

Natal insect experience with *Arabidopsis thaliana* plant genotypes influences plasticity in oviposition behavior

Sean F. Ryan¹ & M. Gabriela Bidart-Bouzat^{2*}

¹Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556, USA, and ²Department of Biological Sciences, Bowling Green State University, Bowling Green, OH 43403, USA

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Abstract

Previous studies have shown that insect experience with secondary chemicals present in different plant species can induce behavioral changes in female oviposition preferences. However, there is a lack of information on whether insect experience with intraspecific plant variation may influence oviposition behavior. The prediction that experience with plant genotypes would affect the oviposition behavior of two crucifer insect pests was tested using a wild ecotype of *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae) (Col-0) and two of its genetically modified lines (35S:ESP and *tgg1 tgg2*), which differ in their glucosinolate hydrolysis profiles. Choice oviposition assays were performed using both naïve and experienced females of the specialist *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) and the generalist *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae). In addition to oviposition preferences, the effect of plant genetic lines was assessed on insect traits related to development, growth, survival, and fecundity. Experience with different natal treatments (i.e., artificial diet and plant genotypes) led to changes in oviposition behavior of the diamondback moth *P. xylostella*; however, this effect was dependent on the specific genetic lines included in dual-choice oviposition assays. In addition, for both moth species, experience led to female oviposition choices that would have maximized fitness of their offspring. In summary, this article suggests that insect experience with plant genotypes varying in their secondary metabolites can influence subsequent oviposition behavior. This outcome may have implications for plant-insect coevolution and integrated pest management.

Introduction

Phenotypic plasticity, defined as the capacity of organisms to modify their phenotypes in response to different environments, is an important factor modulating phenotypic responses of interacting species (Agrawal, 2001). Several studies of species interactions have focused on how genetic variation in one species influences the phenotype of another interacting species. For example, intraspecific variation in plant secondary chemicals (with roles in defense) is known to affect phenotypic or behavioral traits of insect herbivores, as well as their patterns of colonization and herbivory in the field (Bidart-Bouzat et al., 2005; Bidart-Bouzat & Kliebenstein, 2008; Sun et al., 2009; Crutsinger

et al., 2010). However, information is lacking regarding the effect of insect experience with plant genotypes differing in their hydrolysis profiles, and how this may affect plasticity of insect oviposition behavior. Experience may modify oviposition choices of insect herbivores and this can have implications for the co-evolutionary dynamics of plant-insect associations.

In contrast to the lack of knowledge on the role of insect experience with different genotypes of a plant species, there is evidence that insect experience with different host plant species during insect larval, pupal, or early-adult stages can induce female oviposition preferences for particular host plants (Liu et al., 2005; Shikano & Isman, 2009). This can be due to physiological responses of insects to sensory stimulation by certain chemicals present in different plant species, which may result from habituation (Akhtar et al., 2009). For example, the crucifer specialist moth *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) is adapted to feed and oviposit on members of the

*Correspondence: M. Gabriela Bidart-Bouzat, Department of Biological Sciences, 511 Life Sciences Building, Corner of Merry and N College, Bowling Green State University, Bowling Green, OH 43403, USA. E-mail: gbidart@bgsu.edu

Brassicaceae (Reddy et al., 2003), but is repelled by volatiles of other non-host plants, such as those released by *Chrysanthemum morifolium* Ramat (Asteraceae) (Liu et al., 2005). However, studies have shown that females with prior experience with non-host plant species or their extracts were no longer repelled by these volatiles, or showed an induced preference for them (Liu et al., 2005; Zhang et al., 2007; Wang et al., 2008). Similar results have been observed in naïve *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) moths after exposure to the defense-related chemical latex present in the non-host plant *Hoodia gordonii* (Masson) Sweet ex Decne. Naïve *T. ni* females exposed to latex during their last instar accepted this previously oviposition deterrent compound (Chow et al., 2005; Shikano & Isman, 2009).

The effect of particular stimuli present in an insect's natal or early-adult environment on subsequent host preferences has been explained by various hypotheses, such as the Hopkins host selection principle (Hopkins, 1916), the chemical legacy hypothesis (Corbet, 1985), and the natal habitat preference induction (NHPI) hypothesis (Davis & Stamps, 2004). The former two hypotheses focus on the stage where the induction of preference can occur (pre-imaginal vs. post-eclosion, respectively). In contrast, NHPI states that pre-dispersal experiences with cues found in an organism's natal environment can induce changes in preferences for these cues when that organism is dispersing into new habitats. Therefore, NHPI provides a broader and more comprehensive hypothesis for studies testing whether the phenomenon of induction of preferences exists in general, as opposed to the other hypotheses focusing on a particular developmental stage when these changes in preference may or may not occur.

Since the initial observation of this phenomenon (the induction of preferences) over a century ago, multiple examples supporting the NHPI hypothesis have been provided across a diversity of taxa including a number of insect orders (Davis & Stamps, 2004). Changes in habitat/host preferences have been frequently attributed to species-specific physical or chemical cues present in the natal environment (Payne et al., 2000; Wang et al., 2008). For example, natal habitat type (i.e., chaparral vs. oak woodland) appears to influence dispersal and reproductive sites in the brush mice (Mabry & Stamps, 2008). Habitat preference has also been found to be important for the Lepidoptera (Shreeve et al., 2004; Thomas et al., 2001). In addition, for insect species, variation in the secondary chemistry of their host plants has been considered a predominant elicitor of changes in insect behavior associated with experience.

Even though there has been an increase in the number of studies exploring preferences of organisms induced by

natal host environments, the effect of different genotypes of a single host plant species on the behavioral plasticity of insect herbivore species remains virtually unknown. In this study, an experimental approach was used to evaluate whether insect experience with plant genotypes of *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae) differing in their secondary chemistry influences oviposition preferences of two common insect pests of cruciferous plants: the specialist *P. xylostella* and the generalist *T. ni*. *Arabidopsis thaliana* produces glucosinolates, which are secondary metabolites that play a role in chemical defense against insect herbivores. Upon tissue damage by insect herbivory, glucosinolates are hydrolyzed by myrosinase (β -thioglucoside glucohydrolase, TGG) enzymes, which lead to the formation of isothiocyanates (ITCs), nitriles, and other hydrolysis products (Halkier & Gershenzon, 2006). Both intact glucosinolates and their hydrolysis products, most notably ITCs, can be toxic to a wide range of plant attackers including pathogens and mammalian and insect herbivores (Louda & Mole, 1991; Rask et al., 2000). Whereas glucosinolates and their hydrolysis products are known to deter herbivory by generalist insects, they can also act as attractants and feeding/oviposition stimulants for specialist herbivores (Renwick et al., 2006; Mumm et al., 2008; Sun et al., 2009). For this study, we selected genetically modified (GM) lines of *A. thaliana* differing in their glucosinolate hydrolysis profiles. These GM lines express natural variability in the type of hydrolysis products found in natural populations of *A. thaliana*, while maintaining the same genetic background and other phenotypic characteristics of the wild type from which these lines were derived. Thus, the use of these GM lines is ideal to address ecological questions under both laboratory and field conditions. In addition, the effect of variation in *A. thaliana* glucosinolate hydrolysis profiles on fitness-related traits of the two selected lepidopteran herbivores was evaluated. This study provides insight in how variability in one species may drive adaptive responses in other interacting species.

Materials and methods

Selected plant genetic lines and growth conditions

Arabidopsis thaliana is a self-fertilizing annual plant with a short-life cycle. As a member of the Brassicaceae, it is closely related to numerous agriculturally important crop species such as broccoli, mustard, and cabbage. To evaluate the effects of different plant genotypes on *P. xylostella* and *T. ni* oviposition responses and preferences, three genotypes of *A. thaliana* were selected: the wild type Col-0 and two genetically modified lines, the transgenic line 35S:ESP and the double myrosinase knockout *tgg1 tgg2*. Wild type Col-0 seeds were obtained from the Arabidopsis

Biological Resource Center (Ohio State University, Columbus, OH, USA), and the myrosinase double-mutant *tgg1 tgg2* and 35S:ESP lines were propagated from an original stock kindly donated by Dr. Daniel Kliebenstein (University of California, Davis, CA, USA). The two GM lines share the same genetic background of wild type Col-0, from which they were derived, but differ from it in their glucosinolate hydrolysis profiles (Barth & Jander, 2006). The predominant glucosinolate hydrolysis products spontaneously formed in wild-type Col-0 rosette leaves are ITCs (Lambrix et al., 2001). The double knockout *tgg1 tgg2* contains two mutations (T-DNA insertions) that abolish the activation of two major genes involved in the synthesis of myrosinase enzymes, which are needed for the production of hydrolysis products (Barth & Jander, 2006). The transgenic line 35S:ESP used in this study expresses nitriles, a different type of hydrolysis product that is naturally formed in another *A. thaliana* ecotype (i.e., Ler). In contrast to Col-0, rosette leaves of Ler predominantly produce simple nitriles due to the presence of a functional ESP gene that encodes for the epithiospecifier protein (ESP), which is non-functional in the wild type Col-0. The transgenic line 35S:ESP overexpresses the ESP cDNA from the Ler accession under the control of the cauliflower mosaic virus 35S (CaMV35S) promoter in Col-0, and thus, glucosinolates are primarily hydrolyzed into nitriles instead of the corresponding ITCs (Burow et al., 2006). However, even though Col-0 and 35S:ESP significantly differ in their predominant glucosinolate hydrolysis profile, the intact glucosinolate profiles, myrosinase activity, developmental, and morphological characteristics do not differ between the two genotypes (Burow et al., 2006).

Plants used for rearing larvae for the proposed experiments were grown in 10-cm pots containing ProMix BX potting soil (Premier Horticulture, Quackertown, PA, USA), and the plants used in the choice experiment were grown in 98-well flats. All seeds were cold-stratified at 5 °C for 5 days, and then pots were moved into growth chambers with temperature set at a 23/20 °C day/night cycle and a L10:D14 photoperiod. Plants from all treatments were rotated daily to avoid position effects within chambers.

Selected insect species and rearing conditions

Plutella xylostella and *T. ni* are two common insect pests of crucifer plants that have wide geographic distributions. Both *P. xylostella* and *T. ni* have a short-life cycle, high dispersal ability, and high reproductive capacity (Harcourt, 1954; Shikano & Isman, 2009). These life-history characteristics have likely facilitated these species to become devastating pests of cruciferous crops and to evolve resistance to numerous natural and synthetic pesti-

cides (Ratzka et al., 2002). A major difference between *P. xylostella* and *T. ni* is the taxonomic breadth of their host species. Although *P. xylostella* is adapted to feed exclusively on plants of the Brassicaceae family, *T. ni* is a generalist insect that feeds on plants from over 20 plant families (Wittstock et al., 2003).

Plutella xylostella and *T. ni* individuals used in this experiment were obtained from a commercial supplier (Benzon's Research, Carlisle, PA, USA), which has been rearing these species on artificial diets for several years. Larvae of both species were reared on artificial diet obtained from the same supplier at a constant room temperature of 28 °C and a L14:D10 photoperiod. Larvae were randomly assigned to four different natal treatment groups: a 'naïve' (or 'not experienced') group and three 'experienced' groups. Naïve females of both species were reared from egg to adult on artificial diet, whereas females used in the 'experienced' treatment groups were reared on artificial diet during their first two (*P. xylostella*) or three instars (*T. ni*), and then completed their cycles (i.e., last two instars, pupation, and adult eclosion) on plants from each of the three previously described *A. thaliana* genotypes (i.e., Col-0, 35S:ESP, and *tgg1 tgg2*). This experimental setting allowed these species to experience different natal environments during both pre-imaginal (larva and pupa) and imaginal (adult) stages, where changes in the behavioral plasticity of host preferences may occur (Liu & Liu, 2006; Shikano & Isman, 2009).

Two *P. xylostella* larvae were placed on individual 4-week-old plants (in 10-cm pots). Larvae of *T. ni* were placed individually on plants grown in 4.5-cm cells contained in 50-well flats. Each *T. ni* larva was moved to a new plant when more than 75% of the foliage was consumed. Movement of larvae among plants was restricted to within each pot/flat cell (no-choice) by covering each pot or flat cell with plastic containers with mesh lids. Larvae were checked daily for pupation. At pupation, each *P. xylostella* pupa was placed in a 2-ml microcentrifuge tube by carefully sliding the leaf with the attached pupa into the tube and plugging it gently with a piece of mesh. Each tube also had a 1-mm hole drilled in the bottom end for aeration. This method allowed the individuals to further experience the host plant genotypes during pupation and adult eclosion (in addition to the larval stage exposure), and prevented the two emerging adults per plant from mating on, or having oviposition experience with, the natal plant genotype. This was not necessary for *T. ni* because, due to its large size compared to *P. xylostella*, each larva was housed separately in a different pot (one plant per pot), and thus there was no risk of having any accidental mating. It should be noted that although *T. ni* did not eclose in a microcentrifuge tube, the enclosing

space was proportionally similar to that of *P. xylostella* due to the large differences in body size. In addition, this difference in the protocol for rearing these species was applied only during the pupal stage. As larvae, both species freely interacted with the plant. As with *P. xylostella*, each *T. ni* pupa was checked daily for adult eclosion. As they eclosed, both male and female moths were removed from plants and placed in a plastic mating cage (45 × 35 × 35 cm) with an ca. 1:1 sex ratio. Individuals placed in each mating cage underwent the same pre-imaginal and imaginal treatment (same natal plant genotype) and were provided with a 10% (wt/vol) sucrose solution. Females were allowed to mate for 1 or 2 days prior to being used in the oviposition choice experiment for *P. xylostella* and *T. ni*, respectively. These time periods allowed for successful mating, as was estimated in preliminary trials.

Oviposition preference assays

To test whether natal experience with the plant genotypes had an effect on oviposition preferences, females subjected to each of the four previously described natal treatments (artificial diet, Col-0, 35S:ESP, or *tgg1 tgg2*) were used in dual-choice oviposition assays. For naïve females, oviposition assays included all possible pairings of the three natal plant genotypes (i.e., Col-0 vs. *tgg1 tgg2*, 35S:ESP vs. *tgg1 tgg2*, and Col-0 vs. 35S:ESP). However, for experienced females, assays included only pairs of genotypes that contained their natal genotype. All females were used only once for each assay. Each assay was replicated 21–42× using *P. xylostella* females and 6–20× using *T. ni* females from each treatment group. The lower number of replications for *T. ni* was due to the high mortality of this species when reared on the Col-0 genotype.

Mated females of *P. xylostella* were placed individually in plastic storage bins (35 × 32 × 32 cm) containing two 4-week-old plants from different genotypes, placed 50 cm from each other with equal distance from where females were allowed to enter the cage (through a small hole drilled in the side of each storage bin). Plants used in these trials were matched for size. One leaf of equal size from each plant was damaged along the mid-vein with a pair of forceps to promote the release of glucosinolate hydrolysis products. Each female was allowed to oviposit freely between the two plants for 12 h in total darkness. No food was provided during this time. The female was then removed and the eggs laid on each plant were counted. A similar protocol was followed up for *T. ni* with the exception that the cages used were constructed from Styrofoam and lined with aluminum foil, as these materials considerably prevented egg laying on non-plant surfaces. For *T. ni*, each female was allowed to oviposit for 18 h. The longer

time interval used for *T. ni* was based on preliminary tests, which indicated that this species required longer to oviposit under the preset laboratory conditions. Due to the longer assay and larger body size of *T. ni* females, a 10% sucrose solution was also provided, as recommended in a previous study (Shikano & Isman, 2009).

No-choice experiments

To evaluate whether exposure of *P. xylostella* and *T. ni* to the different natal treatments had an effect on their performance, fitness-related traits were measured for individuals used in the oviposition preference assays. These traits included survivorship to adulthood, time to pupation, and adult weight. Time to pupation is considered an indirect measure of fitness, because larvae with longer times to pupation are more vulnerable to disease and predation (Clancy & Price, 1987), and adult weight is significantly correlated with fecundity in many lepidopteran species (Honěk, 1993). Time to pupation was estimated only for females, from the time they were moved from artificial diet into a natal plant genotype treatment group to pupation (i.e., development of the last two instars). Adult weight was recorded for both naïve and experienced females used in the oviposition choice experiment. Survivorship of individuals reared on the various plant genotypes (during the two instars) was estimated as the proportion of both males and females that survived to adulthood over the total number of larvae used for each treatment. In addition, to assess whether exposure to the various natal treatments influenced egg number laid by females, we counted the eggs laid by each female on each plant genotype.

Statistical analysis

To test the main prediction that female oviposition is influenced by the natal environment (i.e., solely artificial diet or experience with different plant genotypes), we performed weighted least squares (WLS) regression analyses on an oviposition preference index (OPI). This index was calculated as $(X - Z)/(X + Z)$, where X and Z represent the number of eggs laid by each female on each of the two genotypes (X and Z) used in each dual oviposition assay. The use of an oviposition index is useful because it allows one to transform a categorical variable into a quantitative one that can be analyzed using an analysis of variance (ANOVA) approach. This is more appropriate than using a G-test or χ^2 test on the original choice data, because the numbers of eggs laid by a female on the respective options in a dual-choice assay are not independent (i.e., each female lays eggs on both plant genotypes offered in choice assays), and these tests assume that there has to be only one observation per subject for each observation to be independent from each other. The OPI values range from

–1 to 1, with values closer to 1 indicating most eggs were laid on X, and those closer to –1 indicating most eggs were laid on Z. Weighted least squares regression was more appropriate than ANOVA in this case, to account for overdispersion of the data and the variability in the total number of eggs laid by each female, which was entered as a weight factor in the regression model. This method also allowed us to estimate the pre-planned contrasts between the effects of female experience with a particular plant genotype (Col-0, 35S:ESP, or *tgg1 tgg2*) vs. no experience (artificial diet) directly from the parameter estimates (using dummy variables coding). OPI was transformed if necessary to satisfy regression model assumptions of normality and homoscedasticity (square-root or rank transformations). In addition, we estimated 95% confidence intervals and performed two-tailed t-tests to test for the null hypothesis ‘OPI = 0’ within each natal treatment level.

A one-way ANOVA was performed to evaluate the effect of larval diet (i.e., solely artificial diet or different plant genotypes during the last two larval stages) on fitness-related traits of the two moth species, including adult weight, time to pupation, and total number of eggs laid. Adult survivorship among plant genotypes was compared using Fisher’s exact tests for both moth species. All statistical tests were performed using SAS version 9.1 (SAS Institute, Cary, NC, USA) and data were rank-transformed to stabilize the variance and minimize heteroscedasticity. Differences among treatment means were compared using Fisher’s least significant difference (LSD) multiple comparisons. For these post-hoc multiple comparisons, a Bonferroni correction was used (corrected $\alpha' = 0.016$).

Results

Oviposition preference assays

For *P. xylostella*, the effect of experience with the *A. thaliana* genotypes during the last two instars until adult eclosion was dependent on both the natal treatment and the specific genotypes presented in the choice assays. Results from WLS regression analyses showed that overall the effect of experience on oviposition preference indices (OPIs) was statistically significant when females were exposed to the choice between Col-0 and 35S:ESP plants ($F_{2,86} = 4.19$, $P = 0.018$; Figure 1A). This is an important result, because this choice assay included the two genotypes that differ in their types of glucosinolate hydrolysis products (i.e., ITCs in Col-0 vs. nitriles in 35S:ESP). The effect of experience on OPIs was also corroborated by significant contrasts between the OPIs of each of the ‘experience’ treatment groups (Col-0 and 35S:ESP) and the ‘no

experience’ group reared on artificial diet (i.e., one contrast between OPIs of moths reared on artificial diet vs. those reared on Col-0, and a second contrast between OPIs of moths reared on artificial diet vs. those reared on 35S:ESP; Figure 1A). Naïve females showed a trend toward an innate preference for Col-0 when given a choice between this genotype and 35S:ESP (as indicated by the mean OPI being different from zero, although marginally significant). However, this trend was no longer found in females that had experienced Col-0 or 35S:ESP earlier in their environments. In addition, even though the overall effect of natal treatment was not significant for the other two choice assays (Col-0 vs. *tgg1 tgg2* and 35S:ESP vs. *tgg1 tgg2*), contrasts between OPIs of naïve females (reared on artificial diet) and those of females that experienced either Col-0 (Figure 1B) or 35S:ESP (Figure 1C) as natal genotypes showed a marginally significant preference toward these genotypes. These results suggest that females’ natal experiences with either Col-0 or 35S:ESP led to a change in female oviposition preferences from no preference in naïve females to a preference for either Col-0 or 35S:ESP (Figure 1B and C). These female oviposition choices for either Col-0 or 35S:ESP vs. *tgg1 tgg2* were corroborated by estimating 95% confidence intervals and t-tests, which indicated that these OPIs were significantly different from zero (Figure 1B and C).

In contrast to these previous results, the effect of natal experience with different plant genotypes for *T. ni* moths was not statistically significant in either of the three types of dual-choice assays performed (Figure 2A–C). Even though results showed some changes in mean OPIs, these were not significant, likely due to the low sample sizes resulting from low survival of this insect species when reared on the plant genotypes Col-0 and 35S:ESP.

No-choice experiments

Overall, the effect of natal treatment on fitness-related traits was markedly different between herbivore species, with host genotype having little to no effect on *P. xylostella* but pronounced effect on *T. ni*. For *P. xylostella*, there was no significant difference in survivorship or adult weight for insects reared on different diets (i.e., artificial diet or plant genotypes during the last two instars) (Figure 3A and C). The only effect that the natal treatment had on this species was a decrease in time to pupation for females raised on the nitrile-producing 35S:ESP (Figure 3B). In contrast, for *T. ni*, plant genotype had a significant effect on all measures related to their performance: survivorship, adult weight, and time to pupation (Figure 3D–F). Survivorship of *T. ni* significantly differed among all genotypes, with the highest number of larvae surviving to adulthood

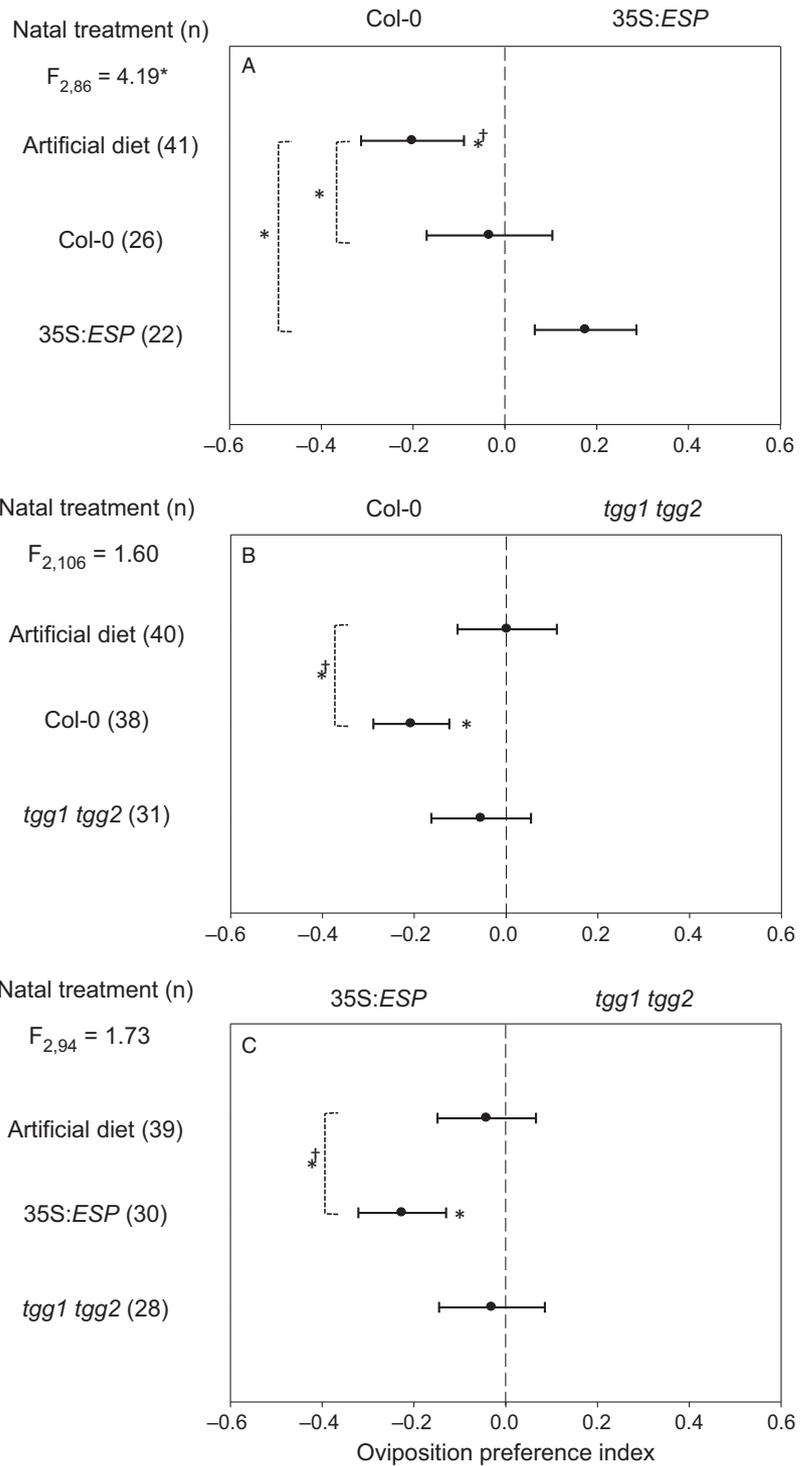


Figure 1 Mean (\pm SE) oviposition preference indices (OPIs) of *Plutella xylostella* females used in three dual-choice assays including *Arabidopsis thaliana* genotypes that vary in their glucosinolate hydrolysis profiles: (A) Col-0 vs. 35S:ESP, (B) Col-0 vs. *tgg1 tgg2*, and (C) 35S:ESP vs. *tgg1 tgg2*. Naïve females were reared on artificial diet and experienced females on each of the three plant genotypes. Sample sizes are indicated in parenthesis. Asterisks on brackets to the left of error bars indicate significant contrasts between OPIs of naïve females (reared on artificial diet) and those of females reared on each of the plant genotypes, asterisks on the right of error bars indicate that the OPI mean is significantly different from zero, based on two-sided t-tests using 95 and 99% confidence intervals (* $P < 0.05$; † $P = 0.07$).

on the double-mutant *tgg1 tgg2*, followed by the nitrile-producing 35S:ESP, and finally, by the ITC-producing Col-0 (Figure 3D). Time to pupation (last two larval stages) was also significantly different among all treatment levels, with longest developmental times found for *T. ni*

larvae reared on 35S:ESP and shortest on *tgg1 tgg2* lines (Figure 3E). In addition, adult *T. ni* females raised on *tgg1 tgg2* plants and artificial diet weighed significantly more than females reared on Col-0 and 35S:ESP plants (Figure 3F).

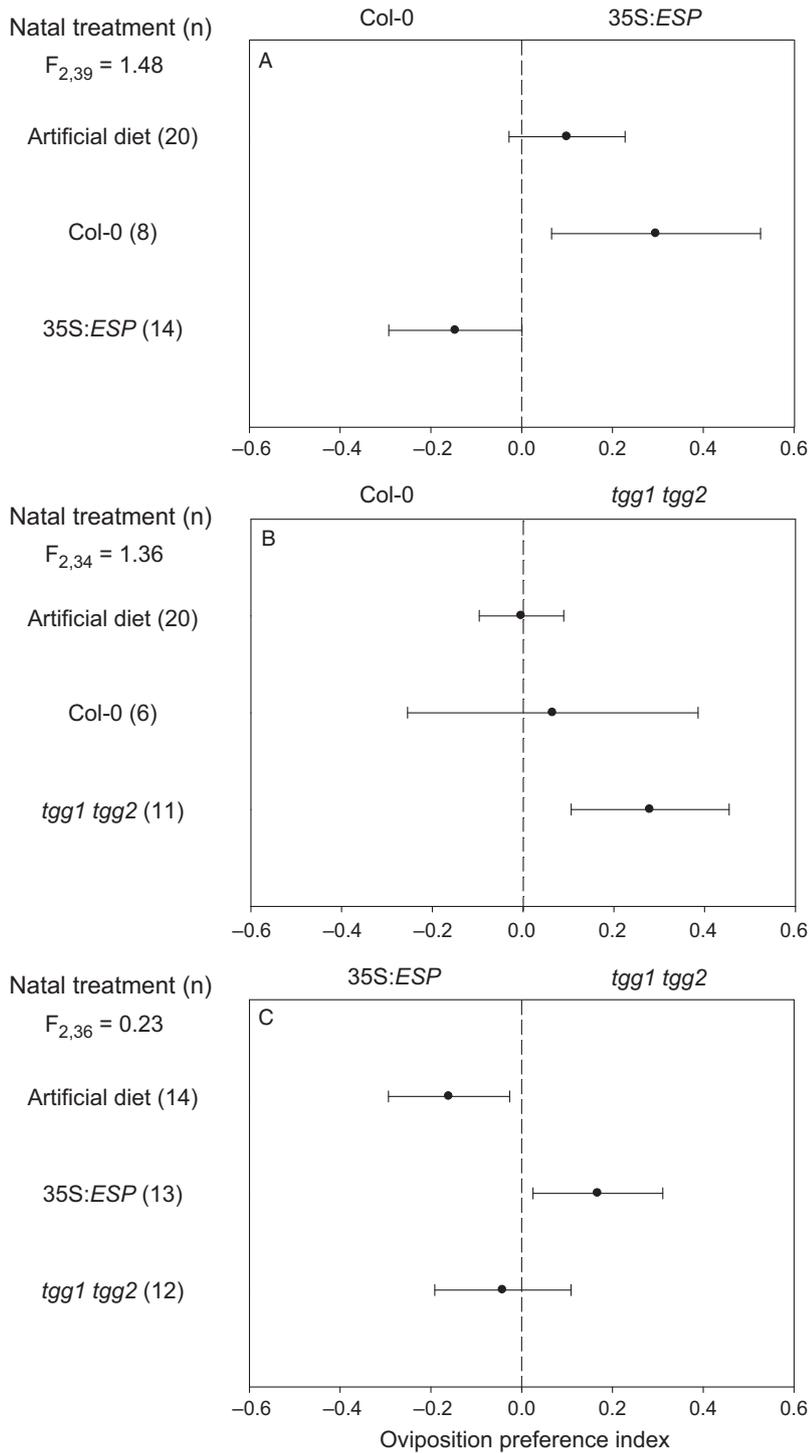


Figure 2 Mean (\pm SE) oviposition preference indices (OPIs) of *Trichoplusia ni* females used in three dual-choice assays including *Arabidopsis thaliana* genotypes that vary in their glucosinolate hydrolysis profiles: (A) Col-0 vs. 35S:ESP, (B) Col-0 vs. tgg1 tgg2, and (C) 35S:ESP vs. tgg1 tgg2. Sample sizes are indicated in parenthesis.

The effect of natal treatment also significantly influenced the total number of eggs laid by females of both moth species (*P. xylostella*: $F_{3,300} = 27.37$, $P < 0.0001$; *T. ni*: $F_{3,115} = 7.00$, $P < 0.001$). *Plutella xylostella* females

raised on any of the plant genotypes (i.e., Col-0, tgg1 tgg2, and 35S:ESP) laid significantly more eggs in all choice assays than females that were reared on artificial diet (Table 1). Conversely, *T. ni* females raised on Col-0 and

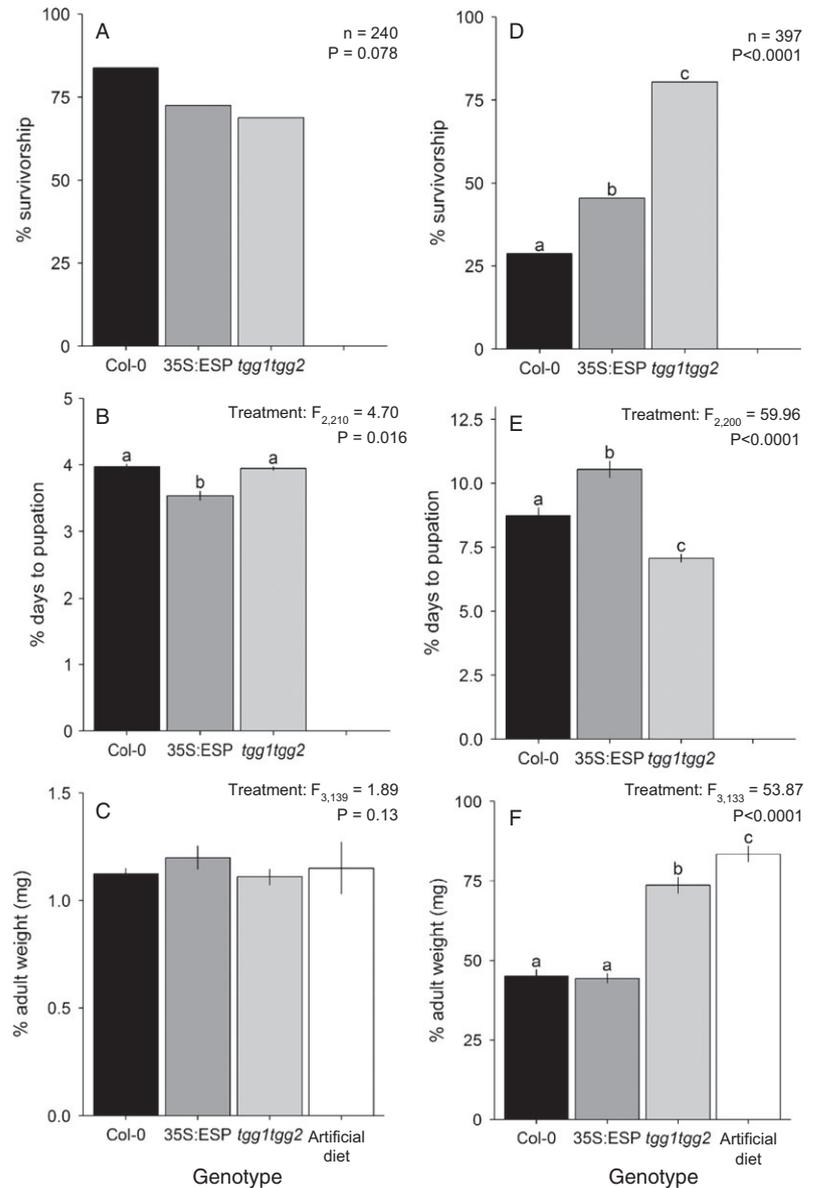


Figure 3 Fitness-related traits (mean \pm SE) of *Plutella xylostella* (A–C) and *Trichoplusia ni* (D–F) reared on artificial diet or different *Arabidopsis thaliana* genotypes during their last two larval stages. Adult survivorship was compared among plant genotypes using Fisher's exact test. Adult weight and time to pupation of moths reared on the different diets were compared using protected LSD pairwise comparisons. Different letters on bars within a panel indicate significant differences among treatment means ($P < 0.05$).

35S:ESP (lines producing hydrolysis products) laid significantly fewer eggs than those reared on artificial diet (Table 1).

Discussion

This study provides experimental evidence that insect experience with different natal treatments can influence plasticity of female oviposition behavior and fitness-related traits in lepidopteran species. There is ample evidence that defense-related secondary chemicals produced by different plant species, such as glucosinolates and their hydrolysis products, influence oviposition of insect herbivores (Halkier & Gershenson, 2006). A few studies have

begun to explore how oviposition preferences are influenced by variation in the secondary chemistry of *A. thaliana* (de Vos et al., 2008; Sun et al., 2009). However, to our knowledge, the effect of natal insect experience with plant genotypes (differing in their glucosinolate hydrolysis profiles) on subsequent oviposition behavior has not yet been investigated. In this study, it was demonstrated that experience with different natal treatments, including artificial diet and three plant lines of *A. thaliana* sharing the same genetic background (Col-0) but differing in their glucosinolate hydrolysis profiles, had a significant effect on oviposition choices of the specialist *P. xylostella*. Conversely, experience with the same plant genotypes did not significantly influence oviposition behavior of the generalist

Table 1 Mean (\pm SE) total number of eggs laid by *Plutella xylostella* and *Trichoplusia ni* females reared on artificial diet or one of three *Arabidopsis thaliana* genotypes

Species of moth	Artificial diet	Col-0	35S:ESP	<i>tgg1 tgg2</i>
<i>P. xylostella</i>	29.58 \pm 1.65a (125)	48.75 \pm 2.94b (64)	50.91 \pm 3.86b (55)	57.2 \pm 3.98b (59)
<i>T. ni</i>	50.93 \pm 6b (54)	17.79 \pm 3.22a (14)	16.30 \pm 2.39a (27)	31.35 \pm 6.12ab (23)

Means followed by different letters are significantly different (LSD pairwise comparisons: $P < 0.05$). Sample sizes in parenthesis.

moth *T. ni*. However, this result was likely influenced by the low sample sizes used for this species oviposition trials and, thus, it does not necessarily reflect species-specific responses. For *P. xylostella*, the role of experience appeared to increase oviposition preferences for those *A. thaliana* lines producing hydrolysis products, such as nitriles and isothiocyanates. Our results support previous findings related to the significance of these volatiles as oviposition cues mediating plant-insect interactions.

In a previous study, de Vos et al. (2008) found that naïve females of the crucifer specialist *Pieris rapae* (L.) exhibited an innate preference for the ITC-producing line Col-0 over the nitrile-producing line 35S:ESP. In our study, *P. xylostella* also appeared to have an innate preference for Col-0 over the 35S:ESP line; however, it was interesting that natal experience with these genotypes altered this innate preference. Experience with Col-0 led to a lack of preference for either Col-0 or 35S:ESP (Figure 1A). Similarly, even though naïve *P. xylostella* females did not show a preference for either Col-0 or 35S:ESP when paired with the double myrosinase knockout (*tgg1 tgg2*), this trend changed after females experienced either Col-0 or 35S:ESP in their natal environments. In other words, experience with either Col-0 or 35S:ESP appeared to cause females to prefer ovipositing on either of these genotypes rather than on *tgg1 tgg2* plants, which have impaired glucosinolate hydrolysis. Lack of preference for wild type Col-0 over the mutant *tgg1 tgg2* by naïve females is consistent with previous studies that explored only the innate preferences of *P. xylostella* and *P. rapae*. However, for *P. xylostella*, this preference was affected by the amount of plant damage; that is, when plants were severely damaged, *P. xylostella* moths preferred the wild type Col-0 over *tgg1 tgg2* (de Vos et al., 2008; Sun et al., 2009). Results from the present study suggest that increased preferences for certain plant genotypes induced by experience may provide a plausible explanation for why insects may be choosing chemotype-specific plant genotypes regardless of the level of plant damage.

In contrast to specialist herbivores, it is commonly assumed that generalist insects are not attracted to, or even repelled by, secondary plant metabolites (Wittstock et al., 2003). Even though results for the generalist *T. ni*

appeared to be consistent with this assumption, we cannot make any conclusions in terms of this species' oviposition preferences, as results could have been associated to the low sample sizes due to high mortality of this species on the plant genotypes Col-0 and 35S:ESP, rather than to species-specific responses. For *P. xylostella*, however, results appear to indicate a preference for plant genotypes producing hydrolysis products over artificial diet or the myrosinase double knockout, which is defective in glucosinolate hydrolysis. These results are in accordance with previous studies showing that genotypes producing hydrolysis products, such as ITCs, can serve as oviposition cues for the specialist *P. xylostella* (Renwick et al., 2006; Sun et al., 2009).

Little is known about the underlying mechanism for the induction of host preference proposed by the NHPI hypothesis (or the Hopkins host selection principle). Previous studies, however, have shown that experience with volatile cues inducing preferences in the larvae of *Spodoptera littoralis* Boisduval (Carlsson et al., 1999), *Manduca sexta* L. (Saxena & Schoonhoven, 1978), and *P. rapae* (Zhou et al., 2009) appeared to be due to increased sensitivity of chemoreceptors. Cunningham & West (2001) and Cunningham (2012) have indicated that 'learning' is involved in changing female oviposition preferences for different host species, and further proposed that 'a fitness cost inherent in the olfactory mechanism could constrain the evolution of insect host selection'. Studies with *Drosophila melanogaster* Meigen have provided compelling evidence for the importance of natal environment in shaping adult olfactory preferences. For instance, Sambandan et al. (2008) found that roughly 50% of the phenotypic variation in adult olfactory behavior of *D. melanogaster* was attributable to gene-environment interactions. Similarly, *D. melanogaster* flies that were deprived of odors in their post-eclosion environment led to a reduction in odor sensitivity. However, as the chemical complexity of the post-eclosion environment increased, greater sensitivity to various odors and the ability to discriminate between them was found (Iyengar et al., 2010). Whether the experience-induced changes in preferences observed for *P. xylostella* females were the result of an increase in sensitivity to intra-specific differences in host plant chemistry is not known,

as the underlying mechanisms for induced plasticity in preference of host chemistry has remained poorly understood in lepidopteran insect herbivores.

Even though we could not compare responses of *P. xylostella* and *T. ni*, in terms of oviposition preferences, we found pronounced fitness-related differences between these two species when reared on artificial diet or on the different plant genotypes. The potential adaptive value of experience-induced plasticity in oviposition preferences could be explained by the preference-performance hypothesis, which predicts that induced preferences will aid in matching adult preference to larval performance as well as in standardizing selective pressures across generations (Stamps, 2001). Assuming that adult performance is indicative of offspring performance, changes in female oviposition preferences induced by experience may lead to an increase in offspring performance or at least prevent a decline in offspring fitness. In this study, fitness-related traits of *T. ni* females, such as survivorship and adult weight, were highly negatively affected by the Col-0 and 35S:ESP genotypes, which produce ITCs and nitriles, respectively. Therefore, we could predict that if these species do not perform well on these genotypes, they may lack a preference for ovipositing on them, which in turn would prevent a decrease in their offspring fitness; however, this prediction would need to be re-tested with larger sample sizes. In contrast, performance of the specialist *P. xylostella* was not or only minimally affected by genotypes producing hydrolysis products and, actually, experience enhanced female oviposition preferences for these genotypes. These results were also in accordance with oviposition choices of both species, in terms of total number of eggs laid on each host genotype. That is, *P. xylostella* females had significantly higher oviposition success when reared on plant genotypes (vs. artificial diet), and *T. ni* laid significantly more eggs on artificial diet than on the plant genotypes. These results could provide support for the preference-performance hypothesis and may suggest that experience-induced plasticity of oviposition preference could be adaptive; however, more research is needed and, specifically, transgenerational studies would be required to confirm this conclusion.

Intraspecific variation in the quality and quantity of plant secondary chemicals has been shown to influence plant utilization patterns by insect herbivores in the field (Bidart-Bouzat & Kliebenstein, 2008), herbivore community structure (Newton et al., 2009), and biodiversity (Poelman et al., 2009). However, the mechanisms underlying herbivore-species specific preferences for particular plant genotypes are poorly understood (Barbour et al., 2009). The finding that insect experience with different natal treatments (i.e., artificial diet or plant geno-

types) can influence insect oviposition preferences, provides a possible mechanism for previously observed variation in patterns of plant use and structuring of insect communities within plant populations. Furthermore, it is possible that this effect of experience may extend up the trophic ladder. Induced preferences for phytochemicals have already been observed in numerous parasitoid wasps (Turling et al., 1993; Storeck et al., 2000), along with innate preferences for chemicals that vary at the intraspecific plant level (Gandolfi et al., 2003; van Emden et al., 2008). For example, when the braconid wasp *Diaeretiella rapae* (McIntosh) was given a choice between purified compounds, such as isothiocyanate (3-isothiocyanatoprop-1-ene) and nitrile (4-isothiocyanatobut-1-ene), in a Y-tube olfactometer test, their preference was dependent upon the plant species fed by their host, the aphid *Brevicoryne brassicae* (L.) (Pope et al., 2008).

Results reported here may have implications not only for the evolutionary ecology of chemically mediated plant-insect interactions but also for pest management, as *A. thaliana* is a member of the economically important Brassicaceae and shares the same chemical defense system with many crucifer crop species (Bidart-Bouzat & Kliebenstein, 2008). Different natal insect experiences of an insect pest may influence oviposition rates, which in turn may affect plant damage levels in both natural and agricultural systems. Our results are in accordance with previous studies performed with *D. melanogaster*, which provided evidence on the important role of the natal environment in shaping adult olfactory preferences (Sambandan et al., 2008; Iyengar et al., 2010). In our study, moths exposed to plant genotypes in their natal environments could have developed an increased olfactory sensitivity or discriminatory ability compared to moths reared in an artificial environment. Information on the potential effects of experience with different natal environments is therefore important for selecting a more effective integrated pest management (IPM) strategy, particularly against devastating crucifer pests, such as *P. xylostella* or *T. ni*. Pre-dispersal experiences should also be considered when evaluating the use of genetically modified crop species, as our results showed that experience with genetically modified plant genotypes differing in their hydrolysis profiles influenced oviposition behavior of an important insect pest. Overall, the integration of the role of experience, plasticity of traits of interacting species, and the contribution of intraspecific plant variation in shaping these plastic responses should provide a more comprehensive framework for future research related to the evolutionary ecology of plant-insect interactions and potential applications for IPM.

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References

- Agrawal AA (2001) Phenotypic plasticity in the interactions and evolution of species. *Science* 294: 321–326.
- Akhtar Y, Shikano I & Isman MB (2009) Topical application of a plant extract to different life stages of *Trichoplusia ni* fails to influence feeding or oviposition behaviour. *Entomologia Experimentalis et Applicata* 132: 275–282.
- Barbour RC, Baker SC, O'Reilly-Wapstra JM, Harvest TM & Potts BM (2009) A footprint of tree-genetics on the biota of the forest floor. *Oikos* 118: 1917–1923.
- Barth C & Jander G (2006) Arabidopsis myrosinases TGG1 and TGG2 have redundant function in glucosinolate breakdown and insect defense. *Plant Journal* 46: 549–562.
- Bidart-Bouzat MG & Kliebenstein DJ (2008) Differential levels of insect herbivory in the field associated with genotypic variation in glucosinolates in *Arabidopsis thaliana*. *Journal of Chemical Ecology* 34: 1026–1037.
- Bidart-Bouzat MG, Mithen R & Berenbaum MR (2005) Elevated CO₂ influences herbivory-induced defense responses of *Arabidopsis thaliana*. *Oecologia* 145: 415–424.
- Burow M, Markert J, Gershenzon J & Wittstock U (2006) Comparative biochemical characterization of nitrile-forming proteins from plants and insects that alter myrosinase-catalysed hydrolysis of glucosinolates. *FEBS Journal* 273: 2432–2446.
- Carlsson MA, Anderson P, Hartlieb E & Hansson BS (1999) Experience-dependent modification of orientational response to olfactory cues in larvae of *Spodoptera littoralis*. *Journal of Chemical Ecology* 25: 2445–2454.
- Chow JK, Akhtar Y & Isman MB (2005) The effects of larval experience with a complex plant latex on subsequent feeding and oviposition by the cabbage looper moth: *Trichoplusia ni* (Lepidoptera: Noctuidae). *Chemoecology* 15: 129–133.
- Clancy KM & Price PW (1987) Rapid herbivore growth enhances enemy attack: sublethal plant defense remain a paradox. *Ecology* 68: 733–737.
- Corbet SA (1985) Insect chemosensory responses: a chemical legacy hypothesis. *Ecological Entomology* 10: 143–153.
- Crutsinger GM, Strauss SY & Rudgers JA (2010) Genetic variation within a dominant shrub species determines plant species colonization in a coastal dune ecosystem. *Ecology* 91: 1237–1243.
- Cunningham JP (2012) Can mechanism help explain insect host choice? *Journal of Evolutionary Biology* 25: 244–251.
- Cunningham JP & West SA (2001) Host selection in phytophagous insects: a new explanation for learning in adults. *Oikos* 95: 537–543.
- Davis JM & Stamps JA (2004) The effect of natal experience on habitat preferences. *Trends in Ecology and Evolution* 19: 411–416.
- van Emden HF, Storeck AP, Douloumpaka S, Eleftherianos I, Poppy GM & Powell W (2008) Plant chemistry and aphid parasitoids (Hymenoptera: Braconidae): imprinting and memory. *European Journal of Entomology* 105: 477–483.
- Gandolfi M, Mattiacci L & Dorn S (2003) Preimaginal learning determines adult response to chemical stimuli in a parasitic wasp. *Proceedings of the Royal Society of London B* 270: 2623–2629.
- Halkier BA & Gershenzon J (2006) Biology and biochemistry of glucosinolates. *Annual Review of Plant Biology* 57: 303–333.
- Harcourt DG (1954) The Biology and Ecology of the Diamondback Moth, *Plutella maculipennis* Curtis, in Eastern Ontario. PhD Dissertation, Cornell University, Ithaca, NY, USA.
- Honěk A (1993) Intraspecific variation in body size and fecundity in insects – general relationships. *Oikos* 66: 483–492.
- Hopkins AD (1916) Economic investigations of the scolytid bark and timber beetles of North America. USDA, Program of Work for the U.S. Dept. of Agriculture 1916/1917: 353.
- Iyengar A, Chakraborty TS, Goswami SP, Wu CF & Siddiqi O (2010) Post-eclosion odor experience modifies olfactory receptor neuron coding in *Drosophila*. *Proceedings of the National Academy of Sciences of the USA* 107: 9855–9860.
- Lambrix V, Reichelt M, Mitchell-Olds T, Kliebenstein DJ & Gershenzon J (2001) The *Arabidopsis* epithiospecifier protein promotes the hydrolysis of glucosinolates to nitriles and influences *Trichoplusia ni* herbivory. *Plant Cell* 13: 2793–2807.
- Liu SS & Liu TX (2006) Preimaginal conditioning does not affect oviposition preference in the diamondback moth. *Ecological Entomology* 31: 307–315.
- Liu SS, Li YH, Liu YQ & Zalucki MP (2005) Experience-induced preference for oviposition repellents from a non-host plant by a specialist herbivore. *Ecology Letters* 8: 722–729.
- Louda S & Mole S (1991) Glucosinolates: chemistry and ecology. *Herbivores: Their Interactions with Secondary Plant Metabolites*, Vol. 1: The Chemical Participants (ed. by GA Rosenthal & MR Berenbaum), pp. 123–164. Academic Press, San Diego, CA, USA.
- Mabry KE & Stamps JA (2008) Dispersing brush mice prefer habitat like home. *Proceedings of Royal Society of London B* 275: 543–548.
- Mumm R, Burow M, Bukovinszkyne-Kiss G, Kazantzidou E, Wittstock U et al. (2008) Formation of simple nitriles upon glucosinolate hydrolysis affects direct and indirect defense against the specialist herbivore, *Pieris rapae*. *Journal of Chemical Ecology* 34: 1311–1321.
- Newton EL, Bullock JM & Hodgson DJ (2009) Glucosinolate polymorphism in wild cabbage (*Brassica oleracea*) influences the structure of herbivore communities. *Oecologia* 160: 63–76.
- Payne RB, Payne LL, Woods JL & Sorenson MD (2000) Imprinting and the origin of parasite-host species associations in

- brood-parasite indigobirds, *Vidua chalybeate*. *Animal Behavior* 59: 69–81.
- Poelman EH, van Dam NM, van Loon JJA, Vet LEM & Dicke M (2009) Chemical diversity in *Brassica oleracea* affects biodiversity of insect herbivores. *Ecology* 90: 1863–1877.
- Pope TW, Kissen R, Grant M, Pickett JA, Rossiter JT & Powell G (2008) Comparative innate responses of the aphid parasitoid *Diaeretiella rapae* to alkenyl glucosinolate derived isothiocyanates, nitriles, and epithionitriles. *Journal of Chemical Ecology* 34: 1302–1310.
- Rask L, Andreasson E, Ekblom B, Eriksson S, Pontoppidan B & Meijer J (2000) Myrosinase: gene family evolution and herbivore defense in Brassicaceae. *Plant Molecular Biology* 42: 93–113.
- Ratzka A, Vogel H, Kliebenstein DJ, Mitchell-Olds T & Kroymann J (2002) Disarming the mustard oil bomb. *Proceedings of the National Academy of Sciences of the USA* 99: 11223–11228.
- Reddy GVP, Tabone E & Smith MT (2003) Mediation of host selection and oviposition behavior in the diamondback moth *Plutella xylostella* and its predator *Chrysoperla carnea* by chemical cues from cole crops. *Biological Control* 29: 270–277.
- Renwick JAA, Haribal M, Gouinguene S & Städler E (2006) Isothiocyanates stimulating oviposition by the diamondback moth, *Plutella xylostella*. *Journal of Chemical Ecology* 32: 755–766.
- Sambandan D, Carbone MA, Anholt RRH & Mackay TFC (2008) Phenotypic plasticity and genotype by environment interaction for olfactory behavior in *Drosophila melanogaster*. *Genetics* 179: 1079–1088.
- Saxena KN & Schoonhoven LM (1978) Induction of orientational and feeding preferences in *Manduca sexta* larvae for an artificial diet containing citral. *Entomologia Experimentalis et Applicata* 23: 72–78.
- Shikano I & Isman MB (2009) A sensitive period for larval gustatory learning influences subsequent oviposition choice by the cabbage looper moth. *Animal Behavior* 77: 247–251.
- Shreeve TG, Dennis RLH & Van Dyck H (2004) Resources, habitats and metapopulations – whither reality? *Oikos* 106: 404–408.
- Stamps JA (2001) Habitat selection by disperses: integrating proximate and ultimate approaches. *Dispersal* (ed. by J Clobert, E Dauchin, AA Dhont & JD Michaels), pp. 230–242. Oxford University Press, New York, NY, USA.
- Storeck A, Poppy GM, van Emden HF & Powell W (2000) The role of plant chemical cues in determining host preference in the generalist aphid parasitoid *Aphidius colemani*. *Entomologia Experimentalis et Applicata* 17: 297–304.
- Sun JY, Sonderby IE, Halkier BA, Jander G & de Vos M (2009) Non-volatile intact indole glucosinolates are host recognition cues for ovipositing *Plutella xylostella*. *Journal of Chemical Ecology* 35: 1427–1436.
- Thomas JA, Bourn NAD, Clarke RT, Stewart KE, Simcox DJ et al. (2001) The quality and isolation of habitat patches both determine where butterflies persist in fragmented landscapes. *Proceedings of the Royal Society of London B* 268: 1791–1796.
- Turling TCJ, Wäckers FL, Vet LEM, Lewis WJ & Tumlinson JH (1993) Learning of host-finding cues by hymenopterous parasitoids. *Insect Learning: Ecological and Evolutionary Perspectives* (ed. by DR Papaj & AC Lewis), pp. 51–173. Chapman and Hall, New York, NY, USA.
- de Vos M, Kriksunov KL & Jander G (2008) Indole-3-acetonitrile production from indole glucosinolates deters oviposition by *Pieris rapae*. *Plant Physiology* 146: 916–926.
- Wang H, Guo WF, Zhang PJ, Wu ZY & Liu SS (2008) Experience-induced habituation and preference towards non-host plant odors in ovipositing females of a moth. *Journal of Chemical Ecology* 34: 330–338.
- Wittstock U, Kliebenstein DJ, Lambrix V, Reichelt M & Gershenzon J (2003) Glucosinolate hydrolysis and its impact on generalist and specialist insect herbivores. *Integrative Phytochemistry: From Ethnobotany to Molecular Ecology* (ed. by JT Romeo), pp. 101–126. Elsevier, Amsterdam, The Netherlands.
- Zhang P, Liu S, Wang H & Zalucki MP (2007) The influence of early adult experience and larval food restriction of responses toward nonhost plants in moths. *Journal of Chemical Ecology* 33: 1528–1541.
- Zhou DS, Wang CZ & van Loon JJA (2009) Chemosensory basis of behavioural plasticity in response to deterrent plant chemicals in the larva of the small cabbage white butterfly *Pieris rapae*. *Journal of Insect Physiology* 55: 788–792.