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Phenol Contents, Oxidase Activities, and the Resistance of Coffee to the Leaf Miner *Leucoptera coffeella*

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Abstract We examined the role of phenolic compounds, and the enzymes peroxidase and polyphenol oxidase, in the expression of resistance of coffee plants to *Leucoptera coffeella* (Lepidoptera: Lyonetiidae). The concentrations of total soluble phenols and chlorogenic acid (5-caffeoylquinic acid), and the activities of the oxidative enzymes peroxidase (POD) and polyphenol oxidase (PPO), were estimated in leaves of *Coffea arabica*, *C. racemosa*, and progenies of crosses between these species, which have different levels of resistance, before and after attack by this insect. The results indicate that phenols do not play a central role in resistance to the coffee leaf miner. Differences were detected between the parental species in terms of total soluble phenol concentrations and activities of the oxidative enzymes. However, resistant and susceptible hybrid plants did not differ in any of these characteristics. Significant induction of chlorogenic acid and PPO was only found in *C. racemosa*, the parental donator of the resistance genes against *L. coffeella*. High-performance liquid chromatography (HPLC) analysis also showed qualitative similarity between hybrids and the susceptible *C. arabica*. These results suggest that the phenolic content and activities of POD and PPO in response to the attack by the leaf miner may not be a strong evidence of their participation in direct defensive mechanisms.

Keywords Coffee · Insect resistance · *Leucoptera coffeella* · Peroxidase · Polyphenol oxidase · Phenolics · Chlorogenic acid

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Introduction

Variation in the concentration of plant phenolics and increased activities of oxidative enzymes in response to insect attack are common phenomena in plants (Felton et al., 1994; Bi et al., 1997; Constabel and Ryan, 1998; Stout et al., 1998; Constabel et al., 2000). The relationship between reduced growth of Lepidopteran larvae and phenolic compounds in attacked plants has been described in several reports (Hartley and Firn, 1989; Stamp 1990; Felton et al., 1992b; Horwath and Stamp, 1993; Stamp and Yang, 1996). However, studies of plant metabolic pathways that are activated in response to insect attack are relatively recent, and the results are far from conclusive.

Phenolic oxidation catalyzed by polyphenol oxidase (PPO) and peroxidase (POD) is potentially a plant defense mechanism against herbivorous insects. Covalent bonds formed between oxidized phenols (quinones) and leaf proteins can alter the availability of amino acids (Felton et al., 1989). Alkylation of amino acids, especially essential amino acids, can result in reduced nutritional value of plant proteins for insects (Felton et al., 1992a; Stout et al., 1998). Also, quinones can be directly toxic to herbivores (Duffey and Stout, 1996), and the cyclic reduction of oxidized phenols can form reactive oxygen species ($^{\bullet}OH$, H_2O_2, and $O_2^{\bullet-}$), that damage essential nutrients and/or complex molecules, such as lipids, proteins, and nucleic acids (Bi and Felton, 1995).

The leaf miner, *Leucoptera coffeella* (Guérin-Méneville, 1842) (Lepidoptera: Lyonetiidae), the main insect pest of coffee plants in Brazil, is also an important primary pest in other coffee-producing countries. It is a microlepidopteran, in which butterflies only lays eggs in the adaxial leaf surface during the night. After a period of approx. 5 d, larvae arise directly from eggs to the leaf mesophyll, without contact with the external environment (Parra 1985). Larvae feed exclusively on palisade parenchyma cells, which in coffee leaves are formed by a single layer of cells (Deddeca 1957). On average, after 10 d the larva leave the leaf mesophyll to pupate, which lasts approx. 5 d. Adults survive on average 10 d under laboratory conditions (Parra 1985). Leaves attacked by this leaf miner usually fall and, depending on the defoliation intensity, yield loss, may account for 50% losses in coffee production (Paulini et al., 1976; Thomaziello et al., 1979).

The genus *Coffea* includes two commercially important species, both susceptible to *L. coffeella*: *C. arabica* L. and *C. canephora* Pierre. Seventy percent of commercial coffee plantations use Arabic-type plants, because of the superior quality of their beans (Pereira 2000).

A strategy for the development of cultivars resistant to leaf miners has been the transfer of resistance genes from the wild species *C. racemosa*, via successive back crosses to *C. arabica*. Although resistance to this pest is conferred by two complementary and dominant genes, little is known about the biochemical nature of the resistance (Guerreiro-Filho et al., 1999). There have been few studies on the biochemical nature of resistance of coffee plants to the leaf miner. Recently, Guerreiro-Filho and Mazzafera (2000) studied plants from 13 *Coffea* Species and *C. arabica/C. racemosa* hybrids, and concluded that resistance is not related to leaf concentrations of caffeine. The differences observed in the leaf structures of *C. arabica*, *C. racemosa*, and susceptible and resistant hybrids are not related to their resistance to leaf miners (Ramiro et al., 2004). However, Ramiro et al. (2004) observed reduced insect growth on leaves of resistant plants. This suggests that chemical substances in the palisade parenchyma might interfere with feeding and the consequent development of leaf miners.

Chlorogenic acid is a general term used to identify a series of esters formed by quinic acid with cinnamic acids (Clifford 1985). Among several isomers found in coffee,

5-caffeoylquinic acid is the most abundant in *C. arabica* seeds (Clifford 1985) and leaves (Mazzafera and Robinson, 2000). This phenol is susceptible to the action of PPO and POD and can be rapidly converted by PPO into chlorogenoquinone, a highly reactive molecule capable of bonding covalently to other molecules, principally proteins, making them less available for assimilation by the digestive tract of insects (Felton et al., 1989). The quantity of constitutive PPO in coffee leaves is relatively high compared to that found in other species. However, induction of this enzyme by mechanical wounding and by treatment with methyl jasmonate is limited (Mazzafera and Robinson, 2000).

Our objective was to analyze the physiological importance of phenolic oxidation and its role in resistance of coffee plants to attack by *L. coffeella*, in *C. arabica*, *C. racemosa*, and in some of their hybrids presenting different levels of resistance.

Methods and Materials

Plant Material

Analyses were carried out on healthy leaves of mature coffee plants maintained under field conditions at the Experimental Center of Instituto Agronômico (IAC), Campinas (SP), Brazil. Plants received the same agricultural practices, such as fertilization and weed control. Plants were not subjected to drought or cold stress, and did not receive any form of chemical treatment to control pests in order to prevent any detrimental effect on the experiments. Plants were divided into four groups: (1) *C. arabica* (susceptible); (2) *C. racemosa* (resistant); (3) susceptible hybrids; and (4) resistant hybrids. The hybrids belong to two progenies of advanced generations of crosses between *C. arabica* and *C. racemosa*, obtained through genetic breeding to produce coffee plants resistant to the leaf miner *L. coffeella*.

Five individuals from each group were chosen by using plant vigor and resistance or susceptibility as selection criteria. They were as follows: *C. arabica* cultivar Obatã IAC-1669-20, lot 100 (C 400, C 215, C 393, C 576, C 569); *C. racemosa* (H6608-1, H6611-1, H6593-1, H6593-3, IAC 5057); *C. arabica* × *C. racemosa*—resistant (H14954-7, H14954-29, H14954-37, H14954-45, H14954-46); *C. arabica* × *C. racemosa*—susceptible (H13685-1-10, H13685-1-2, H14949-14, H13376-8, H13685-1-26). The alphanumeric designators in parenthesis refer to the identification of each individual plant.

Resistance Evaluation

Insects were produced in rearing cages, using the method described by Katiyar and Ferrer (1968) and adapted by Parra (1985). Cages made with voile tissue and containing infested plants were maintained in a room with high humidity, and a 10% aqueous sucrose solution was offered to insects by wetting a filter paper placed inside of the cage. Coffee plant resistance was evaluated according to Guerreiro-Filho (1994), who validated the method by comparing laboratory evaluations with nursery and field evaluations. Here, healthy mature leaves were collected from the field, with their peduncles immersed in distilled water in assay tubes. Leaves were exposed overnight to insect oviposition in the cages. The next day, using a stereo microscope, a place on the adaxial leaf surface containing three eggs was chosen, and a leaf disk (1 cm diam) was removed with a cork borer. Nine disks were obtained for each plant, and they were transferred into a plastic box containing foam in the bottom. The box was closed with transparent glass and placed under dim laboratory light at room temperature (25 ± 2°C). Humidity inside the boxes was maintained by adding

distilled water to the foam. Evaluation of the degree of resistance was made after 10 d of infestation using a scale from 1 to 4 (Guerreiro-Filho et al., 1999): 1 = point lesions (resistant); 2 = small filiform lesions (moderately resistant); 3 = large irregular lesions (moderately susceptible); 4 = large rounded lesions (susceptible). The experimental model adopted for data analysis was a fully randomized allocation, with nine replications (disks), and with the factors "group" and "plants" being analyzed by nested analysis of variance (ANOVA).

Biochemical Analyses

Biochemical analyses consisted of comparative analyses between infested and uninfested leaves in different stages of insect development. For extraction of total soluble phenols, 20 healthy mature leaves were collected from each plant in the field. Ten leaves were infested in the insect rearing cages as previously described, whereas 10 others remained uninfested. Except for exposure to insects, all leaves were maintained under the same conditions. In the case of leaves exposed to leaf miners, eggs deposited on one side of the central leaf vein were eliminated with a scalpel, leaving only those deposited on the other side. Eggs were eliminated under a stereo microscope (Guerreiro-Filho et al., 1999). Infested leaves were maintained in humid boxes until 2 d after eclosion of the caterpillars. The uninfested leaves were maintained in separate boxes under the same conditions and for the same period of time. After eclosion, a 25 mm^2 leaf disk was removed from each side of the central vein of the leaves (sides with and without lesions). On the infested sides, leaf disks were taken from undamaged areas, near the lesions. Soon after they were removed, the disks were individually identified, weighed, and maintained on ice for further extraction. For phenol extractions, each leaf disk was placed into 3 ml ethanol, in screw-top vials, and maintained in a water bath at 50°C until the leaf tissue was completely discolored. Total soluble phenols (Swain and Hillis, 1959) and chlorogenic acid (5-caffeoylquinic acid) (Mazzafera 1999) were determined in this extract. Chlorogenic acid was used as a standard to build a calibration curve ($y = 0.01028x + 0.043$, $R = 0.9936$) to measure total soluble phenols.

A chromatographic profile of the phenolic compounds was obtained with a Shimadzu high-performance liquid chromatography (HPLC), equipped with a diode array detector. Detection of compounds was monitored between 190 and 400 nm. The detector signal was acquired with a workstation, using the Shimadzu Class VP system. An LC18 reverse phase column (Supelco) was used for separation. The separation gradient was 0–70% methanol in 0.5% sodium acetate for 25 min, progressing from 70% to 100% in 25–26 min, and maintained at 100% methanol until 35 min. The solvent flow was 1 ml/min. Quantification of chlorogenic acid for each treatment was made by calculating the areas of the graphs at 326 nm and compared to those obtained with pure chlorogenic acid (Sigma, St. Louis, MO, USA). The elution time of chlorogenic acid was 10 min. Data were analyzed by using a factorial model (infestation group) in a completely randomized experimental design, with five replications (plants). Chlorogenic acid concentration indices were transformed using the equation $y = \log(x + 1)$.

To determine POD and PPO activities, leaves were removed from each plant, placed into leaf-miner-infested cages, and transferred into humid chambers. They were analyzed 1 d (1 DAE) or 4 d (4 DAE) after eclosion of the larva. Controls were uninfested leaves maintained in the humid chambers during the same periods as the infested leaves. Freshly sampled leaves were also collected for analysis. The leaves of five plants were used for each group.

As eclosion of coffee leaf miner eggs occurs approximately 5 d after egg laying (Parra 1985), leaf segments 1 and 4 DAE were evaluated 7 and 10 d, respectively, after the leaves were collected from the field.

Enzymes were extracted with 100 mM sodium phosphate buffer, pH 7, containing 5 mM dithiotreitol (DTT) and polyvinylpolypyrrolidone (1:10 w/v) in a Polytron homogenizer (Kinematica AG, USA). Leaves were cut into small pieces and submitted to a 10-sec pulse at speed 3, followed by an additional 20 sec at speed 5. Part of the extracts was transferred into Eppendorf tubes, and centrifuged in an Eppendorf bench-top centrifuge at 14,000 rpm for 15 min. The supernatant was collected for protein concentration determination (Bradford 1976). Extracts were stored at −80°C for later analysis. POD and PPO measurements followed the methods used by Mazzafera et al. (1989). POD activity was measured with 5 μM guaiacol and 0.05% H_2O_2 in 100 mM K-phosphate buffer, pH 7, following the absorbance variation at 420 nm (25°C, 30 min, in the absence of light). PPO activity was measured with 7 μM chlorogenic acid and 45 mM glycine at 100 mM K-phosphate buffer, pH 7, following the absorbance variation at 420 nm (30°C, 30 min, in the absence of light). Data were analyzed by using the factorial model (days after eclosion populations) in a fully random experimental design, with five replications (plants). Rates were transformed using the equation $y = \log(x + 1)$.

ANOVA was determined with the statistical program SANEST (Zonta and Machado, 1992), and the means compared with the Tukey test, at 5% probability.

Results

Damage to Leaf Disks

The expression of resistance by plants is shown in Table 1. ANOVA confirmed the existence of significant differences in the expression of resistance to leaf miners among the coffee groups. However, there were no significant differences among plants belonging to the same group. Comparison of means by the Tukey test did not show significant differences between *C. arabica* and the susceptible hybrids, or between *C. racemosa* and the resistant hybrids.

Table 1 Level of resistance (scale of 1 to 4, with 1 being very resistant) to coffee leaf miner in plants sampled from four groups evaluated for the type of lesion in leaves

Groups	Individuals evaluated[a]					
	1	2	3	4	5	Mean
Coffea arabica	4.0	4.0	4.0	4.0	4.0	4.0a
Coffea racemosa	1.7	2.4	1.2	2.1	2.4	2.0b
Susceptible hybrids	2.7	4.0	2.9	3.2	2.7	3.0a
Resistant hybrids	1.6	1.0	1.1	1.0	1.0	1.1b
$F_{groups} = 49.41^{**}$						
$F_{Plant\ (groups)} = 0.37^{ns}$						

[a] Mean of nine leaf disks evaluated in the laboratory.

**Significant at the 1% probability level; n.s., not significant at the 5% probability level.

Total Soluble Phenols

Feeding by the coffee leaf miner did not significantly alter the phenol levels in leaves, either on the damaged side of the leaves, or on the side from which the eggs were removed, in each group of plants (Table 2). However, infested leaves showed a trend toward a decrease in phenolic compounds in all populations, although the decrease was not significant. Infested leaves of *C. arabica*, *C. racemosa*, and susceptible hybrids had 19.8%, 15.2%, and 17.0% decreases in phenol concentrations, respectively, compared to uninfested leaves. Resistant hybrids showed little change (3.0%) when infested (Table 2). This decrease in phenolic content was significant only in the means of the treatments (Table 2), demonstrating that these plants, in general, responded to feeding damage by the leaf miners with a reduction in the total soluble phenols in the leaves.

On average, phenolic compounds represented 5.3% of the fresh weight of the leaves of *C. arabica*, 4.8% in the susceptible hybrids, 5.1% in the resistant hybrids, and 2.3% in *C. racemosa*. The high phenolic content of *C. arabica* leaves seems to be a characteristic of the species, as *C. racemosa*, the resistant parent, showed significantly lower content compared with plants from the other groups (Table 2).

Qualitative Comparison of Phenolic Compounds

HPLC analysis did not reveal qualitative differences in the phenolic compounds in infested leaves compared to uninfested leaves (data not shown). On the other hand, *C. racemosa* had a considerably different elution pattern compared to the other genetic groups (Fig. 1). In this species, a large peak was detected at 20 min, but its identity could not be established. It showed two peaks of maximum absorbance at 270 and 326 nm (data not shown).

The leaf contents of chlorogenic acid were significantly different in all treatments. Uninfested leaves of *C. racemosa* had approximately six times less chlorogenic acid than *C. arabica* and the hybrid populations, which did not differ significantly from each other (Table 3).

Chlorogenic acid concentration showed a similar tendency to that observed for total phenols in the hybrids and in *C. arabica*, i.e., a reduced content in infested tissues. There was a 54.9% reduction in the quantity of chlorogenic acid on the infested side of *C. arabica* leaves compared to uninfested leaves. In the susceptible hybrids, reduction in phenol concentration was less pronounced (27.7%), but it was significant. In the resistant hybrid, there was a 19.4% reduction in chlorogenic acid in infested leaves; however, this trend was not significant. On the other hand, opposite to what was observed for total phenols, *C. racemosa* responded to leaf miner attack with a 66.6% increase in chlorogenic acid concentration.

Table 2 Mean concentrations of phenolic compounds [mass equivalents (mg) of chlorogenic acid per G fresh leaf] in leaves of *C. arabica* and *C. racemosa* and of two hybrids derived from a cross between these species

Groups	UL	USL	IL	Mean*
C. arabica	60.9 ± 10.9	49.2 ± 7.1	48.8 ± 4.6	52.9a
C. arabica × *C. racemosa* (S)	53.2 ± 13.9	45.7 ± 5.4	45.1 ± 5.5	48.0a
C. arabica × *C. racemosa* (R)	52.5 ± 2.1	49.9 ± 3.8	51.0 ± 2.9	51.2a
C. racemosa	24.9 ± 9.6	24.2 ± 10.3	20.6 ± 7.8	23.0b
Mean**	47.9A	42.2AB	41.5B	

Mean values for groups (*) and treatments (**) when followed by the same letter, they are not significantly different (Tukey, 5%). S: susceptible; R: resistant; UL: uninfested leaves; USL: uninfested side of the leaf; IL: infested side of the leaf.

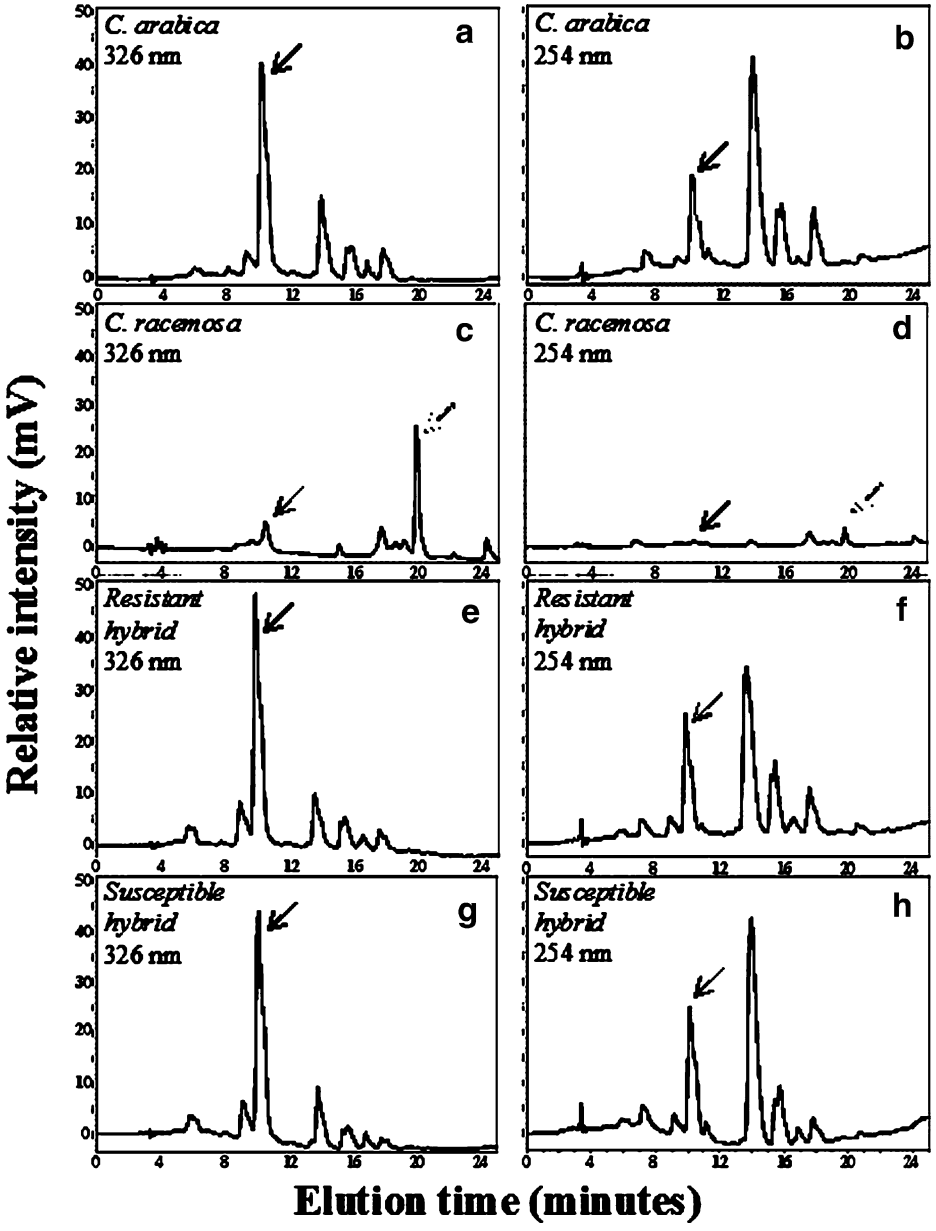

Fig. 1 HPLC chromatogram profiles of alcoholic extracts of leaves of *Coffea arabica* (a, b), *C. racemosa* (c, d), and resistant (e, f) or susceptible (g, h) hybrids to *Leucoptera coffeella*. Profiles obtained at 326 nm (a, c, e, g) and 254 nm (b, d, f, h) are shown. Solid arrows indicate chlorogenic acid and dashed arrow a possible chlorogenic acid isomer in *C. racemosa*

Table 3 Mean concentrations of chlorogenic acid (μg/g fresh leaf) in *C. arabica* and *C. racemosa*, and in two hybrids derived from crosses between them, expressing different levels of resistance to the coffee leaf miner (*Leucoptera coffeella*)

Groups	UL	USL	IL
C. arabica	2092 ± 411a A	1522 ± 229b A	944 ± 202c B
C. arabica × *C. racemosa* (S)	1949 ± 337a A	1674 ± 197b A	1409 ± 182b A
C. arabica × *C. racemosa* (R)	1935 ± 201a A	1833 ± 178a A	1559 ± 222a A
C. racemosa	355 ± 18b B	431 ± 164b B	591 ± 126a C

Values for each group followed by the same capital letter and treatment followed by the same small letter were not significantly different (Tukey 5%). S: susceptible; R: resistant; UL: uninfested leaves; USL: uninfested side of the leaf; IL: infested side of the leaf.

Evaluation of Oxidative Enzyme Activity

POD activities, measured soon after leaves were collected, were higher in *C. arabica* and the resistant progeny than in the susceptible hybrid. Lowest activities were observed in *C. racemosa*, which had up to 15 times less activity.

Under insect infestation, the groups responded differently to treatments 1 and 4 DAE. There was no induction of POD activity due to insect attack in any of the populations at 1 DAE (Table 4). At 4 DAE, leaf miners provoked a 72.4% increase in POD activity in *C. arabica* and 89.6% in the susceptible hybrids. POD activity induced by *L. coffeella* was positively correlated with the type of lesion provoked by feeding activity (Table 1, $R = 0.46$, $T = 2.17$, $P < 0.05$). In these plants, the damage caused by insect feeding was characterized by a massive and localized destruction of a large number of palisade cells (Ramiro et al., 2004).

PPO activity in recently collected leaves varied significantly among populations (Table 5). *C. racemosa* leaves had twice the activity of *C. arabica* leaves, and was fivefold higher than leaves from hybrid plants. The constitutive PPO concentrations in resistant and susceptible hybrids were lower than in the parental species. At 1 DAE, leaf miner attack did not induce PPO activity in any of the populations. At 4 DAE, PPO was significantly induced by insect feeding only in *C. racemosa*. Activity was lower in leaves of resistant and susceptible hybrids, in all the treatments, when compared to the parents.

Table 4 Mean peroxidase activity (ΔA_{470}/hr/μg protein) in recently collected, infested, and uninfested coffee leaves, 1 or 4 days after eclosion of the leaf miner larvae

Groups	RCL	1 DAE		4 DAE	
		UL	I	UL	I
C. arabica	0.569 ± 0.116b A	0.363 ± 0.088c B	0.397 ± 0.078c B	0.566 ± 0.111b A	0.976 ± 0.240a A
Susceptible hybrids	0.212 ± 0.049c B	0.457 ± 0.090b AB	0.432 ± 0.129b B	0.355 ± 0.091b B	0.673 ± 0.194a B
Resistant hybrids	0.676 ± 0.097a A	0.591 ± 0.118a A	0.627 ± 0.192a A	0.550 ± 0.183a A	0.720 ± 0.144a B
C. racemosa	0.047 ± 0.013c C	0.065 ± 0.020bc C	0.097 ± 0.022b C	0.155 ± 0.024a C	0.168 ± 0.052a C

Values for each group followed by the same capital letter and treatment followed by the same small letter were not significantly different (Tukey 5%). DAE: days after eclosion of the leaf miner larvae; RCL: recently collected leaves; UL: uninfested leaves; I: infested leaves.

Table 5 Mean polyphenol oxidase activity (ΔA_{470}/hr/μg protein) in recently collected, infested and uninfested coffee leaves, 1 or 4 days after eclosion of the leaf miner larvae

Groups	RCL	1 DAE		4 DAE	
		UL	I	UL	I
C. arabica	0.155 ± 0.028a B	0.128 ± 0.015a B	0.132 ± 0.029a B	0.155 ± 0.043a B	0.153 ± 0.045a B
Susceptible hybrids	0.075 ± 0.023a C	0.055 ± 0.019a C	0.070 ± 0.023a C	0.080 ± 0.025a C	0.106 ± 0.029a C
Resistant hybrids	0.068 ± 0.017a C	0.057 ± 0.020a C	0.074 ± 0.027a C	0.048 ± 0.015a C	0.095 ± 0.028a C
C. racemosa	0.303 ± 0.077b A	0.350 ± 0.104b A	0.366 ± 0.054b A	0.336 ± 0.087b A	0.521 ± 0.176a A

Values for each group followed by the same capital letter and treatment followed by the same small letter were not significantly different (Tukey 5%). DAE: days after eclosion of the leaf miner larvae; RCL: recently collected leaves; UL: uninfested leaves; I: infested leaves.

Discussion

Resistance of *C. racemosa* to the leaf miner *L. coffeella* was attributable to expression of two dominant and complementary genes that were transferred to *C. arabica* by successive backcrosses of the interspecific hybrids to the later parent (Guerreiro-Filho et al., 1999). Among the hybrids obtained from these backcrosses, we selected one resistant and one susceptible to the leaf miner to study the role played by phenols and oxidative enzymes in resistance. Although the defensive role of phenolic compounds and oxidative enzymes appear to be more evident in other plant–insect interactions (Felton et al., 1989; Duffey and Stout, 1996), our results suggest that phenol content apparently does not play a central role in the resistance of coffee to the coffee leaf miner. We found differences between parental species in total soluble phenol concentration, chlorogenic acid content, and oxidative PPO activity; however, the hybrids, did not differ for any of these characteristics.

Data obtained here show that feeding by *L. coffeella* is correlated with quantitative alterations in phenol metabolism and in the activity of oxidative enzymes in coffee leaves. In general, plants responded to feeding damage by reducing the total soluble phenols in the leaves (Table 2). High phenol concentration seems to be characteristic of *C. arabica*, whereas *C. racemosa*, the donor of resistance genes, showed significantly lower concentration. However, the values of hybrids were similar to *C. arabica*. Additionally, the chromatographic profiles of the hybrids were similar to *C. arabica*.

C. racemosa showed a large peak on HPLC, not detected in other plants used in this study. Although speculative, this peak might be an isomer of chlorogenic acid because it showed a maximum absorption peak at 326 nm, a characteristic wavelength for this group of compounds (Clifford 1985). Identification of chlorogenic acid isomers has been carried out mostly in *C. canephora* and *C. arabica* (Clifford 1985; Clifford et al., 2003). The only report on *C. racemosa* indicated the presence of 5-caffeoylquinic acid as the main isomer in seeds (Clifford et al., 1989).

The peak was absent in the hybrids and *C. arabica*, suggesting that it might not be related to leaf miner resistance. However, insect attack in *C. racemosa* was correlated with a significant induction of chlorogenic acid and PPO. Consequently, this compound is being isolated for identification and to determine its participation in the resistance of *C. racemosa* to *L. coffeella*.

The significant increase in chlorogenic acid and PPO activity observed only in insect-infested *C. racemosa* suggests a strategy to increase the availability of substrate for the production of quinones via enzyme activity. However, this does not seem to be the key factor in the defense of resistant hybrids against the leaf miner, because neither chlorogenic acid induction nor increased PPO activity was observed in these plants.

In a recent report, Melo et al. (2006) showed that constitutive PPO activity was higher in leaves of *C. racemosa* than *C. arabica*, and that it was induced by methyljasmonate in the former species, but not by mechanical damage. Also, two other hybrids from these species showed PPO activity close to the *C. arabica* values. Additionally, the phenol content was higher in *C. arabica*. In support of our results, infestation of the two hybrids and *C. arabica* plants with *L. coffeella* did not cause an increase in PPO activity, although a discrete drop of the constitutive activity was also observed in *C. arabica* leaves after insect exposure. Melo et al. (2006) also studied several other coffee species, but did not find a relationship between PPO activity and phenolic contents with resistance to coffee leaf miner or coffee leaf rust disease. These authors concluded that coffee resistance may be related to the oxidative potential of the tissue regarding the phenolic composition rather than simply to a higher PPO activity.

Unexpectedly, recently collected leaves of *C. arabica* showed higher constitutive levels of POD than either infested or uninfested leaves. We do not have a reasonable explanation for this decrease in activity after insect exposure except to speculate that it might be related to leaf detachment. This was not observed for other coffee populations. However, it is interesting to note that the resistant hybrid also showed high constitutive POD activity, but did not decrease after detachment. Leaf detachment does not affect resistance level as shown by Guerreiro-Filho (1994), who compared data obtained from infested coffee plants in the field and nursery with data from the leaf test used here.

Induction of POD due to attack by the leaf miner occurred in *C. arabica* and in the susceptible hybrids. Stout et al. (1994) suggested that the plant response to damage could be related to the type of tissue attacked and to the duration (persistence) and/or magnitude of the lesion. The ratio between damage intensity and response magnitude was almost always positive, indicating that the number of damaged cells influenced the response.

Our results showed a significant correlation between the amount of damage caused by caterpillars (level of resistance) and POD activity. Nevertheless, this differential induction of peroxidase may not be a reaction to insect attack, but rather an effort to replace damaged tissue, because this enzyme is responsible for lignification and suberization in vegetal tissues (Goldberg et al., 1985). Additionally, POD activity might also be related to oxidative stress developed during the larvae feeding (Bi and Felton, 1995).

According to Stout et al. (1994), the induction of PPO and POD in response to insect feeding is not definitive evidence that oxidative enzymes are directly involved in plant defenses to restrict insect attack, because of the complex relationship between these enzymes and several chemical compounds in the cell, and their multiple biological activities.

The action of phenols in biological systems seems to be influenced by several different factors, such as cell concentration, environmental conditions, structural variations, and the degree of specificity between the organisms involved (Appel 1993). *L. coffeella* has high specificity for coffee. It feeds exclusively on cells of the palisade parenchyma in susceptible *C. arabica* (Ramiro et al., 2004). Studies to evaluate phenols, carbohydrates, proteins, and other compounds in the palisade tissue of *C. arabica* and *C. racemosa* might bring important information for understanding of the resistance mechanism to this pest.

Assuming that resistant hybrids inherited the two resistance genes from *C. racemosa* (Guerreiro-Filho 1994), these results for phenolic content and POD/PPO activity are not strong evidence to suggest their participation in a direct defense mechanism of coffee

against *L. coffeella*. However, coinduction of chlorogenic acid and oxidative enzymes was observed in *C. racemosa*, indicating that the two resistance genes transferred to *C. arabica* (Guerreiro-Filho 1994) may not be related to such a mechanism.

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