December 5-7, 1999.

Pritsch,C., G. Muehlbauer, W.R. Bushnell , D.A. Somers and C.P. Vance. 1999. Fungal development and induction of defense response genes during early infection of wheat spikes by *Fusarium graminearum*. Molecular Plant-Microbe Interactions 13:159-169.

Hilburn, K.L.B., W.R. Bushnell and R.J. Zeyen. 2000. Toward a plant suspension cell assay for eukaryotic antifungal protein constructs used in cereal transformation. Phytopathology 90:535. (Poster presented at the National Meetings of the American Phytopathological Society, New Orleans, August, 2000)