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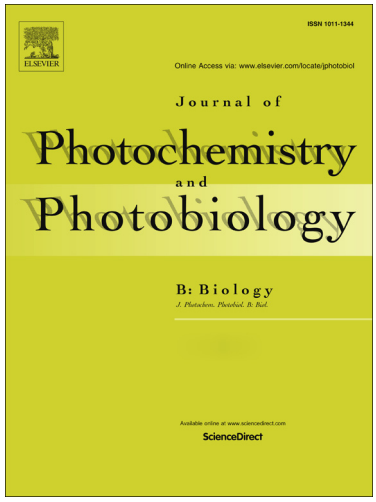

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Impact of UV-A radiation on the performance of aphids and whiteflies and on the leaf chemistry of their host plants

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ABSTRACT

Ultraviolet (UV) radiation directly regulates a multitude of herbivore life processes, in addition to indirectly affecting insect success *via* changes in plant chemistry and morphogenesis. Here we looked at plant and insect (aphid and whitefly) exposure to supplemental UV-A radiation in the glasshouse environment and investigated effects on insect population growth. Glasshouse grown peppers and eggplants were grown from seed inside cages covered by novel plastic filters, one transparent and the other opaque to UV-A radiation. At a 10-true leaf stage for peppers (53 days) and 4-true leaf stage for eggplants (34 days), plants were harvested for chemical analysis and infested by aphids and whiteflies, respectively. Clip-cages were used to introduce and monitor the insect fitness and populations of the pests studied. Insect pre-reproductive period, fecundity, fertility and intrinsic rate of natural increase were assessed. Crop growth was monitored weekly for 7 and 12 weeks throughout the crop cycle of peppers and eggplants, respectively. At the end of the insect fitness experiment, plants were harvested (68 days and 18-true leaf stage for peppers, and 104 days and 12-true leaf stage for eggplants) and leaves analysed for secondary metabolites, soluble carbohydrates, amino acids, total proteins and photosynthetic pigments. Our results demonstrate for the first time, that UV-A modulates plant chemistry with implications for insect pests. Both plant species responded directly to UV-A by producing shorter stems but this effect was only significant in pepper whilst UV-A did not affect the leaf area of either species. Importantly, in pepper, the UV-A treated plants contained higher contents of secondary metabolites, leaf soluble carbohydrates, free amino acids and total content of protein. Such changes in tissue chemistry may have indirectly promoted aphid performance. For eggplants, chlorophylls *a* and *b*, and carotenoid levels decreased with supplemental UV-A over the entire crop cycle but UV-A exposure did not affect leaf secondary metabolites. However, exposure to supplemental UV-A had a detrimental effect on whitefly development, fecundity and fertility presumably not mediated by plant cues as compounds implied in pest nutrition -proteins and sugars- were unaltered.

Keywords: Plant-insect interactions; UV-blocking covers; Insect pests; Pepper; Eggplant

Highlights:

- Supplemental UV-A causes a reduction in pepper stem height
- Aphids benefit from changes in pepper metabolites under supplemental UV-A
- There is a detrimental effect of UV-A radiation on whitefly performance
- UV-mediated changes appear to be highly dependent on each plant-insect complex

1. Introduction

Aphids and whiteflies are two of the most important pests worldwide, not only because of the direct damage they cause, but also because their alimentary habits involve transmission of plant viruses (Hull, 2002). Ultraviolet (UV) radiation plays a major role in herbivores, including insect pests, by modifying their orientation toward potential hosts, flight activity, alighting, arrestment, feeding behavior and interaction between sexes (Raviv and Antignus, 2004; Johansen *et al*., 2011). Aphids (Hemiptera: Aphididae) and whiteflies (Hemiptera: Aleyrodidae) are among the most studied insects concerning their flight behaviour. Aphids have been reported to reduce their flight activity and ability to disperse in UV-deficient environments (Díaz and Fereres, 2007; Döring and Chittka, 2007). Moreover, a decrease in fecundity and population density has been also demonstrated (Antignus *et al*., 1996; Chyzik *et al*., 2003; Díaz *et al*., 2006; Kuhlmann and Müller, 2009a; Paul *et al*., 2011; Legarrea *et al*., 2012). Conversely, UV radiation stimulates whitefly migration (Mound, 1962; Coombe, 1982). Among new integrated pest management strategies, UV-absorbent photoselective nets have been successfully tested in field situations by reducing the impact of insect vectors and plant pathogens on protected crops (Díaz and Fereres, 2007; Weintraub, 2009; Legarrea *et al*., 2012).

Knowledge on the effects of UV-B on plant growth and chemistry (nutritional characteristics relevant to insects) has been developed due to past concerns about ozone depletion (Ballaré *et al*., 1996; Hunt and McNeil, 1999; Mackerness, 2000; Jansen, 2002; Comont *et al*., 2012; Mewis *et al*., 2012). In contrast, understanding of the effects of the UV-A fraction of the solar spectrum on plants and insect pests is very limited. Whilst UV-A radiation is unaffected by ozone depletion, it is a significant component of the solar spectrum affected by latitude, altitude and cloud cover. It is also often absent from the glasshouse/horticultural environment. New environmental concerns suggest that understanding UV-A impacts on plants could be important given that predictions by the United Nations Environment Programme suggest that there will be a higher incidence of cloud free periods, particularly in southern Europe and the Mediterranean Basin. This will result in higher exposure of crops to ambient UV-A radiation (WMO, 2010). Only a few authors have considered UV-A impacts on plant growth (Tezuka *et al*., 1994; Jayakumar *et al*., 2003, 2004; Verdaguer *et al*., 2012). The latter work shows that radiation in the UV-A range produces alterations in leaf morphology and anatomy of several plants, with the most characteristic response mainly observed in the adaxial epidermal cells, which were thicker and longer than those grown without UV-A.

There are no known studies that have focused on how UV-A influences the relationship between phytophagous insects and their plant hosts but there is large body of material published on UV-A plant pollinator interactions (Stephanou *et al.*, 2000; Petropoulou *et al.*, 2001; Dyer and Chittka, 2004). Furthermore, research on spider mites by Sakai and Osakabe (2010) concluded that *Tetranychus urticae* Koch (Acari: Tetranychidae) exploits UV-A information to avoid ambient UV-B radiation. At the same time other work on *Panonychus citri* McGregor (Acari: Tetranychidae) suggested that eggs were tolerant to UV-B radiation

and females successfully oviposited on the upper side of leaves exposed to UV-B via artificial lamps (Fukaya *et al*., 2013).

Our knowledge on the effects of UV-B on plant-insect interactions would suggest that typical plant responses would include the accumulation of UV-screening metabolites, increased leaf thickness and trichome density or reduction in cell elongation (Smith *et al*., 2000; Paul and Gwynn-Jones, 2003; Liu *et al*., 2005; González *et al*., 2009; Kulhmann and Müller, 2009a). These impacts have implications for host success because such physical and biochemical traits affect host acceptance and success of future insect progeny (Vänninen *et al*., 2010; Paul *et al*., 2011)

Understanding of the indirect effects of UV-A on insects *via* plants remains limited to what we know about current practices in horticulture. On one hand, the horticulture industry traditionally grows crop species under glass or plastic with opaque or lowered UV radiation environments. However, evidence suggests that supplemental UV-A may improve plant growth, yield and quality. For example, a combination of visible radiation and UV-A at a particular ratio may be highly suitable for enhanced growth of soybean seedlings (Middleton and Teramura, 1993). Similar findings have been observed on the yield of *Phaseolus mungo* L., which was improved with UV-A exposure (Jayakumar *et al*., 2003). UV cladding materials have been shown to also have positive effects on crop growth by increasing stem length, leaf toughness or trichome density (Hunt and McNeil, 1999; Kittas *et al*., 2006; Kuhlmann and Müller, 2009a, 2010; Paul *et al*., 2011). There is also evidence that UV transmitting environments could produce food plants commercially with increased human health benefits (Tsormpatsidis *et al*., 2011).

In this study, we hypothesise that UV-A is central to the trophic relationships between these two global pests -aphids and whiteflies- and their plant hosts. We grew the horticultural hosts *Capsicum annuum* L. (pepper) and *Solanum melongena* L. (eggplant) and their respective insect pests, the green peach aphid *Myzus persicae* Sulzer (Hemiptera: Aphididae) and the whitefly *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) in the presence and absence of UV-A radiation. We targeted how UV-A impacts the success of insects *via* population growth. In tandem with direct effect of UV-A, we also assessed how UV-A exposure indirectly affects insects *via* changes in plant chemistry. Correlations between the different responses found in leaf chemicals analysed and plant sensitivity to UV-A are considered.

2. Methods and materials

2.1. Plant propagation

Experiments were undertaken in a glasshouse facility at the Institute of Agricultural Sciences of CSIC (Madrid, Spain) (40° 26’ 23’’ N, -3° 41’ 14’’ W) at a temperature of 23:20±2 °C (day:night), a photoperiod of 14:10 (light:dark) and 70-80% RH. *C. annuum* cv California Wonder (Ramiro Arnedo S.A., La Rioja, Spain) and *S. melongena* cv Black beauty (Batlle, S.A., Barcelona, Spain) seeds were germinated in pots with a mixture of soil:vermiculite

(1:1). For both species, three seeds were placed in each pot and thinned to one post germination. Plants were watered three times a week using 20-20-20 (N:P:K) Nutrichem 60 fertiliser (Miller Chemical & Fertilizer Corp., Pennsylvania, USA) at a dose of 0.25 g L^{-1}.

2.2. UV-A treatments

UV-A radiation was supplied by two Osram Ultra-Vitalux UV lamps (Osram GmbH, Munich, Germany). Lamps were switched on and off with no gradual transition for a photoperiod of 14 hours every day throughout the entire length of experiments. The lamps emitted no UV-C radiation and produced radiation levels representative of typical sunny summer day conditions in the centre of the Iberian Peninsula (Gutiérrez-Marco *et al*., 2007; Häder *et al*., 2007). However, it should be emphasised that our aim here was to expose plants and insects to UV-A under glasshouse conditions rather that simulate UV-A outdoors. The lamps used were heavily weighted for UV-A emission so throughout the text we will refer to the treatment as UVA+ (supplemental UV-A). A set of two 1 x 1 x 1m (L x H x W) cages were covered by filters. As a positive control that allowed UV-A radiation transmission but blocked UV-B radiation (Table 1), the upper side of one cage was covered with a 200 µm thickness film (Solplast S.A., Murcia, Spain). The four lateral sides were covered to a 50 cm height with a UV-transparent net T 50 mesh (Polysack Plastic Industries Ltd., Nir Yitzhak, Israel) to permit airflow inside the cage. The remaining upper 50 cm were covered with plastic film. For the suppressed UV-A radiation treatment, a 200 µm thickness Antivirus UV-blocking film (Solplast S.A., Spain) and a UV-absorbing Optinet 50 mesh (Polysack Plastic Industries Ltd., Nir Yitzhak, Israel) were used. Optical properties (transmitted radiation) of the UV-opaque and UV-transparent films were analysed at the CSIC Torres Quevedo Institute (Madrid, Spain) using a double monochromator Lambda 900 UV/Visible/NIR spectrophotometer (PerkinElmer Life and Analytical Sciences Ltd., Connecticut, USA). The main difference between both filters was that the UV-opaque film blocked UV-A transmission (315-400 nm) and the UV-transparent film allowed UV-A transmission, as seen in Figure 1. Lamps were hung at a distance of 1 m above the plant canopy. Irradiance per second was measured daily above cage and at canopy level as well as on the abaxial side of the leaves and through the leaves with clip-cages where insects were monitored with an ALMEMO 25904S radiometer (Ahlborn GmbH, Holzkirchen, Germany). The radiation received by the plants (irradiance) under both treatments is shown in Table 1. The UV daily doses were 71.67 KJ m^{-2} d^{-1} UV-A and 0.55 KJ m^{-2} d^{-1} UV-B for treatment UVA+, and 1.76 KJ m^{-2} d^{-1} UV-A and 0.10 KJ m^{-2} d^{-1} UV-B for treatment UVA-. Daily UV-A radiation inside the cage covered by the blocking film was very low (1.76 KJ m^{-2} d^{-1}) hence this treatment was called UVA- (near zero UV-A). A fourty-fold increase in UV-A transmittance at the plant canopy level inside the regular cage was measured when compared to the cage covered by the UV-absorbing barrier (1.422 vs. 0.035 W m^{-2}) (Table 1). Low levels of UV-B radiation inside both experimental treatments were detected although represented less than 1% of the light received by our plants (0.011 W m^{-2} in treatment UVA+ and 0.002 W m^{-2} in treatment UVA-) (Table 1).

It should again be noted that the experimental set up was used to evaluate how supplemental UV-A affects plant-insect interactions and performance in the glasshouse environment. The

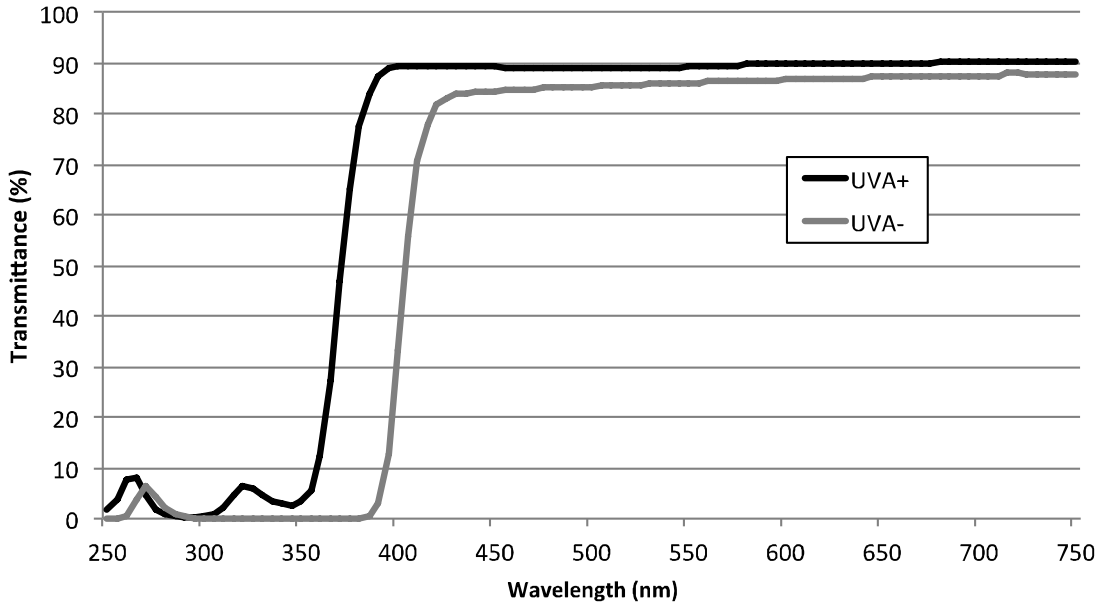
focus was on crop production and this study was not designed to simulate outdoor environmental conditions, hence any extrapolation of findings to field conditions should be done with caution.

Table 1. Radiation conditions at canopy level outside and inside the experimental cages (UVA+ and UVA- treatments), on the abaxial side of the leaves and through the leaves with clip-cages where insects were monitored. Transmission percentages represent radiation transmitted inside both cages in relation to the same level outside cages.

	Treatment UVA+			Treatment UVA-		
	PAR [a]	UV-A [b]	UV-B [b]	PAR	UV-A	UV-B
Canopy level outside cage	515.0 (112.8)	11.722	0.561	505.0 (110.6)	11.290	0.575
Canopy level inside cage	441.8 (96.8)	1.422	0.011	334.6 (73.3)	0.035	0.002
Abaxial side of leaves w/ clip-cage	25.3 (5.5)	0.083	0.002	21.8 (4.8)	0.003	0.002
Through the leaves w/ clip-cage	-	0.030	0.002	-	0.000	0.000
Transmission inside cage (%)	85.79	12.13	1.96	66.26	0.31	0.35

[a] µmol m^{-2} s^{-1} (W m^{-2}), [b] W m^{-2}

Figure 1. Total transmittance from 250 to 750 nm of the UV-transparent (UVA+) and UV-opaque (UVA-) plastic films measured by a double monochromator spectrophotometer.



2.3. Insect exposure and maintenance

M. persicae was continuously reared on pepper plants in a climate chamber at 23:18 °C (day:night), 60-80% RH, and a photoperiod of 16 h and *B. tabaci* Q biotype was reared on eggplants in greenhouse facilities at an average temperature of 23:20°C (day:night), 70-80%

RH and a photoperiod of 16 h. Both species were synchronised prior to assays to ensure that individuals were the same age.

2.3.1. Aphid introduction

Pepper plants were infested by *M. persicae* at the 10-true leaf stage. One single wingless aphid adult was placed in a clip-cage on the abaxial side of the youngest fully developed leaf of each pepper plant and allowed to produce nymphs for 24 hours. Surplus nymphs were removed leaving three nymphs per plant, which were monitored until adulthood stage. When the first nymph reached the adult stage, the other two were removed. Offspring from the remaining insect was monitored by removing nymphs daily for an equal number of days to the pre-reproductive period. The parameters pre-reproductive period (d), effective fecundity (Md), intrinsic rate of natural increase (r_m=0.738*($\log_e Md$)/d), mean relative growth rate (RGR=r_m/0.86) and mean generation time (Td=d/0.738) were calculated (n=19).

2.3.2. Whitefly introduction

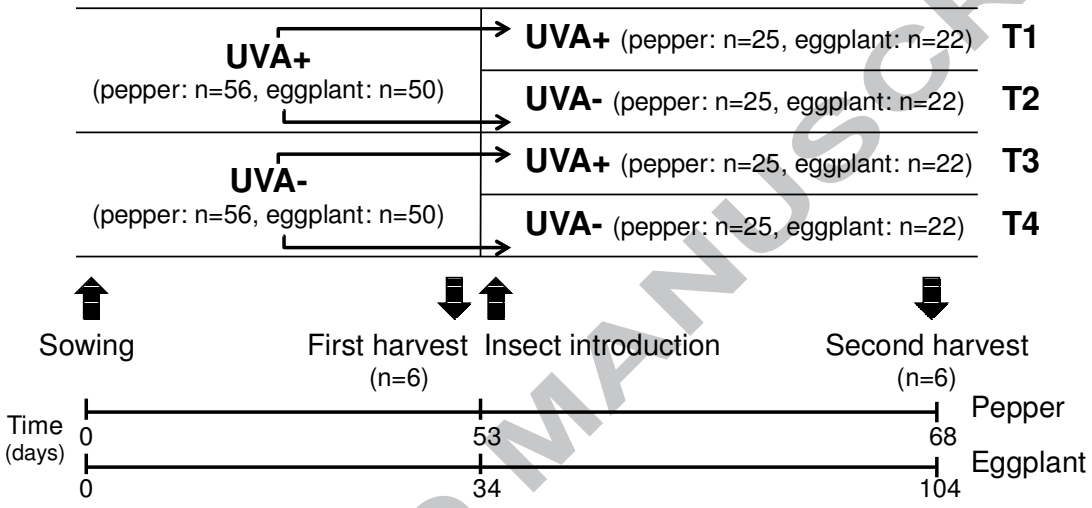
Eggplants were infested by whiteflies at the 4-true leaf stage. Ten pairs of adult whiteflies were left to produce eggs inside clipcages on the abaxial side of the youngest fully developed leaf of each plant for 24 hours and 10 eggs were monitored until adult emergence. A newborn female and male were placed on a new leaf and their offspring monitored for 30 days. Pre-reproductive period, larvae viability, female fecundity and fertility were studied (n=16).

2.4. Experimental design

Pots with seeds were placed inside cages and plants were grown from seeds under two different radiation regimes, either with supplemental (UVA+) or near zero UV-A radiation (UVA-). At a 10-true leaf stage (53 days) for peppers and 4-true leaf stage (34 days) for eggplants, half of the plants of each cage were moved from the UVA+ to the UVA- treatment and vice versa. Some of the plants were infested by aphids (n=19) or whiteflies (n=16) to study the performance of insects. In this way, we had four UV-A treatments: positive control UVA+/UVA+, plants grown under supplemental UV-A radiation for the entire growth cycle; negative control UVA-/UVA-, plants grown at near zero UV-A radiation for the entire growth cycle; UVA+/UVA-, plants grown under supplemental UV-A radiation before insect introduction and at near zero UV-A after insect introduction; and UVA-/UVA+, plants grown at near zero UV-A radiation before insect introduction and under supplemental UV-A after insect introduction. Figure 2 represents a timeline diagram of the experimental procedure. Stem height, and leaf length and width were monitored weekly using a ruler (n=6). The relationship between our measurements and actual leaf area (cm^2) was calculated by scanning 10 leaves of different stages of each plant species and contouring them with Adobe Acrobat software (Pepper: 0.66±0.01. Eggplant: 0.73±0.01). Experiments were repeated twice over one year. Leaf material harvested throughout the experiment was either snap-frozen and maintained at -80°C or air-dried 70°C as relevant for further analyses.

Figure 2. Timeline diagram of the experimental design, showing the four different UV-A treatments (T1: UVA+/UVA+, plants grown under supplemental UV-A radiation for the

entire growth cycle; T2: UVA+/UVA-, plants grown under supplemental UV-A radiation before insect introduction and near zero UV-A after insect introduction; T3: UVA-/UVA+, plants grown near zero UV-A radiation before insect introduction and under supplemental UV-A after insect introduction and T4: UVA-/UVA-, plants grown near zero UV-A radiation for the entire growth cycle), dates of insect infestation to study the performance of aphids and whiteflies and plant harvests for peppers and eggplants. The arrows refer to the moment when half of the plants of each treatment were moved from treatment UVA+ to UVA- and vice versa.



2.5. Plant harvesting

Plants from the two species were harvested at two different growth stages for determining biomass and content of chemical compounds (Figure 2). Plants were harvested from each of the treatment cages at the 10-true leaf stage (53 days after sowing) for peppers plants and 4-true leaf stage (34 days after sowing) for eggplants (n=6). All leaves from each plant were collected for subsequent chemical analyses. Further plants from the treatments were harvested at 18-true leaf stage for peppers (68 days after sowing) and at 12-true leaf stage for eggplants (104 days after sowing). This involved plants from each treatment including those infested with insects and those not (as above, n=6).

2.6. Plant biochemical analysis

2.6.1. Secondary metabolites

Frozen samples were subsequently freeze-dried for 48 hours and leaf material homogenised with a pestle and mortar. Samples were analysed for secondary metabolites by extraction in 70% methanol of freeze-dried samples (100 mg), as described by Comont *et al.* (2012). Supernatants were dried using a Savant SpeedVac SPD121P vacuum centrifuge (Thermo Scientific, Massachusetts, USA) before re-suspension in 500 µL 70% methanol. The solid-phase extraction was performed using a Sep-Pak Vac 500 mg C18 column (Waters Ltd., Elstree, UK) before vacuum centrifugation of the sample to complete dryness. Dried pellets

were suspended in 500 µL 100% methanol and analysed *via* high pressure liquid chromatography (HPLC) with a system comprising a Waters 515 pump, a Waters 717plus autosampler, a Waters 996 photodiode array detector and a Waters C_{18} Nova-Pak radial compression column (C_{18} 4.0 µm, 8.0x100mm cartridge) (Waters Ltd., Elstree, UK) with an injection volume of 30 µL and a flow rate of 2 mL min^{-1}. The mobile phase consisted of 5% acetic acid (solvent A) and 100% methanol (solvent B) with a linear gradient from 5 to 75%, B in A, over 35 min. Peak integration was performed using the Empower software. Liquid chromatography-mass spectrometry (LC-MS) was performed to identify the major compounds. A Thermo Finnigan LC-MS system (Finnigan Surveyor LC pump plus, PDA plus detector, Finnigan LTQ linear ion trap) (Thermo Scientific, Massachusetts, USA) and a Waters Nova-Pak C_{18} 4.0 µm, 3.9x100 mm column was used with an injection volume of 10 µL and a flow rate of 1 mL min^{-1}. The mobile phase consisted of purified water-0.1% formic acid (solvent A) and MeOH-0.1% formic acid (solvent B) with a linear gradient from 5 to 65%, B in A, over 60 min. Phenolics were characterised by UV absorption spectra, MS fragmentation patterns in negative ion mode and comparison with standards and previously reported data in the literature (Clifford *et al*., 2003; Stommel *et al*., 2003; Marín *et al*., 2004; Park *et al*., 2012).

2.6.2. Soluble sugars

Air dried samples (100 mg) were extracted in 3 mL of distilled water at 80 °C three times. Extracts were centrifuged for 10 min at 10,000 rpm. Supernatants were retained, combined and frozen until the analysis. Then 50 µL of sample were added to 950 µL of a buffer comprising 5 mM H_2SO_4 with a 5 mM crotonic acid internal standard. Samples were analysed *via* HPLC comprising a Jasco LG-980-02 ternary gradient unit, a Jasco PU-1580 pump, a Jasco AS-1555 sampler and a Jasco RI-2031 detector (Jasco Ltd., Essex, UK). Injection volume was 25 µL. Sugars were identified by comparison with an internal library of standard compounds (Comont *et al*., 2012).

2.6.3. Free amino acid and proteins

Freeze-dried plant material (100 mg) was extracted in 4 mL of boiling distilled water for 25 minutes. Extracts were allowed to cool and a 1.5 mL aliquot was centrifuged to clarify the solution, following the methodology described by Winters *et al*. (2002). Amino acid absorbance was measured at 570 nm using an Ultrospec 4000 UV/Vis spectrophotometer (GE Healthcare, Buckinghamshire, England). Histidine was used for the calibration curve as most amino acids have the same response. Total proteins were extracted from 100 mg of freeze-dried sample by grinding in 1.8 mL Mclivaine buffer pH 7 containing 50 mM ascorbic acid, and 0.2 mL 20% lithium dodecyl sulphate. Protein content was analysed by the Lowry protein assay (Lowry *et al*., 1951) following precipitation of protein in extracts with 20% trichloroacetic acid, 0.4% phosphotunstic acid and resuspension in 0.1 M NaOH. Absorbance was measured at 700 nm with a µQuant microtitre plate reader spectrophotometer (Bio-Tek Instruments Inc., Winooski, USA). Protein contents were determined against a bovine serum albumin calibration curve.

2.6.4. Photosynthetic pigments

Chlorophyll *a*, chlorophyll *b*, chlorophylls *a+b* and carotenoid contents were analysed in freeze-dried sample extracts. Leaf material (50 mg) was extracted in 80% acetone and supernatants were diluted 1:15 in 80% acetone with absorbance measured at 470, 646.6, 663.6 and 750 nm using an Ultrospec 4000 UV/Vis spectrophotometer (GE Healthcare, Buckinghamshire, England). Pigment contents were determined using equations by Lichtenthaler (1987) and Porra *et al.* (1989).

2.7. Data analysis and statistics

Data were transformed when necessary with either $\sqrt{(x + 0.5)}$, x^2, Ln (x + 1) or 2*arcsin $\sqrt{x}$ in the case of percentage data to decrease heteroscedasticity and improve normal distribution. All the parameters were then analysed using IBM Statistics SPSS 21.0 software (SPSS, 2013) with one-way ANOVA followed by *t*-test ($p \leq 0.05$) to assess differences prior to exchange of plants or pairwise comparison for least significant differences (LSD) ($p \leq 0.05$) to test differences after the exchange of plants. If data did not follow a normal distribution, a non-parametric Kruskal-Wallis *H* or Mann-Whitney *U* test ($p \leq 0.05$) was performed. Stem height and leaf area over the crop cycle (repeated measures over time) were assessed with ANOVA univariate repeated measures analysis ($p \leq 0.05$) using SuperANOVA v. 1.11 software for Macintosh (Abacus Concepts, 1989).

3. Results

3.1. Plant height and leaf area

Addition of UV-A to pepper plants over the entire plant growth cycle (UVA+/UVA+) caused a significant reduction in plant height (Treatment: F=15.399, 3 df, p<0.001. Time: F=137.122, 6 df, p<0.001. Time x Treatment: F=7.311, 8 df, p<0.001). By 68 days, plants grown with supplemental UV-A were 57% shorter compared to plants grown at near zero UV-A (23.9 cm vs. 37.7 cm) (Supplementary Figure 1). Pepper leaf area appeared lower with UV-A but not significantly different (Treatment: F=2.618, 3 df, p=0.068. Time: F=262.928, 6 df, p<0.001. Time x Treatment: F=1.271, 8 df, p=0.267) when compared to the near zero UV-A treatment (Supplementary Figure 1).

Eggplants exposed to UV-A were shorter from 84 days onwards although not significantly (Treatment: F=0.018, 3 df, p=0.997. Time: F=311.450, 11 df, p<0.001. Time x Treatment: F=1.575, 29 df, p=0.042). By the end of the experiment, plants exposed to supplemental UV-A during their entire cycle were 23% shorter than plants that had been grown at near zero UV-A (50.5 cm vs. 62.2 cm) (Supplementary Figure 1). For leaf area no significant effects were observed with UV-A (Treatment: F=0.191, 3 df, p=0.901. Time: F=262.753, 11 df, p<0.001. Time x Treatment: F=1.528, 29 df, p=0.054) (Supplementary Figure 1). Later addition of UV-A when insects were introduced to plants (53-68 days for aphids and 34-104 days for whiteflies) did not alter the height or leaf area responses observed above.

345 *3.2. Insect responses*

346

347 For aphids, the pre-reproductive period (*d*) from birth to adult stage was similar in all
348 treatments (H=2.656, 3 df, p=0.448) (Table 2). However, effective fecundity (*Md*) was
349 significantly higher (F=2.888, 70(3) df, p=0.042) in early supplemental UV-A treatment
350 scenario compared to the near zero UV-A treatment (UVA-/UVA-) (Table 2 and Figure 3).
351 This latter treatment lowered intrinsic rate of natural increase (r_m: F=2.974, 70(3) df,
352 p=0.037) as well as mean relative growth rate (*RGR*: F=2.974, 70(3) df, p=0.037) when
353 compared to pepper plants exposed to UV-A during early growth (UVA+/UVA-, Table 2).
354 UV-A treatment after insect infestation had no effects on aphid fecundity and development
355 (Figure 3).

356

357 The response of whiteflies to UV-A exposure was different to that of aphids. The pre-
358 reproductive period (*d*) from birth to adult stage was significantly shortened by two days
359 (H=10.409, 3 df, p=0.015) at near zero UV-A during insect development on plants (UVA-
360 /UVA- and UVA+/UVA-) (Table 2). Direct exposure of whiteflies to supplemental UV-A on
361 plants raised at near zero UV-A (UVA-/UVA+) significantly lowered fecundity -egg
362 numbers- compared to all other treatments (F=13.256, 60(3) df, p<0.001) (Table 2 and Figure
363 3). Moreover, egg numbers were significantly lower in treatments UVA+/UVA+ and UVA-
364 /UVA+, 47% and 123% respectively, when compared to insects maintained on plants raised
365 at near zero UV-A over the entire experiment (UVA-/UVA-). Supplemental UV-A exposure
366 also lowered egg fertility (F=6.254, 60(3) df, p=0.001) (Table 2). This resulted in a
367 significantly lower (F=14.380, 60(3) df, p<0.001) number of larvae in the treatments where
368 insects were exposed to UV-A, regardless of the previous conditions in which eggplants were
369 raised (treatments UVA+/UVA+ and UVA-/UVA+, Table 2). UV-A treatment after insect
370 infestation had a negative impact on whitefly fecundity, fertility and development (Figure 3).

371

372 Table 2. Life parameters of *Myzus persicae* and *Bemisia tabaci* raised under four different
373 UV-A radiation regimes. Different letters stand for statistical differences ($p \leq 0.05$).

374

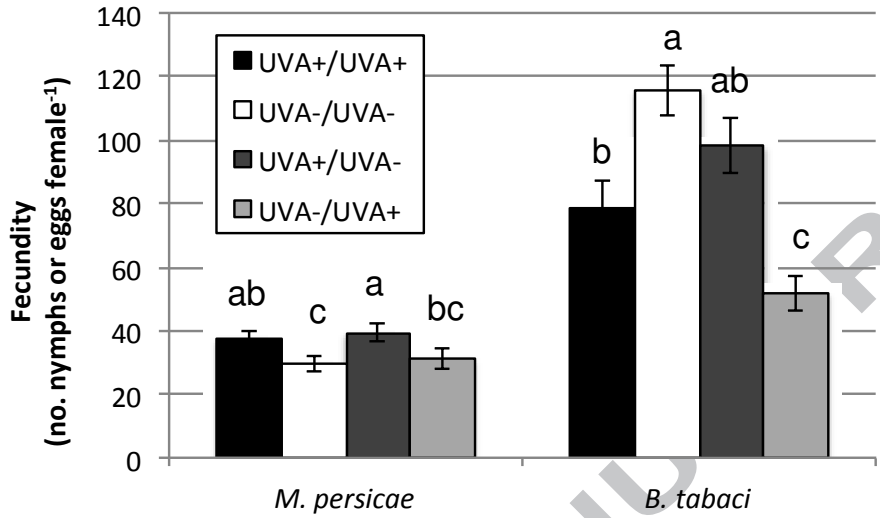
Insect	Parameters	UVA+/UVA+		UVA-/UVA-		UVA+/UVA-		UVA-/UVA+	
M. persciae	*d* [a]	8.89±0.15		8.71±0.17		8.63±0.14		8.74±0.15	
	Md [b]	37.53±2.57	ab	29.71±2.41	c	39.32±2.88	a	31.26±3.18	bc
	Td [c]	12.05±0.20		11.80±0.23		11.70±0.19		11.84±0.20	
	r_m [d]	0.298±0.006	ab	0.284±0.007	b	0.310±0.006	a	0.283±0.010	b
	RGR [e]	0.346±0.007	ab	0.330±0.008	b	0.361±0.007	a	0.329±0.011	b
B. tabaci	Viability [f]	72.43±10.48		81.38±8.37		77.86±8.78		75.71±6.61	
	d	26.99±0.89	a	24.40±0.48	b	24.66±0.46	b	26.94±0.84	a
	No. eggs	78.69±8.12	b	115.69±7.90	a	98.06±8.72	ab	51.88±5.58	c
	No. larvae	50.69±7.22	b	87.44±8.25	a	73.81±9.54	a	25.94±3.25	c
	Fertility [f]	60.30±4.91	b	73.48±3.51	a	72.12±4.10	a	50.31±4.23	b

375 [a] days, [b] effective fecundity, [c] mean generation time, [d] intrinsic rate of natural increase, [e] mean
376 relative growth rate, [f] %

377

378 Figure 3. Comparison between *M. persicae* and *B. tabaci* fecundity, showing the number of

nymphs and eggs per female on peppers and eggplants, respectively, under four different UV-A radiation regimes. Bars refer to standard errors and different letters stand for statistical differences ($p \leq 0.05$).



3.3. Biochemical responses to plant and insect UV-A exposure

3.3.1. Secondary metabolites

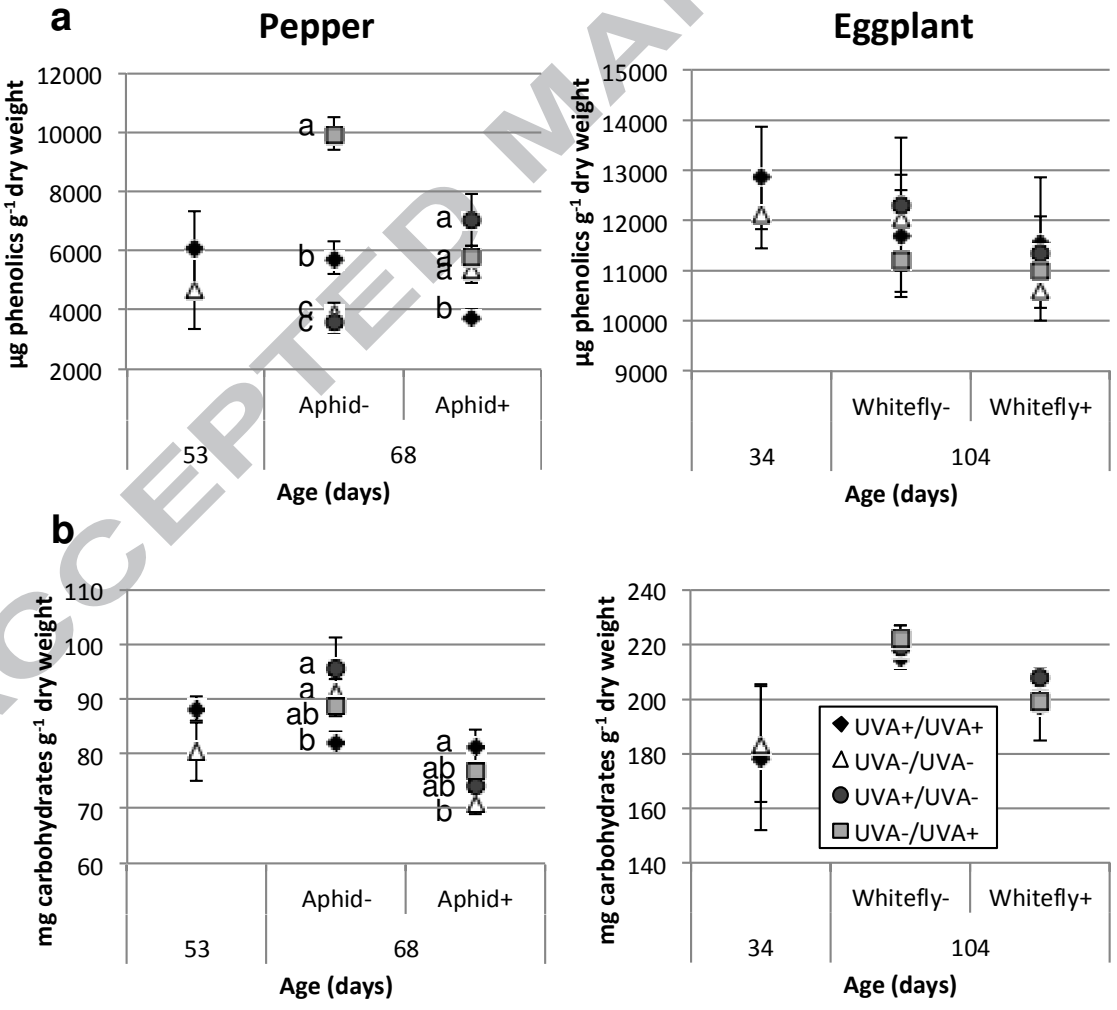
HPLC and LC-MS analysis revealed that there were two hydroxycinnamic acids and four flavonoids identifiable in pepper leaves. Analysis of eggplants revealed phenolics belonging to three classes (chlorogenic acid isomers, hydroxycinnamic acid amide conjugates and isochlorogenic acid isomers), as well as 3-*O*-feruloylquinic acid, which were determined based on HPLC elution times, UV spectra and LC-MS fragmentation data (Supplementary Table 1). Two kaempferol-hexosides with UV absorption maxima at 265 and 349 nm were also identified on the basis of their MS_2, however signals were too low to permit effective quantification of these compounds.

Secondary metabolites were increased in peppers by longer term UV-A exposure (68 days) but this depended on time of harvest and whether plants were simultaneously exposed to insects. Total content was similar under both UV-A regimes at 53 days (t=0.947, 10 df, p=0.366) (Figure 4a). However, when plants were harvested at 68 days, the four main flavonoid contents of pepper plants previously exposed to UV-A and later moved to a near zero UV-A regime (UVA+/UVA-) were comparable to levels found in those that had been grown entirely without UV-A radiation (UVA-/UVA-). This implies that phenolic expression declined when UV-A radiation was withdrawn. Pepper plants grown initially without UV-A and subsequently transferred to UV-A (UVA-/UVA+) also showed phenolic levels that were significantly higher than plants continuously grown under supplemental UV-A (UVA+/UVA+) (Compound 2: F=3.987, 20(3) df, p=0.022. Compound 3: F=5.229, 20(3) df, p=0.008. Compound 4: F=11.145, 20(3) df, p<0.001. Compound 5: F=20.618, 20(3) df, p<0.001. Compound 6: F=35.214, 20(3) df, p<0.001. Total: F=29.945, 20(3) df, p<0.001) (Figure 4a). Results for pepper suggest rapid acclimation to UV-A with aphid introduction

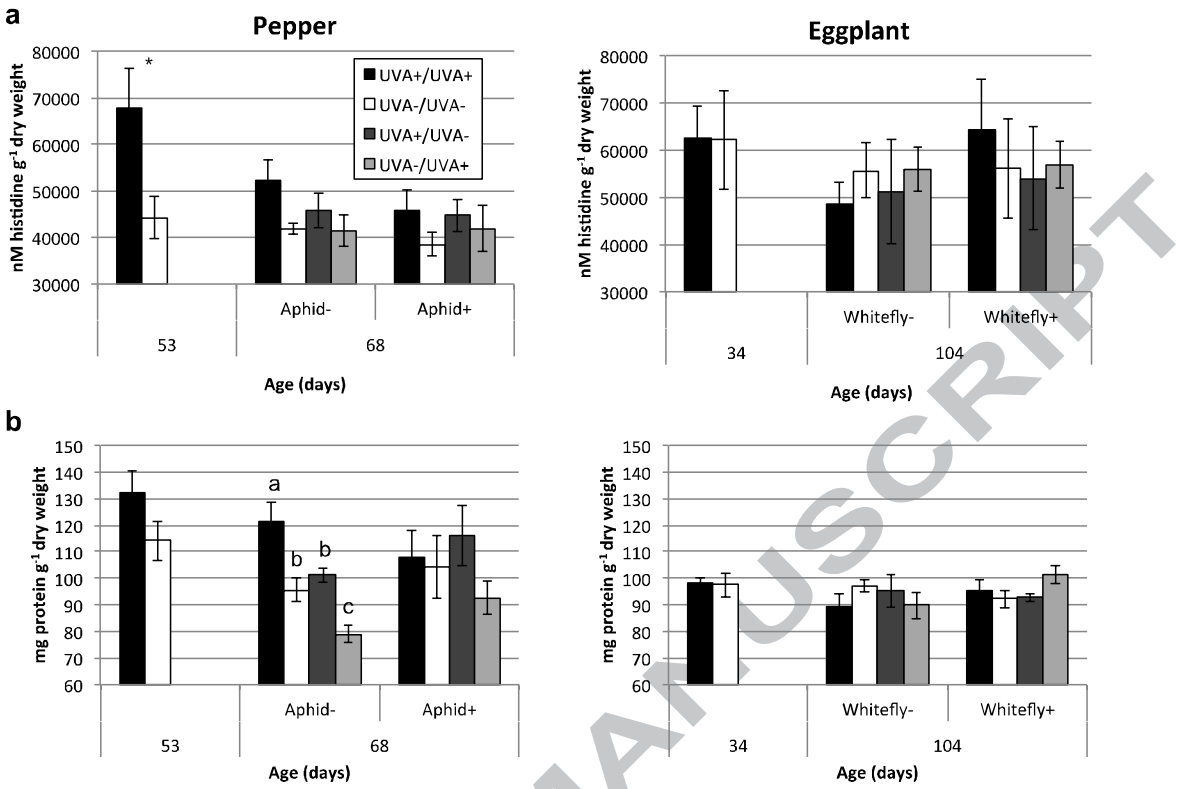
and damage influencing flavonoid profiles, as significantly higher levels were found in plants exposed to supplemental UV-A early but withdrawn from this treatment (UVA+/UVA-) (Compound 4: F=4.632, 20(3) df, p=0.013. Compound 5: F=7.755, 20(3) df, p=0.001. Compound 6: F=7.884, 20(3) df, p=0.001. Total: F=10.546, 20(3) df, p<0.001) (Figure 4a). N-caffeoylputrescine content in both uninfested and infested plants did not differ significantly.

Addition of UV-A radiation did not affect eggplant phenolic expression after the first harvest (34 days) prior to whitefly infestation (t=0.697, 10 df, p=0.502) (Figure 4a). In contrast to pepper plants, eggplant phenolic compounds were unaffected by treatment over the duration of the experiment (F=0.306, 20(3) df, p=0.821) (Figure 4a). As seen in Figure 4a, whitefly infestation did not appear to influence these patterns (F=0.193, 20(3) df, p=0.900).

Figure 4. Total phenolic (a) and soluble carbohydrate content (b) of pepper and eggplant leaves grown under four different UV-A radiation and two herbivore regimes, and harvested at two dates. Bars refer to standard errors and different letters stand for statistical differences ($p \leq 0.05$).



427
428 *3.3.2. Soluble carbohydrates*
429
430 Data showed different carbohydrate profiles with species and treatments. Polymer content
431 was similar under all treatments at any harvest time for both species. Polymer content was
432 very high in eggplant leaves. Significantly lower levels of total non-structural sugars
433 (raffinose, sucrose, glucose and fructose) were observed in uninfested pepper plants grown
434 under treatment UVA+/UVA+ at 68 days (F=3.484, 20(3) df, p=0.035). Raffinose and
435 glucose in particular were significantly higher following treatment UVA-/UVA+ (Raffinose:
436 F=3.440, 20(3) df, p=0.036. Glucose: F=5.365, 20(3) df, p=0.007). For infested plants, total
437 non-structural levels were similar (F=1.205, 20(3) df, p=0.334) although sucrose content was
438 significantly higher in treatments where aphids were grown under supplemental UV-A
439 (F=3.227, 20(3) df, p=0.044). No differences were found at any date in eggplant non-
440 structural sugars. When total sugar content was analysed, UVA+/UVA+ level was lowest in
441 uninfested peppers (F=4.622, 20(3) df, p=0.013) but highest in infested plants (F=3.402,
442 20(3) df, p=0.038) (Figure 4b). Carbohydrate levels under herbivory were lower than those
443 observed in uninfested peppers possibly due to aphid feeding (Figure 4b). Conversely, no
444 differences were found among treatments on eggplants samples both uninfested and infested
445 by whiteflies (Figure 4b).
446
447 *3.3.3. Free amino acid and proteins*
448
449 At 53 days, pepper plants exposed to supplemental UV-A had significantly higher levels of
450 free amino acids (t=2.755, 10 df, p=0.020). However, this trend was not significant at 68 days
451 in uninfested peppers (F=1.871, 20(3) df, p=0.167) (Figure 5a). Infested plants had a lower
452 level compared to uninfested plants possibly due to *in situ* aphid feeding activity but no
453 differences could be found between different radiation regimes (F=0.609, 20(3) df, p=0.617)
454 (Figure 5a). A similar pattern was observed for total protein content with a significantly
455 higher amount in plants continuously grown under supplemental UV-A at 68 days
456 (F=15.062, 20(3) df, p<0.001) (Figure 5b). No differences were observed between treatments
457 in eggplants for free amino acids (34 days: t=0.291, 10 df, p=0.777. 104 days uninfested:
458 F=0.255, 20(3) df, p=0.857. 104 days infested: F=0.217, 20(3) df, p=0.883) and total proteins
459 (34 days: t=0.245, 10 df, p=0.812. 104 days uninfested: F=0.783, 20(3) df, p=0.517. 104 days
460 infested: F=1.634, 20(3) df, p=0.213) when exposed to UV-A and/or feeding by whiteflies
461 (Figure 5a and b).
462
463 Figure 5. Free amino acids expressed as histidine (a) and total protein (b) content of pepper
464 and eggplant leaves grown under four different UV-A radiation and two herbivore regimes,
465 and harvested at two dates. Bars refer to standard errors and asterisks stand for statistical
466 differences ($p \leq 0.05$).



467
468
469 *3.3.4. Photosynthetic pigments*
470
471 There was no significant effect of UV-A exposure on pepper plant photosynthetic pigments
472 either at any harvest time or under aphid herbivory (Supplemental Table 2). In contrast,
473 eggplant leaves exposed to supplemental UV-A had lower chlorophyll content radiation at 34
474 days (Chlorophyll *a*: t=-2.531, 10 df, p=0.030. Chlorophylls *a*+*b*: t=-2.426, 10df, p=0.036)
475 and under whitefly infestation at 104 days (Chlorophyll *a*: F=4.613, 20(3) df, p=0.013.
476 Chlorophyll *b*: F=3.887, 20(3) df, p=0.024. Chlorophylls *a*+*b*: F=4.994, 20(3) df, p=0.010)
477 (Supplemental Table 2). Carotenoids also showed significant accumulation at near zero UV-
478 A (34 days: t=-2.630, 10 df, p=0.025. 104 days uninfested: F=3.803, 20(3) df, p=0.026. 104
479 days infested: F=4.467, 20(3) df, p=0.015). Contents were highest for treatment UVA-/UVA-
480 and mixed treatments where plants received both radiation regimes had intermediate contents
481 (Supplemental Table 2). Chl *a*/*b* ratio was statistically equal in all treatments, ranging from
482 2.3 to 2.5 in peppers and from 2.7 to 2.9 in eggplants.
483
484
485 **4. Discussion**
486
487 In the present work we investigated the effects of UV-A radiation on two key global pests,
488 the aphid *M. persicae* and whitefly *B. tabaci* and their host plants, pepper and eggplant. Our
489 aim was to determine how UV-A in the glasshouse environment influences plant growth and
490 chemistry, and insect performance. This work was undertaken in cages placed in a glasshouse

facility where plants received UV-A radiation via artificial lamp sources. Although the glass of the facility and filter-covered cages absorbed a considerable amount of radiation we cannot neglect at least some natural UV reaching the plants. In particular a higher UV:PAR ratio may have occurred at the start and end of each day because lamps were already switched on early in the morning and after sunset. These diurnal changes in the UV:PAR ratio might have influenced plant chemistry and insect response. However, UV irradiance reaching the plant canopy was predominantly originating from the lamps (70 %) because sunlight was partially filtered by greenhouse glass. Most (99%) of the UV radiation received by plants and insects in the UVA+ treatment was UV-A. However, we must acknowledge the possibility of a small amount of UV-B irradiance, well below ambient UV-B levels, present during our experiments (Table 1). Considering our 14h photoperiod, our plants received 71.67 KJ m^{-2} d^{-1} of UV-A while only 0.55 KJ m^{-2} d^{-1} of UV-B, which is 0.76% of the total UV irradiance. Therefore, we assume that any changes observed in plants and insects under the UVA+ treatment were predominantly elicited by UV-A. To our knowledge, this is the first study that has looked at supplemental UV-A effects on plant-insect interactions in the glasshouse environment, as opposed to previous research mainly focused on UV-B impacts (Hunt and McNeil, 1999; Kittas *et al*., 2006; Kuhlmann and Müller, 2009a, 2010; Paul *et al*., 2011).

For both plants species studied, the supplemental UV-A treatment appeared to alter the size and morphology over the entire crop cycle. Although plants had similar numbers of leaves, pepper internodes were significantly shorter, similarly as previously reported in other plant species (Kuhlmann and Müller, 2010; Comont *et al*., 2012). For eggplants, plant height appeared shortened but there were no significant effects on height or leaf area. This contrasts with previous work focussing on enhanced UV-B impacts on reduced leaf area (Kittas *et al*. 2006). In the current study, chlorophyll and carotenoid contents were lowered in eggplant with UV-A treatment at both harvest dates and under whitefly infestation, as found on buckwheat or quinoa with supplemental UV-B (Gaberščik *et al*., 2002; González *et al*., 2009). A reduction in chlorophyll has been proposed as an indicator of UV sensitivity (Smith *et al*., 2000).

The relevance of components of leaf chemistry was measured in order to try to interpret the insect responses observed. Phenolic patterns in peppers changed in response to UV-A and under herbivory. No secondary metabolite differences were observed during the earlier harvest at 53 days prior to insect introduction but were apparent at 68 days. As expected, 5-*O*-caffeoylquinic acid and flavonoid contents were significantly induced with enhanced UV-A (Gaberščik *et al*., 2002, Izaguirre *et al*., 2007; Mahdavian *et al*., 2008; Kulhmann and Müller, 2009a, 2009b, 2010). In the absence of aphids at 68 days, evidence showed how plants grown at near zero UV-A but later moved to a UV-A regime (treatment UVA-/UVA+) had higher level of leaf secondary metabolites, which even exceeded the levels found in UV-A treated plants over the entire crop cycle (UVA+/UVA+). This readiness of peppers to induce 'sunscreen' compounds might be correlated with UV tolerance (Middleton and Teramura, 1993; Harborne and Williams, 2000). Meanwhile, the flavonoid contents of plants grown with supplemented UV-A but subsequently moved to near zero UVA- declined rapidly to levels comparable to the control treatment UVA-/UVA- after stress recovery. Hence the

effect of UV-A was not cumulative over time (*cf.* Comont *et al*., 2012). Besides UV-shielding metabolites, elevated contents of phenolics have been proposed as antifeedants or digestibility reducers (Ballaré *et al*., 1996; Paul and Gwynn-Jones, 2003). Flavonoid levels are thought to be an important factor in herbivore nutrition and they may be partially induced by the same signaling pathway as UV protection, in which the jasmonic acid plays a key role (Mackerness, 2000; Stratmann, 2003; Demukra *et al*., 2010; Mewis *et al*., 2012). Pepper phenolics were affected by aphid feeding as seen previously in tobacco (Izaguirre *et al*., 2007). Whether the flavonoids detected acted also as a defense against *M. persicae* needs further investigation but results suggest aphid damage influencing their accumulation compared to uninfested peppers. Indeed one of the flavonoids present in our samples, luteolin-7-*O*-(2-apiosyl)glucoside, has been previously proposed as a deterrent compound against the leafminer fly species *Liriomyza trifolii* Burgess (Diptera: Agromyzidae) in sweet pepper leaves (Kashiwagi *et al*., 2005). Phenolics found in eggplants were mainly hydroxycinnamic acids, with 5-*trans*caffeoylquinicacid as the major compound (Stommel *et al*., 2003). As opposed to peppers, no significant increases in secondary metabolites were observed with UV-A or whitefly infestation in eggplants. However, induction of several flavonoids has been stated to protect tissues from UV damage in this species (Toguri *et al*., 1993). Past research has shown that eggplants already have high constitutive defences. Exposure to high UV-B irradiances did not influence phenolic accumulation, leaf area and Chl *a*/Chl *b* ratio (Smith *et al*., 2000; González *et al*., 2009). These results altogether may indicate a high tolerance to UV irradiance in this species possibly related to its ancestral origin from tropical regions.

Total non-structural carbohydrates were lowest in uninfested peppers grown under UV-A during the complete duration of the experiment (68 days) compared to all other treatments. Comont *et al*. (2012) also reported reductions in sucrose, glucose and fructose contents on *Arabidopsis thaliana* L. following UV-B treatment although contrasting results have been obtained on maize leaves (Barsig and Malz, 2000). However when insects were introduced, sucrose content was significantly higher in treatments where *M. persicae* was grown under UV-A. This might agree with previous research done under UV-B stress where higher soluble sugar content, mainly sucrose, was observed under addition of UV-B (González *et al*., 2009). Carbohydrate accumulation may have affected aphid fitness because sucrose is a strong feeding stimulant and the major component of the phloem sap of plants (Mittler *et al*., 1970; Srivastava and Auclair, 1971). Indeed when UV-A was withdrawn, adults produced less progeny with lower growth rates. By contrast, eggplant soluble sugars were unaffected by UV-A and total levels were similar at every harvest time and under whitefly herbivory, displaying another reliable indicator to UV tolerance (González *et al*., 2009).

Amino acids are the major nitrogen source for aphids. In our work, we observed significantly higher free amino acids in pepper leaves exposed to UV-A radiation, suggesting that such plants could be preferred by insects. Amino acids are an essential dietary component for *M. persicae* growth (Dadd and Krieger, 1968) that has a mainly nutritive role in aphid feeding (Srivastava and Auclair, 1975; Weibull, 1987). Nitrogen content is thought to act as a feeding stimulant for insects (Schoonhoven *et al*., 2006), being higher when high radiation intensities

579 are present in the environment (Roberts and Paul, 2006). It is likely that phloem quality under
580 supplemented UV-A conditions had a richer composition that may have triggered a positive
581 plant-mediated effect on *M. persicae* development and fecundity. Moreover, free amino acids
582 levels were unsurprisingly lower under herbivore attack due to aphid feeding. It should be
583 emphasized that here we focussed on the chemical composition of entire pepper leaves and
584 this may not necessary reflect that in the phloem sap (Kehr, 2006). Further studies should be
585 conducted to find out if the observed changes in leaf chemistry due to supplemental UV-A
586 radiation are reflective of the chemical changes in the phloem sap, extracted by stylectomy
587 (Kennedy and Mittler, 1953) or via leaf incisions (Milburn, 1970).
588
589 There were no differences according to UV-A in protein and free amino acid content in
590 eggplants. Very little is known about the impact of UV radiation on the composition of free
591 amino acids in phloem sap, but the same trend has been observed in other species of the
592 family Brassicaceae such as broccoli, where authors reported similar contents except for
593 increased proline under low UV-B compared to high levels of UV-B (Kulhmann and Müller,
594 2009a, 2010).
595
596 The addition of UV-A to the environment had complex effects on aphids. Mainly, an indirect
597 plant-mediated impact on *M. persicae* effective fecundity was observed. The effective
598 fecundity measured was higher in early UV-A treatment scenarios compared to the near zero
599 UV-A treatment (UVA-/UVA-). This latter treatment also resulted in lowered intrinsic rate of
600 natural increase and mean relative growth rate when compared to the scenario where plants
601 had only been exposed to UV-A during early growth (UVA+/UVA-). This may indicate that
602 alterations in tissue chemistry occurred prior to aphid infestation and contributed to its
603 performance. The reduction in the population growth without UV-A exposure is in agreement
604 with findings previously reported for several aphid species (Antignus *et al*., 1996; Chyzik *et*
605 *al*., 2003; Díaz *et al*., 2006; Kuhlmann and Müller, 2009a; Paul *et al*., 2011; Legarrea *et al*.,
606 2012). The pre-reproductive period from birth to adult stage was similar for all treatments. In
607 contrast, results provided evidence that supplemental UV-A exposure had an impact on the
608 fitness of whiteflies, this contrasted with aphids. The pre-reproductive period was
609 significantly increased by two days with supplemental UV-A during insect growth on plants
610 regardless of the radiation regime before insect introduction (treatments UVA+/UVA+ and
611 UVA-/UVA+). Exposure of whiteflies to UV-A on plants raised at near zero UV-A (UVA-
612 /UVA+) significantly lowered the number of eggs compared to near zero UV-A for the entire
613 crop cycle (UVA-/UVA-). There was no statistically significant difference in the number of
614 eggs between treatments UVA-/UVA- and UVA+/UVA-, which supports the hypothesis that
615 this effect was not mediated by host cues as it did not depend on the UV-A regime the plants
616 had been grown under before whitefly infestation. This resulted in a significantly lower
617 fertility in the treatments where UV-A was supplemented during insect growth (Table 2).
618
619 When whiteflies were subjected to supplemental UV-A treatments, eggplants received
620 radiation at the same time although the chemical compounds involved in whitefly nutrition
621 that we analysed (free amino acids and sugars) were unaffected by supplemental UV-A. UV-
622 A radiation inside the clip-cages where insects were monitored was 0.00 W m^{-2} in the

623 treatment UVA- vs. 0.03 W m^{-2} in the treatment UVA+, a difference that may not be
624 sufficient to conclude that UV-A had a direct impact on whitefly performance. However, the
625 floor of the cages was aluminium and reflected part of the UV radiation into the clip-cages in
626 the supplemental UV-A treatment. Radiation transmitted through the leaves could reach the
627 ventral part of the whitefly nymphs and the radiation reflected by the floor reaching the
628 abaxial side of the leaves could irradiate the dorsum of whiteflies (Table 1). While results
629 indicate a possible negative effect of UV-A which cannot be explained by changes in plant
630 chemicals measured, we cannot dismiss the possibility of an effect triggered by aspects of
631 host plant chemistry that were not measured. Further work to isolate direct from plant-
632 mediated effects of UV-A radiation on whitefly performance should be conducted in the
633 future by irradiation of insects under a free-plant environment.
634
635 The effect of UV on the life processes of whiteflies has been little studied. Traditionally
636 research has focused on flight behavior in host choice assays, with more whiteflies being
637 trapped under environments with UV radiation (Antignus *et al*., 1996; Costa and Robb, 1999;
638 Kuhlmann and Müller, 2009a), but to the best of knowledge, for the first time its performance
639 has been tested under different UV-A regimes. In past studies, it is likely that whiteflies were
640 driven by the radiation spectrum rather than by the plant chemistry as they tested orientation
641 and alighting (Kuhlmann and Müller, 2009b), whereas in our work insects were caged and
642 forced to feed on each plant. Whiteflies showed an explicit tendency to grow slower under
643 the UV-A source after insect infestation. This might be explained by the mechanism by
644 which UV radiation triggers a migratory behaviour (Mound, 1962; Coombe, 1982). However,
645 the absence of UV might have extended the mating period so whiteflies fed and laid eggs
646 over a greater period at near zero UV-A radiation.
647
648 Allocation of UV-A-shielding compounds responsible for physicochemical defense involved
649 some constrains on peppers, as plant growth decreased under high UV-A conditions. The
650 UV-induced phenolic pattern in pepper contrasted with lack of changes observed in
651 eggplants. In addition, this latter species also showed other characteristics present in plants
652 tolerant to high UV irradiances, such as no changes in leaf area and content of soluble
653 carbohydrates irrespective of UV-A exposure. We hypothesise that these findings might be
654 related to a high tolerance to UV-A. UV-A radiation altered the chemical composition of
655 pepper plants, with consequences to pest fitness. It is clear that UV-A enriched pepper
656 nutritional quality for aphids. In contrast for whiteflies, there was a direct negative effect of
657 UV-A rather than via tissue quality. As a whole, results reported in the two complexes
658 suggest that UV-mediated changes are highly dependent on the plant and insect studied.
659 Nevertheless, we believe that UV-absorbing nets might be a useful tool against aphids
660 without detrimental effects on crops. Further knowledge is needed to unravel the complete
661 role of UV-A radiation in plant-insect interactions, and to elucidate whether these responses
662 present interactions with effects occurring as a consequence of other fractions of the solar
663 spectrum.
664
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666

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References

Abacus Concepts, 1989. SuperANOVA. Abacus Concepts, Berkeley, CA, USA.

Antignus, Y., Mor, N., Joseph, R.B., Lapidot, M., Cohen, S., 1996. Ultraviolet-absorbing plastic sheets protect crops from insect pests and from virus diseases vectored by insects. Environ. Entomol. 25, 919-924.

Ballaré, C., Scopel, A.L., Stapleton, A.E., Yanovsky, M., 1996. Solar ultraviolet-B radiation affects seedling emergence, DNA integrity, plant morphology, growth rate, and attractiveness to herbivore insects in *Datura ferox*. Plant Physiol. 112, 161-170.

Barsig, M., Malz, R., 2000. Fine structure, carbohydrates and photosynthetic pigments of sugar maize leaves under UV-B radiation. Environ. Exp. Bot. 43, 121-130.

Chyzik, R., Dorinin, S., Antignus, Y., 2003. Effect of a UV-deficient environment on the biology and flight activity of *Myzus persicae* and its Hymenopterous parasite *Aphidius matricariae*. Phytoparasitica 31, 467-477.

Clifford, M.N., Johnston, K.L., Knight, S., Kuhner, N., 2003. Hierarchical scheme for LC-MS identification of chlorogenic acids. J. Agric. Food Chem. 51, 2900-2911.

Comont, D., Winters, A., Gwynn-Jones, D., 2012. Acclimation and interaction between drought and elevated UV-B in *A. thaliana*: differences in response over treatment, recovery and reproduction. Ecol. Evol. 2, 2695–2709.

Coombe, P.E., 1982. Visual behavior of the greenhouse whitefly, *Trialeurodes vaporariorum*. Physiol. Entomol. 7, 243-251.

Costa, H.S., Robb, K.L., 1999. Effects of ultraviolet-absorbing greenhouse plastic films on flight behavior of *Bemisia argentifolii* (Homoptera: Aleyrodidae) and *Frankliniella occidentalis* (Thysanoptera: Thripidae). J. Econ. Entomol. 92, 557-562.

Dadd, R.H., Krieger, D.L., 1968. Dietary amino acid requirements of aphid *Myzus persicae*. J. Insect Physiol. 14, 741-764.

Demukra, 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 Physiol. 152, 1084-1095.

Díaz, B.M., Biurrun, R., Moreno, A., Nebreda, M., Fereres, A., 2006. Impact of ultraviolet-blocking plastic films on insect vectors of virus diseases infesting crisp lettuce. HortScience 41, 711-716.

Díaz, B.M., Fereres, A., 2007. Ultraviolet-blocking materials as a physical barrier to control insect pests and plant pathogens in protected crops. Pest Technol. 1, 85-95.

Döring, T., Chittka, L., 2007. Visual ecology of aphids –a critical review on the role of colours in host finding. Arthropod-Plant Interact. 1, 3-16.

Dyer, A.G., Chittka, L., 2004. Bumblebee search time without ultraviolet light. J. Exp. Biol. 207, 1683-1688.

Fukaya, M., Uesugi, R., Ohashi, H., Sakai, Y., Sudo, M., Kasai, A., Kishimoto, H., Osakabe, M., 2013. Tolerance to solar ultraviolet-B radiation in the citrus red mite, an upper surface user of host plant leaves. Photochem. Photobiol. 89, 424-431.

Gaberščik, A., Voncina, M., Trošt, T., Germ, M., Björn, L.O., 2002. Growth and production of buckwheat (*Fagopyrum esculentum*) treated with reduced, ambient, and enhanced UV-B radiation. J. Photochem. Photobiol. B, Biol. 66, 30-36.

González, J.A., Rosa, M., Parrado, M.F., Hilal, M., Prado, F.E., 2009. Morphological and physiological responses of two varieties of a Highland species (*Chenopodium quinoa* Willd.) growing under near-ambient and strongly reduced solar UV-B in a lowland location. J. Photochem. Photobiol. B, Biol. 96, 144-151.

Gutiérrez-Marco, E., Hernández, E., Camacho, J.L., Labajo, A., 2007. Analysis of UVB values in the centre of the Iberian Peninsula. Atmos. Res. 84, 345-352.

Häder, D.-P., Lebert, M., Schuster, M., del Ciampo, L., Helbling, E.W., McKenzie, R., 2007. ELDONET – A decade of monitoring solar radiation on five continents. Photochem. Photobiol. 83, 1-10.

Harborne, J.B., Williams, C.A., 2000. Advances in flavonoid research since 1992. Phytochemistry 55, 481-504.

Hull, R., 2002. Matthews' Plant Virology. 4th edn. Academic Press, New York, USA.

Hunt, J.E., McNeil, D.L., 1999. The influence of present-day levels of ultraviolet-B radiation on seedlings of two Southern Hemisphere temperate tree species. Plant Ecol. 143, 39-50.

Izaguirre, M.M., Mazza, C.A., Svatoš, 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*. Ann. Bot. 99, 103-109.

Jansen, M.A.K., 2002. Ultraviolet-B radiation effects on plants: induction of morphogenic responses. Physiol. Plant. 116, 423-429.

Jayakumar, M., Amudha, P., Kulandaivelu, G., 2003. Changes in growth and yield of *Phaseolus mungo* L., induced by UV-A and UV-B enhanced radiation. J. Plant Biol. 46, 59-61.

Jayakumar, M., Amudha, P., Kulandaivelu, G., 2004. Effect of low doses of UV-A and UV-B radiation on photosynthetic activities in *Phaseolus mungo* L. J. Plant Biol. 47, 105-110.

Johansen, N.S., Vänninen, I., Pinto, D.M., Nissinen, A.I., Shipp, L., 2011. In the light of new greenhouse technologies 2: Direct effects of artificial lighting on arthropods and integrated pest management in greenhouses crops. Ann. App. Biol. 159, 1-27.

Kashiwagi, T., Horibata, Y., Mekuria, D.B., Tebayashi, S., Kim C.-S., 2005. Ovipositional deterrent in the sweet pepper, *Capsicum annuum*, at the mature stage against *Liriomyza trifolii* (Burgess). Biosci. Biotechnol. Biochem. 69, 1831-1835.

Kehr, J., 2006. Phloem sap proteins: their identities and potential roles in the interaction between plants and phloem-feeding insects. J. Exp. Bot. 57, 767-774.

Kennedy J., Mittler T., 1953. A method of obtaining phloem sap via the mouth parts of aphids. Nature 171, 528.

Kittas, C., Tchamitchian, M., Kasoulas, N., Kariskou, P., Papaioannou, C.H., 2006. Effect of two UV-absorbing greenhouse-covering films on growth and yield of an eggplant soilless crop. Sci. Hort. 110, 30-37.

Kuhlmann, F., Müller, C., 2009a. Development-dependent effects of UV radiation exposure on broccoli plants and interactions with herbivorous insects. Environ. Exp. Bot. 66, 61-68.

Kuhlmann, F., Müller, C., 2009b. Independent responses to ultraviolet radiation and herbivore attack in broccoli. J. Exp. Bot. 60, 3467-3475.

Kuhlmann, F., Müller, C., 2010. UV-B impact on aphid performance mediated by plant quality and plant changes induced by aphids. Plant Biol. 12, 676-684.

Legarrea, S., Betancourt, M., Plaza, M., Fraile, A., García-Arenal, F., Fereres, A., 2012. Dynamics of nonpersistent aphid-borne viruses in lettuce crops covered with UV-absorbing nets. Virus Res. 165, 1-8.

Lichtenthaler, H.K., 1987. Chlorophylls and carotenoids: pigments of the photosynthetic biomembranes. Meth. Enzymol. 148, 350-382.

Liu, L.X., Xu, S.M., Woo, K.C., 2005. Solar UV-B radiation on growth, photosynthesis and the xanthophyll cycle in tropical acacias and eucalyptus. Environ. Exp. Bot. 54, 121-130.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265-75.

Mackerness, S.A.-H., 2000. Plant responses to ultraviolet-B (UV-B: 280-320 nm) stress: what are the key regulators? Plant Growth Regul. 32, 27-39.

Mahdavian, K., Kalantari, K.M., Ghorbanli, M., Torzade, M., 2008. The effects of salicylic acid on pigment contents in ultraviolet radiation stressed pepper plants. Biol. Plantarum 52, 170-172.

Marín, A., Ferreres, F., Tomás-Barberán, F.A., Gil, M.I., 2004. Characterization and quantitation of antioxidant constituents of sweet pepper (*Capsicum annuum* L.). J. Agric. Food Chem. 52, 3861-3869.

Mewis, I., Schreiner, M., Nguyen, C.N., Krumbein, A., Ulrichs, C., Lohse, M., Zrenner, R., 2012. UV-B irradiation changes specifically the secondary metabolite profile in broccoli sprouts: Induced signaling overlaps with defense response to biotic stressors. Plant Cell Physiol. 53, 1546-1560.

Middleton, E.M., Teramura, A.H., 1993. The role of flavonol glycosides and carotenoids in protecting soybean from ultraviolet-B damage. Plant Physiol. 103, 741-752.

Milburn, J.A., 1970. Phloem exudation from castor bean: induction by massage. Planta 95, 272-276.

Mittler, T., Dadd, D, Daniels, S.C., 1970. Utilization of different sugars by the aphid *Myzus persicae*. J. Insect Physiol. 16, 1873-1890.

Mound, L.A., 1962. Studies on the olfaction and colour sensitivity of *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae). Entomol. Exp. App. 5, 99-104.

Park, S., Jeong, W.Y., Lee, J.H., Kim, Y.-H., Jeong, S.W., Kim, G.-S., Bae, D.W. Lim, C.-S., Jin, J.S., Lee, S.J., Shin, S.C., 2012. Determination of polyphenol levels variation in *Capsicum annumm* L. cv. Chelsea (yellow bell pepper) using liquid chromatography-tandem mass spectrometry. Food Chem. 130, 981-985.

Paul, N.D., Gwynn-Jones, D., 2003. Ecological roles of solar UV radiation: towards an integrated approach. Trends Ecol. Evol. 18, 48-55.

Paul, N.D., Moore, J.P., McPherson, M., Lambourne, C., Croft, P., Heaton, J.C., Wargent, J.J., 2011. Ecological responses to UV radiation: interactions between the biological effects of UV on plants and on associated organisms. Physiol. Plant. 145, 565-581.

Petropoulou, Y., Georgiou, O., Psaras, G.K., Manetas, Y., 2001. Improved flower advertisement, pollinator rewards and seed yield by enhanced UV-B radiation in the Mediterranean annual *Malcolmia maritima*. New Phytol. 152, 85-90.

Porra, R.J., Thomson, W.A., Kriedemann, P.E., 1989. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophyll *a* and *b* extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. Biochim. Biophys. Acta 975, 384-394.

Raviv, M., Antignus, Y., 2004. UV radiation effects on pathogens and insect pests of greenhouse-grown crops. Photochem. Photobiol. 79, 219-226.

Roberts, M.R., Paul, N.D., 2006. Seduced by the dark side: integrating molecular and ecological perspectives on the influence of light on plant defence against pests and pathogens. New Phytol. 170, 677-699.

Sakai, Y., Osakabe, M., 2010. Spectrum-specific damage and solar ultraviolet radiation avoidance in the two-spotted spider mite. Photochem. Photobiol. 86, 925- 932.

Schoonhoven, L.M., van Loon, J.J.A., Dicke, M., 2006. Insect-Plant Biology. 2nd edn. Oxford University Press, Oxford, UK.

Smith, J.L., D.J. Burritt, D.J., Bannister, P., 2000. Shoot dry weight, chlorophyll and UV-B absorbing compounds as indicators of a plant's sensitivity to UV-B radiation. Ann. Bot. 86, 1057-1066.

SPSS statistical package, 21.0 version, 2013. SPSS Inc., Chicago, USA.

Srivastava, P.N., Auclair, J.L., 1971. Influence of sucrose concentration on diet uptake and performance by the pea aphid, *Acyrthosiphon pisum*. Ann. Entomol. Soc. Am. 64, 739-743.

Srivastava, P.N., Auclair, J.L., 1975. Role of single amino acids in phagostimulation, growth and survival of *Acyrthosiphon pisum*. J. Insect Physiol. 21, 1865-1871.

Stephanou, M., Petropoulou, Y., Georgiou, O., Manetas, Y., 2000. Enhanced UV-B radiation, flower attributes and pollinator behaviour in *Cistus creticus*: a Mediterranean field study. Plant Ecol. 147, 165-171.

Stommel, J.R., Whitaker, B.D., 2003. Phenolic acid content and composition of eggplant fruit in a germplasm core subset. J. Am. Soc. Hortic. Sci. 128, 704-710.

Stratmann, J., 2003. Ultraviolet-B radiation co-opts defense signaling pathways. Trends Plant Sci. 8, 526-533.

Tezuka, T., Yamaguchi, F., Ando, Y., 1994. Physiological activation in radish plants by UV-A radiation. J. Photochem. Photobiol. B, Biol. 24, 33-40.

Toguri, T., Umemoto, N., Kobayashi, O., Ohtani, T., 1993. Activation of anthocyanin synthesis genes by white light in eggplant hypocotyl tissues, and identification of an inducible P-450 cDNA. Plant Mol. Biol. 23, 933-946.

Tsormpatsidis, E., Ordidge, M., Henbest, R. G. C., Wagstaffe, A., Battey, N.H., Hadley, P., 2011. Harvesting fruit of equivalent chronological age and fruit position shows individual effects of UV radiation on aspects of the strawberry ripening process. Environ. Exp. Bot. 74, 178-185.

Vänninen, I., Pinto, D.M., Nissinen, A.I., Johansen, N.S., Shipp, L., 2010. In the light of new greenhouse technologies 1: Plant-mediated effects of artificial lighting on arthropods and tritrophic interactions. Ann. App. Biol. 157, 393-414.

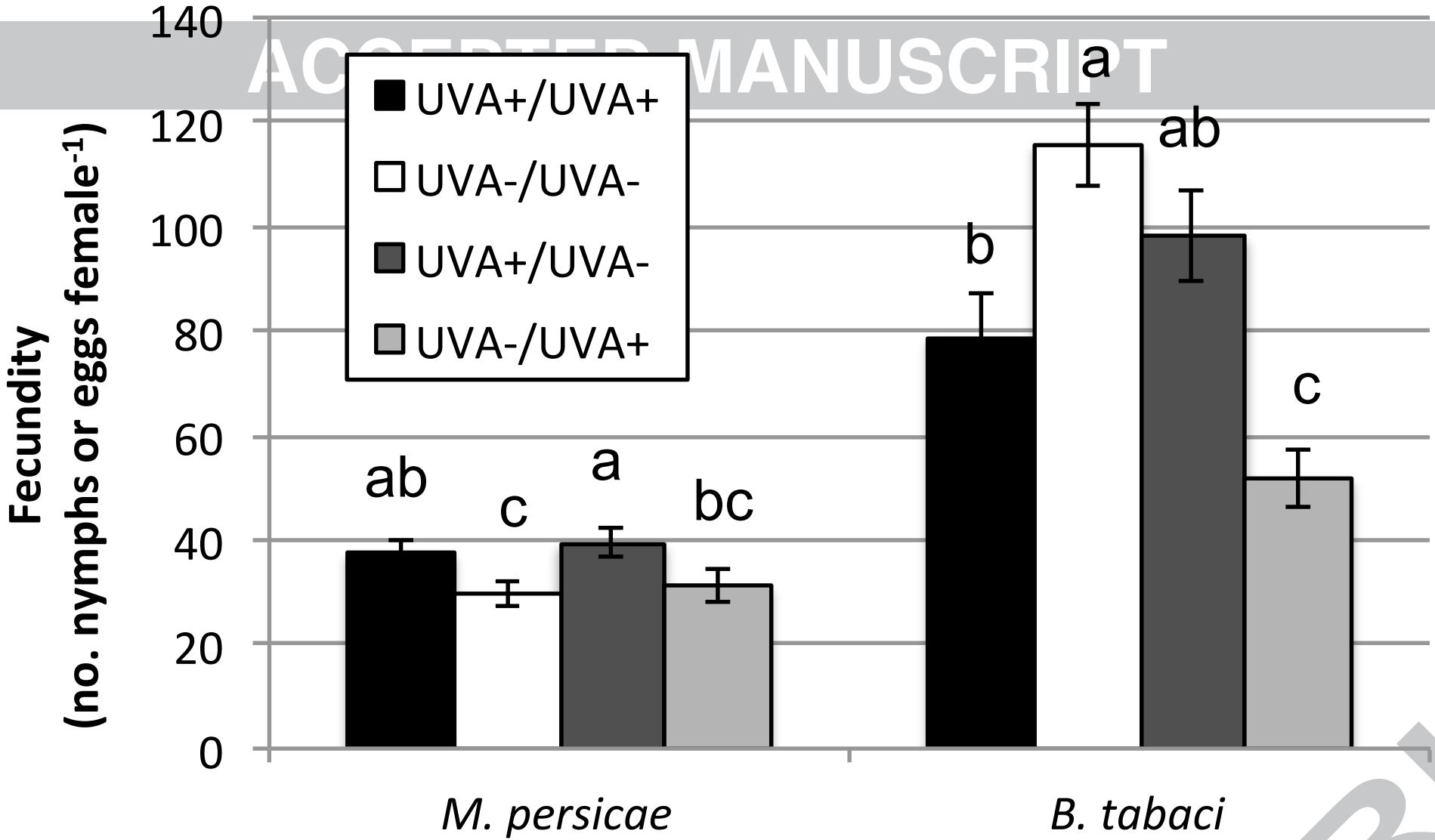
Verdaguer, D., Llorens, L., Bernal, M., Badosa, J., 2012. Photomorphogenic effects of UVB and UVA radiation on leaves of six Mediterranean sclerophyllous woody species subjected to two different watering regimes at the seedling stage. Environ. Exp. Bot. 79, 66-75.

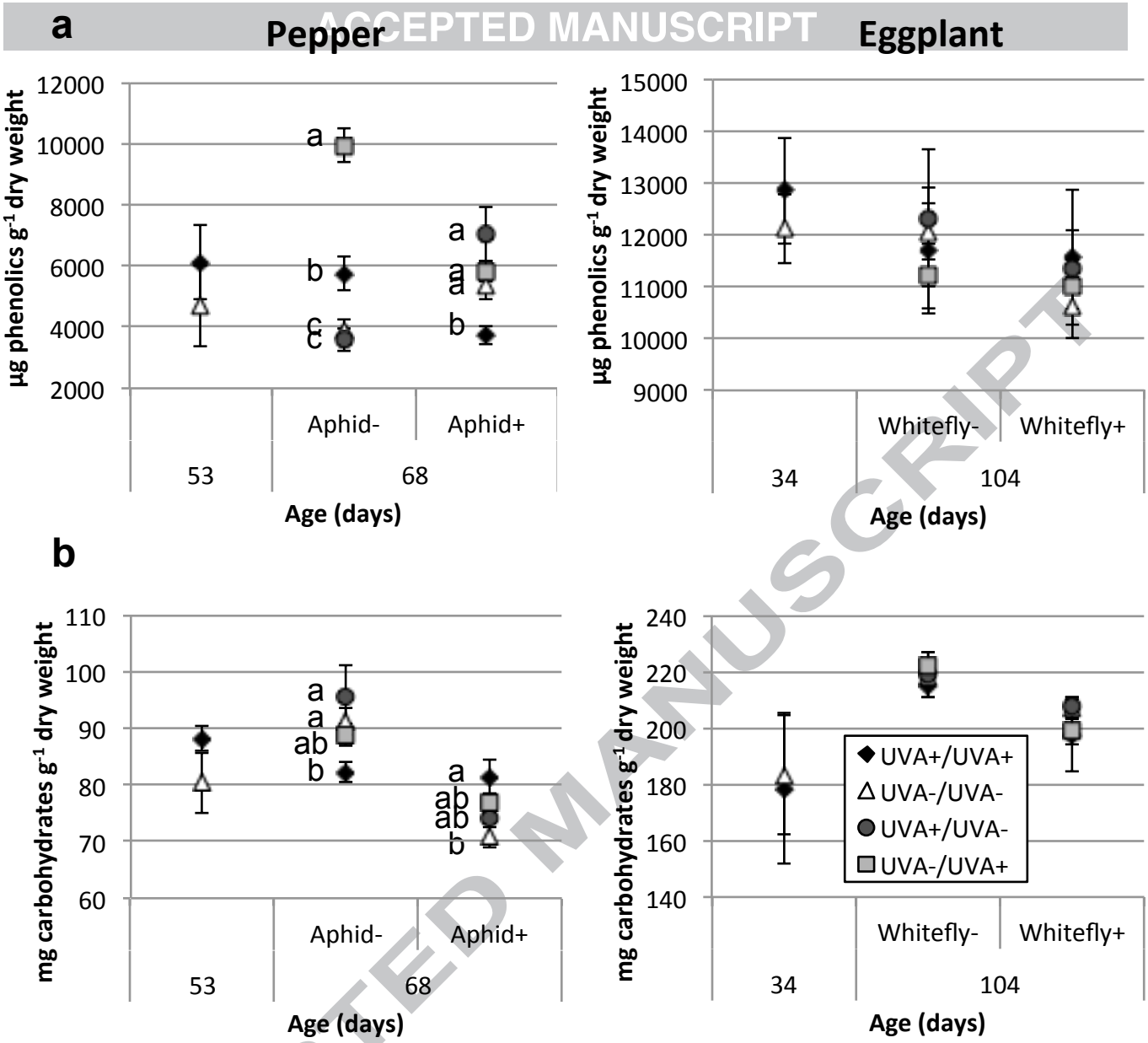
Weibull, J., 1987. Seasonal changes in the free amino acids of oat and barley phloem sap in relation to plant growth stage and growth of *Rhopalosiphum padi*. Ann. App. Biol. 111, 729-737.

Weintraub, P.G., 2009. Physical control: an important tool in pest management. In: Ishaaya, I., Horowitz, A.R. (Eds.), Biorational Control of Arthropod Pests. Springer Science, Berlin, Germany, pp. 317-324.

Winters, A., Lloyd, J.D., Jones, R., Merry, R.J., 2002. Evaluation of a rapid method for estimating free amino acids in silages. Anim. Feed Sci. Technol. 99, 177-187.

WMO, 2010. Scientific assessment of ozone depletion: 2010. World Meteorological Organization, Global Ozone Research and Monitoring Project, Executive summary, Geneva, Switzerland.





925 Highlights:

926 • Supplemental UV-A causes a reduction in pepper stem height
927 • Aphids benefit from changes in pepper metabolites under supplemental UV-A
928 • There is a detrimental effect of UV-A radiation on whitefly performance
929 • UV-mediated changes appear to be highly dependent on each plant-insect complex

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