Wheat Scab in Soft Red Winter Wheat in Indiana in 1986 and Its Relation to Some Quality Measurements

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ABSTRACT

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A severe outbreak of wheat scab in the northern portion of Indiana during the 1986 growing season was associated with prolonged wet weather during and after anthesis. The combined effects of scab and other head and foliar diseases greatly reduced yield and test weight. Samples of grain from throughout Indiana were analyzed to determine the incidence of scab, germination, test weight, and concentration of deoxynivalenol (DON), a toxin produced by *Gibberella zeae*. Grain samples from 43 of 44 counties had scab. Scabby kernels averaged 2.9% by weight and test weight averaged 821 g/L (54.8 lb/bu). Kernel infection by *G. zeae* averaged 23%. DON was detected in 88% of the samples, and the mean concentration was 0.6 parts per million (ppm). Nine percent of the samples had DON $\geq$ 2 ppm. For the 1986 crop, a level of scab $\geq$ 2%, a test weight $\leq$ 809 kg m^{-3} (54 lb/bu), or seed germination $\leq$ 80% could be used as criteria for a decision to analyze a grain sample chemically for DON.

Recent outbreaks of wheat (*Triticum aestivum* L.) scab, caused primarily by *Gibberella zeae* (Schwein.) Petch have been reported from Minnesota (22), Nebraska, Kansas (3,15,20), the mid-Atlantic states (13), and Canada (1) with accompanying production of deoxynivalenol (DON) and rarely zearalenone (4). Although wheat scab is not commonly epidemic in Indiana, serious outbreaks were reported as early as 1891 (2), 1919, and 1928 (16), and there have been sporadic outbreaks since then. In 1986, we observed early severe symptoms of wheat scab in Lafayette, Indiana, and received reports of severe scab from wheat growers in northern and central Indiana. As a consequence, we surveyed the crop to provide timely information for growers, seed dealers, and the grain industry.

Our objectives were to determine the amounts of DON in the wheat crop and to determine the relationships among test weight, visible scab, kernel infection by *G. zeae*, seed germination, and DON concentrations. Some previous investigators (15,20) have reported a correlation of DON with scab kernels and infection of hard winter wheat by *G. zeae*, but correlations were not always high in every year, at every location, or for all grain quality parameters. In related studies, the effects of scab and subsequent fungicide seed treatment on germination, stand establishment, and yield were investigated (6,7).

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MATERIALS AND METHODS

Collection of samples. Samples were obtained from a variety of sources including county agents, farmers, and operators of grain elevators and feed mills. Samples were taken from 44 of 92 Indiana counties, predominantly in northern and central Indiana. A few came from southern Indiana where scab did not appear to be a problem in 1986. Samples, usually 1–2.5 kg, were taken from bins, trucks, and combines. They were carried by hand or posted to us shortly after sampling and stored at 4 C until tested. Samples were split on a Precision Divider (Dean Gamet, Minneapolis, MN). A 250-g sample was taken for scab determination, and the remainder of the grain was split equally. One-half of the grain was ground to the espresso setting, the finest setting on a Grindmaster coffee mill (model 495, Louisville, KY), mixed in a plastic bag, and a 50-g sample was split by a riffle for chemical analyses. The unground remainder was used for various tests.

Determination of disease and grain quality parameters. Three subsamples of 100 kernels from each sample were observed on a black background with ×3 magnification for kernels that were: 1) shrunken and white (chalky), 2) white, but not shrunken, 3) shrunken, but not white, or 4) normal. Kernels in each class were counted and weighed, and percentages of each class were calculated by number and by weight. Kernels were considered scabbed if they were all white or white with pink, whether they were shrunken or not. The percent of kernel infection was determined by placing 50 kernels from each sample on malt-salt agar (6% NaCl) and on potato-dextrose agar (PDA) containing 100 ppm of Tergitol NPX (Union Carbide) and 30 ppm of chlortetracycline (added to molten agar). The kernels were submerged in 1% NaOCl for 1 min, shaken to remove excess liquid, and plated. Test weights of samples were determined with a 473-ml (1-pint) kettle after removal of dockage. To determine seed germinability, 50 seeds from each sample were submerged in 1% NaOCl for 1 min, placed on moistened filter paper in petri dishes, incubated at 10 C for 5 days to break potential dormancy, and placed at 22–24 C for 6 days. A seed was considered germinated if it had both a root and a shoot.

Fungal identification. Fungi were usually identified by cultural characters on the initial isolation plate after 1 wk. Mass transfers were made from representative cultures of *G. zeae* and *Fusarium* spp. They were grown under cool-white fluorescent light for 12 hr per day from a 40-watt lamp placed 25 cm above water agar culture plates that contained autoclaved brome grass leaves or on PDA culture plates. The cultures were examined after 1 and 2 wk at 22–24 C and identified according to Nelson et al (18). Booth (5) and Burgess and Liddell (8) were also consulted.

Chemical analyses. A thin-layer chromatography method approved by the Association of Official Analytical Chemists (AOAC) was used to determine the concentration of DON in grain samples (11). Each sample was analyzed once.

RESULTS

Each measurement will be discussed separately and compared to other measurements, particularly DON. We were interested in finding relationships between grain quality parameters and concentration of DON at $\geq$ 2 ppm, which the U.S. Food and Drug Administration terms the "level of concern" in grain.

Visual inspection of kernels. Samples from 43 of the 44 counties surveyed had scab. Only four of the 75 samples had no scab. There was much less scab in samples from southwestern Indiana, but southern Indiana was not extensively

sampled. Using weight of scabby kernels as a percentage of total grain weight, samples ranged from 0 to 16.6% with a mean of 2.9% (Fig. 1A). Non-chalky shrunken kernels, not counted as scabby, averaged 2.0% by weight. These small kernels probably resulted from other diseases, most probably Septoria leaf and glume blotch and leaf rust. A few samples collected early in the harvest had kernels that fluoresced a dull yellow-green under near-UV light (approximately 365 nm). The fluorescence was not the typical bright green-yellow fluorescence (21) associated with the growth of *Aspergillus flavus* Link:Fr. and appeared similar to that reported by Kulik (14). Several fluorescing samples analyzed for aflatoxins by the AOAC TLC method (21) were negative. The kernels that fluoresced were usually very small and did not yield *A. flavus*.

Kernel infection. Ninety-six percent of the samples were infected by *G. zeae*. Frequency of infection among samples ranged from 0 to 86% with a mean of 23.2% (Fig. 1D). Most scabbed kernels, as well as kernels that were shrunken but not chalky, yielded *G. zeae*. It is not unusual to isolate *G. zeae* from apparently sound kernels. Eight of 10 isolates identified as *Fusarium graminearum* Schwabe (anamorph of *G. zeae*) produced fertile perithecia of *G. zeae* on autoclaved brome leaves on water agar within 2 wk. The ready production of perithecia indicated they were type II (12), although the cultures were mass-spored. When 23 isolates of *Fusarium* not resembling typical *F. graminearum* were cultured on diagnostic media, 43% proved to be *F. graminearum*, 30.4% were *F. acuminatum* Ellis & Everh., 13% were *F. tricinctum* (Corda) Sacc., 8.6% were *F. sporotrichoides* Sherb., and 4.3% were *F. moniliforme* J. Sheld. *Fusarium acuminatum* was distinguished from *F. avenaceum* (Fr.:Fr.) Sacc. by the presence of chlamydospores. All of the species have been reported from wheat kernels (10,22); all are mycotoxigenic, and most have the potential to produce trichothecenes that are more toxic than DON (17).

One sample had 4% *A. flavus* (group species). *Aspergillus glaucus* (group species) was not isolated and *Penicillium* species were isolated from about 2% of the kernels in six samples. Of the fungi other than *Fusarium* that were isolated from grain, most were species of *Alternaria* (usually *A. alternata* (Fr.:Fr.) Kiessl.) and *Epicoccum*.

Test weight. Average test weight was 821 kg m^{-3} (54.8 lb/bu), much lower than the 869–899 kg m^{-3} test weights that are typical for soft red winter wheat produced in Indiana. Only 42% of the samples had a test weight of 839 kg m^{-3} (56 lb/bu) or more (Fig. 1B).

Seed germination. Germination of surface-disinfected seed was not seriously reduced in the laboratory, except for a few samples. Eighty-three percent of the samples germinated above 80%; 47% of the samples germinated above 90% (Fig. 1C). Samples with low germination were usually associated with a modest frequency of infection by *G. zeae* (Table 1).

Chemical analysis. DON was detected in 88% of the wheat samples with an average concentration of 0.6 ppm and a range of 0–4 ppm (Fig. 1). Nine percent of the 75 samples had ≥ 2.0 ppm. A hand-threshed sample, not included in the survey, had 10.3 ppm. Samples from 43 of the 44 counties represented in the survey contained DON. Samples from eight of nine southern counties did not exceed 0.4 ppm DON, but one sample from southern Indiana had 4.0 ppm DON. All of the other samples that had ≥ 2.0 ppm DON were obtained from northern and north-central counties.

DON was confirmed in one sample by mass spectrophotometry, and zearalenone was found in both of two samples tested at levels of 33 and 318 parts per billion (ppb). No other trichothecenes were found in two samples that were positive for DON.

Correlations. The correlations between the various characteristics mea-

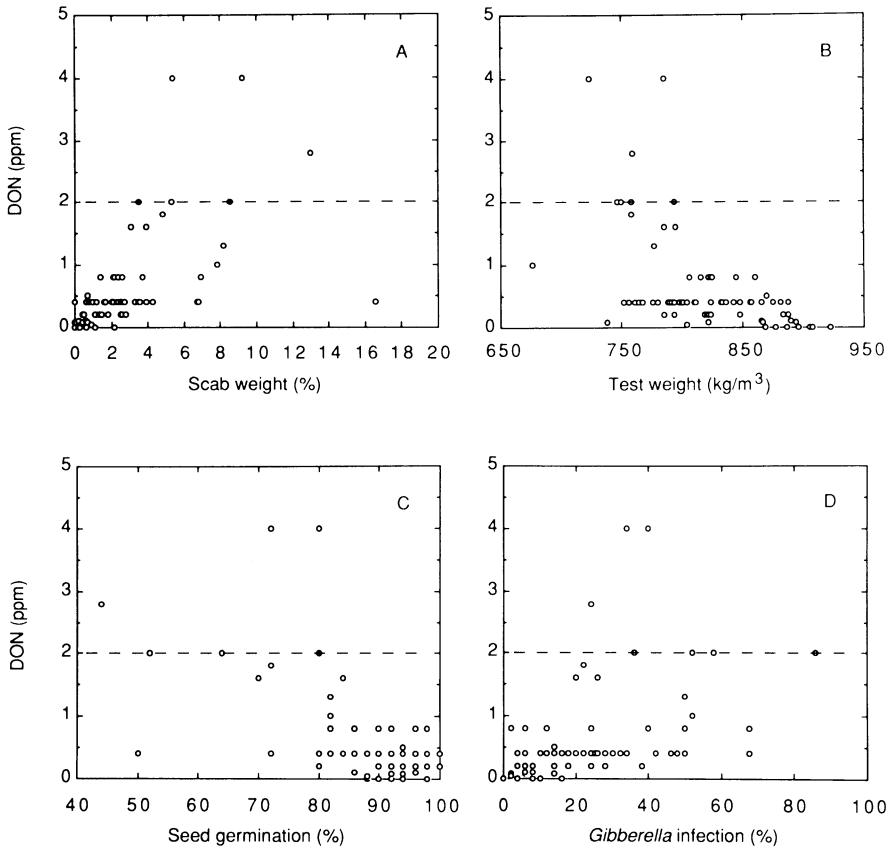

Fig. 1. Relation between various grain quality parameters in wheat samples collected in 1986 from throughout Indiana and the level of deoxynivalenol (DON) in the grain. **(A)** Percentage of of scabby kernels by weight, **(B)** test weight (kg m^{-3}), **(C)** percent of seed germination, and **(D)** percent of infection by *Gibberella zeae*.

Table 1. Correlation coefficient (r) matrix for various parameters related to scabby grain in 75 wheat samples collected from Indiana during the summer of 1986

	Percent scab (weight)[a]	Percent scab (no.)[b]	Test weight (kg m^{-3})	Seed germination (%)	Infection by *G. zeae* (%)[c]	DON (ppm)[d]
Percent scab (weight)	1.00[e]					
Percent scab (no.)	0.98	1.00				
Test weight (kg m^{-3})	−0.65	−0.69	1.00			
Seed germination (%)	−0.71	−0.69	0.45	1.00		
Infection by *G. zeae* (%)	0.70	0.72	−0.62	−0.46	1.00	
DON (ppm)	0.59	0.65	−0.52	−0.62	0.44	1.00

[a] Percentage by weight of visibly scabby kernels in the sample.
[b] Percentage of number of visibly scabby kernels in the sample.
[c] Percentage of kernels in the sample that yielded *Gibberella zeae* upon plating.
[d] Deoxynivalenol concentration.
[e] All correlations are significant at $P = 0.01$.

sured on the scabby wheat samples were all significant at $P = 0.01$ (Table 1). Not unexpectedly, the highest correlation was between scabby kernels as a percentage of weight in the sample and as a percentage of number in the sample. Because of the shriveling associated with these two parameters, each of them was negatively correlated with test weight. Laboratory germination likewise decreased as the percentage of scabby kernels increased.

Generally, the samples with the largest amounts of infection by *G. zeae*, scab, and DON had the lowest test weight. The reverse was true for seed germination. Because of the number of exceptions, probably caused by rusts and leaf and spike infections by *Stagonospora nodorum* (Berk.) Castellani & E. G. Germano, test weight was not a reliable indicator of DON content. However, among samples with $\geq$ 2.0 ppm DON, the highest test weight was 794 kg m^{-3} (53 lb/bu). Of samples with no DON, the lowest test weight was 869 kg m^{-3} (58 lb/bu).

Samples that germinated at 90% or above were usually associated with a high test weight and low amounts of *Gibberella* infection. DON content in samples tended to increase as the percentage of scabby kernels increased, whether expressed as percentage by weight or by number. As expected, the greater the frequency of infection by *G. zeae*, the greater the level of DON, but the correlation coefficient was only 0.44, indicating that conditions other than the frequency of kernels infected in a seed sample determines the level of toxin production.

DISCUSSION

Wheat diseases were prevalent and severe throughout Indiana in 1986, owing to exceptionally wet weather that began in early May (9). These included leaf and stem rusts, *Septoria tritici* and *Septoria nodorum* blotches (the latter on both leaves and heads), and tan spot. Wet weather during and after anthesis in the northern part of the state promoted a severe outbreak of scab in addition to these other diseases.

Scab is not only of concern for its effect on the grain that is used for food or feed but also for the grain that is used for seed. The laboratory studies reported here showed that even after seed was cleaned to remove shriveled and scabby kernels, there could be a high incidence of *Gibberella* infection. To investigate the effect of severe scab on field germination and stand establishment, we conducted fungicide seed treatment experiments in 1987 and again in 1988 with a severely scabbed lot of Caldwell wheat (6,7). The test weight of this sample was only 734 kg m^{-3} (49 lb/bu). When seed was sown in soil in the greenhouse, emergence was only 57%, and several fungicides seed treatments improved emergence significantly (7). Emergence of untreated seed was somewhat greater in the field (69%), and none of the fungicide treatments improved emergence significantly. Likewise, fungicide seed treatments had no effect on the number of stems per unit area at the time of harvest, yield, or test weight. The same lot of scabby seed was used to repeat the experiment in 1988, in comparison with a more sound seed lot harvested in 1987 (6). None of the fungicide seed treatments improved stand establishment over the untreated control, nor was there any difference between the two seed lots. None of the fungicides improved yield or test weight over the untreated control.

Correlations between visible kernel characters, for example mold damage or scab, can be significant but may not be sufficiently high so as to allow reliable prediction of the amount of DON in a sample (15,20). In this study, we obtained a modest but significant correlation between DON and scab incidence ($r = 0.65$) and a smaller, but still significant, correlation between DON and kernel infection by *G. zeae* ($r = 0.44$). More chemical analysis and larger samples may have resulted in a better correlation. However, it is also likely that because isolates of *Gibberella zeae* vary in their ability to produce DON (19) and the amount of growth in a kernel varies in scabbed kernels, precision in prediction of DON content cannot be expected. Our studies suggest that there are thresholds above which chemical analysis for DON should be undertaken. For example, if the amount of scab in a sample was greater than 2% by weight, there was a good chance that the DON concentration was $\geq$ 2.0 ppm (Fig. 1A).

Of all of the characters measured, the amount of scab appears the most definitive and the most diagnostic for DON content, as is bright green yellow fluorescence for aflatoxin in corn (21). Test weight, although complicated by foliar and other diseases, could also be used as a criterion for DON analysis if it is confirmed that there are scabbed kernels in the low test weight sample. We believe that a test weight of less than 809 kg m^{-3} (54 lb/bu) or seed germination below 80% would warrant a DON analysis. Kernel infection by *G. zeae* was less predictive of DON content than test weight or scab damage and appears to be of limited use.

The preponderance of *G. zeae* in the grain samples we analyzed indicates that it was the major cause of scabbed wheat in 1986 in Indiana, as is indicated by other studies in North America (1,22). It seems unlikely that the other fusaria found in this study, some of which are more mycotoxigenic than *G. zeae*, grew sufficiently to produce these more potent toxins in soft red winter wheat grain, but an intensive analysis of scabbed samples for these more toxic mycotoxins may be warranted.

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