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Effects of ambient UV-B radiation on soybean crops: Impact on leaf herbivory by *Anticarsia gemmatalis*

J.A. Zavala, A.L. Scopel and C.L. Ballaré*
*IFEVA, Consejo Nacional de Investigaciones Científicas y Técnicas, Av. San Martín 4453, C1417 DSE,
Buenos Aires, Argentina; *Author for correspondence (e-mail: ballare@ifeva.edu.ar; fax: (54) 11-4514-8730)*

Abstract

Replicated field experiments with large plastic filters were carried out in Buenos Aires (Argentina, 34° S) to study the impacts of current levels of solar UV-B radiation ($\lambda \leq 315$ nm) on soybean (*Glycine max* L.) crops and their interactions with chewing insects, in particular the soybean worm *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae). Solar (near-ambient) UV-B induced changes in the leaves that reduced their attractiveness to *A. gemmatalis* larvae in laboratory "choice" bioassays. When the *A. gemmatalis* larvae were forced to consume leaves from field plots that received solar UV-B, they grew slightly less rapidly and suffered more mortality than their counterparts fed with leaves from plots covered with polyester films that excluded the UV-B component of sunlight. Exposure of the larvae themselves to ambient UV-B under a soybean canopy during the feeding trials did not lower their life expectancy. At the whole canopy level, we found that solar UV-B exclusion resulted in a two-fold increase in the number of leaf lesions inflicted by various species of chewing insects that naturally invaded the field plots. Leaves from canopies exposed to solar UV-B showed significantly higher levels of soluble phenolics and lower levels of lignin than leaves that developed in canopies covered by polyester films. No differences in specific leaf mass, leaf nitrogen or hemicellulose content were detected between the control and the solar-UV-B exclusion treatments. Our results are consistent with the idea that present-day solar UV-B has an important regulatory influence on the interactions between plants and phytophagous insects.

Introduction

Over the last few years there has been a marked increase in the interest for studying the effects of UV-B radiation (280–315 nm) on plants and their implications for plant-consumer interactions in natural and managed ecosystems (reviews in Paul et al. (1997); Ballaré et al. (1999); Paul (2000)). Various research groups have studied the impacts of UV-B on the concentrations of leaf compounds with potential effects on insect herbivores, and have examined the relationships between changes in leaf chemistry and herbivore performance in laboratory experiments. These laboratory studies have shown that UV-B radiation can alter leaf characteristics that could be expected to influence herbivorous insects, such as total leaf N

(Hatcher and Paul 1994; Lindroth et al. 2000), available carbohydrates and fiber (Lindroth et al. 2000), free amino acids (Salt et al. 1998), defense-related phenylpropanoid derivatives (McCloud and Berenbaum 1994; Grant-Petersson and Renwick 1996; Lavola et al. 1998), proteinase inhibitors (Stratmann et al. 2000), and cyanogenic activity (Lindroth et al. 2000).

The impacts of UV-B-induced alterations in leaf quality on insect grazers have been tested in no-choice and choice bioassays that have commonly involved lepidopteran larvae. No-choice bioassays indicated that caterpillars fed UV-B-treated leaves eat less tissue, gain less weight (Grant-Petersson and Renwick 1996), develop more slowly and suffer greater mortality (McCloud and Berenbaum 1994)

than counterparts reared on foliage from plants not exposed to UV-B; however, the response of the insects (in terms of food intake, growth rate and development) can vary with developmental stage and from species to species (McCloud and Berenbaum 1994, 2000; Grant-Petersson and Renwick 1996). Moreover, Lindroth et al. (2000) have shown recently that the consequences of plant exposure to UV-B on insect performance can vary between populations within a particular species of plant host. Choice bioassays have also been carried out with a few combinations of insects and host plants. Laboratory experiments by Lavola et al. (1998) showed that winter moth larvae preferred leaves from plants exposed to UV-B over leaves collected from plants grown without UV-B. In contrast, field experiments with natural enemies of *Datura ferox* (several species of coleopteran insects), *Gunnera magellanica* (moth larvae) and soybean (thrips) showed that when the insects were offered leaves from plants exposed to solar UV-B along with leaves from plants not exposed to solar UV-B, they consistently showed a preference for the latter (Ballaré et al. 1996; Rousseaux et al. 1998; Mazza et al. 1999). The mechanisms behind these responses are not clear (see, e.g., Lavola et al. (1998)).

Other studies have explored the effects of UV-B radiation on the herbivores themselves. Bothwell et al. (1994) were able to show that direct damaging effects of solar UV-B on chirominoid larvae can be large enough to cause a significant reduction in the grazing pressure on diatoms in artificial freshwater ecosystems. The susceptibility of insects to UV-B also varies from species to species: for example McCloud and Berenbaum (2000) showed in a greenhouse study with *Plantago lanceolata* and two species of lepidopteran caterpillars that supplemental UV-B from lamps affected the performance of the generalist herbivore *Trichoplusia ni*, but had no effects on the specialist herbivore *Precis coenia*. UV radiation can also elicit behavioral responses in insects. The impacts of UV-A (315 – 400 nm) have been studied in detail in several systems: many species of insects are attracted by UV-A, and UV-A filtration can lead to reduced population densities of phytophagous insects in the field (e.g., Antignus et al. (1996)). The effects of UV-B are much less documented. Mazza et al. (1999) have recently shown that exclusion of solar UV-B leads to a marked increase in the density of thrips in soybean crops; several UV-B manipulation experiments suggested that thrips detect and can actively avoid UV-B even against a background of very strong UV-A and visible light.

The experiments reported in this paper were carried out to study the effects of ambient UV-B radiation on soybean crops grown in Buenos Aires, Argentina (34° S), focusing on the impacts of solar UV-B on the interaction between soybean plants and chewing insects. Attention was directed to the effects on one of the most important pests of soybean crops in the Rolling Pampas, the soybean worm (or velvetbean caterpillar) *Anticarsia gemmatalis*. Specifically we addressed the following questions:

1. Does solar UV-B affect herbivory by chewing insects in the field?
2. Does solar UV-B induce changes in the leaves that affect their attractiveness to *A. gemmatalis* larvae?
3. Do the changes in leaf characteristics induced by solar UV-B affect the performance (growth and survival) of *A. gemmatalis* larvae?

Materials and methods

Plant culture

Soybean (*Glycine max* L.) plants were grown in the experimental fields of IFEVA, University of Buenos Aires/CONICET (34°35′ S, 58°29′ W), during the summer of 1995–1996. The plants were grown in individual 10-liter pots under 1.2×1.2-m plastic films that either had very high transmittance over the whole UV spectrum (0.02-mm thick polyethylene film, Rolopac, Buenos Aires), or selectively attenuated the UV-B component of sunlight (0.1-mm thick clear polyester) (Mazza et al. 1999). Both films transmitted more than 88% of the photosynthetically-active radiation (400–700 nm). The polythylene film transmitted more than 80% of the incident quanta throughout the UV-B and UV-A bands. The clear polyester blocked essentially all radiation below 310 nm (see transmittance spectrum in Ballaré et al. (1996) and a typical spectral irradiance curve in Mazza et al. (2000)). The spectral properties of the films were stable under the conditions of our study. Each individual plot was surrounded by an almost continuous soybean canopy, which greatly reduced the contribution of sidelight. The filters were kept at a short distance ($\sim$ 5 cm) from the upper-canopy leaves. UV-B at-

tenuation by the polyester films, measured with a broad-band UV-B detector (SUD/240/W attached to a IL-1700 research radiometer; International Light, Newburyport, MA; peak spectral response at 290 nm; half-band width = 20 nm) was found to be consistently greater than 95% at the center of the plots. All leaves used in the bioassays (youngest fully expanded leaves) were collected from plants grown near the center of the plots. The plastic filters could have had some influence on the canopy microclimate compared with a no-filter condition; however, this should not affect the comparison between UV-B treatments. Other studies (Ballaré et al. 1996), as well as spot temperature measurements throughout the present study taken with an infrared thermometer (Therm2228-1/I-9628, Ahlborn, Holzkirchen, Germany), failed to detect any consistent differences in soil or canopy temperature between UV-B treatments.

There were two sets of field plots with four replicates of the UV-B treatments. In one of them (sowing date: 5 December 1995), we grew soybean plants of four classic cultivars (CNS, Williams, Lincoln and Essex). By planting several genotypes we generated a diverse source of leaf material for the feeding experiments, but we did not attempt to document consistent differences among these soybean lines in their responses to solar UV-B. The second set of field plots (sowing date: 12 February 1996; cv. PI 227687) was simply used to provide a soybean canopy environment for a feeding trial carried out in the field between 8 and 20 March 1996. In both cases we used a relatively high density (60 plants m^{-2}) to obtain a high rate of leaf production per unit filter area in the first set of plots, and a well-developed soybean canopy in the second set of plots, which was planted very late in the season. The plants were watered daily to maintain the soil near field capacity. The canopies, although generated by containerized plants, had a structure that was comparable with that of commercial soybean fields planted in narrow rows. The height of the canopies in March was 80 cm for the mix of varieties (early sowing), and 50 cm for PI 227687 (late sowing). By that time the PI 227687 canopy was almost closed, but light interception was not complete; approximately 10% of the ground area received direct sunlight at midday.

Plant damage

In the first set of plots we obtained a coarse estimate of the intensity of the damage to leaf tissue inflicted by natural populations of chewing insects on 3 January 1996, 29 days after sowing (plants with five trifoliate leaves). Damage intensity was assessed by counting the number of lesions (holes in the leaf lamina) in all 5 leaves (20 randomly selected plants per treatment).

Insects

All the bioassays and feeding experiments were carried out with larvae of *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae). This is one of the most important insect pests in commercial soybean crops in Argentina. The larvae for the experiments were obtained from a laboratory colony maintained at the IMIZA, INTA, Castelar, Buenos Aires, Argentina, where insects are raised at 28 °C under continuous artificial light and larvae are fed on a standard artificial diet (Greene et al. 1976) from egg hatch to pupation.

Survivorship (no-choice) experiment

A population of neonate larvae was divided into 12 groups of 30 larvae each. Six groups were fed for nine days with leaves from soybean plants grown under near ambient UV-B (leaf-type treatment: UV-B$_L$) and the other six groups on leaves developed under UV-B exclusion (leaf type treatment: −UV-B$_L$). Each replicate group was placed in a 17 × 13 × 7-cm plastic box in the laboratory at room temperature. Leaves were replaced daily; care was taken to use leaves of similar age (youngest fully-expanded) and with no obvious signs of previous herbivory. Dessication of the leaf tissue was prevented by keeping the petioles in a matrix of water-saturated cotton.

On the ninth day of the experiment, the surviving larvae from each leaf-type treatment were randomly distributed to eight boxes (11–13 larvae per box), each covered with a UV-B transparent film (0.02-mm thick polyethylene film, Rolopac, Buenos Aires). The 16 boxes were distributed under the foliage of the PI 227687 soybean crop (second set of field plots described above). Half of the plots were covered with UV-B transparent polyethylene film (environment treatment = UV-B$_E$), and the other half with polyester filters that selectively removed the UV-B component of solar radiation (environment treatment = −UV-B$_E$). There were four replicate plots of each UV-B environment, and each plot had one feeding box from each leaf type treatment. The number of surviving larvae in each box was recorded and the

leaves replaced daily. The weights of individual caterpillars were determined every 2 or 3 days until pupation.

Preference (choice) experiments

In these experiments, larvae kept in feeding boxes were allowed to choose between soybean leaves that had developed with or without solar UV-B. Before the choice bioassays the larvae were reared on one of the following diets: (i) a standard artificial diet (Greene et al. 1976) (ii) soybean leaves that expanded in the field under UV-B-excluding filters, or (iii) soybean leaves that expanded under near-ambient UV-B.

There were eight (independent) replicate boxes of each experiment, and each box had five larvae. The leaf area was measured at the beginning of the choice experiment ($\sim$ 100 cm^2 of leaf area per UV-B treatment per box), and again 24 h after using an area-meter (LiCor- 2000 Lincoln, NE, USA). The experiments were carried out during three consecutive days.

Leaf characteristics

Leaves were sampled on 5 January 1996 at midday for determinations of crude phenolics using alcoholic extraction at low temperature and specific leaf mass, as described by Rousseaux et al. (1998). We took three 0.3-cm^2 discs from the youngest fully-expanded leaf (at least two plants per plot and per soybean cultivar). Samples for determinations of leaf N, hemicellulose and lignin content were taken on 11 March 1996. The apical leaflet was harvested from the youngest fully-expanded leaf available at the time of harvest. There was one combined sample per plot. Chemical analyses were performed as described in Rousseaux et al. (1998).

Statistical analyses

In the survivorship experiments we used survivorship analysis (SAS LIFE-TEST; SAS (1989)) to compare mean life expectancies between groups and for multiple comparisons involving more than two groups, as described in Hardin et al. (1996). We also used an univariate repeated-measures analysis of variance (Winer 1971; Arnold 1981) to make comparisons between caterpillar growth curves produced by different treatments. The data from the preference experiments were analyzed using analysis of variance (ANOVA).

Table 1. Effects of solar UVB on leaf damage caused by chewing insects in soybean crops. The dependent variable was the number of leaf lesions per plant; meansurements were taken on 3 January 1996, 29 days after sowing. Data were arc-sin transformed for analysis, and back-transformed for presentation.

Treatment	Lesions per plant	Treatment effect[a]
near-ambient UV-B	15.6	P = 0.012
attenuated UV-B	31.9	

[a]The difference among cultivars in damage levels was also significant (P = 0.006), but the cultivar x treatment interaction was not (P > 0.05).

The fraction of leaf area consumed was arc-sin transformed prior to analysis.

Results

Plant damage

Crop damage assessments, carried out in mid-summer on the plants of the first set of plots (cvs. CNS, Essex, Lincoln and Williams), indicated greater damage by chewing insects under −UV-B filters than in the near-ambient UV-B control plots (Table 1). On the average, plants that received no UV-B had two times more leaf lesions than those grown under near-ambient UV-B levels. Inspection of the crops indicated the presence of adult leaf beetles (*Diabrotica speciosa*), larvae of several lepidopteran species and at least two species of grasshoppers. No attempt was made to establish the contribution of the different insect species to the total damage shown in Table 1. All subsequent experiments were carried out with standard populations of the soybean worm, *A. gemmatalis*.

No-choice experiment. Survivorship of A. gemmatalis

Soybean leaves collected from plants that grew either exposed (UV-B$_L$) or not exposed (−UV-B$_L$) to solar UV-B were used to feed *A. gemmatalis* larvae in a laboratory bioassay. Survivorship during the first nine days of the feeding trial was significantly lower for larvae reared on leaves from the UV-B$_L$ treatment than for larvae fed leaves from the −UV-B$_L$ treatment (Log-Rank: chi-square = 4.319, df = 1, P <0.05; Figure 1). Mean survival times were 5.7 d and 6.6 d for UV-B$_L$ and − UV-B$_L$ caterpillars, respectively.

To evaluate potential interactions between foliage quality and ambient UV-B levels (see, e.g., McCloud

Table 2. Results of the log-rank test for global hypothesis of equality of survival distributions for *Anticarsia gemmatalis*, and results of same test performing pariwise rankings of data using only two groups at a time.

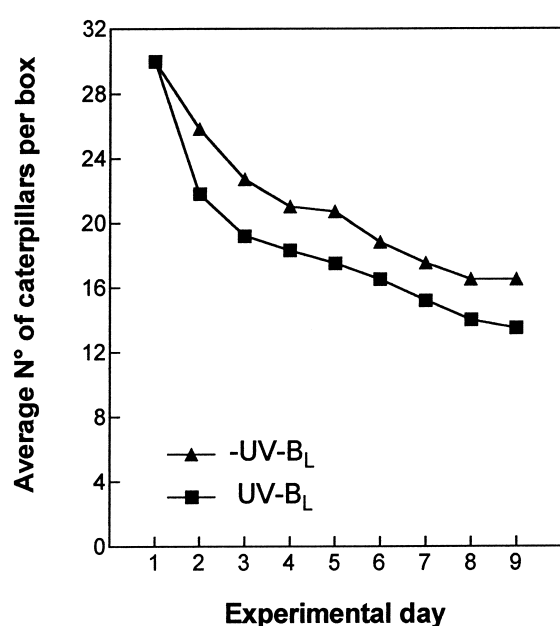|	Chi-Square	d.f.	P
Global	12.2901	3	< 0.001
Pairwise comparisons			
$-UV\text{-}B_L \times -UV\text{-}B_E$ vs $-UV\text{-}B_l \times UV\text{-}B_E$	1.6314	1	0.2015
$-UV\text{-}B_L \times -UV\text{-}B_E$ vs $UV\text{-}B_L \times -UV\text{-}B_E$	2.8166	1	0.0933
$-UV\text{-}B_L \times -UV\text{-}B_E$ vs $UV\text{-}B_L \times UV\text{-}B_E$	2.4236	1	0.1195
$-UV\text{-}B_L \times UV\text{-}B_E$ vs $UV\text{-}B_L \times -UV\text{-}B_E$	9.7803	1	0.0018
$-UV\text{-}B_L \times UV\text{-}B_E$ vs $UV\text{-}B_L \times UV\text{-}B_E$	8.6613	1	0.0033
$UV\text{-}B_L \times -UV\text{-}B_E$ vs $UV\text{-}B_L \times UV\text{-}B_E$	0.0054	1	0.9417

Figure 1. Survivorship schedules of *Anticarsia gemmatalis* larvae reared on leaves from soybean plants grown in the field under either near-ambient UV-B (leaf type treatment: $UV\text{-}B_L$) or attenuated UV-B (leaf type treatment: $-UV\text{-}B_L$; n = 6 groups of 30 caterpillars in each treatment; Log-Rank: chi-square = 4.319, df = 1, P < 0.05).

and Berenbaum (1994)), we took the bioassay boxes to the field at the end of day nine, and placed them under soybean canopies that were covered with either UV-B-excluding polyester films ($-UV\text{-}B_E$ condition) or UV-B-transparent polyethylene filters ($UV\text{-}B_E$ condition). This created a leaf diet (L) by environment (E) combination, with four replicates of each L × E combination. The global hypothesis of equality among treatments in survival distributions was rejected based on the log-rank test (P <0.001; Table 2; survival curves in Figure 2). Mean survival times

were 6.2 d for $-UV\text{-}B_L \times - UV\text{-}B_E$, 6.5 d for $-UV\text{-}B_L \times UV\text{-}B_E$, 5.5 d for $UV\text{-}B_L \times UV\text{-}B_E$, and 5.3 d for $UV\text{-}B_L \times UV\text{-}B_E$ (Figure 2). The results of the log-rank procedure for multiple comparisons of survival distribution, which performs pairwise rankings of data using only two groups at a time (Hardin et al. 1996), are shown in (Table 2). Mean survival time in treatment $- UV\text{-}B_L \times UV\text{-}B_E$ was significantly higher than in the treatments where the insects were fed leaves from plants exposed to solar UV-B (i.e., $UV\text{-}B_L \times -UV\text{-}B_E$, and $UV\text{-}B_L \times UV\text{-}B_E$ combinations). A factorial analysis of the number of dead larvae during the experimental period revealed a significant main effect of the leaf diet on insect mortality (P <0.0001), whereas the main effect of the UV-B environment and the interaction term were not significant (P = 0.607 and P = 0.797, respectively).

The larvae reared on leaves from the $-UV\text{-}B_L$ condition grew faster and had slightly greater weight at pupation than their counterparts reared on leaves from the $UV\text{-}B_L$ condition (Figure 3; repeated measurements analysis F = 4.77; d.f. = 1,42; P = 0.03). The average relative growth rates during the course of the experiment were 0.056 d^{-1} and 0.050 d^{-1} for the $-UV\text{-}B_L$ and $UV\text{-}B_L$ diets, respectively. The weights at pupation were 209 ± 10 mg and 183 ± 4 mg for the $-UV\text{-}B_L$ and $UV\text{-}B_L$ diets, respectively (t-test, P = 0.03, n = 8 boxes).

Preference (choice) experiments

Soybean leaves obtained from plants that had been exposed to contrasting levels of solar UV-B during development were tested in laboratory choice-bioassays employing *A. gemmatalis* larvae previously grown on an artificial diet. In three consecutive days the larvae consumed significantly more leaf area from
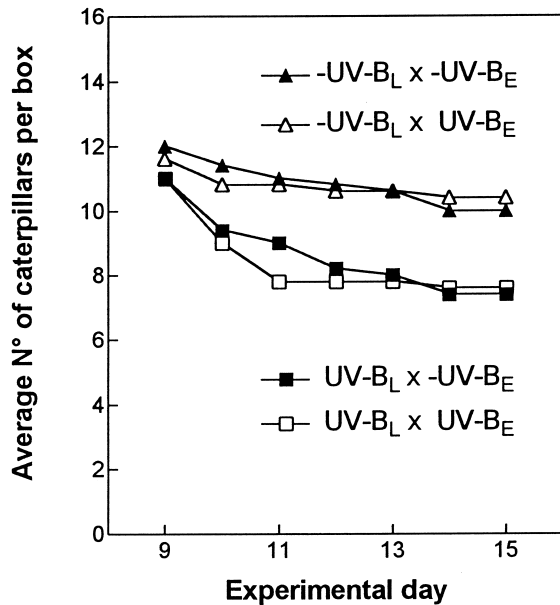
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Figure 2. Survivorship schedules of *Anticarsia gemmatalis* larvae reared on leaves from soybean plants grown in the field under either near-ambient UV-B (leaf type treatment: UV-B$_L$) or attenuated UV-B (leaf type treatment: −UV-B$_L$), when the feeding boxes were transferred to the field (on day 9) and placed under soybean canopies that were covered either with UV-B transparent films (ambient treatment: UV-B$_E$), or with filters that selectively removed the UV-B component of sunlight (ambient treatment: −UV-B$_E$). See statistics in (Table 2). At the time of the experiments (mid March), the height of the canopies was 50 cm (cv. PI 227687; sowing date: 12 February), and approximately 10% of the ground area received direct sunlight at midday.

leaves developed under UV-B attenuation than from leaves grown under near-ambient UV-B, which were offered to the insects in the same feeding boxes (Figure 4). A similar pattern of preference was detected in caterpillars that had been fed with leaves developed under attenuated UV-B before the choice bioassay (Figure 4). Interestingly, caterpillars reared on leaves from plants exposed to solar UV-B before the experiment had a different behavior in the choice bioassay. On the first day of the test the larvae consumed more tissue from leaves developed under near ambient UV-B than from leaves grown under UV-B attenuation. However, after the second test day, the insects preferred leaves that grew under attenuated UV-B over leaves grown under near ambient UV-B (Figure 5).

Leaf characteristics

Exposure to near ambient UV-B resulted in significantly higher levels of leaf phenolic compounds as determined from UV-absorbance measurements in crude alcoholic extracts obtained in mid summer. No significant differences between UV-B treatments were found in leaf N, specific leaf mass or hemicellulose content, whereas the percent lignin content was higher in the plants grown under attenuated UV-B (Table 3).

Discussion

The observation of increased herbivory by chewing insects under filters that specifically attenuated the UV-B component of sunlight (Table 1) accords with previous results from UV-B filtration experiments in natural and cultivated plant communities (Ballaré et al. 1996; Rousseaux et al. 1998, 2001; Mazza et al. 1999). Similarly, other workers have reported reduced herbivory or insect densities under enhanced UV-B conditions in the field (Gwynn-Jones et al. 1997; Salt et al. 1998), although in at least one plant species of the Swedish Lapland (*Vaccinium myrtillus*), UV-B supplementation resulted in increased herbivory (Gwynn-Jones et al. 1997). Moreover, increased leaf consumption in leaves exposed to UV-B in a controlled-environment experiment was reported by Lavola et al. (1998), who studied the impacts of UV-B irradiation on silver birch consumption by winter moth larvae.

The effects of UV-B on herbivores may be direct or indirect. Direct effects of UV-B on *A. gemmatalis* growth and survival were not detected in the present experiments, with caterpillars feeding underneath a growing soybean canopy (Figure 2). Of course our results do not exclude the possibility of direct UV-B effects on insect performance under more exposed conditions, as documented by (McCloud and Berenbaum 1994, 2000) in experiments involving UV-B supplementation with lamps. Indirect effects of UV-B (i.e., those mediated by changes in the soybean plants) appeared to be of great importance for *A gemmatalis*, both in the laboratory (Figures 1 and 4) and (Figure 5) and in the feeding bioassay carried out under a soybean canopy in the field (Figure 2) and (Figure 3).

A limited number of controlled-environment studies have demonstrated that treatment of plant tissue with UV-B radiation reduces the growth rate and/or

Table 3. Characteristics of soybean leaves grown under near-solar (UV-B) or attenuated UV-B or attenuated UV-B radiation (−UV-B). Values represent the average of 4 independent blocks; means followed by different letters in a column are significantly different at P ≤ 0.05. Determinations of specific leaf mass and methanol soluble phenolics were made in early summer, in coincidence with the observations on natural herbivory levels in the field (Table 1). Other determinations were carried out on leaves harvested for the feeding bioassays (Figures 2, 3, 4 and 5). The significant effect of solar UV-B on extractable phenolics persisted throught the growing season (not shown).

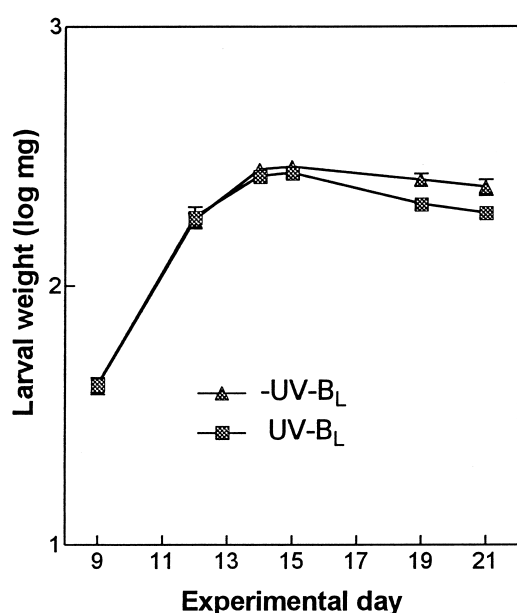| Treatment	Specific leaf mass (g/m^2)	Phenolic compounds (absorbance at 305 nm/ disk)	Leaf N concentration(g/m^2)	Hemicellulose (%)	Lignin (%)
−UV-B	42.9 a	1.07 b	1.15 a	23.4 a	16.1 a
UV-B	48.3 a	1.21 a	1.38 a	27.2 a	11.6 b

Figure 3. Weight of *Anticarsia gemmatalis* larvae reared on leaves from soybean plants grown either under near ambient UV-B (leaf type treatment: UV-B$_L$) or with the UV-B component excluded (leaf type treatment: −UV-B$_L$). Caterpillars began to pupate on day 21; n = 8 boxes (11 to 13 caterpillars per box) for each leaf type treatment. Vertical bars indicate ± 1 S.E. when the S.E. was bigger than the symbol.

on insects or act as feeding deterrents (see (Bergvinson et al. 1994; McCloud and Berenbaum 1994; Grant-Petersson and Renwick 1996). In addition, some of these compounds, such as psoralens, can induce defense genes whose products may have additional negative effects on herbivores (Parsons and Hadwiger 1998). Wheeler and Slansky (1991) showed that the relative growth rates of *A. gemmatalis* larvae fed on artificial diets were consistently depressed when the diet was supplemented with soybean foliage extracts. Several isoflavonoids have been described from soybean foliage that reduce herbivore performance (reviewed by Kogan and Fischer (1991)). For example, the occurrence of the isoflavones coumestrol and diazdein in soybean foliage extracts was related to reduced consumption by cabbage loopers *Trichoplusia ni* (Neupane and Norris 1990). The pterocarpan (phytoalexin) glyceollin has been found to deter feeding by coleopteran herbivores (Hart et al. 1983; Fisher et al. 1990). Coumestrol and other isoflavones such as phaseol have been also associated with reduced larval biomass and higher mortality in *Pseudoplusia includens* (Caballero et al. 1986; Kogan and Paxton 1983). Although we have not identified the specific compounds that account for the increase in the level of soluble phenolics in our soybean crops (Table 3), the evidence summarized above suggests that upregulation of the phenylpropanoid pathway by exposure of plants to current levels of UV-B may play an important role in antiherbivore defense.

A second explanation, which may be complementary to the one just described, is that exposure to solar UV-B induces changes in physical properties of the leaf tissues, reducing their acceptability and/or digestibility. For example, increases in specific leaf mass have been reported in response to UV-B treatments in several species (e.g., Adamse and Britz (1992); Ballaré et al. (1996)), and specific leaf mass
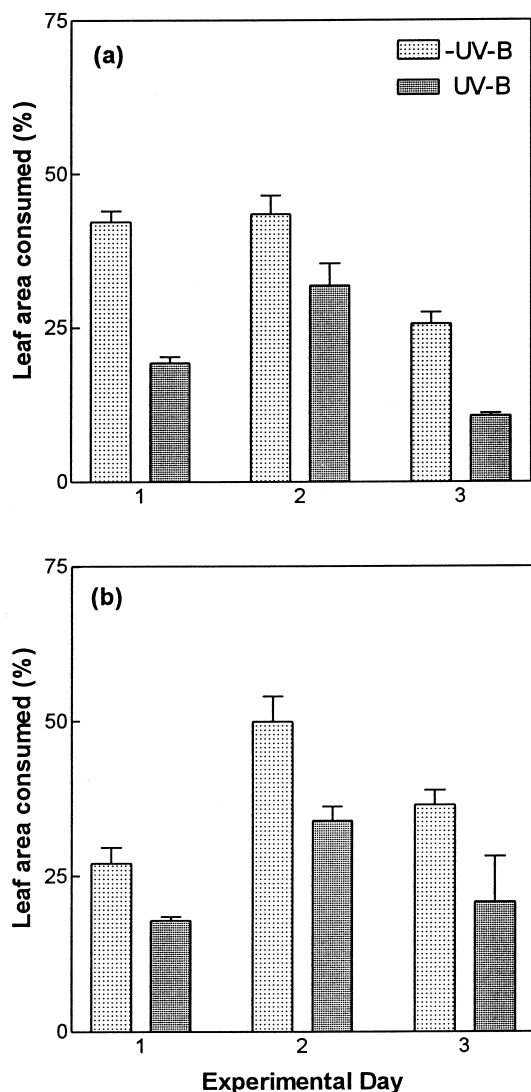
survivorship of moth larvae (e.g., (McCloud and Berenbaum 1994; Grant-Petersson and Renwick 1996); our study is the first to show that larval growth and survival can be reduced by the changes in foliage quality induced by near-ambient levels of solar UV-B. There are various potential explanations for this plant-mediated effect of UV-B radiation on insect performance. The first one is based on the fact that UV-B radiation increases the accumulation of methanol-soluble phenolic compounds in many species, including soybean (Mirecki and Teramura 1984; Mazza et al. 2000), and the present study, Table 3). Several UV-inducible phenolic derivatives can have toxic effects

Figure 5. Effects of leaf exposure to solar UV-B on tissue consumption by early instar *Anticarsia gemmatalis* larvae previously fed leaves from the UV-B$_L$ treatment. Other conditions were as explained in legend to (Figure 4). Arc-sin-transformed data are shown; thin bars indicate ± 1 S.E.; significance of the UV-B effect: day 1 (11 March) $P < 0.05$, day 2 n.s., and day 3 $P < 0.005$.

son, specific leaf mass was not affected by UV-B (Table 3).

A third possibility is that UV-B enhances plant defenses induced by mechanical damage, such as the accumulation of protease inhibitors. Many plants (Ryan 1990), including soybean (e.g., McManus and Burgess (1995)), produce inhibitors of insect proteolytic enzymes, which can retard insect growth and development. Recently, Stratmann et al. (2000) reported that high doses of UV-B radiation enhance the wound-induced accumulation of a proteinase inhibitor in tomato leaves, but it is not known whether a similar synergic effect takes place in response to solar UV-B radiation. In conclusion, therefore, although a negative effect of solar UV-B on foliage quality and performance of moth larvae has been reported by various authors, the underlying mechanisms can involve more than one response, and they have not been unequivocally identified in any experimental system. As discussed by Paul et al. (1997) and McCloud and Berenbaum (2000), given the complexity of host-insect interactions, it is unlikely that a single, general mechanism can explain the responses of a wide variety of organisms.

Our findings demonstrate that, at least in the short term, the changes in plant tissue induced by ambient UV-B can affect insect growth and survival. Do the

Figure 4. Effects of leaf exposure to UV-B on tissue consumption by early instar *Anticarsia gemmatalis* larvae. The soybean plants were grown in the field under either near ambient (UV-B$_L$) or attenuated (–UV-B$_L$) UV-B radiation. The choice bioassays were carried out in the laboratory. The caterpillars had been fed a standard artificial diet (a) or soybean leaves from the –UV-B$_L$ treatment before the experiment (b). Arc-sin-transformed data are shown; thin bars indicate ± 1 S.E.; significance of the UV-B effect: (a) day 1 (13 March) $P < 0.001$, day 2 $P < 0.05$, and day 3 $P < 0.005$; (b) day 1 (11 March) $P < 0.05$, day 2 $P < 0.005$, and day 3 $P < 0.05$.

is highly correlated with tissue toughness (Arnone et al. 1995). In addition, there is evidence indicating that UV-B can increase the lignin content in some tissues (Rozema et al. (1997), but see Gherke et al. (1995)). Solar UV-B did not increase lignin content in our experiment, and at least during the early part of the sea-
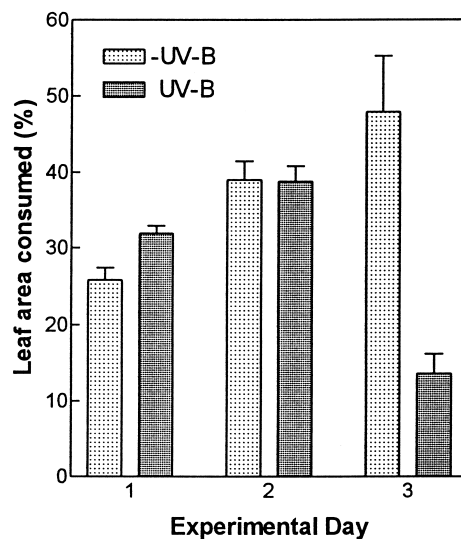
insects avoid leaves that have developed under high levels of solar UV-B? Previous field choice experiments have suggested that this is indeed the case (Ballaré et al. 1996; Rousseaux et al. 1998; Mazza et al. 1999). Our laboratory experiments with *A. gemmatalis* larvae showed greater consumption from leaves that were not exposed to UV-B compared with leaves that developed under near-ambient UV-B levels (Figure 4). Hatcher and Paul (1994) reported that the consumption rate of pea leaf tissue by moth larvae was reduced by previous exposure of plants to UV-B. They suggested that the UV-B-induced increases in N concentration in leaf tissue increased the efficiency with which larvae utilized their food, leading to a concomitant reduction of quantity of leaf material consumed. Slansky and Wheeler (1989) showed that *A. gemmatalis* can compensate for changes in N content in the host tissue by altering their food consumption rate, even if this compensatory feeding response leads to higher consumption of toxic secondary chemicals (Slansky and Wheeler 1992). This compensation response is not a plausible mechanism to explain the greater consumption of leaves grown under attenuated UV-B in our bioassays. First, we did not detect a significant effect of UV-B on total leaf N levels (Table 3). Second, since in our choice experiments the leaves from both UV-B treatments were offered to the insects in the same feeding boxes, and the insects were highly mobile, the observed differences in consumption (Figure 4) are likely to reflect primarily the food selection behavior of the caterpillars. Whether the selection decisions are based on rough physical properties of the leaves (e.g., toughness), or on more subtle recognition features that are altered by solar UV-B is presently unknown. It is intriguing that the choice behavior appeared to depend on the insects' previous experience (cf. Figure 4 and Figure 5), suggesting that the preference for tissue not exposed to UV-B is not hardwired into the insects' behavior.

The conclusion that emerges from this and other recent studies of UV-B effects on plant-insect interactions is that current levels of solar UV-B can have large impacts on herbivory (Ballaré et al. 1999). A better knowledge of the mechanisms involved can help to understand the functional roles of solar UV-B in terrestrial ecosystems, and may also have practical implications to improve crop resistance to insect pests.

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