Production of Zearalenone and Deoxynivalenol in Commercial Sweet Corn

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ABSTRACT

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Deoxynivalenol (vomitoxin) was identified in 33 commercial sweet corn (*Zea mays*) hybrids inoculated with *Gibberella zeae*. The concentration ranged from 20 to 500 μg/g of infected plant tissue and increased in diseased tissue as disease severity ratings increased. Zearalenone (F-2 mycotoxin) was identified in only seven of the 29 inoculated hybrids, with the concentration ranging from 0.4 to 270 μg/g in infected tissue. All three isolates of *G. zeae* used in the inoculation tests produced deoxynivalenol, but only isolate W-8 produced zearalenone. Mold development in ears of inoculated sweet corn hybrids varied from less than 25 to more than 75% of the ear infected.

Additional key words: ear rot, *Fusarium roseum* f. sp. *graminearum*

Naturally occurring toxic metabolites of *Fusarium* sp. have been shown to affect the health of animals (1,2,4,6,8, 11–13,16,18). Zearalenone (F-2 mycotoxin) (15,17) and deoxynivalenol (vomitoxin) (18,19) are two mycotoxins commonly associated with cereal grain infected by *F. roseum* f. sp. *graminearum* (Schw.) Snyder & Hansen (teleomorph: *Gibberella zeae* (Schw.) Petch). Zearalenone in infected grain causes estrogenic mycotoxicoses that include enlargement of the uteri and mammary glands, vulvar swelling, testicular atrophy, and vaginal prolapse in affected animals (10,11,15). Deoxynivalenol is a cytotoxic trichothecene responsible for emesis and feed refusal in swine, hens, and rats (1–3,12,18,19).

Although neither zearalenone nor deoxynivalenol appears to be mutagenic or carcinogenic, Marasas et al (7) raised the possibility of a cocarcinogenic effect. The toxic effects of either mycotoxin on human health have not been determined, but zearalenone and deoxynivalenol have occurred in South Africa in raw and prepared food used for human consumption (5–9), and the incidence of both mycotoxins was high in areas of Transkei with high rates of esophageal cancer (7). The chronic effects of ingesting low amounts of zearalenone and deoxynivalenol over long periods are not known, nor has there been a report of possible additive or synergistic effects.

Research described in this paper has two objectives: to determine whether the popular sweet corn (*Zea mays* L.) hybrids vary in susceptibility to *G. zeae* and to determine whether the mycotoxins deoxynivalenol and zearalenone are produced in infected grain.

MATERIALS AND METHODS

Field plots consisted of two rows each of 33 commercial sweet corn hybrids (listed in Table 1), 25 plants per row, replicated four times. Ten ears were inoculated in each replicate by inserting toothpicks infested with *G. zeae* into the center of the ear through the husks. Controls were inoculated with noninfested toothpicks. The sweet corn hybrids were inoculated as the silks turned brown,

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between 75 and 84 days after planting.

Toothpicks used for inoculation were boiled four or five times in distilled water to remove any toxic substances, autoclaved in potato-dextrose broth, and stored for 2–3 days. Thirty to 50 toothpicks were placed in each petri plate (100 × 15 mm) on potato-dextrose agar and inoculated with a 4-mm plug from a 4-day-old culture of *G. zeae*. Toothpicks were crisscrossed to provide adequate aeration, and cultures were grown under a 12-hr cycle of dark and light (5,550 lux) at room temperature (23 ± 2 C) until heavy mycelial growth was evident (2–3 wk). Three isolates of *G. zeae* (B507, B601, and W-8) collected in Michigan were used. B507 and B601 were isolated from rotted stalks of corn and W-8 from an infected head of wheat. Each of the isolates produced fertile perithecia on toothpicks. Thirty-two varieties were inoculated with B507, 21 with B601, and 29 with W-8.

All of the hybrids were harvested 115 days after planting and immediately rated for disease severity. Disease ratings were made on a scale of 0–5 as indicated in the tables.

The harvested ears were air-dried on greenhouse benches. A modification of the method described by Pathre and Mirocha (14) was followed for the extraction of zearalenone and deoxynivalenol. Extractions were made only from infected portions of ears, and the infected grain from ears within a replicate was pooled to obtain a 50-g sample (dry wt) for analysis. The grain was extracted twice by being blended in a Waring Blendor for 5 min with 250 ml of methanol:water (60:40). The methanol was removed under reduced pressure in a rotary evaporator at 50 C. Thirty milliliters of a saturated sodium chloride solution was added to the remaining aqueous fraction and extracted three times with ethyl acetate (100 ml each time). The ethyl acetate fraction was passed through anhydrous sodium sulfate and the preparation evaporated to dryness as described before. The solids were dissolved in 1–2 ml of methanol and passed through an LH-20 column (1.5 × 45 cm) using 100% methanol as the solvent at a flow rate of 21 ml/hr. Deoxynivalenol was eluted in the 40–55 ml fraction and zearalenone in the 55–65 ml fraction. Fractions were concentrated and spotted on Whatman LHP-K TLC plates and developed in chloroform: methanol:water (90:10:2).

Zearalenone was visible under longwave and shortwave ultraviolet light. Deoxynivalenol was visible after being sprayed with 50% sulfuric acid in methanol and heated on a hot plate for 5 min. Concentrations were determined by comparison with standards of known concentration of a deoxynivalenol preparation (The Myco-Lab, Chesterfield, MO 63017) and a known standard of zearalenone preparation (Sigma Chemical Co., P.O. Box 14508, St. Louis, MO 63178). Absence of a spot at the R_f value of the standard specifies a concentration of zearalenone at less than 50 ng/g and deoxynivalenol at less than 100 ng/g. Confirmation of the presence of zearalenone and deoxynivalenol in randomly selected samples was made by selected ion monitoring of their TMS derivatives on a gas chromatograph/mass spectrometer (Finnigan model 3200) interfaced to a Riber SADR data acquisition and control system.

RESULTS AND DISCUSSION

Disease development in sweet corn hybrids varied considerably following their inoculation with *G. zeae* (Table 1). Disease ratings on individual ears within a hybrid ranged from 0 to 5, suggesting some degree of genetic heterogeneity within a variety. No variety was completely resistant to *G. zeae*, and only five of the hybrids had disease ratings <2.0 (11–25% of the ear moldy). These were Harris Seneca Star, Harris Golden Cup, Sun NK 199, Asgrow Commanche, and Asgrow Apache (Table 1). In preliminary studies, each of the isolates was pathogenic on Michigan corn hybrid 3102-B; deoxynivalenol was produced in infected grain by each isolate, but only W-8 produced zearalenone in detectable quantities (*unpublished data*). Disease development in these experiments may not correlate well with natural infection because the husks, a possible physical barrier to infection, were bypassed by the inoculation method used (large biomass inserted on toothpick).

Disease development was no more severe on late than on early maturing hybrids (Table 1). Mean disease ratings were 3.0, 3.4, and 3.8 for isolates B507,

Table 1. Disease ratings and concentrations of deoxynivalenol and zearalenone in 33 commercial sweet corn hybrids inoculated with *Gibberella zeae*

	Ears				
Hybrid	Maturity (days)	Disease rating[a]	Infected (%)	Deoxynivalenol (μg/g)[b]	Zearalenone (μg/g)[b]
Harris Seneca Star	69	1.7	100	160	···
Harris Bell Ringer	82	2.7	100	180	90
Harris Northern Bell	74	3.0	100	370	···
Harris Silver Queen	94	3.1	77	520	···
Harris Belle Gold	···	2.3	97	335	···
Harris Golden Gleam	90	3.6	100	150	0.4
Harris Gold Cup	82	1.9	93	120	45
Harris Seneca Scout	90	3.2	100	330	180
Sun Sugar Loaf	82	4.2	100	270	···
Sun NK 199	83	1.9	100	230	···
Sun Reliance	73	3.4	100	450	···
Sun Sugar Time	85	4.8	100	410	···
Sun Honeycomb	81	2.3	100	280	···
Sun Yukon	75	3.5	92	230	···
Sun Golden Nectar	87	3.1	100	375	···
Twilley Gold Crest	···	2.6	100	120	···
Asgrow XP 2548 W	86	4.2	100	400	···
Asgrow XP 2547 BC	70	4.4	100	215	···
Asgrow XP 2532	76	2.3	100	20	···
Asgrow XP 2527	80	2.6	100	110	180
Asgrow XP 2513 BC	88	4.9	83	370	···
Asgrow XP 2500	80	2.6	100	90	···
Asgrow Merit	80	3.1	100	135	···
Asgrow Guardian	79	2.4	97	320	0.4
Asgrow Commance	72	1.4	100	40	···
Asgrow Calico	77	3.8	100	320	···
Asgrow Aztec	68	4.1	100	300	···
Asgrow Apache	80	1.7	100	55	···
Asgrow Cherokee	79	2.5	88	270	270
Asgrow Commander	86	4.1	100	370	···
Ferry Morse Mellow Yellow	···	3.2	100	160	···
Ferry Morse Bonanza	86	4.8	100	240	···
Ferry Morse Style Pak	80	4.6	100	370	···

[a] Scale of 0-5 where 0 = no infection, 1 = less than 10% of the ear moldy, 2 = 11-25%, 3 = 26-50%, 4 = 51-75%, and 5 = 76-100%.
[b] Extractions were made only from infected grain. Concentrations were determined on Whatman LHP-K TLC plates by comparison with standards of known concentration.

Table 2. Disease ratings and average mycotoxin concentrations for three isolates of *Gibberella zeae* used to inoculate sweet corn hybrids

Isolate	Disease rating[a]	Deoxynivalenol[b] (μg/g)	Zearalenone[c] (μg/g)
W-8	3.4	126–344	0.4–270
B601	3.8	346–452	ND[d]
B507	3.0	165–267	ND

[a] Scale 0–5 where 0 = no infection, 1 = less than 10% of the ear moldy, 2 = 11–25%, 3 = 26–50%, 4 = 51–75%, and 5 = 76–100%.
[b] Per gram of infected grain.
[c] Found in only seven hybrids of 29 inoculated.
[d] Not detected.

W-8, and B601, respectively (Table 2).

Deoxynivalenol was produced consistently in each of the inoculated varieties and generally at high levels (Table 1). Increased concentrations of deoxynivalenol were positively correlated with increased disease ratings. At a disease rating of 0–1, the concentration was zero; 1–2, 100–140 μg/g of infected grain; 2–3, 175–245 μg/g; 3–4, 276–331 μg/g; and 4–5, 300–354 μg/g. The production of deoxynivalenol in all of the sweet corn varieties and by each of the isolates of *G. zeae* suggests that deoxynivalenol is a common metabolite of *G. zeae*, even though the toxin is difficult to isolate in quantity from cultures grown in a defined medium (14). Cultures of *G. zeae* grown on autoclaved corn, rice (*Oryza sativa* L.), or small grains generally yield deoxynivalenol and sometimes zearalenone (14).

Zearalenone was detected only in ears inoculated with *G. zeae* isolate W-8 and in only seven of the 29 hybrids inoculated. The concentration of zearalenone varied from less than 1 μg/g of grain in Asgrow Guardian and Harris Golden Gleam to 180 μg/g and greater in Harris Seneca Scout, Asgrow Cherokee, and XP2527 (Table 1). Zearalenone was produced only in hybrids that matured in 79 days or more, but not in all late-maturing hybrids.

Because zearalenone was produced in varying amounts in only a few hybrids, the genetic makeup of the host may significantly influence the production of this mycotoxin. Under normal production practices, a significant amount of the pollination would result from intraspecific crosses. In these experiments, the proximity of the varieties could have led to extensive interspecific crosses resulting in increased genetic heterogeneity within a variety.

The data presented here suggest that potential additive or synergistic effects between zearalenone and deoxynivalenol could be avoided by selecting varieties that support only the production of deoxynivalenol. It is not known whether a similar condition prevails in field corn or other feeds intended for animals or in food products such as wheat (*Triticum aestivum* L.) that are intended for human consumption.

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