JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Caffeine and Resistance of Coffee to the Berry Borer *Hypothenemus hampei* (Coleoptera: Scolytidae)

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The role of caffeine as a chemical defense of coffee against the berry borer *Hypothenemus hampei* was investigated. No positive correlation was observed between resistance and caffeine content in experiments in which seeds from several coffee species presenting genetic variability for the alkaloid were exposed to adult insects. The same was observed in an experiment with coffee seeds that had their caffeine content doubled by imbibition with caffeine aqueous solutions. Other experiments showed that the attractiveness to insects was not related to the caffeine content of mature fruits. These results indicate that *H. hampei* has evolved an adaptation to handle the toxic effects of caffeine.

KEYWORDS: **Caffeine; *Coffea*; coffee species; *Hypothenemus hampei*; plant insect resistance**

INTRODUCTION

The coffee berry borer *Hypothenemus hampei* Ferrari is one of the most important pests of coffee plantations (*1*). The pest is distributed worldwide, causing severe economic loss. The adult female oviposits the eggs in the fruits, and 3−4 weeks later the larva arise. Both larvae and adult female can penetrate the fruit, usually by the apical part, opposite the peduncle, and migrate directly to the endosperm, the feeding tissue. The larvae become adult after a month and mate (*2*). The endosperm becomes perforate, losing weight and quality in terms of appearance. It is estimated that a 100% infestation would account for a 21% weight loss of pulped coffee berries (*3*).

H. hampei is a monofagous insect, attacking only species of the *Coffea* genus (*4*). Considering the geographical origin of the coffee species, it has been suggested that *Coffea canephora* (Robusta coffee) was the original host of *H. hampei* (*5*). *C. canephora* is the second most cultivated coffee species, representing ∼25% of the coffee traded in the world. *Coffea arabica* is responsible for almost 75%. Indeed, among several coffee species, *C. canephora* has long been indicated as the most susceptible to this pest (*6*). According to Paulini et al. (*7*) this susceptibility might be explained by the long period between flowering and harvest, fruits at different maturation stages, and the cultivation of *C. canephora* in warmer regions than *C. arabica*, permitting more insect generations. In addition, they also suggested that the thin exocarp and endocarp, and the lower water content of the endocarp, would facilitate penetration. Coste (*8*) observed that *Coffea liberica* and *Coffea excelsa* fruits, with thicker exocarp and endocarp, were less infested than several *C. canephora* cultivars. However, susceptibility variations among cultivars of these species have been described in the literature (*6*−*8*).

H. hampei infestation may be divided into two distinct phases: the attack, characterized by insect attraction to the coffee fruits (usually mature fruits), and pulp perforation/penetration, when the insect reaches the endosperm for feeding and reproduction (*9*). During the first phase, factors such as fruit color (green, immature; red, mature) and size would play a role in attracting the insects (*10*). In the second phase, the contents of soluble sugars, chlorogenic acid, caffeine, and volatile compounds might be important for the success of the infestation. Regarding the fruit color, previous studies have shown the preference of *H. hampei* for mature red fruits rather than green or yellow-green (beginning of maturation) (*11*, *12*). Other studies carried out also indicated that volatile compounds would partially explain the choice of red cherries by *H. hampei* females (*9*). This was confirmed by studies under controlled conditions using gas chromatography (*13*, *14*). Except for these few studies no other investigations concerning the role of coffee endosperm chemical constituents in the attack by *H. hampei* have been reported.

The first direct evidence of the alkaloid caffeine as a chemical defense in plants was given by Nathanson (*15*), who used *Lycopersicon esculentum* × *Manduca sexta* as a model system. Recently, caffeine was shown to be an efficient repellent and toxicant against slugs and snails (*16*, *17*). The same protective role was also suggested for plants containing caffeine (*18*, *19*). Frischknecht et al. (*20*) detected caffeine at 40 mg/g in very young leaflets and estimated that in terms of carbon allocation, this might represent 15% of the carbon burnt in the respiratory process. In mature leaves the caffeine content drops to <8 mg/g (*21*−*23*). Because soft plant tissues do not offer any mechanical resistance against insect herbivory, Frischknecht et al. (*20*) concluded that caffeine might be an effective defense in young

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10.1021/jf0347968 CCC: $25.00
Published on Web 10/24/2003

coffee plant leaves. Although it had been considered a problem in coffee plantations (*24*), interspecific coffee hybrids attacked by leaf cut ants showed low caffeine content (*21*). Several papers showed that coffee species present a considerable genetic variability for caffeine content in the leaves (*22*, *23*, *25*); however, a detailed study using this information could not establish a relationship between the alkaloid content and the resistance to the leaf miner *Perileucoptera coffeella* (*26*).

Gutierrez-Martinez and Ondarza (*14*) obtained organic extracts from different parts of coffee plants and measured the kairomone activity in *H. hampei*. The largest catch of insects was observed with the methylene chloride extract, and caffeine was identified as one of the components of this fraction. The results were confirmed with ethanolic solutions prepared with pure caffeine. Caffeine is not volatile, and although not confirmed so far, the authors suggested that caffeine volatility perhaps is increased under natural cell conditions by becoming soluble in essential oils or other types of substances within the coffee cell.

However, no relationship was evident for *H. hampei* when data from the literature on insect resistance and endosperm caffeine content were compared (*27*). Because the material analyzed for pest resistance was not the same as used in the caffeine determinations, this sort of comparison may not be taken as definitive. Therefore, the role of caffeine in coffee fruits as a protectant against *H. hampei* was investigated in this study. In a first experiment, seeds of several coffee species presenting variability for caffeine were exposed to insect attack. Two other experiments were planned to evaluate the attraction of insects by mature fruits from such species. In a fourth experiment seeds were soaked with aqueous caffeine solutions in order to increase their alkaloid contents and offered to insects. The rationale of the experiments was to establish a significant negative correlation between caffeine and damage/attraction, that is, to characterize resistance, high caffeine content should be associated with low damage/attraction.

EXPERIMENTAL PROCEDURES

Caffeine Determination. Coffee material dried at 80 °C for 2 days was ground in a mortar with a pestle and extracted according to the method of Guerreiro Filho and Mazzafera (*26*). Caffeine in the insect feces was extracted with 0.3 mL of 0.02 N H_2SO_4 in Eppendorf tubes in a boiling water bath for 60 min. After centrifugation, the supernatant was reserved for analysis. Caffeine was determined by high-performance liquid chromatography (HPLC) using a Shimadzu chromatograph system, equipped with an SCL-10Avp system controller, an FCV-10Ai/FCV-10ALvp pump, and an SPD-M10Avp diode array detector. The signals from the detector were acquired by a workstation using ClassVP software (Shimadzu). The alkaloid was separated in a reversed phase C_{18} column (Bio-Rad Bio-Sil C18 HI90-5S) using a 30 min gradient of 0−45% of methanol in 0.5% aqueous sodium acetate, at a flow rate of 1 mL/min. The eluting compounds were detected at 280 nm.

***H. hampei* Rearing.** *C. arabica* fruits infested with adult insects were used to start the rearing. These fruits were mixed with *C. arabica* seeds that had been moistened previously with water to facilitate the penetration of the insect. This prewetting was done by the immersion of the seeds for 12 h. Insects reared in this way were used to increase the population. Nine females and one male were transferred to 200 mL glass flasks containing 100 seeds, capped with silk-screen cloth, and maintained at 25 °C. This sex ratio was used because it is usually observed inside the coffee fruits (*14*). Female insects could be separated because they are larger than males.

Resistance Evaluation. The resistance level of 12 coffee species was evaluated in this experiment (*C. arabica* cv. Mundo Novo and cv. Catuaí; *C. canephora* cv. Robusta, cv. Kouillou, cv. Apoatã; *C. congensis* cv. Uganda; *C. eugenioides*; *C. dewevrei*; *C. liberica*; *C. racemosa*; *C. kapakata*; *C. brevipes*; *C. humilis*; *C. stenophylla*; and *C. salvatrix*). One hundred seeds from each plant were weighed and exposed to 10 adult insects (9 females and 1 male) as described before, and the attack was evaluated 6 months later by weighing the seeds again. The weight difference was expressed as percent weight loss. Caffeine was also determined in these seeds.

Table 1. Caffeine Content in Seeds and Level of Resistance (Mean + SD) to *H. hampei* in Cultivars and *Coffea* Species

coffee species	cultivar	plants analyzed	weight loss (%)	caffeine (mg/g)
C. arabica	Mundo Novo	3	22.7 ± 5.6	12.2 ± 0.5
	Catuaí Vermelho	3	37.7 ± 8.4	12.8 ± 0.5
C. canephora	Robusta	3	46.8 ± 6.51	13.1 ± 2.4
	Kouillou	3	24.2 ± 7.01	17.4 ± 0.5
	Apoatã	3	48.9 ± 3.67	14.0 ± 0.1
C. congensis	Ugandae	3	49.7 ± 3.26	15.8 ± 1.1
C. eugenioides		3	18.5 ± 8.24	3.1 ± 0.2
C. dewevrei		3	16.8 ± 3.05	6.4 ± 0.8
C. liberica		3	12.6 ± 6.55	11.1 ± 1.4
C. racemosa		3	10.4 ± 3.7	4.9 ± 1.7
C. kapakata		3	9.7 ± 3.37	5.5 ± 1.5
C. brevipes		1	34.7	4.9
C. humilis		1	15.7	13.3
C. stenophylla		1	9.8	13.7
C. salvatrix		1	2.7	2.1

Table 2. Distribution of Insects in Preference Tests among Mature Fruits of Coffee Species and Caffeine Content in Their Seeds

coffee species	exptl replicate			total[a]		caffeine (mg/g)
	I	II	III	obsd	expected	
C. arabica cv. Catuaí Vermelho	3	4	4	11	9	13.2
C. canephora cv. Robusta	7	8	8	23	9	14.5
C. congensis cv. Uganda	5	3	4	12	9	16.8
C. kapakata	1	1	1	3	9	5.9
C. eugenioides	1	1	1	3	9	3.2
C. stenophylla	2	2	2	6	9	13.7
C. humilis	2	2	1	5	9	13.3
Total	21	21	21			

[a] χ^2 = 14.5; degrees of freedom = 6; probability = 0.245.

Insect Attraction to Mature Fruits. An experiment was carried out with *C. arabica* cv. Catuaí Vermelho, *C. canephora* cv. Robusta, *C. kapakata*, *C. eugenioides*, *C. stenophylla*, *C. congensis* cv. Uganda, and *C. humilis* to assess the relative attraction of *H. hampei* adults to mature fruits, using an adaptation of the method by Ticheler (*9*). The bottom sides of Petri dishes were divided into seven compartments, each containing two mature fruits of each coffee species. The dishes were lined with silk-screen cloth, and on this fabric in the center of the dish, 21 females were released. Then the dishes were lined with brown paper to decrease the light incidence, and the number of insects on each fruit was recorded 30 min later. Female adults were used because males lack searching behavior (*14*). According to the results obtained in this experiment, a second one was carried out using 20 insects and mature fruits from *C. arabica* cv. Catuaí Vermelho, *C. canephora* cv. Robusta, and *C. kapakata*, which were tested in pairs. These experiments were repeated three times.

Imbibition of Seeds with Caffeine. An experiment was carried out to evaluate the effect of caffeine using *C. arabica* cv. Catuaí Vermelho and *C. canephora* cv. Apoatã. Fifty seeds were taken for caffeine analyses from six samples of 200 seeds each, identified by CV1−CV6 (Catuaí Vermelho) and AP1−AP6 (Apoatã), and the remaining seeds were used to determine seed mass. Then the six samples were immersed with six caffeine aqueous solutions (0, 0.01, 0.1, 0.5, 1.0, and 2.0%). Seeds were imbibed for 24 h in 200 mL of each solution and left to dry in filter paper. Caffeine was determined in 50 imbibed seeds, and the 100 remaining seeds were used to evaluate the insect attack, as described before in the experiment for resistance evaluation. The feces collected from the flasks used to keep the insects were used for caffeine determination.

Table 3. Distribution of Insects in Preference Tests among Mature Fruits of Three Coffee Species and Caffeine Content in Their Seeds

combination[a]	no. of insects	insect distribution in experiments I[b]	II	III	mean	caffeine (mg/g)	χ^2	*P*	df
RO × RO	20	12:7	12:8	10:10	11.3:8.3	14.5 × 14.5	0.23	0.636	1
RO × KA	20	15:5	16:4	15:5	15.5:4.7	14.5 × 5.9	3.02	0.078	1
RO × CV	20	13:6	15:5	13:6	13.7:5.7	14.5 × 13.2	1.72	0.185	1
KA × KA	20	12:8	10:9	11:8	11.0:8.3	5.9 × 5.9	0.19	0.667	1
KA × CV	20	5:9	6:14	6:13	5.7:12.0	5.9 × 13.2	1.16	0.282	1
CV × CV	20	10:10	9:10	7:12	8.7:10.7	13.2 × 13.2	0.10	0.747	1

[a] CV, *C. arabica* cv. Catuaí Vermelho; RO, *C. canephora* cv. Robusta; KA, *C. kapakata*. [b] Some results did not sum to 20 because the insect preference was not evident.

RESULTS AND DISCUSSION

The first experiment was carried out to evaluate the resistance against insect attack (**Table 1**). The data obtained for caffeine in the seeds are in agreement with previous studies carried out with the same plants (*23*, *25*, *28*). The weight loss in coffee seeds exposed to *H. hampei* was higher in the Apoatã and Robusta cultivars of *C. canephora*, supporting previous results in the literature (*6*−*8*). The close results of these cultivars might be explained by the fact that Apoatã was genetically selected from Robusta in a breeding program for nematode resistance (*29*). On the other hand, the Kouillou cultivar from *C. canephora* showed less weight loss, which was opposite the results obtained by Ticheler (*9*), who observed higher penetration of fruits of this cultivar by *H. hampei*. Cv. Kouillou was among the plants (*C. kapakata*, *C. stenophylla*, *C. liberica*, and C. *racemosa*) that were less damaged (weight loss) by the insects. The lowest weight loss was observed with *C. salvatrix*. *C. congensis* fruits were damaged at the same level observed for *C. canephora* vars. Apoatã and Robusta. An intermediary group of species was formed by the other species. Some large variations observed among plants of the same species (*C. liberica* and *C. eugenioides*) might be due to the fact that, except for *C. arabica*, the other coffee species are allogamous (*30*).

Therefore, it was not possible to obtain a significant negative statistical correlation ($r = 0.53$) between caffeine and insect damage. Although the *C. canephora* cultivars showed the highest caffeine content, their seeds were the most infested by *H. hampei*. Another contrasting result was observed with *C. eugenioides* and *C. racemosa*, which had large variations in terms of insect attack although the caffeine contents were practically the same in the three plants of each species.

The results of the second experiment on the attraction of adult females to mature fruits showed a clear preference for *C. canephora* cv. Robusta, followed by *C. arabica* cv. Catuaí Vermelho and *C. congensis* cv. Uganda (**Table 2**). The caffeine content in the seeds of these fruits did not correlate negatively with the insect attraction ($r = 0.61$). This experiment was repeated three times with very similar results. Therefore, a second experiment was carried out to explore more carefully this preference, and mature fruits from *C. arabica* cv. Catuaí Vermelho, *C. canephora* cv. Robusta, and *C. kapakata* were tested in pairs. The preference for *C. canephora* cv. Robusta was confirmed (**Table 3**). Again, no correlation was observed with the caffeine content.

Gutierrez-Martinez and Ondarza (*14*) suggested that caffeine was one of the components responsible by the kairomone effect of a methylene chloride extract obtained from mature coffee fruits. However, there was no statistical difference between insects attracted by caffeine dissolved in ethanol or ethanol in a field experiment. Highest trapping was observed with the methylene chloride extracts. Caffeine is not volatile, and it does not seem plausible to us that the alkaloid becomes more volatile by interacting with essential oils of the coffee plant. On the other hand, Mathieu et al. (*31*) showed that volatile terpenes, sesquiterpenes, and oxygenated compounds are released by coffee fruits at different stages of ripeness and suggested that they might be involved in the attraction of females of the coffee berry borer.

Table 4. Effect of Imbibition of *C. arabica* Cv. Catuaí Vermelho and *C. canephora* Cv. Apoatã Seeds with Caffeine Solutions on *H. hampei* Infestation and Caffeine Content in the Collected Feces

coffee species	caffeine solution[a] (%)	increase of caffeine[b] (%)	weight loss[c] (%)	infested seeds (%)	caffeine in the feces (mg/g)
Catuaí Vermelho	0.00	2.7	22.3	45.0	4.7
	0.01	−4.4	23.7	45.6	4.0
	0.10	11.6	22.5	48.3	3.9
	0.50	24.6	21.8	47.2	4.4
	1.00	64.2	27.9	45.6	4.2
	2.00	125.5	34.7	35.6	4.6
Apoatã	0.00	4.0	55.7	37.8	4.9
	0.01	16.0	52.7	27.2	5.4
	0.10	19.5	52.6	27.8	5.1
	0.50	20.1	55.1	39.4	4.9
	1.00	72.9	51.4	31.7	4.5
	2.00	68.5	56.0	31.7	5.1

[a] Aqueous caffeine solutions used for seed imbibition. [b] Increase of caffeine in relation to the initial content in the seeds of Catuaí Vermelho (12.7 mg/g) and Apoatã (13.8 mg/g). [c] Means of three replicates.

Giordanengo et al. (*13*) showed the preference of *H. hampei* for mature fruits is probably due to emission of specific volatiles. Olfactometry tests showed that *C. canephora* cv. Robusta was highly attractive to the insect females, whereas *C. arabica* cv. Mundo Novo and *C. kapakata* were moderately attractive (*32*). Although we did not try to correlate the preference of the insects with seed composition, it was observed that the development of the insect stopped in *C. kapakata* when the first larval stage was attained, suggesting the presence of toxic constituents in the seeds (*32*). Considering these results and those obtained in this work with weight loss, *C. kapakata* might be considered to be resistant to *H. hampei* because of two mechanisms, antixenosis (nonpreference) and antibiosis (poor development due to seed composition). Indeed, the experiments in **Table 1** on weight loss and in **Tables 2** and **3** on insect attraction support this suggestion.

The method used to enhance the caffeine content of the coffee seeds by imbibition with caffeine aqueous solutions was very satisfactory, although a nonregular increase was observed with Apoatã seeds (**Table 4**). At 20 °C the coffee seed takes ∼60 h for full imbibition (*33*, *34*). At the temperature of the experiments (25 °C) at least a 50% imbibition was attained, and it is possible that inner layers of the seeds were not imbibed.

Curiously, taking in account that seeds of Catuaí Vermelho and Apoatã have ~12−14 mg/g of caffeine (**Table 1**) and that seeds imbibed with 2% caffeine solution showed, respectively, increases of 125 and 70% of the alkaloid (**Table 4**), at least a 50% imbibition probably occurred in these seeds.

The increased caffeine content in these seeds did not change the weight loss caused by *H. hampei* in both species because no statistical differences were observed in any evaluated parameter (**Table 4**). In general, the weight loss was similar to that observed in the first experiment carried out to evaluate resistance (**Table 1**). In the imbibition experiment the percentage of infested seeds was also determined, and it was negatively correlated with weight loss. Although Apoatã seeds were less infested than Catuaí Vermelho, they had greater weight loss. Caffeine seemed to be metabolized to an extent by the digestive tract of the *H. hampei* larva and adults (**Table 4**) because the alkaloid concentration did not vary significantly in the insect feces even from those infesting seeds imbibed with 2% caffeine solution.

Although our data showed a lack of positive correlation between caffeine and resistance to *H. hampei*, the results of weight loss and mature fruit preference allow one to conclude that the *C. canephora* cultivars, Apoatã and Robusta, are more susceptible than the other species used in this study.

Proteins and amino acids, which have nitrogen as a component, are the most limiting macronutrients required by insects for development and reproduction (*35*). Caffeine has four nitrogen atoms in the molecule, and part of the alkaloid ingested by *H. hampei* might have some importance as a nutritional factor. However, caffeine content does not seem to be a determinant for insect attractiveness because coffee species with low caffeine content were also well infested. Therefore, the conclusion of the present study is that, indeed, there is variation for resistance against the attack of *H. hampei* in the *Coffea* germoplasm, but caffeine cannot be associated with the resistance. In addition, it seems that *H. hampei* has evolved an adaptation to avoid the toxic effects of caffeine.

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Received for review July 17, 2003. Revised manuscript received September 20, 2003. Accepted September 24, 2003. This work was funded by Fundação de Amparo à Pesquisa do Estado de São Paulo − FAPESP (Grant 95/5267-5). We thank CNPq-Brasil for research fellowships.

JF0347968