Susceptibility of Maize to *Gibberella zeae* Ear Rot: Relationship to Host Genotype, Pathogen Virulence, and Zearalenone Contamination

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ABSTRACT

Cullen, D., Caldwell, R. W., and Smalley, E. B. 1983. Susceptibility of maize to *Gibberella zeae* ear rot: Relationship to host genotype, pathogen virulence, and zearalenone contamination. Plant Disease 67:89-91.

Susceptibility to *Gibberella zeae* ear rot differed significantly between certain inoculated corn (*Zea mays*) inbreds (A619, A632, A634, and Mo17) and single-cross hybrids (A619 × A632 and Mo17 × A634). Significant differences ($P = 0.05$) in the pathogenicity of two *G. zeae* isolates were also observed. Zearalenone concentrations in infected ears varied according to corn line and fungus isolate. The less virulent isolate produced relatively more zearalenone in culture and in diseased ears. Estimates of disease severity (percent ear rot) were good predictors of zearalenone contamination and may be useful in selecting desirable corn lines.

Zearalenone, an estrogenic mycotoxin, is commonly associated with corn (*Zea mays* L.) molded by *Gibberella zeae* (Schw.) Petch (anamorph *Fusarium graminearum* Schwabe) (1,2,9). Reports of zearalenone-induced hyperestrogenism, particularly in swine, are well documented (9,11,13). Contamination may occur before harvest and/or in storage (1,3,4,11,13).

Accepted for publication 17 June 1982.

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0191-2917/83/01008903/$03.00/0

In laboratory studies using autoclaved cereal substrates, zearalenone production is favored by high kernel-moisture content (45%) and low temperatures (12–14 C) (1,2,5,10). Production in culture varies considerably between isolates of *G. zeae*, but it is unclear whether such variability is expressed in the field (1,5,9).

Caldwell and Tuite (3) in 1970 in Indiana reported that, although corn ears inoculated with *G. zeae* developed detectable levels of zearalenone (5 μg/g), these levels were insufficient to cause hyperestrogenism. The influence of specific corn genotypes on zearalenone production and the relationship between observable disease severity and resultant zearalenone content have not been investigated.

We report the results of field trials in which the accuracy of disease severity ratings as predictors of zearalenone contamination and the effect of host genotype on disease development and zearalenone production were tested.

MATERIALS AND METHODS

Using the toothpick-inoculation method (14), field trials were conducted at the university farms at Hancock, WI, during the 1979 growing season. Six different corn entries, two single-cross hybrids (Mo17 × A634 and A619 × A632), and four inbreds (A619, A632, A634, and Mo17) represented the subplots of a split-plot design (7). Main plots consisted of inoculations with two *G. zeae* isolates that produced either high (42 mg/L) or low (1.3 mg/L) levels of zearalenone in Hidy's fermentation medium (6). Each

isolate-variety combination was replicated 30 times in each of four blocks (120 replicates). Ears were inoculated 10 days following 90% silking by inserting mycelium-infiltrated toothpicks into ears as described by Young (14). After about 3 mo, husks were removed and the extent of visible fungal colonization relative to total ear length (percent ear rot) was estimated. Harvested ears were shelled, dried to 15% moisture content, and stored at −20 C.

For analysis of zearalenone concentration, the entire shelled sample from each subplot was thoroughly mixed and a 300-g subsample was ground in a Wiley mill. Duplicate 50-g samples of ground meal were extracted with ethyl acetate and purified by acetonitrile:petroleum ether partitions (8). Purified extracts were applied to silica gel G thin-layer plates and developed with toluene:ethyl acetate:formic acid (5:4:1, v/v) in unlined tanks (2). Zearalenone was quantified by comparison with pure external standards under long (366 nm) and short (254 nm) wavelength ultraviolet light.

Angular transformations of percent ear rot data were used for analysis of variance. Duncan's least significant differences were calculated to allow mean comparisons between corn lines inoculated with the same or different isolates (7).

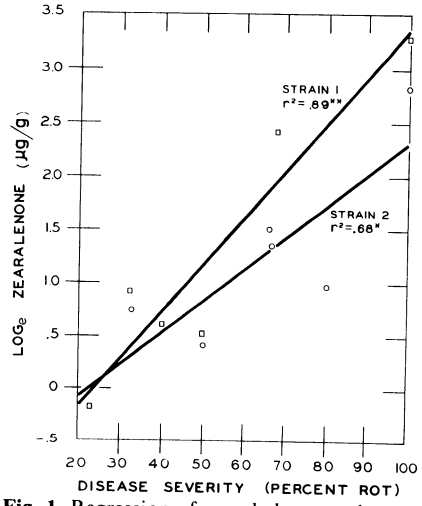

Fig. 1. Regression of sample log-zearalenone content on disease severity (percent ear rot). Each data point represents mean zearalenone content for a single corn line inoculated with one of two *Gibberella zeae* isolates: isolate 1, □; isolate 2, O. Details of sampling and analytical procedures are described in Materials and Methods. For isolate 1, $Y = 0.044X - 1.04$, $r^2 = 0.89$; isolate 2, $Y = 0.029X - 0.625$, $r^2 = 0.68$, where Y = log-zearalenone content and X = percent ear rot. F ratio significance for regression analysis of variance; $*P = 0.05$, $**P = 0.01$.

RESULTS AND DISCUSSION

The severity of Gibberella ear rot differed significantly among corn entries tested (Table 1). Inbreds were consistently more susceptible than hybrids. Disease severity ranged from 23% (A619 × A632) to nearly 100% (Mo17) of ear length rotted.

The low zearalenone-producing isolate of *G. zeae* (strain 2) produced more disease (overall mean of 66% ear rot) than the higher zearalenone producer, isolate 1 (overall mean of 52% ear rot). In hybrid Mo17 × A632 and inbreds A632 and A634, the difference in disease severity between isolates was significant ($P = 0.05$) (Table 1).

Levels of zearalenone per gram of ground sample ranged from 0.9 to 2.1 µg/g in the hybrids and from 1.7 to 26.5 µg/g in the inbreds (Table 1). The low levels of zearalenone detected in the hybrids corroborates Caldwell and Tuite's report (3) that inoculated ears of a single-cross hybrid contained less than 5 µg/g zearalenone. Feed containing 1–5 µg/g of zearalenone per gram is reported to be estrogenic to swine (9); however, such feed may be rejected because of a refusal compound(s) produced concomitantly with zearalenone by *G. zeae* on maturing corn ears (13).

Zearalenone content of isolate 1 and isolate 2 molded samples averaged 7.4 and 5.1 µg/g, respectively. For inbreds A619 and Mo17, the difference between isolates was significant ($P = 0.05$) (Table 1). Higher zearalenone production by isolate 1 was also observed in culture, ie, 42 versus 1.3 mg/L. These findings demonstrate that differences among isolates in their ability to produce zearalenone in culture are expressed in the field.

Isolate difference in pathogenicity and toxin production may play an important role in the natural occurrence of zearalenone toxicoses. The sporadic nature of estrogenic outbreaks (9,11,13) may be due, in part, to the occasional effects of virulent, high-producing isolates of *G. zeae*.

Estimates of disease severity correlated well with zearalenone content. Regressions of log-zearalenone concentration on disease severity (percent ear rot) gave significant coefficients of determination (r^2) (Fig. 1). The regression equation for isolate 1 molded samples was $Y = 0.044X - 1.04$, $r^2 = 0.89$, where Y is predicted log-zearalenone content and X is disease severity (percent ear rot). The standard deviation of Y, Y_s, about the regression line was 0.486. The equation for isolate 2 was $Y = 0.029X - 0.625$, $r^2 = 0.68$, $Y_s = 0.513$. The F ratios for equations describing isolates 1 and 2 were 32.0 (degrees of freedom [df] = 1,4) and 8.32 (df = 1,4), respectively. Regression line Y intercepts were not significantly different at the 5% level (F = 2.62; df = 1,9) (12). Slopes of the lines differed significantly ($P = 0.05$) (F = 9.32; df = 1,8). Because zearalenone is physiologically active at 1–5 µg/g (Y = 0.0–1.6), ears that are more than 25% rotted may induce hyperestrogenism in swine.

The general applicability of these regressions would require confirmation in additional growing areas and seasons, although visual estimation of disease severity should suffice in selecting desirable corn lines.

Table 1. Disease severity and zearalenone content of corn lines inoculated with *Gibberella zeae*

Corn line	Isolate 1		Isolate 2	
	DS[y]	Zearalenone[z]	DS	Zearalenone
A619 × A632	28.6 a	0.9 A	35.2 abc	2.1 A
A634 × Mo17	34.6 ab	2.1 A	45.3 d	1.5 A
A632	39.4 bcd	1.8 A	54.8 e	3.6 A
A634	45.3 cd	1.7 A	62.4 e	2.7 A
A619	56.0 e	10.9 B	54.6 e	3.0 A
Mo17	90.0 f	26.5 C	90.0 f	14.8 B

[y] Disease severity (DS) presented as angular transformations of percent ear rot. Means followed by the same letter within or between columns are not significantly different ($P = 0.05$).
[z] Zearalenone content of field samples expressed in µg/g. Means followed by the same capital letter within or between columns are not significantly different ($P = 0.05$).

ACKNOWLEDGMENTS

This work was supported by the College of Agricultural and Life Sciences, University of Wisconsin, Madison. Project No. 5110. We thank Steve Vicen for the preparation of graphics, and Cindy Morris for assistance with statistical analyses.

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