Occurrence of fusarium head blight and deoxynivalenol (vomitoxin) in two samples of Manitoba wheat in 1984

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Fusarium graminearum Schwabe was identified as the causal agent of fusarium head blight on Sinton hard red spring wheat and Coulter amber durum wheat grown on a farm south of Winnipeg in 1984. Deoxynivalenol (vomitoxin) was found in the grain at levels of 12.6 ppm in the hard red spring and 9.6 ppm in the amber durum. Other *Fusarium* mycotoxins (zearalenone, diacetoxyscirpenol, T-2 toxin and HT-2 were not present. It appears that a corn/wheat rotation and rains at anthesis favored the development of the disease.

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On a identifie *Fusarium graminearum* Schwabe comme la cause de la fusariose du blé rouge dur de printemps, Sinton et du ble dur ambré Coulter cultivés sur une ferme au sud de Winnipeg en 1984. On a trouvé 12.6 ppm de vomitoxine dans le blé dur rouge de printemps et 9.6 ppm dans le blé durum ambré. Zéaralénone, diacétoxycirpénol, T-2 et HT-2, d'autres mycotoxines fusariennes, Btaient absentes. Il semble que la rotation maïs/blé et de la pluie lors de l'anthèse ont favorisé le développement de la maladie.

Introduction

Fusarium graminearum Schwabe has previously been reported to occur on cereal grains in Manitoba at low levels (Gordon, 1952), being much more frequent in Eastern Canada. This species of *Fusarium* has been associated with seedling blight, stalk and cob rot of corn, and head blight of wheat, barely and oats. It is also a known producer of deoxynivalenol (DON vomitoxin) in the field. In 1980 this mycotoxin caused much concern when found in Eastern wheats (Trenholm *etal.*, 1981).

This is the first documented occurrence of *F. graminearom* caused fusarium head blight and DON production in the prairie provinces. A field of Sinton hard red spring wheat (*Triticum aestivum* L.) and one of Coulter amber durum wheat (*T. durum* Desf.) in southeastern Manitoba were affected. Some of the conditions leading to this occurrence are examined.

Methods

Subsamples of the harvested grains were obtained, surface sterilized in 0.3% sodium hypochlorite solution for one minute, air-dried under a laminar flow hood and plated onto potato dextrose agar (PDA) to isolate the pathogen(s). Incubation was for seven days at 22°C under a 12 hr. on/off cycle of fluorescent and long-wave UV lights. *Fusarium* species identification was done by single spore isolation onto PDA and carnation leaf agar to observe macro- and micro-morphology. Cultures were also sent to the Agriculture Canada Biosystematics Research Institutes, Ottawa, for confirmation of identity.

Subsamples of 50g were prepared for preliminary screening by thin-layer chromatography (TLC) for zearalenone and for several trichothecenes, viz., DON, diacetoxyscirpenol (DAS), T-2 toxin (T-2) and HT-2 toxin (HT-2), by the procedures of Scott *et al.* (1978) and Takitani *et al.* (1979). Further subsamples were prepared for gas chromatography/mass spectometry (CG/MS) by the procedures of Romer *et al.* (1978) and Scott *et al.* (1981). The final extracts were treated with heptafluorobutyryl-imidazole, and the heptafluorobutyrate (HFB) derivative mixture injected in *n*-hexane: benzene 9:1 containing 100Mg/M1 methoxychlor as an internal standard.

Aliquots of 2 μL were analyzed using a Hewlett-Packard 5985 B GC/MS system equipped for splitless capillary injection and negative-ion chemical ionization using methane (Rothberg *et al.* 1983). A 12-m silica capillary column coated with OV-101 was run with helium at 230°C. Mass spectra were obtained with ion source temperature of 100°C, and with 1.00cm 3/Min methane giving an ion source pressure of 10^{-4} Torr.

Data on field history was obtained from the grower's records. Weather data was obtained from the records at Environment Canada of five locations closest to the outbreak area.

Results and discussion

Subsamples of Sinton wheat had 68% of seeds infected with four species of *Fusarium. F. graminearum* comprised 90% of the isolates with *F. poae* (Peck) Wollenw., *F. sporotrichioides* Sherb, and *F. oxysporum* Schlecht.emend. Snyder and Hansen accounting for the remaining 10%. The Coulter amber durum had 53% of seeds infected by five species of *Fusarium. F. graminearum* accounted for 92% of the Fusaria, *F. sporotrichioides* for 4%. *F. poae* 2%. F. *oxysporum* 1% and *F. avenaceum* (Fr.) Sacc. 1%.

Initial TLC screening indicated the presence of high (1 ppm) levels of DON in both wheat samples. Although no other mycotoxins were found at this time, samples were re-assayed for the trichothecenes using GC/MS because of the high toxicity of some of these toxins and because of the moderate sensitivity of the spray reagents used.

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Table 1. Summary of agronomic management data of fields of fusarium head-blighted Sinton hard red spring and Coulter amber durum wheats in southeastern Manitoba in 1984.

	Sinton	Coulter
Seeding date	April 20	May 5
Type of seed	Registered seed	Certified seed
Seed source	previous year's crop	previous year's crop
Harvest date	August 15	August 27
Crop Rotation 1983:	Corn (entire field)	1983: Glenlea wheat 24.3 hectares; corn 16.2 hectares
1982:	Sugar beets (entire field)	1982: Corn 24.3 hectares; sugar beets 16.2 hectares
Seed Treatment	Unspecified fungicide for smut control	none
Tillage	Disced in fall, then again in the spring	
Fertilizer Autumn:	43.5 kg actual N/hectare of anhydrous ammonia	
Seeding:	32.6 kg actual N/hectare + 8.2 kg/hectare actual potash and phosphate	

Only DON tri-HFB and the methoxychlor internal standard were found at the characteristic retention times in the injected samples, examining the selected-ion chromatograms for the following characteristic masses (analyte, m/z): methoxychlor, 381; DAS HFB, 542; T-2 HFB, 542; DON tri-HFB, 670 and HT-2 di-HFB 8 16. The presence of DON as the tri-HFB derivative in both samples was confirmed by selected-ion monitoring and co-chromatography of the ions at m/z 884, 670, 630 and 458 characteristic of DON. This mycotoxin was present at 12.6 ppm and 9.6 ppm in the Sinton hard red spring and Coulter amber durum wheats, respectively.

Agronomic practices are given in Table 1. Of potential significance is the growing of corn on the affected fields within the past two years, because the presence of a corn-wheat rotation has been suggested as a main cause of fusarium head blight in Ontario (Teich and Nelson, 1984).

Present in the Sinton and Coulter wheats were shrivelled, chalky white kernels known as "tombstone" kernels. This kernel type constituted 14.7% of the Sinton and 6.4% of the Coulter by weight after combine harvesting. According to Simmonds (1968) this kernel does not develop beyond the early milk stage (1-2 weeks after anthesis) and probably becomes infected prior to this developmental stage. Bechtel et *al* (1985), based on structural studies, concluded that such shrivelled kernels had their development arrested two to three weeks after anthesis. An infection at anthesis could have progressed in two weeks to the level where seed development was seriously affected, producing "tombstone" kernels. Accordingly, both the Sinton and Coulter wheats were likely infected during their respective periods of anthesis, when wheat heads are most susceptible to *Fusarium* sp. (Pugh 1933, Andersen 1948, Sutton 1982) and which coincided with periods of recorded moisture.

Anthesis of the two wheats is estimated to have been about June 20 to 27 for the Sinton, and July 7 to the 14 for the Coulter (E. Czarnecki, personal communication).

During the estimated period of anthesis of the Sinton wheat, five nearby weather stations, Altona, Emerson, Greenridge, Morris 1 and Morris 2, recorded weekly totals of 24.4, 25.6, 27.3, 27.0 and 36.2 mm of precipitation, respectively. The total precipitation in June was 140 mm. During the estimated anthesis time of the Coulter amber durum wheat, the same weather stations recorded weekly totals of 37.0, 36.2, 33.6, 20.0, and 15.4 mm of precipitation. This one week period accounted for over half of the July rainfall, which totalled 44.5 mm. The mean maximum temperature for July of the three stations recording temperature was 26.6°C. High winds and 50 mm of rain were recorded by the grower during August 5-8, after which negligible rainfall occurred. The mean maximum temperature for August of the three stations was 28.4°C. Pugh *et al.* (1933) demonstrated warm temperatures increased the percentage of wheat head infections by *F. graminearum*, therefore the relatively high temperatures experienced in July and August likely aided the disease.

D. Leisle (personal communication) estimated the Sinton would have been at the early dough stage and the Coulter at the milk stage by August 5. The wheat heads would still have been susceptible to infection at this stage of development (Hart et *al.* 1984, Pugh et *al.* 1933) and a series of infections could have occurred up to and during this period as weather conditions permitted (Atanasoff, 1920). Hart et *al.* (1984), reported that production of DON was dependent on the hours of head wetness and not the stage of kernel development. Therefore DON production could also have occurred whenever conditions were suitable. One condition that may have an influence on DON production is temperature. Greenhalgh et *al.* (1983), reported *F. graminearum* grown on corn and rice at 19.5°C mainly produced zearalenone, while at 25°C both DON and zearalenone were formed, and at 28°C mainly DON.

Conclusion

Rains during anthesis combined with ready inoculum from crop debris of the previous two years resulted in fusarium head blight, and with it the production of "tombstone" kernels and of the mycotoxin DON. There is a possiblity that a series of infections occurred, up to and including the time the kernels were at the early dough (Sinton) or milk stage (Coulter).

Avoiding a corn-wheat rotation appears to be of prime importance, even in a region such as Manitoba where the risk of fusarium head blight is usually very low. Proper crop rotation is currently the most effective control measure known, and should ensure that fusarium head blight caused by *F. graminearum* continues to be generally infrequent in Manitoba.

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