Changes in the Distribution of Trichothecenes and Zearalenone in Maize with Gibberella Ear Rot During Storage at Cool Temperatures

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ABSTRACT

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Substantial levels of deoxynivalenol (3,799–4,139 ppb), 15-acetyldeoxynivalenol (746–818 ppb), and zearalenone (870–1,500 ppb) were detected in the severely rotted kernels from one side of ears of a commercial maize hybrid (DeKalb XL-12) that had been wound-inoculated 7 days after silk emergence with strain NRRL 13188 of *Gibberella zeae*. Storage of the remainder of the ears at 12 or 16 C for 10 wk produced no change in the concentration of deoxynivalenol recovered, whereas 15-acetyldeoxynivalenol disappeared and zearalenone increased 1.7- to 2.3-fold. At harvest, a trace of zearalenone was detected in samples of the sound kernels from ears showing typical symptoms of Gibberella ear rot; after storage, 64–132 ppb were detected. There was no evidence for expansion of the rotted areas or growth of fungal mycelium during the storage period.

Mycotoxicoses associated with red ear rot of maize (15), caused by *Gibberella zeae* (Schw.) Petch. (anamorph = *Fusarium graminearum* Schw.), occur in temperate regions of the world when: 1) above-average rainfall occurs at silking and ears become infected with *G. zeae* (8,17,18), 2) harvest is delayed or absent and infected maize is exposed to cool, wet conditions (4,7,17), or 3) infected ears are stored in open cribs or grain is left uncovered in piles and exposed to rainfall and low temperatures (3,4,11). Evidence supporting either of the latter scenarios is based on observations of red ear rot in the field, reports of mycotoxicoses, and the results of laboratory culture experiments. Zearalenone-induced mycotoxicoses (estrogenism) in swine have often resulted from ingesting ear corn that has overwintered in open cribs (12). However, the effect of cool storage temperature on levels of zearalenone and trichothecenes in diseased maize ears has not been reported.

This report describes the mycotoxin profiles in visibly rotted and sound kernels sampled from maize ears at harvest and after exposure to cool temperatures in a controlled-environment facility.

The mention of firm names or trade products does not imply endorsement or recommendation by the U.S. Department of Agriculture over other firms or similar products not mentioned.

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MATERIALS AND METHODS

A commercial loose-husked dent maize hybrid (DeKalb XL-12) was grown to maturity (96 days) in a controlled-environment facility (Biotron, University of Wisconsin) under conditions described by Caldwell and Tuite (5). The photoperiod was 14 hr, with temperatures at 30 ± 1 C (day) and 20 ± 1 C (night) and humidity at 82 ± 3%. Seven days after the silks emerged, ears were wound-inoculated with strain NRRL 13188 of *G. zeae*, using three infested toothpicks inserted equidistant 3–4 cm from the ear tip. This strain, which was isolated in 1975 in Ohio by R. W. Caldwell from maize with Gibberella ear rot, produced zearalenone and the trichothecenes deoxynivalenol (DON) and 15-acetyldeoxynivalenol (15-AcDON) in culture as well as Gibberella ear rot in preharvest maize grown in the Biotron (2).

At harvest, the average moisture content of the sound kernels sampled was 15–16%. The analytical methods of Bennett et al (2) were used to determine levels of trichothecenes and zearalenone for three distinguishable zones of 20 ears showing typical symptoms of Gibberella ear rot: zone I = severely rotted kernels, zone II = lightly to moderately rotted kernels, and zone III = sound kernels. Kernels removed from one side of each ear were analyzed for mycotoxins. Twenty ears were randomly divided into four groups (A, B, C, and D) of five ears each. Kernel samples from each group were pooled according to the ear zone from which they were removed and were analyzed for mycotoxins at harvest. The boundaries of each zone were marked with pins inserted between opposing rows of kernels. The ears were then suspended from the ceiling of a temperature cabinet and incubated for 10 wk at either 16 ± 1 C (groups A and B) or 12 ± 1 C (groups C and D), with 85 ± 5% relative humidity. After this period of incubation, the remaining kernels from each of the three distinguishable zones on the ear were removed separately and pooled as above and analyzed for mycotoxins. Results are reported as the average value of assays on two samples.

RESULTS AND DISCUSSION

The distribution of mycotoxins in the ears at harvest was similar to that reported by Bennett et al (2). Substantial levels of DON (3,799–4,139 ppb), 15-AcDON (746–818 ppb), and zearalenone (870–1,500 ppb) were detected in the severely rotted kernels (Table 1). Lightly to moderately damaged kernels had substantially less DON (243–384 ppb), 15-AcDON (23–29 ppb), and zearalenone (13–18 ppb). Samples of the sound kernels were free from trichothecenes, but zearalenone was detected in two samples (estimated 6 and 8 ppb). The distribution of zearalenone was similar to that reported for maize that had been inoculated with *G. zeae* and harvested from field plots (1,3,9) or an environmental chamber (2).

Postharvest cool-temperature incubation of the ears produced no significant changes in levels of DON but was linked to decreased levels of 15-AcDON and increased levels of zearalenone. Moreover, low levels of zearalenone (64–132 ppb) were detected in apparently sound kernels. There was no evidence for expansion of the rotted areas or growth of fungal mycelium during the storage period. The zearalenone in the sound kernels could have arisen in situ, produced by hyphae or by diffusion or translocation from other severely infected parts of the ear. The former is suggested by Bennett et al (2), who reported that 64% of sound kernels from wound-inoculated ears on plants grown in the Biotron were infected. The movement of zearalenone into kernels seems unlikely because at maturity a suberized black closing layer forms at the base of the kernel and prevents further movement of metabolites into it (16). Christensen and Kaufmann (6) observed that *Fusarium* spp. require a moisture

Table 1. Levels of zearalenone and the trichothecenes deoxynivalenol (DON) and 15-acetyldeoxynivalenol (15-AcDON) in maize with Gibberella ear rot at harvest and after postharvest incubation for 10 wk at 12 or 16 C

Zone[a]	Mycotoxin	Levels (ppb)[b]		
			Postharvest incubation (10 wk)	
		At harvest	16 C	12 C
I	DON	3,799	3,375	···
		4,139	···	3,420
	15-AcDON	818	98	···
		746	···	101
	Zearalenone	870	1,962	···
		1,500	···	2,538
II	DON	384	386	···
		243	···	203
	15-AcDON	29	0	···
		23	···	0
	Zearalenone	18	390	···
		13	···	176
III	DON	0	0	···
		0	···	0
	15-AcDON	0	0	···
		0	···	0
	Zearalenone	Trace	132	···
		Trace	···	64

[a]Defined by Bennett et al (2): zone I = severely rotted kernels (dull brownish red with no bright yellow aleurone layer), zone II = lightly to moderately rotted kernels (at least a portion of the bright yellow aleurone layer present), zone III = sound kernels (all bright yellow).
[b]Each value is the average of two composite samples from groups of five ears each.

content of at least 22–23% on a wet weight basis to grow in starchy cereal grains. Sherwood and Peberdy (14) detected nearly 13,000 ppb of zearalenone in autoclaved wheat grains (moisture content = 23–37%) inoculated with *G. zeae* and incubated at 12 C for 10 wk. In our research, the average moisture content of the sound kernels from individual ears ranged between 18 and 22% after postharvest cool-temperature incubation. This is at the lower limit permitting growth and mycotoxin formation by *G. zeae*.

Although there have been reports that trichothecene contamination of maize may occur if ears stored in exposed cribs or left to overwinter in the field are exposed to cool, rainy weather (10,19), there is no indication that levels of DON increase during such weather. Scott et al (13) reported a decline in DON from 1.56 μg/g on 7 July to 0.11 μg/g on 18 July in Ontario winter wheat as grain ripened before harvest (20–27 July). This was a period of seasonally warm temperatures for the region. Maximum production of DON by *F. graminearum* strain NRRL 5883 occurred at 30 C, whereas only small amounts of DON were produced at 15 and 20 C (20). In our test, levels of zearalenone in maize with Gibberella ear rot increased as a result of exposure to cool temperatures that prevented growth, but levels of the trichothecenes DON and 15-AcDON did not.

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