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IDENTIFICATION AND SYNTHESIS OF A KAIROMONE MEDIATING HOST LOCATION BY TWO PARASITOID SPECIES OF THE CASSAVA MEALYBUG *Phenacoccus herreni*

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Abstract—Two encyrtid species, *Acerophagus coccois* and *Aenasius vexans*, parasitoids of the cassava mealybug *Phenacoccus herreni* use a contact kairomone from the body surface of their host as a host-location stimulant. The kairomone was synthesized and identified as *O*-caffeoylserine based on a combination of chromatographic methods. The synthetic compound was determined to be active.

Key Words—*O*-Caffeoylserine, *Acerophagus coccois*, *Aenasius vexans*, Hymenoptera, Encyrtidae, Homoptera, Pseudococcidae.

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INTRODUCTION

The cassava mealybug, *Phenacoccus herreni* William & Cock (Homoptera: Pseudococcidae), is an important pest of cassava in South America (Bellotti et al., 1983; Noronha, 1990). Two encyrtid parasitoids, *Acerophagus coccois* Smith and *Aenasius vexans* Kerrich (Hymenoptera: Encyrtidae), are being studied to control *P. herreni* populations at the International Center for Tropical Agriculture (CIAT) in Cali, Colombia. *A. coccois* is considered a generalist because it parasitizes different mealybug species among the Pseudococcidae family. *A. vexans* is a specialist, parasitizing only *P. herreni* (Dorn et al., 2001).

During host selection, parasitoids use a variety of cues to assess the quality of their hosts, such as shape, surface structure, and both external and internal semiochemicals (waxy secretions and honeydew) (Lewis and Martin, 1990; Vinson, 1991; Godfray, 1994). The structure and quantity of these semiochemicals influence host acceptance by parasitoids and vary according to species, age or stage of development, size, condition, and diet of the parasitoid host (Vinson, 1991; Powell, 1992; Takabayashi and Takahashi, 1993; Röse et al., 1997). One important class of these subtances used by parasitic Hymenoptera to identify their hosts is kairomones (Lewis and Martin, 1990). For example, in the California red scale *Aonidiella aurantii* Maskell (Hemiptera: Diaspididae), a kairomone isolated from their cover and identified as *O*-caffeoyltyrosine, the ester of caffeic acid and tyrosine, mediates host recognition and induces oviposition by the parasitic wasp *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae) (Hare et al., 1993; Millar and Hare, 1993).

In cassava mealybug *Phenacoccus manihoti* Matile-Ferrero (Homoptera: Pseudococcidae), a serious pest of cassava in Africa (Neuenschwander et al., 1990; Le Rü et al., 1991), preliminary analyses indicated the presence of a compound composed of a serine and a caffeic acid moiety, probably corresponding to *O*-caffeoylserine (Calatayud, unpublished data). This compound was also detected in *P. herreni*; however, it could not be identified in the mealybug because it was always hydrolyzed during the purification process. It was, therefore, necessary to synthesize the ester and then verify whether the synthetic compound was similar to the compound assumed to be *O*-caffeoylserine. Because this putative compound is similar to the contact kairomone *O*-caffeoyltyrosine, important in another parasitoid–homopteran interaction as reported by Hare et al. (1993) and Millar and Hare (1993), we also hypothesized that this substance would act as a contact kairomone for the cassava mealybug parasitoids. Therefore, the objective of this study was to confirm the identification of *O*-caffeoylserine in *P. herreni* after its synthesis and to study its influence on host-location behavior of *A. coccois* and *A. vexans*.

METHODS AND MATERIALS

Insect Rearing. The cassava mealybug, *P. herreni*, was reared at CIAT on 30 to 40-cm potted cassava plants (cv. CMC 40) in a glasshouse at 27–33°C and 12L : 12D photoperiod. Adult females, the stage most parasitized by *A. coccois* and *A. vexans* (Seligmann, 1998), were collected from the colony for chemical analysis and bioassay.

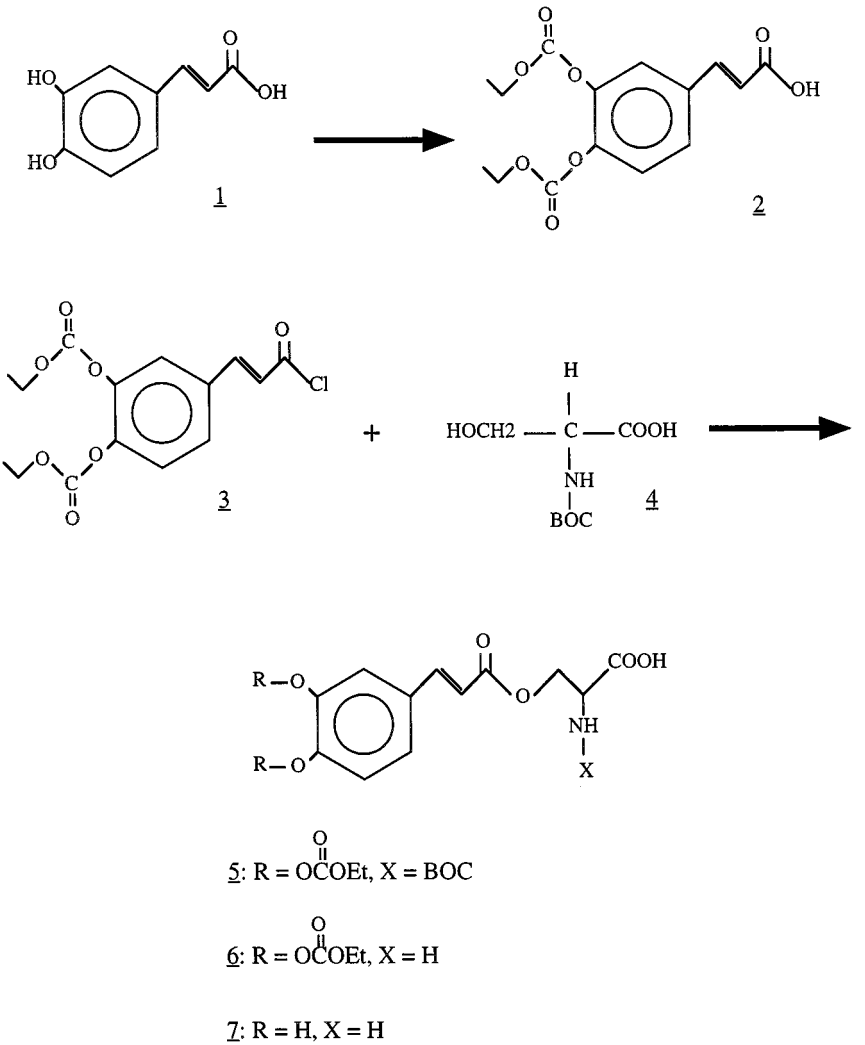
Both parasitoids species, *A. coccois* and *A. vexans*, were reared continuously on mealybug-infested cassava plants (cv. CMC 40) as described above for the cassava mealybug. Emerging adults (both males and females) were placed for 48 hr in transparent polystyrene tubes (15 × 1.5 cm) containing honey droplets as a food source. The wasps were sexed, and females were selected for bioassay.

Synthesis of Kairomone (*Figure 1*). The method used to synthesize *O*-caffeoylserine was that described by Millar and Hare (1993), for synthesizing *O*-caffeoyltyrosine. Caffeic acid (**1**) (10 g, 55 mmol) was added to ethyl chloroformate to produce 13.17 g (73.9% yield) of 3,4-diethoxycarbonyl-caffeic acid (**2**). ^{1}H NMR ($CDCl_3$): ∂ 7.72 (d, 1H, $J = 15.9$ Hz, C<u>H</u>COOH), 7.24–7.46 (3H, m aromatic), 6.42 (d, 1H, $J = 15.9$ Hz, olefinic H), 4.28 (2 overlapped quartets, 4H, $J = 7.1$ Hz, CH_2s), 1.33 (2 overlapped triplets, $J = 7.1$ Hz, methyls).

The acid (**2**) (13.17 g, 40.6 mmol) was stirred with 36.5 ml of thionyl chloride to obtain 10.23 g (73.7% yield) of 3,4-diethoxycarbonyl-caffeoyl chloride (**3**). ^{1}H NMR ($CDCl_3$): ∂ 7.73 (d, 1H, $J = 15.6$ Hz, olefinic H), 7.23–7.48 (3H, m aromatic), 6.56 (d, 1H, $J = 15.6$ Hz, olefinic H), 4.30 (overlapped quartets, 4H, $J = 7.1$ Hz, CH_2s), 1.36 (overlapped triplets, 6 H, $J = 7.1$ Hz, methyls). Then 1.71 g (5 mmol) of the chloride (**3**) was coupled with the *N-t*-BOC-(L)-serine (**4**) (1.03 g, 5 mmol; Sigma Chemical Co., St. Louis, Missouri) to produce 2.39 g (quantitative) of the compound (**5**). ^{1}H NMR ($CDCl_3$): ∂ 7.70 (d, 1H, $J = 16.2$ Hz, olefinic H), 7.22–7.58 (3H, m aromatic), 6.40 (d, 1H, $J = 15.9$ Hz, olefinic H), 5.62 (br. d, 1H, NH), 4.50 (m, 1H, NH-C<u>H</u>), 4.26 (br. quartet, 4H, C<u>H</u>$_2$s), 3.75 (m, 2H, -O-C<u>H</u>$_2$-CH-), 1.27–1.39 (m, 9H, *t*-BOC methyls, 6H, methyls).

After stirring the compound (**5**) (752 mg) with a mixture of methylene chloride and trifluoroacetic acid, the resulting dicarbonate (**6**) was treated with methanolic ammonium hydroxide. *O*-Caffeoylserine (**7**) was purified by TLC using a migration solvent of *n*-butanol–water–AcOH (4 : 1 : 1) for a total yield of 328 mg (87%). Synthesized *O*-caffeoylserine was used to confirm the presence of the compound in *P. herreni* and bioassayed at several concentrations to determine its biological activity on *A. coccois* and *A. vexans*.

To validate the synthetic method, *O*-caffeoyltyrosine was also synthesized, substituting the *N-t*-BOC-(L)-serine with *N-t*-BOC-(L)-tyrosine. The chloride (**3**) (1.71 g, 5 mmol) was coupled with the *N-t*-BOC-(L)-tyrosine (1.41 g, 5 mmol; Sigma) to produce 2.41 g (quantitative) of the compound with the following NMR



5: R = OCOEt, X = BOC

6: R = OCOEt, X = H

7: R = H, X = H

FIG. 1. Scheme of the various molecules obtained during synthesis of *O*-caffeoylserine.

data. ^{1}H NMR ($CDCl_3$): ∂ 7.75 (d, 1H, $J = 15.9$ Hz, olefinic H), 6.95–7.49 (6H, m aromatic), 6.53 (d, 1H, $J = 15.9$ Hz, olefinic H), 5.00 (br. d, 1H, NH), 4.58 (m, 1H, NH-CH), 4.30 (br. quartet, 4H, CH_2s), 3.11 (m, 2H, benzyl CH_2), 1.39 (br. s, 9H, *t*-BOC methyls), 1.34 (t, 6H, $J = 6.5$ Hz, methyls). From 536 mg of this compound, *O*-caffeoyltyrosine was obtained for a total yield of 245 mg (81%). Proton NMR spectra were obtained on an Advance DPX 200 Bruker NMR spectrometer at 200 MHz.

Isolation and Identification of O-Caffeoylserine in Cassava Mealybug. Adult females (2.4 g) were crushed in 5 ml of 70% ethanol. After 8 hr at 10°C, the extract was centrifuged at 21,000 *g* for 3 min, and the aqueous supernatant was washed twice with chloroform to remove lipids. The lyophilisate of the supernatant was suspended in 500 μl of 70% ethanol and banded on to a reverse-phase TLC plate (RP-18, F_{254s}, pre-coated sheet 20 × 20 cm, Merck), developed with acetonitrile–water (50 : 50), and visualized using UV at 254 nm. Two bands with R_f values of 0.75 and 0.69 (shown to contain the putative *O*-caffeoylserine by autoanalysis) were recovered by scraping the plate and extraction in 70% ethanol. After 8 hr at 10°C and centrifugation in 21,000 *g* for 3 min, the supernatants were lyophilized. The lyophilisate was suspended in 70% ethanol and submitted to the same TLC purification as above, but developed in *n*-butanol–2-propanol–water (60 : 20 : 20). Three bands with R_f = 0.81, 0.73, and 0.67 (shown to contain the putative *O*-caffeoylserine by autoanalysis) were recovered as above, let stand at 10°C for 8 hr, centrifuged, and lyophilized. A mixture of compounds including the putative *O*-caffeoylserine remained in the extract. It was not possible to repeat this TLC purification because the putative *O*-caffeoylserine disappeared, probably due to hydrolysis.

The compound was first identified by LC-MS from a portion of the extract. LC-MS experiments were performed on a Finnigan TSQ 700 under atmospheric pressure chemical ionization (APCI) as described elsewhere (Renukappa et al., 1999). In this case, a reversed-phase column (Grom-Sil 120 ODS-5, 250 × 4.4 mm, 5 μm, Grom, Germany) was used. Separation was achieved by using 0.1% aqueous trifluoroacetic acid in a linear gradient of 5–100% with acetonitrile over 35 min and a constant flow of 0.1ml/min.

In the second part, known quantities of the synthetic *O*-caffeoylserine were analyzed, alone or added to the remaining extract, by HPLC and autoanalysis. Samples were analyzed by RP18-HPLC by using the Pico-Tag method from Waters after amino acid derivatization with phenyl isothiocyanate (PITC). The compounds were identified by their retention time and quantified by their UV absorption at 240 nm.

Samples were submitted also to ion-exchange chromatography on an automatic amino acid analyzer (Beckmann 6300). Amino acids were detected by ninhydrin reaction, identified by their retention time and wavelength ratio, and quantified by their absorption at 570 nm.

Determination of O-*Caffeoylserine as Host-Location Kairomone.* To verify that *O*-caffeoylserine is present on the body surface of *P. herreni*, 500 adult females (representing about 500 mg of total fresh weight) without prior maceration were soaked in 500 μl of aqueous 70% ethanol for a final proportion equivalent to 1 μl of extract per individual. Insects without wounds were used, and the suspension was not stirred to limit leaching of internal insect constituents. The suspension was left at 10°C for five days. An aliquot of 100 μl was collected

each day and dried for autoanalysis of amino acids. This experiment was run in triplicate.

To confirm the function of *O*-caffeoylserine as a contact kairomone, 100 mealybugs were soaked in 100 μl of 70% aqueous ethanol at 10°C for three days, pipetted, and used for bioassay. The same experiment was done with pure hexane and bioassayed as a negative control. Because the ethanolic suspension also contained free amino acids, a solution duplicating the composition and concentration of these compounds was also bioassayed. The natural host (i.e., adult female of *P. herreni*) was bioassayed as the positive control. Synthetic *O*-caffeoylserine was bioassayed at several concentrations to confirm its function of as a host-location kairomone for mealybug parasitoids.

Bioassays. Wasps initially investigate mealybugs by palpating with their antennae as they walk from one edge of the mealybug body to the other. They then turn ≈90° and repeat this behavior one or more times. This behavior, previously observed in several Aphelinidae species, has been termed "drumming and turning" by Luck et al. (1982). After drumming and turning, *A. vexans* and *A. coccois* insert their ovipositors inside the host body in most cases, indicating that this behavioral event characterizes their host location. Therefore, the number of drummings and turns was recorded for each wasp observed in the following experiments. The bioassay was conducted on mealybugs or on treated cotton balls, which mimic the mealybug body. For cotton balls, 1 μl of test solution was pipetted onto cotton balls approx. 2.0 mm diameter. One insect or treated cotton ball was placed inside a single glass vial bioassay arena (5 mm diam. × 10 mm high), and for treated cotton balls the solvent was allowed to evaporate. One gravid female was added, and the vial was sealed with a cotton plug. Wasp behavior was observed for 15 min with a 6× dissecting microscope. Bioassays were conducted in the same greenhouse where the parasitoids were reared, between 1000 and 1500 hr at 25–35°C. The natural host (adult females of *P. herreni*) and several solutions were bioassayed as described above.

Five replicate trials of five wasps were bioassayed, with new insects or fresh cotton balls prepared for each trial. Wasps were used only once. The proportion of total drummings and turns on each mealybug or cotton ball was calculated and analyzed by ANOVA after applying $\sqrt{(X+1)}$ transformation. Fisher's (protected least significant difference PLSD) test was used following ANOVA to compare means. Statistical tests were performed with Statview software (Abacus Concept).

RESULTS

Identification of O-*Caffeoylserine in Cassava Mealybug.* LC-MS analysis of *P. herreni* extracts revealed a compound with a molecular weight of 268.3 $(M+H)^+$,

close to that calculated from the formula of *O*-caffeoylserine, corresponding to $C_{12}H_{13}NO_6$, which is 267.2.

To confirm the identity of *O*-caffeoylserine in *P. herreni* extracts, the compound was synthesized according to the method of Millar and Hare (1993), which was used to synthesize another ester, *O*-caffeoyltyrosine. The proton NMRs (CD_3OD) of the two synthetic esters were readily interpreted.

For the *O*-caffeoylserine, we obtained: 7.43 (d, 1H, $J = 15.9$ Hz, olefinic H), 6.78–7.06 (3H, m aromatic), 6.29 (d, 1H, $J = 15.8$ Hz, olefinic H), 5.09 (br s, 1H), 4.10 (m, 1H), 3.79 (q, $J = 11.1$ Hz, 2H). Except for protons lost in exchange in CD_3OD, these NMR data corresponded to those obtained by Lin et al. (1999), confirming the synthesis of this ester.

For the *O*-caffeoyltyrosine, we obtained: 7.77 (d, 1H, $J = 15.9$ Hz, olefinic H β to carbonyl), 7.41 (d, 2H, $J = 8.54$ Hz, aromatic H, tyrosine), 7.04–7.19 (4H, m from aromatic H of tyrosine and caffeic acid), 6.84 (d, 1H, $J = 8.14$ Hz, meta H, caffeic acid), 6.50 (d, 1H, $J = 15.83$ Hz, olefinic H α to carbonyl), 3.82 (dd, 1H, $J = 9.1$, 4.24 Hz, $C\underline{H}NH_2$), 3.35 (dd, 1H, $J = 14.12$, 4.10 Hz, benzylic H), 3.04 (dd, 1H, $J = 14.5$, 9.1 Hz, benzylic H). These NMR data corresponded to those obtained by Millar and Hare (1993), validating the synthesis method.

The retention time of synthetic *O*-caffeoylserine on a reverse-phase HPLC column matched that of the peak at 18.9 min from the *P. herreni* extract. This peak increased in area when the *P. herreni* extract was augmented with 0.4 nmol of synthetic *O*-caffeoylserine (Figure 2).

The retention time of synthetic *O*-caffeoylserine on an ion-exchange column also matched that of the compound isolated from *P. herreni* (chromatograms not shown). This result, together with RP-HPLC data, confirms the identifiy *O*-caffeoylserine in *P. herreni* extracts.

Determination of O-*Caffeoylserine as Host-Location Kairomone.* When mealybugs were soaked in 70% ethanol without prior maceration and submitted to autoanalysis, the *O*-caffeoylserine amounted to 0.026 nmol/mg (approx. 0.03 nmol/mg) or 5.2% (in moles) (Table 1). Compared to the other nitrogenous compounds analyzed, this was not major. Alanine and glutamic acid were much better represented among the compounds analyzed, at 28.4 and 17.1% (in moles), respectively. Furthermore, this concentration of *O*-caffeoylserine was the maximum possible when mealybugs were soaked in 70% aqueous ethanol. In fact, this concentration varied over time (Figure 3) from a minimum of 0.011 ± 0.002 nmol/mg (mean $\pm$ SE, $N = 3$) on day 1 to a maximum of 0.032 ± 0.002 nmol/mg on day 3 and declining to 0.027 ± 0.004 nmol/mg on day 5, probably due to hydrolysis.

The fresh weight of an adult female is about 1 mg on CMC 40. According to the extraction proportion, 1 μl of extract was obtained per individual or per 1 mg of fresh weight. Therefore, 0.03 nmol/mg is equivalent to 0.03 nmol/μl. Synthetic *O*-caffeoylserine was bioassayed based on this concentration (at 0.03 nmol/μl and 10 times higher or 2, 4, or 8 times lower).

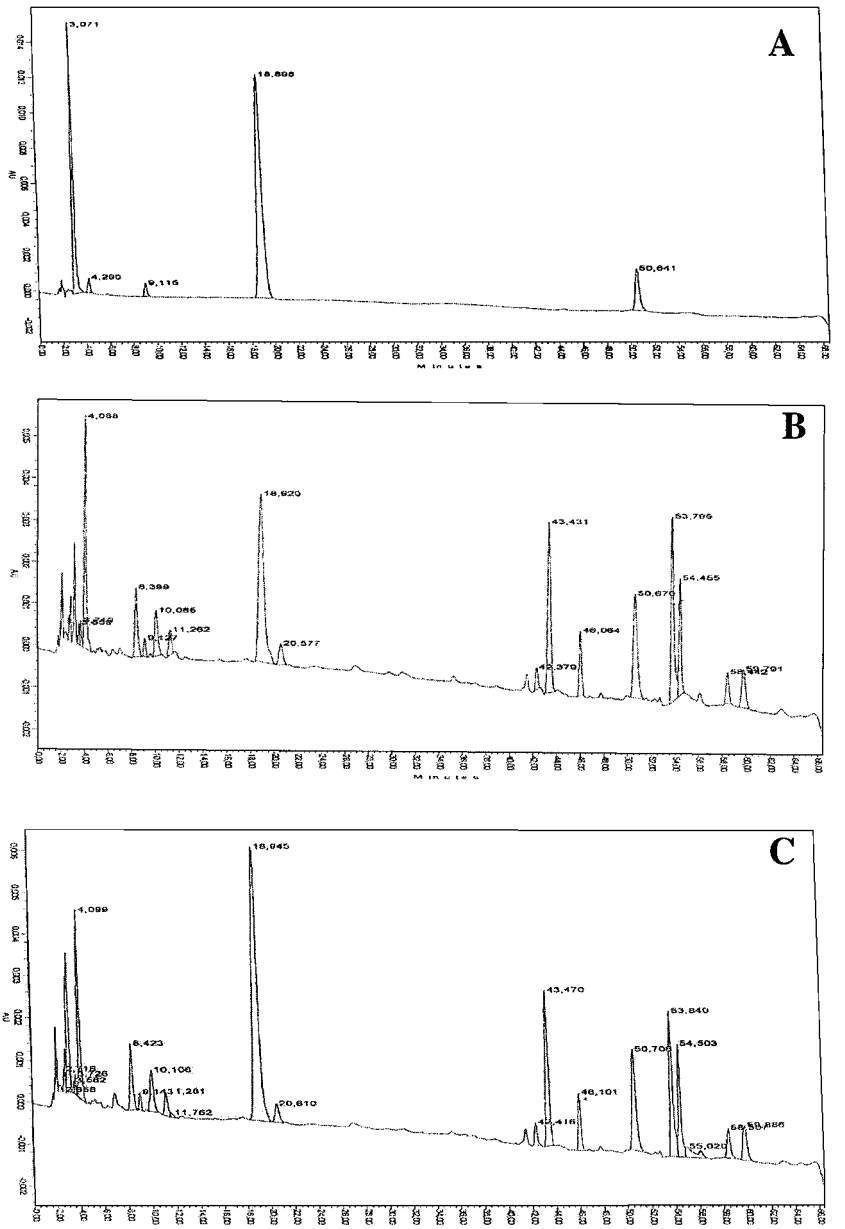


FIG. 2. HPLC chromatograms of (A) the synthetic *O*-caffeoylserine alone (at 2 nmol/10 μl); (B) an extract of *P. herreni* showing the unknown peak at 18.9 min; and (C) the same extract analyzed in (B) augmented with 0.4 nmol of synthetic *O*-caffeoylserine.

TABLE 1. CONTENT AND COMPOSITION OF FREE NITROGENOUS COMPOUNDS IN MEALYBUGS

Free nitrogenous compounds	Concentration (pmol/1 mg)	% (mol)
Asp	30 ± 5	5.9 ± 0.05
Thr	14 ± 2	2.9 ± 0.86
Ser	20 ± 5	3.9 ± 0.25
Asn	8 ± 2	1.5 ± 0.05
Glu	86 ± 25	17.1 ± 1.80
Gln	13 ± 4	2.6 ± 0.40
Pro	24 ± 5	4.7 ± 0.10
Gly	34 ± 7	6.7 ± 0.11
Ala	143 ± 14	28.4 ± 2.41
Val	19 ± 2	3.8 ± 0.30
Met	9 ± 1	1.8 ± 0.10
Ile	7 ± 1	1.4 ± 0.09
Leu	8 ± 1	1.6 ± 0.03
Tyr	13 ± 2	2.7 ± 0.20
Phe	2 ± 1	0.3 ± 0.03
O-Caffeoylserine	26 ± 7	5.2 ± 0.36
Try	22 ± 6	4.4 ± 0.30
Orn	2 ± 1	0.3 ± 0.07
Lys	4 ± 2	0.7 ± 0.02
His	11 ± 4	2.1 ± 0.44
Arg	11 ± 4	2.0 ± 0.45

[a] Values are (mean ± SE, $N = 3$).

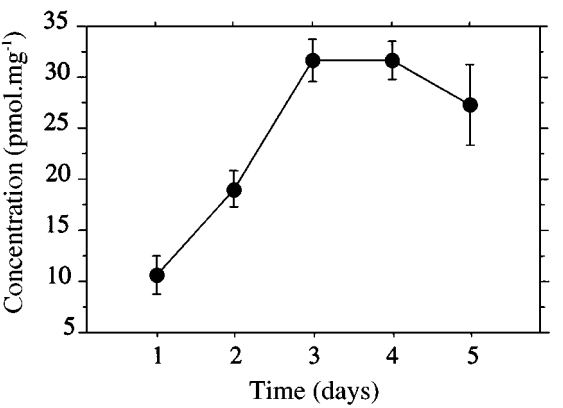


FIG. 3. Concentration of *O*-caffeoylserine in extracts from mealybugs (mean ± SE, $N = 3$) soaked without prior maceration in 70% ethanol as a function of time at 10°C.

For both parasitoid species, the extracts from mealybugs soaked in 70% ethanol were significantly more stimulating for host location than those in hexane (Figure 4), but no oviposition probing was observed. Moreover, these ethanolic extracts showed similar host-location stimulations when *P. herreni* was bioassayed. No stimulation was noted with ethanol alone, suggesting that the mediation of host location was in fact due to compound(s) present in ethanolic extracts of

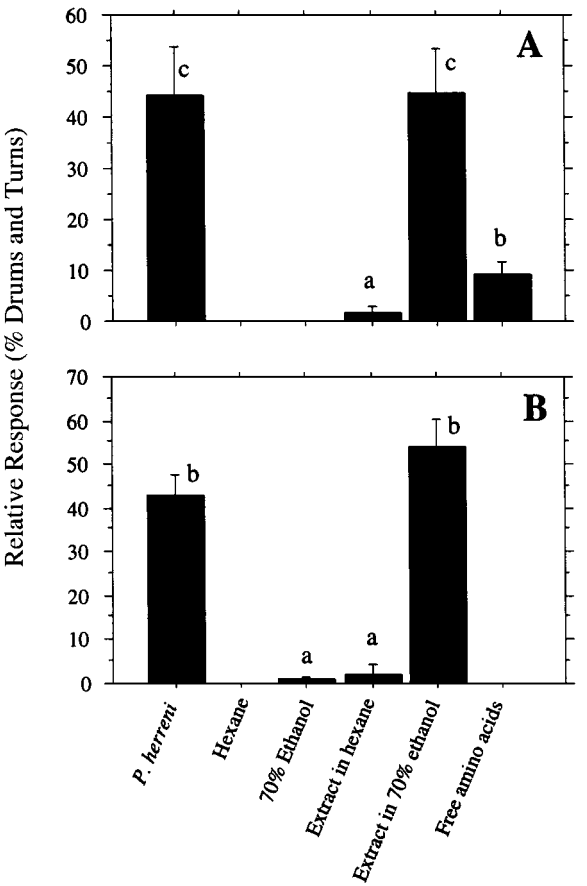


FIG. 4. Percent drums and turns observed for *A. coccois* (A) and *A. vexans* (B) on *P. herreni* and on cotton balls treated with solvent alone (hexane or 70% aqueous ethanol), extracts from mealybugs suspended without prior maceration in hexane or in 70% ethanol, or a mixture of free amino acids using the same concentration and composition as in Table 1. Bars indicate the mean (±SE; five trials with five wasps each). Means with the same letter are not significantly different at 5% level (Fisher's PLSD test following the ANOVA, $P < 0.05$). No statistical comparison was possible for means = 0%.

P. herreni and well solubilized in 70% aqueous ethanol. A mixture of free amino acids, similar in concentration and composition to the one obtained in Table 1, showed no stimulating effects as compared to the extracts of *P. herreni* in ethanol, indicating the presence of other compound(s) in the extracts, different from free amino acids, that have a stimulatory function for host location by *A. coccois* and *A. vexans*.

Among the concentrations in *O*-caffeoylserine tested, 0.03 nmol/μl was highly attractive for *A. coccois* (Figure 5A). Nevertheless, no difference was found between 0.03 and 0.015 nmol/μl. After removing the carbonate-protecting groups

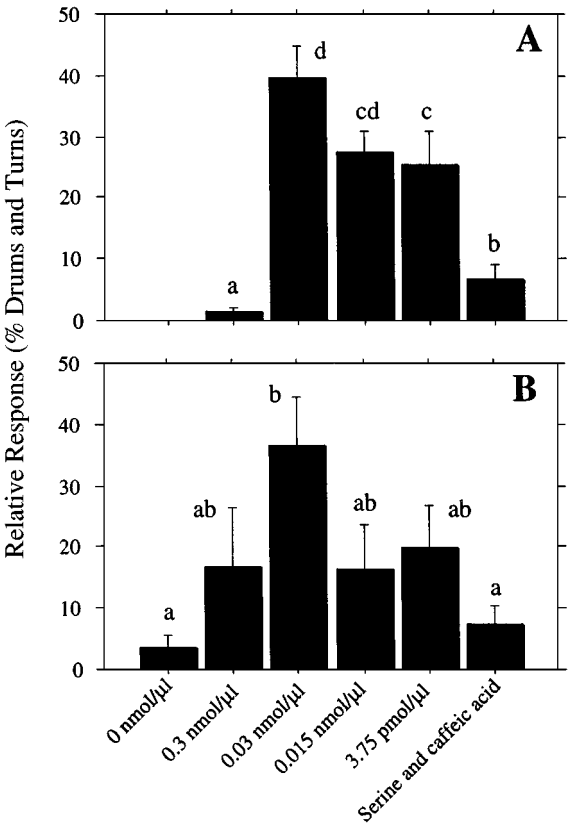


FIG. 5. Percent of drums and turns observed for *A. coccois* (A) and *A. vexans* (B) on cotton balls treated with synthetic *O*-caffeoylserine at several concentrations or with mixture of serine and caffeic acid at 0.03 nmol/μl each compound. Bars indicate the mean ($\pm$SE; five trials with five wasps each). Means with the same letter are not significantly different at 5% level (Fisher's PLSD test following the ANOVA, $P < 0.05$, except for *A. vexans* (B), $P > 0.05$ for ANOVA). No statistical comparison was possible for means = 0%.

(ultimate phase in Figure 1), using Millar and Hare's (1993) synthesis method, there is inevitably a partial ester cleavage, resulting in the presence of free serine and caffeic acid in the synthetic *O*-caffeoylserine solution. Therefore, a mixture of these compounds was also bioassayed at 0.03 nmol/μl, i.e., at the same concentration of *O*-caffeoylserine where the wasps responded better (Figure 5). It appeared that the *A. coccois* female wasps were less stimulated by the hydrolysis products of the *O*-caffeoylserine (caffeic acid and serine) confirming that the ester, *O*-caffeoylserine, is the biologically active compound. Moreover, for this parasitoid species, the ester also induced oviposition probing because 24% (i.e., 6 of the 25) of the wasps bioassayed at 0.03 nmol/μl of *O*-caffeoylserine probed the cotton ball with their ovipositor. Oviposition probing was not observed with the other concentrations of *O*-caffeoylserine tested.

Synthetic *O*-caffeoylserine at 0.03 nmol/μl elicited more drumming and turning of *A. vexans*, but differences were not observed among the concentrations tested (Figure 5B, $P = 0.0547$ for ANOVA), indicating no dosage-dependent response in this parasitoid species. Nevertheless, *O*-caffeoylserine did induce a stimulating effect in this parasitoid species at 0.03 nmol/μl because the wasps exhibited significantly higher drummings and turns than when the solvent alone or the mixture of serine and caffeic acid were bioassayed.

DISCUSSION

The identification of *O*-caffeoylserine in *P. herreni* was confirmed. To our knowledge, this represents the first report of *O*-caffeoylserine isolated from biological material, although this ester was recently synthesized by Lin et al. (1999). Without prior maceration, *O*-caffeoylserine amounted to approx. 0.03 nmol/mg of mealybug fresh weight, representing 5.2% (in moles) of the nitrogenous compounds analyzed (Table 1). Although it can be assumed that the procedure used (i.e., soaking the mealybugs in 70% ethanol for three days at 10°C) is sufficient to leach out a number of internal insect constituents, some arguments support the fact that the method tends to dissolve surface rather than internal constituents and that *O*-caffeoylserine should, in fact, amount to 0.03 nmol/mg on the body surface of *P. herreni* females in the adult developmental stage. First, when mealybugs were crushed, *O*-caffeoylserine amounted to about 9.5 nmol/mg of mealybug fresh weight, i.e., approx. 916 times higher, being much more concentrated than that obtained without maceration. Furthermore, when whole insects were soaked in ethanol, the concentration of *O*-caffeoylserine reached a maximum at three days and was three times higher than on day 1 (Figure 3). If the procedure were efficient in leaching out internal insect constituents, the concentration would increase much more than three times based on the internal concentration of *O*-caffeoylserine mentioned above. Figure 5 shows that both parasitoid species

responded better to 0.03 nmol/μl of *O*-caffeoylserine. This concentration, equivalent to 0.03 nmol/mg of mealybug fresh weight, is similar to that found when mealybugs were soaked in 70% ethanol for three days at 10°C. Given the fact that higher biological activity was obtained at this concentration and that it is the maximum that can be extracted by soaking unmacerated insects in ethanol, we suggest that *O*-caffeoylserine amounts 0.03 nmol/mg on the body surface of adult *P. herreni* females.

The presence of free amino acids in the sample supports the idea that some internal constituents can be leached out as well (Table 1). However, this is more likely due to honeydew secretions containing free amino acids that could stick to the body surface of neighboring mealybugs, given their proximity under mass-rearing conditions. *O*-Caffeoylserine was not found in the honeydew secretion of *P. herreni*.

Using several concentrations of synthetic *O*-caffeoylserine, it was confirmed that this compound influences host-location behavior of two parasitoid species of the cassava mealybug: *A. coccois* and *A. vexans*. Perhaps because of its generalist nature, this was more clearly shown with *A. coccois* due to the fact that the dosage-dependent response was well observed, with higher biological activity at 0.03 nmol/μl (Figure 5A). Although no such response was observed for *A. vexans*, *O*-caffeoylserine also was stimulatory at 0.03 nmol/μl (Figure 5B). In conclusion, for both parasitoid species, 0.03 nmol/μl is the concentration of *O*-caffeoylserine that induces more drummings and turns of wasps (approx. 40% of relative response among the concentrations bioassayed, Figure 5). This concentration, equivalent to 0.03 nmol/mg, is similar, to what can be extracted from the body surface of mealybugs, induces significant drummings and turns (approx. 40% of relative response as compared to the natural host, Figure 4), and indicates that synthetic *O*-caffeoylserine is as active as the chemical isolated from the body surface. All these results are consistent with *O*-caffeoylserine being a contact kairomone that mediates host-location behavior by the two cassava mealybug parasitoids.

Except for *A. coccois* at 0.03 nmol/μl of *O*-caffeoylserine, oviposition probing was not observed with any of the extracts bioassayed, regardless of parasitoid species. It may be that the female wasp behavior for oviposition probing is influenced by other factors such as color and texture of the mealybug body. In fact, in preliminary experiments we observed that for both parasitoid species, texture influenced wasp behavior for host location more than color (Calatayud, unpublished data). Some wasps inserted their ovipositor inside the untreated cotton plug, whereas oviposition probing was not observed with the treated cotton ball inside the glass vial. This was probably due to the fact that the cotton plug was more compact than the cotton ball, suggesting that a good combination between the concentration of *O*-caffeoylserine and texture could enhance oviposition probings. Unfortunately, increasing the pressed texture of the cotton ball was not possible given its size.

O-Caffeoylserine is not provided directly by the host plant; it is either synthesized by the insect itself or with the help of symbiotic bacteria. In fact, it was detected in adult mealybugs reared on holidic diets at 14.9 nmol/mg fresh weight, a higher level than in plant-reared mealybugs (about 9.5 nmol/mg), but not for extracts of host plant (*Manihot esculenta*) leaves. Nevertheless, enzymes commonly involved in the synthesis of phenolic compounds in plants such as phenylalanine or tyrosine ammonia-lyase were not evidenced in *P. herreni* (Calatayud, unpublished data).

The physiological role of *O*-caffeoylserine in the cassava mealybug is not clear. The caffeic acid moiety, as suggested by Hare et al. (1993) for *O*-caffeoyltyrosine, could be a precursor of the quinone compound involved in cuticle sclerotization and tanning. The serine moiety could be a precursor of the bristle (serine polymer) in the ovisacs. The fact that this phenolic compound is present in higher concentration in eggs (at about 26.4 nmol/mg) than in adult females suggests that it may also have a protective function (e.g., antibacterial or antiviral activity) in the insect. All these hypotheses need to be demonstrated explicitly.

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