Evaluation of Wheat (*Triticum aestivum* L.) Phenolic Acids during Grain Development and Their Contribution to *Fusarium* Resistance

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The phenolic acid profiles of six cultivars of wheat with known tolerance to *Fusarium* head blight were evaluated during plant development from anthesis through maturity. Analysis by HPLC of grain at anthesis revealed that *p*-coumaric and ferulic acid were the two principal phenolic compounds present. The effect of these two phenolic acids on *Fusarium* species was evaluated in vitro. Both phenolic acids demonstrated significant reductions ($p < 0.05$) of *Fusarium* species growth at all concentrations tested. Ferulic acid is the primary phenolic acid in grain at all stages of development, and its concentration increased steadily during grain development prior to a 50% decrease during grain ripening. The accumulation of ferulic acid synthesis from anthesis until ~20 days after anthesis appears related with cultivar resistance to *Fusarium*. Concentrations of ferulic acid in the grain were similar at maturity, implying that the end-use quality would be similar for both resistant and susceptible cultivars.

Keywords: *Phenolic; ferulic acid, p-coumaric acid; Fusarium; disease resistance; grain development; quality*

INTRODUCTION

Fusarium head blight (FHB; or scab) is a fungal disease of cereals that causes reduced yields, reduced grain quality, accumulations of hazardous mycotoxins in the grain, and seedling blight when infected grain is used as seed. The principal causal organism of FHB in North America is *Fusarium graminearum* Schwabe, whereas *Fusarium culmorum* (W.G. Smith) Sacc. is predominant in Europe (Parry et al., 1995). *F. graminearum* and *F. culmorum* are non-host specific; that is, they are pathogenic to wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), and other cereals and grasses without showing specialization for any one crop (van Eeuwijk et al., 1995). All cultivars of common wheat (*Triticum aestivum* L.) are at least partially susceptible, and epidemics of FHB have been reported worldwide. FHB outbreaks can be severe when weather is favorable for infection, that is, when warm, wet weather occurs during cereal flowering.

Analysis of *Fusarium*-infected wheat kernels (Pugh et al., 1932; Bechtel et al., 1985) and results of milling investigations (Scott et al., 1983; Young et al., 1984) indicate that fungal mycelia principally reside in the outer coverings of mature grain. This suggests that infection progresses from the exterior of the grain to the interior and that fungal penetration into the endosperm is somewhat limited by the biochemical organization of the outer layers. The outer tissues of mature grain contain high levels of phenolic compounds in the cell walls (Fulcher et al., 1972; Fulcher, 1982), and research suggests that resistance is correlated with grain phenolic acid content at maturity (Assabgui et al., 1993). However, grain susceptibility to *Fusarium* infection is greatest at anthesis, and it is not yet clear if resistance can be related to the phenolic acid content of developing grain.

Phenolic compounds are widespread throughout the plant kingdom, and they have been implicated in disease resistance by a number of researchers (Friend, 1981; Bell, 1981; Matern and Kneusel, 1988; Creasy, 1984; Nicholson and Hammerschmidt, 1992). Phenolic acids have been shown to inhibit the in vitro growth of an assortment of fungal genera (Baranowski et al., 1980; Purushothaman, 1976). The majority of phenolic acids in wheat kernels are bound in cell walls in such forms as *O*-[5-*O*-(*trans*-feruloyl)-α-L-arabinofuranosyl]-(1→3)-*O*-*β*-D-xylopyranosyl-(1→4)-D-xylopyranose (FAXX) (Mueller-Harvey et al., 1986). Insoluble conjugated phenolic compounds, such as diferulate cross-linked FAXX, mechanically strengthen cell walls and may provide structural resistance to invading fungi. The association of phenolic oligopolymers and polymers (lignin) with cell wall carbohydrates in the form of lignin−carbohydrate

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complexes are frequent in the pericarp (Arnason et al., 1992). These compounds appear to be important in controlling the rate and extent of degradation of structural polysaccharides by microorganisms (Wallace et al., 1995).

Breeding for resistance to *Fusarium* is the most practical approach for disease control. Schroeder and Christensen (1963) first proposed that *Fusarium* resistance could be described by two components, type I and type II. Type I resistance operates against initial infection and type II against spread of the pathogen within the host. Type I resistance is thought to involve a large number of characteristics and has not been identified in any existing cereal cultivars. Mesterhazy (1995) has reported that the abilities to resist kernel shriveling after infection (type III), to detoxify deoxynivalenol (type IV), and to maintain yields (type V) are also means of characterizing *Fusarium* resistance. Cereal breeders use these components of resistance to differentiate varietal susceptibility to infection, rating cultivar performance within each type of resistance. However, the mechanisms and means by which these types of resistance are afforded to the grain are neither established nor understood.

Several investigators have examined the cell wall-bound phenolic acids in developing wheat kernels (McCallum and Walker, 1991; Regnier and Macheix, 1996), but studies on the phenolic acid profiles of grain at anthesis are not reported in the literature. Because the most susceptible time for *Fusarium* infection is from plant heading to 10 days after anthesis (DAA), it is important to examine which phenolic compounds are synthesized during this stage of development. The specific objectives of this study were to evaluate (1) the in vitro effect of the principal phenolic compounds present in the grain at anthesis on *Fusarium* species and (2) phenolic acids in developing grains of wheat cultivars that are known to differ in their reaction to *Fusarium* infection to determine if a relationship exists between phenolic acids and resistance.

MATERIALS AND METHODS

Materials. Vanillic, caffeic, syringic, syringaldehyde, *p*-coumaric, ferulic, and sinapic acids were obtained from Sigma Chemical Co. (St. Louis, MO), and vanillin was obtained from Fisher Scientific (Fair Lawn, NJ). Ferulic and *p*-coumaric acids were recrystallized from aqueous methanol (MeOH) before use. All phenolics were subsequently checked for purity by high-pressure liquid chromatography (HPLC). HPLC grade water and MeOH were used for all analyses. Phosphoric acid buffer was made using HPLC grade $NH_4H_2PO_4$ (Fisher Scientific) and H_3PO_4 (Curtin Matheson Scientific, Inc., Houston, TX).

In Vitro Studies. The ability of ferulic acid, *p*-coumaric acid, and their combination to inhibit the in vitro growth of *Fusarium* species isolates was tested by adding various concentrations of phenolic acids to fungal growth media as described by Assabgui et al. (1993) with some modification. Two isolates of *F. graminearum* were collected from two separate locations in Minnesota in two successive years (1994 and 1995). Both isolates were capable of producing trichothecene mycotoxins, such as deoxynivalenol. Isolate Butte 86 was obtained from wheat cultivar Butte 86 grown in Ada, MN, whereas isolate Roblin was obtained from wheat cultivar Roblin grown in Stephens, MN. *F. culmorum* (isolate 89.4, originated from France, screened by Dr. Laurent Saur, INRA, Rennes) capable of nivalenol production was also examined. Neither *F. graminearum* isolate was capable of producing nivalenol.

Table 1. Relative Resistance Ratings of Cultivars Evaluated (Ma et al., 1997)

overall rating	cultivar	type II[a,b]
resistant	Sumai	R
	BacUp	MR
	2375	MS−MR
susceptible	Roblin	S
	Oslo	S
	Wheaton	S

[a] Type II = resistance to pathogen spread within the host. [b] R = resistant; MR = moderately resistant; MS = moderately susceptible; S = susceptible.

The effect of concentration (0, 0.1, 0.2, 0.4, 0.8, and 1.6 mg/mL), five replicates at each concentration, was examined by measuring the area of mycelial radial growth using commercial software installed in a Kontron IBAS image analysis unit (Munich, Germany). Phenolic acids were dissolved in MeOH and added to sterile potato dextrose agar (PDA) by filtration through a sterile 25 mm, 0.2 μm nylon filter (Gelman Sciences, Ann Arbor, MI). For controls, *Fusarium* species isolates were grown on agar media with the corresponding amount of added MeOH and on media alone. Plugs of inoculum (area = 0.76 cm^2) were sectioned from the edges of 6-day-old cultures of *Fusarium* with a cork borer and were inverted onto the center of the Petri plates. Petri plates were incubated at 21 °C with a 12:12 h (light/dark) photoperiod. The area of the resultant fungal mycelium was measured when the control growth reached its maximum area, usually 5−6 days.

Cultivars and Sampling. Six cultivars of hard red spring wheat with known tolerance to scab were grown at St. Paul, MN, in 1997 in a complete block design with three replicates. Cultivars were selected on the basis of their resistance to type II infection (Table 1). All cultivars were sown in two-row plots, 3 m long with 0.3 m between rows, with one row of winter wheat sown between each plot. To define accurately a cultivar's stage of growth, rows were flagged upon heading and 50% anthesis noted. Wheat spike samples were hand-harvested from heading to maturity, with additional sampling at anthesis. All sampling took place between 7 a.m. and 9 a.m. Tillers were cut with scissors ~7.5 cm below the spike and immediately frozen (−20 °C). Samples were subsequently freeze-dried prior to cleaning and analysis. Immature grain samples were hand-threshed and separated or hand-threshed and screened (No. 3.5, 5, and 8, W. S. Tyler, Inc., Mentor, OH) to separate developing kernels from chaff. Mature kernels were cleaned by air classification.

Phenolic Extraction. Cell wall phenolic acids were extracted as described by Sen et al. (1991) with some modification. Grains obtained prior to 7 DAA were directly homogenized in 15 mL of 4 N NaOH with a polytron PT10/35 mixer (Brinkmann Instruments, Westbury, NY), whereas grain samples between 7 DAA and maturity were ground using a Retsch ZM-1 mill (Brinkmann, Germany). Approximately 200 mg (dw) of ground sample was added to 15 mL of 4 N NaOH in a 50 mL Pyrex centrifuge tube (Corning, Corning NY), purged with nitrogen, and shaken for 2 h in the dark with a wrist-action shaker (Burrell model 75, Pittsburgh, PA).

After phenolic acid liberation by alkaline hydrolysis, samples were acidified with ice-cold 6 N HCl to reduce pH to between 1 and 2. Samples were centrifuged at 3000*g* (Beckman GPKR, Palo Alto, CA), and the supernatant was decanted into a 250 mL separatory funnel. The supernatant was extracted with ethyl acetate (3 × 50 mL) with shaking for 10 s, and the mixture was allowed to settle for 5 min between extractions. Ethyl acetate fractions were collected and pooled. The remaining pellet was diluted with 15 mL of distilled H_2O, vortex-disrupted, and recentrifuged at 3000*g*. The second supernatant was re-extracted with ethyl acetate (3 × 50 mL) as before, and all ethyl acetate fractions were pooled.

The phenolic acids-rich ethyl acetate fraction was dried by addition of anhydrous sodium sulfate (Fischer Scientific) and concentrated using a rotary vacuum evaporator (model NE-1,

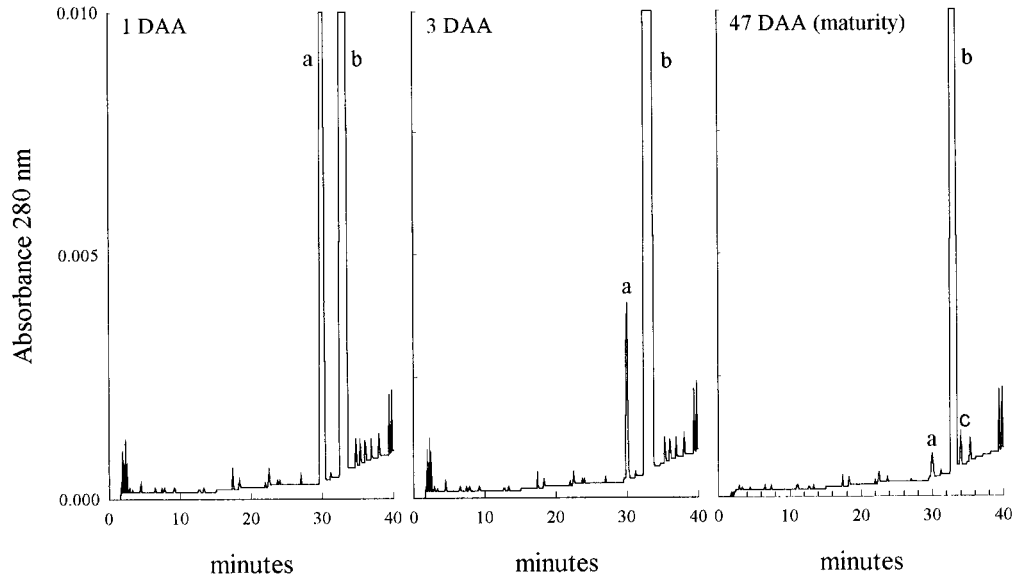

Figure 1. HPLC chromatograms of extracts of cv. 2375 showing changes in the ratio of *p*-coumaric acid and ferulic acid during grain development: (a) *p*-coumaric acid; (b) ferulic acid; (c) sinapic acid.

Wheaton Eyela, Tokyo, Japan) at 35 °C to dryness. The phenolic acids-rich residue was resolubilized in 2.5 mL of MeOH and stored in the dark prior to separation and quantification by HPLC within 24 h of extraction.

HPLC Analysis. All analyses were performed on a Spectra-Physics (San Jose, CA) system consisting of an SP8800 tertiary pump fitted with a 20 μL injection loop, a Spectra 200 programmable wavelength detector, and a Chromjet integrator. An aliquot of the sample suspended in MeOH was diluted with 10 mM phosphoric acid buffer (pH 3.5) to the same concentration as the initial mobile phase (15% MeOH). Samples were next filtered through a 0.2 μm poly(tetrafluoroethylene) (PTFE) filter prior to injection. Separations were achieved on a 5 μm, 250 mm × 4.6 mm, C18 ODS reverse-phase analytical column with a 30 mm × 4.6 mm precolumn of the same material (Beckman, Fullerton, CA). The two-solvent system consisted of MeOH (A) and 10 mM phosphoric acid buffer, pH 3.5 (B), operated at a flow rate of 1.5 mL/min. The phosphoric acid buffer consisted of 10 mM $NH_4H_2PO_4$ adjusted to pH 3.5 with 10 mM H_3PO_4. Phenolic acids were separated by gradient elution as follows: 15% A for 10 min, 15−20% A in 10 min, 20−30% A in 10 min, 30−40% A in 5 min, 40−55% A in 5 min, 55−80% A in 5 min, 80−100% A in 2 min, 100% A for 8 min, 100−15% A in 2 min, and 15% A for 3 min. Detection was measured at 280 nm, which proved to be most appropriate for simultaneous measurement of both hydroxybenzoic and hydroxycinnamic acids. Peak identity was confirmed by comparison with standards of phenolic acids and by spiking of extracts with authentic standards. On the basis of spike recoveries, extraction efficiencies for *p*-coumaric acid and ferulic acid bound in the cell walls were approximately 85 and 87%, respectively.

RESULTS AND DISCUSSION

Phenolic Acids in Developing Grain. For the purpose of this study, only the concentrations of the (*E*)-isomers of phenolic acids were calculated. The occurrence of the (*Z*)-isomers has been well reported (Hartley and Jones, 1977; Yamamoto and Towers, 1985; McCallum and Walker, 1991; Assabgui et al., 1993), and efforts were made in the extraction to minimize the photoisomerization reactions to which phenolic acids are susceptible. Analysis of wheat kernel extracts revealed peaks with relative retention times corresponding to vanillic acid, caffeic acid, syringic acid, vanillin, syringaldehyde, ferulic acid, *p*-coumaric acid, and sinapic acid; and these compounds were separated and eluted in the order listed.

The phenolic acid profiles of developing grain were monitored from anthesis through maturity. Representative chromatograms of wheat grain extracts of cv. 2375 are shown in Figure 1. One day after anthesis, the principal phenolic acids present are ferulic and *p*-coumaric acid. The ratio of ferulic to *p*-coumaric acid at this time is approximately 3:1. Although the concentrations may vary slightly among cultivars, this ratio was representative of the ratio found in all cultivars. As the grain develops, the ratio of ferulic to *p*-coumaric acid begins to increase rapidly (Figure 1). At 3 DAA, the ratio of ferulic to *p*-coumaric acid is 18:1 in cv. 2375. At maturity, the ratio of ferulic to *p*-coumaric acid is approximately 62:1, and sinapic acid has replaced *p*-coumaric acid as the second most predominant phenolic compound in grain (Figure 1). Additionally, in female parts (i.e., ovary tissues) evaluated immediately after heading (2−3 days prior to anthesis), the ratio of ferulic acid to *p*-coumaric acid was approximately equivalent. As anthesis is the time when cultivars are most susceptible to infection, these findings suggest that both ferulic and *p*-coumaric acid may potentially contribute to disease resistance.

In Vitro Inhibition Studies. The addition of ferulic acid, *p*-coumaric acid, and their combination to PDA significantly ($p < 0.05$) reduced growth of *F. graminearum* and *F. culmorum* in vitro at all concentrations tested. A log−log transformation of the data for *F. graminearum* isolate Butte 86 (Figure 2) shows that at higher phenolic acid concentrations, the combined effect of ferulic and *p*-coumaric acid becomes synergistic. This synergistic phenolic acid response was demonstrated for each *Fusarium* species isolate evaluated. From an extrapolation of the transformations, the effective concentration for 50% inhibition of growth (EC_{50}) was determined (Table 2). Similar investigations using different media (mung bean broth, modified Komada's medium) also demonstrated the effectiveness of phenolic acids on growth inhibition (data not shown).

Differences in the EC_{50} values for the two *F. graminearum* isolates (Table 2) demonstrate the potential

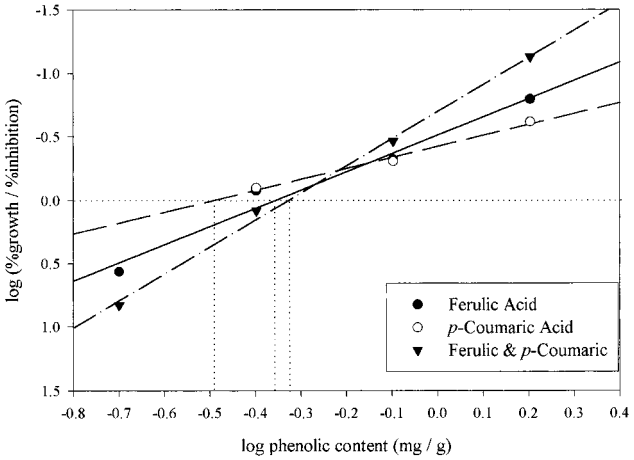

Figure 2. Log−log transformation of the effect of ferulic acid, *p*-coumaric acid, and their combination in equal amounts on the mycelial growth of *F. graminearum* isolate Butte 86. The dotted line shows the derivation of the effective concentration for 50% inhibition of growth (EC_{50}). Regression lines for each phenolic acid treatment are significantly different ($p < 0.05$).

Table 2. Effective Concentration of Phenolic Acids Required for 50% Inhibition of Growth (EC_{50}) of *Fusarium* Species[a]

	EC_{50} (μg/g)		
	ferulic acid	*p*-coumaric acid	ferulic and *p*-coumaric acids
F. graminearum isolate Butte 86	442	316	469
F. graminearum isolate Roblin	668	793	779
F. culmorum	329	559	742

[a] *F. graminearum* isolate Butte 86 values extrapolated from log−log transformation of data shown in Figure 1. Graphical data for *F. graminearum* isolate Roblin and *F. culmorum* not shown.

variability among *F. graminearum* isolates. Our results were relatively similar to those reported by Assabgui et al. (1993) of 0.647 mg/g of (*E*)-ferulic acid. Similar variation should be expected among *Fusarium* species, as demonstrated by the EC_{50} value for the *F. culmorum* isolate. Variation in EC_{50} values may provide a method for classification of fungal pathogenicity or "aggressiveness", but greater sampling of different isolates is necessary to establish this possibility.

The significant in vitro inhibitory effect of phenolic acids on *Fusarium* species suggests these compounds could contribute to disease resistance. Although the majority of phenolic acids in mature grains are reported to be highly cross-linked, esterified, and bound in cereal cell walls, the phenolic acids in undeveloped grains are considerably less modified. McCallum and Walker (1990) reported that the concentration of soluble phenolic acids is greatest in the immature kernel and subsequently decreases during maturity. Phenolic compounds present in kernels at the time of anthesis are likely to mediate and suppress fungal infection in a manner similar to that demonstrated by the in vitro inhibition studies. Although phenolic compounds may become more esterified and cross-linked in the cell walls of developing grain, they are still subject to fungal hydrolases and esterases produced by cereal pathogens. It is possible that the infection process may liberate phenolic acids to levels able to contribute to grain resistance. Dixon and Paiva (1995) have proposed that infection-induced

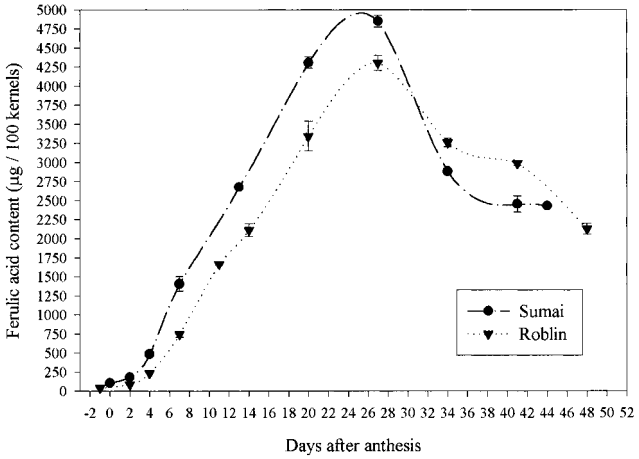

Figure 3. Comparison of ferulic acid synthesis (micrograms per 100 kernels) from anthesis to maturity for cvs. Sumai and Roblin (see Table 1 for cultivar resistance ratings). Standard errors averaged 3% of the mean ($n = 3$ assays).

alkyl ferulate esters and cell wall-bound phenolic esters may act directly as defense compounds or indirectly as precursors for the synthesis of lignin, suberin, and other wound-induced polyphenolic barriers.

Comparison of Ferulic Acid Profiles in Developing Grain. Ferulic acid is the principal cell wall-bound phenolic acid in grain at all stages of development. The rate of ferulic acid synthesis from anthesis to maturity is illustrated for cv. Sumai (resistant) and cv. Roblin (susceptible) in Figure 3. Ferulic acid concentrations reach a peak concentration at ~27 DAA for both cultivars, at which time the concentration of ferulic acid subsequently decreases during grain ripening. There is a change in the accumulation of ferulic acid synthesis after ~7−10 DAA, as seen by the small shoulder in the developmental curve. Cv. Roblin shows an additional shoulder at 40 DAA, possibly corresponding to some late ferulic acid synthesis in the germ. At maturity, the final ferulic acid concentrations in the mature grain were fairly similar among the six cultivars investigated. Phenolic acids are known to contribute to the textural and sensory characteristics of grain products (Maga and Lorenz, 1973; Collins, 1986; Chen and Hoseney, 1995; Shahidi and Naczk, 1995), and their concentrations at maturity may affect the end-use quality of the grain. Because the cultivars investigated had similar ferulate concentrations at maturity, we would not expect the end-use quality with respect to ferulate concentrations to be highly variable.

Several investigators (McCallum and Walker, 1990; Regnier and Macheix, 1996) have reported that phenylalanine ammonia-lyase (PAL) levels are significant during grain development, showing one or two maxima during approximately the first 30 DAA and then decreasing. PAL catalyzes the reductive deamination of phenylalanine to form cinnamic acid and serves as the first committed step in the biosynthesis of phenylpropanoids. Concomitant increases in levels of PAL and phenolic compounds have been demonstrated in many plant tissues (Jones, 1984). Ferulic acid synthesis during the first 27 DAA most likely reflects high PAL activity in the developing grain.

Ferulic acid concentrations decrease by ~50% during grain ripening (~27 DAA through maturity; Figure 3). Several factors contribute to this decrease. First, the rate of endosperm development surpasses the rate of

synthesis of the outer coverings during grain ripening, and the overall phenolic constituent contribution within the kernel is effectively diluted. Second, PAL activity decreases during this time, and at maturity it is difficult to find evidence of PAL activity in the kernel (McCallum and Walker, 1990; Regnier and Macheix, 1996). Finally, peroxidase enzymes present in the outer coverings at this time may induce the formation of covalent cross-links between phenolics and wall polymers (Biggs and Fry, 1987; Kruger and LaBerge, 1974), effectively limiting the extractability of phenolic constituents with alkali treatment. Peroxidases appear to continue to modify and cross-link grain phenolics more tightly into the cell wall during grain storage, as ferulic acid extractability decreased markedly over time. Repeated analysis of samples after 6 months of storage in sealed desiccators showed up to 20% decrease in ferulic acid recovery (e.g., 4850 versus 3900 μg/g for cv. Sumai at 27 DAA).

We observed a small shoulder between 7 and 10 DAA when we added regression lines to the data on ferulic acid concentration versus DAA (Figure 3). McCallum and Walker (1991) have suggested that two pools of ferulic acid synthesis exist and are associated with different tissue development. Simmonds and O'Brien (1981) examined the morphological and biochemical changes in developing wheat and reported that during approximately the first 10−12 days of grain development the testa differentiates from the pericarp in the kernel. The testa is a phenolic-rich layer of maternal tissue that encompasses the entire outer layer of the grain and is ultimately responsible for seed color. Its development corresponds to the time of maximal PAL activity in the pericarp and testa (McCallum and Walker, 1990). Initial ferulic acid synthesis from anthesis to ~7−10 DAA most certainly relates to testa differentiation. For the cultivars investigated, observations by fluorescence microscopy confirmed that the testa was fully differentiated in the kernel by 10−12 DAA.

Subsequently, between approximately 12 and 24 DAA, Simmonds and O'Brien (1981) reported that aleurone differentiation and development occur. Fulcher et al. (1972) reported that the aleurone tissue consists mainly of ferulic acid. This period of aleurone differentiation corresponds with the increased accumulation of ferulic acid shown in Figure 3 between approximately 10 and 27 DAA. It apparently represents the second pool of ferulic acid synthesis proposed by McCallum and Walker (1991). Sections of immature kernels observed using fluorescence microscopy indicated that the aleurone cell wall could first be seen at approximately 10 DAA and was highly differentiated by 27 DAA.

Cv. Sumai (resistant) synthesized higher concentrations of ferulic acid than cv. Roblin (susceptible) over the first 25 DAA (Figure 3). Sumai is a resistant type II cultivar, whereas Roblin is a susceptible type II. The greater ferulic acid synthesis in Sumai during the first 7 days may be one mechanism by which Sumai gains improved resistance to infection. The ferulic acid content in Sumai is twice that of Roblin at 7 DAA (1400 versus 700 μg/100 kernels; Figure 3). The increased synthesis of ferulic acid in Sumai may indicate that the testa layer develops more rapidly and to a greater extent than in Roblin. Greater ferulate synthesis and testa development may contribute to Sumai's high type II resistance by effectively limiting initial fungal penetration and infection. We propose that testa development is one component of type II resistance.

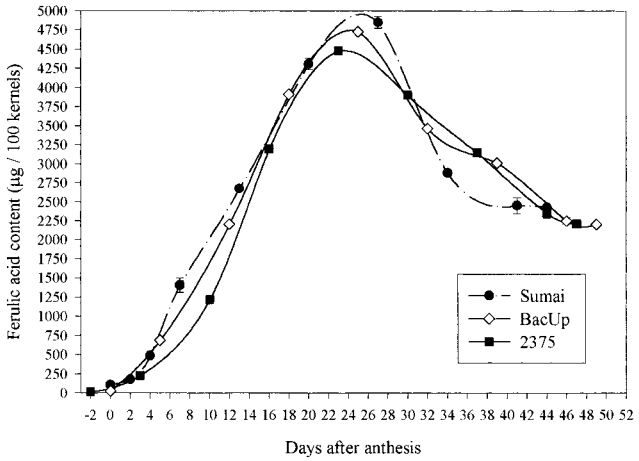

Figure 4. Comparison of ferulic acid synthesis (micrograms per 100 kernels) from anthesis to maturity for resistant cvs. Sumai, BacUp, and 2375 (see Table 1 for cultivar resistance ratings). Standard errors averaged 3% of the mean (n = 3 assays).

In addition, cv. Sumai has a greater concentration of ferulic acid than cv. Roblin from day 10 through day 27, which may further contribute to the grain's type II resistance. If the aleurone differentiates more rapidly in Sumai than in Roblin, secondary fungal proliferation could be further mediated. The grain endosperm is in a soft milk stage during this period of time, which would seemingly provide an ideal fungal growth medium. However, a rapidly developing aleurone layer would provide a secondary means of resistance for fungal mycelium to penetrate to gain access to the liquid endosperm. If fungal mycelia are unable to penetrate and proliferate in the endosperm, it would be reasonable to assume that pathogen spread within the host would be limited. We propose that aleurone development is also a component of type II resistance.

Figure 4 shows how the other two resistant cultivars investigated performed compared to cv. Sumai. Sumai has high type II resistance, whereas cv. BacUp has moderate resistance and cv. 2375 has somewhat less resistance than BacUp. During the first 10 DAA, the relationship between ferulic acid synthesis and resistance is clear. According to ferulate accumulations, the three cultivars rank Sumai > BacUp > 2375, which is how they rank for type II resistance. In addition, all three resistant cultivars rapidly synthesize ferulic acid from 10 to 20 DAA. When the concentrations of ferulic acid are examined from 10 to 20 DAA, it can be seen that 2375 accumulates ferulic acid at the fastest rate according to the regression lines (Figure 4). We hypothesize that one reason these cultivars perform well under scab conditions and can be considered resistant cultivars is because the aleurone tissue differentiates and develops at a rapid rate, limiting fungal penetration into developing endosperm tissue.

Figure 5 shows how the three susceptible cultivars investigated performed. All three cultivars show a considerable lag in ferulic acid synthesis in the first 7−10 DAA when compared to the resistant cultivars (Figure 4). Reduced levels of ferulate synthesis would seemingly provide an opportunity for infection to occur. Secondary ferulate synthesis for these cultivars between days 12 and 20 also lags that of the three resistant cultivars (see Figures 4 and 5), providing an opportunity

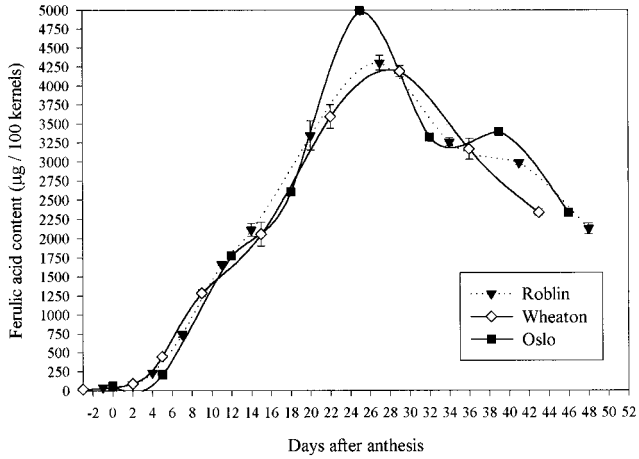

Figure 5. Comparison of ferulic acid synthesis (micrograms per 100 kernels) from anthesis to maturity for susceptible cvs. Roblin, Wheaton, and Oslo (see Table 1 for cultivar resistance ratings). Standard errors averaged 3% of the mean ($n = 3$ assays).

for fungal growth in the developing endosperm. We would predict that they have poor type II resistance from these findings, and data from actual FHB outbreaks support this conclusion. Interestingly, the slope of the regression line for cv. Oslo indicates rapid ferulate synthesis between days 18 and 25. However, ferulate synthesis this late in development would likely have less effect on mediating infection. In severe cases of FHB, infection occurs much earlier in the development of the grain.

In conclusion, disease resistance is a multifaceted mechanism in cereals, and many components contribute to the cultivar's overall resistance. Plant phenolic compounds have long been associated with the role of disease resistance in plants. We have demonstrated that the principal phenolic acids in grain at anthesis, ferulic and *p*-coumaric acid, behave synergistically and effectively inhibit the growth of *Fusarium* species in vitro. Initial production of phenolic acids is localized in the testa and pericarp of kernels as early as anthesis when infection pressure is most critical. For the cultivars investigated, we were able to effectively relate their susceptibility to *Fusarium* infection with the biosynthesis of ferulic acid. Phenolic acid synthesis ultimately relates to cellular differentiation within the kernel and the development of distinct morphological and biochemical constituents. Our findings suggest that the rate and extent of phenolic acid synthesis can be a useful predictor of a cultivar's susceptibility to *Fusarium* infection.

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