

Genetic variation of alkaloid production in *Conium maculatum* after reassociation with the specialist moth *Agonopterix alstroemeriana*

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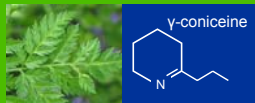
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1. The players

Conium maculatum, a Eurasian weed naturalized in North America, contains high concentrations of piperidine alkaloids (γ -coniceine, coniine, conhydrinone) that may act as chemical defenses. In the United States, *C. maculatum* was largely free from herbivory until approximately 30 years ago, when it was reassociated via accidental introduction of a monophagous European herbivore, the oecophorid caterpillar *Agonopterix alstroemeriana*. At present, *A. alstroemeriana* is found in a continuum of re-association times and intensities with *C. maculatum* across the continent. Studies in biological control and invasion biology rarely determine whether plants reassociated with a significant herbivore from the area of indigeneity increase their chemical defense investment in areas of introduction.

Conium maculatum



- Native to Eurasia
- Introduced to US in the 1800s
- Consumed by few herbivores
- Contains piperidine alkaloids

Agonopterix alstroemeriana



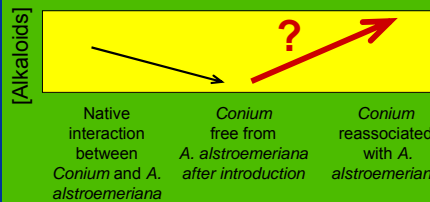
- Native to Europe
- Introduced to Eastern US in 1973
- *C. maculatum* is its sole host
- Potential biocontrol agent



C. maculatum free from herbivory in the mid-west (IL) and under a severe *A. alstroemeriana* pressure in the Pacific Northwest (WA). Damage by *A. alstroemeriana* may result in complete defoliation.

2. Hypothesis

Alkaloid concentrations of *C. maculatum* in the invasive area are expected to increase after reassociation with its specialist moth. This increase should be correlated with the length of reassociation.



3. Experimental design

A common garden design with *C. maculatum* from three locations in the U.S. (Illinois, Washington and New York) that have experienced different lengths of reassociation with *A. alstroemeriana* was conducted to determine whether variation in alkaloid production is genetic. Seeds collected in IL, WA and NY were grown in a greenhouse at UIUC. When plants were 3-months-old foliage was sampled for chemical analyses. Additionally, seedlings were planted in an experimental field. We recorded the natural colonization of *A. alstroemeriana* on *C. maculatum* by counting the number of leaf rolls at the end of the season. A bioassay with larvae from our laboratory colony raised on IL, NY and WA foliage was conducted to determine the effects of secondary chemistry on insect fitness.



Reassociation times between *C. maculatum* and *A. alstroemeriana* in the regions studied. Years of *A. alstroemeriana* introduction: 1973 (NY), 1985 (WA) and 1993 (IL)

Greenhouse common garden

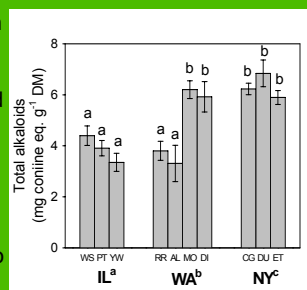
1. Foliar alkaloids, N

Field common garden

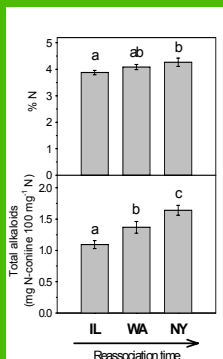
2. Natural infestation
3. Bioassay

4. Alkaloid concentrations

Total alkaloid production in *C. maculatum* was positively correlated with reassociation time between *C. maculatum* and *A. alstroemeriana*, with the longest historical association with *A. alstroemeriana* (NY) had highest alkaloid content; WA plants were intermediate, with the highest amount of variability among sites, and IL plants had lowest alkaloid concentrations. High variance among WA plants (with alkaloid concentrations similar to IL in 2 sites and to WA in 2 other sites) may be a result of transitioning from low alkaloid levels resulting from costs associated with investments in alkaloids in the absence of consistent herbivory, to a highly resistant state where variability has been stabilized due to selection by *A. alstroemeriana*.



Alkaloid concentrations in *C. maculatum* from 3 or 4 sites within IL, WA and NY. Different letters indicate significant differences at $p < 0.05$ ($n = 3-10$)

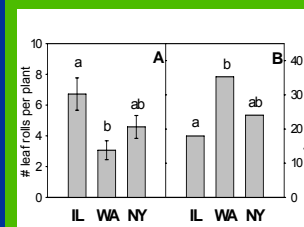


Because alkaloids may be affected by plant N availability we analyzed N concentrations in *C. maculatum* foliage. N was slightly higher in NY compared to IL. However, NY plants invested 50% more N to alkaloid synthesis than did plants from IL, suggestive of selection for higher constitutive alkaloid levels under longer reassociation times between the plant and its herbivore.

N concentrations and % of N invested to alkaloid production in *C. maculatum* from IL, WA and NY plants, with increasing reassociation times. Different letters indicate significant differences at $p < 0.05$ ($n=17-32$)

5. Natural colonization and bioassay

The number of leaf rolls present on *C. maculatum* grown in the field common garden was higher in IL plants compared to WA plants (A). Larvae raised on foliage from IL experienced significantly lower mortality than larvae raised on foliage from WA (B), consistent with the differences in leaf roll abundance in the field. NY plants experienced intermediate levels of mortality and number of leaf rolls.



(A) Number of *A. alstroemeriana* leaf rolls on IL, WA and NY plants in a field common garden ($n=36-46$). (B) Mortality of larvae raised on *C. maculatum* foliage in the laboratory ($n=54-102$). Letters indicate significant differences at $p < 0.05$

6. Discussion

Prolonged reassociation between *C. maculatum* and its specialist moth may increase toxicity of this noxious weed in its introduced range. In a previous experiment we documented differences in alkaloid concentrations among plants from IL, WA and NY collected *in situ* (Castells et al. 2005). Here we show that at least part of these differences, higher alkaloids in those regions with longer reassociation times (NY > WA > IL), have a genetic basis. Plants with higher alkaloid concentrations experienced less damage by *A. alstroemeriana*. At the same time, a decrease in fitness was found when larvae were raised on high alkaloid plants. All of these results are suggestive of strong directional selection exerted by *A. alstroemeriana* on *C. maculatum* chemistry.

References

- Castells E, Berhow MA, Vaughn SF, Berenbaum MR (2005). Geographical variation in alkaloid production in *Conium maculatum* populations experiencing differential herbivory by *Agonopterix alstroemeriana*. *J. Chem. Ecol.* 31(8): 1693-1709
- Castells E, Berenbaum MR. (2006) Laboratory rearing of *Agonopterix alstroemeriana*, the defoliating poison hemlock (*Conium maculatum* L.) moth, and effects of piperidine alkaloids on preference and performance. *Environ. Entomol.* 35: 607-615

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