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# GEOGRAPHIC VARIATION IN ALKALOID PRODUCTION IN *Conium maculatum* POPULATIONS EXPERIENCING DIFFERENTIAL HERBIVORY BY *Agonopterix alstroemeriana*

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**Abstract-***Conium maculatum*, a Eurasian weed naturalized in North America, contains high concentrations of piperidine alkaloids, which act as chemical defenses against herbivores. In the United States, *C. maculatum* was largely free from herbivory until approximately 30 years ago, when it was re-associated via accidental introduction with a monophagous European herbivore, the oecophorid caterpillar *Agonopterix alstroemeriana.* At present, *A. alstroemeriana* is found in a continuum of re-association time and intensities with *C. maculatum* across the continent; in the Pacific Northwest, *A. alstroemeriana* can cause severe damage, resulting in some cases in complete defoliation. Studies in biological control and invasion biology have yet to determine whether plants re-associated with a significant herbivore from the area of indigeneity increase their chemical defense investment in areas of introduction. In this study, we compared three locations in the U.S. (New York, Washington and Illinois) where *C. maculatum* experiences different levels of herbivory by *A. alstroemeriana* to determine the association between the intensity of the interaction, as measured by damage, and chemical defense production. Total alkaloid production in *C. maculatum* was positively correlated with *A. alstroemeriana* herbivory levels; plants from New York and Washington, with higher herbivory levels, invested two and four times more N to alkaloid synthesis than did plants from Illinois. Individual plants with lower concentrations of alkaloids from a single location in Illinois experienced more damage by *A. alstroemeriana*, suggestive of a preference on the part of the insect for plants with less chemical defense. These results suggest that *A. alstroemeriana* may act either as a selective agent or inducing agent for *C. maculatum* and increase its toxicity in its introduced range.



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**Key Words**-Insect-plant interactions, *Conium maculatum*, *Agonopterix alstroemeriana*, chemical defenses, alkaloids, γ-coniceine, coniine, conhydrinone, evolution, herbivory.



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## INTRODUCTION

Flowering plants and the insects that eat them collectively constitute almost half of all terrestrial species (Berenbaum, 1995) and the interactions between plants and herbivorous insects are of central importance in determining community structure in a wide range of terrestrial systems. Thus, perturbations that affect interactions can have profound impacts within these terrestrial systems. Among the perturbations of increasing concern globally are the introduction and incorporation of non-native species into communities within which they have not evolved. Once established, invasive plants can cause direct economic damage by reducing crop yields and livestock growth and indirect damage by altering community composition via displacement of native species (Pimentel et al., 2000; Vitousek et al., 1996). Invasive species can influence the evolution of native species via niche displacement, competitive exclusion, introgression, hybridization, and even extinction (Mooney and Cleland, 2001).

Because the rate at which invasive species enter and become established in the U.S. has been increasing (Mooney and Cleland, 2001), there is considerable interest within the scientific community in understanding the dynamics of invasion. Characterizing "invasibility," however, has proved difficult (Crawley, 1987; Rejmanek and Richardson, 1996; Reichard and Hamilton, 1997). One factor widely acknowledged to play a role in establishment of invasive plants is that transport of a species out of its native habitat generally results in a reduction in herbivory by coevolved specialist insects. Indeed, the idea that plant populations in areas of indigeneity are regulated by herbivores underlies the practice of classical biological control of weeds (Huffaker, 1959; Willis et al., 2000). The



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fact that plants tend to grow taller and produce more seeds in areas of introduction than in areas of indigeneity has been attributed in part to release from herbivorous natural enemies (Crawley, 1987). Blossey and Nötzold (1995), in proposing what they call the evolution of increased competitive ability (EICA) hypothesis based on optimal defense theory (Zangerl and Bazzaz, 1992), argue that invasiveness results from changes in biomass allocation patterns; in areas of introduction, where herbivores are absent, genotypes with reduced resource allocation to herbivore defense and increased resource allocation to competitive abilities are favored. Although preliminary tests of this hypothesis with purple loosestrife (*Lythrum salicaria*) were suggestive (Blossey and Nötzold, 1995) and have been confirmed in other systems (Siemann and Rogers, 2001; Blair and Wolfe, 2004), additional tests failed to document intraspecific variation in herbivore resistance according to plant origin (Willis et al., 1999; Stastny et al., 2005) and a broader survey suggested that differences in sizes of plants in indigenous vs. non-indigenous habitats may represent plastic phenotypic variation rather than evolutionary change (Willis et al., 2000). More recently, a comprehensive comparison of size, fecundity and leaf areas of nonindigenous and indigenous populations of *Hypericum perforatum* provided compelling evidence of the capacity for rapid contemporary evolution of these traits in invasive species (Maron et al. 2004a).

In general, little quantitative and ecologically relevant information is available on phytochemical changes in plants that occur after introduction into a non-indigenous area and release from interactions with longtime insect associates (Daehler and Strong, 1997; Willis et al., 1999; Siemann and Rogers, 2003; Maron et al., 2004b). Perhaps equally importantly, little information is available on phytochemical changes that ensue when coevolved herbivore associates resume interacting with a host plant in a non-indigenous



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area. This scenario is of no small consequence in that classical biological control involves reconstructing such plant-herbivore associations in the area of introduction. The possibility exists that, in the area of introduction, a newly resumed interaction will differ dramatically in its dynamics from such interactions in the area of indigeneity, given the differences in the structure of the surrounding community. Understanding the selective impact of re-associated herbivores on the chemistry of their host plants in areas of introduction is thus of interest not only in the context of understanding the basic dynamics of plant-insect interactions but also in predicting potential trajectories of classical weed biological control programs.

A system in which the chemical consequences of reassociation with a coevolved herbivore may be thoroughly examined involves the interaction between *Conium maculatum* (L.) (Apiaceae) (poison hemlock), a Eurasian weed, and its monophagous associate *Agonopterix alstroemeriana* (Clerck) (Lepidoptera: Oecophoridae), a leaf-rolling European caterpillar known only to feed on *C. maculatum*. *C. maculatum* is an herbaceous Eurasian biennial that is extensively naturalized in temperate North America, as well as in other parts of the world, including Australia, New Zealand, and South America (Parsons, 1976; Holm et al., 1979). The weed is generally regarded as noxious; all aerial parts are poisonous to livestock and to humans (Sperry et al., 1964; Widner, 1984; Markham, 1985; Hannam, 1985; Jessup et al., 1986; Panter et al., 1988; Panter and Keeler, 1989). The toxicity of *C. maculatum* to vertebrates is attributable primarily to its production of relatively high concentrations of coniine and related piperidine alkaloids, including methylconiine, coniceine, and conhydrine (Fairbairn, 1971). Its tendency to invade fields of alfalfa and other forage crops has led to livestock death through contamination of green-



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chopped hay (Kubik et al., 1980; Panter et al., 1988). Due to its toxicity, as well as to its rank odor and profuse growth, *C. maculatum* is frequently a target of eradication programs in populated areas.

Relative to other introduced weed species, *C. maculatum* is attacked by few insect herbivores. In an extensive survey of poison hemlock in southern California, Goeden and Ricker (1982) reported "amazingly few insect species or individuals thereof. A clear majority, 16 (70%) of the 20 [*sic*] phytophagous insect species found on this weed were rare and were only encountered as a few individuals at one or two sites." Of the relatively few native insect species that have colonized the plant extensively throughout its range, the majority are species that feed generally on native and introduced plants in the Apiaceae; these species include *Papilio zelicaon* Lucas (Goeden and Ricker, 1982), *P. polyxenes asterius* Stoll (Feeny et al., 1985; personal observations) (Lepidoptera: Papilionidae), and *Euleia fratria* (Loew) (Diptera: Tephritidae) (Berenbaum, 1981; personal observations). The most abundant insect associate of the plant in California until recently has been an aphid, *Hyadaphis foeniculi* (Passerini), accidentally introduced from Europe, where it also feeds on poison hemlock (Goeden and Ricker, 1982).

*Agonopterix alstroemeriana*, a leaf-rolling oecophorid caterpillar monophagous on *C. maculatum* throughout its native range in Europe was first reported on *C. maculatum* populations in the U.S. in Tompkins County, New York in 1973 (Berenbaum and Passoa, 1983). *A. alstroemeriana* was subsequently reported in 1983 in northern California, Oregon, and Utah and by 1987 was established in mesic areas of Washington, Idaho, and Colorado (Powell and Passoa, 1991), where it remains reliably "collectible in large numbers" (Western Society of Weed Science *et al.*, 1995). An adult *A. alstroemeriana* collected in



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1990 near Columbus, Ohio, marked the first appearance of this species in the Midwest (Powell and Passoa, 1991). In 1993, the existence of established populations of *A. alstroemeriana* in central Illinois was confirmed (Berenbaum and Harrison, 1994); McKenna et al. (2001) reported substantial populations of this insect at several sites throughout Champaign County, Illinois.

Thus, throughout its range in North America, populations of *C. maculatum* populations exist with varying histories of re-association with a specialist herbivore from its area of indigeneity. This continuum of association allows us to test whether a specialist insect (and potential biological control agent) may act as a selective agent on the defense chemistry of its hostplant. In this study, we set out to determine whether chemical defense production by *C. maculatum* changes in response to re-association with a principal herbivore from its area of indigeneity.

## MATERIALS AND METHODS

*Sampling.* Study sites were located in Champaign County, Illinois (40.109 N, 88.203 W), Tompkins County, New York (42.443 N, 76.503 W), and Whitman County, Washington (46.733 N, 117.161 W), USA. These locations were selected because they represent a continuum in time of association with and intensity of herbivory by *A. alstroemeriana* on *C. maculatum,* with longer association and higher levels of herbivory in Washington and New York and shorter association and lower levels of herbivory in Illinois. In June 2003, we selected four sites located more than 2 km apart within each region. *A. alstroemeriana* larvae can be found on *C. maculatum* for a short period of time, between 30



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and 45 days, with some differences in life cycle timing among the regions studied. Thus, *A. alstroemeriana* larvae can be found from late April to early June in IL (Berenbaum and Harrison, 1994), from early May to mid-June in NY (Berenbaum and Passoa, 1983), and from early June to mid-July in WA (Gary Piper, personal observation). *C. maculatum* sampling was thus conducted in early June in IL and NY and in late June in WA to coincide with the presence of *A. alstroemeriana* larvae. Plants are in approximately the same developmental stage across regions when *A. alstroemeriana* is abundant (early to mid-flowering). At each site within region we estimated *A. alstroemeriana* damage levels. Five levels of intensity were identified: Level 0, *A. alstroemeriana* leaf rolls absent; Level 1, leaf rolls present but no visible leaf damage; Level 2, minor defoliation, with up to one-quarter of leaf area damaged (as estimated by eye); Level 3, mild defoliation, between one-quarter and one-half of leaf area damaged; Level 4, major defoliation, with more than one-half of leaf area damaged; and Level 5, complete defoliation, with mostly of leaf area damaged.

In each region we randomly sampled between 12 and 20 *C. maculatum* plants per site. Two subsamples of green foliage per plant, one for alkaloid analyses and one for nitrogen and leaf water content analyses, were placed separately in Eppendorf tubes and frozen *in situ* on dry ice. Samples were shipped on dry ice to our UIUC laboratory and stored at –80 ºC until analyzed.

*Plant extraction and chemical analyses.* Leaf material (ca 200 mg FW) was ground using a ball mill and extracted on a shaker for 1 hr with 1.5 ml of acidified methanol (70% MeOH 30% 0.1 N HCl). After centrifuging, 1 ml of the resulting extract was concentrated



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to approximately 0.2 ml on a centrifugal evaporator (Jouan RC 10.10), extracted with hexane to remove non-polar compounds, and placed back in the centrifugal evaporator to remove the residual hexane. The remaining solution was then basified with 10 M NaOH; these were extracted in 200 µl hexane with 0.01% hexadecane. Alkaloids were analyzed by flame ionization detection on a gas chromatograph equipped with capillary column (Alltech EC-1, 30 m, 0.23 mm) coupled with an autosampler (HP 5890). Hexadecane was used as internal standard. The 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. (±)-coniine (Sigma) was used as a standard. Alkaloids concentrations were expressed as coniine equivalents on a dry weight basis and per mg of nitrogen. Total alkaloid concentrations were calculated by adding the concentrations of each individual alkaloid.

Total nitrogen was measured to estimate the relative resource investment in defense compounds among the three regions studied. A subsample of fresh leaf material was weighed, oven-dried at 60 ºC overnight and weighed again to obtain the FW/DW ratio. Samples (10 plants per site) were then ground and analyzed for total nitrogen in an elemental combustion analyzer (Costech Instruments ECS 4010).

*Isolation and identification of alkaloids.* Leaves of *C. maculatum* were collected during March 2004 at Phillips Tract Experimental Station located at 5 km of Urbana, IL, USA. Fresh leaves (200 g FW) were extracted in a blender with 70% methanol 30% 0.1 N HCl and filtered through Whatman 1 filter paper. The residue was re-extracted two more times; fractions were bulked together (1 l in total) and filtered through reversed-phase C18 (Baker; 40 µm) previously rinsed with methanol to remove non-polar compounds. The



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eluate containing the alkaloids was concentrated by rotary evaporator at low temperature (max 45 ºC) until the volume was reduced by half; the eulate was then partitioned with chloroform three times to further remove non-polar compounds. In a separation funnel, the extract was basified with 10 M NaOH and a liquid-liquid partition was conducted five times with chloroform to extract the alkaloids. The bulked chloroform fractions were mixed with 20% HCl in MeOH and rotavaped down to obtain a mixture of alkaloids in hydrochloride form. Bulk alkaloids were resuspended in ethanol : 0.1 N HCl (1:1), basified with 10 M NaOH and extracted three times with a small volume of chloroform. The individual alkaloids were isolated using a 25 x 2.5 cm silica gel (Merk; 32-63 μm) gravity column eluted with 150 ml of chloroform : ethanol : $NH_3OH$ (70:30:1) (Leete and Olson, 1972) at ca 1 ml $min^{-1}$. The fractions (2 ml each) were monitored by spotting 5 μl on a TLC silica gel plate (Baker; 250 μm) and sprayed with Dragendorff (Jungreis, 1985) or 0.2% ninhydrin reagents (Sigma). Although the individual alkaloids did not completely separate, we could obtain fractions with more than 95% purity for the two major alkaloids to be identified by NMR (RT 6.5 and RT 10) as explained below. The low concentration of the alkaloid RT 12.0 did not allow us to obtain enough pure material for structure elucidation. Coniine identity (RT 5.6) (Figure 1) was established by comparison with authentic material (Sigma).

*LC-ESI-MS Analysis for hemlock alkaloids.* Samples were run on a Finnigan-Thermoquest LCQ LC-MS system (AS3000 autoinjector, P4000 HPLC pump, UV6000 PDA detector, LCQ mass spectrometer) (San Jose, CA) all running under the Xcaliber 1.2 software system. The MS was run with the ESI probe in the positive mode. The column was a 3 mm x 150 mm Inertsil reverse phase C-18, ODS 3, 3 micron column (Metachem,



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Torrance, CA) with a Metaguard guard column. The source inlet temperature was set at 220 $^{o}$C and the sheath gas rate was set at 90 arbitrary units. The MS was optimized for the detection of the hemlock alkaloids by using the autotune feature of the software while infusing a solution of coniine in with the effluent of the column and tuning on an atomic mass unit of 128 $[M+H]^{+}$. The solvent systems were A: water with 0.1 M ammonium acetate, and 0.25% acetic acid, and B: methanol with 0.1 M ammonium acetate, and 0.25% acetic acid. The column was equilibrated with 2% B at a flow rate of 0.3 ml $min^{-1}$. After injection the column was held at the initial conditions for 2 min, then developed with a linear gradient to 40% B over 23 min. and then to 50% B over the next 10 min. The column effluent was monitored at 210 nm in the PDA detector. The software package was set to collect mass data between 150-1000 AMUs. Generally the most significant sample ion generated under these conditions were $[M+1]^{+}$.

*GC-MS analysis for hemlock alkaloids.* Gas chromatography-mass spectrometry (GC-MS) was performed using a Hewlett-Packard (HP) 6890 GC system attached to a HP 5972A Mass Selective Detector. The column used was a fused silica HP-5MS capillary (0.25-µm film thickness, 30 m X 0.25 mm ID). The GC operating parameters were as follows: splitless injection mode; temperature programmed from 50 to 315 ºC at 5 ºC $min^{-1}$ with a 10-min initial temperature hold; He carrier gas flow rate at 1.1 ml $min^{-1}$, with the injector temperature set at 250 ºC. Spectra were compared with known standards or by computer with the Wiley/NBS Mass Spectral Registry (McLafferty and Stauffer, 1989).

*NMR Analysis of hemlock alkaloids.* $^{1}$H, COSY, DEPT, HSQC, and $^{13}$C-NMR spectra were obtained with a Bruker (Billerica, MA, USA) Avance 500 spectrometer equipped with a 5mm inverse broadband Z-gradient probe ($^{13}$C NMR, 125 MHz; $^{1}$H, 500



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MHz). NMR spectra were recorded in methanol-d4, which served as the internal reference ($^{13}$C, 49.0ppm, $^{1}$H, 3.30ppm). The data were analyzed using the Advanced Chemistry Development, Inc., SpecManager 1D Processor and the HNMR and CNMR Predictor software suite (Toronto, ONT.)

*Structure confirmation of γ-coniceine (RT 6.5).* Positive ion LC-ESI-MS showed the presence of one major compound with a large mass ion $[M+H]^+$ of m/z 126. Prominent diagnostic GC-mass spectral ions and their relative intensities are as follows: EI-MS [m/z (%)]: 125 ($M^+$, 13), 124 (10), 110 (32), 97 (100), 96 (43), 82 (10), 70 (19), 55 (10), 54 (10). $^{1}$H-NMR δ ($CD_3OD$): 3.65 (2H, bs); 2.82 (2H, m); 2.62 (2H, m); 1.90 (2H, m); 1.86 (2H, m); 1.71 (2H, m); 1.02 (3H, t, J = 7.4Hz). $^{13}$C-NMR δ ($CD_3OD$): 45.9; 41.0; 30.6; 20.3, 20.3; 17.9; 7.4. The $^{1}$H-NMR and $^{13}$C-NMR spectra identify a compound with a composition of $C_8H_{15}N$ consistent with the structure of γ-coniceine (Asensio et al., 1993; Fukuda, 1991) (Figure 1).

*Structure confirmation of conhydrinone (RT 10).* Positive ion LC-ESI-MS showed the presence of one major peak with a large mass ion $[M+H]^+$ of m/z 142. Prominent diagnostic GC-mass spectral ions and their relative intensities are as follows: EI-MS [m/z (%)]: 141 ($M^+$, 2), 98 (100), 84 (4), 70 (8), 55 (65). $^{1}$H-NMR δ ($CD_3OD$): 4.04 (1H, dd, J = 12.3Hz, J = 3.3Hz); 3.39 (1H, m); 2.99 (1H, m); 2.63 (1H, m); 2.38 (1H, m); 1.91 (2H, m); 1.67 (2H, m); 1.54 (1H, m); 1.09 (3H, t, J = 7.2Hz). $^{13}$C-NMR δ ($CD_3OD$): 207.4; 64.4; 44.9; 32.6; 27.1; 23.2; 23.0; 7.4. The $^{1}$H-NMR showed signals for an isolated ethyl group adjacent to a carbonyl carbon. The downfield proton is consistent with a proton attached to the carbon in the ring that is both adjacent to the nitrogen and attached to the side chain bearing the ketone. All assignments for conhydrinone could be obtained from the COSY



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spectrum. The $^{13}$C-NMR revealed the presence of a keto carbonyl at 207.4 ppm. All the assignments were obtained from DEPT and HSQC experiments. The spectra match that of a compound with a composition of $C_8H_{15}NO$ corresponding to conhydrinone (Leete and Olson, 1972) (Figure 1).

*Information on an unknown alkaloid (RT 12).* Positive ion LC-ESI-MS showed the presence of one major compound with a large mass ion $[M+H]^+$ of m/z 126 and several minor contaminating TIC peaks. The retention time of this compound in the GC is significantly longer than of γ-coniceine; on this basis we conclude the compound has a different chemical structure. Prominent diagnostic GC-mass spectral ions and their relative intensities are as follows: EI-MS [m/z (%)]: 125 ($M^+$, 1), 124 (6), 110 (18), 97 (100), 96 (31), 82 (9), 69 (7), 55 (15). Examination of purified fractions containing this peak by NMR and IR yielded conflicting data as to the exact chemical structure of this compound. At this time we can say it appears to be a coniceine isomer with the exact location of the double bond undetermined.

*Herbivory and alkaloids.* To determine whether *C. maculatum* chemistry is associated with differences in *A. alstroemeriana* abundance, we randomly selected 29 plants from one site in Champaign County, IL ("Yard waste site") in June 2003. For each plant we counted the number of leaf rolls; two leaf samples per plant were then taken and alkaloids were analyzed as described. The number of leaf rolls was used as an estimate of herbivory intensity assuming a proportional relationship between the number of larvae and the number of leaf rolls made during larval development.

*Statistical analyses.* All statistical analyses were performed using Statistica 6.0 (Statsoft, Tulsa, USA). A one-way analysis of variance with "site" nested in "region" (IL,



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NY, and WA) was performed to examine differences among populations in total alkaloid content, individual alkaloid content, and nitrogen content. A *t*-test was conducted to analyze the relationship between herbivory level and total alkaloid and N concentrations. Post-hoc comparisons for "region" or "herbivory level" were conducted using *Tukey's HSD* test. The association between alkaloids and intensity of *A. alstroemeriana* herbivory was tested by conducting a simple regression analysis between total alkaloid concentrations and number of leaf rolls, and a multiple regression analysis with coniine, γ–coniceine, conhydrinone and RT12 as independent variables and leaf roll number as the dependent variable. Data were log transformed to fit normality when necessary.

## RESULTS

*Herbivory. A. alstroemeriana* herbivory levels on *Conium maculatum* were lower in Illinois compared to New York and Washington, ranging from absence of leaf rolls (level 0) to minor defoliation (level 2) (Figure 2). In New York, herbivory damage ranged from minor defoliation (level 2) to total defoliation (level 5). However, in New York, especially at the Etna site (ET), the sampling was conducted earlier in the season compared to the other regions, as shown by the number of early instars found, and thus the actual damage to plants may be higher over the course of the season than that estimated here. In Washington, the damage inflicted by herbivores was extremely severe, ranging from major (level 4) to complete defoliation (level 5) (Figure 2) and plants in many stands were found to be totally desiccated as a result of the damage by *A. alstroemeriana*.



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*Plant Chemistry.* We found a total of four alkaloids in *Conium maculatum* foliage: coniine, γ–coniceine, conhydrinone and an unknown alkaloid (RT12) (Table 1). Not all compounds were present in every plant and when present their concentrations were highly variable among individuals. Total alkaloid concentrations in plants expressed on a dry weight basis varied among the three regions (ANOVA $P < 0.05$, and were lower in Illinois than in New York and Washington (Figure 2, Table 1). These lower levels reflected primarily lower concentrations of γ-coniceine, the major alkaloid in all three regions, constituting 80%, 91% and 89% of the total alkaloids in Illinois, New York and Washington, respectively. Plants in New York and Washington, although not different in total alkaloid content, differed in the concentrations of conhydrinone and RT12, with lower values of both compounds in New York (Table 1). Coniine was present in only about 16% of the plants growing in Illinois; in these plants, however, coniine reached such high concentrations that it constituted the second most abundant alkaloid after γ-coniceine. Coniine was not present either in New York or Washington (Table 1). Alkaloid concentrations expressed by unit N were significantly lower in Illinois and higher in Washington, partially due to differences in leaf N concentrations among regions (Figure 3). Total alkaloid concentrations were significantly higher in plants under increasing levels of *A. alstroemeriana* herbivory (Figure 4). No significant trends were found for herbivory levels relative to N concentrations (Figure 4).

*Herbivory and alkaloids.* The number of leaf rolls in plants from Illinois, used as an estimate of *A. alstroemeriana* herbivory, was marginally negatively correlated with total alkaloids (Figure 5a). In a multiple regression analysis the number of leaf rolls was



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significantly correlated with γ–coniceine (Figure 5b) but no significant relationships were found for coniine, conhydrinone or RT12 (data not shown).

## DISCUSSION

Poison hemlock has been established in Illinois for over 100 years (Vasey, 1861; Patterson, 1876; Jones and Fuller, 1955). Despite the fact that the plant is locally extremely abundant, intermittent inspection over the last decade has consistently revealed few insect associates of this plant and little leaf damage by herbivores (personal observation). This same pattern has been documented in other parts of the United States--by Berenbaum (1981) in central New York and by Goeden and Rickers (1982) in southern California. The failure of insects to colonize this plant in large numbers over historical time may be attributable to its formidable array of chemical defenses. However, populations of poison hemlock in several localities in the United States are now experiencing unprecedented levels of herbivory due to colonization by and population growth of *A. alstroemeriana*. It is of great ecological and evolutionary interest to monitor changes in plant secondary compounds that may accompany this colonization. In this study, the chemistry of poison hemlock populations largely free from *A. alstroemeriana* (Illinois populations) was compared with that of populations in which *A. alstroemeriana* has become successfully established (New York and Washington populations) in order to determine under field conditions whether these herbivores, touted as potential biocontrol agents (www.bio-control.com, www.integratedweedcontrol.com), could reduce plant fitness and at the same time select for chemically based resistance factors (Berenbaum et al., 1986).



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Changes in plant resource allocation between growth and chemical defenses driven by herbivore selective pressures have been discussed by the "optimal defense" hypothesis (Zangerl and Bazzaz, 1992) and the derivative "evolution of increased competitive ability" hypothesis, formulated in the context of invasion biology (Blossey and Nortzold, 1995). According to these theoretical frameworks, when a plant species invade a new habitat where its native herbivores are absent, those genotypes with higher investments in growth and/or reproduction and reduced investments in defense are expected to have higher fitness and to increase their frequencies in the population. A logical corollary to these hypothesis is that defense investments should increase in the area of introduction commensurate with increases in herbivory, either by newly colonizing native species or by re-association with introduced enemies from the area of indigeneity (as in the case of biological control). With the re-association between the plant and the herbivore, such is the case of *C. maculatum* and *A. alstroemeriana*, the genotypes with increased levels of chemical defense should be favored by selection, particularly if, as is suggested in our study, high levels of alkaloid defense are deterrent to *A. alstroemeriana*. In our study we found that *C. maculatum* plants from populations experiencing high levels of *A. alstroemeriana* herbivory (New York and Washington) had higher alkaloid concentrations in foliage than those plants under lower levels of herbivory (Illinois). Because alkaloid concentrations in other species tend to increase with higher plant N availability (Gershenzon, 1984), we also analyzed N concentrations in *C. maculatum* foliage to determine whether variation in the geographic pattern in *C. maculatum* alkaloid production could be related to differences in soils among regions. Nitrogen concentrations in the three populations varied significantly, with higher concentrations in New York and lower concentrations in Washington, but these differences



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were not consistent with the observed differences in alkaloid concentrations. Plants from New York and Washington invested respectively two and four times more N to alkaloid synthesis compared to plants from Illinois; in Washington populations, higher allocation of N to alkaloids was accompanied by lower total N concentration in foliage, which is suggestive of selection for higher constitutive or inducible alkaloid levels under more intense herbivory. Mechanistically, this pattern may result from greater mortality on or avoidance of high levels of alkaloids by *A. alstroemeriana*; the negative relationship between the number of leaf rolls, and thus herbivory intensity, and the total alkaloid concentrations or γ-coniceine concentrations in Illinois plants is consistent with an increase in larval mortality with higher alkaloid concentrations.

Other environmental factors, such as water availability, could contribute to the variation in the concentrations of alkaloids in *C. maculatum* across the United States. As well, the different levels of alkaloid concentrations observed may reflect differential induced responses to herbivory (Castells et al., in prep.). Future studies, including a common garden experiment, will be necessary to determine definitively whether the geographic differences in alkaloids are genetically based and the result of a selection response to herbivory.

Although it was not deliberately introduced into the United States for biological control, *A. alstroemeriana* has demonstrated great potential for systematic use as a biocontrol agent for poison hemlock. In the western United States, this insect has quickly become established naturally in infested locations and as well has established itself when it has been intentionally released (G. Piper, personal communication). Where it is established, it causes severe injury, including complete defoliation and destruction of inflorescences



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(Western Society of Weed Science et al., 1995). The value of a biological control agent, however, must be assessed not only by its ecological effect on population sizes but also by its evolutionary impact on its target host plants. This study suggests that successful biological control agents may have the potential to alter the chemistry of their host plant, leading to increased allelochemical content and potentially greater toxicity to livestock and humans who mistakenly ingest it.

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TABLE 1. TOTAL ALKALOID CONCENTRATIONS (mg $g^{-1}$ DM), INDIVIDUAL ALKALOID CONCENTRATIONS (mg $g^{-1}$ DM) AND ALKALOID RELATIVE ABUNDANCE (%) IN *Conium maculatum* FROM ILLINOIS (IL), NEW YORK (NY) AND WASHINGTON (WA)

| | **Retention time (min)** | **IL** | **NY** | **WA** |
|---|---|---|---|---|
| Total alkaloids | | 2.49[a] | 4.07[b] | 6.48[b] |
| Coniine | 5.7 | [1.1[a]] [(50.9%)] | 0[b] | 0[b] |
| γ-coniceine | 6.5 | 2.0[a] (80.8%) | 3.96[b] (97.3%) | 5.94[b] (91.7%) |
| Conhydrinone | 10.0 | 0.22[a] (9.02%) | 0.08[a] (1.96%) | 0.37[b] (5.72%) |
| RT12 | 11.9 | 0.07[a] (2.73%) | 0.03[b] (0.68%) | 0.16[c] (2.51%) |

*Different letters indicate significant differences among regions at P < 0.05 by tukey's post-hoc comparisons test. Values of coniine shown in brackets indicate the average*



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*concentrations for the 16% of plants from the Illinois population that contain those alkaloids*



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FIGURE LEGENDS

FIG. 1. Major piperidine alkaloids of *Conium maculatum* from Illinois, New York and Washington.

FIG. 2. Total alkaloid concentrations (mean ± SE) of *C. maculatum* plants by sites in each region. In Illinois (IL): Railroad (RR), Windsor Rd (WS), Phillips Tract (PT) and Yard Waste (YW). In New York (NY): Coy Glenn (CG), Elm St (EL), Dump (DU) and Etna (ET). In Washington (WA): Railroad (RR), Albion (AL), Moscow (MO) and Ditch (DI). Averaged *A. alstroemeriana* herbivory levels for each site, are indicated inside the graph bars (see a description of each level in the Materials and methods). Different letters indicate significant differences among regions at $P < 0.05$ by *Tukey's post-hoc* comparisons test.

FIG. 3. N concentration and total alkaloid concentrations (mean ± SE) expressed on N basis of *C. maculatum* growing in Illinois (IL), New York (NY) and Washington (WA). Different letters indicate significant differences at $P < 0.05$ by *Tukey's post-hoc* comparisons.

FIG. 4. Mean of N and total alkaloid concentrations at different *A. alstroemeriana* herbivory levels, from 0 to 5 with increasing levels of herbivory, as described in Materials and Methods. For total alkaloids, the back transformed mean is shown. Different letters indicate significant differences at $P < 0.05$ by *Tukey's post-hoc* comparisons.



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FIG. 5. A) Simple regression between total alkaloid concentrations and number of leaf rolls of 29 *C. maculatum* plants from Illinois. B) Correlation between the residuals of γ-coniceine and the residuals of leaf rolls number after performing a multiple regression analyses with the independent variables coniine, γ-coniceine, conhydrinone and RT12.



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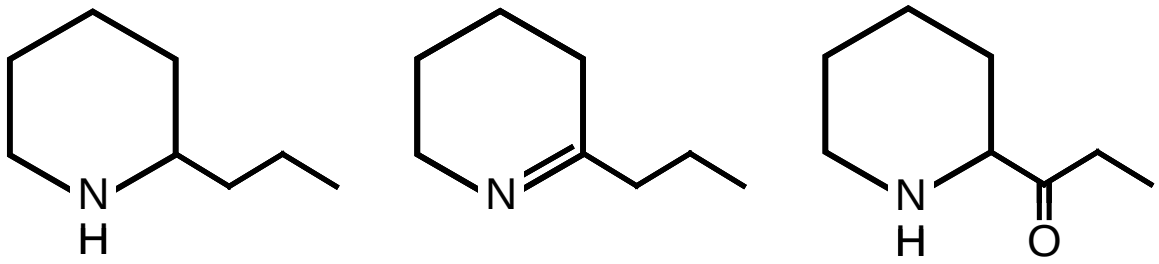
FIG. 1




Coniine γ-coniceine Conhydrinone



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FIG. 2

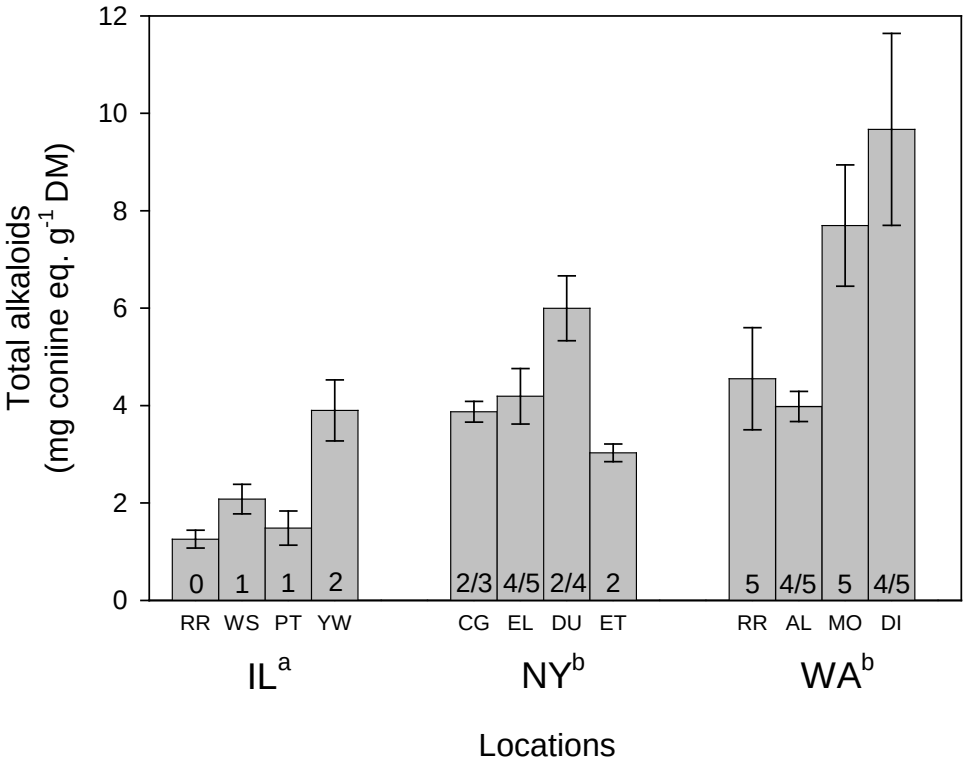




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FIG. 3

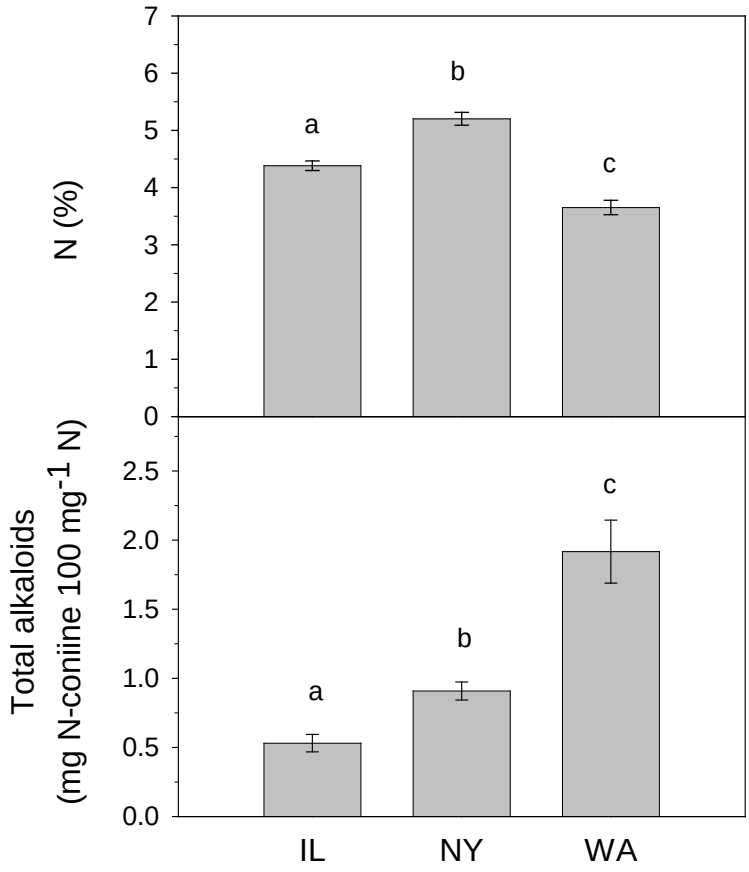




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FIG. 4

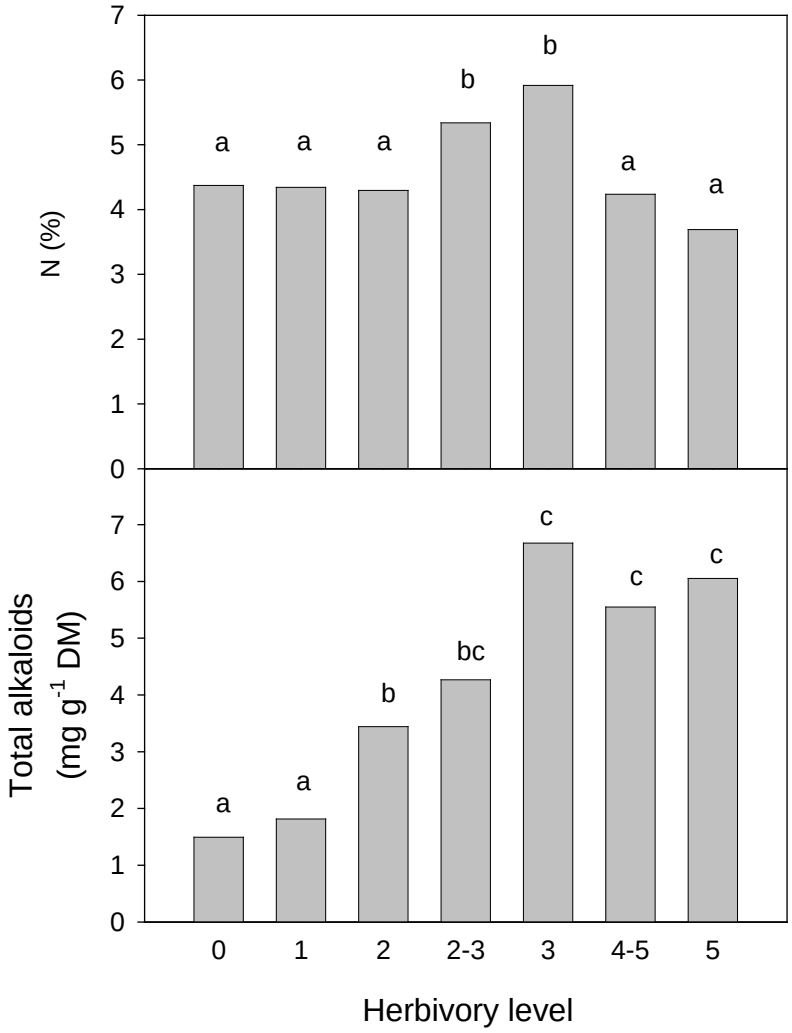




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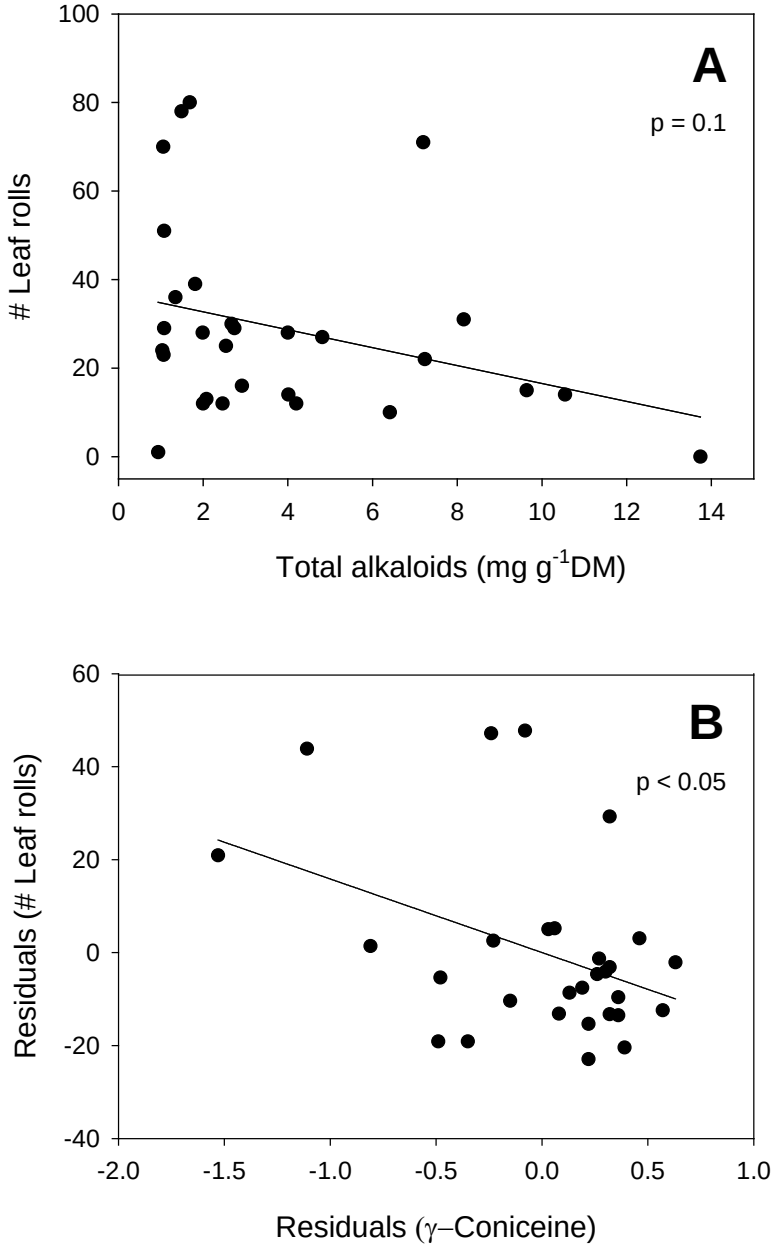
FIG. 5



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