Castells E, Berenbaum M (2008) Host plant selection by a monophagous herbivore is not mediated by quantitative changes in unique plant chemistry: *Agonopterix alstroemeriana* and *Conium maculatum.* **Arthropod-Plant Interactions** 2:43–51
DOI 10.1007/s11829-008-9032-9

Host plant selection by a monophagous herbivore is not mediated by quantitative changes in unique plant chemistry: *Agonopterix alstroemeriana* and *Conium maculatum*

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Running title: Host plant selection and unique chemistry

Key words: *Agonopterix alstroemeriana*, Apiaceae, *Conium maculatum*, host plant selection, Lepidoptera, Oecophoridae, oviposition, piperidine alkaloids, Specialization, terpenes

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2 Type of article: Original research

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Abstract

Host plant selection by ovipositing females is a key process determining the success of phytophagous insects. In oligophagous lepidopterans, host-specific plant secondary chemicals are expected to be dominant factors governing oviposition behavior; distinctive compounds can serve as high-contrast signals that clearly differentiate confamilial hosts from non-hosts increasing the accuracy of host quality evaluation. *Agonopterix alstroemeriana* (Clerk) (Lepidoptera: Oecophoridae) and *Conium maculatum* L. (Apiaceae) form an extremely specialized plant-herbivore system, with *A. alstroemeriana* monophagous on *C. maculatum*, a plant with few other insect herbivores at least in part due to its virtually unique capacity among plants to produce piperidine alkaloids. Here we have studied the response of *A. alstroemeriana* oviposition to unique host plant secondary metabolites, piperidine alkaloids, and widespread compounds, mono- and sesquiterpenes, in a concentration-dependent fashion. Rates of oviposition were negatively correlated with *Z*-ocimene concentrations. To confirm the deterrent properties of this monoterpene for *A. alstroemeriana* oviposition, we conducted a choice experiment using artificially damaged *C. maculatum* plants, with higher emission of volatiles, and undamaged control plants. Damaged plants were less preferred as oviposition sites compared to the controls. The lack of association between oviposition and piperidine alkaloids, defenses unique to *Conium* species, suggests that quantitative changes of these species-specific chemicals do not play a predominant role in host selection by the monophagous *A. alstroemeriana*.

Key words *Agonopterix alstroemeriana*, Apiaceae, *Conium maculatum*, host plant selection, Lepidoptera, Oecophoridae, oviposition, piperidine alkaloids, specialization, terpenes

Introduction

Host plant selection by ovipositing females is a key process critical for the survivorship, performance, and fitness of their offspring, especially in those species with low mobility of immature stages such as many Lepidoptera (Thompson and Pellmyr, 1991). Because plants have evolved a wide array of toxic chemical defenses against herbivores, the mechanism underlying host choice is expected to be highly related to the plant chemical composition (Honda, 1995; Jaenike, 1990). The ability to discriminate among plant chemical patterns varies depending on the degree of specialization (Janz and Nylin, 1997; Bernays, 2001; Egan and Funk, 2006; Wee and Singer, 2007). The Neural Limitation hypothesis predicts that, while generalists evaluate host suitability among different species with diverse chemistries, monophagous or oligophagous insects should have a greater capacity to differentiate host plant chemistry at the intraspecific level (Jaenike, 1990; Janz and Nylin, 1997). A narrow range of hosts, with reduced diversity of plant secondary chemicals, allows for greater efficiency in evaluating host suitability compared to generalists (Janz and Nylin, 1997; Bernays, 2001). Indeed, specialists are expected to select host plants and assess suitability by using a particular plant compound or mixtures of compounds that function as reliable signals for host recognition (Feeny, 1992; Bernays, 2001). By being receptive to a set of high-contrast signals specific to their host plants that clearly stand out from non-host compounds, specialists might increase the speed and accuracy of host detection and appropriately evaluate host quality (Bernays, 2001).

Host breadth shows a general association with the nature of chemical cues used for host plant recognition. Pharmacophagous species (i.e., those that sequester plant

allelochemicals for their own defense) display a high degree of specificity for and reliance upon those compounds that are sequestered (Pereyra and Bowers 1988, Haribal and Feeny, 1998; Haribal et al., 1998; Nishida and Fukami, 1989; Papaj et al., 1992; Sachdev-Gupta et al., 1993). Aucubin-like iridoid glycosides, for example, found not only in plantaginaceous host plants but in at least four other families, are oviposition cues for the specialist buckeye butterfly *Junonia coenia* (Pereyra and Bowers, 1988). Similarly, aristolochic acids, characteristic of a broad range of species in the family Aristolochiaceae, are oviposition kairomones for *Battus philenor* (Nishida and Fukami, 1989; Papaj et al., 1992; Sachdev-Gupta et al., 1993). Glucosinolates, ubiquitous among brassicaceous host plants, regulate the oviposition rate for *Pieris brassicae* and other pierids, with oviposition increasing up to an optimal concentration, above which it remains the same or decreases (Huang and Renwick, 1993).

Although many lepidopterans can be stimulated to oviposit in response to a single class of host-specific compounds (Honda, 2005), such compounds tend to be widely distributed within host plant genera or families. Whether insects primarily respond to compounds restricted to only a single host plant in a concentration-dependent fashion is an open question, at least in part because few species are strictly monophagous. Under the Neural Limitation hypothesis, host selection by an extreme specialist is expected to be determined primarily by compounds restricted to its host plant, as opposed to ubiquitous compounds (Bernays 2001). The Eurasian weed *Conium maculatum* L. (Apiaceae) and its monophagous associate *Agonopterix alstroemeriana* (Clerck) (Lepidoptera: Oecophoridae) form a very tight plant-herbivore association suitable for examining this question. *A. alstroemeriana* is monophagous on *C. maculatum* and is its only consistent

consumer throughout its range, although a few other insect species occasionally feed on the plant as an alternative host (Berenbaum, 1981; Goeden and Ricker, 1982). Host and herbivore have a well-matched life cycle; both species require cold temperatures to attain sexual maturity, and the short larval stage of *A. alstroemeriana*, lasting approximately six weeks during late spring, coincides with the bolting and flowering of *C. maculatum* before the plant completes reproduction in early summer (Berenbaum and Passoa, 1983; Castells and Berenbaum, 2006). Within the Apiaceae, *C. maculatum* is absolutely unique in its ability to produce the piperidine alkaloids coniine, γ-coniceine, methylconiine, conhydrinone and related compounds (Fairbairn, 1971; Berenbaum, 2001). Indeed, γ-coniceine and its relatives are known from only two additional genera, in the monocotyledonous Araceae and Aloaceae (Dring et al., 1984; Dictionary of Natural Products, 2007). As acetylcholine agonists, coniine and other piperidine alkaloids are demonstrably toxic and/or repellent to both invertebrates and vertebrates (Bowman and Sanghvi, 1963; Sperry et al., 1964; Fairbairn, 1971; Panter and Keeler, 1989; Wink et al., 1998; Birkett et al., 2004; Castells and Berenbaum, In press).

The toxicity and uniqueness of *C. maculatum* alkaloids within its family suggest that these compounds could serve as a distinctive signal for *A. alstroemeriana* when evaluating host plant quality. Compounds of the piperidine type are volatile in the free-base form, which might facilitate long-distance host orientation and recognition by *A. alstroemeriana*. However, whether alkaloids are stored in the plant as nonvolatile salts (and thereby available as potential contact kairomones) or volatile bases (and thereby available as potential long-distance orientation kairomones) is not known. Here we aimed to determine whether in this tightly coevolved plant-herbivore system *A. alstroemeriana*

host plant selection is mediated by unique plant secondary metabolites, as expected by the Neural Limitation hypothesis, or otherwise by widespread compounds. We have focused on mono- and sesquiterpenes as ubiquitous compounds because these volatile essential oil constituents are frequently involved in orientation and recognition of host plants by generalist ovipositing females (Renwick and Chew, 1994; Honda, 1995) and are present in many families of angiosperms and gymnosperms (Gershenzon and Croteau 1991). *C. maculatum* plants growing in the field and subject to *A. alstroemeriana* oviposition were analyzed for piperidine alkaloids, monoterpenes and sesquiterpenes. A multiple regression analysis was performed to determine what compounds were quantitatively correlated with oviposition levels.

Materials and methods

Oviposition of A. alstroemeriana *in natural conditions*

This study was conducted at the UIUC Phillips Tract Experimental Field, 7 km from Urbana, Champaign County, IL, USA. *C. maculatum* grows naturally in the area, forming dense patches. Beginning in late March, plants were regularly checked for the presence of *A. alstroemeriana* eggs. On April 27, 2004, when the presence of eggs had been detected for more than a week but no larvae were yet present, we randomly selected 32 *C. maculatum* individuals within a *ca* 40 m^2 area. On each plant, the youngest fully expanded leaf was selected. The number of eggs per leaf was counted *in situ* and four leaf tissue samples per leaf (*ca* 200 mg FW each) were placed in pre-weighed 2 ml Eppendorf tubes in dry ice and transported to the laboratory. Tubes were weighed again to obtain leaf fresh mass and stored at –80^o C for chemical analyses. A subsample per leaf was

used to estimate water content. To have an estimate of leaf size, leaves were cut under the first pair of leaflets on the study plants, dried at 60^{o} C and weighed. Leaf size was additionally estimated by measuring the diameter of the stem 1 cm below the first leaflets using a digital caliper. Diameter and leaf dry mass were significantly correlated (R^2 = 0.86, $p < 0.001$) and thus diameter is a good non-destructive measure for leaf mass.

Chemical analyses

Alkaloid analyses were conducted following Castells et al (2005). A sample of frozen leaf (*ca* 200 mg FW) was ground for 10 sec. with a glass bead in a Wig-L-Bug grinding mill (Crescent Dental, Chicago, IL), extracted on a shaker with 1.5 ml of 70% MeOH 30% 0.1 N HCl for 2 h, and centrifuged. The supernatant was evaporated down using a rotary evaporator (Jouan RC 10.10) and partitioned with hexane (x 3) to remove nonpolar compounds. Alkaloids were transformed to the free base form by raising the pH with 10 M NaOH and extracted with hexane containing 0.01% (v/v) hexadecane as internal standard. Stored volatile compounds were extracted by grinding frozen foliage with 1 ml of hexane containing 0.01% (v/v) hexadecane as internal standard. Alkaloids and volatiles were analyzed by a flame ionization detector (FID) on a gas chromatograph equipped with a capillary column (Alltech EC-1, 30 m, 0.23 mm) coupled with an autosampler (HP 5890). Samples were run with the following temperature program: initial temperature 50 ºC, ramp 5 ºC min^{-1} up to 105 °C, ramp 35 ºC min^{-1} up to 290 ºC, 5 min at 290 ºC. For alkaloids, (±)-coniine (Sigma) was used as a standard at 0.05 % (v/v), and concentrations were estimated as coniine equivalents per unit leaf dry mass. Individual alkaloids were identified by comparison with authentic material (coniine) or

by their retention time (γ-coniceine and conhydrinone, Castells et al., 2005). Prominent diagnostic GC-mass spectral ions and their relative intensities of an unknown alkaloid are as follows: EIMS 70 eV, *m/z* (rel. int.): 153 (M+, 1), 124 (7), 110 (19), 98 (10), 97 (100), 96 (30), 82 (8), 69 (5), 55 (14), 41 (18). This analysis was performed using a gas chromatograph (Agilent 6890 N) coupled with a mass spectrometer (Agilent 5975 inert XL) run at the temperature program detailed above. For volatiles, terpinolene (TCI America, 81% purity) at 0.05 % (v/v) was used as a standard and concentrations were estimated as terpinolene equivalents by unit leaf dry mass. Mass spectral data for volatile compounds were obtained by injecting 1 µl of extract on an Agilent 6890N gas chromatograph system attached to an Agilent 5975 inert XL mass spectrometer run from 50ºC to 300ºC at 5 ºC min^{-1} on a capillary column (Agilent HP-5, 30 m, 0.25 mm). Kováts Retention indices (RI) were determined using AMDIS 2.64 (NIST, Gaithersburg, MD) by comparison with a set of hydrocarbons (C9-C36, Restek). Mono- and sesquiterpenes were tentatively identified by their RI and mass fragmentation spectra in comparison with NIST 05 database and literature (Adams, 2001) (Table 1).

Stored and released volatiles after wounding

The profile of stored volatile compounds and volatiles released after mechanical damage was determined in five *C. maculatum* plants growing in the greenhouse. Stored volatiles were extracted by grinding 100-150 mg FW leaf in 0.5 ml of hexane using a Wig-L-bug grinding mill and analyzed on a GC-FID as explained above. To determine the release of volatiles after damage *ca* 1 g FW leaf per plant was chopped and placed into a 30 ml glass vial covered with aluminum foil. Headspace volatiles were analyzed by solid-phase

microextraction (100 µm polydimethylsiloxane SPME) (Supelco) by inserting the fiber through the foil and exposing it for 5 min. The fiber was then retracted and immediately injected into the GC-FID using the temperature program detailed above. Tentative identification of volatiles for extracted and headspace analysis was conducted by comparison of samples injected at GC-FID and GC-MS. Relative abundance of the main volatiles was calculated from the peak areas obtained by GC-FID (Table 2).

Oviposition choice experiment

To bioassay the effects of plant volatiles on oviposition preference we conducted a choice experiment offering to *A. alstroemeriana* adults undamaged and damaged *C. maculatum* plants. Wounded leaves release stored volatile compounds (Gershenzon and Croteau 1991); increased odor from *C. maculatum* foliage was immediately manifested after mechanical damage was inflicted (EC personal observation). By conducting this experiment we aimed not to mimic the effects of herbivore damage on volatile induction in natural conditions, but rather to take advantage of the volatile release after damage to design a choice experiment with low- and high-volatile emission plants.

A. alstroemeriana larvae were collected at the Yard Waste Recollection site in Champaign County, IL (USA) and raised in the laboratory on *C. maculatum* foliage until pupation. Pupae were transferred to a 1-l plastic container with paper towel (15-20 individuals per container) and kept at room temperature and a photoperiod of 16:8 h (L:D). When moths emerged, a vial of honey water in hummingbird solution (Perky Pet Products, Denver, CO) was placed into the container and replaced every 3-4 days. Because *A. alstroemeriana* is univoltine, a cold treatment was necessary in order to

obtain sexually mature moths. The protocol used to break diapause is explained in Castells and Berenbaum (2006). In brief, on June 16, approximately one month after the moths emerged, the containers with moths were transferred into a cold room at 4^{o} C and 8:16 h (L:D). Two months later, on August 16, the containers with moths were enclosed in a Styrofoam box, removed from the cold room and allowed to warm up gradually to room temperature over 4 hours. Approximately 150 moths, both males and females, were transferred into three screen-wire cages (30 x 30 x 30 cm) provided with honey water and a leaf of *C. maculatum*. Moths started to lay eggs after 7 to 10 days.

The experiment was initiated three weeks after the moths were removed from the cold room to ensure that most females had mated. We set up two screen-wired cages (55 x 45 x 45 cm) in a greenhouse with temperature and photoperiod at equivalent to spring conditions in central Illinois (25 C 16:8 h L:D). The cages were placed 3 m away from each other to avoid possible effects on volatiles coming from outside each cage. In each cage we placed 20 females and 10 males. Honey water with hummingbird solution was provided. Two potted *C. maculatum* plants grown from seed in the greenhouse and measuring approximately 20-25 cm tall with 3-6 leaves were placed in each cage. Just before dusk (approx. 19:00), one of the plants was damaged by cutting with scissors the tips along the leaf edges. This method allowed extensive damage all over the plant without greatly altering leaf shape and area, even though we could not fully control the possible responses of *A. alstroemeriana* moths to changes in leaf morphology. The following morning, plants were removed from the cage, the eggs counted, and leaf area measured using a Delta T area meter (Delta T Devices, Cambridge, England). Average area for all leaves within a plant was 499 cm^2. A new pair of plants was placed every

evening in each cage for five consecutive days (20 plants in total), alternately switching the position of the damaged and undamaged plant inside the cage to avoid any behavior related to the plant orientation within the greenhouse. Care was taken to pair plants with similar size and appearance. Oviposition was calculated as the number of eggs per unit of leaf area.

Statistical analyses

A multiple regression model was constructed to relate oviposition to chemical composition of foliage. First, a correlation analyses was performed for the following variables measured in 30 *C. maculatum* leaves sampled in the field: number of *A. alstroemeriana* eggs, leaf DW, concentrations of the alkaloids coniine, γ-coniceine, conhydrinone and an unknown compound, concentrations of the monoterpenes β-myrcene, *Z*-ocimene and *E*-ocimene, and concentrations of the sesquiterpenes β-caryophyllene, germacrene D and β-sesquiphellandrene. For those variables showing significant correlation coefficients with number of eggs (leaf DW and *Z*-ocimene) a multiple regression analysis was then performed. The ANOVA assessed whether the independent variables, taken together, were significantly associated with the dependent variable. To estimate the relative contribution of each independent variable in the prediction of the dependent variable, the "Beta" or standardized regression coefficient was used. The "B" or regression coefficient assessed the relationship of each independent variable with the dependent variable when all other independent variables were held constant. Finally, the *t*-values and *p*-values gave an estimate of the statistical significance

for the relation between the dependent variable and each independent variable when the other independent variables were taken into account.

The relative abundance of volatiles between stored and released volatile compounds after damage was analyzed by a repeated measure ANOVA with type of analysis (extracted *vs.* headspace) as the repeated variable. For the oviposition choice experiment, a repeated measure ANOVA was performed, with cage as the independent variable and treatment (control *vs.* damage) as the repeated variable. Spatial orientation of control/damaged plants in the greenhouse showed no significant effects on oviposition (ANOVA, $p = 0.63$) and was excluded from the analysis. All analyses were conducted using Statistica 6.0 (Statsoft, Tulsa, OK).

Results

Chemical analyses of the acidic-methanolic extraction revealed the presence of four piperidine alkaloids: coniine, γ-coniceine, conhydrinone and an unknown compound. No alkaloids were detected in the hexane extraction, indicating that alkaloids in the plant were present only in the non-volatile form. The hexane extraction showed the presence of three main monoterpenes and three main sesquiterpenes tentatively identified as β-myrcene, *Z*-ocimene, *E*-ocimene, β-caryophyllene, germacrene D and β-sesquiphellandrene (Table 1). Other terpenes found at trace concentrations were not included in the analysis. Volatile profiles for terpenes stored in foliage and emitted after leaf wounding were qualitatively similar (Table 2). Only two compounds showed relatively different abundance: β-myrcene, which was significantly higher in the emitted

volatiles, and germacrene D, which was significantly higher using the extraction method. Piperidine alkaloids were not detected in the headspace analysis.

C. maculatum growing in the field had an average of 10.3 ± 1.3 (mean ± SE) *A. alstroemeriana* eggs per leaf. The number of eggs laid by *A. alstroemeriana* was positively correlated with leaf DW and negatively correlated with the concentration of the monoterpene *Z*-ocimene (Table 3, Table 4, Fig. 1). No other monoterpenes, sesquiterpenes, or piperidine alkaloids were significantly correlated with oviposition (Table 3). A multiple regression analysis for leaf DW and *Z*-ocimene as independent variables showed that both factors taken together were significantly correlated with oviposition, and that leaf DW had a relatively higher contribution than *Z*-ocimene on determining oviposition, as estimated by the standardized regression coefficient (Table 4). Oviposition density was not affected by leaf DW, indicating that, although larger leaves received more eggs, the density of eggs was not affected by leaf DW.

The oviposition choice experiment with control and artificially damaged plants showed that *A. alstroemeriana* females had a preference for undamaged plants over damaged plants (ANOVA, $F = 6.02$, $p < 0.05$) (Fig. 2). This trend was independent of the cage used (ANOVA, $F = 0.26$, $p = 0.62$). No interaction between treatment (control/damaged) and cage was found (ANOVA, $F = 0.90$, $p = 0.36$).

Discussion

C. maculatum and *A. alstroemeriana* form an extremely specialized interaction; *C. maculatum* experiences little damage from generalist herbivores, but the monophagous leafroller *A. alstroemeriana* is consistently associated with the plant throughout its native

(and much of its non-indigenous) range (Berenbaum, 1981; Goeden and Ricker, 1982; Berenbaum and Passoa, 1983). The presence of unique secondary compounds in *C. maculatum*, the piperidine alkaloids, differentiates this species from all other apiaceous plants, many of which are host plants for other *Agonopterix* species (Hodges, 1974). The use of host-specific compounds during host selection should confer an advantage for this extreme specialist herbivore (Bernays, 2001). Here we have compared the oviposition response of *A. alstroemeriana* to quantitative changes of host plant unique secondary metabolites, the piperidine alkaloids, and widespread secondary metabolites, mono- and sesquiterpenes. That oviposition by *A. alstroemeriana* was not correlated with *C. maculatum* alkaloid concentrations suggests that moths do not depend on assessing amounts of these chemicals to evaluate host plant quality. The fact that alkaloids were stored in the plant as hydrochlorides, and thus in a non-volatile form, could limit the detectability of alkaloid concentrations by *A. alstroemeriana* moths at long distances when they are searching for a suitable host; nonetheless, many if not most of taxonomically restricted host plant recognition chemicals utilized by lepidopterans act as contact kairomones (Renwick and Chew, 1994). Thus, we cannot completely reject the involvement of alkaloids as oviposition signals. Other possibilities should be further explored, especially those related to the use of more complex cues by *A. alstroemeriana* such as the occurrence of different responses to particular alkaloids or the use of specific alkaloid ratios as indicators of host plant suitability.

The lack of correlation between alkaloid concentrations and oviposition level provides relevant information about how selection on *C. maculatum* by *A. alstroemeriana* might be occurring. Castells et al. (2005) found evidences of selection by *A.*

alstroemeriana for plants with low levels of alkaloids. Herbivory on *C. maculatum* individuals in field conditions, estimated as the number of leaf rolls per plant, was negatively correlated with alkaloid concentrations within a population (Castells et al 2005). Additionally, geographical variation in plant chemistry across US, where *C. maculatum* is invasive, was correlated to the intensity of reassociation with *A. alstroemeriana*; regions where plants sustained higher levels of *A. alstroemeriana* herbivory, Washington and New York States, had also increased levels of alkaloids compared to the Midwest, where populations were largely free from herbivores (Castells et al., 2005). Because differences in alkaloid concentrations among regions are genetically based and thus subject to selection (Castells and Berenbaum, unpublished), these geographical changes might reflect an increase in the frequency of those plant genotypes that invested more resources to alkaloids due to herbivore selective pressure. Our findings show that host choice by *A. alstroemeriana* during oviposition is unresponsive to alkaloid concentrations; however, selection could be taking place by differential larval mortality rates due to alkaloid ingestion.

While host-specific secondary metabolites from *C. maculatum*, piperidine alkaloids, were not correlated with *A. alstroemeriana* oviposition, a widespread volatile compound proved to be deterrent; number of eggs laid by *A. alstroemeriana* was negatively correlated with *Z*- ocimene concentrations of *C. maculatum* plants growing in the field. To test whether increased volatiles could deter *A. alstroemeriana* oviposition we conducted a laboratory choice experiment using intact and damaged *C. maculatum* plants in order to obtain individuals with low and high-volatile emission. When undamaged and mechanically damaged *C. maculatum* plants were offered to gravid

female moths as oviposition sites, damaged plants, which released stored volatile compounds, were less likely to be used for oviposition compared to undamaged plants. This pattern is consistent with the negative correlations between the monoterpene *Z*-ocimene and oviposition found in the field, and thus both experiments point to volatile compounds as deterrents for *A. alstroemeriana* oviposition.

The role of volatiles as oviposition deterrents has been described for many plant-insect systems (Gershenzon and Croteau, 1991; Renwick and Chew, 1994; Honda, 1995, Pare and Tumlinson 1999). Such volatiles may be avoided by conspecifics seeking to reduce intraspecific competition (De Moraes et al., 2001) and they are also associated with the indirect plant defense of attracting predators (De Moraes et al., 2001; Kessler and Baldwin, 2001; Gershenzon and Dudareva, 2007). Although no data are available on the kairomonal properties of volatile compounds toward *C. maculatum* predators or parasitoids, ocimene elicits electrophysiological responses in a parasitic wasp, *Microplitis croceipes* (Park et al., 2001) and attracts predatory mites (Scutareanu et al., 1997). *A. alstroemeriana* moths might tend to avoid plants releasing higher amounts of volatiles. Indeed, in the Midwest US, *A. alstroemeriana* is subject to intense predation by the predatory wasp *Euodynerus foraminatus* (Hymenoptera) (McKenna et al., 2001) and thus top-down selective pressures could decrease *A. alstroemeriana* fitness and influence oviposition patterns.

In contrast with expectations based on the Neural Limitation hypothesis, which predicts that host-specific compounds will be play a dominant role during oviposition (Bernays, 2001), quantitative changes in host-specific chemicals were not related to host selection by the monophagous *A. alstroemeriana*. Oviposition behavior was evidently

mediated by concentrations of a widely distributed volatile compound. Although the extreme rarity of piperidine alkaloids make them highly reliable cues, dependence upon such specific cues may be either ecologically costly or physiologically difficult to engineer. Until more examples of chemically mediated host-finding in extreme specialists are chemically characterized, however, a critical evaluation of these possible explanations will not be possible.

Acknowledgments

We thank Arthur R. Zangerl for his invaluable support and advice during this study. This work was supported by a Fulbright-MECD fellowship (Spain) awarded to E.C.

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7

Table 1. Tentative identification of mono- and sesquiterpenes from *C. maculatum* based on molecular mass, RI in comparison with literature, and mass fragmentation spectra.

Compounds	$[M^+]^a$	RI[b]	RI lit.[c]	EIMS 70 eV, *m/z* (rel. int.)
β-myrcene	136	992	991	94 (9), 93 (94), 92 (10), 91 (20), 79 (13), 77 (13), 69 (64), 67 (11), 53 (14), 41 (100)
Z-ocimene	136	1039	1037	105 (14), 93 (100), 92 (40), 91 (40), 80 (14), 79 (34), 77 (29), 53 (18), 43 (13), 41 (30)
E-ocimene	136	1049	1050	121 (17), 93 (100), 92 (25), 91 (45), 80 (37), 79 (40), 77 (31), 53 (20), 43 (15), 41 (35)
β-caryophyllene	204	1425	1419	41 (100), 55 (49), 69 (62), 77 (42), 79 (64), 91 (70), 93 (97), 105 (52), 120 (43), 133 (73)
Germacrene D	204	1489	1485	41 (40), 55 (28), 77 (24), 79 (31), 81 (38), 91 (53), 93 (27), 105 (62), 119 (38), 161 (100)
β-sesquiphellandrene	204	1529	1523	161 (37), 133 (31), 120 (31), 93 (62), 92 (36), 91 (47), 77 (33), 69 (100), 55 (45), 41 (80)

[a]Estimated from EI mass spectra using NIST MS Search 2.0
[b]HP-5 column
[c]DB-5 column (Adams 2001)

Table 2. Comparison of relative abundance of the most abundant mono- and sesquiterpenes stored in *C. maculatum* foliage and emitted after leaf wounding (n = 5). Significant *p*-values from a repeated measure ANOVA are shown in bold.

	% abundance		Statistics	
Compounds	Stored	Released	*F*	*p*-value
β-myrcene	10.5	34.6	40.85	< **0.01**
Z-ocimene	27.2	26.9	0.002	0.96
E-ocimene	13.5	14.6	1.75	0.25
β-caryophyllene	3.8	4.6	4.17	0.11
Germacrene D	26.1	10.5	11.63	< **0.05**
β-sesquiphellandrene	19.0	8.8	6.07	0.06

Table 3. Correlation coefficients between number of *A. alstroemeriana* eggs found in a *C. maculatum* leaf, its leaf dry weight and concentrations of piperidine alkaloids (coniine, γ-coniceine, conhydrinone, unknown alkaloid), monoterpenes (β-myrcene, *Z*-ocimene, *E*-ocimene), and sesquiterpenes (β-caryophyllene, Germacrene D, β-sesquiphellandrene). N=30. Significant correlations at $p < 0.05$ are indicated in bold.

		# Eggs	Leaf DW	coniine	γ-coniceine	Conhydrynone	Unknown alk	β-myrcene	*Z*-ocimene	*E*-ocimene	β-caryophyllene	Germacrene D
	Leaf DW	**0.73**										
ALKALOIDS	coniine	-0.08	-0.05									
	γ-coniceine	-0.17	-0.30	-0.14								
	Conhydrinone	-0.04	0.11	0.02	**-0.63**							
	Unknown alk.	-0.19	-0.34	-0.22	0.01	-0.09						
MONOTERPENES	β-myrcene	-0.13	-0.07	-0.12	-0.11	0.04	-0.05					
	Z-ocimene	**-0.49**	-0.33	-0.12	0.04	-0.09	-0.15	**0.39**				
	E-ocimene	-0.24	-0.07	0.03	0.23	-0.21	-0.01	0.20	0.25			
SESQUITERPENES	β-caryophyllene	-0.31	-0.24	-0.26	0.22	-0.01	-0.17	0.10	**0.41**	-0.21		
	Germacrene D	-0.13	-0.23	-0.10	-0.01	0.05	**0.40**	-0.13	-0.12	**0.46**	-0.17	
	β-sesquiphellandrene	-0.29	-0.10	-0.05	0.08	-0.12	-0.14	0.18	**0.50**	**0.43**	-0.06	-0.04

Table 4. Multiple regression analysis for number of *A. asltroemeriana* eggs as a dependent variable and leaf DW and *Z*-ocimene concentrations as independent variables. Beta = standardized regression coefficient, B = regression coefficient. More details about this analysis can be found in the Materials and Methods. Significant *p*-values are shown in bold at $p < 0.05$.

ANOVA (overall goodness of fit)

	SS	df	MS	*F*	*p*-value
Regression	1062.25	2	531.12	20.6	< **0.001**
Residual	695.21	27	25.74		

Regression results

	Beta	SE of Beta	B	SE of B	*t*-value	*p*-value
Intercept			1.12	4.01	0.28	0.78
Leaf DW	0.64	0.12	7.09	1.42	4.99	< **0.001**
Z-ocimene	-0.27	0.12	-57.34	26.62	-2.15	**0.04**

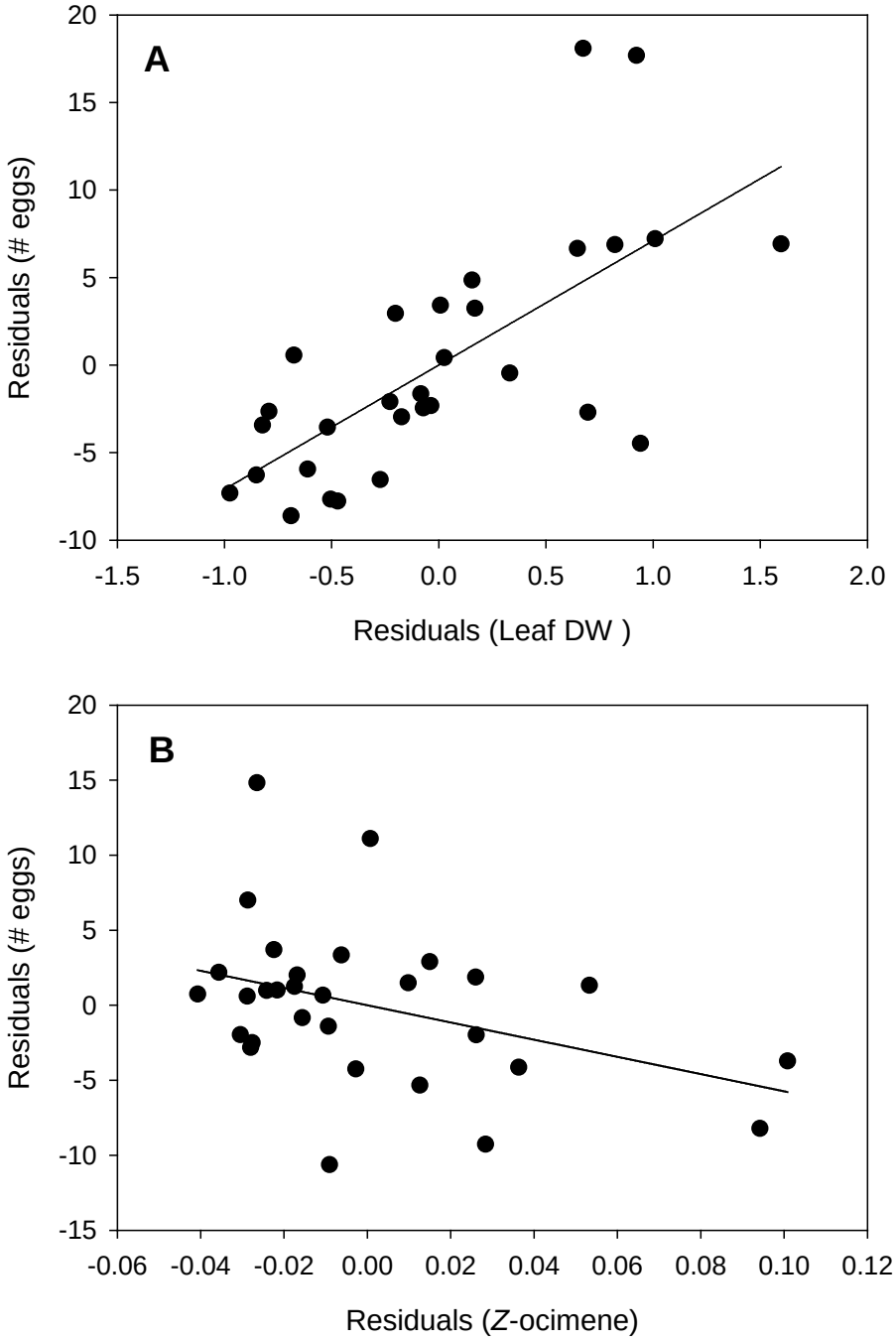
Figure captions

Figure 1. Partial regression for number of *A. alstroemeriana* eggs with (A) leaf DW and (B) *Z*-ocimene concentrations. Partial regression plots, constructed using the residuals of each variable obtained from a multiple regression analysis, show the relationship between the dependent variable (number of eggs) and each independent variable when subtracting the effect of the other independent variable included the model. Correlation coefficients and statistical significances of partial correlations are found in Table 3.

Figure 2. Oviposition choice by *A. alstroemeriana* when presented an intact and an artificially damaged *C. maculatum* plant for 5 consecutive days (mean ± SE). The experiment was conducted with two cages set independently and plants were replaced every day.

1 Figure 1

2



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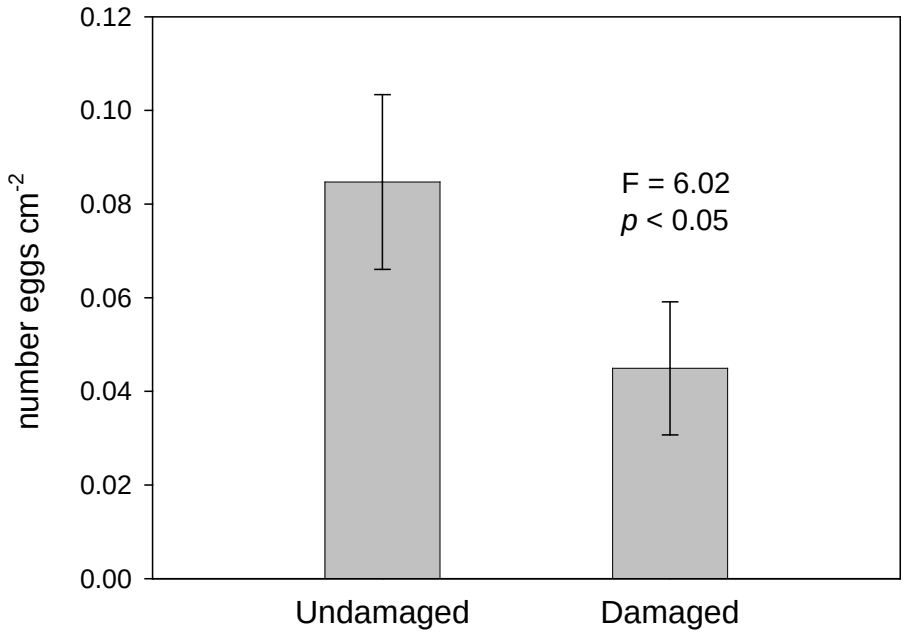
1 Figure 2

2



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