Z-ocimene, but not alkaloids, deters oviposition of the specialist moth *Agonopterix alstroemeriana* on *Conium maculatum* (Apiaceae)

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**Introduction**

Host plant choice by phytophagous insects is highly determined by plant chemical composition. Volatile compounds released by the plant can stimulate or deter oviposition depending on the nature of the compound, their concentrations and the relative abundance of each individual compound compared to the blend composition. The ability of herbivores to select a host by discriminating among different chemical patterns has been related to the evolutionary trend to maximize larval survivorship and performance. *Conium maculatum* (Apiaceae), an Eurasian weed naturalized in North America, contains high concentrations of piperidine alkaloids, such as γ-coniceine, conine and conhydrinone, unique compounds within the Apiaceae that are toxic for livestock and humans and might also confer resistance against phytophagous insects. One of the few herbivores that consistently consumes *C. maculatum* is the monophagous moth *Agonopterix alstroemeriana* (Oecophoridae). *A. alstroemeriana* and *C. maculatum* form a tightly coevolved plant-herbivore system (Fig. 1,2). Indeed, previous studies (Castells et al. 2005) suggest that piperidine alkaloids from *C. maculatum* could be a result of the directional selection exerted by *A. alstroemeriana*.

**Materials and Methods**

We determined the oviposition pattern of *A. alstroemeriana* on *C. maculatum* in relation to plant chemical composition and volatile emission. Experiment 1 We counted the number of *A. alstroemeriana* eggs in 32 *C. maculatum* individuals growing in natural conditions. These plants were analyzed for piperidine alkaloids, and mono- and sesquiterpenes by GC-FID. Compounds were identified by its RI and mass spectra in comparison with literature and databases. Experiment 2 In order to determine the correlation between oviposition and plant volatile emission we conducted a choice experiment offering to gravid *A. alstroemeriana* females undamaged (control) plants and artificially damaged plants, with have increased volatile emission. The choice assay was conducted in a greenhouse for 5 consecutive days in two separate cages. Headspace analysis was conducted for damaged and undamaged plants to detect volatile emission using SPME.

**Results**

**Experiment 1** Concentrations of alkaloids from *C. maculatum* are shown in Table 1. No alkaloids were detected in the hexane extraction, indicating that alkaloids present in the plant were only in the non-volatile hydrochloride form.

The number of *A. alstroemeriana* eggs was positively correlated with leaf size and negatively correlated with the concentration of the monoterpene Z-ocimene (Table 2, Figure 3). No other monoterpenes, sesquiterpenes or pipideridine alkaloids were significantly correlated with oviposition.

**Experiment 2** Artificially damaged *C. maculatum* plants grown in the greenhouse significantly increased the emission of volatile compounds while damaged leaves released significant amounts of 5-endo-myrcene, Z-ocimene, E-ocimene, 5-caryophyllene, germacrene D, and the unknown sesquiterpene. Piperidine alkaloids were absent in the headspace analysis for control and artificially damaged plants, which confirms that alkaloids of *C. maculatum* are stored as salts. The oviposition choice experiment with control and artificially damaged plants showed that *A. alstroemeriana* females had a preference to oviposit on the undamaged plants, which released less volatile compounds in comparison to the damaged plants (Fig. 4).

**Discussion**

Plant secondary chemistry is highly involved in plant host preference by herbivores. In a tightly coevolved plant-herbivore system, such as the one formed by the *Agonopterix alstroemeriana* and *Conium maculatum*, a preference for less defended individual plants might increase the insect offspring fitness. However, we did not found a correlation between oviposition and plant alkaloid concentrations. This lack of correlation suggests that *A. alstroemeriana* moths do not actively select for lower alkaloid *C. maculatum* individuals, and that the higher presence of *A. alstroemeriana* on less defended plants as previously found (Castells et al. 2005) could be caused by a higher mortality of larvae due to alkaloid ingestion. Oviposition, however, was negatively correlated to the monoterpene Z-ocimene. This result is in accordance to the laboratory choice assay where damaged plants were less likely to be used as oviposition sites compared to undamaged plants. Because the release of plant volatiles is dramatically increased after damage, the lower oviposition rate on plants with higher Z-ocimene concentrations and higher emission of volatiles suggests an avoidance behavior of *A. alstroemeriana* moths for those plants where competition by other larvae might be increased. In conclusion, monoterpenes are more important than alkaloids in determining *A. alstroemeriana* oviposition patterns. Unique plant compounds are not involved in plant host preference in a tightly coevolved plant-herbivore system.

**Table 1. Concentrations of alkaloids and sesquiterpenes for 32 plants growing in natural conditions in Urbana, IL (USA). Alkaloids were only detected in the methanolic extraction. These results indicate that alkaloids are found in the salt form and thus are not volatile in natural conditions.**

**Table 2. Correlations between number of *A. alstroemeriana* eggs found in a *C. maculatum* leaf, the leaf size estimated by its dry weight, and the concentrations monoterpene, sesquiterpene and piperidine alkaloids. Significant correlations at \(p < 0.05\) are indicated in bold. Cuts in red show the significant correlations with oviposition.**

**Fig. 1** The Apiaceae Conium maculatum, which contains unique piperidine alkaloids (γ-coniceine is shown in figure), is almost exclusively consumed by the specialist moth *Agonopterix alstroemeriana*. Plant and insect form a tight coevolved system.

**Fig. 2** A. alstroemeriana exert a directional selective pressure on *C. maculatum*. Larvae (red arrows) attack leaves and flower buds causing substantial damage.

**Fig. 3** Partial regressions for number of *A. alstroemeriana* eggs with (A) leaf size and (B) Z-ocimene concentrations from a multiple regression analysis.