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Changes in Phenolic Concentrations during Recurrent Selection for Resistance to the Mediterranean Corn Borer (*Sesamia nonagrioides* Lef.)

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Recurrent selection has been reported as successful for improving maize resistance against corn borers. This study was conducted to determine if phenolics concentration in maize changes during recurrent selection to improve stalk resistance to the Mediterranean corn borer. Three cycles of selection [EPS12(S)C0, ESP12(S)C2, and EPS12(S)C3] from the maize synthetic population EPS12 and test crosses to inbred lines A639, B93, and EP42 were field grown and artificially infested with Mediterranean corn borer larvae, and the pith tissues were sampled for biochemical analyses. Two major simple phenolic acids [*p*-coumaric (*p*-CA) and *trans*-ferulic (*E*-FA) acids] were identified in free and cell-wall fractions, whereas four isomers of diferulic acid (DFA) (8−5′l, 5−5′, 8−o−4′, and 8−5′ benzofuran form) were present in the cell-wall bound fraction. The selection cycles EPS12(S)C0 and EPS12(S)C3 showed less damage and higher cell wall phenolics concentrations than the cycle EPS12(S)C2. In addition, higher concentrations of total DFAs were associated with shorter tunnel length and lower numbers of larvae per stem. The current study shows new and concrete evidence that the cell-wall bound phenolics could have a determinative role in the resistance to the Mediterranean corn borer, although future development of recurrent and divergent selection cycles will clarify this point.

KEYWORDS: ***Zea mays*; *Sesamia nonagrioides*; recurrent selection; phenolic compounds**

INTRODUCTION

Maize, *Zea mays* L., grown in northwestern Spain and the Mediterranean area is mainly exposed to Mediterranean corn borer, *Sesamia nonagrioides* (Lefèbvre) (Lepidoptera: Noctuidae), damage (*1*–*3*). Maize resistance to this borer has largely focused on the second generation of this pest, which feeds in the pith tissues, reducing plant growth and yield. Additionally, stem damage by borers increases plant lodging and helps the attack by other insect pests and microorganisms such as fungi or viruses (*4*).

Recurrent selection has been reported as successful for improving maize resistance against corn borers (*5, 6*). The BS9 is a maize population derived from the Iowa Stiff Stalk Synthetic and improved through recurrent selection for first and second generations of the European corn borer, *Ostrinia nubilalis* (Hübner) (*7*). To better understand host resistance to European corn borer, phytochemical changes over these cycles of selection have been studied. Buendgen et al. (*8*) investigated changes in the cell-wall composition of leaf sheaths and observed negative correlations between cell-wall constituents and European corn borer feeding damage. Nevertheless, the high concentrations of neutral and acid detergent fiber, cellulose, lignin, and ash in a high-silica population of maize (WFISIHI) did not render this population more resistant to second-generation European corn borer than the BS9 population, which had lower levels of these cell-wall constituents. In the presence of phenolic−carbohydrate complexes, fiber strength may increase, providing a tougher physical barrier to restrict insect penetration and render nutrients within the tissue less accessible (*9*). Bergvinson et al. (*9*) have pointed out that phenolic acids, in particular diferulic acids (DFAs), have increased over the cycles of selection in the population BS9 to render maize tissues more resistant through fortification of cell walls.

An intrapopulational recurrent selection program for improving Mediterranean corn borer resistance in the maize synthetic population EPS12 was initiated in the 1990s. The intensity of selection applied was 10%, selecting 10 families, of 100, with shortest stem tunnel length and yield above the 100-family mean. At this time three cycles of S_1-progeny selection have been completed in EPS12, and the direct response of the EPS12 population to reduce tunnel length damage, while maintaining

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yield, has been evaluated (*10*). Differences among cycles of selection were significant for tunnel length, and the linear decrease for this trait (-1.80 cm cycle^{-1}) achieved during selection accounted for most of these differences. In general, resistance to Mediterranean corn borer was improved, whereas yield was maintained (*10*).

Resistance may involve one or several mechanisms (physical as well as chemical) that could interact to determine the level of resistance for each genotype. Nevertheless, phenolic acids have been deeply studied as a line of defense against corn insect pests feeding on different tissues (*11–14*). Previous studies evaluated the role of hydroxycinnamic acids contents in maize pith and leaf sheaths in the resistance against the Mediterranean corn borer (*15, 16*). The amount of these compounds in the pith was correlated with the resistance level in the genotypes, with the resistant inbreds having the highest concentrations (*15*). In addition, significant negative correlations were found between larvae weight reared on leaf-sheaths and diferulic acid content (*16*). The genotypes used on those studies were inbred lines with diverse background and perhaps some particular mechanisms of defense, but work with populations, more closely related, could remove the background differences.

On the basis of these reports and the availability of a recurrent selection program to improve stalk resistance to the Mediterranean corn borer, the objectives of the present study were (1) to find out if three cycles of recurrent selection for Mediterranean corn borer resistance in the EPS12 maize population resulted in correlated changes in the phenolic concentrations in the pith and, therefore, could be related with the damage caused by this borer and (2) to determine if this relationship is consistent in crosses of cycles to specific testers.

MATERIALS AND METHODS

Plant Materials and Experimental Design. The S_1 recurrent selection program used to improve resistance of EPS12 against Mediterranean corn borer attack started in 1993. In 1994, 100 S_1 progenies were evaluated under artificial infestation with eggs of *S. nonagrioides*, and the 10 lines that showed the shortest stem tunnel length and yield above the mean of the 100 families were selected. In 1995 selected families were recombined, and the first cycle of recurrent selection EPS12(S)C1 was established in 1996. In a similar way, EPS12(S)C2 and EPS12(S)C3 were obtained in 1999 and 2002, respectively. Unfortunately, EPS12(S)C1 seeds were accidentally mixed with seeds from another maize synthetic, and they could not be included in the present study. Nevertheless, the selection process was not affected because S_1 families were obtained from EPS12(S)C1-Syn1 before recombination to obtain EPS12(S)C1 (*10, 17*). In 2002, seeds from the synthetics EPS12(S)C0, EPS12(S)C2, and EPS12(S)C3 were multiplied and test-crossed to inbred lines A639, B93, and EP42. The inbred lines A639 and B93 were reported as resistant to Mediterranean corn borer (*18, 19*) and EP42 was reported as susceptible (*19*). Further details about the selection program are included in ref *10*.

The three cycles of selection, EPS12(S)C0, EPS12(S)C2, and EPS12(S)C3, and test-crosses to A639, B93, and EP42 were grown at Pontevedra, a location in northwestern Spain (42° 25′ N, 8° 38′ W, and 20 m above sea level) in 2003 and 2004. The experimental design was a randomized complete block design with three replicates. Each experimental plot was hand-planted and consisted of four rows spaced 0.80 m apart with 25 two-plant hills spaced 0.21 m apart. Plots were overplanted and thinned, obtaining a final density of ≈60000 plants ha^{-1}.

To define accurately the genotype's silking time, plots were checked until 50% of plants showed silks. Eggs were obtained from a local population harvested the winter previous to infestations and reared in the laboratory according to the method described by Eizaguirre and Albajes (*20*). At silking, 10 plants were infested with a mass of about 40 eggs of Mediterranean corn borer, enough to guarantee damage, and placed between the shank of the main ear and the stem (*21*). According to previous studies the best time to collect samples for analyses was 30 days after silking (*15, 16*). At this time the fourth above-ground internode was hand-harvested. Five to eight noninfested plants were collected and pooled from each plot depending on the amount of tissue of each genotype. The pith was obtained by manual elimination of the rind and immediately frozen (−20 °C). Chemical analyses were performed in triplicate for each plot. At harvest, 10 infested plants were dissected, and tunnel lengths (centimeters) and number of larvae per stalk were measured as part of the evaluation of the selection program (*10*).

Extraction of Phenolic Compounds. Two fractions of phenolic compounds were extracted from the pith tissue samples: free phenolics and insoluble cell-wall bound phenolics. Extraction was based on a procedure previously described (*22*) with minor modifications. Freeze-dried pith samples were ground in a Pulverisette 14 (Fritsch GmbH) rotor mill with a 0.75 mm screen, and 1 g of material was extracted with 30 mL of 80% methanol. The suspension was homogenized for 30 s with a Heidolph mixer (Heidolph Instruments GmbH & Co. KG) before being centrifuged at 1000*g* for 10 min. After centrifugation, the pellet contained the insoluble cell-wall bound phenolics and the supernatant, the soluble phenolics.

The supernatant for free phenolic acids was concentrated in a Speed Vac (Savant Instruments, Holbrook, NY) to 20 mL. The aqueous solution was acidified using 6 N HCl to pH 2.0 before extraction with 20 mL of ethyl acetate. The ethyl acetate extract was reduced to dryness in a Speed Vac at medium settings without a radiant cover, and the resulting precipitate was resuspended in 3 mL of HPLC grade methanol. This solution was used to determine free phenolic compounds.

The pellet containing ester-bound phenols incorporated in the cell wall was then shaken in 20 mL of 2 N NaOH under nitrogen flow and darkness for 4 h. Digested samples were neutralized with 6 N HCl, and the pH was lowered to 2.0. After centrifugation, the supernatant was collected and the pellet washed twice with distilled water (10 mL each). Supernatants were pooled and then extracted twice with ethyl acetate (40 mL each). Collected organic fractions were combined and reduced to dryness using a Speed Vac for 4 h. The final extract was dissolved in 3 mL of HPLC methanol. All of the extracts were stored at −20 °C prior to HPLC analysis.

HPLC Analysis. Standards and samples were filtered through a 2 μm pore poly(tetrafluoroethylene) filter (Chromatographic Specialties, Brockville, ON) before analysis. Analyses were performed using a 2690 Waters Separations Module (Waters, Milford, MA) equipped with a 996 photodiode array detector (Waters) with a Waters YMC ODS-AM narrow-bore column (100 × 2 mm i.d.; 3 μm particle size). Elution conditions with a mobile phase system of acetotrinile (solvent A) and trifluoroacetic acid (0.05%) in water (solvent B) were as follows: initial conditions 10:90 (A/B), changing to 30:70 in 3.5 min, then to 32:68 in 6.5 min, then to 100:0 in 4 min, then isocratic elution with 100:0 for 4.5 min, finally returning to the initial conditions in 3 min. The mobile phase flow rate was 0.3 mL/min, and the total analysis time was 21.5 min. The sample injection volume was 4 μL, and the elution profiles were monitored online by UV absorbance at 325, 280, and 254 nm. Retention times were compared with freshly prepared standard solutions. Standards of the most common phenolics (caffeic acid, chlorogenic acid, ferulic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, and vanillic acid) were purchased from Sigma (St. Louis, MO). The identities of diferulates were confirmed by comparison with the autenthic 5−5′ standard and/or retention time and UV spectra previously published (*23*).

Statistical Analysis. Combined analyses of variance (ANOVA) for phenolic compounds concentration were computed with the PROC GLM procedure of SAS, version 9.1 (*24*). Year, replication, and their interactions were considered to be random factors. The sums of squares due to genotypes were orthogonally partitioned into cycles, test crosses to A639, test crosses to B93, test crosses to EP42, and between groups. The sums of squares of the genotype × environment interaction were similarly partitioned. Comparisons of means were made by the least significant difference method (LSD). Simple linear regressions among phenolic compounds and tunnel length and number of larvae per stem were made with the PROC REG procedure of SAS (*24*).

Table 1. Means for Tunnel Length (Centimeters), Number of Larvae per Stem, and Concentration (Micrograms per Gram of Dry Weight) of the Major Phenolics Identified in the Pith of the Three Cycles of S_1 Recurrent Selection[a] Grown in Pontevedra in 2003 and 2004

	cycles of S1 recurrent selection			
phenolic compound[b]	EPS12(S)CO	EPS12(S)C2	EPS12(S)C3	LSD ($P \leq 0.05$)
Free Phenolics				
p-CA	14.00a	14.00a	16.35a	
E-FA	5.60a	8.10a	6.75a	
Cell-Wall Phenolics				
p-CA	7096.0a	6566.8a	7412.1a	
E-FA	2925.9a	2523.2b	2873.5a	247.97
DFA 8−5′l	26.50a	16.14b	32.15a	9.54
DFA 5−5′	40.75a	38.22a	54.35a	
DFA 8−o−4′	77.50a	70.44a	83.10a	
DFA 8−5′b	74.80a	68.28a	70.80a	
DFAt[c]	219.55ab	193.08b	240.40a	38.46
Damage Traits				
tunnel length	59.00a	61.72a	55.10a	
larvae per stem	1.52ab	2.03a	1.13b	0.83

[a] Means within a row followed by the same letter are not significantly different ($P \leq 0.05$). Cycle EPS12(S)C1 was not included in this study because it was lost. [b] Phenolic compounds: *p*-CA, *p*-coumaric acid; *E*-FA, *trans*-ferulic acid. Diferulates: DFA 8−5′l (linear form); DFA 8−o−4′; DFA 8−5′b (benzofuran form). [c] DFAt: total diferulate content.

Table 2. Cycle Mean Concentrations (Micrograms per Gram of Dry Weight) (Boldface Font) and Mean Concentrations of the Major Phenolics Identified in the Pith of Three Cycles of S_1 Recurrent Selection[a] Test-Crossed to A639, B93, and EP42, Grown in Pontevedra in 2003 and 2004

	free phenolics		cell-wall bound phenolics						
genotype	*p*-CA	*E*-FA	*p-CA*	*E-FA*	DFA 8−5′l	DFA 5−5′	DFA 8−o−4'	DFA 8−5′b	DFAt[b]
cycle mean	14.78B	6.82A	7051.9BC	2788.9B	25.45AB	44.81B	77.40BC	71.47 A	219.12AB
test crosses to A639									
EPS12(S)C0 × A639	18.55a	12.00a	8640.6a	3383.9a	28.93a	56.40a	95.41a	90.26a	271.00a
EPS12(S)C2 × A639	22.25a	10.55a	8575.7a	3066.8a	30.45a	59.70a	83.40a	72.70a	246.25a
EPS12(S)C3 × A639	23.15a	6.70a	8204.6a	3065.0a	22.60a	61.95a	81.65a	67.75a	233.95a
LSD ($P < 0.05$)									
cycles × A639 mean	21.37A	9.75A	8473.6A	3171.9A	27.33A	59.35A	86.82AB	76.90A	250.40A
test crosses to B93									
EPS12(S)C0 × B93	13.18a	9.00a	6617.7a	2254.7a	16.25a	34.55a	59.75a	67.40a	177.95a
EPS12(S)C2 × B93	13.60a	7.65a	6587.9a	2274.0a	18.95a	42.89a	66.60a	67.10a	195.54a
EPS12(S)C3 × B93	13.90a	10.65a	6428.8a	2392.1a	19.40a	41.05a	68.05a	74.65a	203.15a
LSD ($P < 0.05$)									
cycles × B93 mean	13.56B	9.10A	6544.8C	2306.9C	18.20B	39.5B	64.80C	69.72A	192.21B
test crosses to EP42									
EPS12(S)C0 × EP42	24.80a	15.30a	6882.7a	2879.4a	21.10a	50.80a	85.00a	72.75a	229.65a
EPS12(S)C2 × EP42	15.00a	7.70a	7911.0a	3078.7a	24.55a	44.37a	92.55a	81.20a	242.67a
EPS12(S)C3 × EP42	14.75a	12.40a	7424.3a	3090.0a	26.80a	53.79a	98.78a	80.85a	260.22a
LSD ($P < 0.05$)									
cycles × EP42 mean	18.18AB	11.80A	7406.0B	3016.0AB	24.15AB	49.65AB	92.11A	78.27A	244.18A
LSD ($P < 0.05$) means	5.09		720.58	286.28		11.24	13.98		40.34

[a] Means within a column followed by the same lowercase or uppercase letter are not significantly different ($P \leq 0.05$). Cycle EPS12(S)C1 was not included in this study because it was lost. Phenolic compounds: *p*-CA, *p*-coumaric acid; *E*-FA, *trans*-ferulic acid. Diferulates: DFA 8−5′l (linear form); DFA 8−o−4′; DFA 8−5′b (benzofuran form). [b] DFAt, total diferulate content.

RESULTS AND DISCUSSION

In the current study, two major simple phenolic acids, *p*-coumaric acid (*p*-CA) and *trans*-ferulic acid (*E*-FA), were identified in both free and cell-wall fractions, whereas four isomers of diferulic acid (8−5′l, 5−5′, 8−o−4′, and 8−5′ benzofuran form) were present, particularly in the cell-wall bound fraction (**Tables 1** and **2**; **Figure 1**). Jointly with the simple monomers most of these isomers have been previously identified in the pith and sheath of maize (*15, 16*). Only the concentrations of the (*E*)-isomers of phenolic acids were quantified. Efforts were made in the extraction to minimize the photoisomerization reactions to which phenolic acids are susceptible (*25, 26*). Other free phenolics could not be identified with the standards used.

The lowest concentrations of free *p*-CA (13.60 μg/g of dry weight) and free *E*-FA (5.60 μg/g of dry weight) were quantified in the test cross EPS12(S)C2 × B93 and the cycle EPS12(S)C0, respectively, whereas the highest concentrations of free *p*-CA (22.25 μg/g of dry weight) and free *E*-FA (15.30 μg/g of dry weight) were quantified in the test crosses EPS12(S)C2 × A639 and EPS12(S)C0 × EP42, respectively (**Tables 1** and **2**). No significant differences were found between cycles for both free forms, suggesting that these compounds were not handled by selection for resistance. Previous studies showed comparable concentrations of these compounds in the pith of diverse inbred lines, but significant differences between resistant and susceptible genotypes to the Mediterranean corn borer were found (*27*). Nevertheless, in relation with their functionality in resistance,

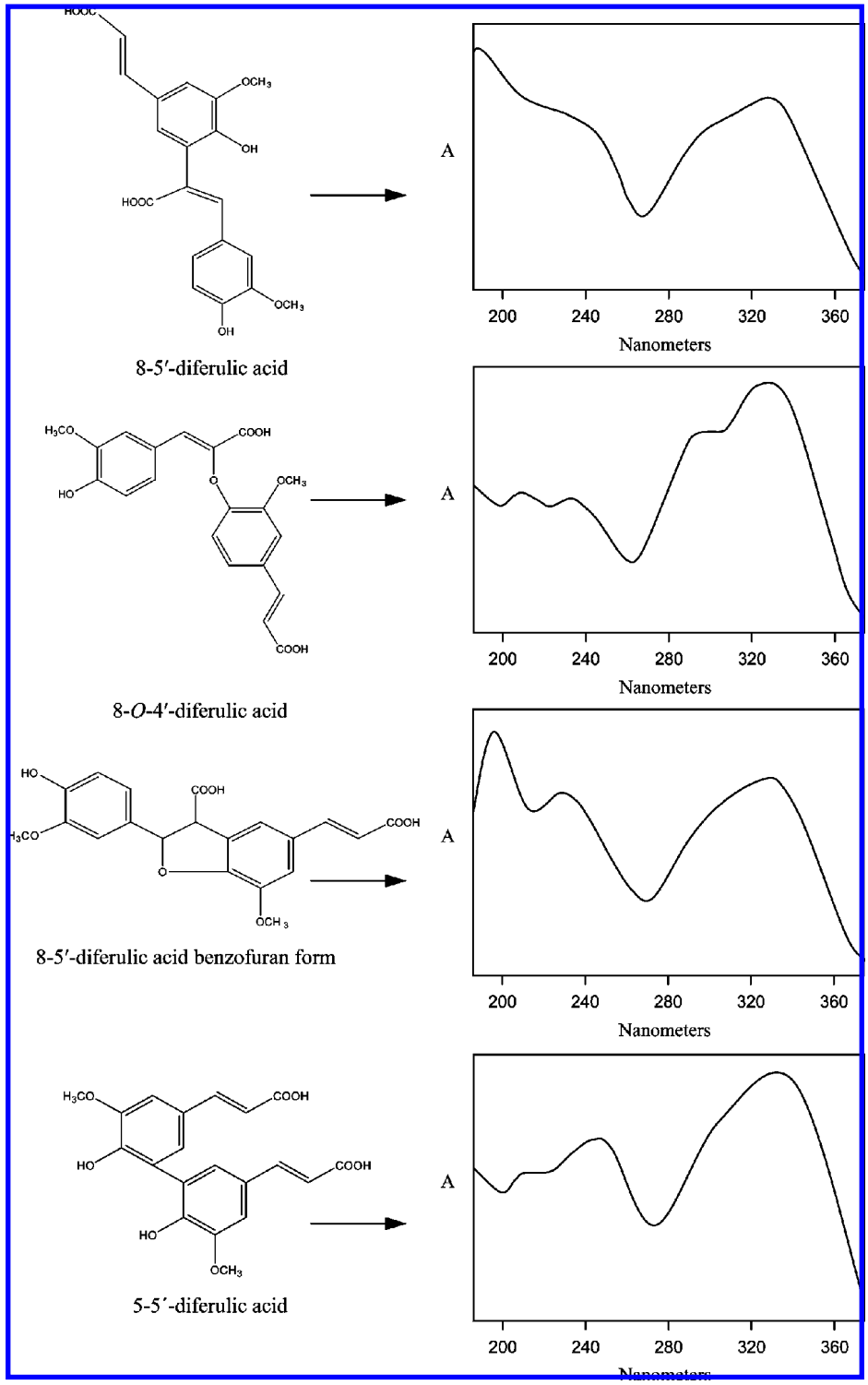

Figure 1. Absorption spectra and chemical structures of ferulic acid dehydrodimers (DFASs) occurring in the pith of maize cell walls.

the same authors suggested that these free forms could perform as a pool from which cell-wall bound forms are produced.

From an antibiotic approach, some studies have shown that free *p*-CA and free *E*-FA have no effect on the European corn borer (*28*), whereas later research showed a significant negative correlation between free *E*-FA concentration and European corn borer larval growth (*29*). Previous evaluations of the effect of four phenols (*p*-CA, *E*-FA, *p*-hydroxybenzaldehyde, and vanillin) on the growth of Mediterranean corn borer larvae showed that these compounds are not toxic to this borer in their free form under laboratory conditions (*30*). No significant changes between cycles of selection or test crosses for resistance to Mediterranean corn borer in the concentration of these compounds in the present evaluation suggest that free phenolics have no functionality as part of the resistant mechanisms to this borer in the maize synthetic EPS12. Additionally, no relationship with damage traits in the regression analysis was found.

The highest levels of *p*-CA and *E*-FA were released from alkaline treatment of the cell-wall bound phenolic fractions (**Tables 1** and **2**). The highest (8640.6 μg/g of dry weight) and lowest concentrations (6428.8 μg/g of dry weight) of cell-wall bound *p*-CA were quantified in the test crosses EPS12(S)C0 × A639 and EPS12(S)C3 × B93, respectively (**Table 2**), whereas the highest (3383.9 μg/g of dry weight) and lowest concentra-

tions (2254.7 μg/g of dry weight) of cell-wall bound *E*-FA were quantified in the test crosses EPS12(S)C0 × A639 and EPS12(S)C0 × B93, respectively (**Table 2**). Although *p*-CA was found to be the predominant species of the cell-wall phenolics in the pith of the genotypes tested, in accordance with previous studies (*15*), no significant differences were shown between cycles or test crosses. However, the cycle EPS12(S)C2, which showed the highest susceptibility to the Mediterranean corn borer attack, presented the lowest concentration for *E*-FA (**Table 1**).

Regarding the diferulate concentrations, the highest (32.15 μg/g of dry weight) and lowest (16.14 μg/g of dry weight) concentrations of DFA 8−5′ were quantified in the cycles EPS12(S)C3 and EPS12(S)C2, respectively, whereas for the other three isomers and total diferulate content (DFAt) the highest concentrations [from 61.96 μg/g of dry weight (DFA 5−5′) to 271.00 μg/g of dry weight (DFAt)] were quantified in the crosses to tester A639 and the lowest concentrations [from 34.55 μg/g of dry weight (DFA 5−5′) to 177.95 μg/g of dry weight (DFAt)] were measured on the crosses to tester B93 (**Tables 1** and **2**). DFA 8−5′ and DFAt showed the same trend of variation as *E*-FA, with significantly lower concentrations in the cycle EPS12(S)C2 than in the other cycles. In addition, the other isomers of diferulic acid showed a nonsignificant decrease in EPS12(S)C2 compared to EPS12(S)C0 and EPS12(S)C3. However, no significant differences in the diferulates concentrations were observed in the crosses to testers.

In the evaluation of the response of the EPS12 maize synthetic to recurrent selection for resistance to borers, Sandoya et al. (*10*) observed significant differences among cycles ($P < 0.01$) for tunnel length, EPS12(S)C3 being the least damaged cycle. The crosses to testers A639 and B93 showed similar decreases with significant differences between crosses to cycle EPS12(S)C0 and crosses to cycles EPS12(S)C2 and EPS12(S)C3. Besides, the number of larvae per stem showed a comparable trend, although this trait could be influence by natural infestation. On that study eight different environments, including damage by Mediterranean corn borer and European corn borer, were included; however, in the present study just two environments for Mediterranean corn borer damage were considered. Attending to these results, we found that the trend of variation for tunnel length and number of larvae per stem in the selection cycles showed the opposite trend of variation that we get for all of the cell-wall bound phenolics concentrations (**Table 1**). The selection cycles EPS12(S)C0 and EPS12(S)C3 showed less damage and higher cell wall phenolics concentrations than the cycle EPS12(S)C2, with more damage and lower concentrations (**Table 1**).

The concentration of cell-wall phenolics was not significantly different between cycles EPS12(S)C0 and EPS12(S)C3; nevertheless, although a previous study showed an increase of DFAt by recurrent selection for improving the resistance to European corn borer in the leaf tissue, this increase was not significantly different from cycle 0 after five cycles of selection were completed (*9*). In addition, a similar trend of variation was shown for DFAt in mature leaf tissues, with a decrease in the concentration in cycle 2 and an improvement along successive cycles (*9*). During for the development of new cycles of selection, the same increase in the cycle EPS12(S)C3 was observed in the present study.

Concerning the crosses to testers, no clear trend of variation in relation to damage was observed in the current study (data not shown), and, in addition, no significant differences were found for the phenolic concentrations (**Table 2**). Those results could be expected because the differences in range between cycles are always reduced in the crosses to testers with independence of the concentrations in the testers. Furthermore, inbred lines A639, B93, and EP42 were used in the evaluation of the selection program mainly because they represent different heterotic groups (Reid, Lancaster, and humid Spain heterotic groups, respectively). These genotypes could have a mechanism of resistance completely different against the Mediterranean corn borer and not related with the phenolic concentration, making unclear the relationship between the cell-wall phenolics and the resistance in the crosses.

Table 3. Simple Linear Regressions of Damage Traits on Phenolics Concentrations in the Pith of Three Cycles of S_1 Recurrent Selection Evaluated in 2003 and 2004

dependent variable	independent variable	intercept	*b* coefficient	Pr > *F*	R^2
tunnel length	DFA 8−o−4′	98.40	−0.516	0.10	0.97
tunnel length	DFAt	88.68	−0.138	0.10	0.97
larvae per stem	DFAt	5.70	−0.019	0.01	0.99
larvae per stem	DFA 8−o−4′	7.04	−0.071	0.01	0.99
larvae per stem	*p*-CA (cell-wall bound)	8.97	−0.001	0.04	0.99
larvae per stem	DFA 8−5′l	2.94	−0.055	0.06	0.99

It is interesting to note that the crosses to A639 showed significantly higher concentration than the cycles for the *p*-CA, *E*-FA, and DFA 5−5′, whereas crosses to B93 showed lower concentrations for all cell-wall bound phenolics, but significant for only *E*-FA (**Table 2**). Inbreds A639 and B93 will be evaluated in the future for cell-wall bound phenolic concentration to determine if the increase or decrease in crosses concentration could be related with the contents in the inbreds per se; in this sense, dominant effects in the inheritance of the phenolic concentration could be working.

The variability among cycles for tunnel length and larvae per stem was explained mostly by the concentrations of DFA 8−o−4′ and the total diferulic acid content (DFAt) ($P \leq 0.10$, $R^2 = 0.97$; and $P \leq 0.01$, $R^2 = 0.99$, respectively) (**Table 3**). Higher concentrations of DFAs are associated with shorter tunnel length and lower number of larvae per stem, reinforcing the possible role of DFAs in the resistance to the Mediterranean corn borer. From a structural approach, *E*-FA is esterified to arabinose subunits of arabinoxylan chains, and jointly with its dimers (DFAs) could cross-link arabinoxylans chains to each other and to lignin, enhancing the cell-wall strengthening and stiffening (*31*–*33*). In agreement with current results, the content of isomers of dehydrodiferulic acid in corn leaves was highly and negatively correlated across genotypes of maize with field leaf damage by the European corn borer (*34*), the southwestern corn borer, and the sugar cane borer (*14*). Regarding the pith tissue, DFA levels were negatively related with the number of tunnels by European corn borer (*9*). Furthermore, in relation with Mediterranean corn borer resistance, significant negative correlations were found between larva weight reared on leaf sheaths of diverse genotypes evaluated and the diferulic acid content of these genotypes (*16*).

In summary, the present study shows new and concrete evidence that the cell-wall bound phenolics could have a determinative role in resistance to the Mediterranean corn borer. On the other hand, to check if an increase in the resistance to this borer in the synthetic population EPS12 could be clearly related with an effective increase in the cell-wall bound phenolics concentrations, new selection cycles would be available in the future (three years per recurrent selection cycle). Furthermore, a selection program focusing on differences in the diferulate concentrations instead of differences due to the tunnel

length was initiated with the purpose of confirming the direct role of DFAs in resistance to Mediterranean corn borer larvae.

NOTE ADDED AFTER ASAP PUBLICATION

Tables 1 and **2** of the original posting of July 26, 2008, have been slightly modified with the posting of August 7, 2008.

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