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Original Article

Soybean resistance to stink bugs (*Nezara viridula* and *Piezodorus guildinii*) increases with exposure to solar UV-B radiation and correlates with isoflavonoid content in pods under field conditions

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ABSTRACT

Solar UV-B radiation (280–315 nm) has a significant influence on trophic relationships in natural and managed ecosystems, affecting plant–insect interactions. We explored the effects of ambient UV-B radiation on the levels of herbivory by stink bugs (*Nezara viridula* and *Piezodorus guildinii*) in field-grown soybean crops. The experiments included two levels of UV-B radiation (ambient and attenuated UV-B) and four soybean cultivars known to differ in their content of soluble leaf phenolics. Ambient UV-B radiation increased the accumulation of the isoflavonoids daidzin and genistin in the pods of all cultivars. Soybean crops grown under attenuated UV-B had higher numbers of unfilled pods and damaged seeds than crops grown under ambient UV-B radiation. Binary choice experiments with soybean branches demonstrated that stink bugs preferred branches of the attenuated UV-B treatment. We found a positive correlation between percentage of undamaged seeds and the contents of daidzin and genistin in pods. Our results suggest that constitutive and UV-B-induced isoflavonoids increase plant resistance to stink bugs under field conditions.

Key-words: daidzin; genistin; isoflavonoids; plant–insect interactions; pod feeders.

INTRODUCTION

Solar ultraviolet-B (UV-B) radiation (280–315 nm) is an important modulator of plant interactions with consumer organisms, including pathogens and herbivorous insects, in terrestrial ecosystems (reviewed in Caldwell *et al.* 2003; Ballaré *et al.* 2011; Kuhlmann & Müller 2011; Ballaré 2014). Filtering out UV-B radiation from the light received by plants under field conditions frequently results in increased levels of insect herbivory (reviewed in Caldwell *et al.* 2003; Caldwell *et al.* 2007; Bidart-Bouzat & Imeh-Nathaniel 2008; Ballaré *et al.* 2011; Kuhlmann & Müller 2011; Ballaré *et al.* 2012). Although some insects can directly perceive and respond to ambient UV-B radiation, as has been shown for soybean thrips (*Caliothrips phaseoli*) (Mazza *et al.* 2002, 2010), most of the effects of UV-B radiation on plant–insect interactions are likely to be indirect (i.e. mediated by the effects of UV-B on the quality of plant tissues). These effects may include variations in leaf phenolics (McCloud & Berenbaum 1994; Mazza *et al.* 2000; Rousseaux *et al.* 2004; Izaguirre *et al.* 2007; Kotilainen *et al.* 2009; Kuhlmann & Müller 2009, 2010), cyanogenic compounds (Lindroth *et al.* 2000), diterpene glycosides (Dinh *et al.* 2013) and defence-related proteins such as trypsin proteinase inhibitors (Stratmann *et al.* 2000; Izaguirre *et al.* 2003; Stratmann 2003; Demkura *et al.* 2010). The effects of solar UV-B radiation on plant metabolomics are attracting attention in the context of potential utilization in agriculture for improving plant resistance to pests and pathogens (Ballaré *et al.* 2012) and food quality (Jansen *et al.* 2008; Ballaré *et al.* 2011; Schreiner *et al.* 2012; Wargent & Jordan 2013; Williamson *et al.* 2014).

Soybean (*Glycine max*), the most important legume crop in South and North America, is attacked by both the southern green stink bug (*Nezara viridula* L.) and the redbanded stink bug (*Piezodorus guildinii* W.). Temperate regions of Brazil, Argentina and the southern U.S. are invaded by *N. viridula* and *P. guildinii*. These species are part of the stink bug complex, which is of major economical importance in all soybean-growing regions (Panizzi & Slansky 1985; Aragon *et al.* 1997). *P. guildinii* has become a major stink bug pest in Louisiana soybeans (Temple *et al.* 2009) and causes more damage per insect than other stink bug species (Correa-Ferreira & de Azevedo 2002). Stink bugs can puncture most aboveground plant parts with their piercing-sucking mouthparts, but preferentially feed on young developing seeds (McPherson *et al.* 1994). Seed feeding can cause seed abortion or deformation, and decreases

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germination, emergence and survival of plants (Daugherty *et al*. 1964; Todd & Turnipseed 1974). Reductions in yield caused by stink bug attack have been documented, mainly as a consequence of reduced seed quality for commercialization (Todd & Turnipseed 1974; McPherson *et al*. 1979; Musser *et al*. 2011).

Soybean plants can respond to insect damage by up-regulating defences that may deter potential herbivores or decrease the intensity of tissue damage (Underwood *et al*. 2000; Zavala *et al*. 2008, 2009). Isoflavonoids are one of the main chemical defences against insect attack in soybeans, and the concentration and composition of this group of compounds can vary considerably in response to genetic and environmental factors (Carrao-Panizzi & Kitamura 1995). Unlike flavonoids, which are widely distributed in the plant kingdom, the occurrence of isoflavonoids is almost entirely restricted to plants belonging to the Fabaceae (Heinonen *et al*. 2002). The isoflavonoid glycoside genistin extracted from soybean foliage was reported to negatively affect the behaviour and physiology of *Anticarsia gemmatalis* and *Trichoplusia ni* caterpillars (Hoffmann-Campo *et al*. 2001). Work with stink bugs demonstrated that *N. viridula* attack induced increased levels of isoflavone glycosides (daidzin and genistin) in soybean seeds, and that stink bugs preferred to feed on seeds with low levels of isoflavonoids (Piubelli *et al*. 2003a,b). UV-B radiation is known to induce isoflavonoid accumulation in the leaves of common bean (*Phaseolus vulgaris*) (Beggs & Wellman 1994) and can reduce the damage caused by folivorous insects such as *A. gemmatalis* and *C. phaseoli* on soybean plants (Mazza *et al*. 1999; Zavala *et al*. 2001). However, little is known about the effects of solar UV-B radiation on the phenolic profiles of reproductive structures, which are the main targets of stink bugs, and no experiments have been carried out to evaluate the responses of piercing-sucking insects that feed on pods and seeds to metabolic changes induced by solar UV-B radiation.

In the experiments reported in this study, we tested the effects of ambient UV-B radiation on the colonization of soybean crops by natural populations of *N. viridula* and *P. guildinii*. We conducted field experiments in Buenos Aires (34° Southern Latitude), manipulating the levels of UV-B radiation received by soybean crops by means of UV-B-absorbing filters. We found that soybean crops exposed to ambient UV-B were less attacked by stink bugs than those grown under attenuated UV-B levels. Choice experiments demonstrated that exposure to ambient UV-B radiation induced changes in the plants that made them less attractive to stink bugs. Plant exposure to solar UV-B increased the contents of several isoflavonoids (daidzein, genistein) and isoflavonoid glycosides (daidzin, genistin) in soybean pods, but not in seeds. At harvest, soybean crops grown under attenuated UV-B had significantly higher numbers of unfilled pods and damaged seeds than those grown under ambient UV-B radiation. Across cultivars and UV-B treatments, we found a negative correlation between the contents of daidzin and genistin in pods and the percentage of damaged seeds. Our results, along with evidence emerging from other studies, show that plant exposure to solar UV-B radiation can induce changes in the plant metabolome that improve plant resistance to insect pests, with beneficial effects on plant health and crop yield.

MATERIAL AND METHODS

Plant culture

The experiments were carried out in the experimental fields of the University of Buenos Aires (34°35′S, 58°29′W), Buenos Aires, Argentina. Soybean (*G. max*) seeds were sowed in rows in replicated field plots. The distance between rows was 15 cm; plant density was 60 m^{-2}. There were two planting dates: November 13 2010 (experiment I, spring season) and February 11 2011 (experiment II, summer season). In both experiments, there were five true replicates (independent plots) of each genotype x UV-B treatment combination (see below). The plots were watered as needed and weeds controlled manually.

UV-B treatments

Soybean of the cvs Williams [maturity group (MG) III], Dekalb CX458 (MG IV; referred to as DK458 in this paper), Charata-76 (MG VII) and A5308 (MG V) were allowed to emerge and grow in the field under 3×4.2 m aluminum frames covered with either clear polyester films (Mylar-D; Dupont, Wilmington, DE, USA; 0.1 mm thick), which virtually cut off all UV radiation below 320 nm without affecting longer wavelengths (Attenuated UV-B treatment, UVB−), or 'Stretch' films (Bemis Co., Minneapolis, MN, USA; 0.025 mm thick), which had very high transmittance over the whole UV and visible spectrum ('Ambient' treatment, UVB+). The spectral characteristics of the filters are described in detail in Mazza *et al*. (2002). This experimental set-up has been used in previous experiments to evaluate the effects of ambient UV-B radiation on plant growth and plant–insect interactions (Rousseaux *et al*. 1998, 2001; Mazza *et al*. 1999, 2000, 2013; Zavala *et al*. 2001; Robson *et al*. 2003). The degree of UV-B attenuation at the center of the UVB− plots (measured with a broad-band UV-B detector SUD/240/W attached to IL-1700 research radiometer; International Light, Newburyport, MA, USA; peak spectral response at 290 nm; half-bandwidth = 20 nm) was >95%. Peak levels of photosynthetically active radiation (PAR) at midday, measured with a Li-Cor quantum sensor (Li-188B; Li-Cor, Lincoln, NE, USA), were around 1900 μmol m^2 s^{-1}, and were not significantly different between filter types. Average canopy temperatures under UVB+ and UVB− filters, measured with an IR thermometer, were always within <1° C of each other, and in no case were consistent differences in temperature detected between filter treatments. The height of the filters was adjusted periodically to maintain them approximately 10 cm above the upper-canopy leaves. Filters were changed one or two times during the course of the growing season because the plastics tended to deteriorate and accumulate dust. All measurements were performed on the plants located at the centre of the plots, in order to minimize potential effects of unfiltered diffuse radiation.

Insect survey and choice experiments

Plants at our field site were colonized by natural populations of *N. viridula* L. and *P. guildinii* W. (Hemiptera: Pentatomidae). The effect of solar UV-B on soybean attractiveness to natural stink bug colonization was tested in field and laboratory experiments. In both experiments (I and II), beginning at the R5 growth stage (beans were green and beginning to develop) (Fehr *et al*. 1971) and continuing for 5 d, we monitored the natural development of stink bug populations by sampling 1 m of row from each plot. We counted adults and nymphs >6 mm, since this size represents the lower limit at which stink bug nymphs are included in injury threshold determinations (Todd & Turnipseed 1974).

Manipulative choice experiments were carried out using adult insects from the natural population. In these experiments, stink bugs were starved for 48 h and then transferred to feeding boxes ($20 \times 35 \times 10$ cm^3) where they were allowed to choose between soybean branches (bearing pods) that had developed under either ambient (UVB+) or attenuated (UVB−) solar UV-B radiation. All the branches for the choice experiments were at the R5 growth stage (beans beginning to develop) and collected from plants grown near the centre of the plots. The tests lasted 15 min and the response variable was the number of insects found sucking on the pods, which was used to evaluate feeding preferences in paired comparisons between treatments. There were 10 replicate boxes of each experiment for each species, and each box had 20 stink bugs. The experiments were carried out during three consecutive days.

Seed damage

Since stink bug attack decreases soybean seed quality (Todd & Turnipseed 1974; McPherson *et al*. 1979), at the end of the cropping season (April 2011), plants from all plots were hand harvested for determination of number of pods with and without seeds, and number of attacked and healthy seeds (see below). Within each experiment, seed samples (four subsamples of 50 seeds each per plot) from both UV-B treatments and all cultivars were collected and stored in paper bags.

Harvested seeds were separated into damaged and undamaged seeds. Damaged seeds were either visually punctured seeds with extensive shriveling or seeds that displayed one or more punctures in the tetrazolium test. The tetrazolium test was performed as described previously (França Neto *et al*. 1998). Seeds were mixed with tetrazolium (0.075%) and heated at 40 °C for 180 min. After this time, seed samples were washed with tap water and seed damage was evaluated. Damaged tissue was considered dead when it showed a white or green coloration after staining with tetrazolium. An intense red ring typically separated dead tissue from active tissue, and in general, a puncture produced by the insect was clearly visible at the centre of the ring.

Isoflavonoid extraction and determination

To determine the effects of solar UV-B radiation on isoflavonoid accumulation, seeds and pods of soybean plants grown under either UVB+ or UVB− treatments were harvested at R6 (Fehr *et al*. 1971). Pods (at least one per plant) from the same position of five different plants of each genotype were collected and pooled to generate one replicate (5 pods per plot and per genotype). All pods were collected near the center of the plots. Seeds and pods were defatted with cyclohexane; extractions were made with methanol. Samples were purified with a C18 Silica Cartridge (Agilent Technologies, Palo Alto, CA, USA) using different mixtures of MeOH–H_2O. 60% MeOH fractions were evaporated and redisolved in MeOH. Aliquots of 5 μL were subjected to high-performance liquid chromatography (HPLC; Agilent 1100 A series) using a reverse-phase octadesyl column (Eclipse XDB-C18 4.6×150 mm, 5 μm). The isoflavonoids were eluted using a mobile phase gradient of 15–60% acetonitrile in 0.1% acetic acid for 60 min at a flow rate of 1 mL min^{-1}. Compounds were measured with the detector set at λ 270 nm. Retention times and quantitative data for daidzein, daidzin, genistein and genistin were obtained by comparison to known standards (all from Sigma-Aldrich, St. Louis, MO, USA). External standard curves were performed for isoflavonoid quantification.

Statistical analysis

Data were analysed with SAS, version 8.02 (SAS, Cary, NC, USA). The number stink bugs per plant, percentage of damaged seeds, percentage of empty pods, and seed and pod isoflavonoid contents in each experiment were analysed by two-way ANOVA. Main factors were Genotype (labelled G in all figures) and UV-B environment (labelled UVB in all figures). Specific least square means comparisons were performed in those cases where the interaction between main factors was statistically significant ($P < 0.05$). Primary data were transformed as needed to meet the assumptions of ANOVA (square root transformation for percentage of empty pods in experiment II and daidzin content, log transformation for daidzein content). Data from the choice experiments were analysed by Wilcoxon signed-rank test.

RESULTS

Insect surveys

In both experiments (I and II), solar UV-B decreased the density of the stink bug populations that invaded our field crops (UVB+ versus UVB− plots; Fig. 1a,b). In the second experiment, there was a significant UV-B × Genotype interaction, essentially because the effect of UV-B lowering insect density was not present in plants of cv DK458 (Fig. 1b).

Seed damage

Plants grown under ambient UV-B radiation had lower numbers of damaged seeds than those grown under

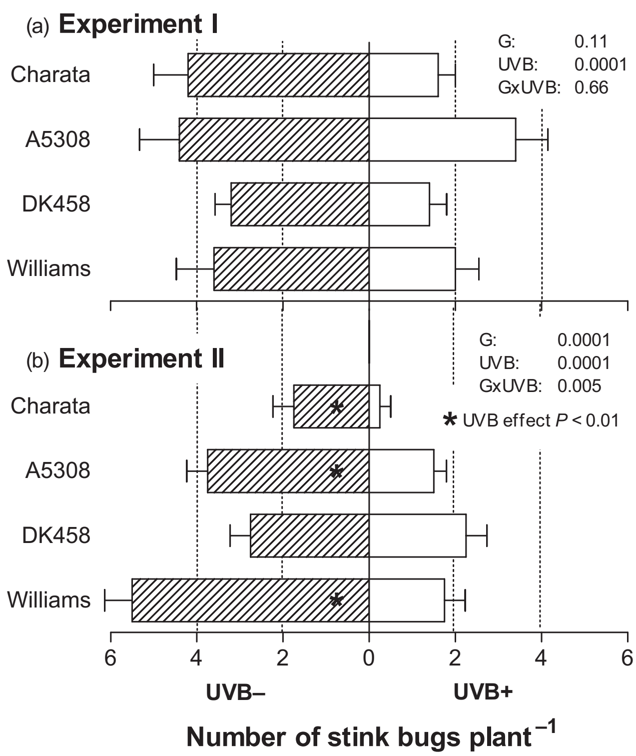

Figure 1. Total number (±SE) of stink bugs (*Nezara viridula* and *Piezodorus guildinii*) that colonized foliage of soybean cultivars grown under attenuated UV-B radiation (UVB−) and ambient UV-B radiation (UVB+): (a) experiment I and (b) experiment II. *P*-values of the main factors genotype (G) and UV-B treatment (UVB) are shown, as well as their interaction (G × UVB). Specific comparisons between UV-B treatments were performed when the interaction term was statistically significant and are indicated with asterisks. Values represent the average of five independent blocks.

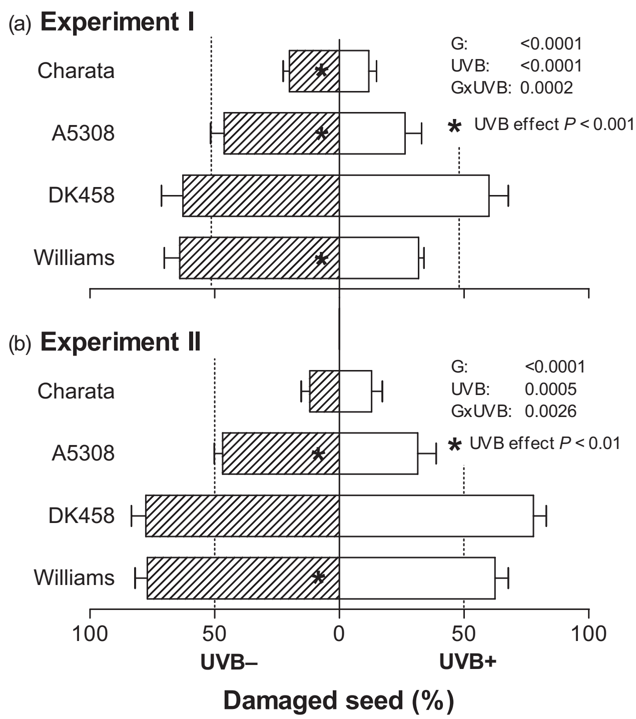

Figure 2. Percent of seed damage (±SE; $n = 5$) in soybean cultivars grown under attenuated (UVB−) or ambient (UVB+) UV-B radiation: (a) experiment I and (b) experiment II. See Fig. 1 legend for details.

attenuated UV-B (Fig. 2a,b). Ambient UV-B radiation decreased the percentage of damaged seeds in cv A5308, cv Williams and cv Charata in experiment I, and cv A5308 and cv Williams in experiment II (Fig. 2a,b). Since stink bug infestations can negatively affect pod filling in soybeans (Bothel *et al*. 2000), we determined the effects of ambient UV-B radiation on the number of empty pods. Plants grown under ambient UV-B radiation showed lower percentage of unfilled pods than those grown under attenuated UV-B radiation in experiment II (Fig. 3b), but no differences were found between treatments in experiment I (Fig. 3a).

Preference (choice) experiments

To test the hypothesis that pods from plants exposed to solar UV-B are less preferred by stink bugs than pods produced by plants grown under attenuated UV-B, we performed binary choice bioassays. In three out of the four cultivars (A5308, Charata and Williams) both stink bug species preferred branches grown under attenuated UV-B over those from the ambient UV-B treatment [*N. viridula* (Fig. 4a) and *P. guildinii* (Fig. 4b)]. No significant effects of UV-B radiation on stink bug preferences were found for branches of cv DK458 (Fig. 4).

Phenolic compounds

Since isoflavonoids in soybean seeds are thought to function as defences against stink bugs (Piubelli *et al*. 2003a), we determined the content of daidzein, daidzin, genistein and genistin in seeds and pods of plants collected from our field plots at R6. In the seeds, there were some differences among cultivars for the glycosides daidzin and genistin, with cv. Williams showing the highest (daidzin: 102 μg g^{-1} and genistin: 194 μg g^{-1}) and cv A5308 the lowest (daidzin: 18 μg g^{-1} and genistin: 22 μg g^{-1}) levels of these compounds. However, isoflavonoid contents in the seeds were not affected by the UV-B treatment. In contrast, in the pods, exposure to solar UV-B radiation caused a general increase in the contents of daidzin, genistin and daidzein in all genotypes (Fig. 5a–c). For genistein accumulation, the effect of UV-B increasing the content of this isoflavonoid was only significant in cv A5308 (Fig. 5d).

To test the relationship between isoflavonoid accumulation and herbivory by stink bugs, we regressed the percentage of damaged seeds from experiments I and II against the contents of daidzein, daidzin, genistein and genistin in pods of plants grown under the two radiation treatments. We

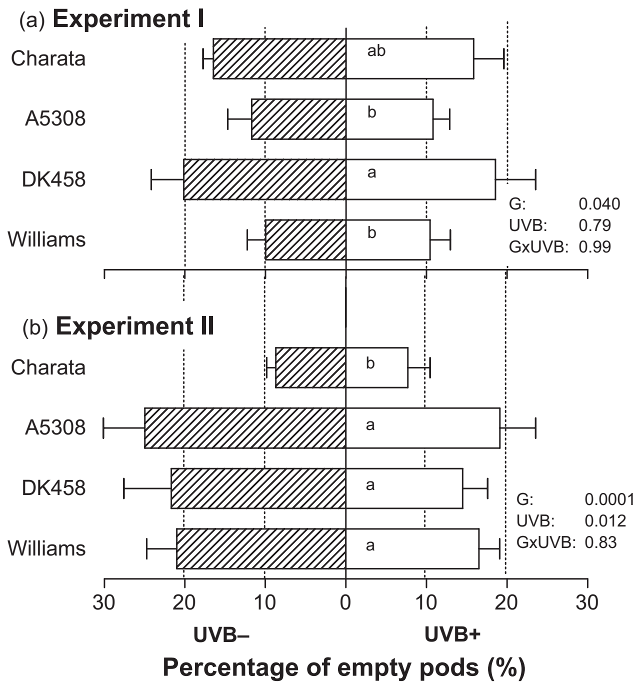

Figure 3. Percentage of empty pods (±SE; $n = 5$) in soybean cultivars grown under attenuated (UVB−) or ambient (UVB+) UV-B radiation: (a) experiment I and (b) experiment II. Different letters indicate genotype differences [Fisher's least significant difference (LSD) test]. See Fig. 1 legend for details.

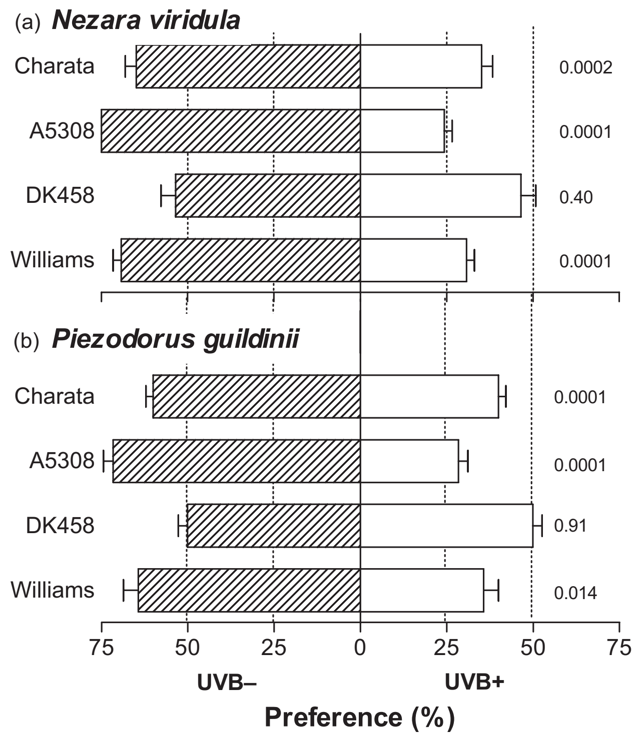

Figure 4. Choice experiments testing the preferences of stink bugs (±SE; $n = 10$) for soybean plants grown under attenuated (UVB−) or ambient (UVB+) UV-B radiation: (a) *Nezara viridula*. (b) *Piezodorus guildinii*. *P*-values of Wilcoxon signed-rank test are indicated at the right of each genotype.

found that an exponential function of daidzin content in pods explained between 40 and 71% of the variation in seed damage across cultivars and radiation treatments (Fig. 6a). A strong and significant negative relationship between isoflavonoid concentration in pods and seed damage was also found for genistin (Fig. 6b), but not for daidzein and genistein (data not shown). Interestingly, the isoflavonoid content of the seeds themselves did not correlate with the level of seed damage.

DISCUSSION

It is well established that solar UV-B radiation can have important effects reducing insect herbivory, although most of the available evidence comes from studies that focused on leaf damage by chewing insects (Caldwell *et al*. 2003; Ballaré *et al*. 2011; Kuhlmann & Müller 2011; Ballaré 2014). Information on the effects on phloem feeders is much less abundant, and limited to a few studies on aphids that feed predominantly on vegetative structures (Kuhlmann & Müller 2010; Hu *et al*. 2013). The experiments reported in this study demonstrate that exposure of soybean plants to solar UV-B radiation decreases colonization and attack by stink bugs, which feed predominantly on pods, and that this UV-B effect is associated with reduced seed damage.

Previous UV-B supplementation studies suggested that enhanced UV-B radiation can produce negative effects on reproductive biology and fruit set of sensitive genotypes of soybean (Teramura *et al*. 1990; Koti *et al*. 2004; Chimphango *et al*. 2007) (e.g. by decreasing pollen production and germination; Koti *et al*. 2004). In the present study, under natural radiation and herbivory pressure, solar UV-B had a positive effect reducing the percentage of damaged seed and empty pods (Figs. 2 and 3), suggesting an indirect effect of solar radiation mediated by reduced insect herbivory. In fact, the effect of solar UV-B could be explained by the reduced stink bug preferences for UV-B-exposed soybean plants in choice experiments, and stink bug damage in the field was inversely correlated with the contents of UV-B-inducible isoflavone glycosides (daidzin and genistin) in soybean pods (Figs. 5 and 6). Although other plant traits could be affected during the experiments, these correlative results suggest that changes in isoflavonoid levels induced by solar UV-B radiation can influence soybean resistance to one of its most important insect pests under field conditions.

Adult stink bugs preferentially feed on young pods and cause seed abortion, increasing the number of empty pods (Todd 1989; McPherson *et al*. 1994). The highest density of stink bugs on soybeans generally occurs during mid- to late pod-fill (R5-R7), when these piercing-sucking insects produce significant damage to developing seeds (McPherson 1996; Bundy & McPherson 2000; Smith *et al*. 2009). We found that developing seeds from pods with high concentrations of the UV-B-inducible isoflavonoids daidzin and genistin were

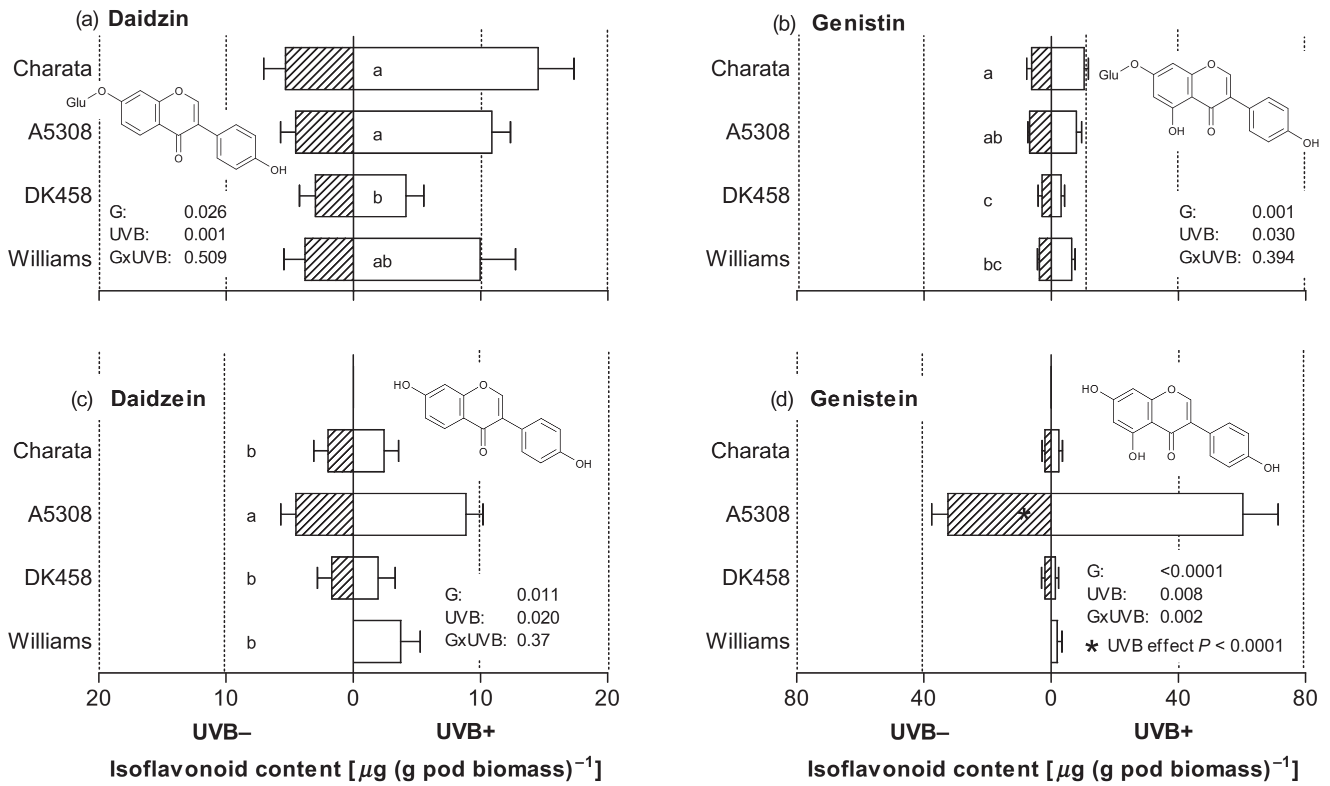

Figure 5. Isoflavonoid contents of pods (±SE; $n = 5$) of the four soybean cultivars grown under attenuated (UVB−) or ambient (UVB+) UV-B radiation: (a) daidzin; (b) genistin; (c) daidzein and (d) genistein. Different letters indicate genotype differences (Fisher's least significant difference (LSD) test]. See Fig. 1 legend for details.

less damaged by stink bugs than those from pods with low isoflavonoid content (Fig. 6a,b). Of note, isoflavonoid concentrations in seeds were not affected by UV-B radiation, suggesting that chemical changes produced in pods were responsible for stink bug behaviour rather than chemical changes in seeds (Figs 4–6).

The flavonoid composition of soybean tissues has been shown to be determined by several genes that influence the various patterns of glycosylation and the interconversion of the flavonol aglycones, daidzein and genistein. Synthesis of flavonol aglycones in legumes is mediated by isoflavone synthase (IFS), which catalyses the unique aryl migration reaction to create daidzein and genistein (Steele *et al*. 1999). Isoflavonoid accumulation in developing soybean seeds is regulated by *CHALCONE SYNTHASE* (*CHS*) *7*, *CHS8* and *ISOFLAVONE SYNTHASE 2* expression (Dhaubhadel *et al*. 2007). In Arabidopsis, *CHS* gene expression has been shown to be induced by UV-B through the UVR8-COP1 pathway (Brown *et al*. 2005). More recent microarray experiments with Arabidopsis demonstrated that the effects of UV radiation on leaf phenolic profiles are positively correlated with changes in the expression of genes involved in the phenylpropanoid biosynthetic pathway: *PAL1* and *PAL2*, *CHS*, *CHALCONE ISOMERASE*, *TT7*, *DFR*, *FLAVONOL SYNTHASE1 and FLAVANONE HYDROXYLASE* (Morales *et al*. 2013). Most of these genes are regulated by UV-B through the UVR8 pathway, and *uvr8* mutants fail to accumulate flavonoids and other phenylpropanoid derivatives in response to UV-B radiation (Demkura & Ballaré 2012). Our results demonstrate that accumulation of isoflavonoids in soybeans is promoted by ambient levels of UV-B radiation under field conditions. Isoflavonoids, as other flavonoid compounds, have high absorbance in the UV region of the solar spectrum and antioxidant properties, and are likely to play a significant role in UV-B photoprotection. In fact, UV-B-induced phenolic compounds can limit the penetration of UV radiation through the epidermis, protecting DNA and other sensitive targets in field-grown soybean plants (Mazza *et al*. 2000). In addition, it is well known that phenolic compounds can also function as defence against herbivory in plants (e.g. Elliger *et al*. 1981; Stamp & Yang 1996; Hoffland *et al*. 2000; Hoffmann-Campo *et al*. 2001; Zavala *et al*. 2001; Piubelli *et al*. 2005; Izaguirre *et al*. 2007; O'Neill *et al*. 2010). Their role as antifeedant molecules could be part of a cost-saving strategy, where compounds possessing multiple ecological roles are induced by diverse stressors. More work in needed to understand how the accumulation of isoflavonoids is regulated by multiple factors under field conditions.

Our results suggest that stink bug attack is regulated by pod defences through pre-ingestive cues rather than post-ingestive effects on insects. Previous work has shown that

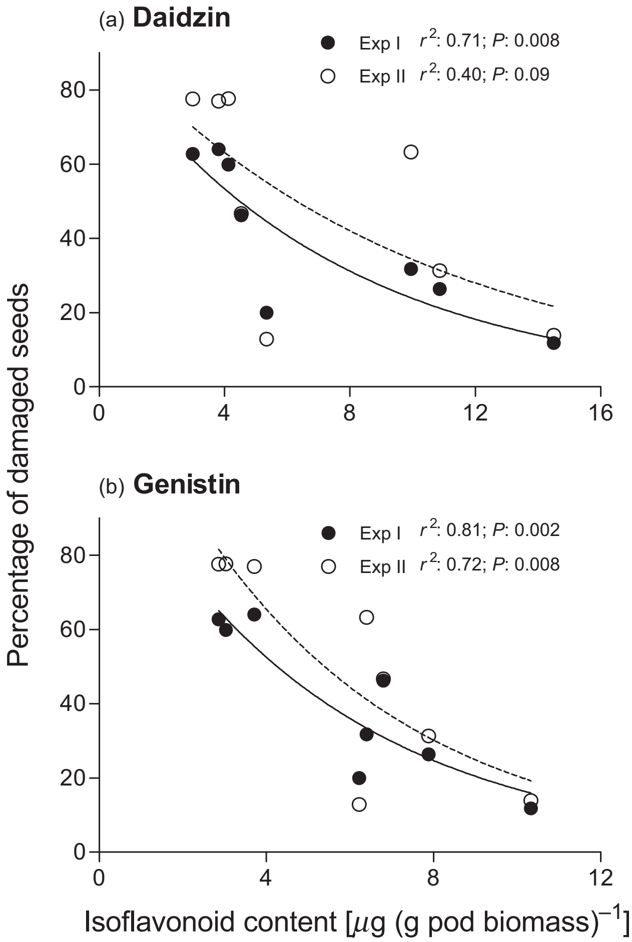

Figure 6. Percentage of seeds damaged by stink bug attack regressed against the content of glycoside isoflavonoid in soybean pods (micrograms per gram biomass) in experiment I (filled symbols, solid line) and experiment II (empty symbols, doted line): (a) daidzin and (b) genistin. Lines represent a regression fitted to an exponential function.

when stink bugs were forced to feed directly on soybean seeds under laboratory conditions, daidzin and genistin accumulation increased. Furthermore, application of methanolic extracts of both isoflavonoids to the seeds resulted in decreased *N. viridula* attack (Piubelli *et al*. 2003a). In our field experiments, pods of three out of four cultivars (Williams, A5308 and Charata) were less preferred by stink bugs, and supported lower seed damage, when plants were exposed to solar UV-B radiation than when plants grew under UV-B-absorbing filters (Figs 1, 2 & 4). It is interesting to note that, in those cultivars, UV-B tended to induce a bigger increase in daidzin accumulation (2.6×, 2.4× and 2.7×, respectively) than in DK458 (1.4×) (Fig. 5), the only cultivar where no differences in stink bug preferences or seed damage between UV-B treatments could be detected. These differences among cultivars add weight to the hypothesis of a causal relationship between UV-B-induced isoflavonoid accumulation in pods and stink bug deterrence.

Another possible explanation for the stink bug preference to feed on soybean pods developed under attenuated UV-B environment could be based upon potential direct effects of solar UV-B radiation on insect behaviour. Stink bugs tend to bask at the canopy surface and seed damage is confined primarily to the upper plant parts (Panizzi & Slansky 1985). Although in previous studies we were able to demonstrate direct behavioural responses of herbivorous insects to solar UV-B radiation (such in the case of the thrips *C. phaseoli*) (Mazza *et al*. 1999, 2002, 2010), we did not find any evidence of a similar effect of solar UV-B radiation on the behaviour of stink bugs using comparable experimental approaches (J. Zavala, C. Mazza and C. Ballaré, unpublished data).

Our study showed that the changes in plant traits induced by solar UV-B can affect stink bug herbivory. Moreover, solar UV-B radiation decreased insect damage to developing seeds in soybean crops, presumably through mechanisms involving UV-B-induced enhancement of isoflavonoid concentrations. However, modern management practices directed to increase crop yield through increasing canopy leaf area index early in the season can substantially reduce the levels of UV-B radiation reaching the plants. High planting densities may eliminate the beneficial effects of solar UV-B radiation on plant immunity, which could have negative consequences on crop health (Ballaré *et al*. 2012). Our study highlights the potential value of taking advantage of the beneficial effects of solar UV-B radiation on plant defences (Ballaré *et al*. 2012; Mazza *et al*. 2013; Wargent & Jordan 2013; Williamson *et al*. 2014). Exploitation of these mechanisms by conventional breeding or biotechnology could help in the development of crop varieties that express high levels of natural plant defences over a wide range of density conditions.

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REFERENCES

Aragon J.R., Molinari A. & Lorenzatti de Diez S. (1997) Manejo integrado de plagas. In *El cultivo de soja en la Argentina* (eds L.M. Giorda & H.E.J. Baigorri), pp. 248–288. INTA y SAGPyA, Argentina.

Ballaré C.L. (2014) Light and plant defense. *Annual Review of Plant Biology* **65,** 335–363.

Ballaré C.L., Caldwell M.M., Flint S.D., Robinson S.A. & Bornman J.F. (2011) Effects of solar ultraviolet radiation on terrestrial ecosystems. Patterns, mechanisms, and interactions with climate change. *Photochemical and Photobiological Sciences* **10,** 226–241.

Ballaré C.L., Mazza C.A., Austin A.T. & Pierik R. (2012) Canopy light and plant health. *Plant Physiology* **160,** 145–165.

Beggs C.G. & Wellman E. (1994) Photocontrol of flavonoid biosynthesis. In *Photomorphogenesis in Plants* (eds A.E. Kendrick & G.H.M. Kronenberg), pp. 733–751. Kluwer Academic Publishers, Dordrecht, the Netherlands.

Bidart-Bouzat M.G. & Imeh-Nathaniel A. (2008) Global change effects on plant chemical defenses against insect herbivores. *Journal of Integrative Plant Biology* **50,** 1339–1354.

Bothel D.J., Russin J.S., Wier A.T., Layton M.B., Mink J.S. & Boyd M.L. (2000) Delayed maturity associated with Southern green stink bug (Heteroptera: Pentatomidae) injury at various soybean phenological stages. *Journal of Economic Entomology* **93,** 707–712.

Brown B.A., Cloix C., Jiang G.H., Kaiserli E., Herzyk P., Kliebenstein D.J. & Jenkins G.I. (2005) A UV-B-specific signaling component orchestrates plant UV protection. *Proceedings of the National Academy of Sciences of the United States of America* **102,** 18225–18230.

Bundy C.S. & McPherson R.M. (2000) Dynamic and seasonal abundance of sink bugs (Heteroptera: Pentatomidae) in cotton-soybean ecosystem. *Journal of Economic Entomology* **93,** 697–706.
Caldwell M.M., Ballare C.L., Bornman J.F., Flint S.D., Bjorn L.O., Teramura A.H., . . . Tevini M. (2003) Terrestrial ecosystems increased solar ultraviolet radiation and interactions with other climatic change factors. *Photochemical and Photobiological Sciences* **2,** 29–38.
Caldwell M.M., Bornman J.F., Ballaré C.L., Flint S.D. & Kulandaivelu G. (2007) Terrestrial ecosystems, increased solar ultraviolet radiation, and interactions with other climate change factors. *Photochemical and Photobiological Sciences* **6,** 252–266.
Carrao-Panizzi M.C. & Kitamura K. (1995) Isoflavone content in Brazilian soybean cultivars. *Breeding Science* **45,** 295–300.
Chimphango S.B.M., Brown C.F., Musil C.F. & Dakora F.D. (2007) Effects of UV-B radiation on seed yield of *Glycine max* and an assessment of F1 generation progeny for carryover effects. *Physiologia Plantarum* **131,** 378–386.
Correa-Ferreira B.S. & de Azevedo J. (2002) Soybean seed damage by different species of stink bugs. *Agricultural and Forest Entomology* **4,** 145–150.
Daugherty D.M., Newstandt M.H., Gehrke C.W., Cavanah L.E., Williams L.F. & Green D.E. (1964) An evaluation of damage to soybeans by brown and green stink bugs. *Journal of Economic Entomology* **57,** 719–722.
Demkura P.V. & Ballaré C.L. (2012) UVR8 mediates UV-B-induced *Arabidopsis* defense responses against *Botrytis cinerea* by controlling sinapate accumulation. *Molecular Plant* **5,** 642–652.
Demkura P.V., Abdala G., Baldwin I.T. & Ballaré C.L. (2010) Jasmonate-dependent and -independent pathways mediate specific effects of solar ultraviolet B radiation on leaf phenolics and antiherbivore defense. *Plant Physiology* **152,** 1084–1095.
Dhaubhadel S., Gijzen M., Moy P. & Farhangkhoee M. (2007) Transcriptome analysis reveals a critical role of *CHS7* and *CHS8* genes for isoflavonoid synthesis in soybean seeds. *Plant Physiology* **143,** 326–338.
Dinh T.S., Galis I. & Baldwin I.T. (2013) UVB radiation and HGL-DTGs provide durable resistance against mirid (*Tupiocoris notatus*) attack in field-grown *Nicotiana attenuata* plants. *Plant, Cell & Environment* **36,** 590–606.
Elliger C.A., Wong Y., Chan B.G. & Waiss A.C. Jr. (1981) Growth inhibitors in tomato (*Lycopersicon*) to tomato fruitworm (*Heliothis zea*). *Journal of Chemical Ecology* **7,** 753–758.
Fehr W.R., Caviness C.E., Burmood D.T. & Pennington J.S. (1971) Stage of development descriptions for soybeans, *Glycine max* (L.) Merrill. *Crop Science* **11,** 929–931.
França Neto J.B., Krzyzanowski F.C. & Costa N.P. (1998) *El test de tetrazolio en semillas de soja*. EMBRAPA-CNPSo, Londrina. (EMBRAPA-CNPSo. Documentos, 117), Brasil, 72 pp.
Heinonen S.M., Wahala K. & Adlercreutz H. (2002) Metabolism of isoflavones in human subjects. *Phytochemistry Reviews* **1,** 175–182.
Hoffland E., Dicke M., Van Tintelen W., Dijkman H. & Van Beusichem M.L. (2000) Nitrogen availability and defense of tomato against two-spotted spider mite. *Journal of Chemical Ecology* **26,** 2697–2711.
Hoffmann-Campo C.B., Harborne J.B. & McCaffery A.R. (2001) Pre-ingestive and postingestive effects of soya bean extracts and rutin on *Trichoplusia ni* growth. *Entomologia Experimentalis et Applicata* **98,** 181–194.
Hu Z.Q., Zhao H.Y. & Thieme T. (2013) Probing behaviors of *Sitobion avenae* (Hemiptera: Aphidae) on enhanced UV-B irradiated plants. *Archives of Biological Science Belgrade* **65,** 247–254.
Izaguirre M.M., Scopel A.L., Baldwin I.T. & Ballaré C.L. (2003) Convergent responses to stress: solar ultraviolet-B radiation and *Manduca sexta* herbivory elicit overlapping transcriptional responses in field-grown plants of *Nicotiana longiflora*. *Plant Physiology* **132,** 1755–1767.
Izaguirre M.M., Mazza C.A., Svatos A., Baldwin I.T. & Ballaré C.L. (2007) Solar ultraviolet-B radiation and insect herbivory trigger partially overlapping phenolic responses in *Nicotiana attenuata* and *Nicotiana longiflora*. *Annals of Botany* **99,** 103–109.
Jansen M.A.K., Hectors K., O'Brien N.M., Guisez Y. & Potters G. (2008) Plant stress and human health: do human consumers benefit from UV-B acclimated crops? *Plant Science (Shannon, Ireland)* **175,** 449–458.
Koti S., Reddy K.R., Kakani V.G., Zhao D. & Reddy V.R. (2004) Soybean (*Glycine max*) pollen germination characteristics, flower and pollen morphology in response to enhanced ultraviolet-B radiation. *Annals of Botany* **94,** 855–864.
Kotilainen T., Venäläinen T., Tegelberg R., Lindfors A., Julkunen-Tiitto R., Sutinen S., . . . Aphalo P.J. (2009) Assessment of UV biological spectral weighting functions for phenolic metabolites and growth responses in silver birch seedlings. *Photochemistry and Photobiology* **85,** 1346–1355.
Kuhlmann F. & Müller C. (2009) Development-dependent effects of UV radiation exposure on broccoli plants and interactions with herbivorous insects. *Environmental and Experimental Botany* **66,** 61–68.
Kuhlmann F. & Müller C. (2010) UV-B impact on aphid performance mediated by plant quality and plant changes induced by aphids. *Plant Biology* **12,** 676–684.
Kuhlmann F. & Müller C. (2011) Impacts of ultraviolet radiation on interactions between plants and herbivorous insects: a chemo-ecological perspective. *Progress in Botany* **72,** 305–347.
Lindroth R.L., Hofmann R.W., Campbell B.D., McNabb W.C. & Hunt D.Y. (2000) Population differences in *Trifolium repens* L. response to ultraviolet-B radiation: foliar chemistry and consequences for two lepidopteran herbivores. *Oecologia* **122,** 20–28.
McCloud E.S. & Berenbaum M.R. (1994) Stratospheric ozone depletion and plant-insect interactions: effects of UV-B radiation on foliage quality of *Citrus jambhiri* for *Trichoplusia ni*. *Journal of Chemical Ecology* **20,** 525–539.
McPherson R.M. (1996) Relationship between soybean maturity group and the phenology and abundance of stink bugs (Heteroptera: Pentatomidae): impact on yield and quality. *Journal of Entomological Science* **31,** 199–208.
McPherson R.M., Newsom L.D. & Farthing B.F. (1979) Evaluation of four stink bug species from three genera affecting soybean yield and quality in Louisiana. *Journal of Economic Entomology* **72,** 188–194.
McPherson R.M., Todd J.W. & Yeargan K.V. (1994) Stink bugs. In *Handbook of Soybean Insect Pests* (eds L.G. Higley & D.J. Boethel), pp. 87–90. Entomol. Soc. Amer., Lanham, MD.
Mazza C.A., Zavala J.A., Scopel A.L. & Ballaré C.L. (1999) Perception of solar UVB radiation by phytophagous insects: behavioral responses and ecosystem implications. *Proceedings of the National Academy of Sciences of the United States of America* **96,** 980–985.
Mazza C.A., Boccalandro H.E., Giordano C.V., Battista D., Scopel A.L. & Ballaré C.L. (2000) Functional significance and induction by solar radiation of ultraviolet-absorbing sunscreens in field-grown soybean crops. *Plant Physiology* **122,** 117–125.
Mazza C.A., Izaguirre M.M., Zavala J.A., Scopel A.L. & Ballaré C.L. (2002) Insect perception of ambient ultraviolet-B radiation. *Ecology Letters* **5,** 722–726.
Mazza C.A., Izaguirre M.M., Curiale J. & Ballaré C.L. (2010) A look into the invisible: ultraviolet-B sensitivity in an insect (*Caliothrips phaseoli*) revealed through a behavioural action spectrum. *Proceedings of the Royal Society B* **277,** 367–373.
Mazza C.A., Gimenez P.I., Kantolic A.G. & Ballaré C.L. (2013) Beneficial effects of solar UV-B radiation on soybean yield mediated by reduced insect herbivory under field conditions. *Physiologia Plantarum* **147,** 307–315.
Morales L.O., Brosché M., Vainonen J., Jenkins G.I., Wargent J.J., Sipari N., . . . Aphalo P.J. (2013) Multiple roles for UV RESISTANCE LOCUS8 in regulating gene expression and metabolite accumulation in Arabidopsis under solar ultraviolet radiation. *Plant Physiology* **161,** 744–759.
Musser F.R., Catchot A.L., Gibson B.K. & Knighten K.S. (2011) Economic injury levels for southern green stink bugs (Hemiptera: Pentatomidae) in R7 growth stage soybeans. *Crop Protection* **30,** 63–69.
O'Neill B.F., Zangerl A.R., Dermondy O., Bilgin D.D., Casteel C.L., Zavala J.A., . . . Berenbaum M.R. (2010) Impact of elevated levels of atmospheric CO_2 and herbivory on flavonoids of soybean (*Glycine max* Linnaeus). *Journal of Chemical Ecology* **36,** 35–45.
Panizzi A.R. & Slansky F.J. (1985) Rev. of phytophagous pentatomids (Hemiptera: Pentatomidae) associated with soybeans in the Americas. *The Florida Entomologist* **68,** 184–214.
Piubelli G.C., Hoffmann-Campo C.B., de Arruda I.C., Franchini J.C. & Lara F.M. (2003a) Flavonoid increase in soybean as a response to *Nezara viridula* injury and its effect on insect-feeding preference. *Journal of Chemical Ecology* **29,** 1223–1233.
Piubelli G.C., Hoffmann-Campo C.B., de Arruda I.C. & Lara F.M. (2003b) Nymphal development, lipid content, growth and weight gain of *Nezara viridula* (L.) (Heteroptera: Pentatomidae) fed on soybean. *Neotropical Entomology* **32,** 127–132.
Piubelli G.C., Hoffmann-Campo C.B., Moscardi F., Miyakubo S.H. & De Oliveira M.C.N. (2005) Are chemical compounds important for soybean resistance to *Anticarsia gemmatalis*? *Journal of Chemical Ecology* **31,** 1509–1525.
Robson T.M., Pancotto V.A., Flint S.D., Ballaré C.L., Sala O.E., Scopel A.L. & Caldwell M.M. (2003) Six years of solar UV-B manipulations affect growth

of sphagnum and vascular plants in a Tierra del Fuego peatland. *The New Phytologist* **160,** 379–389.

Rousseaux M.C., Ballaré C.L., Scopel A.L., Searles P.S. & Caldwell M.M. (1998) Solar ultraviolet-B radiation affects plant-insect interactions in a natural ecosystem of Tierra del Fuego (southern Argentina). *Oecologia* **116,** 528–535.

Rousseaux M.C., Scopel A.L., Searles P.S., Caldwell M.M., Sala O.E. & Ballaré C.L. (2001) Responses to solar ultraviolet-B radiation in a shrub-dominated natural ecosystem of Tierra del Fuego (southern Argentina). *Global Change Biology* **7,** 467–478.

Rousseaux M.C., Julkunen-Tiitto R., Searles P.S., Scopel A.L., Aphalo P.J. & Ballaré C.L. (2004) Solar UV-B radiation affects leaf quality and insect herbivory in the southern beech tree *Nothofagus antarctica*. *Oecologia* **138,** 505–512.

Schreiner M., Mewis I., Huyskens-Keil S., Jansen M.A.K., Zrenner R., Winkler J.B., . . . Kumbein A. (2012) UV-B-induced secondary plant metabolites: potential benefits for plant and human health. *Critical Reviews in Plant Sciences* **31,** 229–240.

Smith J.F., Luttrell R.G. & Greene J.K. (2009) Seasonal abundance, species composition, and population dynamics of stink bugs in production fields of early and late soybean in south Arkansas. *Journal of Economic Entomology* **102,** 229–236.

Stamp N.E. & Yang Y.L. (1996) Response of insect herbivores to multiple allelochemicals under different thermal regimes. *Ecology* **77,** 1088–1102.

Steele C.L., Gijzen M., Qutob D. & Dixon R.A. (1999) Molecular characterization of the enzyme catalyzing the aryl migration reaction of isoflavonoid biosynthesis in soybean. *Archives of Biochemistry and Biophysics* **367,** 146–150.

Stratmann J.W. (2003) Ultraviolet-B radiation co-opts defense signaling pathways. *Trends in Plant Science* **8,** 526–533.

Stratmann J.W., Stelmach B.A., Weiler E.W. & Ryan C.A. (2000) UVB/UVA radiation activates a 48 kDa myelin basic protein kinase and potentiates wound signaling in tomato leaves. *Photochemical and Photobiological Sciences* **71,** 116–123.

Temple J.H., Leonard B.R., Davis J. & Fontenot K. (2009) Insecticide efficacy against red banded stink bug, *Piezodorus guildinii* (Westwood), a new stink bug pest of Louisiana soybean. *Midsouth Entomologist* **2,** 68–69.

Teramura A.H., Sullivan J.H. & Lydon J. (1990) Effects of UV-B radiation on soybean yield and seed quality: a 6-year field study. *Physiologia Plantarum* **80,** 5–11.

Todd J.W. (1989) Ecology and behaviour of *Nezara viridula*. *Annual Review of Entomology* **34,** 273–292.

Todd J.W. & Turnipseed S.G. (1974) Effects of southern green stink bug damage on yield and quality of soybeans. *Journal of Economic Entomology* **67,** 421–426.

Underwood N., Morris W., Gross P. & Lockwood I.I.I.J.R. (2000) Induced resistance to Mexican bean beetles in soybean: variation among genotypes and lack of correlation with constitutive resistance. *Oecologia* **122,** 83–89.

Wargent J.J. & Jordan B.R. (2013) From ozone depletion to agriculture: understanding the role of UV radiation in sustainable crop production. *The New Phytologist* **197,** 1058–1076.

Williamson C., Zepp R., Lucas R., Madronich S., Austin A.T., Ballaré C.L., . . . Bornman J.F. (2014) Solar ultraviolet radiation in a changing climate. *Nature Climate Change* **4,** June 2014. doi: 10.1038/NCLIMATE2225; in press.

Zavala J.A., Scopel A.L. & Ballaré C.L. (2001) Effects of ambient UV-B radiation on soybean crops: impact on leaf herbivory by *Anticarsia gemmatalis*. *Plant Ecology* **156,** 121–130.

Zavala J.A., Casteel C.L., DeLucia E.H. & Berenbaum M.R. (2008) Anthropogenic increase in carbon dioxide compromises plant defense against invasive insects. *Proceedings of the National Academy of Sciences of the United States of America* **105,** 5129–5133.

Zavala J.A., Casteel C.L., Nabity P.D., Berenbaum M.R. & DeLucia E.H. (2009) Role of cysteine proteinase inhibitors in preference of Japanese beetles (*Popillia japonica*) for soybean (*Glycine max*) leaves of different ages and grown under elevated CO_2. *Oecologia* **161,** 35–41.

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