Zearalenone Production in Field Corn in Indiana

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ABSTRACT

A study was made to determine if zearalenone (RAL) was produced by isolates of *Fusarium roseum*, including cultivars Culmorum and Graminearum, in dent corn in the field in amounts sufficient to cause estrogenic upset in swine (minimal levels 5-10 ppm). Ears were inoculated at silk with six isolates that produced 50 to 1,100 ppm of RAL in autoclaved corn in laboratory storage. Production in the field was absent or ranged up to 2% of that produced in vitro, and amounts never exceeded 5 ppm and almost always were less. Eight isolates of *F. tricinctum* and 12 *F. roseum* isolates were used to inoculate ears of dent corn. *Fusarium tricinctum* and cultivars Equiseti, Avenaceum, and Gibbosum of *F. roseum* were not pathogenic. Ears invaded by Graminearum produced less than 5 ppm of RAL. Ten samples of corn collected from commercial fields in Indiana in 1965 infected with *Gibberella zeae* yielded no more than 0.1 ppm of RAL. Thus, estrogenic upset in swine in Indiana caused by RAL is usually a result of growth by *F. roseum* 'Graminearum' (*G. zeae*) in storage, and not in developing ears. Phytopathology 60:1696-1697.

An estrogenic syndrome is elicited in swine consuming *Gibberella zeae*-infected corn (2, 6, 7) that contains zearalenone (F-2), a resorcylic acid lactone (8).

Estrogenic disturbances usually occur in the spring and early summer and seem to be associated with the feeding of moldy corn from the previous year's crop (2, 4, 5) and not with freshly harvested corn. In 1965, 10 samples of corn from Indiana, 10-100% naturally infected with *G. zeae* in the field, were assayed for zearalenone by the procedure of Caldwell et al. (1). A level of 0.1 ppm, based on 12% moisture corn (wet wt basis), was detected in two samples; the other eight were negative.

To further assess production of zearalenone in the field in Indiana, field inoculations were carried out in 1967. Dent corn, K61 XPrl, was planted 2 weeks apart to permit different ripening periods. Ears were inoculated at 90% full silk with six isolates of *Fusarium roseum* (Lk.) emend. Snyd. & Hans. (included were four isolates able to produce the sexual stage, *G. zeae*) by inserting a toothpick invaded by an isolate into the husk at the tip of the ear. When these same isolates were grown in the laboratory on autoclaved corn kernels for 2 weeks at 24 C followed by 8 weeks at 12 C (6), the following levels of zearalenone in ppm were produced: FR 4 (100), FR 22 (300), FR 25 (50), FR 26 (700), FR 29 (1,100), and FR 31 (300). Seventy-six ears were inoculated with each of the six isolates. The infected corn was harvested the 1st week in November, about 100 days after inoculation. Temperatures during this postinoculation period averaged 16.1 C, 2.3 C below average. Rainfall was 7.8 inches, 0.18 inch below average. The ears were visually rated for disease: 0 = no infection; 1 = 1-25% infection; 2 = 26-50% infection; 3 = 51-75% infection; and 4 = 76-100% infection (Table 1). Samples were shelled from the tip of the ears where infection was heaviest and assayed for zearalenone.

Isolate FR 4 from turf was weakly pathogenic and produced no zearalenone (Table 1). The Culmorum and four Graminearum cultivars of *F. roseum* isolated from grain varied in disease indices, but all produced from 0.2-5.0 ppm of zearalenone (Table 1). The field production was 0.1 to 2% of that produced in the laboratory, and did not usually reach the levels (5-10 ppm) required to incite an estrogenic response in swine (Martin Stob, *personal communication*).

In a second study in 1967, the pathogenicity of eight *F. tricinctum* (Cda.) emend. Snyd. & Hans. and 12 *F. roseum* cultures with varying abilities to produce zearalenone and isolated from a wide variety of substrates was evaluated. A single-cross hybrid, Wf9 × 38-11, was inoculated with toothpicks invaded by the isolates, and after 100 days the ears were rated for disease (Table 2). Only the three *F. roseum* 'Graminearum' cultivars, FR 27, 28, and 33, originating from barley, corn, and wheat, respectively, visibly rotted the ears. All eight *F. tricinctum* isolates were nonpathogenic, including an isolate that could produce zearalenone (1). Ears inoculated with those isolates that produced zearalenone when grown on autoclaved corn kernels, FT 3, FR 3, 10, 21, 23, 27, 28, and 33, were assayed for zearalenone. Only the pathogenic isolates, *F. roseum* 'Graminearum', FR 27, 28, and 33, produced any zearalenone in the developing ears, and the amounts were low, 0.5, 1.1, and 4.0 ppm, respec-

TABLE 1. Pathogenicity and zearalenone production by *Fusarium roseum* isolates

Isolate	Scientific name	Disease index[a]	Zearalenone (ppm)[b]
FR 4 E[c]	*F. roseum*[e]	0.1	0
FR 4 L[d]		0.6	0
FR 22 E	*F. roseum*	0.8	0.5
FR 22 L	'Culmorum'	0.9	3.5
FR 25 E	*F. roseum*	2.2	1.0
FR 25 L	'Graminearum'	2.0	0.2
FR 26 E	*F. roseum*	2.3	3.0
FR 26 L	'Graminearum'	2.8	2.0
FR 29 E	*F. roseum*	0.2	0.2
FR 29 L	'Graminearum'	0.6	5.0
FR 31 E	*F. roseum*	1.0	0.2
FR 31 L	'Graminearum'	1.0	1.0

[a] Per cent of corn ear visibly infected: 0 = no infection; 1 = 1-25% infection; 2 = 26-50% infection; 3 = 51-75% infection; 4 = 76-100% infection.

[b] Average of two replications; ppm based on 12% moisture corn (wet wt basis).

[c] Early-planted corn.

[d] Late-planted corn.

[e] Obtained from H. B. Couch, who isolated it from turf.

TABLE 2. Pathogenicity and host or habitat of *Fusarium tricinctum* and *F. roseum* isolates

Isolate	Scientific name	Host or habitat	Disease index[a]
FT 1	*F. tricinctum*	Soil	0
FT 3[b]	*F. tricinctum*	Bluegrass	0
FT 5	*F. tricinctum*	Bluegrass	0
FT 6	*F. tricinctum*	Soybean seed	0
FT 15	*F. tricinctum*	Red clover	0
FT 16	*F. tricinctum*	Corn kernels	0
FT 17	*F. tricinctum*	Carnation	0
FT 18	*F. tricinctum*	Grass hay	0
FR 3[b]	*F. roseum*	Turfgrass	0
FR 9	*F. roseum*	Bluegrass	0
FR 10[b]	*F. roseum*	Squash fruit	0
FR 11	*F. roseum*	Carnation	0
FR 16	*F. roseum* 'Equiseti'	Soil	0
FR 20	*F. roseum* 'Equiseti'	Soybean seed	0
FR 21[b]	*F. roseum* 'Gibbosum'	Wheat	0
FR 23[b]	*F. roseum* 'Culmorum'	Wheat	0
FR 24	*F. roseum* 'Avenaceum'	Wheat	0
FR 27[b]	*F. roseum* 'Graminearum'	Barley	1.8
FR 28[b]	*F. roseum* 'Graminearum'	Corn	1.9
FR 33[b]	*F. roseum* 'Graminearum'	Wheat root	2.2

[a] Per cent of corn ear visibly infected: 0 = no infection; 1 = 1-25% infection; 2 = 26-50% infection; 3 = 51-75% infection; 4 = 76-100% infection.

[b] Positive for zearalenone production when grown on moist autoclaved corn kernels for 3 weeks at 16 C.

tively. Therefore, zearalenone was produced in the field only by pathogenic isolates of *F. roseum* and in amounts that probably are insufficient to incite an estrogenic response. In addition, since the fungus, as it invades developing ears, often produces a substance (s) that causes swine to refuse to eat infected grain (3), it is not likely that significant amounts of zearalenone would be ingested.

LITERATURE CITED

1. CALDWELL, R. W., J. TUITE, M. STOB, & R. S. BALDWIN. 1970. Zearalenone production among Fusarium species. Appl. Microbiol. 20:31-34.
2. CHRISTENSEN, C. M., G. H. NELSON, & C. J. MIROCHA. 1965. Effect on the white rat uterus of a toxic substance isolated from Fusarium. Appl. Microbiol. 13: 653-659.
3. CURTIN, T. M., & J. TUITE. 1966. Emesis and refusal of feed in swine associated with Gibberella zeae infected corn. Life Sci. 5:1937-1944.
4. KOEN, J. S., & H. C. SMITH. 1945. An unusual case of genital involvement in swine associated with eating moldy corn. Vet. Med. 40:131-133.
5. MCNUTT, S. H., P. PURWIN, & C. MURRAY. 1928. Vulvovaginitis in swine. J. Amer. Vet. Med. Ass. 73:484-492.
6. MIROCHA, C. J., C. M. CHRISTENSEN, & G. H. NELSON. 1967. Estrogenic metabolite produced by Fusarium graminearum in stored corn. Appl. Microbiol. 15: 497-503.
7. STOB, M., R. S. BALDWIN, J. TUITE, F. N. ANDREWS, & K. G. GILLETTE. 1962. Isolation of an anabolic uterotrophic compound from corn infected with Gibberella zeae. Nature 196:1318.
8. URRY, W. H., H. L. WEHRMEISTER, E. B. HODGE, & P. H. HIDY. 1966. The structure of zearalenone. Tetrahedron Letters 27:3109-3114.