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Antennal electrophysiological responses of the giant swallowtail butterfly, *Papilio cresphontes*, to the essential oils of *Zanthoxylum clava-herculis* and related plants

Henry Fadamiro · Li Chen · Clement Akotsen-Mensah · William N. Setzer

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Abstract We used the electroantennogram (EAG) technique to compare the antennal sensitivity of both sexes of the giant swallowtail butterfly, *Papilio cresphontes* to four doses (1, 10, 100, and 1,000 μg) of the leaf essential oils of *Zanthoxylum clava-herculis* and *Ptelea trifoliata* (key host plants) and *Sassafras albidum* (a marginal or non-host plant). The main hypothesis tested was that *P. cresphontes* will show greater olfactory sensitivity to volatiles of the key host plants than to volatiles of the marginal host plant, in particular at low doses. At the lower doses, extract of the key host plant, *Z. clava-herculis* elicited greater EAG responses in both sexes than extracts of the remaining two plants. At higher doses, however, extracts of *P. trifoliata* and *S. albidum* elicited greater EAG responses than extract of *Z. clava-herculis.* These results partly support our hypothesis and may suggest that *Z. clava-herculis* is a more preferred host plant of *P. cresphontes* than *P. trifoliata*. In general, female butterflies showed greater EAG responses than males to the three plant extracts at the higher doses. Preliminary coupled gas chromatography-electroantennogram (GC-EAD) tests revealed four components each from *Z. clava-herculis* and *P. trifoliata* (three peaks common to both extracts) and seven from *S. albidum* (one shared with *Z. clava-herculis*) which elicited GC-EAD activity in *P. cresphontes* females, but the peaks were un-indentified because most were detected in trace amounts. In addition, the chemical composition of the leaf essential oil of *Z. clava-herculis* was analyzed by GC–MS. The leaf essential oils consisted of 25 components, largely menthane monoterpenoids, dominated by limonene and 1,8-cineole, but neither of the two major components elicited significant GC-EAD response in *P. cresphontes*. These results are discussed in relation to host-plant selection in *P. cresphontes.*

Keywords *Zanthoxylum clava-herculis* · Essential oil composition · *Papilio cresphontes* · Electroantennogram · GC-EAD · GC–MS

H. Fadamiro (✉) · L. Chen · C. Akotsen-Mensah
Department of Entomology and Plant Pathology,
Auburn University, Auburn, AL 36849, USA
e-mail: fadamhy@acesag.auburn.edu

W. N. Setzer
Department of Chemistry, University of Alabama in Huntsville,
Huntsville, AL 35899, USA
e-mail: wsetzer@chemistry.uah.edu

Introduction

The giant swallowtail butterfly, *Papilio cresphontes* Cramer (Lepidoptera: Papilionidae) is the largest butterfly in North America with a wingspan of 100–160 mm, and can be found throughout eastern North America and all year round in Florida and the Deep South. An oligophagous butterfly species, members of the Rutaceae serve as host plants for *P. cresphontes*, including *Zanthoxylum clava-herculis* L., *Z. americanum* Mill., *Z. fagara* (L.) Sarg., *Ptelea trifoliata* L., *Amyris elemifera* L., and *Casimiroa edulis* La Llave & Lex (Scott 1992; McAuslane 2009). *P. cresphontes* larvae are also known as "orange dogs" because they feed on the leaves of orange tree, *Citrus* spp. (Scott 1992). Early instar larvae of *P. cresphontes* and other *Papilio* species are generally unable to move from plant to plant, thus host-plant selection is determined mainly by the ovipositing females (Rausher 1979; Schoonhoven et al. 2005).

Host-plant selection by phytophagous insects generally involves two key steps, host finding/location and host

recognition and acceptance (oviposition decision), each step being mediated by different cues. Host-plant location in butterflies can be mediated by visual modalities (optical characteristics of plants), chemical cues (plant volatiles), or both (Visser 1986; Bernays and Chapman 1994; Schoonhoven et al. 2005). Several studies have documented the response of some butterfly species to optical characteristics of plants including color (spectra quality), size, and shape or pattern (Rausher 1978; Papaj 1986; Schoonhoven et al. 2005). Associative learning of color has also been demonstrated for some butterfly species (Traynier 1986; Van Loon et al. 1992). Volatile chemical compounds are also known to mediate long range attraction of some butterflies to host plants (Visser 1986; Feeny et al. 1989; Städler 1992; Schoonhoven et al. 2005). Once they land on host plants, the current evidence suggest that both plant volatiles (Vaidya 1969; Saxena and Goyal 1978; Feeny et al. 1989; Baur et al. 1993; Heinz 2008; Mercader et al. 2008) and non-volatile contact chemicals (Roessingh et al. 1991; Nishida 1995; Haribal and Feeny 2003; Schoonhoven et al. 2005) may play a role in host acceptance and oviposition behavior of butterflies. In one of the classic demonstrations of the role of host-plant volatiles on the oviposition behavior of *Papilio* butterflies, females of *P. demoleus* L. were shown to orient toward a volatile extract of its host *Citrus limettoides* Tan (Rutaceae) and even laid eggs when they were allowed to make contact with the moistened host-plant extract (Saxena and Goyal 1978). On the other hand, several studies have identified contact oviposition stimulants for a number of swallowtail butterflies (*Papilio* spp.) (Nishida 1995; Haribal and Feeny 1998; Carter et al. 1999; Ono et al. 2000; Nakayama et al. 2003; Nakayama and Honda 2004), providing evidence for the role of non-volatile contact chemicals in oviposition behavior. A recent paper also reported on the identification of amine receptors in tarsal chemosensilla of *P. xuthus* L. which may function in chemoreception of oviposition stimulants (Ono and Yoshikawa 2004). Despite the prior work on several *Papilio* species, little is known about host-plant selection in *P. cresphontes*. Specifically, the response of *P. cresphontes* to Rutaceae host plants has not been investigated and it is not known what (if any) roles volatile chemicals may play in host selection and preference.

Two distinct but related objectives were investigated in this study. In the first, we tested the ability of *P. cresphontes* butterflies to perceive the volatile essential oils of *Z. clava-herculis*, *P. trifoliata*, and *Sassafras albidum* (Nutt.). The first two plants are host plants of *P. cresphontes*, whereas *S. albidum* is a host plant of the spicebush swallowtail butterfly, *P. troilus* L. (Nitao et al. 1991; Lederhouse et al. 1992). Based on published reports (e.g., Scott 1992), *Z. clava-herculis* and *P. trifoliata* should be regarded as key hosts and *S. albidum* a marginal (or non-host) host plant for *P. cresphontes*. Since volatile plant compounds (attractants and repellents) are primarily detected in adult insects by antennal olfactory sensilla, we compared the gross antennal sensitivity of both sexes of *P. cresphontes* to the leaf essential oil extracts of the three plant species by using the electroantennogram (EAG) technique. Females were tested since it is the sex primarily involved in host location for oviposition. Males were tested because they are known to search for females around host plants (Rutowski 1991; Scott 1992), suggesting that males may also have evolved ability to respond to host-plant volatiles. The main hypothesis tested was that *P. cresphontes* will show greater olfactory sensitivity (higher EAG response) to volatiles of the key host plants (*Z. clava-herculis* or *P. trifoliata*) than to volatiles of the marginal host plant (*S. albidum*), in particular at low doses. Since females are the sex primarily involved in host-plant location for oviposition and are expected to have evolved greater olfactory sensitivity to host odor than males, we also predicted that *P. cresphontes* females will show relatively greater EAG responses than conspecific males to the host-plant volatiles. Additionally, preliminary tests were conducted using coupled gas chromatography-electroantennogram (GC-EAD) recordings to determine the components of the leaf essential oils of the three plants perceived by *P. cresphontes* females.

In the second objective, we characterized the chemical composition of the leaf essential oil of *Z. clava-herculis* growing in Huntsville, Alabama. This study was conducted based on the results of the first objective which indicated that *Z. clava-herculis* may be a preferred host of *P. cresphontes*. Furthermore, the chemical compositions of the leaf essential oil of *P. trifoliata* (Takaku and Setzer 2007) and *S. albidum* (Kaler and Setzer 2008) have been recently reported. To our knowledge, this report is the first to present the electrophysiological responses of *P. cresphontes* to host-plant volatiles and characterize the chemical identity of *Z. clava-herculis* leaf oils.

Materials and methods

Plant materials

A sampling of mature leaves of *Z. clava-herculis*, *P. trifoliata*, and *S. albidum* from different locations on different trees growing in second growth forest at 187-m elevation on the western foot of Green Mountain, Huntsville, Alabama (34°38′46″ N, 86°33′27″ W) was conducted in 2004. The leaves were collected in the morning (approximately 8:00 a.m.), immediately chopped, and hydrodistilled for 4 h using a Likens–Nickerson hydrodistillation-extraction (choloform) apparatus.

Insects

Papilio cresphontes pupae were purchased from Shady Oak Butterfly Farm (Brooker, FL) and the Butterfly Farmers (La Belle, FL). Upon receipt, butterfly pupae were first placed in a plastic Petri dish (100 × 20 mm) (2 per dish). The dishes were then placed in a plastic cage (30 × 30 × 30 cm) at 25 ± 1°C, LD 14:10 h, and 70 ± 5% RH until emergence. High humidity was maintained by placing a glass beaker (100 mL) loaded with wet paper towel in the cage. Sugar (10% sucrose) solution and water were provided in the Petri dishes as food sources for newly emerged butterflies. Newly emerged butterflies were removed daily and the sex determined by the presence of an ovipositor in the female and the male genitalia. Butterflies used for the electrophysiological recordings were one-day old.

Electroantennogram (EAG) recordings

Electroantennogram bioassays were performed using the procedures described by Chen and Fadamiro (2007). Briefly, glass capillary (1.1 mm I.D.) filled with Ringer solution was used as electrodes. Antennal preparations were made by first cutting the base and distal end of antenna with a scalpel. The reference electrode was connected to the base of the antenna, and the recording electrode was connected to the cut tip of the antenna. Chlorinated silver–silver chloride junctions were used to maintain electrical contact between the electrodes and input of preamplifier. The analog signal was detected through a probe (INR-II, Syntech®, Hilversum, the Netherlands), captured and processed with a data acquisition controller (IDAC-4, Syntech®, the Netherlands), and later analyzed with a software (EAG 2000, Syntech®) on a personal computer. Serial dilutions (0.1, 1.0, 10, and 100 μg/μL) of each essential oil extract were made with hexane. A 10-μL aliquot of each solution was applied to a piece of filter paper (7 × 40 mm, Whatman no. 1). After allowing for solvent evaporation, the impregnated filter paper was inserted into a glass Pasteur pipette (~14 cm in length, Fisher Scientific, Pittsburgh, Pennsylvania, USA) constituting an odor cartridge. The control stimulus was a similar pipette containing a filter paper impregnated with a 10-μL aliquot of hexane. The tip of the pipette was placed about 3 mm into a small hole in the wall of a glass tube (13 cm long, 8 mm diameter) oriented toward the antennal preparation (~0.5 cm away from the preparation). In this way, the stimuli were provided as 0.2 s puffs of air (2 mL) into continuous humidified air stream at 1,000 mL/min generated by an air stimulus controller (CS-55, Syntech®), blown over the excised whole antenna. At least 2 min was allowed between successive stimulations for antennal recovery.

A test series of odorant compounds (essential oil stimuli) of the same dose (1, 10, 100, or 1,000 μg) were applied to butterfly antennal preparations using the following order: hexane control, standard stimulus, odorant stimuli (essential oils), hexane control, and standard stimulus. 100 μg of *cis*-3-hexenol was used as the standard stimulus. Odorant stimuli were presented in a random sequence. In most cases, EAG recordings were obtained from 10 successful antennal preparations per sex but only five successful recordings were obtained from females at the two lower doses (1 or 10 μg). Two recordings (maximal millivolt, mV) to the standard stimulus on a single antenna preparation were averaged as standard EAG amplitude. Similarly, two recordings to the control solvent (hexane) stimulus on a single antennal preparation were averaged as control EAG amplitude. For analysis, EAG response to the solvent control (average of two recordings per antennal preparation) was deducted from the EAG amplitudes elicited by the test extract (or standard stimulus) to obtain corrected EAG responses. Normalization was made by dividing the peak EAG amplitude of the test extract with corresponding standard EAG amplitude. All electrophysiological tests (EAG and GC-EAD) were conducted at 25 ± 1°C, LD 14:10 h, and 70 ± 5% RH. Data were first analyzed by using the standard least squares fit model method (JMP® 7.0.1, SAS Institute 2007) to determine the effects of odorant stimuli (test extracts), dose, and sex on normalized EAG responses of *P. cresphontes*. This model also allowed testing for effects of two-way and three-way interactions among the above three variables. Data were normally distributed and further analyzed by using analysis of variance (ANOVA) followed by Tukey–Kramer HSD comparison test to compare normalized EAG responses of *P. cresphontes* females or males to the test extracts at each test dose ($P < 0.05$; JMP® 7.0.1, SAS Institute 2007). Sexual differences in absolute EAG responses were tested using the Student's t test ($P < 0.05$; JMP® 7.0.1, SAS Institute 2007). Normalized EAG data were not used for determination of sexual differences since the females showed higher responses than males to the standard stimulus (*cis*-3-hexenol).

Coupled gas chromatography-electroantennogram detection (GC-EAD)

A 3-μL sample of diluted essential oil solution (10 μg/μL) was injected into a Shimadzu GC-17A fitted with a nonpolar Rtx®-1MS capillary column (30 m × 0.25 mm with a 0.25-μm film thickness, Restek) with helium as the carrier gas. The GC temperature program started at 90°C for 2 min, increased at 15°C/min to 270°C, and held for

16 min. The column effluent was split 1:1, with one part going to the flame ionization detector of the GC and the other through a heated (220°C) transfer line (Syntech®) into a humidified airstream (1,000 mL/min) directed at an excised butterfly whole antenna. The antenna preparation and EAG setup were as described above. The antennal and FID signals were amplified and recorded simultaneously by Syntech software (GcEadPro, Syntech®). At least 10 successful GC-EAD runs with *P. cresphontes* females were obtained for each plant sample, and traces were overlaid on the computer monitor to determine which GC peaks consistently yielded EAD responses.

GC–MS analyses

The leaf essential oil of *Z. clava-herculis* was subjected to GC–MS analysis on an Agilent system consisting of a model 6890 gas chromatograph, a model 5973 mass selective detector (EIMS, electron energy, 70 eV), and an Agilent ChemStation data system. The GC column was an HP-5 ms fused silica capillary with a (5% phenyl)-methylpolysiloxane stationary phase, film thickness of 0.25 μm, a length of 30 m, and an internal diameter of 0.25 mm. The carrier gas was helium with a column head pressure of 7.07 psi and flow rate of 1.0 ml/min. Inlet temperature was 200°C and MSD detector temperature was 280°C. The GC oven temperature program was used as follows: 40°C initial temperature, hold for 10 min; increased at 3°C/min to 200°C; increased 2°C/min to 220°C. Each sample was dissolved in $CHCl_3$ to give a 1% w/v solution; 1 μL injections using a splitless injection technique were used. Identification of oil components was achieved based on their retention indices (RI, determined with reference to a homologous series of normal alkanes), and by comparison of their mass spectral fragmentation patterns with those reported in the literature (Adams 2007) and stored on the MS library [NIST database (G1036A, revision D.01.00, ChemStation data system (G1701CA, version C.00.01.08))].

Results

Electrophysiological responses of *P. cresphontes* to essential oils

Antennal preparations of *P. cresphontes* in this study lasted for up to 1 h with no noticeable decreases in EAG responses observed over this time period. Standard least squares modeling revealed significant effects of odorant stimuli (test extracts), dose, and sex on EAG responses of *P. cresphontes*, as well as significant stimuli × dose and dose × sex interactions (Table 1). *P. cresphontes* females and males showed a differential and dose-dependent EAG response to the three plant extracts (Fig. 1). At the lower dose of 10 μg, females showed significantly greater EAG responses to *Z. clava-herculis* than to the other two extracts

Table 1 Standard least squares model testing for effects of stimuli (essential oils), dose, sex, and interactions of these variables on normalized EAG responses of *Papilio cresphontes*

Source of variation	DF	Sum of squares	*F*	*P*
Stimuli (essential oils)	2	0.21	5.39	0.0052
Dose	3	19.46	340.78	<0.0000
Sex	1	0.15	7.91	0.0054
Stimuli × Dose	6	1.77	15.53	<0.0001
Stimuli × Sex	2	0.05	1.42	0.2442
Dose × Sex	3	0.41	7.26	0.0001
Stimuli × Dose × Sex	6	0.05	0.42	0.8657

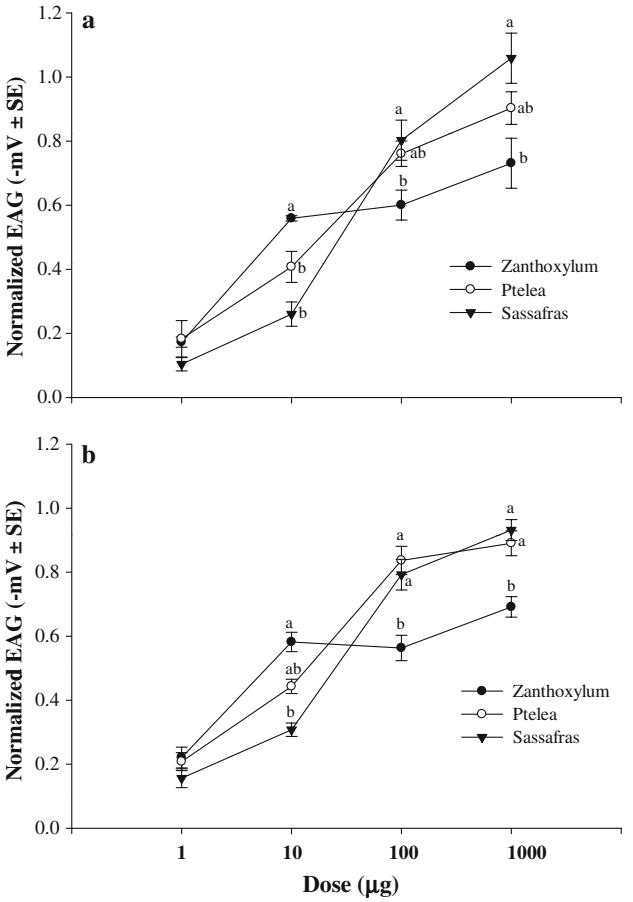

Fig. 1 Electroantennogram (EAG) responses of *Papilio cresphontes* females (**a**) and males (**b**) to a range of doses of three leaf essential oils: *Zanthoxylum clava-herculis*, *Ptelea trifoliata* (key host plants), and *Sassafras albidum* (a marginal or non-host plant). Figure shows mean ± SE normalized EAG ($n = 10$). In this and the next figure, dose indicates amount of each test compound (μg) loaded onto a piece of filter paper in a Pasteur pipette odor cartridge. Means within each dose followed by different letters are significantly different ($P < 0.05$, Tukey–Kramer HSD)

($F = 2.88$, $df = 2$, $P < 0.05$). In contrast, significantly greater EAG responses were recorded to extract of *S. albidum* than to *Z. clava-herculis* extract at the higher doses of 100 µg ($F = 4.46$, $df = 2$, $P = 0.02$) and 1,000 µg ($F = 5.44$, $df = 2$, $P = 0.01$) (Fig. 1a). Similar results were obtained for *P. cresphontes* males, which also showed significantly greater EAG response to *Z. clava-herculis* at the lower dose of 10 µg ($F = 9.70$, $df = 2$, $P = 0.0007$) but showed significantly greater responses to *P. trifoliata* and *S. albidum* at the higher doses of 100 µg ($F = 35.12$, $df = 2$, $P < 0.0001$) and 1,000 µg ($F = 18.21$, $df = 2$, $P < 0.0001$) (Fig. 1b). In general, no significant differences were recorded in the responses of females ($F = 1.07$, $df = 2$, $P = 0.36$) or males ($F = 1.07$, $df = 2$, $P = 0.36$) to the three extracts at the lowest dose of 1 µg. No significant sexual differences were recorded in EAG response to the three extracts at the lowest dose of 1 µg (*Z. clava-herculis*: $F = 0.71$, $df = 1$, $P = 0.41$; *P. trifoliata*: $F = 0.47$, $df = 1$, $P = 0.51$; *S. albidum*: $F = 1.00$, $df = 1$, $P = 0.33$). However, females generally showed greater EAG responses than males to the three extracts at higher doses, although this trend was not always significant (Fig. 2). At the 10 µg dose, females showed significantly greater response than males to two of the extracts, *Z. clava-herculis* ($F = 13.76$, $df = 1$, $P = 0.003$) and *P. trifoliata* ($F = 7.86$, $df = 1$, $P = 0.015$), but no significant sexual difference was recorded to *S. albidum* extract ($F = 1.49$, $df = 1$, $P = 0.24$). In general, similar results were recorded at the 100 µg (*Z. clava-herculis*: $F = 10.28$, $df = 1$, $P = 0.005$; *P. trifoliata*: $F = 3.19$, $df = 1$, $P = 0.09$; *S. albidum*: $F = 9.23$, $df = 1$, $P = 0.007$, Fig. 2a) and 1,000 µg (*Z. clava-herculis*: $F = 5.35$, $df = 1$, $P = 0.03$; *P. trifoliata*: $F = 8.26$, $df = 1$, $P = 0.01$; *S. albidum*: $F = 29.26$, $df = 1$, $P < 0.0001$, Fig. 2b) doses.

Typical GC-EAD traces of *P. cresphontes* females to the three leaf oils are shown in Fig. 3. Preliminary analyses showed four peaks each from *Z. clava-herculis* (peak # 2, 3, 5, and 9) and *P. trifoliata* (peak # 1, 2, 3, and 5), and seven peaks from *S. albidum* (peak # 4, 6, 7, 8, 9, 10, and 11) extracts, which elicited GC-EAD responses in *P. cresphontes* females (Fig. 3). Three of the GC-EAD peaks (peak # 2, 3, and 5) appear common both to *Z. clava-herculis* and to *P. trifoliata* extracts, while only one peak (peak # 9) is shared by *Z. clava-herculis* and *S. albidum.* No GC-EAD peaks are common to *P. trifoliata* and *S. albidum.* The chemical identities of most of these peaks are currently unknown since they are mostly trace components. Preliminary investigation of the GC-EAD active peaks by GC–MS showed that none is a major component of the essential oils. The retention indices of the GC-EAD active peaks are also presented in Fig. 3.

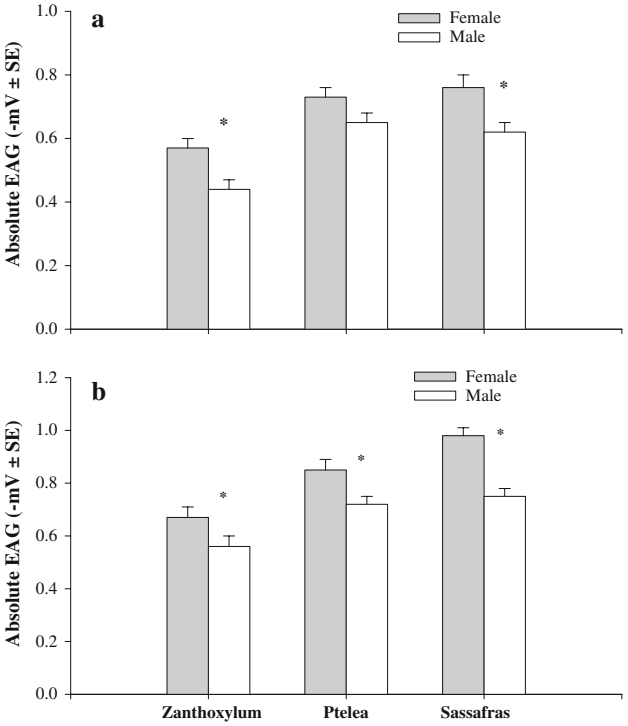

Fig. 2 Sexual differences in the EAG responses of *Papilio cresphontes* to higher doses of three leaf essential oils: *Zanthoxylum clava-herculis*, *Ptelea trifoliata* (key host plants), and *Sassafras albidum* (a marginal or non-host plant). **a** 100 µg and **b** 1,000 µg. Figure shows mean ± SE absolute EAG ($n = 10$). *Significant sexual differences ($P < 0.05$, Student's t test)

Discussion

The results of the EAG tests showed that both female and male *P. cresphontes* responded to extracts of all three plants. The data also showed that both sexes were more responsive to *Z. clava-herculis* (a key host) leaf oil than oils of *P. trifoliata* (a key host) or *Sassafras albidum* (a marginal or non-host) at lower doses. At higher doses, however, extracts of *P. trifoliata* and *S. albidum* elicited greater EAG responses in both sexes than did extract of *Z. clava-herculis*. These results partly support our hypothesis that *P. cresphontes* will show greater olfactory sensitivity to a preferred host (*Z. clava-herculis*) than to a marginal (*S. albidum*) host plant. Similar results have been reported for other *Papilio* species (Baur and Feeny 1994; Mercader et al. 2008). Mercader et al. (2008) reported that the generalist papilionid butterflies, *P. glaucus* L. and *P. canadensis* R & J showed greater EAG responses to their preferred host than to a secondary host or a non-host plant. However, our data in which *P. cresphontes* females and males showed greater EAG responses to the marginal (*S. albidum*) host plant at higher doses than to a key host (*Z. clava-herculis*) is intriguing. It is sometimes difficult to infer behavior from EAG response since the technique

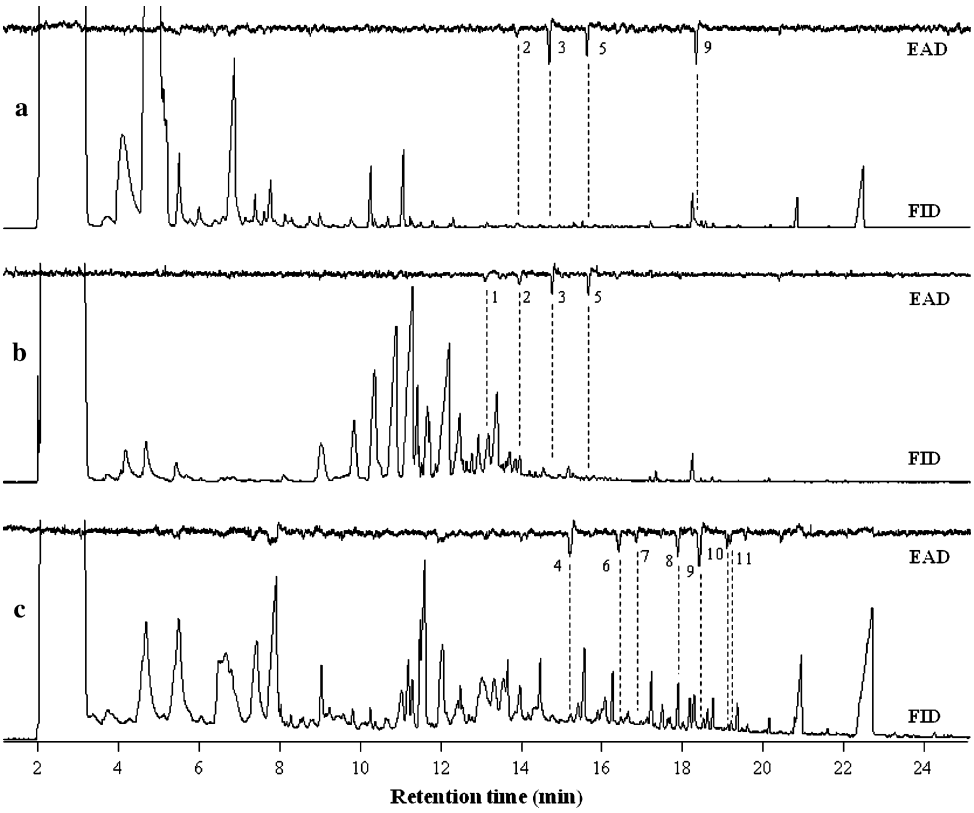

Fig. 3 GC-EAD responses of female *Papilio cresphontes* to three leaf essential oils: **a** *Zanthoxylum clava-herculis* (key host plant), **b** *Ptelea trifoliata* (key host plant), and **c** *Sassafras albidum* (a marginal or non-host plant). The calculated retention indices (RI, DB-1 column) for the GC-EAD peaks are: #1, 1,415; #2, 1,501; #3, 1,555; #4, 1,601; #5, 1,634, #6, 1,766, #7, 1,794; #8, 1,878; #9, 1,918, #10, 2,021, #11, 2,038

cannot distinguish between attractant, repellent, or alternatively stimulatory chemicals. Assuming a correlation between EAG response and behavior, the results showing higher response of *P. cresphontes* to a key host (*Z. clava-herculis*) than to marginal host (*S. albidum*) at lower doses but not at higher doses may suggest that the differential response of *P. cresphontes* to different host plants and its host preference is mediated, at least in part, by quantitative differences in the concentrations of the same or similar volatile components. We propose that the EAG responses to lower doses of the extract may well correspond to attraction, whereas the EAG responses to higher doses may correspond to repellency or behavioral inhibition. Thus, one could predict that the volatiles that mediate attraction of *P. cresphontes* to its host plants will be emitted in relatively low to moderate amounts by *Z. clava-herculis*, but emitted in relatively greater amounts by the other two plants. In other words, plumes containing low to moderate amounts of the volatiles would signal an attractive host, whereas plumes with relatively high amounts would signal a marginal or non-preferred host. This proposal is consistent with the view that the mechanisms of host-plant selection in insects are largely a matter of gradation and balance between chemicals rather than clearly definable and different cues (Schoonhoven et al. 2005). Although little is known about host preference in *P. cresphontes*, the EAG results may suggest that *Z. clava-herculis* is a more preferred host of *P. cresphontes* than *P. trifoliata*, since the butterfly was more sensitive to *Z. clava-herculis* than to *P. trifoliata* at low doses. Behavioral studies are necessary to test this prediction.

Our results, which showed equally high EAG responses of *P. cresphontes* males to the plant extracts, are not surprising given that males search for females around host plants, and thus are expected to have evolved similar sensitivity as females to host-plant chemicals. However, females showed greater EAG responses than males at the higher doses. In contrast, Mercader et al. (2008) reported no significant sexual differences in the EAG responses of *P. glaucus* and *P. canadensis* to host-plant odor. In the study by Mercader et al. (2008) the plant extracts were tested at only one dose (2 mg) making it difficult to compare their results with those of the present study, which evaluated varying doses. Our results, which showed relatively greater EAG responses for females at higher doses, support our prediction, and may be related to possible differences in the antennal morphology of the sexes. Although antennal morphology and sensilla have not been characterized for *P. cresphontes*, the female antenna (42 segments) is slightly longer than the male antenna (38 segments) (personal observation). Since EAG is a total summation of all receptor responses, the sex with the greater number of chemosensilla is likely to show greater EAG response, simply as a function of larger surface area.

Studies on abundance and types of antennal chemosensilla in both sexes of *P. cresphontes* are necessary to confirm this prediction.

The gas chromatographic-mass spectral analyses are summarized in Table 2. A total of 25 compounds were identified in the leaf oil of *Z. clava-herculis*. The major components were limonene (57%) and 1,8-cineole (30%). Other components detected in relatively less amounts include α-thujene, linalool, γ-terpinene, and α-terpineol. The preliminary GC-EAD results showed that *P. cresphontes* responded selectively only to a few components of the extracts: four components each from *Z. clava-herculis* and *P. trifoliata* and seven from *S. albidum.* Three of the GC-EAD active components were common to *Z. clava-herculis* and *P. trifoliata*, and one shared by *Z. clava-herculis* and *S. albidum*. Interestingly, none of the GC-EAD active components was common to *P. trifoliata* and *S. albidum*. However, we were unable to identify the GC-EAD active peaks at this time since most were detected in the extracts in trace amounts. The key host plants for *P. cresphontes* include *Z. clava-herculis*, *P. trifoliata*, *A. elemifera*, *C. edulis*, *Z. fagara*, and *Citrus* spp. (Scott 1992). To our knowledge, it is not currently known what, if any, host volatile chemicals may serve as attractants for *P. cresphontes* to these host plants. *A. elemifera* leaf essential oil is rich in limonene and linalool (Schmidt et al. 2006) and these two monoterpenoids are abundant in *Citrus* leaf oils as well (Attaway et al. 1967; Blanco Tirado et al. 1995; Lota et al. 2001, 2002; Gancel et al. 2002, 2003; Vekiari et al. 2002). Our data on the chemical composition of *Z. clava-herculis* leaf essential oil showed that it was made up of 25 components, largely menthane monoterpenoids. Monoterpene hydrocarbons and oxygenated monoterpenoids were dominant, with very small amounts of sesquiterpenes and simple oxygenated compounds. The most abundant components were limonene and 1,8-cineole, with lesser amounts of α-thujene, linalool, γ-terpinene, and α-terpineol. Limonene has been found in many other *Zanthoxylum* spp. (Setzer et al. 2005a; Boehme et al. 2008), and was reported as an abundant component of *Z. limonella* (Itthipanichpong et al. 2002) and *Z. piperitum* (Jiang and Kubota 2004) fruit essential oils. However, limonene was not detected in the leaf essential oils of many host plants of *P. cresphontes* including *Z. fagara* (Setzer et al. 2005b), *P. trifoliata* (Takaku and Setzer 2007), and *C. edulis* (Miller et al. 2009). Linalool and α-terpineol seem to be ubiquitous components of *Zanthoxylum* leaf oils (Setzer et al. 2005a), and 1,8-cineole is an abundant constituent of *Z. acuminatum* leaf essential oil (Setzer et al. 2005a). α-Thujene and γ-terpinene are common constituents of leaf oils of *Citrus*, also in the Rutaceae (Lota et al. 2000, 2001, 2002; Gancel et al. 2002, 2003).

Table 2 Chemical composition of the leaf essential oil of *Zanthoxylum clava-herculis*

RI	Compound	Composition (%)
862	*cis*-3-Hexenol	0.5
938	α-Thujene	3.0
944	α-Pinene	1.4
980	Sabinene	0.7
981	β-Pinene	0.3
996	Myrcene	0.7
1,026	*p*-Cymene	0.1
1,031	Limonene	57.0
1,035	1,8-Cineole	30.3
1,060	γ-Terpinene	1.7
1,068	*cis*-Sabinene hydrate	0.3
1,099	*trans*-Sabinene hydrate	0.2
1,103	Linalool	5.6
1,106	Nonanal	0.4
1,134	*cis*-Limonene oxide	0.1
1,138	*trans*-Limonene oxide	0.2
1,177	Terpinen-4-ol	0.2
1,192	α-Terpineol	1.8
1,216	*cis*-Sabinene hydrate acetate	0.7
1,350	α-Terpinyl acetate	1.1
1,417	β-Caryophyllene	0.4
1,475	γ-Muurolene	0.3
1,493	Valencene	0.2
1,512	γ-Cadinene	0.2
1,522	δ-Cadinene	0.3

Interestingly, female *P. cresphontes* showed no sensitivity to any of the green leaf volatile or oxygenated monoterpenoid components of the extracts, in contrast to what was observed by Baur and Feeny (1994) for *P. polyxenes*, *P. machaon hippocrates*, and *P. troilus*. The authors found the three *Papilio* spp. to be very sensitive to (*E*)-2-hexenal, (*Z*)-3-hexenyl acetate, linalool, and terpinen-4-ol. It is unlikely that attraction of *P. cresphontes* to *Z. clava-herculis* and other host plants is mediated by limonene, linalool, or 1,8-cineole, since these major components of the extracts did not elicit GC-EAD responses in the females. In contrast, *P. cresphontes* showed sensitivity to some unidentified oxygenated sesquiterpenoids, which were present only in trace amounts. Three of the GC-EAD active components were common to *Z. clava-herculis* and *P. trifoliata* but were not detected in *S. albidum* extract, and only one GC-EAD active component was common to *Z. clava-herculis* and *S. albidum*. These preliminary results may suggest that qualitative differences in the volatile profiles of related plants may also play a role in mediating host preference in *P. cresphontes*, in addition to quantitative differences. Thus, it is possible that some of the seven GC-EAD active components unique to *S. albidum* may

mediate the reduced preference of *P. cresphontes* to this plant by acting as olfactory repellents.

In summary, the results of this study suggest that quantitative as well as qualitative differences in volatile chemical profiles of different host plants may explain the observed host preferences of *P. cresphontes* in the field. Other sensory stimuli, such as non-volatile plant chemicals (contact chemical cues) and visual cues may also contribute to host-plant selection and preference. Future studies will include examination of additional host-plant species by GC–MS and GC-EAD analyses to identify active components of the various host plant. Behavioral bioassays will also be conducted to examine the link between our EAG data and behavior.

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References

Adams RP (2007) Identification of essential oil components by gas chromatography/mass spectrometry, 4th edn. Allured Publishing Corporation, Carol Stream, Illinois

Attaway JA, Pieringer AP, Barabas LJ (1967) A study of the percentage variation in peel and leaf oil terpenes during one season. Phytochemistry 6:25–32

Baur R, Feeny P (1994) Comparative electrophysiological analysis of plant odor perception in females of three *Papilio* species. Chemoecology 5:26–36

Baur R, Feeny P, Städler E (1993) Oviposition stimulants for the black swallowtail butterfly: identification of electrophysiologically active compounds in carrot volatiles. J Chem Ecol 19:919–937

Bernays EA, Chapman RF (1994) Host-plant selection by phytophagous insects. Chapman and Hall, New York

Blanco Tirado C, Stashenko EE, Combariza MY, Martinez JR (1995) Comparative study of Colombian citrus oils by high-resolution gas chromatography and gas chromatography-mass spectrometry. J Chromatogr A 697:501–513

Boehme AK, Noletto JA, Haber WA, Setzer WN (2008) Bioactivity and chemical composition of the leaf essential oils of *Zanthoxylum rhoifolium* and *Zanthoxylum setulosum* from Monteverde, Costa Rica. Nat Prod Res 22:31–36

Carter M, Feeny P, Haribal M (1999) An oviposition stimulant for spicebush swallowtail butterfly, *Papilio troilus*, from leaves of *Sassafras albidum*. J Chem Ecol 25:1233–1245

Chen L, Fadamiro HY (2007) Behavioral and electroantennogram responses of phorid fly *Pseudacteon tricuspis* (Diptera: Phoridae) to imported fire ant *Solenopsis invicta* odor and trail pheromone. J Insect Behav 20:267–287

Feeny P, Städler E, Åhman I, Carter M (1989) Effects of plant odor on oviposition by the black swallowtail butterfly, *Papilio polyxenes* (Lepidoptera: Papilionidae). J Insect Behav 2:803–827

Gancel AL, Olle D, Ollitrault P, Luro F, Brillouet JM (2002) Leaf and peel volatile compounds of an interspecific citrus somatic hybrid [*Citrus aurantifolia* (Christm.) Swing. + *Citrus paradisi* Macfayden]. Flavour Fragr J 17:416–424

Gancel AL, Ollitrault P, Froelicher Y, Tomi F, Jacquemond C, Luro F, Brillouet JM (2003) Leaf volatile compounds of seven citrus somatic tetraploid hybrids sharing willow leaf mandarin (*Citrus deliciosa* Ten.) as their common parent. J Agric Food Chem 51:6006–6013

Haribal M, Feeny P (1998) Oviposition stimulant for the zebra swallowtail butterfly, *Eurytides marcellus*, from the foliage of pawpaw, *Asimina triloba*. Chemoecology 8:99–110

Haribal M, Feeny P (2003) Combined roles of contact stimulant and deterrents in assessment of host-plant quality by ovipositing zebra swallowtail butterflies. J Chem Ecol 29:653–670

Heinz CA (2008) Host plant odor extracts with strong effects on oviposition behavior in *Papilio polyxenes*. Entomol Exp Appl 128:265–273

Itthipanichpong C, Ruangrungsi N, Pattanaautsahakit C (2002) Chemical compositions and pharmacological effects of essential oil from the fruit of *Zanthoxylum limonella*. J Med Assoc Thai 85(Suppl 1):S344–S354

Jiang L, Kubota K (2004) Differences in the volatile components and their odor characteristics of green and ripe fruits and dried pericarp of Japanese pepper (*Xanthoxylum piperitum* DC.). J Agric Food Chem 52:4197–4203

Kaler KM, Setzer WN (2008) Seasonal variation in the leaf essential oil composition of *Sassafras albidum*. Nat Prod Commun 3:829–832

Lederhouse RC, Ayres MP, Nitao JK, Scriber JM (1992) Differential use of lauraceous hosts by swallowtail butterflies, *Papilio troilus* and *P. palamedes* (Papilionidae). Oikos 63:244–252

Lota ML, de Rocca Serra D, Tomi F, Casanova J (2000) Chemical variability of peel and leaf essential oils of mandarins from *Citrus reticulata* Blanco. Biochem System Ecol 28:61–78

Lota ML, de Rocca Serra D, Tomi F, Casanova J (2001) Chemical variability of peel and leaf essential oils of 15 species of mandarins. Biochem System Ecol 29:77–104

Lota ML, de Rocca Serra D, Tomi F, Jacquemond C, Casanova J (2002) Volatile components of peel and leaf oils of lemon and lime species. J Agric Food Chem 50:796–805

McAuslane HJ (2009) Giant swallowtail, orangedog, *Papilio cresphontes* Cramer (Insecta: Lepidoptera: Papilionidae). University of Florida Cooperative Extension Service, document EENY-008 (IN134)

Mercader RJ, Stelinski LL, Scriber JM (2008) Differential antennal sensitivities of the generalist butterflies *Papilio glacus* and *P. canadensis* to host plant and non-host plant extracts. J Lepid Soc 62:84–88

Miller SL, Haber WA, Setzer WN (2009) Chemical composition of the leaf essential oil of *Casimiroa edulis* La Llave & Lex. (Rutaceae) from Monteverde, Costa Rica. Nat Prod Commun 4:425–426

Nakayama T, Honda K (2004) Chemical basis for differential acceptance of two sympatric rutaceous plants by ovipositing females of a swallowtail butterfly, *Papilio polytes* (Lepidoptera, Papilionidae). Chemoecology 14:199–205

Nakayama T, Honda K, Omura H, Hayashi N (2003) Oviposition stimulants for the tropical swallowtail butterfly, *Papilio polytes*, feeding on a rutaceous plant, *Toddalia asiatica*. J Chem Ecol 29:1621–1634

Nishida R (1995) Oviposition stimulants of swallowtail butterflies. In: Scriber JM, Tsubaki Y, Lederhouse RC (eds) Swallowtail butterflies: their ecology and evolutionary biology. Scientific Publishers, Gainesville, Florida, pp 17–26

Nitao JK, Ayres MP, Lederhouse RC, Scriber JM (1991) Larval adaptation to lauraceous hosts: geographical divergence in the spicebush swallowtail butterfly. Ecology 72:1428–1435

Ono H, Yoshikawa H (2004) Identification of amine receptors from a swallowtail butterfly, *Papilio xuthus* L.: cloning and mRNA localization in foreleg chemosensory organ for recognition of host plants. Insect Biochem Mol Biol 34:1247–1256

Ono H, Nishida R, Kuwahara Y (2000) A dihydroxy-γ-lactone as an oviposition stimulant for the swallowtail butterfly, *Papilio bianor*, from the rutaceous plant, *Orixa japonica*. Biosci Biotechnol Biochem 64:1970–1973

Papaj DR (1986) Conditioning of leaf-shape discrimination by chemical cues in the butterfly, *Battus philenor*. Anim Behav 34:1281–1288

Rausher MD (1978) Search image for leaf shape in a butterfly. Science 200:1071–1073

Rausher MD (1979) Larval habitat suitability and oviposition preference in three related butterflies. Ecology 60:501–511

Roessingh P, Städler E, Schöni R, Feeny P (1991) Tarsal contact chemoreceptors of the black swallowtail butterfly *Papilio polyxenes*: responses to phytochemicals from host- and non-host plants. Physiol Entomol 16:485–495

Rutowski RL (1991) The evolution of male mate-locating behavior in butterflies. Am Nat 138:1121–1139

SAS Institute (2007) JMP 7.0.1. Statistical discovery software. SAS Institute Statistical Analysis System, Cary, North Carolina, USA

Saxena KN, Goyal S (1978) Host-plant relations of the citrus butterfly *Papilio demoleus* L.: orientational and ovipositional responses. Entomol Exp Appl 24:1–10

Schmidt JM, Noletto JA, Vogler B, Setzer WN (2006) Abaco bush medicine: chemical composition of the essential oils of four aromatic medicinal plants from Abaco Island, Bahamas. J Herbs Spices Med Plants 12(3):43–65

Schoonhoven LM, Van Loon JJA, Dicke M (2005) Insect-plant biology. Oxford University Press, Oxford, p 412

Scott JA (1992) The butterflies of North America. Stanford University Press, Stanford, California

Setzer WN, Noletto JA, Lawton RO, Haber WA (2005a) Leaf essential oil composition of five *Zanthoxylum* species from Monteverde, Costa Rica. Mol Divers 9:3–13

Setzer WN, Schmidt JM, Eiter LC, Haber WA (2005b) The leaf oil composition of *Zanthoxylum fagara* (L.) Sarg. from Monteverde, Costa Rica, and its biological activities. J Essent Oil Res 17:333–335

Städler E (1992) Behavioral responses of insects to plant secondary compounds. In: Rosenthal GA, Berenbaum MR (eds) Herbivores: their interactions with secondary metabolites: ecological and evolutionary processes, vol II. Academic Press, USA, pp 45–48

Takaku S, Setzer WN (2007) Chemical composition of the leaf essential oil of *Ptelea trifoliata*. J Essent Oil Bear Plants 10:104–108

Traynier RMM (1986) Visual learning in assays of sinigrin solution as oviposition releaser for the cabbage butterfly, *Pieris rapae*. Entomol Exp Appl 40:25–33

Vaidya VG (1969) Investigations on the role of visual stimuli in the egg-laying and resting behaviour of *Papilio demoleus* L. (Papilionidae, Lepidoptera). Anim Behav 17:350–355

Van Loon JJA, Everaarts TC, Smallegange RC (1992) Associative learning in host-finding by *Pieris brassicae* butterflies: relearning preferences. In: Menken SBJ, Visser JH,Harrewijn (eds) Proceedings of 8th international symposium on insect-plant relationships. Kluwer Academic Publishers, Dordrecht, pp 162–164

Vekiari SA, Protopapadakis EE, Papadopoulou P, Papanicolaou D, Panou C, Vamvakias M (2002) Composition and seasonal variation of the essential oil from leaves and peel of a Cretan lemon variety. J Agric Food Chem 50:147–153

Visser J (1986) Host odor perception in phytophagous insects. Annu Rev Entomol 31:121–144