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Aphid orientation and performance in glasshouses under different UV-A/UV-B radiation regimes

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Running headline: *Aphid behaviour under UV radiation*

Key words: *Myzus persicae*, pepper, flight behaviour, life history, Hemiptera, Aphididae, visual cues, landing, *Capsicum annuum*, green peach aphid, Solanaceae, host selection

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Abstract

Visual cues leading to host selection and landing are of major importance for aphids and evidence suggests that flight activity is very dependent on ultraviolet (UV)-A radiation in the environment. At the same time research on insect plant hosts suggest that the UV-B component can deter some pests via changes in secondary metabolite chemistry. Here we examine the potential of UV (UV-A/UV-B) radiation to control insect pests in the glasshouse environment. We first examined artificial exposure to UV-B and the potential to trigger morphological and biochemical modifications in pepper (*Capsicum annuum* L., Solanaceae) with implications for the fitness of green peach aphid, *Myzus persicae* Sulzer (Hemiptera: Aphididae). UV-B caused accumulation of leaf secondary metabolites and soluble carbohydrates, and stimulated photosynthetic pigments. However, UV-B did not impact on foliar protein content and aphid performance was unaffected. Next we studied how altering the UV-A/UV-B ratio environment affected aphid orientation and spatial distribution over time, either directly or by exposing plants to supplemental UV before insect introduction. Aphids directly settled and dispersed on their host pepper plants more readily in the presence of supplemental UV-A and UV-B. In the control treatment with ambient glasshouse UV-A and UV-B, insects remained more aggregated. Furthermore, insects were less attracted to peppers pre-exposed to supplemental UV-A and UV-B radiation. Our results suggest that suppression of UV-A and UV-B inside the protected environment reduces aphid colonization and dispersal. Further, application of moderate exposure of young pepper plants to supplemental UV-B radiation could aid in protection from the colonization by phytophagous insects.

Introduction

Myzus persicae Sulzer (Hemiptera: Aphididae) is a cosmopolitan, polyphagous aphid pest of greenhouses and field crops. More importantly, it is highly efficient as a vector of more than 100 plant viruses; therefore, repeated insecticide applications to lower vector density have constituted the traditional control strategy in the past, causing environmental and energetic costs (Blackman & Eastop, 2007). *Myzus persicae* has a trichromatic visual system with three spectral photoreceptors in the ultraviolet (UV)-A (320-330 nm), blue (440-480 nm), and green (530 nm) regions of the spectrum (Briscoe & Chittka, 2001; Kirchner et al., 2005).

Insect activities leading to host landing comprise several steps with the involvement of visual, olfactory, and tactile cues (Döring, 2014). Within this process, visual cues are of major importance, especially for aphids because their orientation, host finding, and performance is very sensitive to changes in the amount and type of UV radiation present in the environment (Raviv & Antignus, 2004; Döring & Chittka, 2007). Several studies have highlighted how absence of UV in the environment can interfere with the ability of aphids to locate their hosts (Raviv & Antignus, 2004; Johansen et al., 2011; Antignus, 2014), and decreases their performance (Chyzik et al., 2003; Legarrea et al., 2012b). In this sense, visual manipulation of the environment with photoselective screens has resulted in a novel means of aphid and virus control, with positive outcomes for horticultural crops of economic interest (Díaz & Fereres, 2007; Legarrea et al., 2012a; Antignus, 2014).

Besides the direct influence on insects, morphological and chemical alterations of host plants as a consequence of UV exposure are thought to mediate insect responses (Vänninen et al., 2010; Dáder et al., 2014). Changes in plant architecture, leaf thickness, or trichome density could influence insect preference and settling, and biochemical adjustments in the nutritive composition or increased secondary compounds could alter insect feeding and performance, some of these compounds being involved in pest defence (Smith et al., 2000; Jansen, 2002; Kittas et al., 2006; Izaguirre et al., 2007; Kuhlmann & Müller, 2010; Paul et al., 2012; Rechner & Poehling, 2014).

Research into the effects of UV radiation on insects in ecosystems has evolved into two categories including work focused on UV-B radiation (280-315 nm) due to ozone depletion impacts (e.g., Smith et al., 2000; Izaguirre et al., 2007; González et al., 2009; Mewis et al., 2012; Bornman et al., 2015) and research on the role of UV-A (315-400 nm) on visual systems. Evidence suggests that UV-A radiation has direct impacts on insect vision but this wavelength range also affects plant growth and biochemistry (Tezuka et al., 1994; Jayakumar

et al., 2003; Paul & Gwynn-Jones, 2003; Verdaguer et al., 2012; Dáder et al., 2014). There is also some evidence that some insect species use UV-A to avoid harmful UV-B radiation (Sakai & Osakabe, 2010).

In this work we have exposed the system *Capsicum annuum* L. (Solanaceae)-*M. persicae* to various individual or mixed regimes of UV-A and UV-B during a variety of periods ranging from hours to lifespan, to study aphid orientation and life history, as well as pepper physiology. The hypotheses that we considered to control aphid populations are based on how UV radiation affects plant-insect interactions in the glasshouse environment: (1) long-term UV-B application during a sustained period of time directly triggers photochemical modifications in pepper leaf tissue quality that make this host unattractive to aphids and indirectly influence insect performance, (2) the absence of UV-A and UV-B directly reduces aphid alighting, settlement, and dispersal, and (3) the presence of moderate UV-B radiation at an early pepper growth stage indirectly enhances aphid resistance by deterring aphid choice for plants previously grown under those conditions.

Comment [J1]: I think it is a good idea to phrase your hypotheses a bit bolder… - I skipped the coulds and woulds (an hypothesis with 'could' in it is next to impossible to reject!)

Comment [BD2]: We agree.

Materials and methods

Experiments were performed in glasshouse facilities in two locations over several years: the Aberystwyth University (Wales, UK) (52°25'06"N, 4°03'56"W) during summer 2013 and the Institute of Agricultural Sciences of the Spanish National Research Council (CSIC; Madrid, Spain) (40°26'23"N, 3°41'14"W) during springs 2014 and 2015.

Aphid colonies

Two clonal populations of *M. persicae* were established on pepper plants from two virus-free females in the UK and Spain. Individuals were synchronized prior to assays. In the UK, wingless aphids provided by John Innes Centre (Norwich, UK) were reared in a growth chamber at 22 °C, 70% r.h., and L16:D8 photoperiod. In Spain, aphids were reared in a climate chamber at L16(23 °C):D8(20 °C) and 60-80% r.h. Alate aphids were produced by placing 10 apterous adults per plant and developing the colony for 3 weeks to stimulate overcrowding.

Effect of UV-B on *Myzus persicae* life history and pepper leaf chemistry

The first question to address was whether long-term UV-B application could trigger photochemical modifications in pepper that would negatively affect aphid performance on

such plants. The experiment was performed in glasshouse facilities at the Aberystwyth University (Wales, UK) during summer 2013. Glasshouse dimensions were 6 × 3.5 × 3 m and light transmission properties of outer surface were 51% PAR (photosynthetically active radiation), 32% UV-A, and 13% UV-B. *Capsicum annuum* cv. California Wonder (Ramiro Arnedo, Calahorra, Spain) seeds were germinated in 12-cm-diameter pots with John Innes substrate no. 2 (John Innes Manufacturers, Theale, UK). Plants were watered 3× a week.

UV-B treatments

Peppers were grown under ambient glasshouse (control) and supplemental (+UV-B) conditions (Table 1). Controls were established by wrapping Q-panel 313 UV-B tubes (Q-lab Europe, Bolton, UK) in 0.1-mm-thick polyester film Autostat CT4 (MacDermid, Wantage, UK) to cut off wavelengths <320 nm. For +UV-B treatment, tubes were wrapped with 0.1-mm-thick cellulose diacetate film Ultraphan (Modulor, Berlin, Germany) to cut off wavelengths <295 nm. Filters were replaced after 40 h of use. Tubes were suspended at 30 cm high above canopy and switched on and off with no gradual transition for an 8-h photoperiod (10:00-18:00 hours, GMT+0) throughout the entire length of experiment. Irradiance conditions are summarized in Table 1.

Life history experimental design

When peppers reached a stage of eight true leaves after 40 days, half the plants from each treatment were exchanged into the opposite treatment, following Dáder et al. (2014). At this point, insects were introduced to the plants involving four overall treatments: +UV-B/+UV-B, control/control, +UV-B/control, or control/+UV-B (n = 11). Using this design we could determine direct and indirect effects (via plants) of UV-B on aphid performance. One wingless *M. persicae* adult was placed in a clip-cage on the adaxial side of the youngest fully expanded leaf of each plant and allowed to produce nymphs for 24 h. Three nymphs per plant were kept on each plant and monitored until adulthood, and the rest were removed. When the first nymph reached adult stage, the other two were removed. Offspring were counted by removing nymphs daily for an equal number of days to the pre-reproductive period. Duration of the pre-reproductive period (d), effective fecundity (Md), mean generation time (Td = d/0.738), intrinsic rate of natural increase [$r_m = 0.738 \times \ln(Md)/d$], and mean relative growth rate (RGR = $r_m/0.86$) were calculated (Wyatt & White, 1977).

Plant harvesting and chemical analysis

Pepper leaves were harvested to study direct effects of UV regimes on tissue quality prior to aphid introduction at the eight-true-leaf stage and 40 days old, and at the end of the aphid history experiment to study direct and indirect effects at the 14-true-leaf stage and 58 days old. We measured plant height and leaf area with an Area Meter MK2 VM21N 30 (Delta T-Devices, Cambridge, UK). Each repetition corresponded to the harvest of all leaves from one individual plant, which we processed, freeze-dried, and finely ground together. This ground material was subdivided for phenolic, sugar, protein, and pigment analysis (n = 3). Height and leaf area were evaluated at the 14-true-leaf stage to study accumulated UV effects on final size and canopy growth. Chemical compounds were evaluated at the eight-true-leaf stage to know initial tissue quality before placing aphids on those plants.

Secondary metabolites were extracted in 70% methanol (Comont et al., 2012) and analysed with a high pressure liquid chromatography (HPLC) system (Waters, Elstree, UK). 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. Phenolics were characterized by UV absorption spectra and comparison with standards (Clifford et al., 2003; Stommel et al., 2003; Marín et al., 2004; Park et al., 2012). Soluble sugars were extracted in distilled water at 80 °C. Supernatants were diluted 1:20 in 5 mM H_2SO_4 buffer with 5 mM crotonic acid internal standard. Samples were analysed with a HPLC system (Jasco, Essex, UK). Sugars were identified by comparison with an internal library of standards (Comont et al., 2012). Total proteins were extracted in McIlvaine buffer pH 7 containing 50 mM ascorbic acid and 0.2 ml of 20% lithium dodecyl sulphate and then analysed by the Lowry assay (Lowry et al., 1951). Absorbance was measured at 700 nm with a µQuant microtitre plate reader spectrophotometer (Bio-Tek Instruments, Winooski, VT, USA). Protein contents were determined against a bovine serum albumin calibration curve. Chlorophylls a+b and carotenoids were extracted in 80% acetone. Supernatants were diluted 1:15 in 80% acetone with absorbance measured at 470, 647, 664, and 750 nm using an Ultrospec 4000 UV/Vis spectrophotometer (GE Healthcare, Amersham, UK) (Lichtenthaler, 1987; Porra et al., 1989).

Myzus persicae settlement and dispersal in pepper crop under UV-A and UV-B radiation

The second and third questions to answer were whether the absence of UV radiation could directly reduce aphid settling and dispersal, and whether the presence of moderate UV-B at an early growth stage could indirectly enhance aphid resistance by deterring aphid choice for these plants. Experiments were performed in glasshouse facilities at the Institute of

Agricultural Sciences (Madrid, Spain) during springs 2014 and 2015. Glasshouse dimensions were 6.4 × 6 × 4.5 m and light transmission properties of outer surface were 50% PAR, 15% UV-A, and 10% UV-B. *Capsicum annuum* cv. California Wonder (Ramiro Arnedo) seeds were grown in 9-cm-diameter pots with a 1:1 mixture of vermiculite (Asfaltex, Barcelona, Spain) and soil substrate (Kekkilä Iberia, Quart de Poblet, Spain). Plants were watered 3× a week using 20-20-20 (N:P:K) Nutrichem fertilizer (Miller Chemical & Fertilizer, Hanover, PA, USA) at a dose of 0.25 g l^{-1}.

UV-A/UV-B treatments

We tested three treatments: control, +UV-B, and +UV-A/+UV-B. Treatment '+UV-A' was not included in this targeted design as this has already been covered by our previous research (Dáder et al., 2014). UV-A radiation was supplied by Philips TL-K 40W/10-R tubes and UV-B radiation by Philips TL 40W/12 RS tubes (Royal Philips Electronics, Amsterdam, The Netherlands). For the control treatment, UV-A tubes were wrapped with 0.2-mm-thick high-density polyethylene (HDPE) film (Solplast, Lorca, Spain) to cut off wavelengths <400 nm, and UV-B tubes with 0.1-mm-thick polyester film Autostat CT4 (MacDermid) to cut off wavelengths <320 nm. For '+UV-B' treatment, UV-A tubes were wrapped with HDPE film and UV-B tubes with 0.1-mm-thick cellulose diacetate film Ultraphan (Modulor) to cut off wavelengths <295 nm. For treatment '+UV-A/+UV-B', UV-A tubes did not have any additional film whereas UV-B tubes were wrapped with cellulose diacetate film. Filters were replaced after 40 h of use. Tubes were suspended at 1 m high above canopy. Instantaneous irradiance received by plants during the experiments was monitored with an Almemo 25904S radiometer (Ahlborn, Holzkirchen, Germany). A minimal presence of UV-A and UV-B (1%) came from the exterior, therefore the artificial sources provided the majority of UV radiation. This created a UV-deficient environment inside the glasshouse as most UV radiation was filtered by the roof (Table 1). A set of cages (1 × 1 × 1 m) covered with a fine cloth to allow ventilation and light transmission were used for insect release and plant growth. They were rotated to avoid positional effects. Two experiments were designed, exploring the direct effect of the three regimes on aphids and the indirect effect mediated by the peppers grown under the treatments before insect release.

Direct effect of UV-A/UV-B on aphid orientation

Sixteen 5-week-old plants grown in an insect-proof walk-in growth chamber – at L16(23 °C):D8(20 °C) and 60-80% r.h. – were placed in a 4 × 4 disposition inside each of the

three cages (control, +UV-B, and +UV-A/+UV-B). Two hundred alate aphids were released in black tubes at canopy level. Numbers of adults and nymphs on each plant were recorded after 2, 6, 24, or 48 h in separate experiments. Each period of evaluation was repeated 4× (four cages and 64 plant observations per treatment and time of evaluation). Lamps remained switched on continuously for evaluations at 2 and 6 h, and with a 16-h photoperiod for evaluations at 24 and 48 h (Table 1). Experiments were performed during a 4-week period. Climatic conditions during the experiments were (mean ± SE =) 24.6 ± 0.1 ºC and 53.3 ± 0.3% r.h.

Comment [J3]: I added this – is it correct?

Comment [BD4]: Yes.

Distribution patterns of alate aphids were studied with the 'Spatial Analysis by Distance IndicEs' (SADIE) methodology (Perry, 1995, 1998), where each plant was the spatial unit and the count was the mean number of alate aphids on each plant. The spatial pattern of a population is described by the index of aggregation, Ia, which by convention indicates an aggregated sample if Ia>1, a random sample if Ia = 1, and a regular sample if Ia<1 (Perry et al., 1999). SADIE also quantifies the degree to which each count contributes towards the overall degree of clustering of the entire population, providing the positive index of clustering in patches, Vi, and the negative index of clustering in gaps, Vj (Perry et al., 1999). By convention, values > +1.5 indicate patches, and values < –1.5 indicate a gap. Both clustering indices visually indicate the location and extent of clusters in the data so they can be plotted on a map with Surfer v.9.0 software (Golden Software, Golden, CO, USA).

Comment [J5]: Note that I turned the 'unequal' sign around – ok? (I presume you mean 'larger than'...)

Comment [BD6]: Yes.

Indirect effect of UV-A/UV-B exposure of peppers before insect introduction

Seedlings were grown in an insect-proof chamber at L16(23 °C):D8(20 °C) and 60-80% r.h. Three-week-old plants were transferred to cages under each of the three treatments (control, +UV-B, and +UV-A/+UV-B) for two more weeks, and they were exposed to the regimes 3 h a day (08:00-11:00 hours, GMT+1). Irradiance conditions during plant growth are summarized in Table 1. After this growth period, insect choice assays were performed using a set of three cages. Fifteen plants (five of each treatment) were placed alternatively in a 5 × 3 disposition inside each cage. Two hundred alate aphids were released in black tubes at canopy level inside each cage. Numbers of adults and nymphs on each plant were recorded after 2, 6, 24, or 48 h in separate experiments. Each period of evaluation was repeated 6× (six cages and 30 plant observations per treatment and time of evaluation). Choice experiments received standard glasshouse irradiance conditions (Table 1). Standard lamps remained switched on continuously for evaluations at 2 and 6 h, and with a 16-h photoperiod for evaluations at 24 and 48 h. Experiments were performed during a 7-week period. Climatic

conditions during the experiments were (mean ± SE =) 23.1 ± 0.1 ºC and 44.4 ± 0.4% r.h.

Comment [J7]: I added this – is it correct?

Comment [BD8]: Yes.

Statistical analysis

Count data were transformed with either $\sqrt{(x+0.5)}$, x^2, or ln(x+1) in order to decrease heteroscedasticity and achieve normal distribution. If data were expressed as a percentage, the angular transformation 2*(arcsin$\sqrt{x}$) was used. The parameters were analysed using SPSS v.21.0 software (IBM-SPSS Statistics, Armonk, NY, USA). Parametric procedures were used whenever variables followed a normal distribution, with one-way ANOVA followed by least significant difference (LSD) pairwise comparison tests ($\alpha = 0.05$). If data did not follow a normal distribution after transformations, a non-parametric Kruskal-Wallis H-test was applied ($\alpha = 0.05$).

Results

Long-term UV-B altered pepper leaf chemistry but did not affect aphid life history

Pepper height and leaf area were similar among all treatments but leaf chemistry was altered due to long-term UV-B exposure (Table 2). Total soluble carbohydrates were highest in +UV-B/+UV-B compared to the other treatments, total protein content was unaffected, and total phenolic content increased under supplemental UV-B radiation (Table 2). Peppers exposed to supplemental UV-B before aphid introduction and later moved to ambient (treatment +UV-B/control) had comparable levels to those of plants grown under ambient UV-B during the whole cycle (control/control). Pepper plants grown initially without supplemental UV-B and subsequently transferred to UV-B (control/+UV-B) showed similar levels to treatment +UV-B/+UV-B, with plants grown always under supplemental UV-B. Photosynthetic pigments were significantly higher if supplemental UV-B exposure had been applied before aphid introduction (+UV-B/+UV-B and +UV-B/control) compared to treatment Control/+UV-B, but no differences were found when compared to Control/Control (Table 2).

Comment [J9]: ... but not compared to the control/control treatment!?! Rephrase pls

Comment [BD10]: Done.

Aphid performance was not altered by supplemental UV-B exposure. Pre-reproductive period, effective fecundity, mean generation time, intrinsic rate of natural increase, and mean relative growth rate were not different among treatments (Table 3).

Comment [J11]: Where are the data?? You need to add a table with d, Md, Td, r_m, and RGR values for the various treatments (+ the statistical information, which can then be removed from the running text)

Comment [BD12]: We had not included aphid data in a table because growth parameters were non significant among treatments. However now you can find a new table with aphid data and statistics (Table 3) in this final version (page 22). The statistical information has been removed from the running text.

The absence of UV-A and UV-B directly reduced aphid settlement and dispersal

The settlement rate of alates on peppers was lowest under lack of UV radiation (control

treatment) after 24 and 48 h of release (24 h: $F_{2,9}$ = 6.585; 48 h: $F_{2,9}$ = 6.687, both P = 0.017; Figure 1A). After 24 and 48 h, plants had the fewest adults (24 h: H = 6.510, P = 0.039; 48 h: H = 11.289, P = 0.004; Figure 1B) and nymphs (24 h: H = 6.123, P = 0.047; 48 h: H = 11.302, P = 0.004; Figure 1C) under control treatment, and the most under treatment +UV-A/+UV-B. No differences among treatments were found after 2 or 6 h (Figure 1A-C).

The patterns of alates studied with SADIE indicated that aphids were randomly distributed in treatment +UV-A/+UV-B but they remained significantly aggregated in the absence of UV-A and UV-B (control) after 2-48 h (Figure 2). Two and 48 h after release aphids were significantly aggregated in treatment +UV-B (Figure 2).

Moderate UV-B at an early growth stage indirectly decreased aphid colonization of pepper seedlings

Compared to the Control treatment, alate settlement was slower in plants previously grown under +UV-B and +UV-A/+UV-B at 2, 24, and 48 h after release (2 h: H = 8.726, P = 0.013; 24 h: H = 8.972, P = 0.011; 48 h: H = 5.821, P = 0.050; Figure 1D). Fewer adults and nymphs were found on plants grown under the two treatments supplemented with UV-B at 2-48 h after release (adults, 6 h: H = 6.796, P = 0.033; 24 h: H = 9.963, P = 0.007; 48 h: H = 10.594, P = 0.005; nymphs, 2 h: H = 10.499, P = 0.005; 6 h: H = 19.499, P<0.001; 24 h: H = 2.221, P<0.001; 48 h: H =5.286, P<0.001; Figure 1E,F).

Discussion

We examined the performance, settlement, and dispersal of the key aphid species *M. persicae* on a pepper crop under different individual or mixed regimes of UV-A and UV-B radiation during a variety of periods ranging from hours to lifespan. To our knowledge, this is the first attempt to unravel the direct and plant-mediated roles of UV-A and UV-B at the same time on aphid behaviour in glasshouse conditions. As the glass of the facility absorbed a considerable amount of radiation, we cannot neglect the fact that the UV-A/UV-B ratio present in our control conditions did not necessarily represent the normal proportion existing in natural environments, but it constituted an ambient glasshouse treatment for our experiments. Exposure to supplemental UV-B was found to alter pepper leaf chemistry; however, aphid fitness remained similar. In agreement with typical changes reported in the past, supplemental UV-B radiation triggered the accumulation of phenolic compounds, soluble carbohydrates, and photosynthetic pigments in pepper (Smith et al., 2000; Izaguirre et

al., 2007; Mahdavian et al., 2008; González et al., 2009). Accumulation of phenolics and sugars was also found for pepper plants supplemented with UV-A (Dáder et al., 2014). In this study, plant height and leaf area remained unaltered under supplemental UV-B, as opposed to reduced growth under UV-A exposure found by Dáder et al. (2014).

Plant-induced defence to UV-B is believed to be partly similar to the responses induced by insects, in which jasmonic acid plays a key role (Mackerness, 2000). This defence has been associated with increased accumulation of secondary metabolites and anti-nutritive defensive proteins, which may reduce the palatability of plants to herbivores (Izaguirre et al., 2007; Demukra et al., 2010; Mewis et al., 2012). Nevertheless, in this work the addition of UV-B radiation did not result in differences in aphid performance. Our results corroborate previous research highlighting no effect on generalist species such as *M. persicae* under UV-B exposure (Kuhlmann & Müller, 2010; Rechner & Poehling, 2014). Other studies have found a variety of responses for *M. persicae* depending on the host, with enhanced performance under supplemental UV in pepper (Dáder et al., 2014), a neutral response in broccoli (Kuhlmann & Müller, 2010; Mewis et al., 2012), and diminished fitness under UV-deficient enclosures in lettuce (Paul et al., 2012). It is likely that the similar performance of aphids in our experiment may be correlated to the unaltered nutritional nitrogen value of tissue under UV-B exposure. Particularly, amino acids are the major nitrogen source for aphids and an essential nutritive component for *M. persicae* feeding (Dadd & Krieger, 1968; Weibull, 1987). Indeed, *M. persicae* development and fecundity were indirectly enhanced when they fed on peppers with a richer amino acid and protein composition under supplemental UV-A (Dáder et al., 2014).

Our second question focused on whether the absence of UV-A and UV-B radiation directly reduced aphid settlement and dispersal from a pepper crop. *Myzus persicae* appeared to settle and produce more nymphs when they were exposed to treatment +UV-A/+UV-B compared to control. Several aphid species have been reported to orient towards UV-rich environments, and our findings agree with previous studies on how diminished UV directly disrupts movement and dispersal of aphid populations, as well as host finding (Díaz & Fereres, 2007; Döring & Chittka, 2007; Legarrea et al., 2012a). The presence of UV radiation is known to increase aphid fitness, in agreement with our finding of more nymphs in +UV-A/+UV-B (Antignus et al., 1996; Chyzik et al., 2003; Kuhlmann & Müller, 2009; Paul et al., 2012; Legarrea et al., 2012b). Despite the short exposure to treatments, we cannot dismiss possible olfactory responses of aphids triggered by some alterations in the plants due to supplemental UV radiation (Döring, 2014).

According to the spatial distribution by SADIE, *M. persicae* remained aggregated near the borders of control cages. On the contrary, aphids randomly occupied the whole surface under supplemental UV-A and UV-B, similar to *Macrosiphum euphorbiae* (Thomas) in a lettuce crop (Legarrea et al., 2012a). A dual mechanism has been reported for the anti-insect activity of UV-deficient environments. First, the number of insects that invade the enclosed structure is lower due to more UV reflectance emitted by the sky or reflected from the photoselective materials that cover the greenhouses. Second, the light environment created inside alters the normal behaviour of insects, thus resulting in reduced flight activity, which could explain why aphids occupied significantly fewer plants and were aggregated on the control plants in our experimental conditions (Raviv & Antignus, 2004; Antignus, 2014). Aphids sought hosts and oriented better in an environment rich in UV-A and UV-B, had a poor colonization inside the UV-deficient cage, and an intermediate response in treatment +UV-B, so it is likely that both fractions are equally important in direct aphid flight activity.

To test the third hypothesis, pepper seedlings were exposed to the UV regimes at an early stage before studying *M. persicae* preference in choice experiments. It is known that UV radiation influences insects via plant-mediated changes (Vänninen et al., 2010; Johansen et al., 2011). Peppers grown under treatments +UV-B and +UV-A/+UV-B had shorter curled leaves. Peroxidases have been postulated to be responsible for UV-induced morphological changes by lowering indoleacetic acid, which results in reduced cell elongation (Jansen, 2002). In the choice experiments, aphids significantly settled in peppers previously grown in control conditions without supplemental UV. There were no differences between treatments +UV-A/+UV-B and +UV-B, so the presence of UV-B radiation may explain this lower pepper-mediated aphid predilection. Altogether, peppers grown without supplemented UV could have been more attractive targets for aphids, and this outcome might be likely mediated by chemical cues. Additionally, the effects of UV radiation on plant volatile organic compound profiles would help to understand the full impact on aphid behaviour.

Based on life-history and orientation analysis, the variability of responses could be partially due to the experimental light conditions received by the plants during each assay. UV effects are highly dependent on the host plant and insect studied, and may differ between UV-A and UV-B among species. Overall, our data suggest that suppression of UV-A and UV-B may directly reduce aphid colonization and dispersal; however, the application of moderate UV-B at an early growth stage provokes plant chemical responses that have an important indirect role in UV-mediated biotic interactions, such as reduced attack by phytophagous insects. The above may have important practical applications in the future if

understood as a combined means of control. Using UV-B tubes or bulbs to enhance insect resistance of seedlings inside nurseries could be the first step. These lights would be applied at early stages of growth. Later on during the crop season, suppression of UV light with photoselective filters would reduce insect entrance and herbivore attack inside commercial greenhouses. This feasible utilization would intend to obtain resistant plants and avoid insect settlement in two phases. Additional knowledge is needed to untangle the complete role of UV in plant-insect interactions, to achieve a deeper understanding of the direct and indirect effects, and to distinguish whether common or distinctive signalling pathways mediate responses to UV-A and UV-B.

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Figure captions

Figure 1 Mean (± SE) (A,D) settlement rate (%) of alate *Myzus persicae* per cage, (B,E) number of alate aphids per plant, and (C,F) number of nymphs per plant, under the three UV light regimes (+UV-A/+UV-B, +UV-B, and control) after 2, 6, 24, or 48 h of aphid release, in the 'direct' and 'indirect effect' choice experiments. Means (within a time period since aphid release and within a panel) with different letters are significantly different (LSD tests: P<0.05). The asterisk in panel F indicates a significant difference between control and treatments +UV-B and +UV-A/+UV-B, for every period since aphid release (LSD tests: P<0.05).

Figure 2 Classed post maps of the spatial distribution of mean numbers of alate *Myzus persicae* under the three UV light regimes (+UV-A/+UV-B, +UV-B, and control) after 2, 6, 24, or 48 h of aphid release. Dots indicate individual test plants. Dot size and filling represent classes of clustering indices: 0 to +0.99 or 0 to –0.99 (small filled dots; clustering below expectation), +1 to +1.49 or –1 to –1.49 (unfilled dots; clustering exceeds expectation slightly), >+1.5 or <1.5 (large filled dots; more than half as much as expected). Red lines enclosing patch clusters are contours of v = 1.5, blue lines are of v = –1.5. Black lines are zero-value contours, representing boundaries between patch and gap regions. The index of aggregation (Ia), the positive patch cluster index (vi), and the negative gap cluster index (vj) enclosed by a red line are statistically significant (LSD tests: P<0.05). The arrowhead indicates the cardinal north direction.

Comment [J13]: I added this – correct?

Comment [BD14]: Yes

Comment [J15]: I added this – correct?

Comment [BD16]: Not always, only for Figure 1A. If data did not follow a normal distribution after transformations, a non-parametric Kruskal-Wallis H-test was used, for example in 1B, C, D and E (page 10, lines 15-18, 33-34; page 11, lines 2-5).

Comment [J17]: I added this – correct?

Comment [BD18]: Not correct, in this case data did not follow a normal distribution after transformations, and we performed a non-parametric Kruskal-Wallis H-test (page 11, lines 3-5).

Comment [J19]: These are too difficult to see in the figure!! Better use drawn/dotted/dashed lines!

Comment [J20]: Fill out, please

Comment [BD21]: Done.

Table 1 Mean instantaneous irradiance (W m^{-2}) measured throughout the duration of the three types of experiments. For the life-history experiment, radiation was measured directly under the lamps at canopy level. For the orientation assays, radiation was measured inside the experimental cages, inside the glasshouse, and outdoors at canopy level

Experiment				PAR	UV-A	UV-B
Life history			+UV-B	71.950	1.427	1.463
			Control	72.840	1.407	0.078
Orientation	Direct	Cage for aphid release	+UV-A/+UV-B	48.140	1.660	0.115
			+UV-B	30.416	0.360	0.137
			Control	42.451	0.287	0.009
		Glasshouse		134.136	3.653	0.033
		Outdoors		403.282	37.403	0.767
	Indirect	Cage for plant growth	+UV-A/+UV-B	36.980	1.574	0.167
			+UV-B	37.199	0.298	0.114
			Control	39.825	0.364	0.006
		Cage for aphid release		38.512	0.746	0.006
		Glasshouse		143.107	4.342	0.067
		Outdoors		267.396	24.655	0.593

Table 2 Mean (± SE; n = 3) pepper leaf content and plant growth at two growth stages under the four UV-B regimes of the life-history experiment (+UV-B/+UV-B: plants with supplemental UV-B before and after introduction of *Myzus persicae*; control/+UV-B: plants without supplemental UV-B before aphid introduction and with supplemental UV-B after aphid introduction; +UV-B/control: plants with supplemental UV-B before aphid introduction and without supplemental UV-B after aphid introduction; control/control: plants without supplemental UV-B before and after aphid introduction). Means within a row followed by different letters are significantly different (ANOVA followed by LSD tests: $P<0.05$)

Plant age (days)	Parameter	+UV-B/+UV-B	Control/control	+UV-B/control	Control/+UV-B	F	d.f.	P
40 (eight true leaves)	Sugars (mg g^{-1} dry weight)	101.20 ± 6.46a	76.83 ± 1.78b	81.33 ± 1.81b	81.84 ± 7.23b	4.687	3,8	0.036
	Proteins (mg g^{-1} dry weight)	136.77 ± 4.40	121.73 ± 7.04	126.57 ± 2.79	145.74 ± 6.67	3.840	3,8	0.057
	Phenolics (µg g^{-1} dry weight)	15379 ± 460a	10898 ± 195b	10369 ± 67b	14309 ± 1595a	9.795	3,8	0.005
	Chlorophyll a+b (µg ml^{-1})	209.43 ± 3.17a	197.47 ± 8.79ab	208.74 ± 3.63a	185.80 ± 3.53b	4.440	3,8	0.041
	Carotenoids (µg ml^{-1})	40.37 ± 0.15a	38.11 ± 1.29ab	40.28 ± 0.24a	36.67 ± 0.81b	5.488	3,8	0.024
58 (14 true leaves)	Height (cm)	21.73 ± 1.43	24.27 ± 0.15	23.60 ± 0.91	23.87 ± 0.86	1.289	3,8	0.34
	Leaf area (cm^2)	588.90 ± 5.17	555.50 ± 8.90	601.57 ± 12.01	592.80 ± 25.40	1.911	3,8	0.21

Comment [J22]: Add df's to all rows, please

Comment [BD23]: Done.

Table 3 Mean (± SE) life parameters of *Myzus persicae* under the four UV-B regimes of the life-history experiment (+UV-B/+UV-B: plants with supplemental UV-B before and after introduction of *Myzus persicae*; control/+UV-B: plants without supplemental UV-B before aphid introduction and with supplemental UV-B after aphid introduction; +UV-B/control: plants with supplemental UV-B before aphid introduction and without supplemental UV-B after aphid introduction; control/control: plants without supplemental UV-B before and after aphid introduction). Means within a row followed by different letters are significantly different (ANOVA followed by LSD tests: $P<0.05$)

Comment [BD24]: New table with aphid data, according to comment in page 10.

Parameter	+UV-B/+UV-B	Control/control	+UV-B/control	Control/+UV-B	F	d.f.	P
d	9.10±0.10	9.55±0.21	9.10±0.10	9.36±0.20	1.714	3,38	0.180
Md	44.70±1.90	52.45±2.10	46.50±4.08	42.82±3.48	2.260	3,38	0.097
Td	12.33±0.14	12.93±0.28	12.33±0.14	12.69±0.28	1.714	3,38	0.180
r_m	0.31±0.00	0.31±0.01	0.31±0.01	0.29±0.01	0.923	3,38	0.439
RGR	0.36±0.01	0.36±0.01	0.36±0.01	0.34±0.01	0.923	3,38	0.439

Figure 1

Lettering in figures: do *not* use **BOLD** typeface, please

Lines in the six panels are too thick

Make all axes + tick marks black, not grey

Axis labels, panel A: ‘Alate settlement per cage (%)’; panel B: ‘No. alates per plant’; panel C: ‘No. nymphs per plant’

Panels A + D: skip all ‘%’ near the tick marks; panel titles ‘Direct’ and ‘Indirect’: use ‘Sentence style’ (that is, with initial capital only)

Panels C + F, horizontal axis labels: replace 2x ‘Hours’ with ‘Time since aphid release (h)’ <once, centred relative to the width of the whole figure>

Increase font size of numbers along the axes a bit

Make the x-axes linear (so for instance 2 and 6 should be closer together than 24 and 48)

Increase space between graph headers and content, i.e. “INDIRECT” is too close to the “a”

Increase horizontal space between plots a bit

Move labels “A”, “B” and “C” a bit away from the y-axis labels

Comment [BD25]: All suggestions have been considered. We have attached the updated figure as a separated TIFF file.

Figure 2

Lettering in figures: do *not* use **BOLD** typeface, please

The numbers along the axes are illegible!?! Better remove them

Insert a space before and after each '='

'Ia', 'Vi', 'Vj' 'Pa', 'Pvi', and 'Pvj': not italic

Replace '=0.000' with '<0.001' [NB: no space before/after '<' !]

Replace 'Hours' with 'Time since aphid release (h)' <once, centred relative to the width of the whole figure>

If you keep this as a colour figure: the costs for colour print are GBP 150. I think the figure would be clearer if you use different lines and symbols in grey and black.

Comment [BD26]: All suggestions have been considered. We have attached the updated figure as a separated TIFF file.

Comment [BD27]: We have decided to keep the figure as a colour figure.