IDENTIFYING RESISTANCE AND THE RELATIONSHIP BETWEEN SPIKELET SYMPTOMS AND KERNEL INFECTION IN *Fusarium graminearum* INFECTED SOFT RED WINTER WHEAT

Marla Hall, Brenda Kennedy, and Dave Van Sanford
Department of Agronomy, University of Kentucky, Lexington, KY

OBJECTIVES:

1) To screen soft red winter wheat for Type II resistance to *Fusarium graminearum*.
2) To investigate the relationship between visual spikelet infection and kernel infection.
3) To examine the spread of *Fusarium graminearum* through the spike.

INTRODUCTION:

Fusarium head blight (FHB), also known as head scab, caused by *Fusarium spp.*, has been a historically devastating disease of wheat and barley all around the world. In Kentucky, the prevalent cropping system of no till or minimal till wheat production works to influence head scab levels by providing sufficient inoculum levels. Incorporating FHB resistance into soft red winter wheat is considered to be the most effective control strategy.

There are reports of five different modes of resistance to FHB. Type II (resistance to spread within in the spike) is commonly measured in greenhouse inoculation experiments. Type IV (resistance to kernel infection) is less understood but has been researched. Researchers have reported that there are genotypes that have less kernel infection than anticipated, based on FHB values. This experiment was completed to better understand the interaction of spikelet infection and kernel infection due to FHB and thus provide information on the most effective breeding and selection methods.

MATERIALS AND METHODS:

In the fall of 1999, 29 soft red winter wheat lines and 21 F_1's were evaluated in the greenhouse for Type II resistance to *Fusarium graminearum*. The fifty wheat genotypes were planted in the greenhouse on October 11, 1999 in a completely randomized design.

Type II Screening:

Macroconidial suspensions were prepared in the lab from a mixture of eleven different *F. graminearum* isolates. At the time of anthesis a central floret of each spike was marked with a permanent non-toxic pen and inoculated by pipetting 3 µl of the spore suspension containing approximately 1,000 spores. After inoculation, plants were placed in a humidity chamber for three consecutive nights. Plants were moved out of the chamber on the fourth day and scored for disease development on the 21st day post-inoculation. The number of diseased spikelets and the total number of spikelets were recorded for each inoculated spike. The spikelet infection rate was calculated as the percentage of diseased spikelets per total spikelets.

Kernel Assessment:

Each inoculated spike was harvested and the kernels from each spike were plated onto acidified potato dextrose broth agar to quantify the presence of *F. graminearum* in the developed kernels. Seeds from each spike were plated onto the agar according to the spatial arrangement of the spikelets from which they came. The number and position of blank spikelets containing no seed were recorded. Plates were incubated for 7 days at 20˚C. After incubation, those kernels that showed the presence of *F. graminearum* were recorded.

RESULTS AND DISCUSSION:

Type II Resistance:

The 50 genotypes differed in their response to FHB (Table 1). For brevity, only twelve of the 50 genotype means are shown (Table 2). The number of replicates per genotype varied due to low numbers of F_1 seed and the loss of some seedlings due to de-vernalization.

Table 1: ANOVA Tables for Kernel Infection and Spikelet Infection

ANOVA Kernel Infection

Source	df	Sum of Squares	Mean Square	F value	
Genotype	49	52033.47	1061.91	2.11	***
Error	312	156814.99	502.61		
Total	361	208848.45			

*** p<0.001

ANOVA Spikelet Infection

Source	df	Sum of Squares	Mean Square	F value	
Genotype	49	33490.98	683.49	1.76	**
Error	312	121350.11	388.94		
Total	361	154841.10			

** p<0.01

Relationship Between Spikelet Infection and Kernel Infection

From Table 2 we see that some genotypes did have a lower kernel infection rate than expected from their spikelet infection rate. These genotypes would posses type IV resistance based on Mesterhazy's explanation. For example, Glory, which had a spikelet infection rate of 45.6%, had a kernel infection rate of only 30.5% (Table 2).

Effect on Selection

If we set a hypothetical selection criteria of 10% and keep only those genotypes showing less than 10% spikelet infection, 28 genotypes would have been selected. Of those 28 genotypes, 7 actually were above the 10% infection level based on kernel infection data and 4 genotypes would not have been selected based on spikelet infection but should have been selected based on kernel infection data.

Is there a significant difference between kernel infection rate and spikelet infection rate?

A one tailed t test was completed to compare the two overall means. The result from this test revealed that the difference between overall kernel infection mean and overall spikelet infection mean was not significant at the 5 or 10% level. Although the overall means are not different, differences in spikelet infection and kernel infection are noted on an individual genotype mean basis. The correlation coefficient between these two variables was $r = 0.51 (p<0.01)$. The relationship between kernel infection and spikelet infection is moderate.

TABLE 2. COMPARISON OF KERNEL AND SPIKELET INFECTION BY *Fusarium graminearum* IN SEVERAL SOFT RED WINTER WHEAT GENOTYPES AND F_1's.

Pedigree	Kernel Infection	Spikelet Infection	n
Patton/Glory	0.00	6.60	5
Patton/Foster	0.00	3.50	2
Ernie	1.42	3.34	10
Foster/91C-117-32	2.21	5.75	4
Coker 9663/91C-117-32	3.19	11.63	8
Foster	3.72	11.71	7
Patton	4.31	5.30	10
Glory/91C-117-32	6.34	8.38	8
Coker 9663	20.32	15.41	6
91C 117-32	22.51	13.07	8
90C 054-6	23.29	39.20	5
Glory	30.47	45.57	7
Overall Mean	15.55	12.94	50

Injection Inconsistencies

Table 3 provides a breakdown of all observations made in this study. In the first scenario where neither plant symptoms or fungus presence was recorded, the necessary conditions for FHB development did not occur. This could be attributed to nonviable spores, improper environmental conditions, or ill-timed injections. Most likely these escapes are due to injections made prior or past anthesis, the most infectious stage. The next scenario describing visual symptoms but no actual fungus present in the kernels could be accounted for by early senescence fooling the human eye. A white head symptom has also been described in the literature where the wheat head is not actually infected with the fungus but assimilate is shut off to the head thus causing the white head appearance. The final situation that warrants some attention is most troubling. No visual symptoms were noted in the plants but indeed they were infected and the fungus present in the kernels. It is not uncommon to isolate *F. graminearum* from sound looking kernels, yet not only did these kernels look sound but the spikelets looked sound as well. This scenario did not

occur prevalently and could possibly be attributed again to the improper human judgment of symptoms.

TABLE 3. COMPARISON OF SPIKELET INFECTION LEVELS TO KERNEL INFECTION LEVELS

	Plates showing NO fungus present	Plates showing fungus present	Total Observations
Spikelets showing NO symptoms	38 (10.5%)	22 (6.07%)	60 (16.57%)
Spikelets showing symptoms	126 (34.8%)	176 (48.62%)	302 (83.42%)
Total Observations	164 (45.30%)	198 (54.69%)	362

What should n be?

Based on the error mean square contained in this study, 14 replicates would reduce error variance sufficiently to detect a difference of 10% in spikelet infection. Fifty-six replicates are needed to increase the detection level to 5%. To detect a difference of 10% in kernel infection, 16 replicates are sufficient. Eighty-one replicates are sufficient at the 5% detection level. Noting that 56 and 81 replicates are economically non-feasible for most university breeding programs, we recommend that 15 replicates be used in greenhouse experiments with similar levels of experimental variation. Of course the inherent variation within an experiment greatly influences the number of replicates needed. As the variation decreases the number of necessary replicates also decreases.

Spread Through the Spike

Plating the kernels in order, according to their arrangement on the spike, allowed us to follow the spread of the fungus through the spike. Based on the results from the plating data we could reconstruct the presence of the fungus within each spike. From this enormous amount of data it appears that the fungus spirals both up and down the spike infecting florets.