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Relationships between Stalk Resistance and Corn Borers, Agronomic Traits, and Cell Wall Hydroxycinnamates in a Set of Recombinant Inbred Lines from a Maize MAGIC Population

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Abstract: Corn borers are the most important pest affecting maize. Resistance to corn borer attack may compromise plant fitness being detrimental for some important agronomic traits such as yield. Against the attack of this pest, cell wall-bound hydroxycinnamates have been previously described as a possible defense mechanism. In this study, agronomic characterization and cell wall-bound hydroxycinnamates quantification was performed in a subset of Recombinant Inbred Lines (RILs) from a Multiparent Advanced Generation Intercross (MAGIC) population that showed contrasting behavior against corn borer attack. Resistant lines showed greater concentration of p-coumaric acid, the only hydroxycinnamate that could have a role in the resistance in these particular materials. In addition, results indicated that resistant lines showed precocity, low grain moisture at harvest, and reduced plant height, thus, selecting for resistance may be detrimental for yield. In this way, a breeding strategy directly targeting grain yield in order to tolerate corn borer attack would be the recommended one.

Keywords: corn borer; yield; cell wall hydroxycinnamates; RILs; agronomic performance; resistance

1. Introduction

Maize is consumed by a large variety of herbivorous insects that have diverse feeding habits and consume many plant parts. Stem corn borers include diverse lepidopteran insects that feed on the pith of maize producing tunnels that can cause 30% yield losses, equivalent to world-wide losses of 311.3 million tons every year [1]. In the northwest of Spain, the average yield loss is around 15% and is mainly caused by the Mediterranean corn borer, Sesamia nonagrioides Lef. (MCB); the damage produced by the European corn borer, Ostrinia nubilalis (ECB), being less important. Both borers show similar life cycles and herbivorous behavior, but the MCB is more voracious than the ECB [2].

MCB infestation on maize begins at an early phenological stage in this area. After completing their first generation, that usually coincides with the plant juvenile stage, stem borers of the second generation attack the plants during the reproductive stage. The damage produced by second generation larvae causes the most important losses, decreasing forage, stover and grain yields as a consequence. Tunnels produced in the stalk pith interfere with nutrient assimilation moving toward the developing ear and increases the lodging rate [1,3]. Corn borers attack the ears by causing direct damage to kernels and increasing infections by mycotoxin-producing fungus [4,5].
Breeding for increased resistance to borers has been effective for reducing the length of tunnels made by borers but has provoked undesirable changes in agronomic traits [6–8]. Previous research indicated that recurrent selection for resistance to the European corn borer (ECB) resulted in decreased yielding ability [6,9] and changes in important agronomic traits [9]. Klenke et al. [6] found that four selection cycles reduced the damage from attacks by both the first and second generation ECB but decreased grain yield, suggesting that yield should be included in the selection criteria in a selection program. Likewise, recurrent selection to improve resistance to MCB also led to a decrease in yield, even when yield was included in the selection criteria [8].

On the other hand, several cell wall components have been associated with maize resistance to corn borers. Many studies have focused on studying the cell wall as a biochemical and structural barrier against insects [10–12]. In particular, the role of cell wall-bound hydroxycinnamates in cell wall strengthening, stiffening, and fortification has been deeply evaluated. The most important hydroxycinnamates are p-coumaric acid (PCA) and ferulic acid (FA). The accumulation of PCA is considered a relevant indicator of lignin deposition, one of the main components of cell wall fibers, and lignin has been directly related with corn borer resistance by increasing the rigidity of the cell wall [13,14]. On the other hand, FA can undergo dehydrodimerization, and the resulting dimers (diferulates, DFAs) crosslink heteroxylans that enhance cell wall stiffening and strengthening. Additionally, during lignification, FA and diferulic esters form crosslinks through the etherification of the phenolic hydroxy group to lignin polymers, producing a polysaccharide-lignin matrix [15–17]. Taking into account those functions, several studies have pointed out the role of hydroxycinnamates in plant resistance to pests and diseases [10,18–20]. Furthermore, negative correlations were observed between larval weight and DFA concentration in the leaf-sheath of maize [21], and between stem tunnelling by ECB and MCB, and total PCA and DFAs [19,22].

In addition, recurrent selection to improve resistance to corn borer attack has been demonstrated to influence cell wall-bound hydroxycinnamates concentration. Higher concentrations of total DFAs were associated with shorter tunnel lengths and fewer larvae per stem over cycles of selection to MCB resistance in the maize synthetic EPS12 [23]. The same relationship between resistance and DFA concentration was found when DFAs were the primary selection criteria [24].

Studies on the relationship between resistance and agronomic or biochemical traits have usually been carried out using a diverse set of genotypes (hybrids, inbred lines, populations) with different levels of resistance [18,25]. One of the problems of this approach is that each genotype comes from different genetic backgrounds and the relationships between them are not known, which can lead to false associations [26]. On the other hand, to study the relationships between resistance to corn borer and other traits, Recombinant Inbred Lines (RILs) populations derived from biparental crosses have also been used [27–29]. The derived RILs have a common genetic background and are developed randomly, by the single seed descending method, without selection or drift. The main disadvantage of this approach is the limited variability of the biparental populations, with only two parents, and great linkage disequilibrium [30]. To solve these problems, we have evaluated RILs from a MAGIC (Multiparent Advanced Generation Intercross) population of eight parents, with enough variability and reduced linkage disequilibrium, since six recombination cycles have been performed before starting with the RILs development.

Hence, in the current research, agronomic characterization, and cell wall-bound hydroxycinnamates quantification was performed in a subset of Recombinant Inbred Lines derived from a MAGIC population chosen for their contrasting behavior against corn borer attack. The main goals are to deepen the knowledge of: (1) the relationship between resistance to corn borer and the main agronomic traits, and (2) the role of cell wall-bound hydroxycinnamates as a defense mechanism against corn borers using RILs from the same genetic background that have been randomly developed from the MAGIC population.
2. Materials and Methods

2.1. Experimental Design, Plant and Insect Material

Misión Biológica de Galicia’s Maize Genetics and Breeding Group has developed a MAGIC population with 700 RILs (currently, there are approximately 600 lines that can be purchased on request). Details of further developing the MAGIC population was described in Butrón et al. [31] and Jiménez-Galindo et al. [32]. Jiménez-Galindo et al. [32] evaluated 680 RILs for resistance to corn borers and agronomic traits. Based on that evaluation and the seed availability for future evaluations, 56 RILs, presenting extremes values for tunnel length, were selected and classified into two groups according to their resistance to corn borer attack: (1) short tunnel length (resistant); (2) large tunnel length (susceptible).

Selected RILs, along with eight inbred lines chosen as corn borer attack checks, were evaluated for resistance to corn borer and agronomic traits (Supplementary Table S1). The inbred checks included five of the eight MAGIC population founders that are partially resistant to corn borer attack (A509, EP125, EP17, EP86, F473) and inbreds EP42, EP47, and EP80, which are susceptible to corn borer attack.

Selected RILs and checks were evaluated across two years (2016 and 2017) in Pontevedra (42°26′01″ N 8°38′51″ O). Each year, two adjacent trials were conducted with an 8 × 8 simple lattice design. The experimental plot consisted of 15 plants on an area of 2.2 m² and a density of 70,000 plants/ha.

One trial was protected with insecticide, treating the plants every 21 days from approximately 45 days after sowing until harvest; the other was infested with *S. nonagrioides* eggs prior to flowering. Standard procedures were followed for the insect rearing (carried out in Misión Biológica de Galicia) and production of *S. nonagrioides* eggs [33]. Before flowering, 10 plants from each plot were infested with ~40–80 *S. nonagrioides* eggs placed between the sheath and the stem in the internode below the main ear.

2.2. Resistance Trait (Tunnel Length)

At harvest, ten infested plants per plot were collected in infested trials. The stalks were split lengthwise, and the lengths of the tunnels (cm) produced by the larvae were measured.

2.3. Agronomic Traits

Agronomic traits were recorded in both the infested and protected trials.

2.4. Days to Silking/Anthesis

Considered as the time passed from the day of sowing until approximately 50% of the plants showed either pollen (male anthesis) or silks (female silking). They were recorded periodically by the time each plot started to show pollen and silks.

2.5. Plant Height

Considered as the mean plant height (in cm) of five plants per plot. Plant height was measured from the base to the tip of flag leaf after flowering and the average of five plants per plot was presented in this study.

2.6. Lodging

This was calculated as the sum of broken and leaning plants divided by the total number of plants in the plot, expressed in percentage. A plant was considered broken when it was split underneath the main ear and leaned when the stem formed an angle with the ground at less than 45°. It was recorded at harvest.

2.7. Grain Moisture

Expressed as a percentage, was recorded at harvest using a moisture meter Kett (model PM-400) in a sample of 240 cm³.
2.8. Grain Yield

Grain yield was calculated as the weight of grain in Mg ha\(^{-1}\) and adjusted at 14% grain moisture. It was determined by the following Equation (1).

\[
\text{Grain Yield (Mg ha}\^{-1}\) = \frac{\text{Plot weight (kg) \times (100 - Humidity) \times Grain weight of 5 ears (g) \times 10}}{\text{Surface (m}^2\) \times 86 \times \text{Total weight of 5 ears (g)}}
\] (1)

where grain moisture was estimated as described above; surface was calculated as the number of plants per plot multiplied by the space between rows (0.80 m) and the space between plants (0.18 m). The value 86 on the equation corresponds to a constant to adjust the yield at 14% humidity. Yields were calculated per plot and then transformed to Mg ha\(^{-1}\); thus, this refers to a maximum potential yield.

2.9. Biochemical Analysis

The second internode below the main ear was collected from five plants in each plot in protected trials. Samples were collected at 55 days after silking. For each harvested internode, the pith was manually detached and frozen at \(-20^\circ\)C. Then, samples were lyophilized and ground in a Wiley (Arthur H. Thomas, Philadelphia, PA, USA) mill with a 0.75 mm screen before being analyzed. A recently optimized protocol was used for hydroxycinnamate quantification [34]. Phenolic standards ferulic acid and p-coumaric acid were purchased from Sigma-Aldrich Químic SL, Madrid, Spain Sigma. The identities of FA dimers were confirmed by a comparison with the authentic 5–5 standard or published retention times and UV spectra. The total diferulate content (DFAT) was calculated as the sum of the following three identified and quantified DFA isomers: DFA 8–O–4, DFA 5–5, and DFA 8–5. The DFA 8–5 concentrations were calculated as the sum of 8–5-cyclic (or benzofuran)-DFA and 8–5-noncyclic (or open).

2.10. Statistical Analysis

Combined analyses across years were performed for each trait for protected and infested trials using the mixed model procedure of the SAS program (version 9.4) [35]. Means for each trait were calculated based on combined data across years. Lines were considered as fixed effects and years, replicates, and blocks as random effects. The comparison of means was carried out using least significant difference (LSD).

2.11. Contrast Analysis

After a variance analysis, the RILs were, again, qualitatively classified according to their BLUEs (Best Linear Unbiased Estimators) for tunnel length (under infestation) in resistant, susceptible, or intermediate. Resistant and susceptible groups were formed by 20 RILs in each. With the qualitative dataset, including the checks, mean comparisons were performed in order to determine the existence of significant differences for agronomic traits and cell wall-bound hydroxycinnamates between groups of RILs with contrasting values for resistance to corn borer. Discussion will focus on differences among resistant (short tunnel length) and susceptible (large tunnel length) groups.

2.12. Correlation Analysis

Genotypic and phenotypic correlations were performed among tunnel length and agronomic traits using Restricted Maximum Likelihood (REML) according to a published SAS mixed model procedure [36].

2.13. Multiple Linear Regression Analysis

In order to understand the role of cell wall-bound hydroxycinnamates as one of the defense mechanisms against corn borer, BLUEs estimates were used to build multiple linear regression models. The stepwise method following the PROC REG procedure in SAS was used [35]. We have considered as dependent variables the trait involved in stalk resistance.
to corn borer (tunnel length). On the other side, cell wall-bound hydroxycinnamates were considered as independent variables.

3. Results

As a starting point, in means comparison analyses, RILs differ significantly for every trait under study in both infestation conditions with the exception of grain moisture under infestation (Supplementary Tables S1 and S2). Among others, the RILs differed significantly in tunnel length under infestation conditions and that allowed the classification in resistant, medium, or susceptible for contrast analysis (Supplementary Tables S1 and S2).

3.1. Contrast Analysis

RILs were classified in three groups according to their tunnel length under infestation conditions: (1) short tunnel length (resistant, 5–26 cm); (2) medium tunnel length (26–44 cm); (3) large tunnel length (susceptible, 45–63 cm). The RIL of the resistant group with the shortest tunnels (26 cm) differed significantly ($p < 0.05$) from the RIL of the susceptible group with the largest tunnels (45 cm). Even though values for the checks and the medium RILs are included, for the discussion, we will only focus on differences among resistant (short tunnel length) and susceptible (large tunnel length) RILs. According to this classification, significant differences for agronomic traits between groups are also shown in Table 1.

Table 1. Contrast analysis for the large, short and medium tunnel length groups and checks of RILs classified according to resistance to corn borer. Means for agronomic traits and hydroxycinnamic acids with significant differences among groups are included.

<table>
<thead>
<tr>
<th>Tunnel Length (cm)</th>
<th>Large</th>
<th>Medium</th>
<th>Short</th>
<th>Checks</th>
<th>LSD $^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Under Control Condition (Insecticide Protected)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days to Anthesis</td>
<td>67.0 a</td>
<td>65.8 b</td>
<td>63.2 c</td>
<td>67.7 a</td>
<td>0.8</td>
</tr>
<tr>
<td>Days to Silking</td>
<td>68.5 ab</td>
<td>67.4 b</td>
<td>64.1 c</td>
<td>69.3 a</td>
<td>1.4</td>
</tr>
<tr>
<td>Grain Yield ($t \text{ ha}^{-1}$)</td>
<td>5.48 a</td>
<td>5.24 a</td>
<td>4.01 b</td>
<td>5.37 a</td>
<td>0.88</td>
</tr>
<tr>
<td>Grain Moisture (%)</td>
<td>19.3 a</td>
<td>17.8 ab</td>
<td>16.4 b</td>
<td>18.6 ab</td>
<td>2.4</td>
</tr>
<tr>
<td>Plant Height (cm)</td>
<td>181 a</td>
<td>170 b</td>
<td>157 c</td>
<td>181 d</td>
<td>5</td>
</tr>
<tr>
<td>Lodging (%)</td>
<td>13.23 ab</td>
<td>20.67 a</td>
<td>22.01 a</td>
<td>9.19 b</td>
<td>10.69</td>
</tr>
<tr>
<td>PCA (mg/g)</td>
<td>6.43 a</td>
<td>8.07 b</td>
<td>10.0 c</td>
<td>9.71 c</td>
<td>1.58</td>
</tr>
<tr>
<td>FA (mg/g)</td>
<td>1.68 a</td>
<td>1.69 a</td>
<td>1.60 a</td>
<td>2.42 b</td>
<td>0.51</td>
</tr>
<tr>
<td>DFA 5-5 (mg/g)</td>
<td>0.058 a</td>
<td>0.062 a</td>
<td>0.067 a</td>
<td>0.087 b</td>
<td>0.017</td>
</tr>
<tr>
<td>DFA 8-O-4 (mg/g)</td>
<td>0.090 a</td>
<td>0.096 a</td>
<td>0.097 a</td>
<td>0.134 b</td>
<td>0.029</td>
</tr>
<tr>
<td>DFA 8-5-1 (mg/g)</td>
<td>0.064 a</td>
<td>0.068 ab</td>
<td>0.065 a</td>
<td>0.084 b</td>
<td>0.011</td>
</tr>
<tr>
<td>DFA 8-5-b (mg/g)</td>
<td>0.083 a</td>
<td>0.090 a</td>
<td>0.089 a</td>
<td>0.127 b</td>
<td>0.017</td>
</tr>
<tr>
<td>DFA 8-5 (mg/g)</td>
<td>0.153 a</td>
<td>0.158 a</td>
<td>0.148 a</td>
<td>0.211 b</td>
<td>0.026</td>
</tr>
<tr>
<td>DFAT (mg/g)</td>
<td>0.301 a</td>
<td>0.317 a</td>
<td>0.312 a</td>
<td>0.435 b</td>
<td>0.079</td>
</tr>
</tbody>
</table>

| **Under Infestation Condition** | | | | | |
| Days to Anthesis   | 67.6 a | 66.0 ab | 63.2 b | 66 ab | 4.3 |
| Days to Silking    | 69.3 a | 67.3 b | 64.5 c | 69.9 a | 1.2 |
| Grain Yield ($t \text{ ha}^{-1}$) | 5.69 a | 5.59 a | 4.20 a | 5.52 a | 2.12 |
| Grain Moisture (%) | 16.7 a | 15.5 a | 14.8 a | 15.8 a | 5.1 |
| Plant Height (cm)  | 176 a | 162 ab | 146 b | 165 ab | 26 |
| Lodging (%)        | 40.03 a | 43.26 a | 50.40 a | 43.03 a | 30.21 |

$a$. Least significant differences between means at the 0.05 significant level, according to Fisher protected LSD method. Means showing the same letter do not differ significantly. PCA: p-coumaric acid; FA: Ferulic acid; DFA5-5: diferulic acid 5-5; DFA 8-O-4: Diferulic acid 8-O-4; DFA85: Diferulic acid 8-5-Linear; DFA85b: Diferulic acid 8-5-Benzofuran; DFA85: Diferulic acid 8-5; DFAT: Total diferulic acids content.

RILs classified as resistant to corn borer attack, under control conditions, were the earliest (63–64 days to anthesis, silking), the smallest (157 cm) and the driest (16.4%), in addition to producing a lower yield ($4 \text{ t ha}^{-1}$). Furthermore, at a biochemical level, resistant lines presented higher concentrations of PCA (10 mg/g).
Under infestation with MCB eggs, resistant lines were also the earliest, showing similar flowering dates as in controlled conditions, and the smallest (10 cm smaller than the plants in control conditions). Nevertheless, resistant and susceptible RILs did not differ in grain yield and moisture.

3.2. Correlation Analysis

The relationships among agronomic traits, under both infestation conditions, were evaluated through genotypic and phenotypic correlations. Correlation coefficients are shown in Table 2. In this section and further in the discussion, we will focus on correlations above 0.50 (absolute value) highlighted in bold in Table 2. Apart from days to anthesis and silking (rp 0.87 c, 0.93 ui), we did not find any other important phenotypic correlation under both infestation conditions: control (c), under infestation (ui). On the other hand, we found a strong positive genotypic correlation between tunnel length and grain yield (rg 0.56 c, 0.68 ui) and moisture (rg 0.54 c, 1 ui) and between tunnel length and plant height (rg 0.57 c, 0.60 ui) both in infested and control trials. Under infestation, we found strong genotypic correlations between flowering time and grain moisture (rg 0.71, 0.55) and between plant height and grain yield (rg 0.56).

Table 2. Genotypic (above diagonal) and phenotypic (below diagonal).

<table>
<thead>
<tr>
<th></th>
<th>Tunnel Length</th>
<th>Plant Height</th>
<th>Days to Anthesis</th>
<th>Days to Silking</th>
<th>Grain Yield</th>
<th>Lodging</th>
<th>Grain Moisture</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Under Control Condition (Insecticide Protected)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tunnel Length</td>
<td>0.57 *</td>
<td>0.47 *</td>
<td>0.43 *</td>
<td>0.56 *</td>
<td>−0.12</td>
<td>0.54 *</td>
<td></td>
</tr>
<tr>
<td>Plant Height</td>
<td>0.32 *</td>
<td>0.39 *</td>
<td>0.42 *</td>
<td>0.63 *</td>
<td>0.24</td>
<td>0.39 *</td>
<td></td>
</tr>
<tr>
<td>Days to Anthesis</td>
<td>0.27 *</td>
<td>0.18</td>
<td></td>
<td>0.96 *</td>
<td>0.04</td>
<td>0.08</td>
<td>0.37 *</td>
</tr>
<tr>
<td>Days to Silking</td>
<td>0.23 *</td>
<td>0.19</td>
<td>0.87 *</td>
<td>−0.07</td>
<td>0.04</td>
<td>0.34 *</td>
<td></td>
</tr>
<tr>
<td>Grain Yield</td>
<td>0.13</td>
<td>0.20 *</td>
<td>−0.07</td>
<td>−0.13</td>
<td>0.10 *</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>Lodging</td>
<td>−0.09</td>
<td>0.10</td>
<td>−0.04</td>
<td>−0.03</td>
<td>0.18</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>Grain Moisture</td>
<td>0.30 *</td>
<td>0.15</td>
<td>0.25 *</td>
<td>0.24 *</td>
<td>0.16 *</td>
<td>0.13</td>
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</table>

<table>
<thead>
<tr>
<th></th>
<th>Tunnel Length</th>
<th>Plant Height</th>
<th>Days to Anthesis</th>
<th>Days to Silking</th>
<th>Grain Yield</th>
<th>Lodging</th>
<th>Grain Moisture</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Under Infestation Condition</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tunnel Length</td>
<td>0.60 *</td>
<td>0.43 *</td>
<td>0.48 *</td>
<td>0.68 *</td>
<td>−0.04</td>
<td>1 *</td>
<td></td>
</tr>
<tr>
<td>Plant Height</td>
<td>0.43 *</td>
<td>0.32</td>
<td>0.35</td>
<td>0.56 *</td>
<td>−0.11</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>Days to Anthesis</td>
<td>0.13</td>
<td>0.07</td>
<td>0.97 *</td>
<td>0.10</td>
<td>−0.10</td>
<td>0.71 *</td>
<td></td>
</tr>
<tr>
<td>Days to Silking</td>
<td>0.13</td>
<td>0.06</td>
<td>0.93 *</td>
<td>0.04</td>
<td>−0.09</td>
<td>0.55 *</td>
<td></td>
</tr>
<tr>
<td>Grain Yield</td>
<td>0.22 *</td>
<td>0.38 *</td>
<td>0.05</td>
<td>−0.10</td>
<td>0.04</td>
<td>0.44</td>
<td></td>
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<tr>
<td>Lodging</td>
<td>0.02</td>
<td>0.08</td>
<td>−0.01</td>
<td>−0.06</td>
<td>0.17</td>
<td>0.41</td>
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<tr>
<td>Grain Moisture</td>
<td>0.08</td>
<td>0.17</td>
<td>0.19</td>
<td>0.21 *</td>
<td>0.16</td>
<td>0.15</td>
<td></td>
</tr>
</tbody>
</table>

* Significant correlation coefficient because it exceeded twice its standard error. † Tunnel length data comes from trial under infestation condition. Correlations above 0.50 (in absolute value) are highlighted in bold

3.3. Multiple Linear Regression Analysis

In order to understand the role of cell wall-bound hydroxycinnamates as a mechanism of defense against corn borers, we performed multiple linear regression models. We considered tunnel length (under infestation) as a dependent variable and cell wall-bound hydroxycinnamates as independent variables. The best model for tunnel length explained 53% of the variance, mainly by PCA (15%), FA (28%) and DFA 5-5 (5%) and DFAT (4%) (Table 3).
Table 3. Multiple linear regression models (using stepwise selection) of tunnel length on hydroxycinematic acids.

<table>
<thead>
<tr>
<th>Step Wise Selection</th>
<th>Tunnel Length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Step</strong></td>
<td><strong>Variable introduced in the Model</strong></td>
</tr>
<tr>
<td>1</td>
<td>PCA (mg/g)</td>
</tr>
<tr>
<td>2</td>
<td>FA (mg/g)</td>
</tr>
<tr>
<td>3</td>
<td>DFA 5-5 (mg/g)</td>
</tr>
<tr>
<td>4</td>
<td>DFAT (mg/g)</td>
</tr>
<tr>
<td><strong>Model</strong></td>
<td>TUNNEL LENGTH: 42.13−3.08 × PCA + 5.87 × FA + 132.1 × DFA-8-5-1</td>
</tr>
</tbody>
</table>

R^2 partial: percentage of the variance explained by each independent variable; R^2: Total percentage of the variance explained by the model. PCA: p-coumaric acid; FA: Ferulic acid; DFA 5-5: Diferulic acid 5-5; DFAT: total diferulates content.

4. Discussion

In order to achieve an accurate phenotyping, we repeated the evaluation of MAGIC RILs performed by Jiménez-Galindo et al. [32] selecting those presenting extreme values for tunnel length and according to their seed availability for future evaluations, resulting in a total of 56 RILS [32]. From those 56, another selection according to tunnel length under infestation was performed for contrast analysis, resulting in three groups: resistant, intermediate, and susceptible. Further on we will discuss differences between resistant and susceptible RILs.

4.1. Relationship between Resistance to Corn Borer and Main Agronomic Traits

Resistant and susceptible RILs differed in agronomic performance under both infestation conditions. Statistical analyses indicated that RILs presenting greater tunnel length, hence classified as susceptible to corn borer attack, were taller, presented with delayed maturity and had greater grain moisture at harvest. Besides, susceptible RILs yielded significantly more than resistant RILs under controlled conditions. These results are in agreement with previous findings observed in diverse genetic backgrounds [5,37–40].

The relationship between flowering time and resistance to corn borer attack is complex, and results from experimentation may be contradictory since this is a trait dependent on a lot of factors, such as the genetic background or the infestation time. Thereby, our results agree with those obtained by Jimenez-Galindo et al. [41], Santiago et al. [18], and Krakowsky et al. [42], but are opposite to those presented by Ordás et al. [27], Samayoa et al. [39], and Bohn et al. [43].

Late flowering genotypes are related to greater plant height, vigor, and yield; traits that have been negatively correlated to resistance to corn borer attack [27,44]. In northwestern Europe, the damage produced by second generation larvae causes the most important losses, damaging the plants during the reproductive stage [1,3]. An agronomic strategy proposed in order to alleviate the larvae damage is to advance the sowing time producing similar effects of those of early flowering. Early genotypes, with fast growth rate, would be in an advanced developmental stage by the time the MCB infestation peak occurs. Therefore, precocity would favor resistance to corn borer. Along with this, it has been demonstrated that tissue toughness, and cell wall lignification and fortification increase with plant maturity, interfering with the larvae progress.

The relationship between tunnel length and flowering time is clear in the contrast analysis, but it was not observed in correlation analysis in the infection condition. We identified genetic correlations above 0.5 between flowering time and grain moisture. Those results indicate that tunnels produced by the larvae under infestation conditions cause a delay in grain maturation [45] and could suggest that the period between flowering until grain maturation is more decisive in resistance than the period before flowering.
Moving on in our results, we found a high genetic correlation between plant height and tunnel length. Velasco et al. [46] hypothesized that taller plants would provide larger damage extent to the larvae. In an F2 derived from the cross of B73 and Dc811, Krakowsky et al. [42] found positive genetic correlations between stalk tunneling and ear height, which is highly correlated with plant height, indicating that the length of the stalk may be a limiting issue in the larvae-lant interaction. The above is supported to a greater extent if the positive relationship between plant height and days to flowering is taken into account: tall plants presented delayed maturity and greater susceptibility. The same correlations between plant height and tunnel length were found by Samayoa et al. [39] in bi-parental populations derived from the cross of resistant and susceptible inbred lines, and from crosses of a tolerant and sensible line [27,41]. In relation to these observations, several authors have also found QTL co-localization between plant height and stalk tunneling, reporting that favorable alleles increased the values for both traits, indicating an important genomic region in relation to resistance and its influence in agronomic traits [27,39,47]. This association between traits need to be considered in order to breed for borer resistance.

Finally, susceptible RILs yielded significantly more than resistant RILs under protected conditions, and we observed a strong, positive genetic correlation between grain yield and tunnel length under both infestation conditions. Breeding for increased resistance to corn borer attack has been related to decreases in grain yield [3–6], which agrees with our results. In contrast analysis, under infestation, we did not observe differences in grain yield between resistant and susceptible groups, which was contrary to controlled conditions. This could be explained because resistant RILs invest in constitutive defenses against the pest, which may imply a reduction of its yield. Under protected conditions, susceptible RILs do not suffer the attack of the plague producing significantly greater grain yield than resistant RILs, since they do not produce constitutive defenses. However, these differences are no longer significant when the RILs are exposed to the attack of the larvae: resistant RILs maintain their yield because of their defense mechanism and, in contrast, susceptible RILs see their grain yield compromised.

To sum up, greater tunnel length has been related to delayed maturity, which corresponds to a lower development of the plant tissues at the time of the larvae attack and greater grain moisture at the time of harvest. Delayed maturity also correlates with taller plants that present a greater extent to be consumed by the larvae. Lastly, susceptible RILs presented greater grain yield than resistant RILs when not exposed to the pest.

4.2. Role of Cell Wall Hydroxycinnamates as Defence Mechanism against Corn Borers

Resistant and susceptible RILs differed in cell wall-bound hydroxycinnamates content under both infestation conditions. From a biochemical point of view, the cell wall fortification mediated by hydroxycinnamates has been proposed as a defense mechanism against diverse pests [48]. Specifically, Santiago et al. [7] observed significant variation for cell wall phenylpropanoids in maize inbred lines presenting a wide range of susceptibility against *S. nonagrioides* attack. They observed significant differences in FA and DFATs pith concentrations, being greater in resistant inbreds. Subsequently, a successful selection for higher DFATs in maize pith resulted in increased resistance to corn borer attack, as a result of increases in the cell wall stiffening and strengthening by crosslinking hemicellulose chains [24]. However, in the current study, we did not observe an influence of DFATs on tunnel length. Even so, we did find in the regression analysis a positive influence of FA in greater tunnel length, the opposite of what was found by Santiago et al. [7]. There is a dependency relationship between FA and DFAT, since DFAT are a consequence of FA monomer dimerization, which is supported by strong genotypic and phenotypic correlations between those traits [49,50]. In this sense, greater concentrations of FA could indicate lower dimerization, hence lower crosslink, and stiffening. This would explain the major positive effect of FA in the tunnel length regression model.

On the other hand, a higher concentration of PCA monomer in the pith of maize was related to higher resistance to corn borer damage in both the regression model and contrast
analysis. Increases in PCA concentration have previously been observed in both pith [18,19] and rind tissues [18] of maize resistant inbreds. In subsequent studies, Santiago et al. [51] concluded that alleles for increased ester hydroxycinnamates content, affecting one or more hydroxycinnamate compounds, could be associated with increased stem resistance to MCB; PCA being the hydroxycinnamate with the highest contribution to it. Furthermore, in a recent study, Gesteiro et al. [52] selected 10 F_{2.3} families with contrasting values for PCA, DFAT, and tunnel length in order to elucidate how cell wall-bound phenolic affects borer resistance. They observed a negative correlation between tunnel length and PCA concentration, in agreement with our results.

In maize, lignins (primarily syringyl units) are acylated at the γ-position by p-coumarates [53]. Most PCA accretion occurs in tandem with lignification and its accumulation could be considered a relevant indicator of lignin deposition. The fact that, in Gesteiro et al. [8], families showing the highest levels of PCA also showed the highest proportion of subunit S, suggests that the role of PCA in resistance could be associated not only to the lignin content but also to the lignin composition and structure [2]. S lignin, indirectly associated to more PCA acetylation, has been noted in resistance against different biotic stresses [52,54,55].

To sum up, the present study using MAGIC RILS adds to the list of evidence for the potential role of PCA in pest resistance. However, in this vegetal material, stiffening and crosslinking of DFAs does not take a part in the resistance to corn borer attack.

5. Conclusions

Corn borer resistant RILs showed precocity, low grain moisture at harvest, and reduced plant height. We observed that the negative correlation between yield and tunnel length, previously observed in other vegetal materials, is maintained after all of the recombination events in this MAGIC population. Therefore, breeding using the tunnel length criteria should be carried out with caution because this may compromise plant fitness and grain yield. In this case, we recommend a breeding strategy directly targeting grain yield.

Furthermore, taking account of a higher level of PCA acetylation in the selected materials as a secondary criteria could be advisable in future breeding programs, although negative correlations between plant fitness and p-coumarilation need to be addressed.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy11061132/s1, Supplementary Table S1: Means for agronomic traits and cell wall-bound hydroxycinnamates in protected from infestation and under infestation conditions of the RILs evaluated. Supplementary Table S2: Means for agronomic traits under infestation conditions of the RILs evaluated.

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