EVOLUTIONARY ECOLOGY OF *ARABIDOPSIS THALIANA*: INTERACTIONS WITH BIOTIC AND ABIOTIC ENVIRONMENTAL FACTORS

by

Adebobola O. Imeh-Nathaniel

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Committee:

Dr. Maria G. Bidart- Bouzat
Dr. Juan L. Bouzat
Dr. Ron Woodruff
Dr. Dan Pavuk
Dr. Maria Rizzo (Graduate Faculty Representative)
ABSTRACT

Dr. M. Gabriela Bidart-Bouzat, Advisor

Previous studies have demonstrated that plants may be affected by their interactions with both biotic and abiotic environmental factors. An important biotic factor that usually affects the performance of plants is insect herbivory. In addition, abiotic factors such as light, or more specifically ultraviolet B (UVB) radiation, can also affect growth and physiological processes in plants. *Arabidopsis thaliana* is an annual herbaceous plant species with documented defense strategies including resistance and tolerance. Studies have also shown that these plant responses to insect herbivory may be modified by other abiotic factors such as UVB radiation. In a series of common garden experiments, the role of insect herbivory *per se* and in combination with UVB radiation was evaluated. Using a population of recombinant inbred lines of *A. thaliana*, the first experiment (described in Chapter 1) investigated the role of plant trichomes and size on patterns of plant use by an insect community. In this experiment, it was also evaluated whether insect herbivory imposes selection on trichome production and plant size. In a second experiment (described in Chapters 2 and 3), the effect of UVB radiation on plant resistance and tolerance to insect herbivory as well as on their potential fitness costs was assessed. This experiment also evaluated the role of UVB on patterns of plant utilization by insect herbivores and whether this important abiotic factor may influence plant phenotypic responses. Results from these experiments revealed that plant trichomes influenced levels of herbivore damage and plant size had an effect on colonization of plants by insect herbivores. In addition, results from selection analyses revealed that insect herbivores exerted directional selection on trichome density in *A. thaliana*. The second study showed that UVB radiation influenced the expression
of resistance and tolerance as well as their associated fitness costs. Similarly, patterns of plant utilization by insect herbivores and phenotypic responses of plants to insect herbivory were modified by UVB radiation. Overall, these results highlight the importance of evaluating complex environments, including both abiotic and biotic factors, as it relates to the evolution and maintenance of traits related to plant defense against insect herbivory.
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CHAPTER 1

INTRASPECIFIC GENETIC VARIATION IN PLANT SIZE AND TRICHOME DENSITY INFLUENCES PATTERNS OF PLANT USE BY AN INSECT COMMUNITY

ABSTRACT

Intraspecific genetic variation in plant traits can have important consequences for the insect community utilizing plant resources for feeding and reproduction. For example, variation in a plant structural defensive trait such as trichome density can influence levels of herbivore damage. In addition, variability in plant size may affect host choice by insect herbivores since this trait can serve as a visual cue for insects to locate a plant host species as well as provide variable surface areas for insect oviposition and feeding. Despite the importance of these plant traits, little is known regarding how genetic variation may influence plant damage levels and colonization of plants by a natural insect community. Similarly, few studies have evaluated whether insect herbivores impose selection on trichome production and plant size. Results from a common garden experiment revealed the presence of genetic variation in trichome density and plant size in a population of recombinant inbred lines of *Arabidopsis thaliana*. Genetic variation in these traits significantly influenced both colonization of plants by insect herbivores and levels of herbivore damage among plants. Selection analyses revealed directional selection favoring a reduction in trichome density in the absence of insect herbivory (sprayed plots), which may be indicative of a fitness cost related to the production of this defensive trait. In terms of plant size, selection favored larger plants in field plots exposed to insect herbivory. These results corroborate the role of plant trichomes as a resistant trait favored by selection in the presence of
herbivory and emphasize the understudied role of plant size as an important cue in herbivore colonization of plants in the field.
INTRODUCTION

Plants have evolved different morphological structures such as trichomes and thorns, as well as a wide variety of secondary chemical compounds including glucosinolates and phenolics, which act as defense mechanisms against insect herbivore attacks (Levin 1973; Zangerl and Bazzaz, 1992; Strauss and Agrawal, 1999; Rautio et al., 2002). Although chemical responses of plants to insect herbivores have been widely studied (Bennett and Wallsgrove 1994; McConn et al. 1997; Clauss et al. 2006; McConn et al. 1997; Kliebenstein 2004; Bidart-Bouzat et al. 2005), little is known regarding how genetic variation in structural defensive traits may influence plant damage levels and colonization of plants by natural insects. Trichomes are hair-like outgrowths derived from the epidermal cells of plants that may have defensive function against herbivore attacks (Figure 1). Despite potentially important roles of trichomes in plant defense, most of the evidence suggesting that trichomes may be involved in defense were obtained from studies conducted under laboratory conditions using insect herbivores or through simulated herbivory (Traw and Bergelson 2003; Clauss et al. 2006). Thus, these studies may not directly reflect the role of trichomes on natural levels of insect herbivory. In addition, more information is required on how genetic variation in trichome density influence patterns of plant utilization by insect herbivores in natural populations. This is important because the ability of plants to respond to herbivory in an evolutionary manner will mostly depend on the availability of genetic variation in traits related to fitness.

Trichomes occur in different parts of the plants such as stems, leaves, and reproductive organs, and vary within and/or between populations (Strauss and Agrawal 1999; Handley et al. 2005). They may also vary in their structure, ranging from unicellular to multicellular, and
anatomy, which may be branched, unbranched, spiral or hooked (Ouwerkerk et al. 1999; Hulskamp 2000). In addition, trichomes can be characterized as glandular or non-glandular based on their ability to secrete chemical materials such as terpenes, alkaloids, or fatty acid derivatives (Schilmiller et al. 2008; Wang et al. 2008). The presence of trichomes in many plant species has been associated with many physiological and defensive functions. Leaf trichomes may reduce insect herbivory by making leaves less attractive to herbivores, trapping small insects, and reducing mobility and nutritional quality of leaves, which may in turn, influence oviposition rates, egg hatching, larval growth, and survival. Glandular trichomes also produce exudates that may be toxic or serve as repellant to insect herbivores (Perez-Estrada et al. 2000; Simmons et al., 2004; Wang et al. 2004; Handley et al. 2005). In addition to the role of trichomes in defense against herbivores, previous studies have also shown that trichomes may be beneficial to herbivores and pathogens trapping natural enemies of insect herbivores (Turlings and Benrey 1998; Lovinger et al. 2000; Kennedy 2003).

Apart from the role of trichomes in plant-herbivore interactions, there is evidence that they play multiple functions in plants other than that of defense against herbivores. For example, leaf trichomes may serve the purpose of resistance to other abiotic stress such as drought by reflecting light and reducing evapo-transpiration rates or by facilitating condensation of moisture onto the plant surface (Ehleringer 1984; Larcher 2001), as well as removal of excess salt (Gravano et al. 1998) and heavy metals (Sarret et al. 2006). In addition, trichomes may protect living cells from the harmful effects of ultraviolet radiation and extreme temperature changes (Skaltsa et al. 1994; Werker 2000; Agrawal et al. 2004). Since all of these functions
may act in concert or separately in different plant species, experimental approaches are required to determine whether trichomes play an important role in plant resistance to insect herbivory.

Although the presence of trichomes can reduce levels of herbivore damage (Clauss et al. 2006; Handley et al. 2005), less information is known about the role of other phenotypic traits such as plant size on plant utilization patterns by insect herbivores in the field. Plant size is an important phenotypic trait that can affect not only the levels of damage to plants, but also host choice by herbivores. This is because herbivores use both olfactory and visual cues to choose host plants for oviposition (Singer 2004; Costa et al. 2009; Reeves et al. 2009). Several studies have also suggested that larger plants are more likely to be first (and more often) colonized than smaller plants, since plant size may be a reflection of vigor and nutrient availability, which in turn may enhance herbivore performance (Heisswolf et al. 2005; Lastra et al. 2006). It is also possible that bigger plants would be a better choice for oviposition purposes due to their ability to provide a larger surface area for oviposition, especially in herbivores that produce large amounts of eggs. For example, herbivores may deposit a cluster of eggs on a host plant that has a large surface area for oviposition (Wahlberg et al. 2004; Saastamoinen 2007), whereas less eggs per plant may be deposited on plants with smaller surface areas (Courtney 1982). Thus, time (and costs) expended by insects on searching for suitable plants for oviposition may be reduced when insects locate larger sized plants with larger surface areas (Nieminin et al. 2003; Kuussaari et al. 2004; Singer 2004).

In a common garden experiment, the presence of natural variation in trichome density and plant size was assessed in a population of recombinant inbred lines of *Arabidopsis thaliana*. In addition, it was determined whether this variation influenced the patterns of plant use by
insect herbivores in the field (i.e., order of herbivore colonization and levels of herbivore damage). It was also tested whether variation in trichome density, plant size, and herbivore damage levels was significantly associated to plant fitness. Evaluation of such link between the trichome density, levels of herbivore damage, and plant fitness is important because differences in herbivore damage may impose selection for different types of defensive traits. Finally, this experiment evaluated whether selection was acting on plant size and trichome density, and whether insect herbivores were indeed an important selective pressure on these traits. As summarized in Mauricio and Rausher (1997), three conditions should be met for showing that a plant character that influences levels of insect herbivory (e.g., trichome density or plant size), is under selection by the herbivores. First, the character has to be genetically variable (potential to evolve). Second, variation in the studied character must influence levels of herbivore damage, and has to be associated with plant fitness. Lastly, it has to be demonstrated that the presence of insect herbivores indeed influences selection estimates; that is, that upon removal of the selective pressure imposed by insect herbivory, estimates of selection are modified. In this experiment, selection on plant size was measured only in the presence of herbivores but selection on trichomes was evaluated in both the presence and absence of herbivores. Specifically, insect herbivores were removed from half of the field plots, and potential changes in the patterns of selection on trichome density were evaluated by comparing selection gradients for this character in the presence and absence of insect herbivores.
MATERIALS AND METHODS

Study Plant

Arabidopsis thaliana (L.) Heynh (Brassicaceae) commonly known as the mouse ear cress is an annual herbaceous weed, which is native to Europe and Asia but has spread its distribution to North America and Africa. This plant species is naturally found in open or disturbed habitats, sandy soils, riverbanks, roadsides, rocky slopes, and meadows (Baskins and Baskins 1983). Arabidopsis thaliana has a short life cycle lasting between 6-8 weeks from germination to senescence and it is a mostly self-fertilizing species with less than 1% outcrossing rates (Hoffmann et al. 2003). Trichomes in A. thaliana are usually unicellular, branched, and non-glandular (Hulskamp and Kirik 2000). In this study, we used a population of recombinant inbred lines (100 RILs), which was derived from a cross between the ecotypes Columbia (Col-0) and Landsberg (Ler), and advanced to the eighth generation through a single seed descent (Lister and Dean 1993). Arabidopsis thaliana RILs from this cross were used because a previous study showed variation in trichome density between the two parental lines, Col-0 and Ler ecotypes (Mauricio 2005). Therefore, the use of this recombinant inbred line population allows evaluating whether genetic variation in trichome density may influence natural patterns of insect herbivory.

Experimental Design and Plant Growth Conditions

The experimental design consisted of a split-plot with two levels of herbivory treatment (i.e., natural insect herbivores present and excluded) assigned to ten plots distributed in a checkerboard pattern (Figure 2). Each of these treatments was replicated in five plots accounting for a total of ten plots. Naturally occurring insect herbivores were excluded in control plots using an insecticide (Proclaim by Syngenta Inc. USA) at intervals of ten days throughout the
experimental period. Three replicates of the 100 RILs were randomly assigned to each of the 10 plots (total of 3000 plants). For both treatments, mammalian herbivores were excluded by using wire mesh fencing.

Several species of insect herbivores are known to attack *A. thaliana* plants in the field. Bidart-Bouzat and Kliebestein (2008) reported the presence of diamondback moths (*Plutella xylostella*; Plutellidae), striped and western cabbage flea beetles (*Psylloides striolata* and *Psylloides pusilla*, respectively; Chrysomelidae), cabbage aphids (*Brevicoryne brassicae*; Aphididae) and onion thrips (*Thrips tabaci*; Thripidae) as natural herbivores observed feeding on different *A. thaliana* genotypes. Other studies (Mauricio 1998; Mosleh Arany et al. 2005; Arany 2008) have also reported other insect herbivores feeding on field-grown *A. thaliana* plants including the cabbage looper *Tricoplasia ni* (Noctuidae), *Spodoptera exigua* (Noctuidae), European cabbage butterfly *Pieris rapae* (Pieridae), and specialist weevils *Ceutorhynchus atomus* and *C. contractus* (Curculionidae). During this field study, the most common herbivore observed attacking *A. thaliana* plants was the specialist diamondback moth (*Plutella xylostella*). The generalist cabbage looper (*Tricoplasia ni*) and the western cabbage flea beetles *Psylloides pusilla* were also observed feeding on the plants, but causing minor effects.

In terms of growth conditions, seeds were cold-treated for 5 days at 4°C to break dormancy, and then, transferred to growth chambers for germination. Within the chambers, plants were exposed to 10 hours of light and 14 hours of dark cycles, with day and night temperatures of 20°C and 16°C, respectively. Plants were rotated within growth chambers every other day and among chambers every week to avoid chamber and position effects. After five
weeks of growth in the chambers, plants were transplanted to the field (Ecological Research Station, Bowling Green State University, Bowling Green, Ohio).

**Measurement of Plant Traits**

In this experiment, the following plant traits were measured: trichome density, rosette diameter, and total fruit number. Trichome density was measured using digital images of the youngest fully extended rosette leaf per plant at bolting time. Leaf area was estimated using image J software (rsbweb.nih.gov/ij/). Trichome density was calculated as the total number of trichomes (on the upper side of the leaf) divided by leaf area. In order to evaluate if trichomes are inducible in the presence of herbivores, we compared trichome density in sprayed plants to plants exposed to insect herbivores. To estimate plant size, rosette diameter was measured as the widest length of the plant rosette. A previous study has shown rosette diameter to be strongly correlated with rosette area, a measure of plant size (Mauricio 1998).

Fitness was estimated as the lifetime fruit production and relative fitness as absolute fitness divided by the mean absolute fitness per herbivory level. Total fruit production was previously shown to be strongly correlated with total seed production in *Arabidopsis thaliana* (Mauricio and Rausher 1997; Bidart-Bouzat 2004). Since selection operates at a population level, and this selection is based on the differences in fitness between individuals; therefore, the use of relative fitness is more appropriate than using absolute fitness (Corner and Hartl 2004).

To assess whether plant genotype affected the order of colonization of insect herbivores among plants, all plants were inspected daily for insect damage and the numbers of days to first observed herbivore damage on each plant was recorded herein, referred to as colonization. To estimate insect herbivore damage to the plant rosette leaves (i.e., percent area removed by
herbivores), a qualitative leaf damage index adapted from Bidart-Bouzat et al. (2005) was used. This index was calculated by assigning leaves to three size categories based on leaf length (i.e., small: 0-3cm, medium: 3-6cm, and large: 6-9cm), and three damage categories based on the amount of tissue removed (i.e., <25%, 25-75%, and >75).

**Statistical Analysis**

To evaluate the effects of RILs (i.e., genotype) and block on trichome density, rosette diameter, days to first observed herbivore damage, rosette damage, and total number of fruits, analyses of variance using a linear random model (SAS statistical software, version 9.1) were performed. Data was either log- or ranked-transformed to correct for normality and heteroscedasticity, when necessary. In addition, we performed a series of analyses of covariance to infer whether the associations between the response variables: herbivore colonization and damage, and the covariates: trichome density and rosette diameter (estimate of plant size) were affected by the plant genotype (RIL) using the model: \( Y = \text{covariate} + \text{RIL} + \text{covariate} \times \text{RIL} + \text{error} \).

To evaluate patterns of selection on trichome density, plant size, order of herbivore colonization and rosette damage, a regression analysis was used to estimate directional (\( \beta \)) and stabilizing/disruptive (\( \gamma \)) selection gradients. In addition, it was determined whether herbivores were exerting selection on trichome density, by performing analyses of variance on the directional and stabilizing selection gradients to compare patterns of selection on trichome density in the presence and absence of natural herbivores (Lande and Arnold 1983; Mauricio and Rausher 1997). For this purpose, a mixed linear (for directional selection) and quadratic (for stabilizing selection) models were used, considering the herbivory treatment as a fixed effect and
RIL as a random effect. The effect of block and its interactions were removed from this model because they were not significant.

RESULTS

Analyses of variance revealed that all traits measured in this study were highly variable (Table 1). Effects of RIL and herbivory on trichome density were significant (P= <0.0001) and plant responses to herbivory were found to be dependent on the RIL (i.e., Herbivory × RIL interaction: P= 0.0233). In addition, the effect of RIL on plant size, days to herbivore colonization, and rosette damage were all statistically significant.

Analyses of covariance showed that variation in trichome density significantly influenced herbivore damage levels but it did not affect days to colonization of plants by insect herbivores (Figures 3A and 3B). On the other hand, plant size (rosette diameter) significantly influenced both the number days to herbivore colonization and herbivore damage levels (Figures 3C and 3D). In any case, the effect of RIL (plant genotype) was significant in all ANCOVA models. In addition, the relationship between the covariates and both damage and colonization was not significantly affected by RIL (Figures 3A-D). These results suggest that trichome density and plant size may play an important role in plant susceptibility/resistance to insect herbivory as well as in determining patterns of plant colonization by insect herbivores in the field.

Regarding the patterns of selection on variables related to plant size and trichome density, directional regression analyses revealed that relative fitness was inversely associated with trichome density in sprayed plots but not in the plots exposed to insect herbivory (Figure 4). In other words, selection favored genotypes with lower trichome density in sprayed plots but not in
plants exposed to insect herbivores. Conversely, plant fitness was higher as plant size increased (Figure 5), which implies that genotypes that produce larger plants are favored by selection. There was no evidence of stabilizing or disruptive selection acting on either plant size or trichome density. In addition, regression analyses did not indicate any form of selection acting on days to herbivore colonization (Figure 6A); there was a borderline significant selection differential however, indicating directional selection favoring a decrease in rosette damage (Figure 6B).

In order to detect whether insect herbivores are exerting selection on trichome density, we compared general patterns of selection on trichome density in the presence and absence of natural herbivores using both directional and stabilizing gradients of selection (Tables 2A and 2B). Results revealed that the effect of herbivory on directional selection for trichome density (i.e., trichome × herbivory interaction) was significant but the quadratic term for trichome density was not significant, suggesting that insect herbivores are exerting directional selection but not stabilizing selection on trichome density.

**DISCUSSION**

Herbivores are known to be attracted to their host plants through visual and olfactory cues (Costa et al. 2009; Reeves et al. 2009; Singer 2004). These cues include plant volatiles (Costa et al. 2009; Szendrei and Isaacs 2005), plant size (Handley et al. 2005), shape, orientation and plant age, which may in turn influence the type and quantity of volatiles released (Moreau et al. 2003; Vergés et al. 2007; Wentzell and Kliebenstein 2008). This study assessed the role of trichome density and plant size in determining patterns of plant utilization by insect herbivores in
the field. Results from this study showed that larger plants were the first to be colonized by insect herbivores. This result further supports previous studies that have shown that plant size may be important in host preference (Nieminen et al. 2003; Kuussaari et al. 2004; Heisswolf et al. 2005; Lastra et al. 2006). First, larger plants may be colonized in preference of smaller plants because they are visually more conspicuous to ovipositing females when compared with smaller plants. Second, larger plants may be advantageous to herbivores in that they may be more suitable for larval development. This is because larvae movement may be restricted during early instars; thus, herbivores may show more preference for a plant that provides larger surface area and more nutrients (Nieminen et al. 2003; Kuussaari et al. 2004). In addition, the plant vigor hypothesis also predicts that vigorous plants with larger size will be preferred by herbivores because they are more likely to have higher biomass and nutritional quality than smaller plants (Heisswolf et al. 2005; Lastra et al. 2006).

Furthermore, this study experimentally demonstrated the role of trichomes in resistance against insect herbivores in the annual crucifer *A. thaliana*. Herbivore damage levels significantly decreased with increased trichome production. In addition, the exclusion of herbivores resulted in a change in selection on trichome production, demonstrating that insect herbivory is indeed an agent of selection on this character. These results are in agreement with other studies that have shown that the presence of trichomes may lead to a reduction in the levels of insect damage. For example, Mauricio (1988), in a field experiment, demonstrated that leaf trichomes led to a decrease in herbivore damage in 144 natural populations of *A. thaliana*. Similarly, leaf trichomes have been found to be associated with reduced level of herbivory in the closely related *Arabidopsis lyrata* (Handley et al. 2005; Loe et al. 2007), as well as other
members of the Brassicaceae plant family (Lamb 1980; Agren and Schemske 1993). With respect to plant size, larger plants had more damage than smaller plants. This further supports the earlier observation that insect herbivores may link larger size to higher nutritional quality for larval development. Additionally, larger plant size may indirectly imply better larval performance by making them less vulnerable to plant defense traits if, as previously suggested, more resources are allocated to vegetative growth at the expense of allocation to defense (Obeso 2002).

In order to investigate patterns of selection on trichome density, plant size, and plant utilization by insect herbivores, regression analyses of these variables on relative fitness were performed. Directional selection on trichome density was observed, which favored a decrease in the production of this defensive character in sprayed plots. Conversely, no evidence of selection acting on this character was found in plots exposed to insect herbivores. This result suggests the existence of a cost of trichome production in the absence of insect herbivory (Bergelson and Purrington 1996; Mauricio 1998). It is interesting to note that in contrast to what was expected, there was no evidence of directional selection for increase in trichome production in the presence of herbivory. In other words, since results showed that trichomes reduced levels of insect herbivore damage and that there was negative selection acting against herbivore damage, then, it was expected that selection would favor an increase in defense related characters such as trichomes in the presence of herbivores (Valverde et al. 2001). The lack of positive selection for trichome density in the presence of insect herbivores may imply the presence of a cost of resistance; that is, production of trichomes is expensive and the benefit derived in terms of resistance may not compensate for its cost. This may also explain the observed directional
selection for decreased trichome production in the sprayed plots when the benefit of resistance to herbivory was absent. This result was in agreement with a previous study by Mauricio (1998) in which trichome density was negatively associated with relative fitness, indicating the presence of a fitness cost for trichome production (but see Valverde 2001). Another possible explanation for this result could be that the levels of herbivore damage observed were not large enough to reverse the direction of selection on trichome production in the A. thaliana populations used in this study. Alternatively, there may have not been enough time for the A. thaliana population to respond to the presence of natural herbivores (Mauricio and Rausher 1997). This is because genotypes used in this study are recombinant inbred lines, which are not originated from the study area. Therefore, it may require a longer period of time in order to observe a shift in the direction of selection in this population. Anyhow, the direction of selection did show a shift towards positive selection, but the shift was not sufficiently large to be significant. Taken these results together, we predict that the cost of resistance may be acting to slow down the shift in the direction of selection for trichome density and that given a longer period of time under the same environmental conditions this selection gradient may become positive.

In spite of the positive association between plant size and both herbivore damage and colonization, selection analyses revealed that selection favored larger plant size. Selection for larger plant size suggests that mechanisms leading to tolerance of insect herbivory may be favored (Paige 1992; Juenger and Bergelson 2000; Rautio et al. 2002; Freeman et al. 2003; Gonzalez-Teuber and Gianoli 2007); for example, by increased vegetative biomass growth compensating for plant tissue lost to herbivory. This is an alternative strategy of plants to minimize the detrimental effects of herbivory on fitness. Paige and Whitham (1987) have
documented this phenomenon of compensation in the biennial *Ipomopsis aggregata*, which showed a release of apical meristem dominance and the proliferation of lateral branches when browsed by mammalian herbivores like mule deer and elk. This study also demonstrated that insect herbivores impose selection on trichome density, which was documented by a significant effect of insect herbivores in modifying patterns of directional selection for trichome density. To date, only two studies have demonstrated this criterion for trichome density in the field, despite the claim that co-evolution is the main driving force for the evolution of resistance to insect herbivory (Mauricio and Rausher 1997; Valverde et al. 2001). This is because any claim that an environmental factor is having an evolutionary impact on a character must be able to demonstrate the existence of genetic variation in the character of interest, a link between variation in the resistance character and fitness, and most importantly, that the presence of the selective agent is imposing direct selection on the character. Two previous studies (Mauricio and Rausher 1997; Valverde et al. 2001) as well as the present study have experimentally manipulated the presence of the selective agent (insect herbivores) to show how its absence influenced patterns of selection on trichome density. Although this study does not rule out the possibility of other environmental factors simultaneously imposing selection on trichomes, it does support the idea that insect herbivores are indeed important selective agents influencing the evolution of defense structures in plants.
Table 1. ANOVA-F values showing the presence of genetic variation (RIL factor) in measured phenotypic characters.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Trichomes</th>
<th>Plant size</th>
<th>Days to colonization</th>
<th>Rosette damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>20.90***</td>
<td>21.67***</td>
<td>0.08</td>
<td>16.01***</td>
</tr>
<tr>
<td>Herbivory</td>
<td>21.16***</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>RIL</td>
<td>2.47***</td>
<td>3.36***</td>
<td>1.34*</td>
<td>1.81***</td>
</tr>
<tr>
<td>Herbivory x RIL</td>
<td>1.31*</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Significance levels * P<0.05, ** P<0.01, *** P<0.001
Table 2A. Analysis of variance for relative fitness showing the effects of insect herbivores on directional selection for trichomes in *A. thaliana*.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIL</td>
<td>1.61</td>
<td>0.0095</td>
</tr>
<tr>
<td>Herbivory</td>
<td>0.48</td>
<td>0.4900</td>
</tr>
<tr>
<td>Trichome Density</td>
<td>0.03</td>
<td>0.8654</td>
</tr>
<tr>
<td>Herbivory x Trichome Density</td>
<td>3.94</td>
<td>0.0499</td>
</tr>
<tr>
<td>Error</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2B. Analysis of variance for relative fitness showing the effects of insect herbivores on stabilizing/disruptive selection for trichomes in *A. thaliana*.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIL</td>
<td>1.52</td>
<td>0.0211</td>
</tr>
<tr>
<td>Herbivory</td>
<td>0.56</td>
<td>0.4546</td>
</tr>
<tr>
<td>Trichome Density</td>
<td>0.20</td>
<td>0.6538</td>
</tr>
<tr>
<td>Trichome Density sq</td>
<td>0.10</td>
<td>0.7579</td>
</tr>
<tr>
<td>Herbivory x Trichome Density sq</td>
<td>0.18</td>
<td>0.6713</td>
</tr>
<tr>
<td>Error</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 1. Picture of *Arabidopsis thaliana* plant showing the presence of trichomes
Figure 2. A split-plot experimental design with two levels of herbivory (herbivory and sprayed) distributed in a checkerboard pattern among ten plots. Different letters indicate different plots per treatment.
Figure 3. Analysis of covariance showing the relationship between trichome density and days to colonization (A) or rosette damage (B), and the relationship between rosette diameter and either days to colonization (C) or rosette damage (D). * P<0.05, ** P<0.01, *** P<0.001
Figure 4. Directional (β) and stabilizing/disruptive (γ) selection gradients estimated by regression selection analyses on trichome density in the presence (A) and absence (B) of insect herbivores. * P<0.05
Figure 5. Directional (β) and stabilizing/disruptive (γ) selection gradients estimated by regression selection analyses on plant size in the presence of insect herbivores. *** $P<0.001$
Figure 6. Directional (\(\beta\)) and stabilizing/disruptive (\(\gamma\)) selection gradients estimated by regression selection analyses on number of days to first herbivore colonization (A) and insect herbivore damage (B). † P<0.1
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CHAPTER 2

CHANGES IN ULTRAVIOLET B RADIATION MODIFY RESISTANCE AND TOLERANCE IN *ARABIDOPSIS THALIANA* TO INSECT HERBIVORY.

ABSTRACT

Plants have evolved two major mechanisms in response to frequent attacks from insect herbivores. While resistance reduces the levels of damage suffered by plants, tolerance reduces the negative effects of herbivory on plant fitness. Despite their roles in defense and consequent fitness advantage, the evolution of these two characters may be constrained by the fitness costs associated with their production. In addition, these costs may be influenced by variation in environmental conditions. In a common garden experiment, the effects of ultraviolet B (UVB) radiation on plant resistance and tolerance to insect herbivory was evaluated by exposing recombinant inbred lines of *Arabidopsis thaliana* to natural levels of insect herbivory under two levels of UVB radiation (ambient and excluded UVB). Specifically, this study evaluated the existence of genetic variation in plant resistance and tolerance to insect herbivory under variable UVB radiation and whether changes in UVB radiation modify fitness costs associated with tolerance and resistance. Results from this study revealed the presence of genetic variation in plant resistance and tolerance and that this variation is dependent on UVB radiation levels. In addition, there were differences in the fitness cost of plant resistance and tolerance across UVB environmental gradients. No trade-off between resistance and tolerance however, was observed. These results highlight implications of changes in UVB radiation on future evolution of important plant characters involved in plant defense to insect herbivory.
INTRODUCTION

Resistance and tolerance represent two major mechanisms by which plants may reduce the detrimental effects of insect herbivores on their fitness. While resistance directly influences the amount of damage inflicted on plants by their herbivores, tolerance reduces the fitness consequences of damage (Rausher 1992; Strauss and Agrawal 1999; Stowe et al. 2000).

Resistance may be expressed in the form of production of structural barriers such as trichomes, and thorns, or chemical toxins like phenolics and glucosinolates (Levin 1973; McConn et al. 1997; Strauss and Agrawal, 1999; Rautio et al., 2002 Handley et al. 2005; Clauss et al. 2006; Kliebenstein 2004; Bidart-Bouzat et al. 2005). On the other hand, tolerance through compensatory growth enables plants to maintain fitness despite biomass loss resulting from herbivore damage. Compensatory responses may include the removal of apical dominance and proliferation of lateral branches (Paige 1992; Juenger and Bergelson 2000; Rautio et al. 2002; Freeman et al. 2003), extension of flowering period (Pilson and Decker 2002; Freeman et al. 2003), and shifts in resource allocation to different plant parts or plant architecture (Gonzalez-Teuber and Gianoli 2007).

Although herbivores may impose directional selection on resistance and tolerance (Marquis 1992; Valverde et al. 2001), several studies have failed to find this trend in both field and laboratory studies (Mauricio and Rausher 1997; Weinig et al. 2003). One major reason for this observation is the potential presence of fitness costs, which may constrain the evolution of resistance and tolerance (Fineblum and Rausher 1995; Stowe 1998; Pilson 2000). Fitness costs may arise due to potential tradeoffs between defense and other functions, such as growth and reproduction (Herms and Mattson 1992; Belsky et al. 1993; Vandermeijden et al. 1988; Obeso
Consequently, in the absence of herbivory, more resistant or tolerant plants would have lower fitness compared to the most susceptible ones. Yet, in the presence of herbivory, the benefit of resistance or tolerance may be able to offset their associated costs. Thus, the evolution of these characters will depend on the balance between their associated costs and benefits (Obeso 2002).

Multiple environmental factors such as nutrient availability, competition, elevated CO$_2$, and ozone, have been shown to modify the expression of tolerance and resistance to insect herbivore damage (Bolsinger et al. 1992; Bidart-Bouzat et al. 2005; Zvereva and Kozlov 2006; Caldwell et al. 2007; Veteli et al. 2007; Lau and Tiffin 2009). Even though considerable work has demonstrated how environmental factors may influence costs of resistance (Bergelson and Purrington 1996; Prittinen et al. 2003), only few studies have evaluated environmental effects on the costs of tolerance (Agrawal et al. 2004; Stevens et al. 2007). In addition, both empirical and theoretical studies have not shown a consistent pattern on how the environment may modify these costs (Bergelson and Purrington 1996). For example, while some authors have predicted a higher cost of resistance or tolerance under more stressful conditions (Mutikainen et al. 2002), others have predicted a reverse trend (Herms and Mattson 1992). An understanding of how environmental factors may influence genetic variation in tolerance and resistance, as well as their associated costs, may help in predicting evolutionary trajectories of these characters in future changing environment (Tiffin 2000a).

Ultraviolet B (UVB) radiation represents a small fraction of the solar spectrum (290-315 nm). The level of UVB radiation reaching the surface of the Earth has been recently changing due to the depletion of the stratospheric ozone layer, pollution, and cloud cover.
Several studies have evaluated the effect of UVB radiation on plant growth and physiological processes (Hader et al. 2000; Searles et al. 2001), ranging from inhibition of photosynthetic activities to modification of flowering patterns, biomass accumulation, and reproduction (Searles et al. 2001; Tegelberg et al. 2004). With respect to plant chemistry, UVB radiation can increase accumulation of UVB-absorbing compounds such as flavonoids and phenolics, which may in turn influence plant's interactions with higher trophic levels (Agrell et al. 2000; Searles et al. 2001; Hofmann et al. 2003; Izaguirre et al. 2003; Stratmann 2003; Bassman 2004; Izaguirre et al. 2007). For example, Izaguirre et al. (2007) reported that the same phenolics compounds commonly induced in response to insect herbivory were found to be induced in *Nicotiana attenuata* and *Nicotiana longifolia* when exposed to enhanced UVB radiation. Similarly, Stratmann (2003) detected an increase in phenylpropanoids in plant plants exposed to ambient UVB radiation when compared with plants grown under excluded UVB (using light filters) suggesting that UVB radiation may influence plant-insect interactions.

Since variation in UVB levels has been shown to affect different plant characters related to defense, growth and reproduction (Veteli et al. 2003; Bassman 2004; Rousseaux et al. 2004), it is expected that these changes could have an effect on plant resistance and/or tolerance to insect herbivory. Previous studies have focused on the effect of changes in UVB radiation levels on the production of plant secondary chemicals, such as phenolics, which have a role in resistance against insect herbivores (Agrell et al. 2000; Searles et al. 2001; Hofmann et al. 2003; Izaguirre et al. 2003; Stratmann 2003; Bassman 2004; Izaguirre et al. 2007). No information appears to exist however, on the role of UVB on structural defenses, such as trichomes or
characters related to plant tolerance against insect herbivory. With respect to the effects of variable environments on resistance and tolerance, previous studies have shown variable responses of characters related to resistance and tolerance (Ziska and Teramura 1992; Dube and Bornman 1992; Tevini 1993; McDonald et al. 1999; Agrell et al. 2000; Lavola et al. 2000; Hofmann et al. 2003). However, there is a lack of information regarding effects of the UVB environment on potential fitness costs related to plant resistance and tolerance.

This study presents data from a common garden experiment in which a population of recombinant inbred lines (RIL) of Arabidopsis thaliana was exposed to two different levels of UVB radiation (i.e., ambient- and excluded-UVB) and insect herbivory (i.e., sprayed plants and plants exposed to natural levels of insect herbivory). The main goal of this study was to determine whether there was genetic variation in resistance and tolerance to insect herbivory in this A. thaliana RIL population under variable UVB radiation levels, and to assess potential fitness costs associated with plant resistance and tolerance. An understanding of the effects of UVB on plants as well the fitness costs associated with plant response to this environmental factor in terms of resistance and tolerance is important because they may constrain the future evolution of these characters, which in turn may alter the dynamics of plant-insect interactions. For example, if costs increase with enhanced UVB levels, plant resistance and tolerance may be more commonly expressed as inducible strategies in order to minimize their costs (Simms and Rausher 1987, Herms and Mattson 1992; Bergelson and Purrington 1996; Cipollini 1998; Purrington 2000). In addition, the expression of fitness costs across different UVB environments may provide information regarding whether either plant tolerance or resistance are favored over the other, or whether these strategies are likely to be maintained at intermediate levels.
MATERIALS AND METHODS

Study Plant

*Arabidopsis thaliana* (L.) Heynh (Brassicaceae), also known as the mouse ear cress, is an annual herbaceous plant native to Europe and Asia with a wide geographic distribution across North America and Africa. This plant species is naturally found in open or disturbed habitats, sandy soils, riverbanks, roadsides, rocky slopes, and meadows (Baskins and Baskins 1983). *Arabidopsis thaliana* has a short life cycle lasting between 6-8 weeks from germination to senescence and it is mostly a self-fertilizing species with less than 1% outcrossing rates (Hoffmann et al. 2003). Because of the high selfing rates, natural populations seem to consist majorly of a single genotype. However, a significant level of genetic variation exists among different ecotypes in both reproductive and life history characters (Bergelson et al. 1998; Mauricio 1998; Nordborg and Bergelson 1999). In *A. thaliana*, two types of resistance to insect herbivory have been reported. These include structural defenses, such as trichomes, and chemical defenses such as glucosinolates, which are known to vary across populations (Mauricio and Rausher 1997; Valverde et al. 2001; Bidart-Bouzat et al. 2005; Clauss et al. 2006; Loe et al. 2007). In this study, 100 recombinant inbred lines (RILs) derived from a cross between the *A. thaliana* ecotypes Columbia (Col-0) and Landsberg (Ler), and advanced to the eighth generation through a single seed descent were used (Lister and Dean 1993). A RIL population was used in order to assess intraspecific genetic variation in plant resistance and tolerance to UVB radiation.

Experimental Design

A common garden experiment was performed to assess the effects of UVB radiation and plant genotype on tolerance and resistance in *A. thaliana*. This experiment included two UVB
treatment levels (total of 20 plots), which consisted of ambient (UVB+) and excluded (UVB-)
ultraviolet B radiation (Figure 1). Ultraviolet B radiation was excluded from the randomly
assigned UVB- plots using Mylar polyester film. This film transmits radiations only in the
wavelength greater than 315 nm, thereby blocking UVB radiation but transmitting radiations in
UVA regions. For the UVB+ treatment, plots were covered with clear acetate film that allowed
most UVB radiation to pass through (see Figure 2). Half of the plots assigned to each UVB level
were exposed to natural levels of insect herbivory and the other half were excluded from insect
herbivores by spraying the plots with a pesticide (Proclaim by Syngenta Inc. USA) at ten days
interval. Plants excluded from insect herbivory were inspected on a daily basis to ensure there
was no herbivore damage. Each plot was surrounded with a wire-mesh fence to exclude
mammalian herbivory.

One hundred recombinant inbred lines (RILs) of *A. thaliana* were used in this study.
Seeds were initially cold-treated for 5 days at 4°C to break dormancy, and then, transferred to
growth chambers for germination. Within the chambers, plants were exposed to 10 hours of
daylight and 14 hours of night, with day and night temperatures of 20°C and 16°C, respectively.
Plants were rotated every other day within growth chambers and every week among chambers to
avoid chamber and position effects. After five weeks of growth in the chambers, plants were
transplanted to the field (Ecological Research Station, Bowling Green State University, Bowling
Green, Ohio). Two sets of 100 RILs were randomly assigned to each of the 20 plots (total of
4000 plants).
**Estimation of Resistance and Tolerance**

To evaluate the effects of UVB radiation on plant resistance and tolerance, two resistance measures (defined as direct and indirect resistance) and one measure of tolerance were estimated for each RIL. The direct measure of resistance was estimated as trichome density. Trichome density was measured using digital images of the youngest fully extended rosette leaf per plant at bolting time. Leaf area of the selected rosette leaves was estimated using image J software (www.rsbweb.nih.gov/ij). Trichome density was calculated as the total number of trichomes (on the upper side of the leaf) divided by the leaf area. The indirect measure of resistance was estimated by subtracting a proportion of the rosette area damaged from one (i.e., 1- damage). This measurement assumes that the level of herbivore damage sustained is a reflection of the level of resistance (i.e., a plant with a high resistance level will have low levels of herbivore damage and vice versa). This indirect estimate of resistance is important because it accounts for all types of resistance mechanisms present in this plant species (both structural and chemical). Rosette leaf damage (i.e., percent area removed by herbivores on the rosette leaves) was used as a measure of insect herbivore damage, which was estimated as a qualitative leaf damage index (Bidart-Bouzat et al. 2005). This index was calculated by assigning leaves to three size categories based on leaf length (i.e., small: 0- 3cm; medium: 3 - 6cm; and large: 6-9cm), and three damage categories based on the amount of rosette leaf tissue removed (i.e., < 25%, 25-75 %, and > 75%).

To estimate plant tolerance, plant fitness was measured as total fruit number, which is an estimate of lifetime fitness in *A. thaliana*, and further compared among damaged and undamaged plants. Relative fitness was estimated as absolute fitness divided by the mean absolute fitness of
the population of RILs per UVB and herbivory treatment level. Tolerance was estimated by comparing the relative fitness of the damaged plants \((W_D)\) to that of the undamaged plants \((W_U)\). This may be estimated as the ratio of the relative fitness of the undamaged to the damaged plants \((W_D/W_U)\) or as the difference \((W_D-W_U)\) between the relative fitness of damaged and the undamaged plants (Strauss and Agrawal 1999). The choice between these two approaches depends on how the damage data were collected. For example, when damage data are categorical (i.e., presence or absence of damage), an approach using the difference in mean relative fitness would be more appropriate; however, when damage data are continuous, the use of the ratio would be more indicated. This is because tolerance estimated as a ratio is conceptually similar to using a regression coefficient of fitness on damage. This is equivalent to defining tolerance as a reaction norm along a gradient of variable levels of damage, such that the slope describes to what extent plant fitness has been affected by damage (Mauricio and Rausher 1997; Simms and Triplett 1994; Tiffin and Rausher 1999; Strauss and Agrawal 1999). In this study, rosette damage was estimated using a damage index, which vary in a continuous fashion. Therefore, tolerance was estimated as the ratio of the relative fitness of the undamaged to the damaged plants \((W_D/W_U)\). Two approaches were used to evaluate costs associated to resistance and tolerance, as well as the effect of UVB radiation on these costs. First, fitness costs were detected through Pearson’s correlation coefficients as the presence of a negative association between relative fitness of the undamaged plants and either resistance or tolerance under each level of UVB radiation. Second, the existence of a potential trade-off between plant resistance and tolerance was also assessed using correlation analysis.
**Statistical Analysis**

To evaluate the effects of UVB radiation and RIL on indirect resistance, and those of UVB, herbivory and RIL on trichome density, analyses of variance were performed using the PROC GLM procedure (SAS statistical software, version 9.1). Data was either log- or ranked-transformed to correct for normality and heteroscedasticity when necessary. For this purpose, a mixed linear model was used for trichome density, considering UVB radiation and herbivory as fixed effects, and block, RILs and its interactions as random effects. For indirect resistance, herbivory was not included in the model since this measure was estimated only from plants exposed to herbivory. In addition, higher-order block interactions were first included in the model but then excluded because they were not significant. Regarding tolerance, since it was not possible for a plant to be damaged and undamaged at the same time, tolerance was estimated as means for each RIL. In order to show the presence of genetic variation for tolerance, an analysis of covariance was performed including relative fitness as the dependent variable and rosette damage as the covariate using the following model: Relative fitness = Block + RIL + Rosette damage + RIL × Rosette damage + Error. Significant rosette damage × RIL interaction indicates the presence of genetic variation in tolerance.

Cost of resistance was evaluated using Pearson’s correlation coefficients as estimates of potential genetic associations between the RIL means of relative fitness of undamaged plants and the two measures of resistance; that is, trichome density and indirect resistance (1-damage). Relative fitness of the undamaged plants was used based on the premise that in the absence of herbivory, the benefit of being resistant is removed and only the cost will be manifested. Thus, a significant negative genetic correlation coefficient between relative fitness and resistance in the
absence of damage implies the presence of a fitness cost. In the case of tolerance, however, a direct genotypic correlation between the fitness of the undamaged plant and tolerance would be biased (Tiffin and Rausher 1999). This is because using the same data to estimate relative fitness of the undamaged plants and tolerance would introduce an artifactual covariance (Tiffin and Rausher 1999). This artifactual covariance was calculated and subtracted from the estimated covariance between tolerance and relative fitness of undamaged plants. A bootstrap technique was used to generate a 95% confidence limit in order to determine if the negative covariances have confidence limits that do not include zero using a modified version of the SAS macro as described in Stinchcombe (2005). This method compiles the bootstrap distribution of each corrected covariance, and the 2.5 and 97.5 percentile of this distribution formed the 95% bootstrap percentile confidence interval. Thus, if the confidence interval failed to include zero, then the p-value is deemed to be less than or equal to 0.05, and the effect is said to be significant.

RESULTS

Analyses of variance revealed significant UVB effects on both direct (trichome density) and indirect resistance measures as well as genetic variation in these characters’ responses to changes in UVB variation (see Figure 3A, 3B, 3C and Table 1). Overall, exclusion of UVB and herbivores resulted in a decrease in trichome density, although this effect was more pronounced in the sprayed treatment (herbivory excluded) as indicated by a significant herbivory by UVB interaction. In addition, plant responses to UVB radiation in terms of trichome density were found to be dependent on both RIL and herbivory (i.e., significant UVB × RIL interaction) (Table 1). Effects of herbivory on trichome density were found to be marginally dependent on
RIL and the three-way interaction (UVB × Herbivory × RIL) was statistically not significant (Table 1). This non-significant three-way interaction indicates that UVB radiation affected trichomes density in response to herbivory in the same way across the genotypes (Table 1). With respect to tolerance to insect herbivory, a significant genetic variation in tolerance was revealed by a significant RIL × rosette damage interaction in an analysis of covariance using relative fitness as the dependent variable (Figure 3D).

In order to evaluate fitness costs of resistance (both direct and indirect resistance measures) and tolerance, as well as whether variation in UVB radiation influenced these costs, genotypic correlations between relative fitness of the undamaged plants and resistance or tolerance were performed. Overall, results suggest that changes in UVB radiation influence fitness costs associated with both direct and indirect resistance as well as tolerance to insect herbivory. In terms of direct resistance (trichome density), results from genotypic correlations did not reveal any significant trade-offs between relative fitness and trichome density under ambient UVB radiation levels ($r=-0.06$, $P=0.596$); (Figure 4A). However, a marginally significant cost was observed in plants excluded from UVB radiation ($r=-0.17$, $P=0.0875$); (Figure 4B). In terms of resistance, a negative genotypic correlation between relative fitness of the undamaged plants and indirect resistance was detected under ambient UVB, which suggests the presence of fitness cost in this environment ($r=-0.31$, $P=0.0015$); (Figure 4C). Conversely, under the excluded UVB regime, although an inverse trend was observed, the correlation was not significant ($r=-0.13$, $P=0.1889$); (Figure 4D).

In terms of tolerance, results revealed a significant negative genotypic correlation between relative fitness of the undamaged plants and tolerance under both ambient and excluded
UVB radiation levels \(r=-0.68, P<0.0001\); and \(r=-0.36, P=0.0003\); respectively; (Figures 4E and 4F). However, after correcting for the artifactual covariance, the corrected covariance was negative under ambient UVB but not under excluded UVB (Table 3). This result suggests that cost of tolerance appears to be present only under ambient UVB. The significant negative genetic correlation observed under excluded UVB may have been due to the presence of an artifactual covariance as result of using the same data to estimate relative fitness of the undamaged plants and tolerance. Despite the presence of fitness costs for both indirect resistance and tolerance, evaluation of the potential presence of trade-offs between tolerance and trichome density did not reveal any form of trade-offs under ambient UVB \(r=0.13518, P=0.1799\) or excluded UVB \(r=0.014, P=0.888\); (Figures 5A and B). Similarly, evaluation of trade-offs between tolerance and indirect resistance did not reveal any trade-offs under ambient \(r=0.035, P=0.7281\) or excluded UVB \(r=-0.051, P=0.6176\); (Figures 5C and D).

**DISCUSSION**

This study demonstrates that variation in UVB radiation influences resistance and tolerance to insect herbivory in *A. thaliana*. Although previous studies have demonstrated herbivore-induced variation in resistance characters such as trichome density and plant damage levels (Mauricio and Rausher 1997; Weinig et al. 2003; Clauss et al. 2006), no studies to date have shown whether variation in UVB radiation levels may influence intraspecific genetic variation in resistance and tolerance to insect herbivory. This study revealed significant genetic variation in the response of both direct resistance (trichome density) and indirect resistance characters to variation in UVB levels. This significant RIL× UVB interaction is very important
because it indicates a potential for these characters to evolve under future changes in this environmental factor. Another interesting finding from this study includes the significant interaction between UVB and herbivory for trichome density, suggesting that future changes in UVB radiation may influence plant structural defense responses to insect herbivory, which in terms may affect coevolutionary plant-insect associations.

With respect to potential fitness costs associated with resistance, this study failed to find costs associated with trichome production in both ambient and excluded UVB environments (although a marginally significant result was found in excluded UVB plots). Given the detrimental effects of UVB radiation on plant fitness and related characters, the inability to detect a cost for the production of trichomes is not unexpected. This is because previous studies have linked trichome production with plant protection from harmful effects of ultraviolet radiation and extreme temperatures (Skaltsa et al. 1994; Werker 2000; Agrawal et al. 2004). Thus, trichomes may be serving a dual role in plants by protecting them from both herbivores and harmful UVB radiation, thereby reducing the negative fitness consequence of these factors on plants. In terms of indirect resistance, however, a significant cost was detected in the presence of ambient UVB but not under excluded UVB, indicating that UVB radiation modified the cost of indirect resistance in this system. This finding is concordant with previous studies that have demonstrated that environmental factors may modulate cost of resistance (Bergelson and Purrington 1996; Koricheva 2002; Siemens 2003; Osier and Lindroth 2006). For example, Osier and Lindroth (2006) demonstrated that nutrient availability modified cost of resistance in *Populus tremuloides*. Similarly, Siemens (2003) found the cost of resistance to differ in *Arabis perennans* that grew alone and *Arabis perennans* that grew under competition with *Bouteloua*
gracilis. It is also important to note that in this study, the measure of indirect resistance was related to the level of herbivore damage, and thus, it includes potential costs related to both structural and chemical defenses. Since costs related to structural defenses were absent in this study, the presence of this indirect resistance cost may have arisen from the constitutive chemical resistance mechanisms present in A. thaliana (Marak et al. 2003; Cipollini et al. 2003; Sampedro et al. 2011; Stowe and Marquis 2011). For example, Stowe and Marquis (2011) provided empirical evidence for the presence of constitutive costs of foliar glucosinolates in Brassica rapa by artificially selecting for foliar glucosinolates. Their results revealed that lines selected for high foliar glucosinolates showed decrease in number of flowers and mean seeds per fruit. It is also possible that the presence of fitness costs may be associated with the process of induction of chemical resistance (see Cipollini et al. 2003)

With respect to fitness costs associated with tolerance, this study revealed a significant negative genetic correlation between relative fitness of undamaged plants and tolerance under both levels of UVB radiation. After correcting for an artifactual covariance however, which likely resulted from using the same data to estimate relative fitness of the undamaged plants and tolerance, the corrected covariance was only negative in plots exposed to ambient UVB radiation. This result suggests that there is evidence that costs of tolerance may exist under ambient UVB radiation levels. Little information exists on the effects of abiotic environmental changes on the cost of tolerance to insect herbivory and different results have been obtained in terms of how cost of tolerance may vary across environmental gradients. For example, while Stinchcombe (2002) detected a cost related to tolerance to be present under low soil nutrients but not under high nutrient level in the ivy leaf morning glory exposed to deer herbivory, Steven et
al. (2007) failed to find costs of tolerance to differ between low and high nutrient levels in aspen plants exposed to artificial defoliation. In addition, Siemens et al. (2003) found that costs of tolerance to insect herbivory in *Arabis perennans* were present in a non-competitive environment but absent in a competitive environment. The inconsistent patterns in the expression of fitness costs associated with tolerance in these studies suggest that these costs may be dependent on multiple factors including the plant species and the specific selective factor/s acting in each type of environment.

Since theoretical models predict that tolerance and resistance serve the same function, it can be expected that plants should be either resistant or tolerant but not both (Fineblum and Rausher 1995; Tiffin 2000b; Fornoni et al. 2004). Yet, some models have predicted that both resistance and tolerance may be maintained at intermediate levels under certain conditions. These models, as summarized by Fornoni et al. (2004) and Nunez-Farfan et al. (2007), include the cost of tolerance and resistance across environmental gradients, fluctuations in both time and space with respect to insect herbivory, and characters that are under the selective pressures of factors other than herbivory. In addition, the type of damage on plants, such as rosette damage or apical damage, and type of herbivores present may also be exerting different pressures on tolerance and resistance. In this study, trade-offs between resistance and tolerance to insect herbivory were not detected. This result was in agreement with previous studies that have assessed the presence of trade-offs between resistance and tolerance. For example, Tiffin and Rausher (1999) did not detect any trade-offs between resistance and tolerance to foliovory and apical meristem damage in the common morning glory. Likewise, Weinig et al. (2003) did not find any significant trade-offs between tolerance and resistance to apical damage in *A. thaliana,*
suggesting the possibility of these two characters evolving independently of each other. Most of
these studies, however, did not include potential changes in these trade-offs across environments
(but see Stevens et al. 2007).

The inability to detect trade-offs between resistance and tolerance in this study could be
explained by two factors. First, fitness costs for resistance and tolerance varied across
environments. For example, fitness costs of tolerance and indirect resistance were only observed
in plots exposed to ambient UVB but not in plots excluded from UVB. This type of variation in
fitness costs across variable environments may lead to the maintenance of genetic variation in
resistance and tolerance, and thus, to selection for intermediate levels of these characters across
populations (Fornoni et al. 2004). Second, it is possible that defense traits used in this study may
be under selection by selective agents other than insect herbivory (Stowe et al. 2000; Fornoni et
al. 2003; Loe et al. 2007; Nunez-Farfan et al. 2007). For example, enhanced UVB radiation in
the absence of herbivory led to an increase in trichome density. This result therefore suggests
that trichomes may be playing a protective role against UVB radiation (Skaltsa et al. 1994;
Werker 2000; Agrawal et al. 2004) and may be under selection in the presence of UVB radiation.
Nevertheless, a selection experiment will be required to confirm whether UVB radiation is
indeed imposing direct selection on trichome density.

In conclusion, results from a common garden experiment revealed that changes in UVB
radiation influence plant characters related to resistance and tolerance to insect herbivory, and
that this environmental effect is dependent on plant genotype (RIL). This study also showed the
existence of fitness costs associated with plant resistance and tolerance, which were dependent
on the UVB environment; that is, they were present only under ambient UVB radiation.
Although previous studies have not shown a consistent pattern on the expression of fitness cost associated with resistance and tolerance (i.e. whether costs are more pronounced in the more stressful environments or less stressful environments), results from this study support the view that cost of tolerance and resistance may be more pronounced under a more stressful environment (Mutikainen et al. 2002; Sampedro et al 2011). This is because growth and other physiological processes may be compromised if resources decline or conditions become more stressful. In addition, plant responses to UVB may also come at an additional fitness cost, which becomes more pronounced in the presence of insect herbivory. The presence of these fitness costs under enhanced UVB radiation may have important evolutionary implications because selection for higher levels of resistance and tolerance to insect herbivory under enhanced UVB may result in lower fitness, and thus, may constrain the evolution of these characters.
Table 1. ANOVA F-values showing the effects of herbivory, UVB radiation, RILs and their interactions on trichome density (direct resistance) and an indirect resistance estimate (1-herbivore damage).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Trichome Density</th>
<th>Indirect Resistance</th>
<th>Tolerance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>38.59***</td>
<td>1.14</td>
<td>-</td>
</tr>
<tr>
<td>Herbivory</td>
<td>220.48***</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>UVB</td>
<td>73.79***</td>
<td>92.94***</td>
<td>0.55</td>
</tr>
<tr>
<td>Herbivory x UVB</td>
<td>75.74***</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>RIL</td>
<td>0.85</td>
<td>1.27</td>
<td>0.86</td>
</tr>
<tr>
<td>Herbivory x RIL</td>
<td>1.35†</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>UVB x RIL</td>
<td>2.32***</td>
<td>1.39*</td>
<td>0.90</td>
</tr>
<tr>
<td>Herbivory x UVB x RIL</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Significance levels: † P<0.1, * P<0.05, ** P<0.01, *** P<0.001
Table 2. ANCOVA F-values showing the relationship between relative fitness and rosette damage across UVB environments and in each of the UVB environments. A significant association in rosette damage × RIL indicates the presence of genetic variation in tolerance.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Across UVB environments</th>
<th>UVB+</th>
<th>UVB-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>17.50***</td>
<td>25.84***</td>
<td>7.09***</td>
</tr>
<tr>
<td>RIL</td>
<td>1.27*</td>
<td>1.26†</td>
<td>1.33*</td>
</tr>
<tr>
<td>Rosette Damage</td>
<td>3.29†</td>
<td>7.24**</td>
<td>1.50</td>
</tr>
<tr>
<td>Rosette Damage x RIL</td>
<td>1.28*</td>
<td>1.07</td>
<td>1.26†</td>
</tr>
</tbody>
</table>

Significance levels: † P<0.1, * P<0.05, ** P<0.01, *** P<0.001
Table 3. Pearson’s correlation coefficients showing the relationship between relative fitness of undamaged plants and tolerance under ambient (UVB+) and excluded (UVB-) ultraviolet B radiation level. Corrected covariance represents the covariance adjusted for the artifactual covariance.

<table>
<thead>
<tr>
<th></th>
<th>UVB+</th>
<th>UVB-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation coefficient</td>
<td>-0.68*</td>
<td>-0.36*</td>
</tr>
<tr>
<td>Covariance</td>
<td>-0.0474</td>
<td>-0.0151</td>
</tr>
<tr>
<td>Artifactual covariance</td>
<td>-0.0317</td>
<td>-0.0229</td>
</tr>
<tr>
<td>Corrected covariance</td>
<td>-0.0157</td>
<td>0.0079</td>
</tr>
<tr>
<td>95% Confidence interval</td>
<td>(-0.036,0.003)</td>
<td>(-0.004,0.019)</td>
</tr>
</tbody>
</table>

Significance levels for Pearson coefficient of correlation: * P<0.001
Figure 1A. Picture of the experimental plots showing plant arrangements within each plot.

Figure 1B. Schematic representation of experimental plot layout showing two levels of herbivory (herbivory and sprayed) and UVB (ambient and excluded) treatments distributed among twenty plots.
Figure 2. Absorption spectra for Mylar and Clear acetate filters.
Figure 3. Effects of the presence (UVB+) and exclusion (UVB-) of ultraviolet B radiation on trichome density in the presence of herbivory (A), trichome density in the absence of herbivory (B), indirect resistance (C), and tolerance (D) of an A. thaliana recombinant inbred line population. Means of RILs are represented with closed circles and asterisks on lines represent significant differences in phenotypic means between treatments. * P< 0.05, **P<0.001
Figure 4. Genotypic correlations showing fitness costs of trichomes (A and B), resistance (C and D) and tolerance (E and F) in *A. thaliana* plants grown under ambient and excluded UVB radiation. Each point represents genotypic means of RILs. ** P<0.01, *** P<0.001
Figure 5. Genotypic correlations between resistance and tolerance in *A. thaliana* plants grown under ambient and excluded UVB radiation. Each point represents genotypic means of RILs.
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CHAPTER 3

EFFECTS OF ULTRAVIOLET-B RADIATION ON PLASTICITY OF FITNESS-RELATED RESPONSES OF ARABIDOPSIS THALIANA TO INSECT HERBIVORY

ABSTRACT

The amount of ultraviolet-B (UVB) radiation reaching the Earth’s surface has been changing over the past few decades due to the destruction of the stratospheric ozone layer, increased air pollution, and cloud coverage. Current changes in the environment may lead to the evolution of adaptive plastic responses in plants as well as influence interactions of plants with other organisms, such as insect herbivores. Potential evolutionary responses of organisms to environmental change however, will likely depend on the underlying genetic variation that is present in a population. Here, data from a common garden experiment is presented in which recombinant inbred lines (RILs) of Arabidopsis thaliana were exposed to two different levels of UVB radiation (i.e., ambient and excluded UVB) and natural levels of insect herbivory. This study evaluated whether variation in UVB radiation influenced fitness-related traits of A. thaliana genotypes as well as their phenotypic responses to insect herbivory. Results from this study indicate that A. thaliana RILs grown under excluded-UVB were the first to be colonized by insect herbivores and received higher levels of insect damage. Results from this study revealed that variation in UVB and herbivory levels influenced all fitness-related traits measured in a common garden experiment. These traits were affected not only by changes in the UVB environment, but were also genetically dependent, which may have implications for the evolution of these traits in future changing environments. More interestingly, UVB levels influenced plant responses to insect herbivory, in terms of total fruit production and flowering
time. This result is important because it implies that changes in UVB radiation may influence the dynamics of plant-insect interactions.
INTRODUCTION

Multiple environmental changes influencing organisms and their interactions have been witnessed during the past few decades. Such changes in the environment may lead to the evolution of plastic responses, which may be reflected in variation in phenotypic traits related to growth, development, and/or reproduction. These plastic changes represent mechanisms by which organisms can respond, at least in the short term, to challenges imposed by variable or heterogeneous environments (Bradshaw 1965; Travis 1994; Schmitt et al. 1999; Sultan 2000). Phenotypic plasticity is a widely accepted adaptive strategy, particularly useful to sessile organisms like plants, which have limited capacity to escape in space from the potential detrimental effects of currently changing environments (Bradshaw 1965). To predict to what extent such plastic responses may influence the evolutionary trajectory of a species and its interactions with other species, it is imperative to evaluate whether there is genetic variation in plasticity as well as genetic variation in the plastic response to environmental factors. This is essential because the extent, to which a population may evolve in the face of a changing environment, will likely depend on the underlying genetic variation present at the population level.

Ultraviolet-B radiation represents a small fraction of the solar spectrum ranging from 290 to 315 nm. The level of UVB radiation reaching the Earth’s surface has been consistently changing during the last decades due to the depletion of the tropospheric ozone layer, pollution levels, and cloud coverage (Intergovernmental Panel on Climate Change, IPCC 2007). Several studies have evaluated the effect of UVB radiation on plant growth and physiological processes as well as plant chemical responses to this factor, which appear to vary among plant species.
(Hader 2000; Searles et al. 2001a; Searles et al. 2001b). Whereas some studies failed to find significant effects of UVB radiation on plant growth and photosynthetic processes (Searles et al. 2001a; Tegelberg et al. 2004), other studies have shown UVB-induced decreases in leaf area and growth rate (likely as a result of decreases in photosynthetic rate and increases in leaf thickness (Bornman and Teramura 1993; Stratmann 2003). Changes in UVB radiation may also modify flowering patterns, biomass accumulation, and reproductive traits. With respect to plant chemistry, UVB radiation is known to increase accumulation of UVB-absorbing compounds, such as flavonoids and phenolics, which in turn may interfere with plant interactions with their herbivores (Agrell et al. 2000; Searles et al. 2001; Hofmann et al. 2003; Izaguirre et al. 2003; Bassman 2004; Izaguirre et al. 2007). On the other hand, levels of secondary chemicals, such as terpenoids may be unaffected or even decreased in some plant species when exposed to enhanced UVB radiation levels (Singh 1996; Ambasht and Agrawal 1997).

Several studies have evaluated the interactive effect of UVB radiation with other abiotic agents on plant responses. For example, abiotic factors such as elevated CO$_2$ (Ziska and Teramura 1991; McDonald et al. 1999; Agrell et al. 2000; Lavola et al. 2000), nutrient and water stress (Hofmann et al. 2003), temperature changes (Tevini 1993), and heavy metals (Dube and Bornman 1992) have been shown to modify plant response to UVB radiation. On the other hand, only few studies have addressed the interactive effect of UVB radiation with biotic agents such as insect herbivores (Ballare et al. 1996; McCloud and Berenbaum 1999; Caputo et al. 2006; Caldwell et al. 2007). These studies, although informative, have mostly been conducted under laboratory conditions, but see Caputo et al. (2006). More importantly, they did not evaluate intraspecific variation in plant fitness responses to insect herbivory under variable UVB radiation
levels. In general, previous studies have mainly focused on assessing effects of UVB radiation on insect performance and the amount of insect damage suffered by plants. For examples, studies have shown that UVB may affect the nutrient levels of plants thereby leading to poor larval performance, increase in the number of days to pupation and late eclosion (Leonard and Juliano 1995). Additionally, UVB radiation may also induce responses similar to those induced by herbivory, such as production of secondary chemicals, which may be directly toxic to herbivores, and thus, reduce the amount of damage suffered by plants (Agrell et al. 2000; Searles et al. 2001; Hofmann et al. 2003; Izaguirre et al. 2003; Bassman 2004; Izaguirre et al. 2007).

Furthermore, it is not known whether effects of UVB radiation on patterns of insect distribution and damage among plants in the field are genetically dependent. This information, in addition to potential genetic variation in the plant phenotypic response to both UVB radiation and insect herbivory is important because the interactive effect of these factors may lead to natural selection favoring some genotypes over others, which may in turn alter the co-evolution of plant-insect interactions.

This study presents results from a common garden experiment in which recombinant inbred lines (RILs) of *Arabidopsis thaliana* were exposed to two different levels of UVB radiation (i.e., ambient- and excluded-UVB) as well as to the presence and absence of natural levels of insect herbivory. The interactive role of plant genotype, UVB radiation, and insect herbivory was assessed on plant fitness-related responses of the annual plant *A. thaliana*. Patterns of insect distribution and herbivory in the field were also evaluated to assess whether these could explain observed changes in plant phenotypic responses to insect herbivore attacks. Information from this experiment would allow us to make predictions regarding whether changes
in UVB radiation may influence phenotypic responses of plants to a natural insect community, and thus, the evolution of plant-insect interactions in future changing environments.

**MATERIALS AND METHODS**

*Study Plant Species*

*Arabidopsis thaliana* (L.) Heynh (Capparales: Brassicaceae), also known as the mouse-ear cress, is an annual herbaceous plant native to Europe and Asia with a wide geographic distribution across North America and North-western Africa (Baskins and Baskins 1983; Hoffmann et al. 2003). This species is a self-fertilizing plant, characterized by a short life cycle, which is naturally found in open or disturbed habitats (Baskins and Baskins 1983; Hoffmann et al. 2003). Because of the high selfing rates (less than 1% outcrossing rates), natural populations seem to consist majorly of single genotypes. However, significant levels of genetic variation in phenotypic traits exist among different local populations of this species (Bergelson et al. 1998; Mauricio 1998; Nordborg and Bergelson 1999). Recombinant inbred lines of *A. thaliana* (100 RILs) derived from a cross between the ecotypes Columbia (Col-0) and Landsberg (Ler), and advanced to the eighth generation through a single seed descent were used in this study (Lister and Dean 1993). These RILs were used to generate a genetically variable population since the two parental lines (Col-0 and Ler) are known to differ in several characteristics related to growth, reproduction, phenology, and both chemical and structural defenses (Mauricio 2005; Weinig et al. 2003). Thus, genetic and phenotypic variation among these RILs allows for the assessment of intraspecific variation in the response of this plant species to both insect herbivory and UVB radiation.
**Experimental Design**

To address the question of whether UVB radiation can influence plasticity of plant phenotypic responses to insect herbivory, the model plant *Arabidopsis thaliana* was used in a common garden experiment in which the presence/absence of natural insect herbivory and the levels of UVB radiation were manipulated. Specifically, a split-plot design was used to assess the effect of UVB radiation, insect herbivory, and plant genotype on plant phenotypic responses. The two UVB treatment levels consisted of ambient (UVB+) and excluded (UVB-) ultraviolet-B radiation randomly assigned to 20 different plots (i.e., 10 plots assigned to each UVB level). While ultraviolet-B radiation was excluded from the randomly assigned UVB- plots using Mylar polyester film, UVB+ plots were covered with clear acetate film allowing most of the UVB radiation to pass through. Mylar film transmits radiations only in the wavelength greater than 315 nm thereby blocking all the UVB radiation but transmitting radiations in the ultraviolet-A regions. On the other hand, clear acetate film allowed most UVB to pass through. *Arabidopsis thaliana* genotypes consisted of 100 recombinant inbred lines (RILs), which were planted at random within each of the plots. Two sets of 100 RILs were randomly assigned to each of the 20 plots (total of 4000 plants). Insect herbivores were excluded from sprayed plots by spraying with a pesticide (Proclaim by Syngenta Inc. USA) at ten days interval. Plants excluded from insect herbivores were inspected on daily basis to ensure no herbivore damage. Each plot (both herbivory and sprayed plots) was surrounded with a wire-mesh fence to exclude mammalian herbivory.

In terms of growth conditions, seeds were cold-treated for 5 days at 4°C to break dormancy. Prior to transplanting to the field, plants were initially raised in a 50 well flats in
laboratory growth chambers. Within the chambers, plants were exposed to 10 hours of day and 14 hours of night, with day and night temperatures of 20°C and 16°C, respectively. Plants were rotated within growth chambers every other day and among chambers every week to avoid chamber and position effects. After five weeks of growth in the chambers, plants were transplanted to the field (Ecological Research Station, Bowling Green State University, Bowling Green, Ohio).

**Plant phenotypic traits**

In order to assess responses of plant RILs to herbivory under different UVB levels, variables related to phenology (flowering time), plant growth (rosette biomass), and reproduction (total fruit production) were measured in plots with herbivores present and excluded. Similarly, the effects of UVB radiation, and RIL were assessed on days to plant colonization and percent area of rosette leaves removed by insect herbivory. Flowering time was estimated as the number of days required for the first flower per plant to be fully opened. Plant rosette biomass was measured as rosette dry weight by oven drying the rosette leaves for two days at 70°C. Plant fitness was estimated as the total fruit number produced over the plant lifetime, since this measure is highly correlated with total seed production (Bidart-Bouzat 2004). To document the order of colonization of insect herbivores among plants, all plants were inspected daily for insect damage and the numbers of days to first observe herbivore damage on each plant was recorded herein, referred to as colonization.

**Statistical Analysis**

To evaluate responses of plant genotypes to herbivory under different UVB levels, analyses of variance were performed for each of the measured variables, flowering time, rosette
biomass and total fruit production using PROC GLM (SAS statistical software, version 9.1). Data was either log- or ranked-transformed to correct for normality and heteroscedasticity when necessary. For this purpose, a mixed linear model was used, considering the herbivory and UVB treatments as fixed effects, and plant genotype (RIL) as a random effect. In addition, similar analyses were performed on the order of insect herbivore colonization among RILs and plant rosette damage in order to evaluate the effects of UVB radiation, and genotype on patterns of plant utilization by insect herbivores.

RESULTS

Analyses of variance revealed that A. thaliana differed in their fitness-related responses to UVB exclusion and insect herbivory (Table 1). With respect to the number of fruits produced, both UVB exclusion and herbivory significantly affected this trait (see Table 1 and Figure 1A). Exclusion of UVB resulted in a significant increase in total fruit production and herbivory led to a general decrease in the total number of fruits (Figure 1A and B). Additionally, plant responses to herbivory in terms of total number of fruits produced were found to be dependent on UVB radiation levels (Table 1). This significant interaction between herbivory and UVB radiation levels has particular evolutionary significance since future changes in UVB radiation levels may modify plant phenotypic response to their herbivores. The effect of genotype (RIL) on total fruit number was statistically not significant (Table 1). However, separate analyses of variance for each UVB levels (when pooling herbivore treatments) revealed a significant effect of RIL on total fruit number (P= 0.0107 for UVB+; and P= <0.0001 for UVB-), indicating the existence of genetic variation in this trait. All phenotypic responses of plants to UVB radiation were found to be genotype dependent (significant UVB × RIL interaction), but those to insect herbivory were
not (see Table 1). In addition, the three-way interaction UVB × herbivory × RIL was statistically not significant indicating that UVB radiation levels affected total number of fruits produced in response to herbivory across the genotypes in the same manner.

With respect to rosette biomass, UVB exclusion and herbivory significantly affected this trait (Table 1). Exclusion of UVB resulted in a significant increase in rosette biomass, and herbivory led to a general decrease in rosette biomass (Figure 1C and D). Overall, the effect of genotype (RIL) on rosette biomass was statistically not significant (Table 1). However, separate analyses of variance for each UVB levels revealed a significant effect of genotypes on rosette biomass (P= <.0001 for UVB+; and P= <.0001 for UVB-), indicating the existence of genetic variation in this trait. Rosette biomass responses to UVB were found to be dependent on genotype (significant UVB × RIL interactions), but not on herbivory (Table 1). Furthermore, responses of plant to herbivory were not genotype dependent. Analysis of the three-way interaction (i.e., UVB × herbivory × RIL) was not significant indicating that UVB radiation levels affected rosette biomass produced in response to herbivory across the genotypes in the same manner.

Analyses of variance also revealed that UVB, herbivory, and genotype significantly affected the number of days to first flowering (Table 1). Overall, exclusion of UVB radiation resulted in a decrease in the number of days to first flowering, and herbivory led to an increase in this trait value (Figure 1E and F). For this trait, a significant interaction between herbivory and UVB radiation was observed, indicating that plant responses to UVB radiation in terms of flowering time is dependent on herbivory (Table 1). Moreover, while phenological responses of plants to UVB variation were genotype-dependent (significant UVB × RIL interaction),
responses to herbivory did not significantly depend on the genotype (Table 1). Finally, the three-way interaction of UVB radiation, herbivory, and genotype was significant, which implies that UVB radiation differentially affected plant response to herbivory across the genotypes.

Exclusion of UVB led to a significant decrease in the number of days to plant colonization by insect herbivores (Table 1 and Figure 2A). The effects of genotype on days to herbivore colonization were marginally significant, and the effect of UVB radiation on this variable was not dependent on plant genotype (Figure 2A). In addition, changes in UVB radiation significantly affected levels of herbivore damage (Table 1 and Figure 2B). Plants excluded from UVB radiation had more damage than plants exposed to ambient UVB. Although, the effect of RILs was not significant on the levels of insect herbivore damage, separate analyses of variance under the two UVB radiation levels revealed a significant effect of RILs on the levels of herbivore damage (P=0.0055 for UVB+; and P<.0001 for UVB-). In addition, variation in damage levels as a result of differences in UVB radiation levels was also dependent on plant genotype (significant UVB × RIL; Table 1 and Figure 2B).

**DISCUSSION**

Even though several studies have investigated the effects of UVB radiation and insect herbivory on plants, information is lacking regarding the effect of these two important environmental factors on plant fitness-related responses. This study is also of evolutionary significance because the ability of plant populations to evolve and the extent to which a population may evolve in a changing environment will depend on the available genetic variation within such population. In addition, this study revealed a significant UVB × RIL interaction for
all measured variables suggesting the presence of evolutionary potential of these traits to respond to changes in UVB radiation. This significant interaction is important as it may lead to the maintenance of genetic variation under variable environments, and allow populations to evolve in changing environments (Via and Lande 1985; Via and Lande 1987; Gillespie and Turelli 1989).

Overall, the effect of UVB radiation was detrimental to plant fitness-related variables measured in this study. Exclusion of UVB radiation led to an increase in the total number of fruits and rosette biomass produced, as well as a delay in the number of days required for plants to first flower. In the same vein, insect herbivory also had a negative effect on measured phenotypic variables. This result is consistent with other studies that have shown that plants may suffer fitness consequences because of exposure to high levels of UVB (Teramura et al. 1990; Teramura and Sullivan 1991; Grammatikopoulos et al. 1994). This may be through growth inhibition, alteration of photosynthetic enzyme activities, modification of stomata closure, and alteration of growth patterns (e.g., decreases in stem length and leaf area, and changes in flowering phenology) (Teramura 1993; Hader 2000; Searles et al. 2001a; Searles et al. 2001b Bornman and Stratmann 2003). The negative impact of UVB radiation on fitness may also be due to energy costs of synthesizing protective secondary metabolite in response this type of stress (Logemann et al. 2000). In the same manner, insect herbivory may also have negative consequences on plant fitness such as reduced seed production, increased fruit abortion, decreased growth rate, changes in plant architecture, and delayed senescence (Marquis 1992; Bidart-Bouzat 2004; Rudgers and Whitney 2006). It is important to emphasize that this study revealed a significant interaction between herbivory and UVB, indicating an effect of variation in
UVB levels on plasticity of plant-fitness responses to insect herbivory. Thus, future changes in UVB radiation levels may significantly affect plant-insect interactions and the fitness outcome of plants exposed to natural insect communities.

With respect to patterns of plant utilization by insect herbivores, increase in UVB led to a decrease in insect herbivore damage and an increase in the number of days to herbivore colonization. This result may be due to two major reasons. Insect herbivores may detect differences in the levels of UVB radiation and present avoidance behavior to high levels of UVB radiation (Mazza et al. 1999; Mazza et al. 2002). Alternatively, UVB may mediate changes in plant morphology and chemistry, which in turn influences the amount of insect herbivory. For example, previous studies have suggested that trichomes, which are important structural barriers for insect herbivores, may also be involved in the protection of plants against harmful effects of UVB radiation. This protective mechanism is due to the ability of trichomes to reflect radiations in the UVB region (Ehleringer 1984; Karabourniotis and Bornman 1999). There is experimental evidence that trichome production was induced by UVB radiation in this study (see Chapter 2). In addition to induction of physical barriers to herbivores, UVB radiations may also increase the concentration of UVB absorbing compounds such as phenolics, flavonoids, and other secondary chemicals that may directly influence plant utilization by herbivores (Lavola et al. 2003). For instance, Izzaguire et al. (2007) found that UVB radiation induced the same phenolics compounds that were induced by herbivory, suggesting that UVB radiation may trigger the same defense pathway as insect herbivory. Information also exists on effects of UVB radiation influencing concentrations of glucosinolates, a well-studied chemical defense system in the Brassicaceae plant family (Barro et al. 2002; Schreiner et al. 2009). For instance, Schreiner et
al. (2009) found up to six-fold increase in glucotropaolein, the only type of glucosinolates present in nasturtium (*Tropaeolum majus*) after exposure to UVB radiation. Likewise, Baro et al. (2002) observed similar increases in glucosinolate contents in the Ethiopian mustard (*Brassica carinata*) when exposed to UVB radiation. This UVB-mediated increase in glucosinolates has been suggested to be a stress response similar to that observed in plant defense responses to insect herbivory and pathogen attack (Schreiner et al. 2009). Taken together, these findings suggest that UVB radiation may have induced both physical and chemical defenses in our system, which in turn may have pronounced effects on patterns of plant use by insect herbivores.

Intuitively, one would expect that lower herbivory impact (less plant damage) under UVB+ should be associated with enhanced plant fitness in this environment; this was not the case however, in this study. Plants grown under UVB- had more herbivore damage, yet had higher fitness. There are two possible reasons for this observation. First, it is possible that the detrimental effects of UVB radiation are more severe than the effects of herbivory on plant fitness; that is, potential fitness gains due to a reduction in levels of herbivory in UVB+ environments could not compensate for the reduction in fitness due to UVB radiation. Second, there might be a cost associated to plastic responses of plants to UVB radiation. This cost, which may be related to the production of UVB absorbing compounds or due to genetic causes such as potential deleterious effects of genes involved in plasticity (Pigliucci 2001) may account for the lower plant fitness in UVB+ plots even though insect damage was decreased in this environment. Given that the interaction between UVB and insect herbivory was significant and there was a marked decrease in the plasticity of total fruit production (estimate of fitness in this study) in
response to UVB exclusion, it is reasonable to infer that the effects of UVB radiation were likely more detrimental than those of insect herbivory on plant fitness. Nonetheless, potential physiological and genetic costs on plant fitness cannot be discarded, and may also at least partly explain the observed results.

In conclusion, this study addressed how variation in UVB radiation levels may influence potential fitness consequences of insect herbivory in plants. Although overall, UVB radiation had a negative effect on plant fitness, this study revealed the presence of genetic variation in both UVB environments suggesting a potential for plants to evolutionary respond to future changes in UVB radiation levels. It is also important to note that although UVB radiation tended to reduce the amount of herbivore damage inflicted on plants (likely due to increased induction of protective secondary metabolites and physical structures), this reduction was not enough to compensate for the negative effects of UVB radiation on plants.
Table 1. Mixed model analysis of variance showing the effects of UVB radiation, insect herbivory and RIL on plant phenotypic variables of *A. thaliana*.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Flowering date</th>
<th>Fruits</th>
<th>Rosette Biomass</th>
<th>Colonization Damage</th>
<th>Rosette Damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>1.76</td>
<td>22.60***</td>
<td>6.98***</td>
<td>11.49***</td>
<td>0.61</td>
</tr>
<tr>
<td>Herbivory</td>
<td>181.27***</td>
<td>817.48***</td>
<td>235.99***</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>UVB</td>
<td>649.93***</td>
<td>246.48***</td>
<td>16.48***</td>
<td>155.80***</td>
<td>157.43***</td>
</tr>
<tr>
<td>Herbivory x UVB</td>
<td>6.98**</td>
<td>98.98***</td>
<td>0.03</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>RIL</td>
<td>4.00***</td>
<td>1.01</td>
<td>0.71</td>
<td>1.30†</td>
<td>1.28</td>
</tr>
<tr>
<td>Herbivory x RIL</td>
<td>0.92</td>
<td>0.92</td>
<td>0.98</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>UVB x RIL</td>
<td>2.38***</td>
<td>1.45*</td>
<td>17.65***</td>
<td>1.18</td>
<td>2.02***</td>
</tr>
<tr>
<td>Herbivory x UVB x RIL</td>
<td>1.29*</td>
<td>1.03</td>
<td>0.49</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Significance levels †P<0.1, *P<0.05, **P<0.01, ***P<0.001
Figure 1. Reaction norms showing *A. thaliana* phenotypic responses, in terms of total fruit production (A-B), rosette biomass (C-D), and number of days to first flowering (E-F) to variation in UVB radiation levels in herbivory and sprayed plots. Means of RILs are represented with closed circles and represent significant differences in phenotypic means between UVB treatment levels. * P<0.001
Figure 2. Reaction norms showing *A. thaliana* phenotypic responses, in terms of days to herbivore colonization (A), and rosette damage (B). Means of RILs are represented with closed circles represent significant differences in phenotypic means between UVB treatment levels.

* P<0.001
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