

Laboratory Rearing of *Agonopterix alstroemeriana*, the Defoliating Poison Hemlock (*Conium maculatum* L.) Moth, and Effects of Piperidine Alkaloids on Preference and Performance

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ABSTRACT *Conium maculatum* L. (Apiaceae), or poison hemlock, is an invasive plant native to Europe that has become extensively naturalized throughout North America. This species contains piperidine alkaloids, including coniine and γ -coniceine, that are highly toxic to vertebrates. *C. maculatum* was relatively free from herbivores in North America until the accidental introduction 30 yr ago of its monophagous European associate *Agonopterix alstroemeriana* (Clerck) (Lepidoptera: Oecophoridae). At present, *A. alstroemeriana* is widespread across the United States, and in some areas, such as the Northwest, can inflict substantial damage on its host plant, leading to desiccation and death. *A. alstroemeriana* has been used in recent years for the biological control of *C. maculatum*, although its use has been limited by the availability of larvae, which are field-collected from early to mid-spring, and by the lack of available information about its life history and feeding habits. Here we describe a laboratory-rearing protocol incorporating a simulated winter to induce diapause and a semidefined artificial diet that allows the production of multiple generations per year and enabled us to determine the number and duration of *A. alstroemeriana* developmental stages. The development of the artificial diet also permitted studies of preference and performance of *A. alstroemeriana* in relation to hostplant chemistry. Rearing *A. alstroemeriana* on artificial diet supplemented with 1.5% DW coniine had no adverse impact on ultimate instar growth or performance. In a feeding behavior assay, the presence of coniine in the diet increased *A. alstroemeriana* consumption three-fold relative to control diet. This behavioral response contrasts dramatically with that of *Agonopterix clemensella*, a native Apiaceae specialist that does not use *C. maculatum* as a host; of 30 larvae tested, 29 fed exclusively on diets lacking supplemental coniine. The rearing protocol and artificial diet presented here can facilitate further studies of ecological and evolutionary responses of *C. maculatum* after its reassociation with a coevolved herbivore in North America.

KEY WORDS *Agonopterix alstroemeriana*, *Conium maculatum*, rearing, diet choice, alkaloid

Conium maculatum (Apiaceae) is a European plant that has been extensively naturalized in North America and other parts of the world (Parsons 1976, Holm et al. 1979). Throughout its range, it is highly toxic to humans and other vertebrates because of the presence of coniine and other related piperidine alkaloids (Fairbairn 1971, Panter et al. 1988, Panter and Keeler 1989). Although in its native range *C. maculatum* is restricted in distribution primarily to the wet soils of riverbanks, in areas of introduction, this species has colonized a wide range of habitats, including, in the United States, waste places along roads, ditches, cultivated fields, and occasionally riparian forests and floodplains (Pursh 1979, Goeden and Ricker 1982). Because of its toxicity

and its rank odor, *C. maculatum* is at present considered a noxious weed in several states and is subject to control programs, particularly in populated areas.

In recent years, *C. maculatum* has gained attention as a weed of agricultural importance. Its tendency to invade fields of alfalfa and other forage crops (Montegut and Jauzein 1984) has led to livestock death through contamination of green-chopped hay (Panter et al. 1988). *C. maculatum* has also been implicated as a reservoir of plant diseases in agricultural systems. Raju et al. (1980) documented high levels of *Xylella fastidiosa* in poison hemlock plants growing along streambeds in the vicinity of Napa Valley vineyards in California; as the causative agent of Pierce's disease, *X. fastidiosa* contributes to the death of susceptible European and bunch grape cultivars. *C. maculatum* is also host to plant viruses. In Washington, researchers identified gradients of carrot thin leaf virus infection from weed hosts, including *C. maculatum*, into com-

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mercial carrot fields (Howell and Mink 1977). Celery mosaic virus has been isolated from both celery and poison hemlock and is transmissible between infected plants by aphid feeding (Sutabutra and Campbell 1971, Gracia and Feldman 1977). Given the capacity of poison hemlock to harbor plant diseases and the ability of pathogens to spread to agricultural crops, it has been a priority to develop programs to keep these plants at noninjurious levels. Methods for controlling poison hemlock have traditionally included herbicide spraying and regular mowing (Montegut and Jauzein 1984, Vetter 2004), although resistance to herbicides has already been documented (e.g., Chile; Valdes et al. 1973). Historically, poison hemlock has been colonized by remarkably few insects; Goeden and Ricker (1982) reported finding "amazingly few insect species or individuals" in a comprehensive survey of the plant in southern California. Among the handful of native species that have incorporated the plant into their diet, the majority are specialists on host plants in the family Apiaceae (Berenbaum 1981); these include two swallowtail caterpillars, *Papilio zelicaon* in the western United States (Goeden and Ricker 1982) and *Papilio polyxenes* in the eastern United States (Feeny et al. 1985). Rarely do population levels of either of these species reach damaging levels on *C. maculatum*.

In recent years, a monophagous European associate of *C. maculatum*, the poison hemlock defoliating moth *Agonopterix alstroemeriana* (Clerck) (Lepidoptera: Oecophoridae), has attracted interest as a potential biocontrol agent. Reported in the United States for the first time in Tompkins County, NY, in 1973 (Berenbaum and Passoa 1983), *A. alstroemeriana* has successfully colonized *C. maculatum* extensively throughout the western United States and more recently has appeared in scattered locations in the Midwest (Powell and Passoa 1991, Berenbaum and Harrison 1994, McKenna et al. 2001). *A. alstroemeriana* larvae preferentially consume foliage, but after defoliating a plant, they can also consume developing flower buds and fruits. In mesic areas of Washington, Colorado, and Idaho, populations of *A. alstroemeriana* reach high densities, with hundreds of larvae infesting a single plant; at these densities, entire stands can be completely defoliated, resulting in high plant mortality (Western Society of Weed Science 1996; E.C., unpublished data). The host plant specificity of *A. alstroemeriana* and the impact it can have on its hostplant are two features that make this species attractive as a biological control agent. Moreover, in the western United States, this insect has quickly become established wherever it has been intentionally released (Western Society of Weed Science 1995).

Although *A. alstroemeriana* is commercially available in the United States for control of *C. maculatum* (www.bio-control.com, www.integratedweedcontrol.com), remarkably little is known about its biology in either its native (Stainton 1861) or introduced range (Berenbaum and Passoa 1983). To use *A. alstroemeriana* most efficiently as a biocontrol agent, information about its feeding behavior would be of considerable value. While alkaloids from *C. maculatum* are

demonstrably toxic to vertebrates (Fairbairn 1971, Panter et al. 1988, Panter and Keeler 1989), their behavioral and physiological effects on *A. alstroemeriana* are yet to be determined. Behavioral dependence on a host-specific kairomone to initiate feeding is considered a desirable trait in a biological control agent because such dependency reinforces host plant specificity (Zwolfer and Harris 1971), and accordingly, reduces the risk of nontarget impacts (Louda and Stiling 2004). The presence of chemoreceptors in the Klamath beetle *Chrysolina brunsvicensis* specifically responsive to hypericin, an anthraquinone pigment restricted in distribution to *Hypericum* and its relatives (Rees 1969), was regarded as indicative of dependence on *Hypericum* species as host plants and suggested that host shifts after introduction for biocontrol purpose would be unlikely.

In the absence of any information on the effects of poison hemlock alkaloids on *A. alstroemeriana* feeding behavior and physiology, it is difficult to anticipate the effects of phytochemical variation on the establishment and impact of this herbivore on its host plants; concentrations of the piperidine alkaloids present in *C. maculatum* can vary 100-fold among individuals from different populations across the United States (from 0.03% to 3% dry weight) (data from Castells et al. 2005) and high concentrations may be repellent, as they are for other potential herbivores. Although Nitaotao (1987) found no evidence of growth or feeding inhibition in the generalist noctuid caterpillar *Helicoverpa zea* as a result of consuming diet containing 1% coniine, Bernays and Chapman (1977) reported that diet containing 1% dry weight coniine reduced feeding by the polyphagous orthopteran *Locusta migratoria*. The value of *A. alstroemeriana* for biocontrol may also be limited if, as is the case for another depressariine oecophorid introduced into North America, *Depressaria pastinacella*, it can act as a selective agent on the defensive chemistry of its host plant the wild parsnip *Pastinaca sativa* (Berenbaum et al. 1986). Phytochemical analysis of herbarium specimens both in the United States and Europe indicate that increases in furanocoumarin content of *P. sativa* seeds occurred subsequent to the accidental introduction of this oligophagous fruit-feeding specialist (Zangerl and Berenbaum 2005). Intense herbivory by *A. alstroemeriana* may similarly result in increased alkaloid production in weeds that escape total defoliation, with the result that biological control efforts may increase the toxicity of the surviving weeds.

Limiting the study of *A. alstroemeriana* and its interactions with *C. maculatum* is the fact that this species is univoltine; larvae are present in the field only from the end of May through June in the midwestern and northwestern United States (Berenbaum and Passoa 1983). We developed a method for maintaining this species in continuous culture incorporating a simulated winter to induce diapause, thereby allowing us to obtain information, hitherto lacking, on the number and duration of larval instars. Development of a semidefined artificial diet also permitted us to examine the degree of physiological and behavioral

adaptation of *A. alstroemeriana* to the alkaloids in its host plant and to compare the feeding responses of this species to those of its native congener *Agonopterix clemensella*, sympatric throughout its range, that has not yet colonized *C. maculatum*

Materials and Methods

Rearing Protocol. *A. alstroemeriana* larvae were collected from *C. maculatum* stands in Champaign County, IL, and Tompkins County, NY, during May and June 2003. Larvae were reared in the laboratory to the adult stage at room temperature and a photoperiod of 16:8 h (L:D) on *C. maculatum* plants cultivated from seed in a greenhouse. *C. maculatum* seeds were collected in various locations throughout Champaign County, placed on moist organic soil in an incubator set at 25°C and 16:8 h (L:D) until germination (Baskin and Baskin 1990), and transplanted into 250-ml pots in a greenhouse with peat:perlite:drummer soil (1:1:1). Plants were watered and fertilized as needed. At pupation, individuals were sexed (Berenbaum and Passoa 1983) and placed in 750-ml plastic containers on a moist paper towel (≈ 10 individuals/container). After emergence (by the end of June), moths were fed 10% honey water for 2 wk before being transferred to a cold room at 5°C with a 12:12-h (L:D) photoperiod. By the end of August, the photoperiod was shortened to 8:16 h (L:D). Moths were kept in the cold room for 3 mo until mid-October. Before removal from the cold room, the containers with moths were enclosed in a foam box, which was placed at room temperature for 4 h to allow a gradual warm-up.

At least 15 moths per population, both males and females, were placed in each oviposition chamber, a 25-liter bucket provided with a screen lid, a 10- to 15-cm tall *C. maculatum* plant grown from seed in a greenhouse, a paper towel, and a 30-ml plastic cup filled with 10% honey in hummingbird nectar (Gardensong). Commercial hummingbird nectar was used because it contains a preservative that prevents spoilage of the food source. The oviposition cages were kept in a greenhouse with temperature and photoperiod equivalent to spring conditions in central Illinois (25°C, 16:8 h [L:D]). The hummingbird nectar was replaced twice weekly, and each plant was checked for eggs every 4–5 d; after the eggs appeared, the plant was replaced every 4 d. Leaves with eggs were cut and placed in 500-ml capped plastic deli containers at 30°C and 16:8 h (L:D). The containers were gently shaken twice a day to remove the neonates. Thirty neonates from the Illinois population were placed individually in 30-ml plastic cups filled with a piece of *C. maculatum* leaf and 5–6 ml of agar to ensure sufficient moisture. Larvae were checked daily to record mortality or instar change. Leaf material was replaced as needed. Three days after the larvae pupated, individuals were sexed, weighed, and placed individually in capped glass vials under high moisture conditions at 30°C. The date on which adults eclosed was recorded.

Table 1. Plant-based artificial diet for *A. alstroemeriana* modified from Nitao and Berenbaum (1988)

Component	Amount
<i>Conium maculatum</i> foliage	12 g
Distilled water	100 ml
Agar	0.8 g
Sorbic acid	0.2 g
Methylparaben	0.2 g
Ascorbic acid	0.4 g
Streptomycin	0.015 g
10% formaldehyde	0.25 ml
4 M KOH	2.5 ml

Additionally, we developed a plant-based artificial diet. The diet (Table 1) was based on Nitao and Berenbaum (1988), but the nutrient group (group B) and Vanderzant vitamins were replaced by lyophilized *C. maculatum* foliage. The *C. maculatum* leaves used to prepare artificial diet were collected along a railroad right-of-way west of the city of Champaign (the railroad site described in McKenna et al. 2001) during spring 2004. This *C. maculatum* population was selected for its low alkaloid concentrations ($\approx 0.12\%$ DW) (Castells et al. 2005). Leaves were lyophilized, ground, sieved through a 0.5-mm mesh, and stored at -20°C . To prepare the diet, agar (Sigma, St. Louis, MO) and water were heated until boiling. When the solution cooled down to 60–65°C, sorbic acid, methylparaben, ascorbic acid, and streptomycin (Sigma), along with formaldehyde and KOH, were added, followed by the powdered leaf material at 40–45°C. *C. maculatum* was added at lower temperature because it contains piperidine alkaloids in salt form that may be transformed to the volatile free-base form under heat (E.C., unpublished data). The diet was stirred for 2–3 min and poured in 30-ml diet cups. Thirty 1-d-old second instars reared on plant material were transferred to six diet cups (five individuals per cup) filled with 5–6 ml of artificial diet; neonates placed immediately after egg hatch on artificial diet experienced high levels of mortality and accordingly were raised through first instar on plant material. Because previous assays revealed that the use of sealed containers led to high larval mortality, probably because of release of volatiles from the artificial diet, cups were capped with a lid provided with a fine mesh screen (0.3 mm) to allow air circulation. Cups were placed in a closed 20-liter plastic container kept at high humidity to prevent diet desiccation and ventilation was provided with an aquarium pump. Diet was replaced as needed. Larval mortality, date of instar change, and pupation date were recorded.

To distinguish larval instars, head capsule measurements were obtained after each molt. At each instar, three larvae maintained on plant material were dehydrated through an ethanol series (from 70 to 100%) and mounted on a slide on a mixture of Canada balsam and methyl salicylate (3:1). The slides were observed through a Leica MZ12_s stereomicroscope, and images were collected through a Microfire (Optronics) digital camera. The transverse width of the head capsule was measured at its widest point at $\times 10$ magnification

for first instars, $\times 8$ for second instars, and $\times 5$ for third, fourth, and fifth instars.

Feeding Preference Assay. Second and third larvae instars of *A. alstroemeriana* were collected from *C. maculatum* at the Yard Waste Reclamation Site in Champaign County, IL, during May 2004. In contrast with *A. alstroemeriana*, which feeds exclusively on *C. maculatum*, the native congener *Agonopterix clemensella* is reported to feed on >15 native species of Apiaceae (Berenbaum 1982). Second- and third-instar *A. clemensella* larvae for use in feeding preference assays were collected on *Sanicula marilandica* L. during May 2004 in Coles County, IL. Larvae of both species were brought to the laboratory and raised at 25°C and 16:8 h (L:D) on the same host plant species from which they were collected in the field. Early fifth instars within 12 h of the ultimate larval molt were selected for use in the feeding preference assay.

Feeding preferences were tested between two artificial diets containing foliage of *C. maculatum*; whereas one diet contained low levels of piperidine alkaloids occurring naturally in foliage of plants from one population used by *A. alstroemeriana*, a second diet contained levels elevated by the addition of coniine (98%, Sigma) to a concentration known to occur in other populations used by this species (1.5%). Diets were prepared following the protocol used for laboratory rearing, but before the diet reached the gelling temperature, we added either 2 ml of 20% HCl in methanol for the control diet or 0.21 ml of coniine dissolved in 2 ml of 20% HCl in methanol for the high alkaloid diet (for a final concentration of 1.5%). Acidic methanol was used to convert the coniine free-base form as purchased to the nonvolatile hydrochloride form. The diets were poured separately into 24-well plates and kept frozen at -20°C. To conduct the experiment diet cubes were thawed at room temperature and weighed before placing them in 9-cm-diameter plastic petri dishes. In each dish, we placed a single caterpillar and two diet cubes: one control (low alkaloid) and one enriched with 1.5% coniine (high alkaloid) at opposite sides. We conducted 52 replicates for *A. alstroemeriana* and 30 for *A. clemensella*. Petri dishes were wrapped with Parafilm to maintain constant humidity and kept at room temperature with a photoperiod of 18:6 h (L:D). Additional diet cubes from both diet types were weighed, oven-dried at 65°C, and weighed again to estimate water content. Caterpillars were allowed to feed for 56 (*A. alstroemeriana*) or 48 h (*A. clemensella*) to optimize diet consumption.

At the end of each assay, diet cubes were oven-dried at 65°C and weighed. Consumption of each diet cube was calculated by subtracting the final dry weight from the estimated initial dry weight. We also calculated the antifeedant index $[(C - T)/(C + T)]\%$, where C and T represent the amount eaten of the control (low alkaloid) and treatment (high alkaloid) diet cubes, respectively. Positive values for this index indicate nonpreference or antifeedant activity and negative values for this index indicate preference or kairomonal activity.

A. alstroemeriana Performance Bioassay. We checked the effects of two major alkaloids, coniine and γ -coniceine, on *A. alstroemeriana* growth, consumption, and efficiency of ingested and digested food. Semidefined artificial diet, as described above, was enriched with either 1.5% coniine (Sigma) or 1.5% γ -coniceine. γ -coniceine was purified from *C. maculatum* seeds collected at the Yard Waste Reclamation Site in Champaign County, IL. Seeds were extracted with 70% methanol 30% 0.1 N HCl ($\times 3$) and filtered through diatomaceous earth (Celite; Fisher Scientific, Fair Lawn, NJ). The resulting solution was filtered through a Buchner funnel filled with 1 cm of C18 packing for flash chromatography (40- μ m particle diameter; Baker) to remove the nonpolar compounds, concentrated by rotary evaporator at low temperature (max 40°C), and partitioned with CHCl_3 in a separation funnel to further remove nonpolar compounds. The alkaloids were converted from the hydrochloride form to the free-base form by adding 10 M NaOH and then extracted with CHCl_3 ($\times 5$). The bulked chloroform fractions were mixed with 20% HCl in MeOH and concentrated by rotary evaporator 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 by 2.5-cm silica gel (Merck; 32–63 μ m) gravity column eluted with 150 ml of chloroform:ethanol: NH_3OH (70:30:1) (Leete and Olson 1972) at ≈ 1 ml/min. The fractions (2 ml each) were monitored by spotting 5 μ l on a TLC silica gel plate (Baker; 250 μ m) and sprayed with Dragendorff (Jungreis 1985) or 0.2% ninhydrin reagents (Sigma). Fractions containing γ -coniceine were bulked together, transformed to the hydrochloride form by adding 20% HCl in MeOH, dried down under a flow of N_2 , and stored into a desiccator. Purity of γ -coniceine was >95% as detected by gas chromatography. Because γ -coniceine in the hydrochloride form has deliquescent and hygroscopic properties (Cromwell 1956, Fairbairn and Challen 1959), the compound could not be completely dried and retained unknown amounts of water.

A. alstroemeriana larvae collected on *C. maculatum* during spring 2004 at Champaign County, IL, were reared in the laboratory on foliage until they reached the fifth instar. Within 12 h of the ultimate instar molt, 120 fifth instars were weighed and placed individually in 30-ml diet cups. A preweighed ≈ 0.75 -ml diet cube of control (low alkaloids), coniine-enriched, or γ -coniceine-enriched diet was placed in each cup; there were 40 individuals per treatment. Caterpillars were kept in the diet cups until pupation. Pupal weight was recorded 3 d after pupation, together with remaining diet and frass produced. Pupal sex was determined as described in Berenbaum and Passoa (1983).

We measured several standard gravimetric performance indices. These included time to pupation (days), relative growth rate [RGR = (initial weight -

Table 2. Head capsule size for *A. alstroemeriana* at each larval instar raised on plant material (mean \pm SE, $n = 3$)

Larval stage	Head capsule size (μm)
First instar	190 \pm 5.8
Second instar	306.7 \pm 17.6
Third instar	519 \pm 15.3
Fourth instar	716.6 \pm 18.6
Fifth instar	1040 \pm 28.8

final weight) / time to pupation], relative consumption rate [RCR = (initial diet - final diet) / time to pupation], efficiency of conversion of ingested food (ECI = increased weigh/consumption \times 100), efficiency of conversion of digested food [ECD = increased weigh / (consumption - frass) \times 100], and approximate digestibility [AD = (consumption - frass) / consumption \times 100].

Statistical Analyses. Differences in duration of developmental stages for caterpillars reared on plant material and artificial diet, in male and female pupal weights for caterpillars raised on plant material, and amounts of each diet type consumed in the preference bioassay were tested for significance with a *t*-test for dependent samples. A two-way analysis of covariance (ANCOVA) with diet treatment and sex as independent variables and larvae initial fresh weigh as a covariate was used to test differences in *A. alstroemeriana* performance on low versus high alkaloid diets. Statistical analyses were conducted using Statistica 6.0 (StafSoft, Tulsa, OK).

Results

After placing *A. alstroemeriana* adults in winter-simulating conditions for 3 mo, individuals from both Illinois and New York populations laid several hundred eggs. Oviposition began 7 d after removing the moths from the cold room and lasted \sim 1 mo, from 25 October to 22 November. The eggs, which were white, oval in shape, and \approx 0.75 mm in length immediately after oviposition were scattered on the abaxial side of the leaves. After 2-3 d, the eggs became yellowish orange. Approximately 75% of the eggs were viable and hatched within 4-5 d. We identified five

A. alstroemeriana larval stadia by comparing head capsule size (Table 2). After fifth instar, the larvae entered a prepupal stage, in which pink stripes appear on the dorsal side. Twenty-one of the 29 larvae (68.9%) raised on plant material and 7 of the 29 larvae (24.1%) raised on artificial diet survived through pupation (Table 3). The total duration of the larval stage was shorter in the larvae fed plant material (14.7 ± 0.1 d) compared with the larvae fed on artificial diet (23.7 ± 1.1 d; *t*-test, $P < 0.001$; Table 3). In plant-reared larvae, although female pupae seemed to weigh slightly more (15.7 ± 0.53 [SE] mg; $n = 7$) than male pupae (14.2 ± 0.68 ; $n = 13$), this trend was not statistically significant (*t*-test, $P = 0.14$). The total duration of the pupal stage was 11.06 ± 0.13 d ($n = 17$), and 85% of the pupae emerged as adults.

Agonopterix alstroemeriana larvae consumed on average three times more high alkaloid diet than low alkaloid control diet (*t*-test, $P < 0.001$) when given a choice (Fig. 1). The fact that the averaged antifeedant index value was negative (-48.1 ± 8.9) is consistent with a kairomonal (food recognition) function. In contrast, *A. clemensella* displayed an overwhelming preference for the low alkaloid diet (*t*-test, $P < 0.001$; Fig. 1), with an averaged antifeedant index of 97.1 ± 6.7 ; 29 of 30 caterpillars consumed low-alkaloid diet exclusively.

Coniine added to the artificial diet had no effect on *A. alstroemeriana* growth, time to pupation, or relative consumption rate over the duration of the ultimate instar (Table 4; Fig. 2). However, larvae consuming diets high in coniine had significantly lower AD values compared with the controls, leading to higher values of ECI and ECD. Additions of γ -coniceine to the diet had no discernible effect on any performance parameter measured (Fig. 2).

Discussion

Like most depressariine oecophorids (L'vovsky 1975), *A. alstroemeriana* is univoltine. As is the case for *D. pastinacella*, with which it is sympatric throughout much of its range in eastern North America, this species has an obligatory reproductive diapause induced by cold temperatures (Nitao and Berenbaum 1988).

Table 3. Duration of each stage and cumulative survival under laboratory conditions with larvae raised on *C. maculatum* foliage or plant-based artificial diet

Developmental stage	Plant-raised			Artificial diet-raised		
	No. observed (n)	Duration in days (mean \pm SE)	Cumulative survival (%)	No. observed (n)	Duration in days (mean \pm SE)	Cumulative survival (%)
Instar 1	29	3 \pm 0	75.8	—	(3 \pm 0)	—
Instar 2	22	1.9 \pm 0.04	75.8	29	1.85 \pm 0.3	96.5
Instar 3	22	2.1 \pm 0.1	75.8	28	4.4 \pm 0.5	75.8
Instar 4	22	2.0 \pm 0.04	72.4	22	7.3 \pm 1.6	34.4
Instar 5	21	2.7 \pm 1.0	72.4	10	6.0 \pm 1.0	24.1
Prepupae	21	1.9 \pm 0.1	72.5	10	1.1 \pm 0.1	24.1
Pupa	21	—	68.9	7	—	24.1
Total	—	14.7 \pm 0.1	—	—	23.7 \pm 1.1	—

Duration of first instars in artificial diet treatment is shown in parentheses because at this stage, larvae were raised on *C. maculatum* foliage.

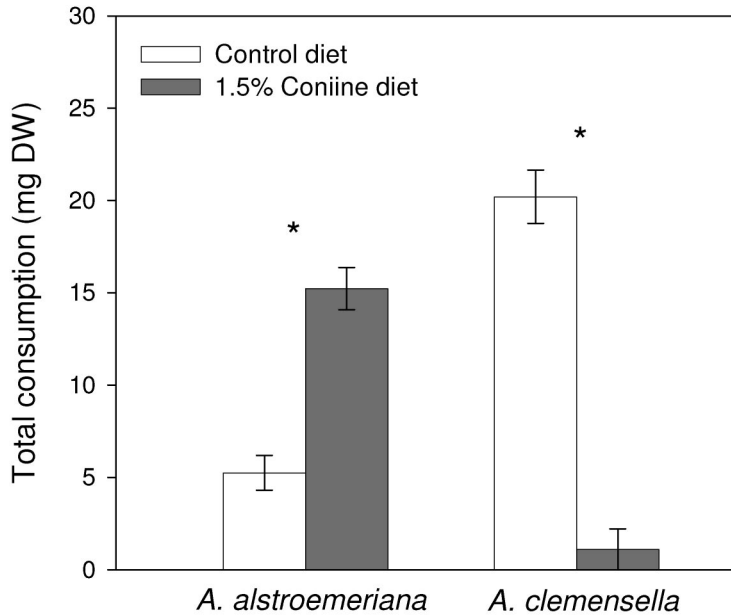


Fig. 1. Consumption of *A. alstroemeriana* and *A. clemensella* fifth-instar larvae of plant-based control diet with low alkaloid concentrations (0.12% DW) and coniine enriched diet (1.5% DW) in a diet choice bioassay. Mean \pm SE, $n = 52$ for *A. alstroemeriana* and $n = 30$ for *A. clemensella*. A t -test for dependent variables was conducted for each species, $*P < 0.001$.

We were able to rear *A. alstroemeriana* in the laboratory continuously by placing the adults, on emergence, in winter-simulating conditions for a 3-mo period. Using the protocol described here, several generations of *A. alstroemeriana* can be reliably produced annually, which should increase their use as biological control agents considerably. While *C. maculatum* can be found as rosettes during fall and as flowering plants during spring and early summer, *A. alstroemeriana* larvae are normally available in the

field only during mid- to late spring; laboratory rearing can increase the availability of caterpillars during times when field populations are low or absent. The development of a semidefined artificial diet also allows rearing of *A. alstroemeriana* through its entire life cycle even when *C. maculatum* is unavailable during late summer and winter. Lyophilized plant material can be kept frozen for several months to be used when necessary, and the small amounts of plant material needed to feed the neonates can be obtained easily from seeds in a greenhouse. Although overall survival on the semisynthetic diet is low in comparison with survival on leaf material in this study, generational survival is nonetheless comparable with that for other candidate lepidopteran biological control agents even when raised on intact plants (e.g., *Episimus utilis*; Martin et al. 2004).

An additional advantage of the use of artificial diet is that it allows for more controlled bioassay of host plant chemical constituents to isolate behavioral and physiological responses to variation in host plant chemistry. Here we examined the effects of coniine, one of the major *C. maculatum* alkaloids (Castells et al. 2005), on feeding behavior of *A. alstroemeriana*. For many oligophagous lepidopterans, unique host plant constituents can serve a kairomonal function (Feeny 1992). Elevated levels of coniine brought about a three-fold increase in consumption of artificial diet in *A. alstroemeriana*, suggestive of kairomonal activity-piperidine alkaloids, may be important in facilitating host plant recognition in that *C. maculatum* is likely the only member of the family Apiaceae to produce these compounds (Fairbairn 1971). That elevated co-

Table 4. Statistical results of the performance bioassay with *A. alstroemeriana* consuming low alkaloid (control) diet, 1.5% coniine diet, or 1.5% γ -coniceine diet

		df	F	P
Time to pupation	Treatment	2	0.31	0.73
	Sex	1	4.50	0.03
	Treatment \times sex	2	0.82	0.43
RGR	Treatment	2	1.09	0.33
	Sex	1	0.54	0.46
	Treatment \times sex	2	0.79	0.45
RCR	Treatment	2	0.05	0.94
	Sex	1	1.79	0.18
	Treatment \times sex	2	0.29	0.74
ECI	Treatment	2	3.93	0.02
	Sex	1	1.07	0.30
	Treatment \times sex	2	0.55	0.57
ECD	Treatment	2	8.50	<0.001
	Sex	1	3.58	0.06
	Treatment \times sex	2	0.63	0.53
AD	Treatment	2	6.69	0.001
	Sex	1	1.87	0.17
	Treatment \times sex	2	0.63	0.53

Significant P values are indicated in bold.
 $N = 40$.

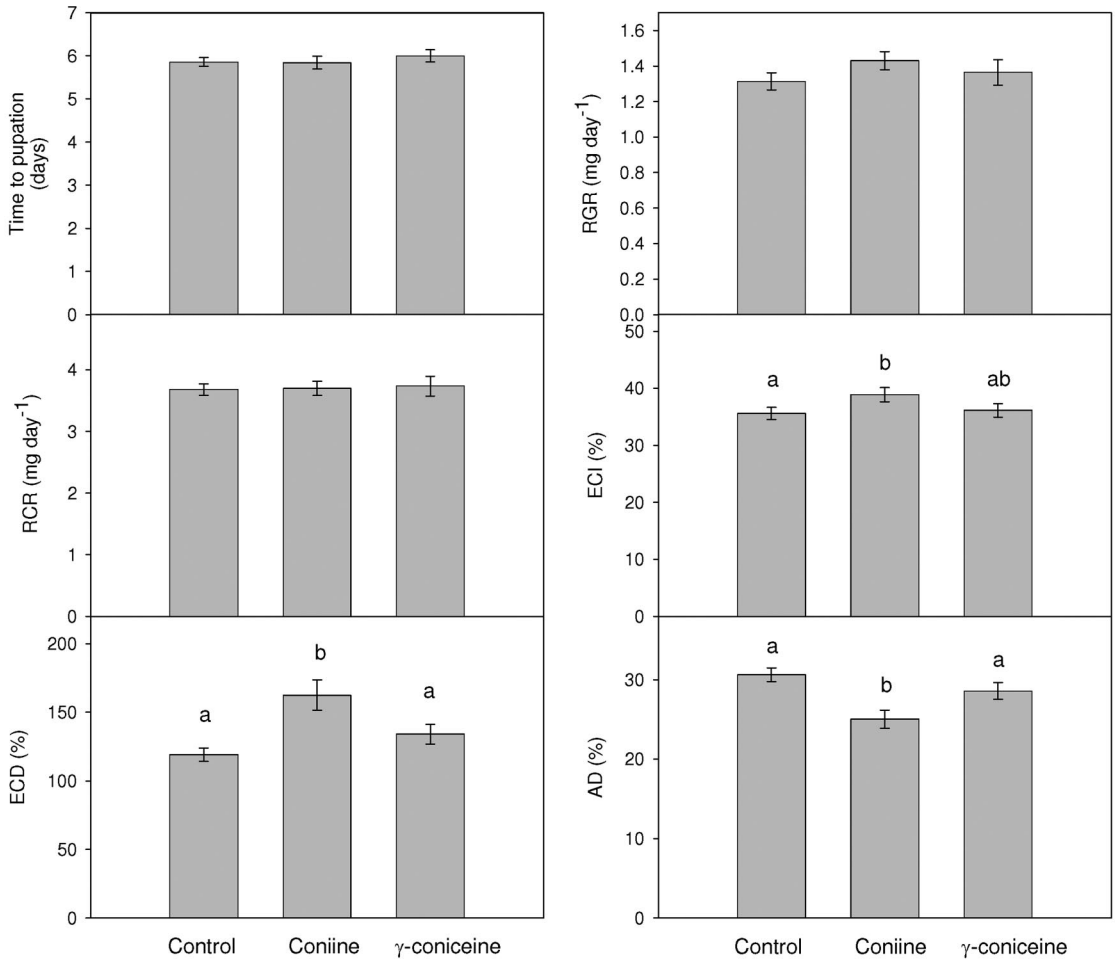


Fig. 2. Effects of coniine and γ -coniceine enriched diet on *A. alstroemeriana* time to pupation, RGR, RCR, ECI, ECD, and AD. Different letters show significant differences among treatments using Fisher least significant difference post hoc comparisons.

niine did not increase the relative consumption rate over the duration of the instar may indicate that its primary action may be to initiate and sustain feeding rather than to accelerate the rate of feeding. Despite the demonstrable toxicity of piperidine alkaloids to vertebrates (Wink et al. 1998, Lopez et al. 1999), no adverse physiological impacts on any performance parameter measured were discernible in *A. alstroemeriana*. This species may in fact benefit from the presence of alkaloids in the diet, both through facilitation of host plant recognition and possible enhancement of defense against vertebrate predators if substantial quantities of unmetabolized alkaloids can be sequestered; preliminary studies indicate that hemolymph of caterpillars raised on coniine-augmented diet does in fact contain coniine (unpublished data).

In contrast, caterpillars of the congener *A. clemensella*, which despite feeding on a wide array of Apiaceae have never been reported on *C. maculatum* (Berenbaum 1982; MB, unpublished data), consistently avoided high levels of coniine; for this closely

related species, coniine seems to have allomonal rather than kairomonal properties. This compound, and possibly co-occurring related piperidine alkaloids (Castells et al. 2005), may account for the failure of *A. clemensella*, which has incorporated other European apiaceous invasive species into its diet (including *Pastinaca sativa* and *Daucus carota*; Berenbaum 1982), to colonize poison hemlock over more than a century of exposure to the plant.

The use of a biological control agent depends on both operational factors, such as ease of handling and availability, and ecological factors—most notably specificity but also the reciprocal selective impact of the association between the herbivore and its host plant. Although in this study, levels of 1.5% alkaloid had no obvious negative effects on *A. alstroemeriana*, the extent of tolerance of this species to these alkaloids remains undetermined; greatly elevated levels of alkaloids may prove toxic, as is the case with other oligophagous species tolerant of host-specific allomones (Blau et al. 1978, Appel and Martin 1992,

Kester et al. 2002). By the same token, the extent to which alkaloid production in North American populations of poison hemlock is genetically variable and therefore available for selection by herbivory is at present unknown. The possibility exists, however, that increased herbivory by *A. alstroemeriana* as a result of expanded biological control efforts might paradoxically increase the alkaloid-based toxicity of surviving plants and thereby increase the noxiousness (and possibly invasiveness) of this weed. A better understanding of the ecology of this noxious weed in North America and insights into more effective management rest on further elucidation of its chemistry and of the behavior and physiology of *A. alstroemeriana*, the species that at present represents the only significant herbivore of this plant throughout much of its North American range.

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References Cited

- Appel, H. M., and M. M. Martin. 1992. Significance of metabolic load in the evolution of host specificity of *Manduca sexta*. *Ecology* 73: 216–228.
- Baskin, J. M., and C. C. Baskin. 1990. Seed germination ecology of Poison hemlock, *Conium maculatum*. *Can. J. Bot.* 68: 2018–2024.
- Berenbaum, M. R. 1981. Furanocoumarin distribution and insect herbivory in the Umbelliferae: plant chemistry and community structure. *Ecology* 62: 1254–1266.
- Berenbaum, M. R. 1982. New hostplant records for *Agonopterix clemensella* (Lepidoptera: Oecophoridae). *J. Lep. Soc.* 36: 160.
- Berenbaum, M. R., and S. Passoa. 1983. Notes on the biology of *Agonopterix alstroemeriana* (Clerck), with description of the immature stages (Oecophoridae). *J. Lepid. Soc.* 37: 38–45.
- Berenbaum, M. R., and T. L. Harrison. 1994. *Agonopterix alstroemeriana* (Oecophoridae) and other lepidopteran associates of poison hemlock (*Conium maculatum*) in east central Illinois. *Great Lakes Entomol.* 27: 1–5.
- Berenbaum, M. R., A. R. Zangerl, and J. K. Nitao. 1986. Constraints on chemical coevolution: wild parsnips and the parsnip webworm. *Evolution* 40: 1215–1228.
- Bernays, E. A., and R. F. Chapman. 1977. Deterrent chemicals as a basis for oligophagy in *Locusta migratoria* (L.). *Ecol. Entomol.* 2: 1–18.
- Blau, P. A., P. Feeny, L. Contardo, and D. S. Robson. 1978. Allylglucosinolate and herbivorous caterpillars: a contrast in toxicity and tolerance. *Science* 200: 1296–1298.
- Castells, E., M. A. Berhow, S. F. Vaughn, and M. R. Berenbaum. 2005. Geographical variation in alkaloid production in *Conium maculatum* populations experiencing differential herbivory by *Agonopterix alstroemeriana*. *J. Chem. Ecol.* 31: 1693–1709.
- Cromwell, B. T. 1956. The separation, micro-estimation and distribution of the alkaloids of hemlock (*Conium maculatum* L.). *Biochem. J.* 64: 259–266.
- Fairbairn, J. W. 1971. The alkaloids of hemlock (*Conium maculatum* L.) (or *Conium maculatum* L.: the odd man out), pp. 361–368. In V. H. Heywood (ed.), *The biology and chemistry of the Umbelliferae*. Academic, New York.
- Fairbairn, J. W., and S. B. Challen. 1959. The alkaloids of hemlock (*Conium maculatum* L.). *Biochem. J.* 72: 556–561.
- Feeny, P., W. S. Blau, and P. M. Kareiva. 1985. Larval growth and survivorship of the black swallowtail butterfly in central New York. *Ecol. Monogr.* 55: 167–187.
- Feeny, P. 1992. The evolution of chemical ecology: contributions from the study of herbivorous insects, pp. 1–44. In G. A. Rosenthal and M. R. Berenbaum (eds.), *Herbivores: their interactions with secondary plant metabolites: ecological and evolutionary processes*, vol. 2. Academic, San Diego, CA.
- Goeden, R. D., and D. W. Ricker. 1982. Poison hemlock, *Conium maculatum*, in southern California—an alien weed attacked by few insects. *Ann. Entomol. Soc. Am.* 75: 173–176.
- Gracia, O., and J. M. Feldman. 1977. Isolation and identification of two celery viruses in Argentina. *Plant Dis. Rep.* 61: 905–908.
- Holm, L., J. V. Pancho, J. P. Herberger, and D. L. Plucknett. 1979. A geographical atlas of world weeds. Wiley, New York.
- Howell, W. E., and G. I. Mink. 1977. The role of weed hosts, volunteer carrots, and overlapping growing seasons in the epidemiology of carrot thin leaf and carrot motley dwars viruses in central Washington. *Plant Dis. Rep.* 61: 217–222.
- Jungreis, E. 1985. Spot test analysis. Wiley-Interscience, New York.
- Kester, K. M., S. C. Peterson, F. Hanson, D. M. Jackson, and R. F. Severson. 2002. The roles of nicotine and natural enemies in determining larval feeding site distributions of *Manduca sexta* L. and *Manduca quinquemaculata* (Haworth) on tobacco. *Chemoecology* 12: 1–10.
- Leete, E., and J. O. Olson. 1972. Biosynthesis and metabolism of the hemlock alkaloids. *J. Am. Chem. Soc.* 94: 5472–5477.
- Lopez, T. A., M. S. Cid, and M. L. Bianchini. 1999. Biochemistry of hemlock (*Conium maculatum* L.) alkaloids and their acute and chronic toxicity in livestock. *A review. Toxicol.* 37: 841–865.
- Louda, S. M., and P. Stiling. 2004. The double-edged sword of biological control in conservation and restoration. *Conserv. Biol.* 18: 50–53.
- L'vovsky, A. L. 1975. Food specialization and seasonal cycles of the broad-winged moths (Lepidoptera: Oecophoridae) of the European part of the U.S.S.R. *Entomol. Rev.* 54: 91–97.
- Martin, C. G., J. P. Cuda, K. D. Awadzi, J. C. Medel, D. H. Habeck, and J. H. Pedrosa-Macedo. 2004. Biology and laboratory rearing of *Episimus utilis* (Lepidoptera: Tortricidae), a candidate for classical biological control of Brazilian peppertree (Anacardiaceae) in Florida. *Environ. Entomol.* 33: 1351–1361.
- McKenna, D. D., A. R. Zangerl, and M. R. Berenbaum. 2001. A native hymenopteran predator of *Agonopterix alstroemeriana* (Lepidoptera: Oecophoridae) in East-Central Illinois. *Great Lakes Entomol.* 34: 71–75.
- Montegut, J., and P. Jauzein. 1984. Problemes d'ombelliferes (Apiaceae) en France. Problemes biologiques et agronomiques. *Med. Facult. Landbouwwetenschappen Rijksuniversiteit Gent.* 49: 1051–1060.
- Nitao, J. K. 1987. A test for toxicity of coniine to a polyphagous herbivore, *Heliothis zea* (Lepidoptera: Noctuidae). *Environ. Entomol.* 16: 656–659.
- Nitao, J. K., and M. R. Berenbaum. 1988. Laboratory rearing of the parsnip webworm, *Depressaria pastinacella* (Lep-

- idoptera: Oecophoridae). *Ann. Entomol. Soc. Am.* 81: 485–487.
- Panter, K. E., R. F. Keeler, and D. C. Baker. 1988. Toxicoses in livestock from the hemlocks (*Conium* and *Cicuta* spp.). *J. Anim. Sci.* 66: 2407–2413.
- Panter, K.E., and R. F. Keeler. 1989. Piperidine alkaloids from poison hemlock (*Conium maculatum*), pp. 109–132. In P.R. Cheeke (ed.), *Toxicants of plant origin*. CRC, Boca Raton, FL.
- Parsons, W. T. 1976. *Noxious weeds of Victoria*. Inkata, Melbourne, Australia.
- Powell, J. A., and S. Passoa. 1991. Rapid colonization of the western United States by the Palearctic moth, *Agonopterix alstroemeriana* (Oecophoridae). *J. Lepid. Soc.* 45: 234–236.
- Pursh, F. 1799. *Flora Americae Septemtrionalis. Historiae Naturalis Classica V.* 104. J. Cramer (ed.). Vaduz, Lichtenstein.
- Raju, B. C., S. F. Nome', D. M. Docampo, A. C. Goheen, G. Nyland, and S. K. Lowe. 1980. Alternative hosts of Pierce's disease of grapevines that occur adjacent to grape growing areas in California. *Am. J. Enol. Vitic.* 31: 144–148.
- Rees, C.J.C. 1969. Chemoreceptor specificity associated with choice of feeding site by the beetle *Chrysolina brunsvicensis* on its foodplant *Hypericum hirsutum*. *Entomol. Exp. Appl.* 12: 565–583.
- Stainton, H. T. 1861. *Depressaria* pt 1. In H. T. Stainton, P. C. Zeller, and J. W. Douglas (eds.), *Natural history of the Tineina*. pp. 256–267. J. van Voorst, London, UK.
- Sutabutra, T., and R. N. Campbell. 1971. Strains of celery mosaic virus from parsley and poison hemlock in California. *Plant Dis. Rep.* 55: 328–332.
- Valdes, R. L., F. G. Cosio, and D. T. Contreras. 1973. Chemical control of weeds and volunteer crop seedlings in established red clover crops. *Malezas Control* 2: 31–40.
- Vetter, J. 2004. Poison hemlock (*Conium maculatum* L.). *Food Chem. Toxicol.* 42: 1373–1382.
- Western Society of Weed Science. 1996. *Western Society of Weed Science. Biological control of weeds in the West*. Bozeman, Montana.
- Wink, M., T. Schmeller, and B. Latz-Bruning. 1998. Modes of action of allelochemical alkaloids: interaction with neuroreceptors, DNA, and other molecular targets. *J. Chem. Ecol.* 24: 1881–1937.
- Zwolfer, H., and P. Harris. 1971. Host specificity determination of insects for biological control of weeds. *Annu. Rev. Entomol.* 16: 157–178.

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