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Resistance of the generalist moth *Trichoplusia ni* (Noctuidae) to a novel chemical defense in the invasive plant *Conium maculatum*

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Running head: Resistance of generalist moth to an invasive plant

Summary

Conium maculatum is an apiaceous species native to Eurasia that is highly toxic to vertebrates due to the presence of piperidine alkaloids, including coniine and γ-coniceine. More than 200 years after invading the United States this species remains mostly free from generalist insect herbivores. The presence of novel chemical defenses in the introduced range could provide invasive species with a competitive advantage relative to native plants. The cabbage looper (*Trichoplusia ni*) is a generalist lepidopteran found throughout the US that occasionally feeds on *C. maculatum*. We evaluated the toxicity of piperidine alkaloids to *T. ni* and determined putative resistance mechanisms, both behavioral and physiological, that allows this insect to develop successfully on *C. maculatum* foliage. *T. ni* larvae raised on diets enriched with coniine and γ-coniceine showed a decrease in consumption and longer development time, but no effects on growth were found at any alkaloid concentration. In a diet choice experiment *T. ni* larvae showed no avoidance of alkaloid-enriched diets, suggesting that the deterrence produced by alkaloids was related to a post-ingestive metabolic response. The ability of *T. ni* to consume diets high in alkaloid content could be due to at least three different mechanisms: 1) a decreased consumption rate, 2) efficient excretion of at least 1/3 of ingested alkaloids unmetabolized in frass, and 3) partial detoxification of alkaloids by cytochrome P450s, as shown by the decreased larval growth in the presence of piperonyl butoxide, a P450 inhibitor. Even though *T. ni* tolerates *C. maculatum* alkaloids, the use of this species as a host plant could be ecologically disadvantageous due to prolonged larval growth and thus increased exposure to predators. Novel plant secondary compounds do not guarantee increased resistance to generalist herbivores.

Introduction

The invasion of exotic species is an important factor affecting biodiversity at a global scale, causing extinction of native species and changes in community structure (Pimentel et al 2000). A relevant aspect of the impact of exotic plants is the change in the interactions between plants and herbivores in the novel environment (Hierro et al 2005, Colautti et al 2004). Even though invasive plants, once in the introduced range, may experience a release of herbivory from their coevolved enemies, they can be colonized by local generalist herbivores (Keane and Crawley 2002). Because in these newly established interactions plants and herbivores have not coevolved, the presence of plant chemical defenses not previously encountered by native herbivores constitute a "novel weapon" that provides the plant with a greater competitive advantage in the introduced range thus facilitating the invasion (Callaway and Ridenour 2004). The ability of an herbivore to switch from a native to an invasive host plant depends on the geographic and temporal range of the plant, as well as the physical and chemical similarities between the invader and its native host plants (Janzen 1968). Thus, chemically unique plants introduced into a novel environment are less likely to be colonized by local herbivores if their chemical distinctiveness confers upon them resistance to natural enemies in the nonindigenous area.

One example of an invasive species with distinctive chemistry that experiences little herbivory after invading a new habitat is *Conium maculatum* (poison hemlock), a species of Apiaceae originally from Eurasia that has invaded many parts of the world, including North and South America, New Zealand and Australia (Parsons 1976, Holm et al 1979). The first records of *C. maculatum* in North America date from the early 1800s

in the eastern US (Nuttall 1818, Pursh 1979). At present, *C. maculatum* is widespread across the US, forming in many locations dense patches in disturbed areas such as roads sides, ditches, or abandoned fields. They also occasionally invade riparian forests and flood plains (Goeden and Ricker 1982). Even though *C. maculatum* has a record of 200 years of potential interaction with the local fauna, only a small number of herbivores have been reported to be consistent consumers (Goeden and Ricker 1982, Berenbaum 1981). Goeden and Ricker (1982) found "amazingly few insect species or individuals thereof" feeding on *C. maculatum* in an extensive survey of *C. maculatum* in California. For a total of 20 phytophagous found in *C. maculatum* populations, only 1 species was common (the aphid *Hyadaphis foeniculi*), 4 were found occasionally (including the generalist caterpillar *Trichoplusia ni* and the Apiaceae specialist *Papilio zelicaon*) and 16 (70%) were very rare. The presence of generalist insects on *C. maculatum* is extremely low compared to other Apiaceae species in the same habitats (Berenbaum 1981). At present, only one insect species is consistently found on *C. maculatum*, the European monophagous specialist *Agonopterix alstroemeriana* (oecophoridae) first reported in the eastern US in 1973 (Berenbaum 1981). Since its appearance *A. alstroemeriana* has extended across the US range causing in some occasions complete defoliation of *C. maculatum* stands (Western Society of Weed Science 1995).

The low variety of consumers on *C. maculatum* has been attributed to the presence of piperidine alkaloids, such as coniine, γ-coniceine or conhydrinone (Fairbairn 1971). *C. maculatum* is one of few apiaceous species that produce alkaloids (Fairbairn 1971). Indeed, piperidine alkaloids are reported only in two families of plants; γ-coniceine and its relatives are known from *C. maculatum* and 6 *Aloe* species (Dring et al

1984). These piperidine alkaloids thus constitute a “novel weapon” in virtually any plant community. Piperidine alkaloids of *C. maculatum* are poisonous to livestock and to humans (Sperry et al. 1964, Panter et al. 1988, Panter and Keeler 1989). The major alkaloids, coniine and γ-coniceine, have long been known to be neurotoxic, interacting with the acetyl choline receptors of the nervous system (Wink et al. 1998) and are implicated in livestock poisonings (Bowman and Sanghvi 1963, Sperry et al. 1964, Panter and Keeler 1989). The toxicity of these compounds to vertebrates suggests a role in chemical defense against herbivores but this hypothesis has been rarely tested. Among the few examples of the toxicity of *C. maculatum* alkaloids are reports of mortality of *Tubifex* worms at 0.002% coniine (Wink et al. 1998), significant antifeedant activity for γ-coniceine in the slug *Deroceras reticulatum* (Birkett et al 2004), and higher mortality and lower pupal weigh of *Papilio zelicaon* raised on *C. maculatum* compared to the alternative host plant *Foeniculum vulgare* (Sims 1980). There is also indirect evidence of the toxicity of *C. maculatum* alkaloids to its monophagous specialist *Agonopterix alstroemeriana*; a negative correlation between the number of leaf rolls and alkaloid concentrations in *C. maculatum* individuals has been found (Castells et al. 2005). In other studies *C. maculatum* alkaloids were innocuous; no effects of coniine on food preference or growth were shown when *Helicoverpa zea* larvae were raised on an alkaloid-enriched diet (Nitao 1987).

T. ni (the cabbage looper) is one of the few generalist herbivores that occasionally consumes *C. maculatum* (Goeden and Ricker 1982, Berenbaum and Harrison 1994), and laboratory trials have shown the ability of *T. ni* to complete its development when raised on *C. maculatum* foliage (EC unpublished data). This tolerance to piperidine alkaloids is

unexpected in a generalist herbivore that has not been historically exposed to these compounds. We aimed to ascertain whether piperidine alkaloids of *C. maculatum* constitute a novel weapon in an invaded habitat. We determined 1) the effects of increasing concentrations of coniine and γ-coniceine on *T. ni* performance during larval stage, and 2) the ability of *T. ni* larvae to recognize and respond to these alkaloids when presented with a diet choice, and 3) the potential mechanisms involved in the ability of *T. ni* to tolerate piperidine alkaloids. In case a decreased performance is shown for insects raised on alkaloid-containing diet, piperidine alkaloids would constitute an efficient defense mechanism for *C. maculatum* during invasion.

Materials and Methods

Chemicals

Coniine hydrochloride was purchased from City Chemical (West Haven, CT, USA). γ-Coniceine was purified from *Conium maculatum* seeds collected in Champaign County, IL, USA. Seeds were ground in a blender and extracted three times with 70% methanol 30% 0.1 N HCl and then filtered through diatomaceous earth (Celite®; Fisher). The extractions were bulked together and filtered through C18 (40 μm particle diameter; Baker) to remove the non-polar compounds. The resulting solution was concentrated by rotary evaporation at low temperature (max 40 ˚C) and partitioned with $CHCl_3$ in a separation funnel to further remove non-polar compounds. The alkaloids were extracted with $CHCl_3$ (x 5) after basifying with 10 M NaOH. The bulked chloroform fractions were

mixed with 20% HCl in MeOH and concentrated in a rotary evaporator to obtain a mixture of alkaloids in hydrochloride form. 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.

In order to separate individual compounds the solution containing bulk alkaloids was poured through a gravity column (25 x 2.5 cm silica gel 32-63 μm, Merck) eluted with 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 reagent (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 in a desiccator. Because γ-coniceine in hydrochloride form has deliquescent and hygroscopic properties (Cromwell 1956, Fairbairn and Challen 1959), the compound could not be completely dried. Although a purity of γ-coniceine higher than 95% was detected by gas chromatography the compound retained unknown amounts of water.

Piperonyl butoxide (PBO) and diethyl maleate (DM) were purchased from TCI America and Sigma, respectively.

Insects and diet

T. ni were obtained from a colony maintained in our laboratory at UIUC. Larvae were reared on a standard artificial diet as described in Nitao and Berenbaum (1988). Briefly,

13 g agar (Sigma) dissolved in 770 mL distilled water was heated until boiling and mixed in a blender with 31.5 g of vitamin-free casein (Sigma), 24 g of sucrose, 27 g of wheatgerm (Kretschmer), 9 g of Wesson's salt mix, 10 g of alphacel and 5 mL of 4 M KOH. When agar cooled down to 60 ˚C the following ingredients were added: 18 g Vanderzant vitamins (Bioserv), 1.6 g of sorbic acid (Sigma), 1.6 g of methylparaben (Sigma), 3.2 g of ascorbic acid (Sigma), 0.12 g of streptomycin sulfate (Sigma), 4 mL of wheat germ oil (Viobin) and 2 mL of 10% formaldehyde. Diet was poured into 30 mL individual cups and allowed to set. To prepare alkaloid-enriched diet, either coniine or γ-coniceine in the hydrochloride form was dissolved in methanol (with a maximum of 0.75% methanol per FW of diet) and added as the last ingredient just before the diet reached the gelling temperature. The control diet was prepared as described for the standard diet but the same amount of methanol used to dissolve the alkaloids was added to the diet.

Experiment 1: Effects of 1% DW coniine and 1% DW γ-coniceine on T. ni *performance on larval development*

Newly hatched *T. ni* neonates (n=150) were transferred individually to 30 mL cups provided with a snap lid and filled with *ca* 4 mL of control diet, 1% DW coniine enriched diet, or 1% DW γ-coniceine enriched diet (50 neonates per treatment). For each cup, diet was weighed to determine the initial fresh weight. Diet from five additional cups of control diet were weighed, oven-dried at 65 ˚C for 48 h and weighed again to estimate water content at the beginning of the experiment. Larvae were kept at room temperature

(*ca* 25 C) until pupation. Pupation date was recorded. The remaining diet and the frass produced were also oven-dried to determine dry weight. Diet consumption was calculated by subtracting the final diet DW to the estimated initial diet DW. Three days after pupation pupae were sexed and oven-dried. Gravimetric performance indices (Scriber and Slansky, 1981) were calculated following the Raubenheimer and Simpson (1994) analysis of covariance:

Relative growth rate; RGR = final pupal weigh / time to pupation

Relative consumption rate; RCR = consumption / time to pupation

Efficiency of conversion of ingested food; ECI = pupal weigh / consumption x 100

Efficiency of conversion of digested food; ECD = pupal weigh / (consumption – frass) x 100

Approximate digestibility; AD = (consumption – frass) / consumption x 100

Experiment 2: Short-term effects of increasing coniine concentrations on T. ni *performance*

We determined the effects of coniine-enriched diets at concentrations ranging from 0.05 to 5% DW on *T. ni* performance. These concentrations were chosen because they comprise the range of alkaloid concentrations found in *C. maculatum* growing in natural conditions (Castells et al. 2005). Neonates of *T. ni* were reared on standard artificial diet until they reached the 5^{th} (ultimate) instar. Larvae were then transferred individually to 30 mL cups with a pre-weighed cube of *ca* 1 g FW of control diet or coniine diets at 0.05, 0.5, 1, 2 or 5% DW coniine. Twenty pre-weighed larvae were used for each treatment.

Seven additional fifth-instars were dried at 65 ˚C for 48 h to estimate initial water content. Larvae were kept at room temperature and allowed to feed for 30 h to optimize diet consumption. Each larva, the remaining diet, and the frass produced were individually oven-dried. Larval growth was calculated as the difference between the final dry weight and the estimated initial dry weight. Consumption was calculated by subtracting the diet DW at the end of the experiment from the estimated initial diet dry weight. Assimilation was calculated as the difference between the diet consumed and the frass produced. The gravimetric performance indices ECI, ECD and AD were calculated as described.

Experiment 3: Extraction of coniine from frass

Ten newly molted fifth instars of *T. ni* were individually placed in 30 mL plastic cups filled with a pre-weighed cube of *ca* 1 g FW of 1% DW coniine-enriched diet. A subsample of initial diet was oven-dried to estimate water content. Larvae were kept at room temperature for 48 h. The diet remaining after consumption was oven-dried at 65 ˚C and weighed. Diet consumption was calculated as the difference between the final and the initial diet. A subsample of the frass produced (*ca.* 150 mg FW) was used to estimate the coniine content. The remaining frass was weighed, oven-dried at 60 ˚C and weighed again to determine water content. Coniine was extracted by homogenizing the frass with 1.5 mL 70% MeOH 30% 0.1 N HCl for 2 min. using a Wig-L-Bug grinding mill (Crescent Dental, Chicago, IL) modified to accommodate 2 mL Eppendorf tubes. Samples were extracted for 1 h on a shaker and concentrated to approximately 200 uL on a centrifugal evaporator (Jouan RC 10.10). The solution was then partitioned with hexane

and basified with 100 uL of 10 M NaOH to transform coniine to a non-protonated free base form. Coniine was extracted in 200 μL hexane with 0.01 % hexadecane and analyzed by flame ionization detection on a gas chromatograph equipped with capillary column (Alltech EC-1, 30 m, 0.23 mm) and 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. The total coniine consumed was estimated by multiplying the diet consumption by the coniine concentration added in the diet, and the total coniine in the frass by multiplying the coniine concentration in the frass by the total amount of frass produced. We calculated the total amount of coniine excreted in the frass as a percentage of the total coniine ingested.

Experiment 4: Diet choice assay

The ability of *T. ni* to distinguish between control diet *vs.* coniine-enriched diet was studied by a choice experiment. Two diet cubes (*ca* 1 g FW), one of control diet and one enriched with coniine at 1, 2 or 5% DW, were placed at opposite sides of a 9-cm diameter plastic Petri dish. Separate experiments were conducted for each coniine concentration, with 36 replicates for the 1% coniine experiment and 30 replicates for the 2% and 5%. A single larva was placed at the middle of the Petri dish, dishes were wrapped with Parafilm to avoid diet desiccation and kept at room temperature (25 C). After 24 h diet was removed and dried at 65 ˚C. Because the larvae tended to consume either the control or

the coniine diet cubes, we did not calculate the antifeedant indeces but rather recorded the choice of diet consumed.

Experiment 5: Effects of enzyme inhibitors PBO or DM with coniine on T. ni *growth*

We assessed the contributions of detoxification enzymes to coniine metabolism by adding to the artificial diet specific inhibitors for cytochrome P450s (piperonyl butoxide; PBO), and gluthatione transferases (diethyl maleate; DM). A preliminary assay was performed to determine the highest concentrations of PBO and DM that did not affect larval growth, testing a range of concentrations from 0.01% to 0.5% FW on 10 larvae per concentration. The absence of toxicity, with no statistically detectable differences in larval growth compared with the control, was achieved with 0.1% FW PBO and 0.05% FW DM. Artificial diets were prepared as described, with the addition of 1% DW coniine and/or either of the inhibitors at the concentrations determined previously as nontoxic. One-hundred twenty newly molted fifth instars raised on standard artificial diet were assigned at random to one of these diet treatments (20 larvae per treatment): 1) control, 2) PBO, 3) DM, 4) coniine, 5) PBO + coniine, and 6) DM + coniine. Larvae were allowed to feed for 24 h at room temperature (25 C). Larval weigh gain was recorded at the end of the experiment.

Statistics

A two-way analysis of variance (ANOVA) with diet treatment (control, coniine or γ-coniceine) and sex as independent variables was conducted to determine the effects of alkaloids on larval performance in Experiment 1. The effects of increasing coniine concentrations in Experiment 2 were assessed by a one-way analysis of covariance (ANCOVA) with initial larval weight as a covariate and coniine concentration as the independent variable (Raubenheimer and Simpson 1994). Post-hoc comparisons were conducted by a Tukey's LSD test. Diet preference (Experiment 4) was analyzed by the non-parametric 2 x 2 table with Fisher's tests. The effects of PBO and DM (Experiment 5) were assessed by a two-way ANCOVA conducted with initial larval weight as a covariate and coniine + inhibitor (PBO or DM) as independent variables. All statistical analyses were performed using Statistica 6.0 (StatSoft, Tulsa, OK).

Results

Relative growth rate (RGR) of *T. ni* larvae raised on alkaloid-enriched diet throughout development was not affected by the consumption (RCR) of 1% coniine or 1% γ-coniceine (Fig. 1). These alkaloids, however, decreased consumption rate and increased the efficiency of conversion of ingested food (ECI) and the efficiency of conversion of digested food (ECD) (Fig 1). Time to pupation was significantly increased in larvae raised on γ-coniceine-enriched diet (Fig 1). Approximate digestibility (AD) was, on average, lower when *T. ni* were raised on alkaloid diet but this effect was only marginally significant (ANOVA, $p<0.074$). Different responses between sexes were found only for relative growth rate and relative consumption rate, with higher rates in males compared to

females (Fig. 1). No significant differences were found between coniine and γ-coniceine. Fifth instars of *T. ni* raised for 30 h on diet enriched with a range of coniine concentrations (from 0.05% to 5%) showed similar responses to the long-term performance experiment. Thus, no effects on larval growth were found at any concentration, while coniine treatment decreased consumption, assimilation efficiency, and AD, and increased ECI and ECD, mainly due to the strong effects of the 5% coniine-enriched diet (Table 2, Fig. 2).

In the diet choice experiment where *T. ni* larvae were presented with a control diet *vs.* coniine-enriched diet (either 1, 2 or 5% coniine DW), no preference was detected for any of the diets (Table 1). The lack of differentiation between control and coniine diets was also suggested by the fact that *T. ni* larvae fed exclusively on a single diet cube, never switching between cubes.

Frass of *T. ni* larvae which fed on coniine-enriched diet contained unmetabolized coniine. The amount of coniine ingested that was excreted in the frass ranged from 21.3% to 33.2%, with an average of 26.8 ± 1.8% (mean ± SE, n = 10). Because the procedure for coniine extraction may not be completely efficient it is likely that this percentage could be even higher. Coniine is also metabolized by *T. ni*. When PBO, an inhibitor of the cytochrome P450s, was added to the diet together with coniine, larval growth showed a significant decrease, even though PBO or coniine alone did not have any effect (Fig. 3). No significant interaction was found between the effect of coniine on larval growth and the effect of DM, a specific inhibitor of the glutathione transferases.

Discussion

Colonization of an invasive plant by native herbivores is expected to be facilitated when there are physical and chemical similarities between its native host plants and the invasive species (Janzen 1968). According to this hypothesis, the distinctiveness of the secondary chemistry of *C. maculatum*, the only Apiaceae species containing piperidine alkaloids, should protect this invasive species against local herbivores in the non-indigenous community. Indeed, *C. maculatum* supports relatively few herbivores even after more than 200-years in the US (Goeden and Ricker 1982). Thus, the ability of the generalist *T. ni* to utilize this plant is surprising. Larvae raised on coniine and γ-coniceine-enriched diets did not show any effects on growth at the concentrations tested (from 0.05 to 5% DW). Only a slight reduction in the development time was detected when larvae were raised at 1% DW coniine, but this did not result in reduced pupal size. The concentrations used for these experiments are representative of the levels of piperidine alkaloids found in *C. maculatum* growing in natural conditions, which may vary as much as 100-fold among individuals from different populations across the US (Castells et al. 2005). Thus, no differences in larval growth should be expected in *T. ni* consuming *C. maculatum* in the field as far as alkaloid concentrations are concerned. The absence of negative effects of coniine and γ-coniceine on *T. ni* performance contrasts with the toxicity caused by the alkaloid nicotine. Although in our experiment no mortality was detected when larvae were raised on diets containing up to 5% DW coniine, *T. ni* larvae died on diet containing nicotine concentrations above 0.064% FW (Krischik et al 1991). Indeed, toxicity of alkaloids against insect herbivores may not be a general pattern. Quinolizidine alkaloids have also found to be innocuous for generalist and specialist Lepidoptera (Stermitz et al 1989).

The ability of *T. ni* to tolerate *C. maculatum* alkaloids can be explained by the presence of both behavioral and physiological resistance mechanisms. Insects use these two broad categories of resistance strategies to cope with noxious secondary metabolites. During behavioral resistance insects avoid exposure of plant allelochemicals by selective feeding, or decrease consumption when ingesting a plant containing toxic allelochemicals (Tallamy 1986), while physiological resistance involves activity of detoxification enzymes (e.g. cytochromes P450s, glutathione transferases or esterases) which modifies the chemical structure of the allelochemical to reduce or eliminate its toxicity, thus allowing excretion of the resulting metabolites (Brattsten 1992). Excretion of unmetabolized allelochemicals may also be a prominent mechanism to eliminate toxic compounds from the body. *T. ni* larvae decreased consumption rates when raised from neonate to pupae on 1% DW coniine or γ-coniceine enriched-diets, thus reducing their daily exposure to alkaloids. A short-term decrease in consumption (48 h trial) was also detected at higher alkaloid concentrations (5% DW). Because no preference for control diet *versus* alkaloid-enriched diet was found in the diet-choice experiment, the decrease in consumption could be more related to metabolic disruptor and not by stimuli of the peripheral sensilla. Indeed, generalists might have neural limitations that restrict their ability to choose a suitable plant host, even if consumption of a particular plant is not favorable in terms of growth and development (Bernays 2001). An unexpected effect of high-alkaloid diet on *T. ni* performance was the higher efficiencies of ingestion and digestion (ECI and ECD) associated with a decrease in consumption rates. No direct evidence suggests that coniine and γ-coniceine enhance food processing; thus, increased

ECI and ECD were most likely caused indirectly by the longer food retention in the gut facilitating increased absorption.

Another mechanism that allows *T. ni* to cope with piperidine alkaloid toxicity is cytochrome P450-mediated metabolism. Cytochrome P450s are involved in the detoxification of a wide array of allelochemicals, including alkaloids (Li et al 2007). Ingestion of coniine-enriched diet resulted in a significant decrease of *T. ni* larval growth when a specific inhibitor of cytochrome P450s, piperonyl butoxide, was added to the diet, while no effects were found for coniine alone. The significant interaction between PBO and coniine demonstrates that P450s prevents the post-ingestive toxic effects of coniine. In contrast to P450s, glutathione transferases do not appear to be involved in the detoxification of coniine. P450s activity is apparently necessary to avoid toxic effects of piperidine alkaloid ingestion. Even though *T. ni* has not coevolved with *C. maculatum* and does not consistently encounter piperidine alkaloids in its diet, P450s from generalists are structurally more flexible and able to accept a wider array of substrates compared to specialists (Li et al 2004). A third mechanism of resistance toward *C. maculatum* alkaloids is an efficient excretion of unmetabolized alkaloids, with more than 27% of the ingested coniine readily excreted into the frass. This value is low compared to excretion of secondary chemicals by the specialists caterpillars *Manduca sexta* and *Papilio polyxenes*, which have been found to eliminate more than 90% and 50% of ingested allelochemicals, respectively, but it is a high value compared to the 1% of the generalist *Spodoptera frugiperda* (fall armyworm) (reviewed in Brattsten 1992)

In general, generalist insects may have a greater capacity to deal with novel plant secondary chemicals than do specialists. *Agonopterix clemensella*, a caterpillar reported

to feed on more than fifteen native species of Apiaceae (Berenbaum 1982), overwhelmingly discriminated against a high coniine diet in a choice test (Castells and Berenbaum 2006) whereas no avoidance or preference was displayed by *T. ni* in response to any of the alkaloid concentrations tested.

Since its introduction, *C. maculatum* has spread conspicuously across US, forming extensive stands that invade cultivated and natural areas. Whether *C. maculatum* remains free from most native herbivores due to the presence of piperidine alkaloids is unclear. From this study we find no evidence that the low presence of *T. ni* on *C. maculatum* in the field can be explained by the toxicity of piperidine alkaloids. Behavioral and physiological resistance mechanisms displayed by the insect seem sufficient to avoid large negative effects on performance. However, consuming *C. maculatum* could also determine some disadvantages. Insects could be more vulnerable to predators because the time spent to complete development is increased. Moreover, detoxification by P450s and alkaloid excretion could determine a metabolic cost that might influence *T. ni* host plant choice. Other toxic secondary compounds in *C. maculatum*, such as furanocoumarins, could be also involved in the low frequency of herbivores in the field.

Final remarks

When a plant species invades a new habitat it re-establishes interactions with the biotic environment in the novel community, which, in most cases, differs from the interactions from the plant's native range. The presence of unique secondary compounds in the invaded community may provide the plant with a "novel weapon" to which

herbivores have not coevolved any resistance, thus increasing the plant's competitive ability (Callaway and Ridenour 2004). This case scenario was tested here for the invasive *C. maculatum*. Even piperidine alkaloids were neverencountered by herbivores in the US prior to *C. maculatum* introduction, the resistance of the native generalist caterpillar *T. ni* was efficient enough to cope with the new secondary metabolites. Novel plant compounds do not guarantee increased resistance to generalist herbivores.

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Figure Captions

Figure 1. Long-term feeding of *T. ni* diet with standard diet (control) or diet enriched with 1% coniine or 1% coniceine from neonate to pupation. A two-way ANOVA analysis was performed with treatment and sex as independent variables. A Tukey's post-hoc test is shown for those dependent variables with a significant main effect. Means and SE are shown (n = 50).

Figure 2. Performance of 5^{th} instar *T. ni* larvae feeding on coniine enriched diets at increasing concentrations over a 30-h period. A one-way ANCOVA was performed with larval initial weight as a covariate (Table 2). Different letters show significant differences ($p < 0.05$) by pairwise comparisons from the least square means using a Tukey's LSD test. The least square means and SE are shown (n = 20).

Figure 3. Effects of A) piperonyl butoxide (PBO, 0.1% DW) and B) dimethyl maleate (DM, 0.05% DW) on larval growth for 5^{th} instar *T. ni* feeding with and without coniine (1% DW). Two-way ANCOVA analyses were performed with the inhibitor presence and coniine presence as independent variables and initial larval weight as a covariate. Results are shown in Table 2. Different letters show significant differences by a Tukey's post-hoc test. Means and SE are shown (n = 20).

Table 1. Feeding preference of 5th instar *T. ni* when presented with a choice between standard diet (control) and a coniine-enriched diet at 1, 2 or 5% DW. A non-parametric test (2 x 2 frequency table) was used to test whether the diet preference was significantly different from a random distribution.

Coniine concentrations	n	Control	Coniine	*p*
1%	36	21	15	0.31
2%	30	14	16	0.50
5%	30	12	18	0.30

Table 2. Analysis of covariance for *T. ni* feeding diets enriched with coniine over a 30-h period. Larval initial weight was used as covariate following Raubenheimer and Simpson (1994). P-values in bold indicate significant differences at $p < 0.05$.

	SS	Df	MS	F	P
LARVAL GROWTH					
Initial DW	3928.69	1	3928.69	709.664	**0.000**
Coniine	17.49	5	3.49	0.632	0.676
Error	625.56	113	5.53		
CONSUMPTION					
Initial DW	10972.44	1	10972.44	329.74	**0.000**
Coniine	879.59	5	175.91	5.28	**0.000**
Error	3760.13	113	33.27		
ASSIMILATED					
Initial DW	3623.37	1	3623.37	275.65	**0.000**
Coniine	1028.45	5	205.69	15.64	**0.000**
Error	1485.34	113	13.14		
ECI					
Initial DW	13.01	1	13.01	3.18	0.077
Coniine	270.47	5	54.09	13.21	**0.000**
Error	462.48	113	4.093		
ECD					
Initial DW	78.14	1	78.14	6.20	0.14
Coniine	2513.23	5	502.64	39.92	**0.000**
Error	1422.68	113	12.590		
AD					
Initial DW	18.13	1	18.13	3.86	0.052
Coniine	649.02	5	129.80	27.66	**0.000**
Error	530.28	113	4.69		

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Figure 1

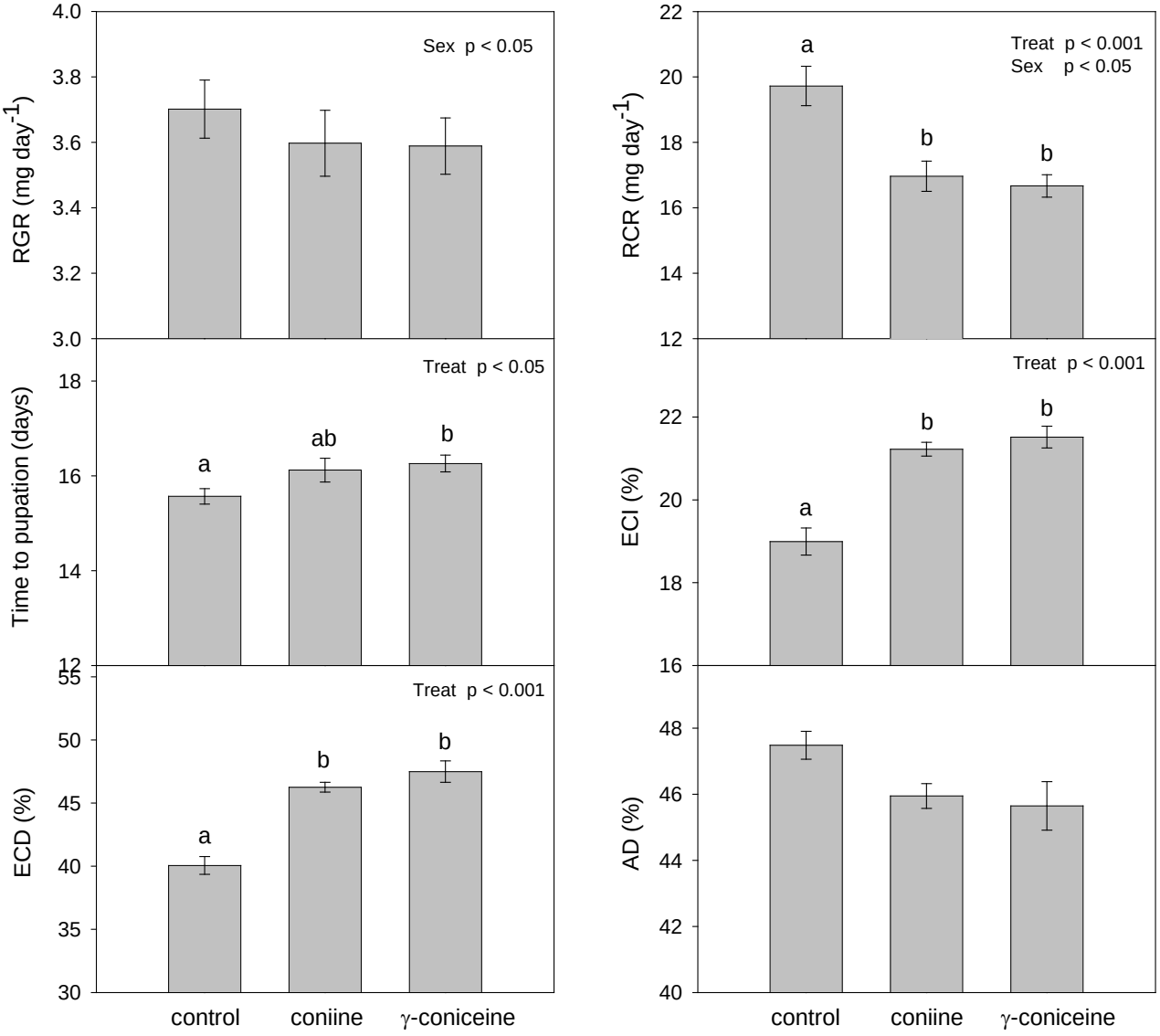

RGR (mg day-1)
Sex p < 0.05
Time to pupation (days)
Treat p < 0.05
a
ab
b
ECD (%)
Treat p < 0.001
a
b
b
RCR (mg day-1)
Treat p < 0.001
Sex p < 0.05
a
b
b
ECI (%)
Treat p < 0.001
a
b
b
AD (%)
control
coniine
γ-coniceine

Figure 2

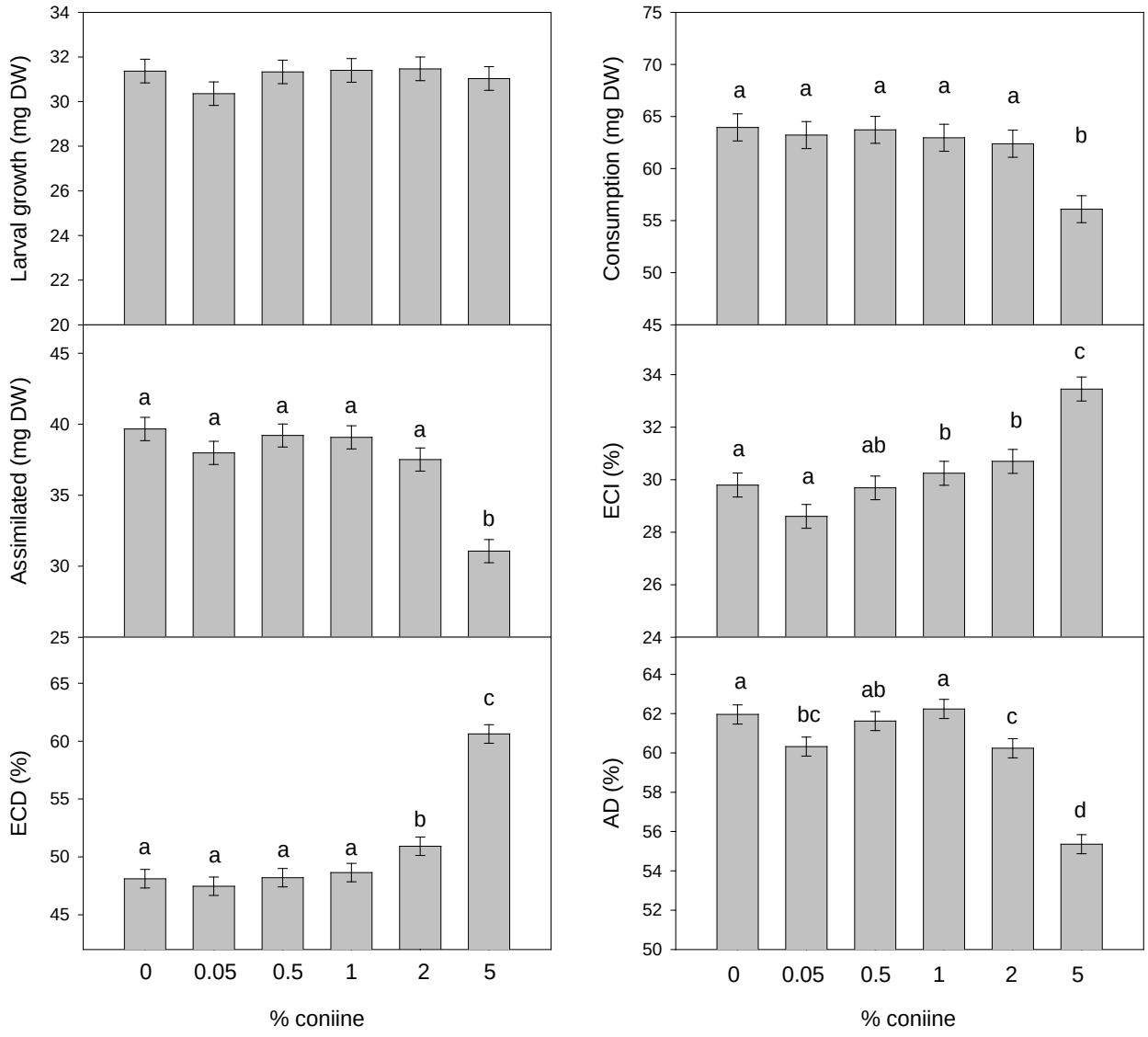
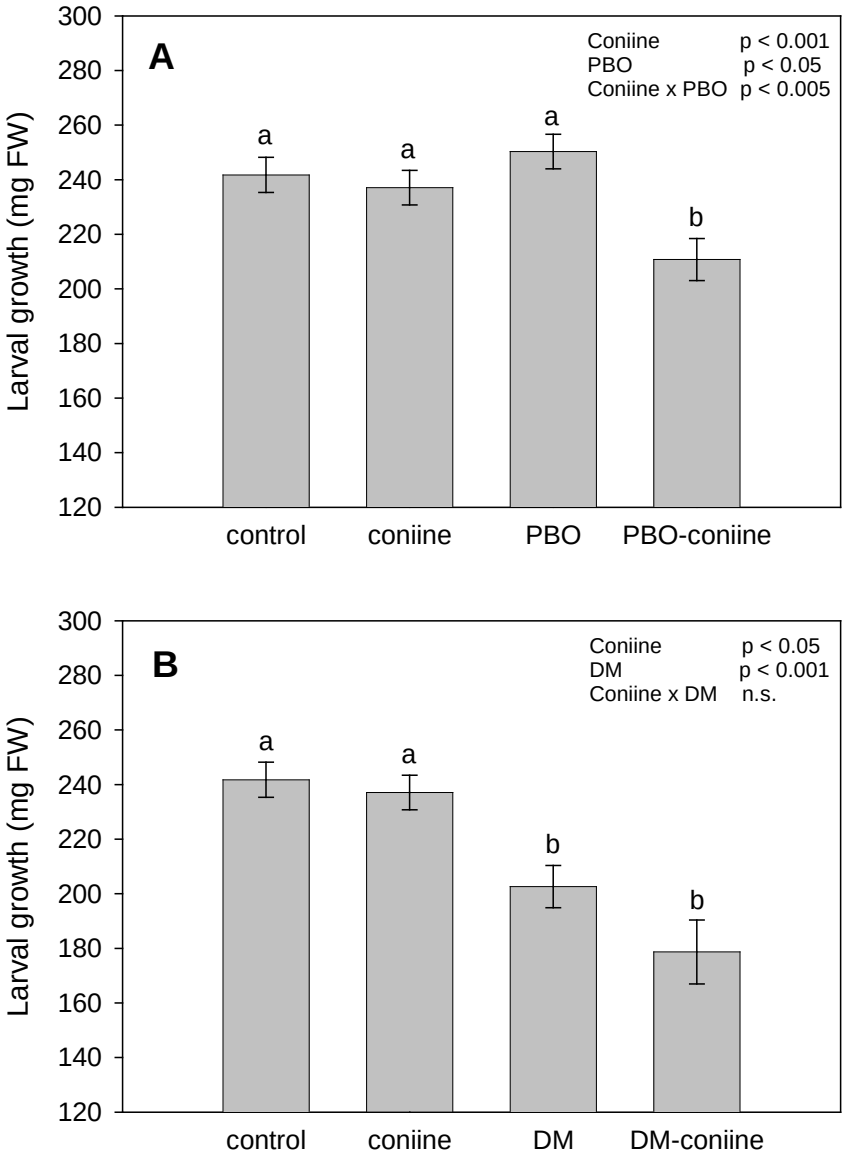

Larval growth (mg DW)
Assimilated (mg DW)
ECD (%)
Consumption (mg DW)
ECI (%)
AD (%)
a a a a a b
a a a a a b
a a a a b c
a a ab b b c
a bc ab a c d
0 0.05 0.5 1 2 5
% coniine

Figure 3


A
Coniine p < 0.001
PBO p < 0.05
Coniine x PBO p < 0.005
Larval growth (mg FW)
300
280
260
240
220
200
180
160
140
120
a
a
a
b
control
coniine
PBO
PBO-coniine
B
Coniine p < 0.05
DM p < 0.001
Coniine x DM n.s.
Larval growth (mg FW)
300
280
260
240
220
200
180
160
140
120
a
a
b
b
control
coniine
DM
DM-coniine