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Author(s): S.T.V. Magalhães, F. L. Fernandes, A. J. Demuner, M. C. Picanço, and R.N.C. Guedes

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Leaf Alkaloids, Phenolics, and Coffee Resistance to the Leaf Miner *Leucoptera coffeella* (Lepidoptera: Lyonetiidae)

S.T.V. MAGALHÃES,[1] F. L. FERNANDES,[1] A. J. DEMUNER,[2] M. C. PICANÇO,[1] AND R.N.C. GUEDES[1,3]

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ABSTRACT Coffee (*Coffea* spp.) alkaloids (caffeine and related methylxanthines) and phenolics (caffeic and chlorogenic acids) have recognized pestistatic/pesticidal activity and mediate insect–plant interactions. The present investigation assessed the resistance of 12 coffee genotypes to the leaf miner *Leucoptera* (=*Perileucoptera*) *coffeella* (Guérin-Méneville & Perrottet) (Lepidoptera: Lyonetiidae) and correlated such results with the leaf content of coffee alkaloids and phenolics that probably play a role in the interaction between coffee and this leaf miner. The levels of chlorogenic and caffeic acid, caffeine, and related methylxanthines were measured and quantified in leaf extracts of these genotypes before and 7 d after their infestation by the leaf miner. Some coffee genotypes (*Coffea canephora* L. and *Coffea racemosa* Lour. and its hybrids with *Coffea arabica* L.) exhibited high pesticidal activity (100% mortality) toward the *L. coffeella*, indicating their antibiosis resistance. However, there was no correlation between this activity and the leaf levels of coffee alkaloids and phenolics. Curiously, infestation by *L. coffeella* leads to a nearly four-fold decline in the leaf levels of chlorogenic acid, which does not affect this pest species but may affect other generalist species. Indeed, chlorogenic acid sprayed on coffee leaves stimulated locomotory activity of the green scale *Coccus viridis* (Green) (Hemiptera: Coccidae), thus minimizing their feeding in contrast with the absence of this polyphenol. Therefore, reduction of chlorogenic acid levels in coffee leaves due to leaf miner infestation seems to also favor infestation by generalist insects, such as the green scale.

KEY WORDS antiobiosis, caffeine, caffeic acid, chlorogenic acid, *Coffea*

Coffee (*Coffea* spp.) is a major commodity in Neotropical America and Africa and the *Leucoptera* leaf miners are major pests of this crop in both regions. *Leucoptera* (=*Perileucoptera*) *coffeella* (Guérin-Méneville & Perrottet) (Lepidoptera: Lyonetiidae) was initially assumed to be the same coffee leaf miner species that occurs in West Africa but was later recognized as two species: *Leucoptera meyricki* Ghesquière and *Leucoptera caffeina* Washburn (Box 1923, Notley 1948, Bradley 1958, Crowe 1964). The true *L. coffeella* is restricted to the Neotropical region (Reis and Souza 1984, Mey 1994). Its presence in Brazil was reported as early as 1851, but periodic population outbreaks of this species started in the 1970s as a likely result of changes in coffee cultivation practices, including frequent fungicide spraying against coffee rust fungus *Hemileia vastratrix* (Berk. et Br.) (Pucciniaceae) (Reis and Souza 1984, Souza et al. 1998, Pereira et al. 2007).

L. coffeella is a monophagous pest species of coffee (Souza et al. 1998, Gallo et al. 2002). Females lay their eggs on the adaxial leaf surface at night, and the newly hatched larvae move directly to the leaf mesophyll where they start to feed on the palisade parenchyma; they remain there throughout larval development (10–40 d) and leave only to pupate (Souza et al. 1998, Pereira et al. 2007). *L. coffeella* should therefore be well-adapted to the main secondary compounds of coffee leaves—alkaloids (caffeine and related methylxanthines) and phenolics (caffeic and chlorogenic acids). That these phytochemicals are particularly abundant in young coffee leaves (Ashihara 2006, Ramiro et al. 2006), the most susceptible to attack by *L. coffeella* (Souza et al. 1998; Pereira et al. 2007; Magalhães et al. 2008a,b) provides support for such expectation. Indeed, recent studies failing to report detrimental effects of caffeine and total soluble phenols against coffee yield loss by this pest species (Guerreiro Filho and Mazzafera 2000, Ramiro et al. 2006) come as no surprise. However, potential beneficial effects of coffee leaf alkaloids and phenolics to the coffee leaf miner and their potential induction by this species were not considered in previous studies, but they were among the objectives of the present investigation.

[1] Departamento de Biologia Animal, Universidade Federal de Viçosa, Viçosa, MG 36571-000, Brazil.
[2] Departamento de Química, Universidade Federal de Viçosa, Viçosa, MG 36571-000, Brazil.
[3] Corresponding author: Instituto Nacional de Ciência e Tecnologia em Interações Planta-Praga (INCT-IPP), Universidade Federal de Viçosa, Viçosa, MG 36571-000, Brazil (e-mail: guedes@ufv.br).

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Coffee resistance to *L. coffeella* remains elusive, particularly within the species *Coffea arabica* L., the main cultivated species in Neotropical America (Souza et al. 1998, Gallo et al. 2002, Ramiro et al. 2006). This management tactic sparked renewed interest with the still unresolved overall inefficiency of biological control agents in containing outbreaks of *L. coffeella* and increasing problems of insecticide resistance in field populations of this insect species (Reis and Souza 1986; Souza et al. 1998; Reis et al. 2000a; Fragoso et al. 2002, 2003; Pereira et al. 2007). Preliminary screenings for reduced leaf miner damage in coffee species reported in local coffee conference meetings suggest potential resistance only in *Coffea* species other than *C. arabica* and *Coffea canephora* L., the two commercially cultivated coffee species (Almeida et al. 2003, Matielo et al. 2003). Based on such preliminary information, the coffee breeding programs in Brazil have focused in introducing leaf miner resistance genes from the species *C. racemosa* Lour., which exhibits only moderate loss of yield by the leaf miner, into *C. arabica* because the crosses are more easily carried out (Guerreiro Filho et al. 1999, Almeida et al. 2003, Matielo et al. 2003). Curiously, the mechanisms and causes of coffee resistance to *L. coffeella* remain elusive despite recent evidence of egg-laying stimulation by the coffee leaf compounds caffeine and *p*-cymene (Magalhães et al. 2008a,b).

The present investigation assessed the resistance of 12 coffee genotypes to *L. coffeella.* Resistance was assessed through the direct effect of the coffee genotypes on the leaf miner, rather than by assessing the damage on the coffee genotypes as in previous studies (Guerreiro Filho and Mazzafera 2000, Ramiro et al. 2006). Results were correlated with the leaf content (and eventual induction) of alkaloids and phenolics likely to play a role in the postingestion interaction between coffee and its pest species. The initial expectation was of neutral or beneficial association between the leaf contents of coffee alkaloids and/or phenolics and insect development. The potential consequence of induction or repression of alkaloid and/or phenolic production in coffee leaves by the leaf miner also was assessed in the generalist green scale, *Coccus viridis* (Green) (Hemiptera: Coccidae), another coffee insect pest (Gallo et al. 2002).

Materials and Methods

Coffee Genotypes, Insects, and Chemicals. Leaves of coffee plants from 12 different genotypes were collected from productive coffee plants (>7 yr old) at the Coffee Breeding Program at the Federal University of Viçosa (Viçosa, MG, Brazil). The 12 coffee genotypes used were as follows: *C. arabica* 'Bourbon Amarelo', *C. arabica* 'Catuaí Vermelho IAC 99′, *C. arabica* 'Mundo Novo IAC 376-4-32′, *C. arabica* 'Oeiras MG 6851′, *C. arabica* 'Topázio', *C. canephora* 'Robusta', the natural (tetraploid) hybrid between *C. arabica* and *C. canephora* called Híbrido de Timor, *Coffea racemosa* (UFV 545 c 28), and four triploid hybrids resulted from the natural cross between *C. racemosa* (UFV 544) and *C. arabica.*

Leaves mined by *L. coffeella* were collected weekly during early morning from field plants of *C. arabica.* The collected leaves were placed in "Gerbox" boxes (11- by 11- by 3.5-cm germination boxes) containing an aqueous solution of 10^{-6} M benzyladenine following Reis et al. (2000b). The pupae were collected and transferred to glass vials until adult emergence.

All reagents used were obtained from Sigma-Aldrich Química Brasil (São Paulo, SP, Brazil), including the following standards used for the chromatographic determinations: caffeic acid (3,4-dihydroxycinnamic acid), chlorogenic acid (5-*O*-caffeoyl-D-quinic acid), caffeine (1,3,7-trimethylxanthine), theobromine (3,7-dimethyxanthine), theophylline (1,3-dimethylxanthine), xanthine (2,6-dihydroxypurine), 3-methylxanthine (2,6-dihydroxy-3-methylpurine), and 7-methylxanthine (2,6-dihydroxy-7-methylpurine).

Biossay of Coffee Resistance to *L. coffeella.* Leaves of each coffee genotype with their petioles inserted through the lid of glass vials (one leaf per vial, 8.0 cm in height by 3.0 cm in diameter) with an aqueous solution of benzyladenine were placed in a wooden cage covered with organza (40 by 40 by 40 cm) and containing 40 adult *L. coffeella* (20 males and 20 females). Groups of one to 10 leaves depending on the coffee genotype and replicate were exposed to the adult insects for 24 h, after which the leaves with six to eight eggs were separated (one per replicate per genotype for the resistance bioassay) and individually maintained in an insect rearing room with controlled environmental conditions (25 ± 2°C, 70 ± 5% RH, and a photoperiod of 12:12 [L:D] h). The leaves were inspected daily, and the number of eggs, larvae, and pupae present throughout development until adult emergence were recorded. Insect mortality also was recorded. Duration of the egg stage, percentage of egg hatch, larval longevity (days), and percentage of larval mortality were recorded. The reproductive parameters of the adult insects were not considered because no adults emerged from the resistant coffee genotypes, on which very high levels of larval mortality took place early in their development.

Extraction, Identification, and Quantification of Leaf Compounds. Coffee leaves were collected before and 7 d after the insect infestation. Four leaf batches of 15 g each were collected from each genotype following the procedures of the coffee resistance bioassay described above. The leaves were dried at 40°C for 2 wk for an average weight loss of 70%. The dried leaves were ground, and 1-g samples were added to 30 ml of methanol and maintained in a water bath for 4 h at 60°C for the phytochemical extraction. The resulting extract was filtered through filter paper, concentrated in a rotatory evaporator, and diluted in methanol for a final volume of 3 ml. The samples were subsequently filtered again under vacuum in a C18 solid phase extractor (ODS C18 cartridge; Agilent Technology, Barueri, São Paulo, Brazil).

The extracts obtained were further diluted to 10 ml with methanol. Aliquots (0.5 ml) were obtained from

each sample and diluted in methanol:water (1:1) to a 10-ml volume, and 2 ml of this solution was further diluted to 10 ml with the same solvent mixture. This last solution was filtered using a filtering unit with PTFE membrane (0.45-μm mesh and 13 mm in diameter). An aliquot of 20 μl of the filtered solution was used for injection in a high-performance liquid chromatograph (HPLC) (model LC-10AD [two-pump] with a SPD-10AV dual detector, Shimadzu, Kyoto, Japan), adjusted to detect alkaloid compounds (i.e., caffeine and related methylxanthines) at $\lambda = 272$ nm in channel 1 and to detect phenolic compounds (i.e., caffeic and chlorogenic acids) at $\lambda = 320$ nm in channel 2. The HPLC also was equipped with a CBM-10A communication system (Shimadzu). The compounds of interest were separated with a RP-18 reverse phase column (Lichrosorb silica, Merck, Rio de Janeiro, Brazil; 250 mm by 4.6 mm by 5 μm) using a methanol: water solution with 1.0 mM HCl in gradient (0.1–7.0 min [17:83%], 7.1–37.0 min [23:77%], and 37.1–40.0 min [100:0%]), at a flow rate of 1.0 ml/min (Daglia et al. 1994).

The chemical standards were individually injected in the column and also injected together for determining the retention times. Increasing concentrations of each standard (1, 5, 10, 20, 100, and 200 μg/ml) were injected in the column for establishing the calibration curve of each standard and eventual quantification in the samples obtained from the coffee leaves by using the external standard method. The quantifications were carried out in triplicate for each of the four replicated leaf batches used in the extraction and collected at the same time with samples for the resistance bioassay already described.

Bioassay of Chlorogenic Acid Against Green Scales. Because the chlorogenic acid was drastically reduced by leaf miner infestation, a bioassay was carried out to test its potential effect on the green scale, a generalist pest species also important in coffee. Leaves of the mid-third of the coffee canopy (fourth pair from the top) from one of the susceptible coffee genotypes (*C. arabica* Catuaí Vermelho) were immersed for 5 s in either one of two solutions: water + surfactant (0.02% calcium dodecylbenzene sulfonate) or water + surfactant (0.02% calcium dodecylbenzene sulfonate) + chlorogenic acid (0.1%, based on the maximum concentration detected in our leaf determinations, i.e., 1,700 ppm). The leaves were subsequently dried for 2 h and then placed on petri dishes (9 cm in diameter). A first-instar green scale was released on the leaf in the center of each petri dish, and after 30 s of contact with the treated leaf, the length of time that each nymph remained immobile (and feeding) or walking was recorded. The recording time was 15 min, and 10 replicates were used for each treatment (i.e., leaf impregnated or not with chlorogenic acid).

Statistical Analysis. The results of the coffee resistance bioassay with *L. coffeella* for each coffee genotype were subjected to a multivariate analysis of variance (ANOVA) and canonical variate analysis by using the procedure CANDISC with the DISTANCE statement from SAS (SAS Institute 2002). This approach was used to determine whether there were significant differences in the overall insect response to the coffee genotypes (maintaining an overall $P < 0.05$) and to determine their relative importance and similarity. These results were subsequently subjected to univariate ANOVA and Tukey's honestly significant difference (HSD) test ($P < 0.05$), whenever appropriate (PROC GLM, SAS Institute 2002). The contents of phenolics and alkaloids of each coffee genotype before and after infestation by *L. coffeella* were individually subjected to a two-way ANOVA (two periods $\times$ 12 genotypes) and subsequently Tukey's HSD test ($P < 0.05$), when appropriate (PROC GLM, SAS Institute 2002). The leaf levels of alkaloids and phenolics were also subjected to a canonical correlation against the leaf miner response to each coffee genotype by using the procedure CANCORR (SAS Institute 2002). The results of locomotory activity of green scales exposed or not to chlorogenic acid on coffee leaves were subjected to Fisher's F test ($P < 0.05$). Assumptions of normality and homogeneity of variance were checked using the procedure UNIVARIATE (SAS Institute 2002), and data transformation was required only for the contents of 7-methylxanthine and theobromine, which were transformed to log (x).

Results

Coffee Genotype Similarity Regarding Resistance to *L. coffeella*. The multivariate ANOVA for leaf miner development indicated significant overall differences among coffee genotypes (Wilks lambda = 0.014; $F_{appr.} = 6.06$; $df_{num,\ den} = 44, 128.20$; $P < 0.0001$). Subsequent univariate analyses of variance carried out for each individual insect trait assessed also indicated significant differences among genotypes ($P < 0.05$), except for incubation time for the eggs to hatch ($F_{11,36} = 0.83$; $P = 0.61$) (Table 1). Only the genotype hybrid UFV 557-04 showed significantly lower eggs hatched ($F_{11,36} = 2.09$; $P = 0.048$) (Table 1). However, greater variation in insect mortality and survival time were observed among coffee genotypes (Table 1).

Canonical variate analysis (CVA) resulted in four canonical axes of which only the first was significant ($P < 0.05$) (Table 2), accounting for 95.95% of the total variance. The variable with greater canonical loadings (pooled within the canonical structure) accounting for most of the divergence among genotypes was larva mortality (Table 2).

The ordination diagram derived from the CVA was made using only the first two axes, which explained 98.98% of the total variance observed among genotypes, but only the first axis was significant at $P < 0.05$. Four genotype clusters were obtained with two major groups of genotypes (Fig. 1). The two clusters containing the commercial varieties of *C. arabica* (i.e., Bourbon Amarelo, Catuaí Vermelho, Mundo Novo, Oeiras, and Topázio) and the hybrid between *C. arabica* and *C. canephora* Híbrido de Timor overlapped and were completely isolated from the other group of

Table 1. Egg incubation period, eggs hatched, larval survival time, and larval mortality of *L. coffeella* reared on 12 coffee genotypes

Genotype	Egg incubation period (d)	Eggs hatched (%)	Larval survival time (d)	Larval mortality (%)
C. arabica Bourbon Amarelo	5.00 ± 0.00a	100.00 ± 0.00a	10.53 ± 0.91a	21.82 ± 5.07e
C. arabica Catuaí Vermelho	4.81 ± 0.20a	92.50 ± 7.50a	8.90 ± 0.41a	47.20 ± 4.31cd
C. arabica Mundo Novo	5.00 ± 0.00a	97.50 ± 2.50a	10.50 ± 0.95a	31.29 ± 8.46de
C. arabica Oeiras	4.94 ± 0.06a	92.50 ± 4.79a	9.50 ± 1.31a	51.39 ± 9.45c
C. arabica Topázio	5.00 ± 0.00a	94.44 ± 5.56a	10.60 ± 0.94a	31.07 ± 8.38de
C. canephora Robusta	4.74 ± 0.19a	90.45 ± 5.52a	3.08 ± 0.17bc	100.00 ± 0.00a
Coffea racemosa	5.03 ± 0.03a	97.50 ± 2.50a	2.03 ± 0.06c	100.00 ± 0.00a
Hybrid UFV 557-02	4.89 ± 0.11a	92.50 ± 4.79a	4.78 ± 0.92b	94.38 ± 3.29b
Hybrid UFV 557-03	5.08 ± 0.08a	95.00 ± 2.89a	1.45 ± 0.18c	100.00 ± 0.00a
Hybrid UFV 557-04	4.75 ± 0.25a	75.91 ± 8.17b	1.58 ± 0.25c	100.00 ± 0.00a
Hybrid UFV 557-06	5.03 ± 0.03a	100.00 ± 0.00a	1.48 ± 0.28c	100.00 ± 0.00a
Híbrido de Timor	4.75 ± 0.25a	100.00 ± 0.00a	10.98 ± 1.05a	26.59 ± 4.02e

Means ± SEM followed by the same letter in a column are not significantly different by Tukey's HSD test ($P < 0.05$).

genotypes. The larval mortality in these two clusters of commercial *C. arabica* and Híbrido de Timor was significantly smaller, and the larvae survived longer than in the other coffee genotypes (Table 1). The other group of genotypes, which was resistant to *L. coffeella*, was formed by *C. canephora* Robusta, *C. racemosa*, and its hybrids with *C. arabica* (Fig. 1). The Hybrid UFV 557-04 was separated even further showing much higher resistance to *L. coffeella* (Table 1; Fig. 1).

Leaf Compounds and Their Association With Coffee Resistance. Among the compounds investigated, caffeic acid, theophylline, and 3-methylxanthine were detected in just a few genotypes and in low concentrations. Therefore, they were not considered in the subsequent analysis. The leaf contents of caffeine, 7-methylxanthine, xanthine, and theobromine did not significantly change before and after the leaf miner infestation ($P > 0.05$), and the interaction between period of determination (i.e., before and 7 d after the insect infestation) and coffee genotype was also not significant for these four alkaloids ($P > 0.05$). Their results were therefore pooled together and subjected to the Tukey's HSD test ($P < 0.05$) to screen for the effect of coffee genotype (Table 3), which showed significance in the two-way analyses of variance. The leaf levels of chlorogenic acid contrasted with those of the alkaloids described above. The interaction among coffee genotypes and period of determination was not significant, nor was the difference among genotypes ($P > 0.05$). Curiously, the leaf levels of chlorogenic acid significantly dropped approximately four-fold after the leaf miner infestation, from 922.95 ± 65.28 to 240.55 ± 65.35 ppm ($F_{1,83} = 14.48$; $P < 0.001$).

Table 2. Canonical loadings (pooled within the canonical structure) for the traits of *L. coffeella* reared on 12 coffee genotypes

Variable	Canonical axis			
	1	2	3	4
Time for eggs to hatch (d)	0.11	0.7	−0.28	0.73
Eggs hatched (%)	0.37	0.04	0.88	0.44
Time for the larvae to die (d)	0.08	0.95	0.26	−0.36
Larva mortality (%)	−0.84	0.09	0.56	0.25
$F_{appr.}$	6.06	1.23	0.58	0.39
df (numerator, denominator)	44, 128.20	30, 100.47	18, 70	8, 36
P	<0.0001*	0.22	0.9	0.92
Square canonical correlation	0.97	0.47	0.18	0.08

* Significant at $P < 0.05$.

The leaf levels of the xanthine alkaloids (levels before and after infestation were pooled for analysis because they were not significantly different at $P < 0.05$) and of the phenolic chlorogenic acid (levels before and after infestation were maintained for analysis) were correlated against the set of insect variables used to assess the coffee genotype resistance to *L. coffeella*. The results of this canonical correlation were not significant (Wilks lambda = 0.011; $F_{appr.} = 0.89$; $df_{num, den} = 24, 8.18$; $P = 0.62$); therefore, no association between coffee resistance to *L. coffeella* and leaf levels of coffee alkaloids and phenolics was observed.

Effect of Chlorogenic Acid in Green Scales. The reduction in the coffee leaf levels of chlorogenic acid with the infestation by *L. coffeella* may have consequences particularly for generalist pest species that attack coffee plants, such as green scale. Chlorogenic acid applied on coffee leaves stimulated locomotory activity on green scales minimizing their feeding on the leaves (14.02 ± 1.03 min walking), in contrast with treatments without this polyphenol (4.63 ± 0.87 min walking) ($F_{1,8} = 7.45$; $P < 0.001$). Therefore, reduction of chlorogenic acid levels in coffee leaves due to leaf miner infestation seems to favor infestation by generalist insects, such as the green scale.

Discussion

There was ample variation in *L. coffeella* resistance among the genotypes tested. However, the trends observed differed somewhat from the previous studies, which were based on assessment of coffee yield loss (Guerreiro Filho et al. 1999, Almeida et al. 2003, Matielo et al. 2003). The commercial varieties of *C. arabica* were fairly susceptible to the leaf miner, as expected, but *C. canephara* Robusta led to 100% mortality of the leaf miner larvae, a result similar to *C. racemosa* and its hybrids, which were also very resistant to this insect pest. One of the hybrids of *C. race-*

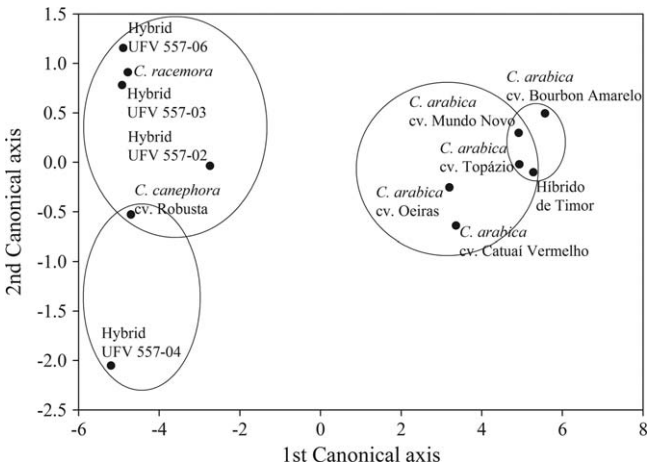

Fig. 1. Ordination (CVA) diagram showing the divergence among coffee genotypes regarding the resistance to *L. coffeella* (see Table 1). Only the first canonical axis is significant ($P < 0.05$) and accounts for 95.95% of the total variance explained. The symbols are centroids of treatments representing the class mean canonical variates. The large circles indicate clusters of treatments that are not significantly different by the approximated F-test ($P < 0.05$), based on the Mahalanobis (D^2) distance between class means.

mosa, hybrid UFV 557-04, was particularly resistant to *L. coffeella* and seems a promising source of resistance for further breeding. Híbrido de Timor, however, performed more closely to *C. arabica* rather than *C. canephora*, which may be a consequence of its genetic background distinct from *C. canephora* Robusta. The relative ease of crossing *C. arabica* and *C. racemosa*, the genotypic variation obtained with these crosses, and the high resistance levels observed in their hybrids reinforce the use of *C. racemosa* as a source of resistance genes against *L. coffeella* for transfer to commercial varieties of *C. arabica*, as has been currently emphasized in some Brazilian coffee breeding programs (Almeida et al. 2003, Matiello et al. 2003).

The resistant coffee genotypes of the current study caused high larval mortality that prevented the completion of larval development. Such high larval mortality occurred rapidly, usually within 5 d of egg hatching. The Hybrid UFV 557-04 acted even faster leading to 100% larval mortality within 2 d of hatching, and also seemed to reduce egg hatching. These results suggest antibiosis as the main resistance mechanism involved, but additional mechanisms may be present as suggested by our previous work on egg-laying preference of *L. coffeella* (Magalhães et al. 2008a,b). The specific cause underlying antibiosis as a coffee resistance mechanism against *L. coffeella* remains elusive.

Coffee resistance to *L. coffeella* was not correlated with the coffee leaf alkaloids and phenolics investigated here, as also reported for total phenols and caffeine in previous studies with other coffee genotypes (Guerreiro Filho and Mazzafera 2000, Ramiro et al. 2006). The results presented here indicate a neutral (or lack of) effect of coffee leaf alkaloids and phenolics on *L. coffeella*, as we predicted previously, because this is a specialist herbivore well adapted to coffee plants. In addition, we observed a nearly four-fold drop in the leaf levels of chlorogenic acid after 7 d of the infestation; therefore, with further larval development than that allowed by Ramiro et al. (2006). As expected, chlorogenic acid favors locomotory activity of green scales, minimizing their feeding. Therefore,

Table 3. Leaf content of caffeine and related methylxanthines from 12 coffee genotypes

Genotype	Caffeine (ppm)	7-Methylxanthine (ppm)	Xanthine (ppm)	Theobromine (ppm)
C. arabica Bourbon Amarelo	767.38 ± 228.28d	4.12 ± 4.12c	4.00 ± 1.40ab	101.16 ± 55.42b
C. arabica cv. Catuaí Vermelho	1,252.62 ± 93.96ab	6.36 ± 6.36ab	4.38 ± 2.00ab	9.92 ± 7.80b
C. arabica Mundo Novo	900.40 ± 198.86cd	0.00 ± 0.00b	3.96 ± 1.24ab	25.64 ± 13.14b
C. arabica Oeiras	1,115.36 ± 62.66bc	5.30 ± 2.80b	4.74 ± 0.20ab	12.74 ± 5.98b
C. arabica Topázio	1,068.56 ± 98.96cd	16.52 ± 9.08ab	5.26 ± 0.54ab	7.80 ± 4.38b
C. canephora Robusta	1,486.04 ± 94.50a	1.80 ± 1.80b	2.42 ± 0.22b	85.60 ± 70.60b
Coffea racemosa	25.40 ± 11.06e	6.68 ± 6.38b	3.58 ± 1.38ab	1.00 ± 1.00b
Hybrid UFV 557-02	22.26 ± 11.08e	0.00 ± 0.00b	3.06 ± 0.78ab	113.78 ± 108.42b
Hybrid UFV 557-03	68.38 ± 55.20e	13.44 ± 5.20ab	5.48 ± 0.72ab	4.58 ± 4.58b
Hybrid UFV 557-04	104.90 ± 32.98e	6.04 ± 6.04b	4.28 ± 1.28ab	46.10 ± 39.48b
Hybrid UFV 557-06	338.80 ± 40.02e	4.70 ± 4.70b	4.68 ± 0.26ab	39.46 ± 19.86b
Híbrido de Timor	3.08 ± 3.08e	25.60 ± 7.44a	6.16 ± 1.34a	503.88 ± 63.68a

Means ± SEM followed by the same letter in a column are not significantly different by Tukey's HSD test ($P < 0.05$).

reduction of chlorogenic acid levels in coffee leaves due to *L. coffeella* infestation seems to also favor infestation by the green scale, a generalist herbivore.

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