Correlation of Kernel (E)-Ferulic Acid Content of Maize with Resistance to *Fusarium graminearum*

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ABSTRACT

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The relationship between (E)-ferulic acid content in kernels of 19 inbreds of maize (*Zea mays*), chosen to represent a taxonomic and latitudinal series showing variation, and resistance to infection by *Fusarium graminearum* was investigated. A negative, significant correlation (1988: $r = -0.68$, $P = 0.002$, and $n = 57$; 1991: $r = -0.71$, $P = 0.002$, and $n = 57$) was found between the amount of ear rot observed in field trials and the amount of (E)-ferulic acid detected in kernels by high-pressure liquid chromatography (HPLC) analysis. Furthermore, fungal growth in vitro was greatly decreased when pure (E)-ferulic acid was added to artificial media; the effective concentration for 50% inhibition of growth (EC_{50}) was 0.65 mg/g. Based on the results of the present study, conventional breeding programs aimed at attaining maize germ plasm resistant to *F. graminearum* should incorporate genotypes of maize containing high concentrations of kernel (E)-ferulic acid.

Fusarium graminearum Schwabe (sexual stage: *Gibberella zeae* (Schwein.) Petch) is the causal organism of a sporadically occurring ear rot of corn (*Zea mays* L.) in cool and humid areas of the world (31). The economic significance of this disease is due to the fact that infected grain may be contaminated with mycotoxins (26). Consumption of contaminated grain by swine can lead to complex reproductive and digestive disorders (26).

Host-plant resistance to *F. graminearum*, and breeding for increased resistance, has been suggested as a feasible means of disease control (16). Phenolic compounds are widespread throughout the plant kingdom, and they or their oxidation products have been implicated in disease resistance (11,13,19,22). Phenolic acids also have been shown to inhibit the in vitro growth and reproduction of a wide array of fungal genera (6,17,20), including *F. oxysporum* Schlechtend.:Fr. f. sp. *radicis-lycopersici* W.R. Jarvis & Shoemaker (18), *F. o.* Schlechtend.:Fr. f. sp. *lycopersici* (Sacc.) W.C. Snyder & H.N. Hans. (17), and *F. moniliforme* J. Sheld. (14).

In previous studies by our research group, we suggested a role for flavonoid metabolism in maize-silk resistance to *F. graminearum* (27). The current study examined the possibility of a phenolic acid-based resistance mechanism in maize kernels. The specific objectives of this study were 1) to determine whether a varietal correlation exists between susceptibility of maize artificially inoculated with *F. graminearum* and levels of (E)-ferulic acid in kernels and 2) to determine the ability of (E)-ferulic acid to inhibit the growth of *F. graminearum* in vitro.

MATERIALS AND METHODS

Fungal cultures. A highly aggressive strain of *F. graminearum*, ARC2, isolated from infected maize in 1988 by A. Bolton, Agriculture Canada, Ottawa, was used in these studies. The culture was maintained on a potato-sucrose agar (PSA; pH 5.4) (3) at 21 C with a 16:8 (light/dark) photoperiod (light intensity at plate level = 2 W/m^2).

Spore suspensions were prepared by spreading sterile distilled water onto 14-day-old cultures of *F. graminearum*. Sterile glass beads were added and agitated to dislodge the spores. The resulting suspension was passed through eight layers of cheesecloth, and the concentration of spores was adjusted to 10^6 spores per milliliter.

Plant material. Nineteen inbreds of maize, chosen to represent a taxonomic and latitudinal series showing variation in resistance to *F. graminearum*, were used in this study. These inbreds were developed from the germ plasm bank at the International Maize and Wheat Improvement Center (CIMMYT), located in El Batan, Mexico (5) (Table 1). This material comprised four broad-based latitudinal gene pools developed by CIMMYT for use in different latitudinal regions. The CIMMYT German maize gene pool (NTR-2) consisted of germ plasm from Mexico, Peru, Bolivia, Pakistan, China, Hungary, and the United States. It was adapted in the temperate regions in latitudes 46–52° N-S of the equator. The gene pool for the northern temperate region gene pool (NTR-1) consisted of materials originating in the U.S. corn belt and was adapted in latitudes 46–52° N-S of the equator. The gene pool for the intermediate temperate region gene pool (ITR) consisted of European maize material and was adapted in latitudes 40–46° N-S of the equator. The gene pool for the southern temperate region gene pool (STR) consisted of germ plasm from the U.S. corn belt and tropical lowlands and highlands and was adapted in latitudes 34–40° N-S of the equator.

In addition to the latitudinal gene pools, three populations of distinct indigenous races of maize from Mexico, two land races from Argentina, and three CIMMYT maize pools were included (Table 1).

Determination of phenolic acid content. The kernel phenolic acid content of the 19 inbreds was determined separately for 1988 and 1991 field trials. At harvest, all visibly noninfected control ears of each inbred within the same row were placed in paper bags and air-dried for 8 wk. Ears from the same row were hulled, and three samples, each consisting of 20 randomly selected kernels, were ground with a Wiley mill equipped with a 1-mm mesh screen. This procedure was repeated for each of the three replicates of the 19 inbreds. Therefore, the kernel phenolic acid content of each inbred replicate represented the mean of three individual phenolic acid-content determinations.

Kernel phenolic acid content was determined by a 4 h base hydrolysis (30). For each analysis, 1 g of maize flour was hydrolyzed in 35 ml of 2 N sodium hydroxide in an Erlenmeyer flask closed with a rubber septum and was purged with nitrogen. The flask was placed on a shaker for 4 h and subsequently acidified to pH 2.0 with 6 N hydrochloric acid. The slurry was centrifuged

at 750 *g* for 10 min, and the supernatant was decanted into a separatory funnel. The pellet was rinsed with 2 × 20 ml of dH_2O and centrifuged at 750 *g* for 10 min after each wash. All supernatants were combined and extracted with 4 × 50 ml of high-pressure liquid chromatography (HPLC)-grade ethyl acetate. The pooled organic layers were combined and extracted with 4 × 50 ml of dH_2O. The pooled organic layers from the final extractions, including any emulsion that formed between the organic and aqueous layers, were collected and dehydrated over sodium sulphate (anhydrous). The resultant extract was roto-evaporated to nearly complete dryness in vacuo at 35 C. Final evaporation was carried out under a stream of nitrogen to prevent oxidation and degradation of the samples.

A Beckman HPLC equipped with a model 165 variable wavelength detector, a model 110A solvent metering pump system, and a model 420 system controller programmer (Beckman, Fullerton, CA) was used to quantitate the phenolic acids found in the extracts. Twenty microliters was injected into the system, and the phenolics were quantified with a C-18 reverse-phase ultrasphere 5-μm ODS 4.6-mm × 25-cm column, an isocratic elution consisting of 80% 20 mM citrate buffer (pH 5.4)/20% methanol, at a flow rate of 1 ml/min and detection wavelength = 280 nm, and an online UV scan from 240 to 400 nm of eluted peaks.

***F. graminearum* ear rot field trials.** Field evaluations of resistance to ear rot were conducted in 1988 and 1991 at the Plant Research Centre, Agriculture Canada, Ottawa, Ontario. The 19 inbreds were planted in a randomized complete block design with three blocks. Each block consisted of 38 single-row plots, each containing 10 plants, to which the maize inbreds and inoculation treatments (inoculation with sterile distilled water or inoculation with a spore suspension) were randomly assigned.

The date on which 50% of the ears from an inbred had silks protruding at least 1 cm was defined as the midsilk date for the inbred. At 7–10 days post-midsilk, all ears of each plant were inoculated with 1 ml of a 10^6 spores per milliliter conidial suspension of *F. graminearum* sprayed directly onto the silk. Control ears were sprayed with 1 ml of sterile distilled water. Ears were covered with a clear polyethylene bag and then a brown paper bag. Both bags were stapled around the stalk of the plant to hold them in place. A small hole was pierced in the polyethylene bag to promote drainage of collecting water from the bag (9). The purpose of the polyethylene bag was to promote humid conditions favorable to fungal infection and growth. The purpose of the paper bag was to reduce overheating due to the greenhouse effect within the polyethylene bag (9).

Ears were harvested 5 wk after inoculation, on the assumption that sufficient time had elapsed to allow adequate incubation of the fungus (9). Percent ear rot was calculated by dividing the total number of kernels that were visibly rotted by the total number of kernels on the ear. When an area of the ear was damaged to the point that separate kernels could not be distinguished, the number of damaged kernels was estimated. The average amount of ear rot was obtained from 10 ears in each block.

Manipulation of phenolic levels in fungal growth media. The ability of (E)-ferulic acid to inhibit the in vitro growth of *F. graminearum* was tested by adding various concentrations of (E)-ferulic acid to fungal growth media. The effect of six concentrations of (E)-ferulic acid (0, 0.1, 0.2, 0.4, 0.8, and 1.6 mg/g), 10 replicates at each concentration, was examined by measuring mycelial radial growth. (E)-Ferulic acid was dissolved in 50% ethanol and added to autoclaved PSA. For controls, the corresponding amount of 50% ethanol was added to agar media. Plugs of inoculum (area = 0.8 cm^2) were sectioned from the edges of 14-day-old cultures of *F. graminearum* with a cork borer and were inverted onto the center of the petri plates. Petri plates were incubated at 21 C with a 16:8 (light/dark) photoperiod (light intensity at plate level = 2 W/m^2). The diameter of the resultant fungal colonies was measured after a period of 6 days.

Statistical analysis. Statistical analysis was performed with Statistical Analysis System software (SAS Institute Inc., Cary, NC).

TABLE 1. *Fusarium graminearum* ear rot and kernel (E)-ferulic acid content of 19 maize (*Zea mays* L.) inbreds

Genotype	Amount of seed (E)-ferulic acid (mg/g)[x]	Amount of *Fusarium* ear rot (%)[y]
Northern temperate region		
NTR-1 3947	1.80 a[z]	12.23 h–j
NTR-1 3962	1.39 fg	44.33 a–c
NTR-2 Canada 4071	1.38 fg	48.15 ab
NTR-2 Germany 4042	1.61 bc	18.75 g–i
NTR-2 Holland 4022	1.43 d–g	47.82 ab
NTR-2 Poland 4064	1.55 c–e	36.32 a–e
NTR-2 Switzerland 4034	1.63 bc	21.60 e–i
Intermediate temperate region		
ITR 3862	1.41 e–g	35.90 a–e
ITR 3878	1.50 c–g	44.25 a–c
Southern temperate region		
STR 3802	1.60 bc	19.37 f–i
STR 3823	1.37 g	33.80 b–g
Argentine landraces		
Cateto C 2030	1.55 b–e	28.03 d–g
Cateto E 2051	1.52 c–f	27.07 d–h
CIMMYT maize pools		
POOL 27	1.37 g	51.40 a
POOL 28	1.58 b–d	34.85 b–f
POOL 30	1.54 b–d	39.17 a–d
Mexican landraces		
Mexico-5	1.64 bc	28.78 c–g
Oaxaca-179	1.56 b–e	3.17 j
Puebla-463	1.67 ab	6.20 ij

[x] Amount of seed (E)-ferulic acid is a mean of nine replicates from 1988 field trials and nine replicates from 1991 field trials.

[y] Amount of *Fusarium* ear rot is a mean of 30 replicates from 1988 field trials and 30 replicates from 1991 field trials.

[z] Means in the same column followed by the same letter indicate no significant difference according to Tukey's studentized range test ($P = 0.05$).

RESULTS

Determination of phenolic acid content. Analysis of maize kernel extracts revealed peaks with relative retention times corresponding to (E)-ferulic acid, (Z)-ferulic acid, (E)-*p*-coumaric acid, and (Z)-*p*-coumaric acid detected in all cultivars of maize. The UV spectra of the peaks corresponded to those of the standard compound. Identity of the phenolic acids was confirmed by GC-MS (gas chromatography-mass spectrometry) of extracts. There was an approximate 3 and 5% conversion of (E)- to (Z)-isomers of ferulic acid standards, even though the sample was protected from UV light and hydrolysis was performed in an atmosphere of nitrogen to prevent oxidation. For the purpose of this study, only the concentrations of the (E)-isomer of ferulic acid and the (E)-isomer of *p*-coumaric acid were calculated.

Standard curves created from a dilution series (100–6.25 μg) of both (E)-ferulic acid and (E)-*p*-coumaric acid were linear. Peak heights were measured as absorbance units from the base line to the top of the peak. The response of the detector to different concentrations of the standard compounds was also linear, justifying the use of peak heights and standard curves to calculate the concentrations of both (E)-ferulic acid and (E)-*p*-coumaric acid in the extracts.

The average amount of kernel (E)-ferulic acid ranged from 1.37 mg/g in inbreds STR 3823 and Pool 27 to 1.80 mg/g in inbred NTR-1 3947 (Table 1).

***F. graminearum*-induced ear rot in field trials.** The correlations between the amount of ear rot observed in field trials and the corresponding amount of (E)-ferulic acid, for 1988 and 1991, are shown in Figures 1 and 2, respectively. Silking dates of the plants within the rows varied, leading to a variation in inoculation times of the maize ears. Therefore, percent ear rot and phenolic acid content of the three replicate rows for each cultivar were determined separately and were treated as individual data points.

A negative, highly significant correlation (1988: $r = -0.68$,

$P = 0.002$, $n = 57$; 1991: $r = -0.71$, $P = 0.002$, $n = 57$) was found between maize kernel (E)-ferulic acid content and amount of ear rot (Figs. 1 and 2). A positive, highly significant correlation was found between maize kernel (E)-*p*-coumaric acid contents and amount of ear rot (1988: $r = 0.18$, $P = 0.002$, $n = 57$; 1991: $r = 0.29$, $P = 0.002$, $n = 57$).

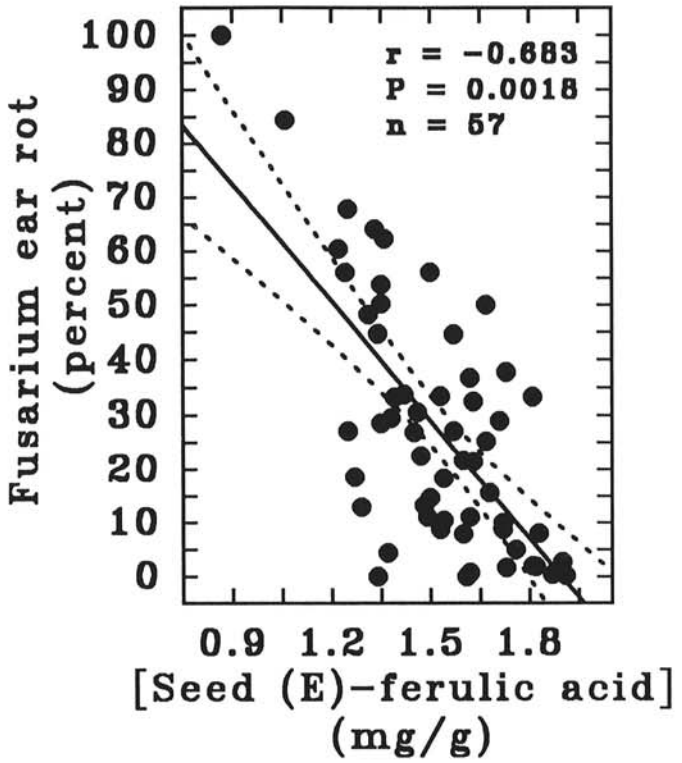

Fig. 1. Correlation between percentage of *Fusarium* ear rot and amount of seed (E)-ferulic acid in 1988 field trials. Dashed lines show the 95% confidence limits. Solid line shows the slope.

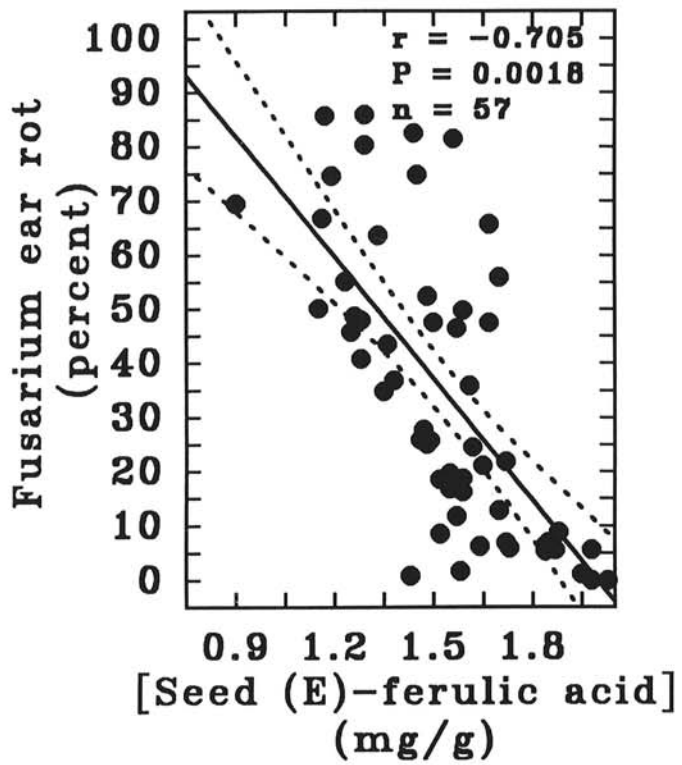

Fig. 2. Correlation between percentage of *Fusarium* ear rot and amount of seed (E)-ferulic acid in 1991 field trials. Dashed lines show the 95% confidence limits. Solid line shows the slope.

Manipulation of phenolic levels in fungal growth media. The addition of (E)-ferulic acid to PSA had significant effects ($P < 0.05$) on the overall growth of *F. graminearum* in vitro at all concentrations tested, causing total inhibition at the highest concentration. Probit analysis of the data resulted in a linear transformation (Fig. 3). Extrapolation from the resultant transformation showed that the effective concentration for 50% inhibition of growth (EC_{50}) of the *F. graminearum* culture was induced at an in vitro concentration of 0.647 mg of (E)-ferulic acid per gram.

DISCUSSION

Maize inbreds with high kernel concentrations of (E)-ferulic acid were significantly more resistant to infection by *F. graminearum* than were those with low kernel concentrations of (E)-ferulic acid. Although the extracts contained (E)-ferulic acid in the highest concentrations, (Z)-ferulic acid and (Z)-*p*-coumaric acid also were found in the extracts, and these compounds also may be implicated in resistance.

The inclusion of germ plasm from a wide geographical range did not result in geographical trends with respect to either the amount of ear rot or the amount of kernel (E)-ferulic acid content. This may be due, in part, to the limited number of genotypes used in this study. Further, the use of germ plasm from a wide geographical range may have introduced variation into the disease data, because of the unknown effect of growing conditions at a high latitude on the physiology of plants adapted for low latitudes.

Although the results of two field trials indicated a significant linear relationship between kernel (E)-ferulic acid content and resistance to *F. graminearum*, it is not known whether this rela-

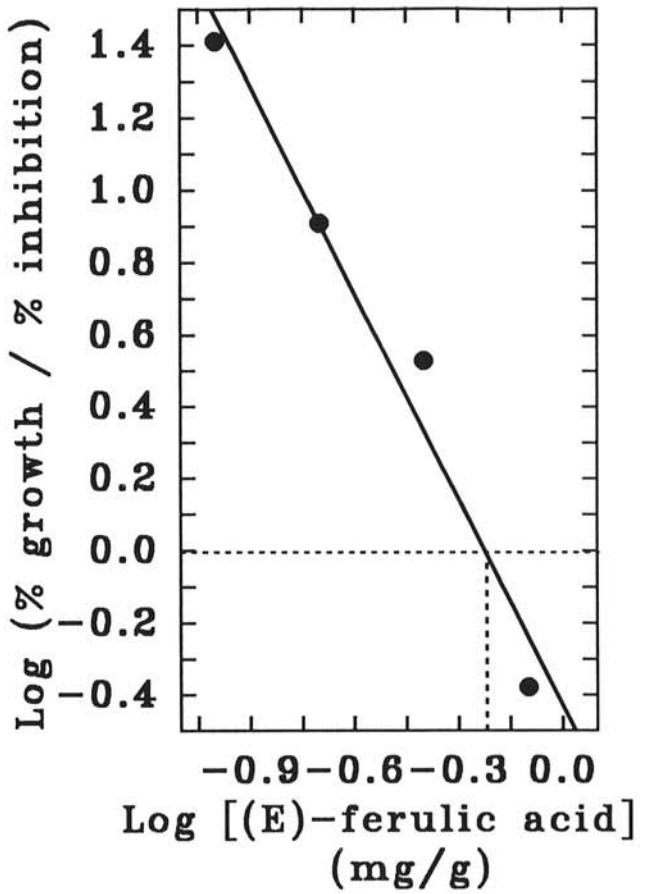

Fig. 3. Probit transformation of the effect of six concentrations of (E)-ferulic acid on mycelial growth of *Fusarium graminearum*. The dashed line refers to the derivation of the effective concentration for 50% inhibition of growth (EC_{50}) Solid line shows the slope.

tionship is causal. The ability of (E)-ferulic acid to inhibit the growth of *F. graminearum* in vitro was demonstrated by experimentally manipulating levels of the phenolic acid in fungal growth media. The EC_{50} of (E)-ferulic acid to *F. graminearum* was calculated to be 0.65 mg/g, well within range of the concentrations of kernel (E)-ferulic acid content inhibiting the growth of *F. graminearum* in vivo. However, this antibiosis of free ferulic acid is not unequivocal evidence of a cause-and-effect relationship.

Although free ferulic acid has growth-inhibiting properties, most of the phenolic acids in maize kernels are found in bound forms. In cereal cell walls, phenolic acids are complexed to hemicelluloses in forms such as FAXX, *O*-[5-*O*-(*trans*-feruloyl)-α-L-arabinofuranosyl]-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-D-xylopyranose (1). These feruloyl arabino xylans are subject to hydrolysis by fungal feruloyl esterases produced by cereal pathogens (23). Thus, growth studies with free ferulic acid may be very relevant. In addition, FAXX is subject to the formation of covalent cross-links created by extracellular peroxidase of the plant cell wall (10,12). The diferulate cross-links that are thus formed mechanically strengthen the cell wall and may provide structural resistance to invading ear rot fungi. These phenolic acid-carbohydrate complexes are abundant in the pericarp (1). A second type of phenolic acid complex involves conjugation to amides in compounds such as feruloyl, diferuloyl putresine, or spermidine. These compounds are located in the aleurone layer and may have direct toxic effects on fungal growth (1). We are currently investigating what role, if any, these compounds may have in limiting fungal growth.

The presence of phenolic acids, especially (E)-ferulic acid, in a wide geographic range of inbreds as cell wall carbohydrate complexes suggests that the role of these substances is constitutive rather than an induced defense. However, phenolics can accumulate rapidly during host-parasite interactions and can mediate defense suppression through inactivation of fungal enzymes or strengthening of plant structural components (2). The possibility that some forms of phenolics accumulate on infection of maize kernels by *F. graminearum* also should be investigated.

A negative, highly significant correlation was observed between levels of maize kernel (E)-ferulic acid content and amount of ear rot. However, a positive, highly significant correlation was observed between levels of maize kernel (E)-*p*-coumaric acid content and amount of ear rot. Such a relationship may be due to the lower conversion of *p*-coumaric acid to ferulic acid in susceptible plants. The continued proliferation of the fungus within its plant host also may be due to the potential tolerance of or insensitivity to the defense mechanisms of the plant it invades (35). Such tolerance may be due to the production of mycotoxins. Two mycotoxins, zearalenone (24,25) and deoxynivalenol (32,33), are produced by *F. graminearum*. It has been reported that production of deoxynivalenol increases as disease severity increases (15), and production of zearalenone is positively correlated with increasing disease severity (7). These studies suggest that deoxynivalenol and zearalenone production would be lower in inbreds resistant to *F. graminearum*. Further, it has been suggested that these mycotoxins may play a role in overcoming host-plant defenses during infection (4,34). Therefore, resistance of maize inbreds to *F. graminearum* may be related to their resistance to fungal mycotoxins or to their ability to detoxify mycotoxins.

Resistance and susceptibility of maize to *F. graminearum* may be influenced by the heritability of phenolic acids. Heritability of phenolics in maize has been documented (8,21), the heritability of (E)-ferulic acid has been examined, and a maternal effect, with pericarp dominance, has been demonstrated (29).

The demonstration of the correlation between ear rot and (E)-ferulic acid in grain at harvest makes selection easier for the breeder. A newly developed quantitative determination analysis of phenolic acids in maize grain (28) greatly reduces analysis time, making the selection process far more convenient and rapid.

Based on the results of the present study, we suggest that conventional breeding programs aimed at attaining maize germ plasm resistant to *F. graminearum* should incorporate inbreds of maize containing high concentrations of kernel (E)-ferulic acid.

LITERATURE CITED

1. Arnason, J. T., Gale, J., Conilh de Beyssac, B., Sen, A., Miller, S. S., Philogène, B. J. R., Lambert, J. D. H., Fulcher, R. G., Serratos, A., and Mihm, J. 1992. Role of phenolics in resistance of maize grain to the stored grain insects, *Prostephanus truncatus* (HORN) and *Sitophilus zeamais* (MOTSCH.). J. Stored Prod. Res. 28:119-126.
2. Bell, A. A. 1981. Biochemical mechanisms of disease resistance. Annu. Rev. Plant Physiol. 32:21-81.
3. Booth, C. 1977. *Fusarium*. Laboratory Guide to the Identification of the Major Species. Commonw. Mycol. Inst. Kew, Surrey, England. 58 pp.
4. Casale, W. L., and Hart, L. P. 1988. Inhibition of ^{3}H-leucine incorporation by trichothecene mycotoxins in maize and wheat tissue. Phytopathology 78:1673-1677.
5. Chiang, H. C. 1978. Pest management in corn. Annu. Rev. Entomol. 23:101-123.
6. Christie, T. 1965. The effects of some phenolic compounds upon the growth of two species of *Phytophthora*. N.Z. J. Agric. Res. 8:630-635.
7. 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 Dis. 67:89-91.
8. Dunn, G. M., Long, B. J., and Routley, D. G. 1981. Inheritance of hydroxamic acids in *Zea mays*. Can. J. Plant Sci. 61:583-593.
9. Enerson, P. M., and Hunter, R. B. 1980. A technique for screening maize (*Zea mays* L.) for resistance to ear mold incited by *Gibberella zeae* (Schw.) Petch. Can. J. Plant Sci. 60:1123-1128.
10. Eraso, F., and Hartley, R. D. 1990. Monomeric and dimeric phenolic constituents of plant cell walls. J. Sci. Food Agric. 51:163-170.
11. Farkas, G. L., and Király, Z. 1962. Role of phenolic compounds in the physiology of plant diseases and disease resistance. Phytopathol. Z. 44:105-150.
12. Fincher, G. B., and Stone, B. H. 1986. Cell walls and their components in cereal grain technology. Adv. Cer. Sci. Tech. 8:207-295.
13. Friend, J. 1977. Phenolic substances and plant disease. Pages 557-588 in: Recent Advances in Phytopathology. Vol. 12. Biochemistry of Plant Phenolics. T. Swain, J. B. Harborne, and C. F. VanSumere, eds. Plenum Publishing Corp., New York.
14. Ghosal, S., Chakrabarti, D. K., and Srivastava, R. S. 1978. Effect of 3 polyphenolic compounds against ear-rot of corn incited by *Fusarium moniliforme* Sheld. Experientia 35:83-84.
15. Hart, L. P., Braselton, W. E., Jr., and Stebbins, T. C. 1982. Production of zearalenone and deoxynivalenol in commercial sweet corn. Plant Dis. 66:1133-1135.
16. Hooker, A. L. 1956. Association of several seedling, root, stalk, and ear diseases in corn. Phytopathology 46:379-384.
17. Ismail, I. M. K., Salama, A.-A., Ali, M. I. A., and Ouf, S. A.-E. 1987. Effect of some phenolic compounds on spore germination and germ tube length of *Aspergillus fumigatus* and *Fusarium oxysporum* f. sp. *lycopersici*. Crytogram. Mycol. 8:51-60.
18. Kasenberg, T. R., and Traquair, J. A. 1988. Effects of phenolics on growth of *Fusarium oxysporum* f. sp. *radicis-lycopersici* in vitro. Can. J. Bot. 66:1174-1177.
19. Ku, J. 1964. Phenolic compounds and disease resistance in plants. Pages 63-81 in: Phenolics in Normal and Diseased Fruits and Vegetables. V. C. Runeckles, ed. Imperial Tobacco Co., Montreal.
20. Li, C. Y., Lu, K. C., Trappe, J. M., and Bollen, W. B. 1969. Effect of phenolic and other related compounds on growth of *Poria weirii in vitro*. Microbios 1:305-311.
21. Lunsford, J. N., Futrell, M. C., and Scott, G. E. 1975. Maternal influence on response of corn to *Fusarium moniliforme*. Phytopathology 65:223-225.
22. Matern, U., and Kneusel, R. E. 1988. Phenolic compounds in plant disease resistance. Phytoparasitica 16:153-170.
23. McCallum, J. A., Arnason, J. T., Taylor, I. E. P., and Towers, G. H. N. 1992. Effects of UV-irradiation on enzymatic digestion of barley cell walls. J. Plant Physiol. 135:641-646.
24. Mirocha, C. J., and Christensen, C. M. 1974. Estrogenic mycotoxins synthesized by *Fusarium*. Pages 129-143 in: Mycotoxins. I. F. H. Purchase, ed. Elsevier Science Publishing Co., Amsterdam.
25. Mirocha, C. J., Harrison, J., Nichols, A. A., and McClintock, M. 1968. Detection of a fungal estrogen (F-2) in hay associated with infertility in dairy cattle. Appl. Microbiol. 16:797-798.
26. Mirocha, C. J., Pathre, S. V., Schauerhamer, B., and Christensen, C. M. 1976. Natural occurrence of *Fusarium* toxins in feedstuff. Appl. Environ. Microbiol. 32:553-556.
27. Reid, L. M., Mather, D. E., Arnason, J. T., Hamilton, R. I., and Bolton, A. T. 1992. Changes in phenolic constituents of maize silk

infected with *Fusarium graminearum*. Can. J. Bot. 70:1697-1702.
28. Sen, A., Miller, S. S., Arnason, J. T., and Fulcher, R. G. 1991. Quantitative determination by high performance liquid chromatography and microspectrofluorimetry of phenolic acids in maize grain. Phytochem. Anal. 2:225-229.
29. Serratos, A., Arnason, J. T., Nozzolillo, C., Lambert, J. D. H., Philogène, B. J. R., Fulcher, G., Davidson, K., Peacock, L., Atkinson, J., and Morand, P. 1987. Factors contributing to resistance of exotic maize populations to maize weevil, *Sitophilus zeamais*. J. Chem. Ecol. 13:751-762.
30. Sosulski, F., Krygier, K., and Hogge, L. 1982. Free, esterified, and insoluable phenolic acids. III. Composition of phenolic acids in cereal and potato flours. J. Agric. Food Chem. 30:337-340.
31. Sutton, J. C. 1982. Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum*. Can. J. Plant Pathol. 4:195-209.
32. Vesonder, R. F., Ciegler, A., and Jensen, A. H. 1973. Isolation of the emetic principle from *Fusarium*-infected corn. Appl. Microbiol. 15:1008-1010.
33. Vesonder, R. F., Ciegler, A., Jensen, A. H., Rohwedder, W. K., and Weisleder, D. 1976. Co-identity of the refusal and emetic principle from *Fusarium*-infected corn. Appl. Environ. Microbiol. 31:280-285.
34. Wang, Y. Z., and Miller, J. D. 1988. Effects of *Fusarium graminearum* metabolites on wheat tissue in relation to Fusarium head blight resistance. J. Phytopathol. 12:118-125.
35. Ward, E. W. B., Unwin, C. H., and Stoessl, A. 1973. Postinfectional inhibitors from plants. VII. Tolerance of capsidiol by fungal pathogens of pepper fruit. Can. J. Bot. 51:2327-2332.