Increasing Plant Defense Mechanisms against Herbivorous Insects by Tailored Narrow-Bandwidth Supplementary Light

Von der Naturwissenschaftlichen Fakultät der

Gottfried Wilhelm Leibniz Universität Hannover

zur Erlangung des Grades
Doktor der Gartenbauwissenschaften (Dr. rer. hort.)

genehmigte Dissertation
von
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2017

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Tag der Promotion: 11.08.2017

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Abbreviations

UV	Ultraviolet radiation (280 nm – 400 nm)
UV-A	Ultraviolet radiation (315 nm – 400 nm)
UV-B	Ultraviolet radiation (280 nm – 315 nm)
m, m²	Meters, square meters
cm, cm²	Centimeters, square centimeters
mm, mm²	Millimeters, square millimeters
µm	Micrometers
nm	Nanometers
var	Variety
i.e.	Latin: *id est* (that is)
et al.	Latin: *et alii* (and others)
vs.	Versus
LED	Light-emitting diode
°C	Degree Celsius
L:D	Photoperiod light to dark
cv	Cultivar
PP	Polypropylen
W	Watts
Lm	Lumen
K	Kelvin
PAR	Photosynthetic active radiation (400 nm – 700 nm)
PWM	Pulse duration modulation
µmol/m^{-2}/s^{-1}	Photon flux density in micromoles per square meter per second
W/m²	Irradiance in watts per square meter
µW/cm²	Irradiance in micro watts per square meter
kJ m^{-2} s^{-1}	Energy in kilo joule per square meter per second
UV/VIS	Ultraviolet/visible
rpm	Revolutions per minute
HPLC	High performance liquid chromatography
ml	Milliliter

µl	Microliters
min	Minute
h	Hour
%	Percentage
Psi	Pound-force per square inch
V	Voltage
L min^{-1}	Liters per minute
ml min^{-1}	Milliliters per minute
m/z	Mass to charge ratio
HPLC-DAD-ESI-MS^n	High-performance liquid chromatography-diode array detector/tandem electrospray ionization mass
HPLC-ESI–MS^2	High-performance liquid chromatography electrospray ionization mass
mg g^{-1} d.w.	Milligram per gram dry weight
µg g^{-1} d.w.	Microgram per gram dry weight
M, mM	Molar, Millimolar
v/v	Percent by volume
GLMM	Generalized linear mixed models
GLM	Generalized linear models
N	Number
Fig	Figure
Tab	Table
SE	Standard error
GS	Glucosinolate
DFG	Deutsche Forschungsgemeinschaft (German research foundation)

Abstract

Different light qualities and quantities can influence the secondary plant metabolism (flavonol glycosides and glucosinolates) and simultaneously alter the plant resistance against herbivorous insects. This study aimed at an approach to enhance plant resistance by increasing the concentrations of flavonol glycosides and glucosinolates in broccoli and Brussels sprout plants, through additional illumination with different light treatments (UV-B, UV-A, violet, blue, green, red) generated with light-emitting diodes (LEDs) or UV-B tubes in climate chamber or greenhouse conditions. Investigated were: plant growth, concentrations of flavonol glycosides and glucosinolates, flavonol absorbance and chlorophyll fluorescence (non-destructive using Dualex), and the associated influence on performance and behavior of generalized as well as specialized phloem-sucking and biting-chewing herbivorous insects. In the climate chamber-setup, specifically quercetin- and kaempferol glycosides remarkably increased under UV-B or violet light, while specific indole glucosinolates increased in plants exposed to UV-B or UV-A radiation depending on quality of the background light . The plant growth in broccoli was reduced under UV-B radiation. The performance of *Brevicoryne brassicae* was lower on UV-B treated plants, but *Myzus persicae* increased its performance on plants under UV-B and UV-A treatments. Green light tended to increase broccoli plant height. *Brevicoryne brassicae* performance was not influenced by longer light wavelengths. In host-choice tests, *B. brassicae* preferred blue-illuminated over control plants. The relative flavonol absorbance showed significantly increased values in broccoli plants treated with UV-B or violet light, while the chlorophyll fluorescence did not differ between the treatments. *Plutella xylostella* reacted with decreased pupa weight on UV-B-treated plants. *Pieris brassicae* showed decreased pupa weight if reared on plants treated with violet light compared to UV-B- and UV-A-treated plants. *Helicoverpa armigera* and *Spodoptera frugiperda* did not react on various light treatments. Furthermore, illumination of plants with four times lower light intensities generated with single LEDs could not influence the insects' performance. In the greenhouse condition with Brussels sprouts as the host plant, *B. brassicae* showed a significant reduction in performance in the UV-A treatment compared to the blue light treatment, which was accompanied by a significant increase in glucosinolate concentrations. The UV-B treatment of Brussels sprouts with LEDs was able to increase the flavonoid concentrations in the plant, but could not affect *B. brassicae* performance indirectly. The reactions were highly plant and insect species specific, and depended on compound classes and chemical structures.

Key words Plant Resistance, Light, Insects

Zusammenfassung

Lichtqualitäten können den sekundären Pflanzenmetabolismus beeinflussen und die Resistenz von Pflanzen gegen herbivore Insekten verändern. Diese Studie hat das Ziel, die Konzentrationen von Flavonolen und Glucosinolaten in Brokkoli und Rosenkohl durch zusätzliche Beleuchtung mit unterschiedlichen Lichtqualitäten (UV-B, UV-A, violett, blau, grün, rot) generiert mit LEDs oder UV-B Leuchtstoffröhren in der Klimakammer und im Gewächshaus zu erhöhen. Untersucht wurden das Pflanzenwachstum, die Konzentrationen von Flavonolglycosiden und Glucosinolaten (HPLC), die Flavonolabsorption und die Chlorophyllfluoreszenz (zerstörungsfrei mit Dualex) sowie die Performance und das Verhalten von spezialisierten und generalisierten phloem-saugenden und beißend-kauenden herbivoren Insekten. In der Klimakammer führte Zusatzbeleuchtung mit UV-B und violettem Licht zur Erhöhung spezifischer Quercetin- und Kaempferolglycoside in Brokkoli, während Indolglucosinolate eine Erhöhung unter UV-B oder UV-A Behandlung zeigten, welche von der Qualität der allgemeinen Hintergrundbeleuchtung abhing. Allerdings war das Wachstum von Brokkoli nach UV-B Behandlung reduziert. *Brevicoryne brassicae* entwickelte sich schlechter auf Brokkoli unter UV-B Behandlung, während *Myzus persicae* sich auf Pflanzen unter UV-B und UV-A Behandlung besser entwickelte. Grünes Licht erhöhte tendenziell die Pflanzenhöhe von Brokkoli. *Brevicoryne brassicae* wurde durch Wirtspflanzenbeleuchtung mit längeren photosynthetisch aktiven Wellenlängen in ihren Entwicklungsparametern nicht beeinflusst aber die Blattlaus präferierte blau-beleuchtete Pflanzen vor Kontrollpflanzen. Die Flavonolabsorption war in UV-B und violett bestrahlten Brokkolipflanzen signifikant erhöht, während sich die Chlorophyllfluoreszenz zwischen den Lichtbehandlungen nicht unterschied. *Plutella xylostella* zeigte reduzierte Puppengewichte auf Pflanzen nach UV-B Behandlung. *Pieris brassicae* erreichte auf violett beleuchteten Pflanzen im Vergleich zu UV-B und UV-A beleuchteten Pflanzen niedrigere Puppengewichte. *Helicoverpa armigera* und *Spodoptera frugiperda* zeigten keine Reaktion auf die unterschiedlichen Lichtqualitäten. Pflanzenbeleuchtung mit einzelnen LEDs und sehr niedrigen Intensitäten führte zu messbaren Reaktionen von Pflanzen aber nicht von Herbivoren. UV-A Beleuchtung im Gewächshaus schränkte auf Rosenkohl die Entwicklung von *Brevicoryne brassicae* ein und ging einher mit höheren Gehalten an Glucosinolaten im Vergleich zu blau-beleuchteten Pflanzen. Die Lichtreaktionen waren bei den Pflanzen und Insekten artspezifisch und die Reaktionen im Gehalt von Metaboliten hingen von der Stoffklasse und der chemischen Struktur ab.

Schlagworte Pflanzenresistenz, Licht, Insekten

General Introduction

General research target of the study

LED-illumination allows specific tailored narrow-band application of different light qualities and intensities for the cultivation of horticultural plants in climate chambers or greenhouses. This is a promising approach for optimal adaptation of light qualities, allowing specific control of light-triggered plant growth and development processes. It has been shown that short-wavelength light (UV) enhanced the production of secondary metabolites in plants and has strong potential to indirectly influence herbivorous insects in their development and behavior (Ahmad et al. 2016; Acharya et al. 2016; Ballare 2014). In addition to the UV triggered influences on herbivorous insects, it has recently been shown that the induction of salicylic acid associated pathogen and nematode resistance in soybean and tomato by illuminating plants with longer wavelength (such as red LED light) is possible (Dhakal et al. 2015; Yang et al. 2015). Hence, tailored LED illumination from short to long wavelengths offers in general a new opportunity in biotechnical plant protection. On the other hand, specific secondary plant metabolites could also increase the nutritional and health value of horticultural crops for humans' consumption (Schreiner et al. 2012). Most studies up to now investigated the influence of UV-radiation on the behavior or the development of herbivorous insects with UV-absorbing plastic films in field trials or with broad spectra UV-lamps in greenhouse or climate chamber set-ups (mixed UV-B and UV-A effect) (Ballare et al. 1996; Rechner & Poehling 2014; Gulidov & Poehling 2013; Caputo et al. 2006; Zavala et al. 2001). An approach for plant illumination with a clear separation between shorter wavelengths (like UV-B and UV-A) in comparison to longer wavelengths (like violet, blue and red) from the photosynthetic active radiation section in the electromagnetic spectrum is still missing. To our knowledge, up to now no studies have investigated the differences between the influence of narrow-bandwidth short-wavelengths (differentiated between UV-B and UV-A) and longer wavelengths (violet to red in the visible spectrum). These wavelengths should be investigated in addition to usual broad spectra light sources in climate chambers or greenhouses with reference to plant growth, metabolic composition and their associated influence on specialized and generalized herbivorous insects with different feeding strategies. Shorter wavelength light (UV) is known to modulate plant metabolism and associated interactions with herbivorous insects (Ballare

2014; Schreiner et al. 2012). How could the different light qualities be perceived by plants and further influence the metabolic composition and the associated interaction with herbivorous insects?

Light and photoreception in plants

Light is an electromagnetic radiation consisting of photons. The visible light (400 nm–780 nm) within the electromagnetic spectrum includes the photosynthetic active radiation (PAR) (400 nm–700 nm) and is bordered on the upper end with the far-red region (780 nm–1 mm) and on the lower end with UV-radiation (100 nm–400 nm). UV-radiation can take with its different wavelengths extremely variable influences on organisms and is separated into three different wavelength regions: UV-A: 315–400 nm, UV-B: 280–315 nm and UV-C: 100–280 nm (Diffey 2002). Light is one of the most essential environmental factors for photosynthesis and is responsible for growth, development and metabolic composition of plants (Ballare 2014). To implement growth and development in response to changes in ambient light conditions, plants have three sets of sensory photoreceptors which include: cryptochromes, phototropins and zeitlupe proteins: to detect the UV-A and blue region of the spectrum, the UV Resistance Locus 8 (UVR8) receptor protein: to perceive UV-B radiation, and phytochromes: to track the red and far-red region of the light spectrum. The diversity among these photoreceptors provides the capacity to detect a number of parameters of the light environment, including the presence, absence, wavelength, intensity, direction and duration of the ambient light signals, resulting in different responses of plants to these various light stimuli (Christie 2007; Christie et al. 2012; Heijde & Ulm 2012; Smith 2000; Quail 2002). Generally, different light qualities are perceived by specific protein-photoreceptors, and conformation alterations induced by absorption of specific photons in these receptors trigger an intracellular signal transduction process that culminates in altered expression of the genes which drive different growth and development, metabolic processes or other biological functions in plants. Especially the secondary plant metabolism could be influenced with different light qualities in terms of quality and quantity (Kendrick & Kronenberg 1994; Sancar 2003; Ballare 2014).

Secondary plant metabolites

Flavonoids

High light intensities and in particular high energy photons of short wavelengths (such as UV-

B) can directly destroy macromolecules (DNA-damage), enhance the production of reactive oxygen species and consequently alter the metabolic composition of a plant in different ways. For example, the induction of phenolic substances like hydroxycinnamic acids and flavonoids. These molecules serve multiple functions, like UV-B screening (they have absorbing properties in the UV-range and regulate photoprotection) and antioxidant regulation to defend plant cells against reactive oxygen species (Agati & Tattini 2010; Agati et al. 2013; Ballare 2014; Schreiner et al. 2012). Plants are able to biosynthesize various phenolic substances, which are not required in the primary processes of energy metabolism and development (such as growth and reproduction) but are very important for defending against biotic and abiotic stresses (Cheynier et al. 2013). Flavonoids are located in vacuoles, in the phloem-sap and even in the chloroplasts itself (Agati et al. 2013; Calatayud et al. 1994). They are grouped into three main classes: flavones, flavonols and anthocyans, which contain aromatic and *O*-heterocyclic rings and are often built with sugar as flavonoid glycosides (Harborne 1991; Harborne & Williams 2000; Kuhlmann & Müller 2011). These phenolic metabolites are synthesized from the aromatic amino acid phenylalanine. Key enzymes in the biosynthesis of flavonol glycosides are phenylalanine ammonium lyase, chalcone synthase and chalcone isomerase. These enzymes drive the phenylpropanoid pathway. The different classes and structures of flavonoids are biosynthesized from enzymes, which catalyze hydroxylation, methylation, glycosylation and acylation. Hydroxylation and methylation reactions synthesize various hydroxycinnamic acids. (Harborne 1991; Harborne & Williams 2000; Schreiner et al. 2012; Weißhaar & Jenkins 1998). Plants of the brassicaceae family contain structurally different non-acylated and acylated flavonol glycosides (kaempferol and quercetin glycosides) as well as hydroxycinnamic acid derivatives. These molecules could be induced with high PAR intensities and with UV-B radiation (Neugart et al. 2013; 2014). Flavonol glycosides have not only photoprotective properties but are also highly involved in plants defense mechanisms against certain herbivorous insects (Ballare 2014; Lattanzio et al. 2000; Calatayud et al. 1994). Flavonoids have feeding deterrent and toxic dietary effects on herbivorous insects (Ballare 2014; Lattanzio et al. 2000; Treutter 2005; Onkokesung et al. 2014). This repellent and development inhibitory effects of various specific flavonoids could be shown in several studies for different insect species with different degrees of specialization as well as different feeding strategies (phloem-sucking *vs.* leaf-biting-chewing herbivore) (Kuhlmann & Müller 2010; Onkokesung et al. 2014; Grant-Petersson & Renwick 1996; Zavala et al. 2001; Caputo et al. 2006). However,

contrasting results are reported in terms of defense efficacy as well as specialization. Some studies could show no negative influence of flavonoids on herbivorous insects or even better development and feeding stimulation, when insects were exposed to host plants or diet with higher concentrations of specific phenolic substances. These substances are able to work as plant defense boost but also quite opposite in the other direction as phagostimulants (Chacon-Fuentes et al. 2015; Caasi-Lit et al. 2007). Hence, no general prediction about the type of reaction across different plant-herbivore systems is possible yet.

Glucosinolates

Glucosinolates are another class of secondary plant metabolites and characteristic for the brassicaceae family. Over 200 compounds belonging to this class have been identified. Depending on the structure of the amino acid precursors, they are divided in aliphatic glucosinolates, indole glucosinolates, and aromatic glucosinolates (Agerbirk & Olsen 2012; Ishida et al. 2014). Structurally they contain an amino acid associated with a variable side chain. In contrary to the first described phenolic metabolites, glucosinolates are not involved in photoprotection nor have antioxidant characteristics (Björkman et al. 2011; Schreiner et al. 2012). These molecules are (like flavonoids) constitutively expressed in the plant (Textor & Gershenzon 2009). Nevertheless, the concentrations of these metabolites in plants could also be increased with UV-B radiation, as already shown for specific aliphatic and indole glucosinolates (Mewis et al. 2012a; Pedras & Zheng 2010). Attack by herbivorous insects destroys the plant cells exposing the glucosinolates to enzymes (such as myrosinases or thioglucosidases) and specific cofactor proteins resulting in toxic products like isothiocyanates, nitriles, epithioalkanes and thiocyanates (Ahuja et al. 2010; Bones & Rossiter 2006; Clay et al. 2009; Rask et al. 2000; Björkman et al. 2011). Moreover, the feeding activity (wounding) of herbivorous insects can lead to syntheses of glucosinolates in wounded cells but also systemically in other parts of the plant, increasing the total concentration of glucosinolates in the plant (Schreiner et al. 2012; van Dam et al. 2003; 2005). The glucosinolate–myrosinase system is presumably evolved to increase the plants defense capacity against certain herbivorous insects (such as generalist herbivores) that cannot handle the compounds or their toxic breakdown products (Arany et al. 2008; Björkman et al. 2011; Bones & Rossiter 1996; Rask et al. 2000). On the other hand, certain specialists have acquired the ability to handle these compounds and even use them as recognition pattern or nutritional

compounds as well as secondary defense compounds against predators or parasitoids. Hence, like the phenolic substances discussed above, glucosinolates could also have positive, negative or neutral effects from a plant defense point of view (Björkman et al. 2011; Redovnikovic et al. 2008; Renwick 2002).

Proteinase inhibitors

Another class of resistance responsible molecules in plants are proteinase inhibitors. Proteinase inhibitors are small molecules that can inhibit the function of proteases. Plants contain proteinase inhibitors that have the potential to bind proteolytic enzymes and inhibit the dietary protein digestion of mostly plant-biting-chewing insects (Joshi et al. 2014). Proteinase inhibitors can increase the resistance level of a plant against herbivorous insects. As true for flavonoids, also UV-B and wounding increased the proteinase inhibitor levels in tobacco and tomato, indicating jasmonic acid depending overlapping of UV-B and usual wound defense responses (Stratmann et al. 2000; Izaguirre et al. 2003; 2007).

Plant resistance and the interaction with biotic and abiotic stressors

Direct plant defense against herbivorous insects involves plant characteristics that affect insect preference finding expression in host plant choice and feeding behavior (antixenosis), or insect performance delineated by parameters such as developmental time, growth and fecundity (antibiosis) resulting in increased plant fitness. These plant features include morphological properties for physical defense such as thorns, spines, trichomes and epicuticular waxes. Furthermore, compounds for chemical defense like secondary metabolites (for example flavonoids and glucosinolates – please see above), digestibility reducing proteins, and antinutritive enzymes are involved in the plants resistance process (Ballare 2014; Gatehouse 2002; Karban & Baldwin 1997; Schoonhoven et al. 2005). All these characteristics could be expressed constitutively in the plant or induced and expressed only after exposure of the plant to biotic (for example attack by herbivorous insects) or abiotic stress factors (such as high radiation intensities or UV-B). The induction of resistance is often not restricted to the site of the stressor and could be systemically expressed in non-stressed healthy parts of the plant. Systemic translocation is based on internal plant displacement of mobile signal compounds, for instance jasmonic acid and its metabolites are main signal compounds mobile

in the phloem. Signals could be distributed over long distances, inducing the expression of defense genes in distal parts of the plant (Howe 2004; Smith et al. 2004; Strassner et al. 2002).

Priming of plants with a specific stressor (such as specific light signals or UV-B radiation) can activate the plants defense, for example with the induction of secondary plant metabolites, before herbivorous insects attack the plant. Plants with already triggered defense can fight strongly against various biotic and abiotic stresses, including herbivorous insects, resulting in benefits for the plants (Pastor et al. 2014). The epicuticular wax coverage of plants is the first barrier against phloem-sucking aphids, it could be altered with UV-B radiation and has potential to influence insects on their host plants (Kuhlmann & Müller 2010). Furthermore, UV-B radiation and the plant feeding activity of insects are able to induce the same gene expression in plants, so that priming with UV-B radiation against a subsequent attack by herbivorous insects becomes possible, and plants which already perceived UV-B radiation can show a stronger and faster defense reaction. This fact also often results in an increase in concentrations of the secondary plant metabolites (such as discussed above) which are able to increase the resistance of a plant against insect pests (Stratmann 2003; Caputo et al. 2006; Zavala et al. 2001; Kuhlmann & Müller 2010; Rechner & Poehling 2014).

The peculiarity of induced defense processes mainly depends on the specific feeding properties of different herbivores. Aphids with their piercing-sucking feeding activity in the phloem create minor tissue damage to plants compared to the leaf-biting-chewing lepidopteran larvae, and activate different phytohormone defense responses in plants. Leaf-biting-chewing or cellular-sucking insects (such as lepidopteran larvae or thrips) are mostly activating the jasmonic acid pathway, while phloem-sucking insects (such as aphids and whiteflies) usually activate gene expression of the salicylic acid pathway (Abe et al. 2008; Hopkins et al. 2009). Furthermore, aphids have been reported to induce the salicylic acid hormone pathway to repress the strong response of the jasmonic acid pathway for their benefit. This repression is a result of an antagonistic “crosstalk mechanism” between the different hormone pathways. Crosstalk reactions could in general not only be antagonistic but synergistic as well, and could lead to weaker or stronger plant defense answers against certain herbivorous insect species, also resulting in different metabolic profiles of infested plants (de Vos et al. 2007; Schweiger et al. 2014; Wasternack 2014; Schreiner et al. 2012). The illumination of plants with different light qualities can have various influences on the

morphological properties of plants and their transcriptome, metabolome, hormone composition and concentration, indirectly influencing the plants defense mechanisms and the performance of herbivorous insects feeding on these plants (Ballare 2014). The primary plant metabolism, the transcriptome, the thickness and the composition of the epicuticular wax layer were not investigated in this study; hence the focus is on the light-induced alterations in the concentrations of secondary plant metabolites.

Research objectives and general methodological approach

This work was carried out to investigate light-mediated effects of different colored high-power LED-panels in addition to different usual broad spectra illumination with fluorescence tubes as background light in a climate chamber (Fig. 1 a + b). Furthermore, UV-B tubes were installed and used for plant illumination (Fig. 2 a). Additionally, variable panels were provided with only one single high-power LED to evaluate effects of lower intensities on young plants in the climate chamber (Fig. 2 b + c).

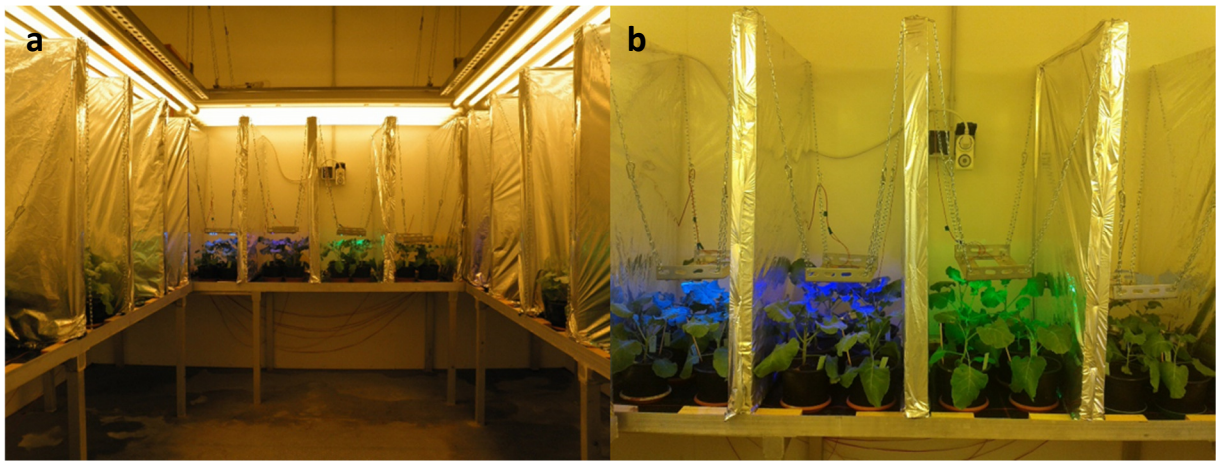

Fig. 1 Climate chamber equipped with broad spectra fluorescent tubes and separated in different independent light compartments (a). Light compartments equipped with high-power LED-panels with UV-A, violet, blue or green wavelengths for broccoli plant illumination (b).

Furthermore, the light-mediated effects of different LED-light treatments were also investigated in addition to sodium vapor lamps in a greenhouse set-up (Fig. 3). Evaluated in the experiments were: plant growth, quantitative contents of specific metabolites (flavonoids and glucosinolates) in host plants (HPLC) or the relative overall flavonol absorbance and chlorophyll fluorescence (Dualex), and the indirect effects of light treatments on the behavior

and performance of herbivorous insects with different degrees of specialization and different feeding strategies.

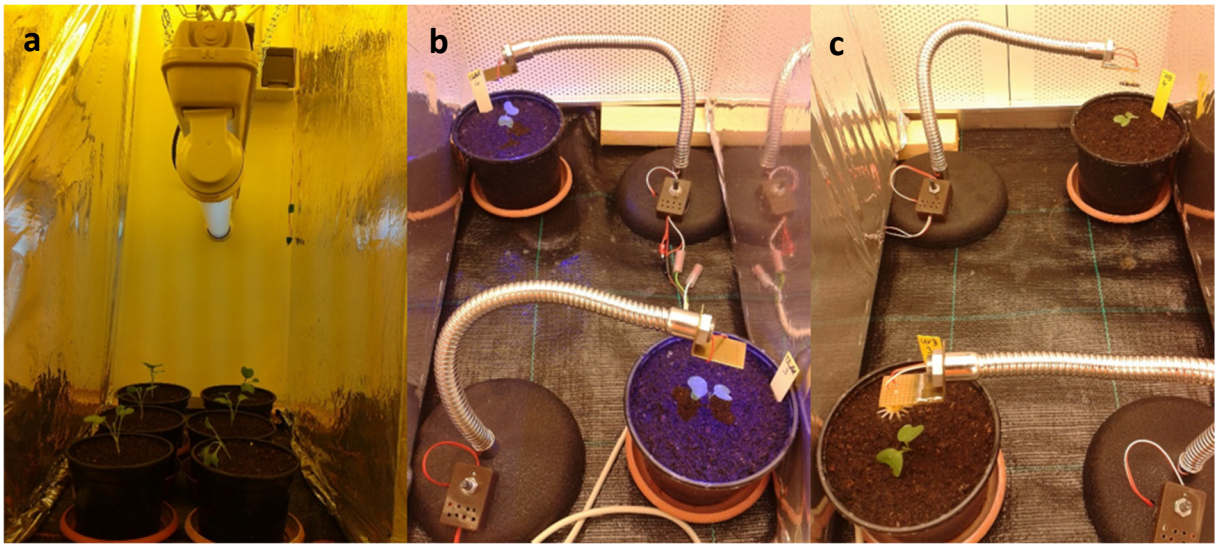

Fig. 2 Compartment in climate chamber with UV-B tube for plant treatments (a). Single high-power LEDs on small panels to directly illuminate young plants with lower light intensities of violet light (b), UV-B (c) or UV-A (*not shown*).

The aim of this research was to increase, by the use of tailored light treatments, specifically secondary plant metabolites that could enhance the plants defense capability and reduce or inhibit the population development of different herbivorous pest insects. Regarding species peculiarities (specialist *vs.* generalist), and most different feeding strategies (phloem-sucking *vs.* biting-chewing) were respected.

Fig. 3 Greenhouse chamber separated in different independent light compartments equipped with sodium vapor lamps and additional specific high-power LED-panels with UV-B, UV-A, blue, or red wavelengths for Brussels sprout plant illumination.

Hence, the impacts of eight different light qualities (UV-B 300 nm, UV-B 310 nm, UV-A 365 nm, UV-A 385 nm, violet 420 nm, blue 470 nm, green 515 nm, and red 660 nm) on two host plants, broccoli (*Brassica oleracea* var. *italica*, cv Monopoly) and Brussels sprouts (*Brassica oleracea* var. *gemmifera*) were explored. Concomitant to light treatments, 35 different secondary plant metabolites (flavonol glycosides and glucosinolates) from the plant tissue were quantitatively analyzed using HPLC. Furthermore, the interaction with different herbivores: (I) two phloem-sucking aphids - the specialized cabbage aphid *Brevicoryne brassicae* and the generalized green peach aphid *Myzus persicae* (both Hemiptera: Aphididae) and (II) four plant-biting-chewing lepidopteran species - two specialized, the cabbage butterfly *Pieris brassicae* (Lepidoptera: Pieridae) and the diamondback moth *Plutella xylostella* (Lepidoptera: Plutellidae) as well as - two generalized species, the cotton bollworm *Helicoverpa armigera* and the fall armyworm *Spodoptera frugiperda* (both Lepidoptera: Noctuidae) were investigated on various light-treated plants.

Research hypotheses of different chapters

In chapter one to four of this study, the emphasis was on the indirect, plant-mediated effects of various light qualities on herbivorous insects on broccoli as a host plant.

The hypotheses of chapter one were (I) different short wavelength light qualities (UV-B – violet) in addition to broad spectra light sources (fluorescence tubes with a high amount of red light) in the climate chamber have various potential to influence secondary plant metabolite concentrations (flavonol glycosides and glucosinolates) and plant growth in broccoli, (II) specialized and generalized aphids (*B. brassicae* and *M. persicae*) could be indirectly affected in their performance via light-induced plant-mediated alterations in broccoli, (III) the different aphid species will react differently in their performance on these plants with light-induced metabolic alterations, and (IV) the infestation with different aphid species of different specialization degrees can also influence the secondary plant metabolism.

In chapter two the broad spectra fluorescence tube light sources in the background contained more blue light and less red light as in chapter one. Chapter two hypothesized that (I) different longer wavelength light qualities (UV-A – green) in addition to broad spectra light sources (fluorescence tubes with a high amount of blue light) in the climate chamber can promote broccoli plant growth, (II) can alter the secondary plant metabolism (flavonol glycosides and

glucosinolates) in broccoli, and (III) different light-induced concentrations of secondary plant metabolites in broccoli can influence *B. brassicae* indirectly in (a) performance and (b) behavior (host-plant choice).

The hypotheses of chapter three were (I) different light qualities (UV-B – violet) in addition to broad spectra light sources (fluorescence tubes with a high amount of red light) in the climate chamber can influence different specialized lepidopteran pest species (the specialists *P. brassicae* and *P. xylostella* and the generalists *H. armigera* and *S. frugiperda*) in their performance on broccoli plants, (II) different light qualities (UV-B – violet) can also affect the relative overall flavonol absorbance and the relative chlorophyll fluorescence in broccoli associated with altered herbivore performance.

Chapter four focused on light intensities and hypothesized that lower intensities of short wavelength light generated with single LEDs in addition to broad spectra light sources (fluorescence tubes with a high amount of red light) in the climate chamber have less potential than high-intensity LED panels to influence (I) plant growths, (II) relative overall flavonol absorbance and relative chlorophyll fluorescence in broccoli and (III) the associated plant-mediated performance of different herbivorous insects (*B. brassicae*, *M. persicae*, *P. xylostella*).

In chapter five the focus was on the indirect, plant-mediated effects of different light qualities on *B. brassicae* on Brussels sprouts as a host plant in a greenhouse setup.

Chapter five aimed at investigating a different plant species and a different experimental approach in a greenhouse setup with sodium vapor lamps as background illumination and Brussels sprouts as a host plant for *B. brassicae*. The hypotheses of chapter five were (I) different narrow-bandwidths light qualities (UV-B – red) in addition to broad spectra light sources (sodium vapor lamps) in the greenhouse have the potential to influence Brussels sprouts plants growths, (II) can increase its secondary metabolite composition (glucosinolates and flavonol glycosides), and (III) can alter its plant defense potential against the cabbage aphid *B. brassicae*.

Chapter 1

Different Narrow-Band Light Ranges Alter Plant Secondary Metabolism and Plant Defense Response to Aphids

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Journal of Chemical Ecology (2016) 42:989–1003

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Abstract

Light of different wavelengths affects various physiological processes in plants. Short-wavelength radiation (like UV) can activate defense pathways in plants and enhance the biosynthesis of secondary metabolites (such as flavonoids and glucosinolates) responsible for resistance against certain herbivorous insects. The intensity of light-induced, metabolite-based resistance is plant- and insect species-specific and depends on herbivore feeding guild and specialization. In this study, broccoli (*Brassica oleracea* var. *italica*) plants were grown for 4 weeks in a climate chamber under conventional fluorescent tubes and were additionally treated with UV-B (310 nm), UV-A (365 or 385 nm), or violet (420 nm) light. The objective was to determine the influence of narrow bandwidths of light (from UV-B to violet) on plant secondary metabolism and on the performance of the cabbage aphid *Brevicoryne brassicae* (a specialist) and the green peach aphid *Myzus persicae* (a generalist). Among flavonol glycosides, specific quercetin- and kaempferol glycosides increased markedly under UV-B, while among glucosinolates only 4-methoxy-3-indolylmethyl showed a 2-fold increase in plants exposed to UV-B and UV-A. The concentration of 3-indolylmethyl glucosinolate in broccoli plants increased with UV-B treatment. *B. brassicae* adult weights and fecundity were lower on UV-B treated plants compared to UV-A or violet light-treated plants. Adult weights and fecundity of *M. persicae* were increased under UV-B and UV-A treatments. When specific light wavelengths are used to induce metabolic changes in plants, the specificity of the induced effects on herbivores should be considered.

Key Words

Resistance, *Brevicoryne brassicae*, *Myzus persicae*, UV, Glucosinolates, Flavonoids, *Brassica oleracea*

Introduction

Light is one of the most important factors regulating plant growth and development. The fine-tuning of plant responses to light depends on quality (wavelength) and quantity (intensity) (Ballare 2014). Plants perceive the energy of photons via specific chromophores linked to proteins; these receptor complexes are cryptochromes and phototropins for UV-A and blue light (Christie 2007; Demarsy & Fankhauser 2009). Various light qualities control different morphological or physiological processes in the plant, i.e., cryptochromes stimulated by blue light control plant growth and leaf size (Ballare 2014). Recently, the UVR8 receptor of ambient UV-B radiation was discovered (Christie et al. 2012; Heijde & Ulm 2012). UVR8 stimulation by ambient intensities of UV-B leads directly to the regulation of genes responsible for enzymes involved in the biosynthesis of secondary phenolic compounds, like hydroxycinnamic acids, kaempferol-, and quercetin-glycosides, that help protect plants from damage caused by UV-B (Mazza & Ballare 2015; Mewis et al. 2012a; Schreiner et al. 2012). Secondary plant metabolites, such as glucosinolates and in particular the phenolic substances, are assumed to also be strongly involved in plant defense against insect herbivores (Ballare 2014; Demkura & Ballare 2012).

The effects of changes in plant metabolism on herbivore performance depend on the plant species but also on the insect species and especially on the insect's degree of specialization on the food source and its feeding strategy. These differences can result in positive, negative, or neutral responses of herbivorous insects to light-induced changes in plant metabolism (Bidart-Bouzat & Kliebenstein 2011; Kuhlmann & Müller 2011; Mewis et al. 2012a; Schreiner et al. 2012).

In the case of artificial UV treatments, studies have not clearly separated UV-induced behavioral changes of herbivores (such as optical-guided host-plant discrimination) and plant-mediated antibiotic or repellent effects. Furthermore, the effects of different light qualities in the short wavelength range, including UV-B, UV-A, violet, and blue light, have not been distinguished. Gulidov & Poehling (2013) and Kuhlmann & Müller (2009b; 2010) showed that, during natural infestations, the cabbage aphid *Brevicoryne brassicae* (Linnaeus) (Hemiptera: Aphididae) preferred to colonize broccoli plants grown under high-UV conditions in open plastic-film greenhouses than under low-UV conditions, but the former authors also found reduced population growth on plants grown under high-UV conditions. High-UV conditions

also reduced insect performance when *B. brassicae* and *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae) and their host plants were kept under control *vs*. UV-supplemented conditions (generated by artificial UV sources) or when *B. brassicae* and its hosts were kept under UV-transmitting *vs.* UV-absorbing plastic films in the field (Caputo et al. 2006; Foggo et al. 2007; Rechner & Poehling 2014).

Varying results have been reported on plant-mediated effects of UV radiation on the green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), which is a generalist. Mewis et al. (2012a) found reduced fecundity of *M. persicae* on UV-B-exposed broccoli sprouts after 5 days of reproduction, while Paul et al. (2012) found increased numbers of aphids on *Lactuca sativa* (Linnaeus) (Asteraceae) plants grown under plastic-films with UV-B and UV-A transmitting properties. Dader et al. (2014) recently reported an increase in numbers of *M. persicae* on pepper and eggplant under artificial UV-A radiation in the greenhouse. In the same study, the population growth of the whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) decreased under UV-A conditions on both hosts although UV-A induced flavonoids in pepper but not in eggplant (Dader et al. 2014). Most studies have used broad-spectrum UV illumination that includes both UV-B and UV-A wavelengths (Kuhlmann & Müller 2009a, 2010; Rechner & Poehling 2014). To our knowledge, other light qualities including wavelengths characteristic of violet light have not been investigated for their potential to induce resistance in plants. LED illumination now makes it possible to produce narrow bandwidths of light and to thereby investigate plant-mediated effects on associated herbivores (Vänninen et al. 2010).

This study investigated the effects of narrow bandwidths of light (from UV-B 310 nm to violet light 420 nm) on broccoli and on broccoli infested with the cabbage aphid *B. brassicae* (a specialist on Brassicales) or the green peach aphid *M. persicae* (a generalist). The objectives were to determine how narrow bandwidths of light affected (I) the development and performance of the two aphids on broccoli plants via the nutritional quality of the host-plant, (II) the morphology of the broccoli plants, and (III) the contents of hydroxycinnamic acids, flavonol glycosides (kaempferol glycosides and quercetin glycosides), and glucosinolates in the broccoli plants.

Materials and Methods

Rearing of insects

Cabbage aphids (*Brevicoryne brassicae*) and a green phenotype ("green clone") of the green peach aphid (*Myzus persicae*) were collected outdoors from broccoli plants at the Leibniz University Hannover, Institute of Horticultural Production Systems, Section of Phytomedicine (N 52° 23`39.22``, E 9° 42`18.86``) in Hannover. The aphids were reared on 4-week-old broccoli plants in a gauze cage with a wooden frame (85 cm × 60 cm × 60 cm); the cages were kept in a climate chamber (20 ± 2°C, relative humidity 65 ± 10%, photoperiod 16:8 h L:D). Additional light treatments were only applied during the experiments and not during aphid rearing. Every week, half of the plants in each cage were replaced to continuously provide a high quality food source. For experiments, adult aphids were randomly collected from these cages.

Plant material and growth conditions

Broccoli plants [*B. oleracea* var. *italica*, cv Monopoly; F1 Hybrid; Syngenta Enkhuizen, Netherlands] were grown under specific light conditions (*see Experimental layout and light treatments*) from seeds in pots (12 cm diameter, 9 cm height, one seedling per pot) containing fertilized soil (Fruhstorfer Erde Type P, Hawita Gruppe, Vechta, Germany). Four-week-old broccoli plants were used for the experiments with aphids.

Experimental layout and light treatments

The experiments were conducted in a climate chamber (Viessmann, 4 m x 3 m x 2.40 m, Allendorf, Germany) with the following conditions: temperature 20 ± 2°C, relative humidity 70 ± 10%, and photoperiod 16:8 h L:D. The climate chamber contained five metal tables that were covered with black mulch film (PP-Gewebe, supplied by Raiffeisen GmbH, Bad Zwischenahn, Germany). On the tables in the chamber, 20 compartments (0.75 m x 0.3 m x 1 m) were separated by wooden frames covered with reflective mulch film (full metal on black film, supplied by Sunup Reflective Films/Star Metal Plating, Escondido, California USA) to avoid light interference from neighboring areas; the tops of the compartments were not closed or covered. The chamber was illuminated with 50 fluorescent tubes (Osram Lumilux Interna, L 58 W / T8, 827, 5200 lm, 2700 K, Munich, Germany), which were mounted 1 m above the tables to provide equal photon flux densities of photosynthetic active radiation (PAR). Additional light treatments with specific narrow-bandwidth wavelengths were generated with

narrowband UV-B tubes (Philips TL 20W/01 RS SLV UV-B Narrowband G13, Hamburg, Germany) or with hexagonal 1 W high-power single-chip LED emitters. The UV-B tubes were located 50 cm above the plants tables. For each LED illuminated compartment, two small aluminum plates (25 cm x 5 cm) were each equipped with three high-power LEDs. The aluminum plates were separated by 5 cm to ensure minimal shading for PAR radiation. The intensities of high-power LEDs were regulated with rotary potentiometers by high-power LED drivers (LED-Slave, PWM Dimmer Onboard, PCB Components, Hildesheim, Germany). The LED panels were located 12 cm above the plants. The peak wavelengths of the LEDs were UV-A 365 nm (H2A1-H365-E), UV-A 385 nm (H2A1-H385), and violet 420 nm (H2A1-H420). The LEDs were supplied by Roithner Laser Technik GmbH, Vienna, Austria. The violet 420 nm LEDs emitted no UV-B and UV-A radiation, only violet light with an intensity of 50 ± 5 µmol m^{-2} s^{-1}. The peak wavelength of the UV-B tubes was 310 nm, and there was also a small peak in the UV-A range at 385 nm (Fig. 1). The radiation spectra of the LEDs and the light tubes were measured with a UV/VIS fiber and a compatible fiber optic spectrometer (AvaSpec 2048-2, supplied by AVANTES, Appeldoorn, The Netherlands) (Fig. 1). PAR was measured with a Licor lightmeter LI-250-A (Lincoln, Nebraska USA) and was adjusted to 100 ± 10 µmol m^{-2} s^{-1} in all treatments (Table 1). The UV-B and UV-A intensities were measured in W/m² and µW/cm², respectively, with a spectra radiometer ALMEMO 2390-5 (Ahlborn Mess- und Regelungstechnik GmbH, Holzkirchen, Germany); so that they were comparable among all treatments, intensities were converted to photon flux density (µmol m^{-2} s^{-1}) based on the spectrum, Planck's constant, and Avogadro's number. For UV-B and UV-A treatments, the energy of the light sources (kJ m^{-2} s^{-1}) was also determined so that the results could be compared with those of other studies (Table 1). Each light compartment contained six broccoli plants, and each light treatment was represented by four replicate light compartments. Thus, the climate chamber contained 120 plants (5 treatments including the control x 4 replicate compartments per treatment x 6 plants per compartment).

Tab. 1 Light intensities provided by the four light treatments with UV-B tubes, LEDs and the control. The background and control illumination was provided by Osram 827 fluorescent tubes

Light treatment	**PAR Osram 827 (400 – 700 nm) [µmol m^{-2} s^{-1}]**	**UV-A (315 – 380 nm) [µmol m^{-2} s^{-1}]**	**UV-A (315 – 380 nm) [kJ m^{-2} d^{-1}]**	**UV-B (290 – 315 nm) [µmol m^{-2} s^{-1}]**	**UV-B (290 – 315 nm) [kJ m^{-2} d^{-1}]**
Control	100 ± 10	0	0	0	0
UV-B 310 nm	100 ± 10	4.3 ± 3	80	**0.13 ± 0.01**	**2.9**
UV-A 365 nm	100 ± 10	**61 ± 3**	**1152**	0.02 ± 0.01	0.03
UV-A 385 nm	100 ± 10	**15 ± 3**	**288**	0.02 ± 0.01	0.03
Violet 420 nm	100 ± 10	0	0	0	0

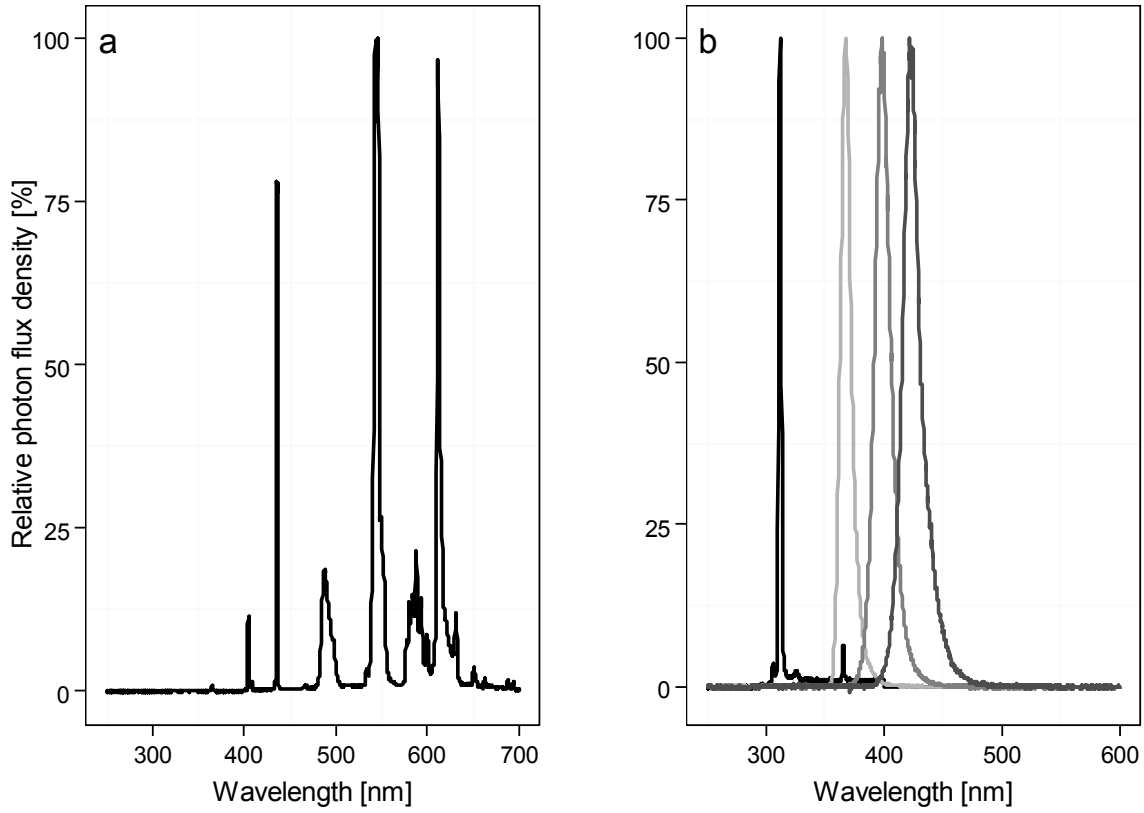

Fig. 1 Wavelength [nm] spectra with the corresponding photon flux density [%] for (a) Osram 827 fluorescent tubes and for (b) UV-B tubes (Philips TL 20W/01 RS SLV UV-B Narrowband G13) or hexagonal 1 W high-power single-chip LED emitters (H2A1-H365-E, H2A1-H385, and H2A1-H420) used in the climate chamber. The spectra were measured from 200 to 800 nm in the dark with a UV/VIS fiber and a compatible fiber optic spectrometer

Developmental parameters of *B. brassicae* and *M. persicae*

For determination of aphid performance, 12 adults of *B. brassicae* or *M. persicae* were carefully placed with a brush on the underside of the second leaf of two of the six plants per light compartment. The plants were infested after 4 weeks of light treatments and the light treatment continued after infestation. To exclude the direct effects of UV radiation on the aphids, the leaf with aphids was enclosed in a 3-cm-diameter clip cage that was impermeable to UV-B and UV-A. The adult aphids were allowed to deposit larvae for 24 h before they were removed. Twelve larvae were permitted to develop per plant (per clip cage). The number of days required for the larvae to develop into adults was recorded (developmental time), and the final weights of 10 adults per clip cage were measured with a microbalance (Type MC 5 Sartorius, Goettingen, Germany). Two adults per plant were kept separately in clip cages on the same plants (second and third leaf), and their offspring were counted every second day to measure fecundity [Md].

Effects of light treatments on plant leaf number, height, and weight without aphid infestation

To determine how the five light treatments affected plant morphology, additional plants were grown without aphids for 4 weeks in the climate chamber. A total of sixteen 4-week-old broccoli plants for each light treatment (four from each replicate) were randomly selected and destructively sampled for biomass analysis. Leaves were counted, and plant height was measured from the main stem base to the top of the plants after 4 weeks of growth. After the harvested plants were kept at 65 °C for 5 days, their dry weights were determined with an electronic balance (Type BP 3100 P, Sartorius, Goettingen, Germany).

Sample preparation

For each light treatment, leaves were collected from eight 6-week-old broccoli plants infested with *B. brassicae* or *M. persicae*, i.e., plants with two infested leaves in a clip cage as described earlier or without aphid infestation from separate plants. A mixed sample of all leaves per plant (excluding stems and midribs) was placed in liquid nitrogen, freeze-dried for 5 days (using a Christ Alpha 1-4 LSC freeze drier), and subsequently ground to a powder (≤ 0.25 mm).

Flavonoid analysis

Flavonoids were analyzed according to Schmidt et al. (2010) with modification. Lyophilized broccoli sprout tissue (0.02 g) was extracted with 600 µl of 60% aqueous methanol on a magnetic stirrer plate for 40 min at 20°C. The extract was centrifuged at 4500 rpm for 10 min at the same temperature, and the supernatant was collected in a reaction tube. This process was repeated twice with 300 µl of 60% aqueous methanol for 20 min and 10 min, respectively; the three supernatants per sample were combined. The extract was subsequently evaporated until it was dry and was then suspended in 200 µl of 10% aqueous methanol. The extract was centrifuged at 3000 rpm for 5 min at 20°C through a Corning® Costar® Spin-X® plastic centrifuge tube filter (Sigma Aldrich Chemical Co., St. Louis, MO, USA) for the HPLC analysis. Each extraction was carried out in duplicate.

Flavonoid composition (including hydroxycinnamic acid derivatives and glycosides of flavonols) and concentrations were determined using a series 1100 HPLC (Agilent Technologies, Waldbronn, Germany) equipped with a degaser, binary pump, autosampler, column oven, and photodiode array detector. An Ascentis® Express F5 column (150 mm × 4.6 mm, 5 µm, Supelco) was used to separate the compounds at a temperature of 25°C. Eluent A was 0.5% acetic acid, and eluent B was 100% acetonitrile. The gradient used for eluent B was 5-12% (0-3 min), 12-25% (3-46 min), 25-90% (46-49.5 min), 90% isocratic (49.5-52 min), 90-5% (52-52.7 min), and 5% isocratic (52.7-59 min). The determination was conducted at a flow rate of 0.85 ml min^{-1} and a wavelength of 320 nm, 330 nm, and 370 nm for hydroxycinnamic acid derivates, acylated flavonol glycosides, and non-acylated flavonol glycosides, respectively. The hydroxycinnamic acid derivatives and glycosides of flavonols were identified as deprotonated molecular ions and characteristic mass fragment ions according to Schmidt et al. (2010) by HPLC-DAD-ESI-MS^n using an Agilent series 1100 ion trap mass spectrometer in negative ionisation mode. Nitrogen was used as the dry gas (10 L min^{-1}, 325°C) and the nebulizer gas (40 psi) with a capillary voltage of -3500 V. Helium was used as the collision gas in the ion trap. The mass optimization for the ion optics of the mass spectrometer for quercetin was performed at *m/z* 301 or arbitrarily at *m/z* 1000. The MS^n experiments were performed in auto up to HPLC-DAD-ESI-MS^3 in a scan from *m/z* 200-2000. Standards (chlorogenic acid, quercertin 3-glucoside, and kaempferol 3-glucoside; Roth, Karlsruhe, Germany) were used for external calibration curves. Results are presented as mg g^{-1} dry weight. Flavonol glycoside

concentration was determined for four light-compartments as replicates per treatment containing two broccoli plants, resulting in eight plants per treatment in total; each replicate sample was measured in duplicate.

Glucosinolate Analysis

Glucosinolate concentration was determined as desulfo-glucosinolates using a modified method according to Wiesner et al. (2013). A 20.0 mg quantity of powdered sample plus 100 µl of 0.1 mM 2-propenyl glucosinolate (BCR-367R, Community Bureau of Reference, Brussels, Belgium) as the internal standard was extracted with 750 µl of 70% (v/v) methanol at 70°C. The preparation was boiled for 10 min and then centrifuged (2250 g) for 5 min at room temperature. The supernatant was decanted, and the residue was re-extracted twice with 500 µl of hot 70% methanol each time. The pooled extracts were loaded onto a mini column containing 500 µl of DEAD-Sephadex A-25 ion-exchanger (acetic acid-activated, Sigma–Aldrich Chemie GmbH, Taufkirchen, Germany) that had been conditioned with 2 M acetic acid and washed with 6 M imidazole formate. After loading, the column was washed with 0.02 M sodium acetate buffer. Finally, 75 µl of an aryl sulfatase solution (Sigma-Aldrich, Steinheim, Germany) was added, and the preparation was incubated overnight. Desulfo-glucosinolates were eluted with water and analyzed by HPLC using a Merck HPLC system (Merck-Hitachi, Darmstadt, Germany) with a Spherisorb ODS2 column (Bischoff, Leonberg Germany; particle size 5 µm, 250 mm x 4 mm). HPLC conditions were as follows: solvent A, MilliQ water; solvent B, 20% v/v acetonitrile in MilliQ water; solvent C, 100% acetonitrile. The 60-min run consisted of 1% (v/v) B (0-2 min), 1% to 20% (v/v) B (2-36 min), 20% (v/v) B (36-41 min), 20% B to 100% (v/v) C (41-43 min), 100% (v/v) C (43-48 min), 100% (v/v) C to 1% (v/v) B (48-50 min), and finally a 10-min hold at 1% (v/v) B (50-60 min). The remaining percentage was applied by solvent A. Determination was conducted at a flow rate of 0.7 ml min^{-1} and a wavelength of 229 nm. Desulfo-glucosinolates were identified based on comparison of retention times and UV absorption spectra with those of known standards. Additionally, desulfo-glucosinolates were previously identified in other *Brassica* species by HPLC-ESI–MS^2 using Agilent 1100 series (Agilent Technologies, Waldbronn, Germany) in positive ionization mode (Krumbein et al. 2005; Zimmermann et al. 2007). Glucosinolate concentration was calculated using 2-propenyl glucosinolate as an internal standard and using the response factor of each individual glucosinolate was calculated relative to 2-propenyl glucosinolate (Brown et al. 2003). Results

are presented as mg g^{-1} dry weight. Glucosinolate concentration was determined in four light-compartments as replicates per treatment containing two broccoli plants, resulting in eight plants per treatment in total; each replicate sample was measured in duplicate.

Statistical analysis

The data were analyzed in R 2.15.2 (R Development Core Team 2008). Graphs were made with the package ggplot2 (Wickham 2009). The effects of the light treatments on plant height, plant dry weight, and aphid adult weight were analyzed using generalized linear mixed models (GLMM) followed by Tukey *post hoc* tests (Pinheiro et al. 2012). The light treatments and aphid infestations were assigned as fixed factors and the light compartments in the climate chamber and the plants as random factors. The effects of aphid infestation and light treatments on plant secondary metabolites (concentrations of flavonoids and glucosinolates) were also analyzed using GLMM and the package lsmeans by estimating least-squares means and differences of contrast. Differences between single light treatments and the control were subsequently determined with a Tukey *post hoc* test. Effects of light treatments were averaged over the levels of infestation, and effects of infestation were averaged over the levels of variant. The total numbers of offspring and the developmental time of aphids as well as the leaf number per plant were analyzed by generalized linear models (GLM) using a log-link together with a quasi-Poisson distribution. The effects of light treatments on the fecundity, the developmental time, and the leaf number were assessed by Tukey *post hoc* tests.

Results

Leaf number, plant height and weight (without aphid infestation)

Leaf number per broccoli plant without aphid infestation was not significantly affected by the light treatments (Fig. 2a). Plant height was lower ($P < 0.01$) for plants treated with UV-B 310 nm than for control plants or plants treated with UV-A 365 nm or UV-A 385 nm (Fig. 2b). Dry weight was lower for plants treated with UV-B 310 nm than for control plants ($P < 0.01$) (Fig. 2c).

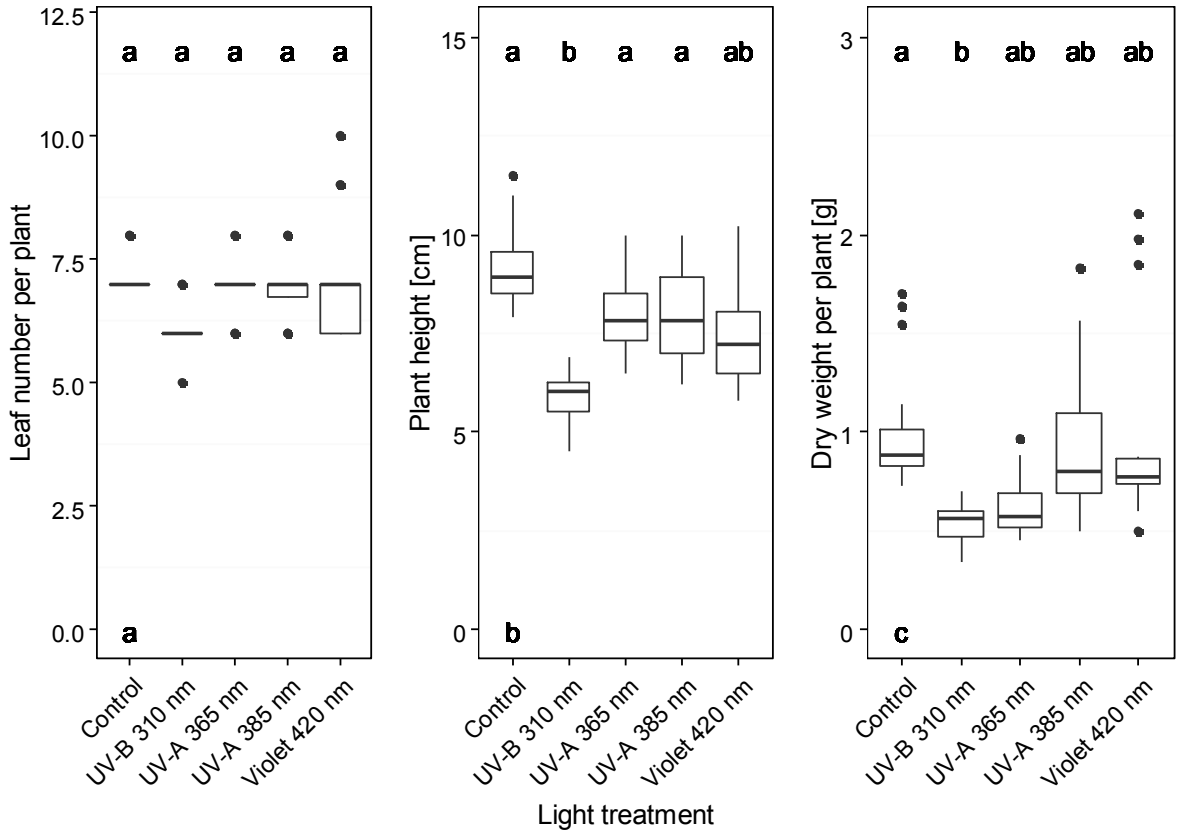

Fig. 2 Leaf number (a), plant height (b), and plant dry weight (c) of 4-week-old broccoli plants grown in a climate chamber and exposed to control lighting with or without one of four light treatments (UV-B 310 nm, UV-A 365 nm, UV-A 385 nm, or violet 420 nm). UV-A 365 nm, UV-A 385 nm, and violet 420 nm were supplied by LEDs. UV-B 310 nm was supplied by UV-B light tubes. Control lighting was supplied by standard fluorescent tubes (400 to 700 nm). Different letters indicate significant differences (GLM (Fig. 2a), GLMM (Fig. 2b, 2c), and Tukey *post hoc* tests at $P < 0.05$; $N = 16$ biological replicates)

Developmental parameters of *Brevicoryne brassicae*

Adult weight of *B. brassicae*

The weight per adult was significantly lower for insects grown on UV-B 310 nm-treated plants compared to those on UV-A 385 nm- or violet 420 nm-treated plants ($P < 0.001$ and < 0.02, respectively). Adult weights on UV-A 365 nm-treated and control plants did not differ and also did not differ on these two treatments *vs.* the other treatments (Fig. 3a).

Fecundity of *B. brassicae*

The fecundity was significantly lower for insects grown on UV-B 310 nm-treated plants compared to those on control plants ($P < 0.01$). *B. brassicae* fecundity was not significantly affected by growth on the UV-B 310 nm-treated plants and the UV-A 365 nm-, UV-A 385 nm-, or violet 420 nm-treated plants (Fig. 3b).

Developmental time of *B. brassicae*

The developmental time did not differ among the treatments (Fig. 3c).

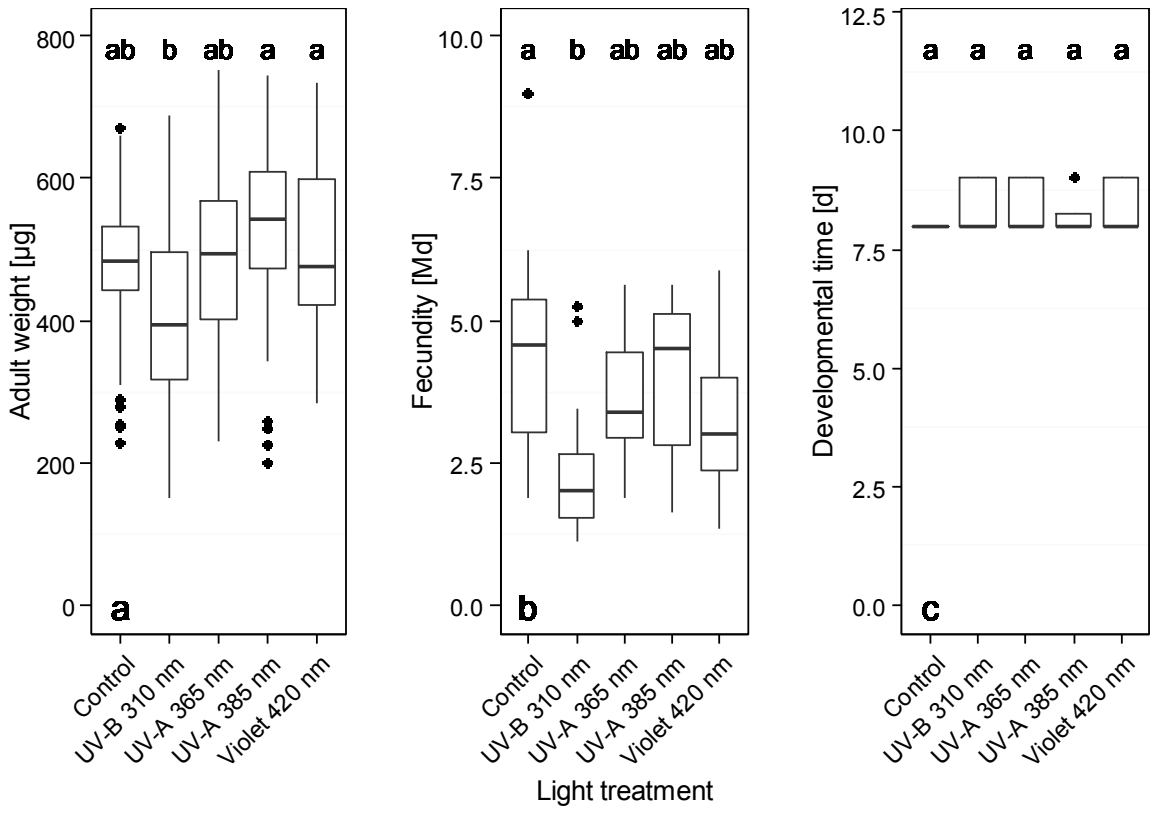

Fig. 3 Adult weight (a), fecundity (b), and developmental time (c) of *Brevicoryne brassicae* kept on broccoli plants that were grown under control lighting with or without one of four light treatments (UV-B 310 nm, UV-A 365 nm, UV-A 385 nm, or violet 420 nm). UV-A 365 nm, UV-A 385 nm, and violet 420 nm were supplied by LEDs. UV-B 310 nm was supplied by UV-B light tubes. Control lighting was supplied by standard fluorescent tubes (400 to 700 nm). The aphids were in clip cages and were not directly exposed to the light treatments. Different letters indicate significant differences (GLMM (Fig. 3a), GLM (Fig. 3b, 3c), and Tukey *post hoc* tests, $P < 0.05$; $N = 8$ biological replicates)

Developmental parameters of *Myzus persicae*

Adult weight of *M. persicae*

The weight per adult was significantly higher for insects grown on UV-B 310 nm- or UV-A 365 nm-treated plants compared to those on control plants ($P < 0.001$ and < 0.05, respectively). The weight did not significantly differ between insects grown on UV-A 385 nm-treated plants *vs.* all other treatments. The weight was significantly less for insects grown on violet 420 nm-treated plants compared to those on UV-B 310 nm- or UV-A 365 nm-treated plants ($P < 0.01$ and < 0.002, respectively) (Fig. 4a).

Fecundity of *M. persicae*

The fecundity was significantly higher for insects grown on UV-B 310 nm- or UV-A 365 nm-treated plants compared to those on control plants or on violet 420 nm-treated plants ($P < 0.001$ for all comparisons). The fecundity was significantly lower for insects on UV-A 385 nm-treated plants compared to those on UV-B 310 nm- or UV-A 365 nm-treated plants ($P < 0.01$ and < 0.05, respectively) (Fig. 4b).

Developmental time of *M. persicae*

The developmental time did not differ among the treatments (Fig. 4c).

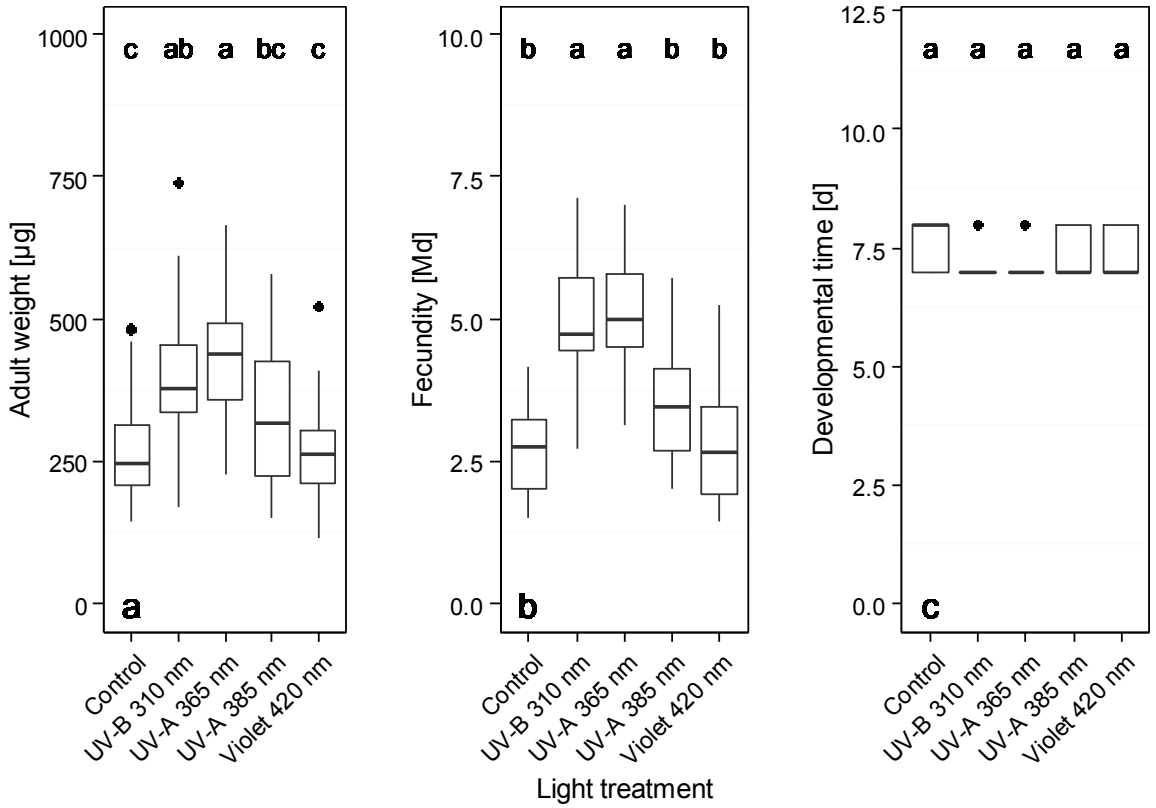

Fig. 4 Adult weight (a), fecundity (b), and developmental time (c) of *Myzus persicae* kept on broccoli plants that were grown in a climate chamber and exposed to control lighting with or without one of four light treatments (UV-B 310 nm, UV-A 365 nm, UV-A 385 nm, and violet 420 nm). UV-A 365 nm, UV-A 385 nm, and violet 420 nm were supplied by LEDs. UV-B 310 nm was supplied by UV-B light tubes. Control lighting was supplied by standard fluorescent tubes (400 to 700 nm). The aphids were in clip cages and were not directly exposed to the light treatments. Different letters indicate significant differences (GLMM (Fig. 4a), GLM (Fig. 4b, 4c), and Tukey *post hoc* tests, $P < 0.05$; $N = 8$ biological replicates)

Secondary plant metabolites

Hydroxycinnamic acids

The following 12 hydroxycinnamic acid derivatives were detected in broccoli plants: caffeoyl quinic acid (3-clorogenic acid), caffeoyl-glucoside, sinapoyl-gentiobiose, feruloyl-glucoside, sinapoyl-glucoside, sinapoyl-feruloyl-triglucoside, sinapoyl-feruloyl-gentiobiose (isomer), disinapoyl-gentiobiose, sinapoyl-feruloyl-gentiobiose, diferuloyl-gentiobiose, trisinapoyl-gentiobiose, and disinapoyl-feruloyl-gentiobiose. Among these, the six considered most relevant to the study are listed in Fig. 5. Sinapoyl-gentiobiose and sinapoyl-glucoside were found only in aphid-infested plants and not in non-infested control plants. Plants infested with

B. brassicae generally contained reduced levels of the monosinaopyl compound sinapoyl-feruloyl-gentiobiose but increased levels of the polysinapoyl compounds disinapoyl-ferululoyl-gentiobiose and trisinapoyl-gentiobiose. Plants infested with *M. persicae* contained increased levels of all sinapic acid-containing hydroxycinnamic acids. The concentration of hydroxycinnamic acids was lowest when broccoli plants were exposed to UV-A light of 365 nm independent of aphid infestation. Interestingly, the concentration of hydroxycinnamic acids in non-infested plants was highest with the UV-A 385 nm treatment, and the concentration in infested plants was highest with the violet treatment. Furthermore, quantities of the monosinapoyl compounds sinapoyl-feruloyl-gentiobiose and sinapoyl-feruloyl-triglucoside did not differ between the control and the UV-A 385 nm treatment in non-infested plants and did not differ between the control and the violet treatment in infested plants. Concentrations of the polysinapoyl compounds disinapoyl-gentiobiose, disinapoyl-feruloyl-gentiobiose, and trisinapoyl-gentiobiose did not differ among the control, UV-A 385 nm treatment, and violet treatment regardless of aphid infestation (Fig. 5).

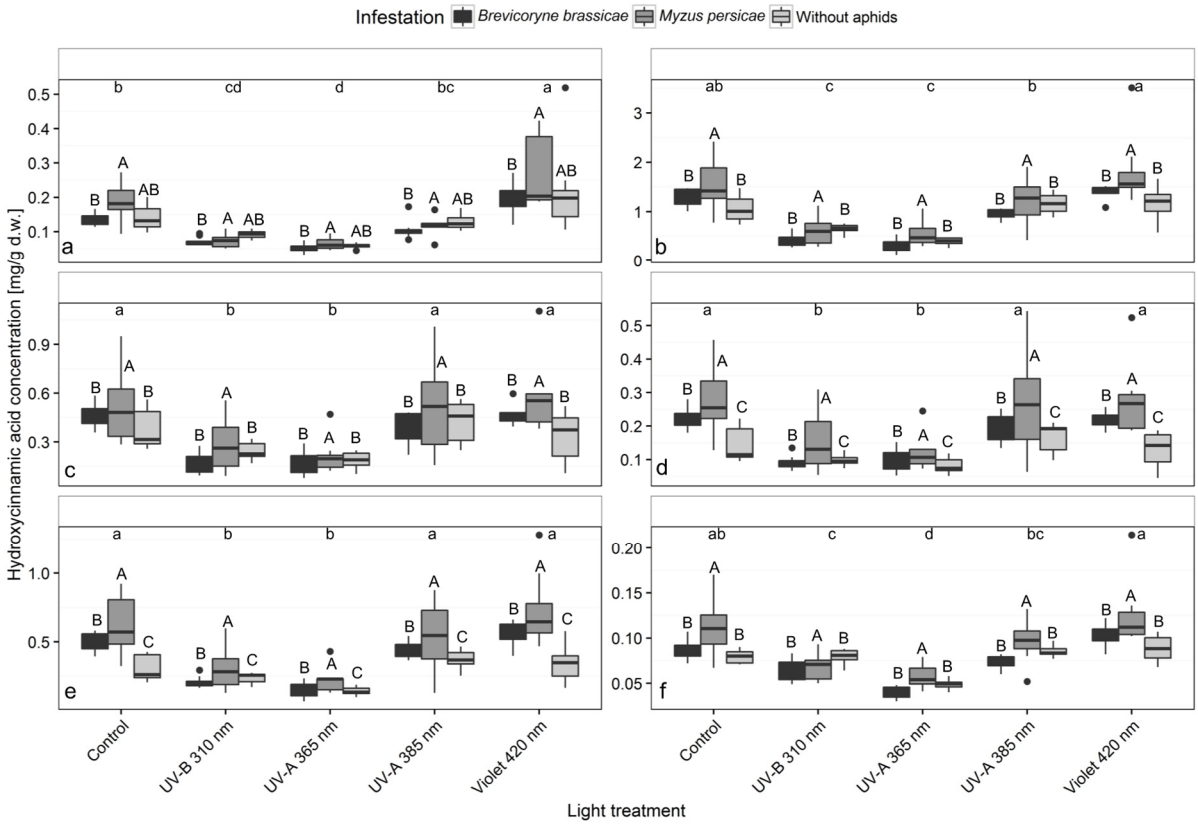

Fig. 5 Concentrations of hydroxycinnamic acids, diferuloyl-gentiobiose (a), sinapoyl-feruloyl-gentiobiose (b), disinapoyl-gentiobiose (c), trisinapoyl-gentiobiose (d), disinapoyl-feruloyl-gentiobiose (e), and sinapoyl-feruloyl-triglucoside (f) in broccoli plants (infested with *Brevicoryne brassicae*, infested with *Myzus persicae*, or non-infested) grown in a climate

chamber and exposed to control lighting with or without one of four light treatments (UV-B 310 nm, UV-A 365 nm, UV-A 385 nm, and violet 420 nm). UV-A 365 nm, UV-A 385 nm, and violet 420 nm were supplied by LEDs. UV-B 310 nm was supplied by UV-B light tubes. Control lighting was supplied by standard fluorescent tubes (400 to 700 nm). Capital Letters indicate significant effects of aphid infestations averaged over the level of variant. Lower case letters indicate significant differences of light treatments averaged over the level of infestation (GLMM and Tukey *post hoc* tests, $P < 0.001$, $N = 8$ biological replicates)

Quercetinglycosides

The less complex non-acylated quercetin-3-*O*-sophoroside-7-*O*-glucoside and the complex diacylated quercetin-3-*O*-hydroxyferuloyl-sinapoyl-triglucoside-7-*O*-diglucoside were found in broccoli plants (Fig. 6).

B. brassicae infestation caused a decrease in the concentration of the sinapic acid monoacylated quercetin glycoside but did not affect the concentration of the non-acylated quercetin glycoside. Interestingly, *M. persicae* infestation caused an increase in the concentration of the non-acylated quercetin glycoside but did not affect the concentration of the sinapic acid monoacylated quercetin glycoside. As was true for the hydroxycinnamic acids, the concentrations of quercetin glycosides were lowest in broccoli plants treated with UV-A 365 nm. The concentrations of quercetin glycosides were highest in UV-B-treated plants except in the case of the sinapic acid monoacylated quercetin glycoside in plants infested with *B. brassicae*. Remarkably, the less complex non-acylated quercetin glycoside was greatly affected by UV-B but not by other light treatments. In contrast, the concentrations of the complex the sinapic acid monoacylated quercetin glycoside did not significantly differ among the control or the UV-B 310 nm, UV-A 385 nm, or violet 420 nm treatments (Fig. 6).

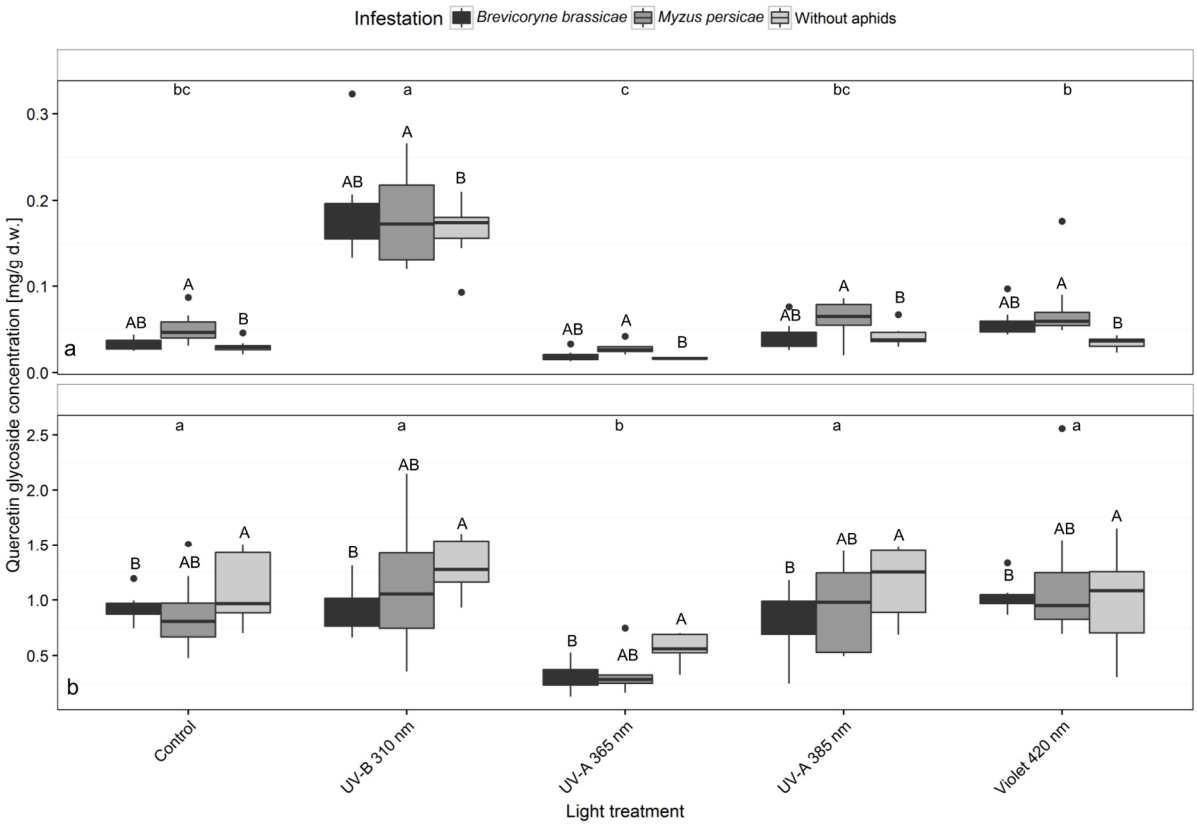

Fig. 6 Concentrations of quercetin glycosides, quercetin-3-*O*-sophoroside-7-*O*-glucoside (a) and quercetin-3-*O*-hydroxy-feruloyl-sinapoyl-triglucoside-7-*O*-diglucoside (b) in broccoli plants (infested with *Brevicoryne brassicae*, infested with *Myzus persicae*, or non-infested) grown in a climate chamber and exposed to control lighting with or without one of four light treatments (UV-B 310 nm, UV-A 365 nm, UV-A 385 nm, and violet 420 nm). UV-A 365 nm, UV-A 385 nm, and violet 420 nm were supplied by LEDs. UV-B 310 nm was supplied by UV-B light tubes. Control lighting was supplied by standard fluorescent tubes (400 to 700 nm). Capital Letters indicate significant effects of aphid infestations averaged over the level of variant. Lower case letters indicate significant differences of light treatments averaged over the level of infestation (GLMM and Tukey *post hoc* tests, $P < 0.001$, $N = 8$ biological replicates)

Kaempferolglycosides

The following 14 kaempferol glycosides were detected in broccoli plants: kaempferol-3-*O*-hydroxyferuloyl-sophoroside-7-*O*-glucoside, kaempferol-3-*O*-caffeoyl-sophoroside-7-*O*-glucoside, kaempferol-3-*O*-sinapoyl-sophoroside-7-*O*-glucoside, kaempferol-3-*O*-sinapoyl-sophoroside-7-*O*-diglucoside, kaempferol-3-*O*-feruloyl-sophoro-side-7-*O*-glucoside, kaempferol-3-*O*-coumaroyl-sophoroside-7-*O*-glucoside, kaempferol-3-*O*-caffeoyl-sophoroside-7-*O*-glucoside (isomer), kaempferol-3-*O*-sinapoyl-hydroxy-feruloyl-triglucoside-

7-*O*-diglucoside, kaempferol-3-*O*-sinapoyl-caffeoyl-triglucoside-7-*O*-diglucoside, kaempferol-3-*O*-sinapoyl-feruloyl-triglucoside-7-*O*-diglucoside, kaempferol-3-*O*-sinapoyl-feruloyl-triglucoside-7-*O*-diglucoside (isomer), kaempferol-3-*O*-sophoroside-7-*O*-glucoside, kaempferol-3,7-*O*-diglucoside, and kaempferol-3-*O*-glucoside-7-*O*-diglucoside. Among these, eight structurally different compounds were considered most relevant to the study (Fig. 7). Kaempferol-3-*O*-hydroxyferuloyl-sophoroside-7-*O*-glucoside, kaempferol-3-*O*-sinapoyl-sophoroside-7-*O*-diglucoside, and kaempferol-3-*O*-caffeoyl-sophoroside-7-*O*-glucoside (isomer) were found only in low concentrations in aphid-infested plants and were not found in non-infested plants.

B. brassicae infestation had almost no effect on the concentration of kaempferol glycosides, which are the main flavonoid glycosides in broccoli. Only the ferulic acid monoacylated kaempferol glycoside was decreased by *B. brassicae* infestation. In contrast, *M. persicae* infestation increased the concentrations of non-acylated di- and triglycosides and diacylated pentaglycosides of kaempferol but decreased or did not affect the concentrations of mono-acylated triglycosides of kaempferol. The concentrations of kaempferol glycosides were lowest in UV-A 365 nm-treated plants and were comparable to the concentrations of quercetin glycosides. The responses of kaempferol glycosides to the light treatments were related to their chemical structures. While the concentrations of the non-acylated diglycoside and the monoacylated triglycosides of kaempferol were highest in the UV-B treatment, the concentration of non-acylated triglycosides of kaempferol was affected by UV-B and violet light. The concentrations of diacylated pentaglycosides of kaempferol were high in non-infested plants treated with UV-A 385 and in infested plants treated with violet light (Fig. 7).

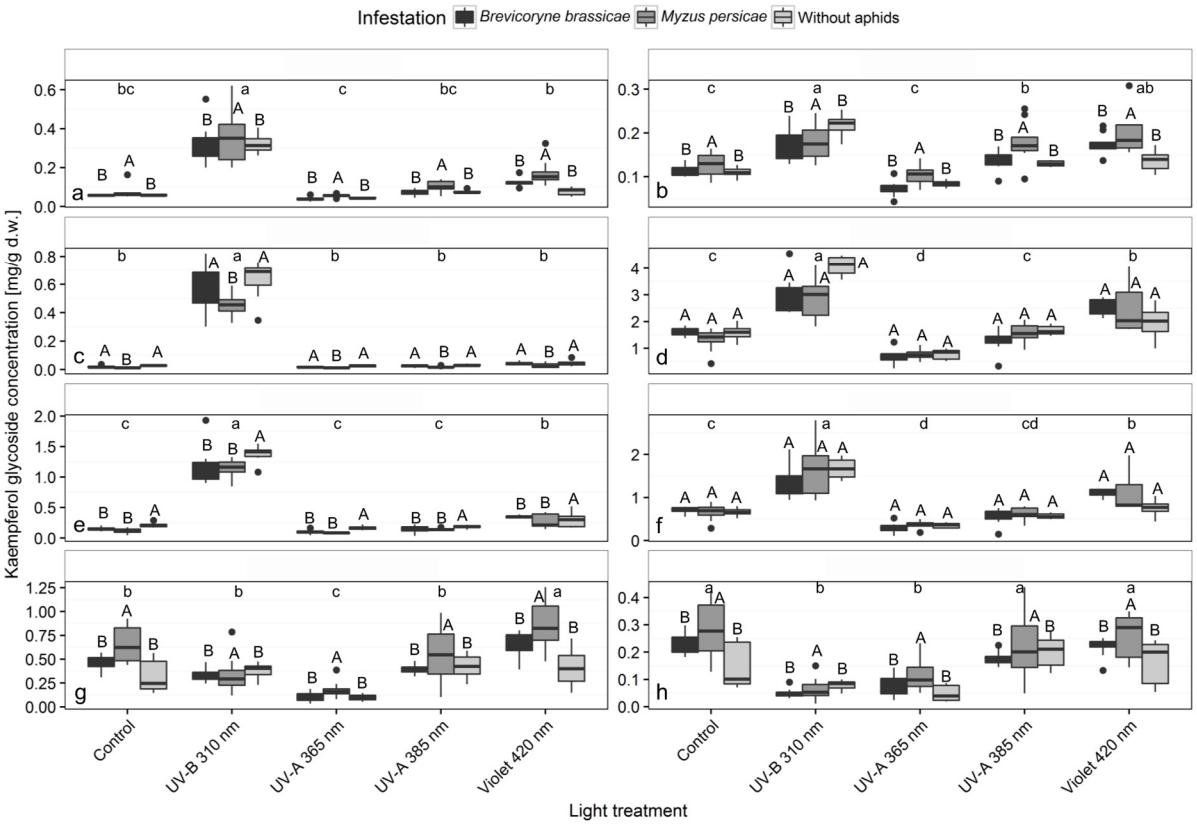

Fig. 7 Concentrations of kaempferol glycosides, kaempferol-3,7-*O*-diglucoside (a), kaempferol-3-*O*-sophoroside-7-*O*-glucoside (b), kaempferol-3-*O*-coumaroyl-sophoroside-7-*O*-glucoside (c), kaempferol-3-*O*-caffeoyl-sophoroside-7-*O*-glucoside (d), kaempferol-3-*O*-feruloyl-sophoroside-7-*O*-glucoside (e), kaempferol-3-*O*-sinapoyl-sophoroside-7-*O*-glucoside (f), kaempferol-3-*O*-sinapoyl-caffeoyl-triglucoside-7-*O*-diglucoside (g), and kaempferol-3-*O*-sinapoyl-hydroxyferuloyl-triglucoside-7-*O*-diglucoside (h) in broccoli plants (infested with *Brevicoryne brassicae*, infested with *Myzus persicae*, or non-infested) grown in a climate chamber and exposed to control lighting with or without one of four light treatments (UV-B 310 nm, UV-A 365 nm, UV-A 385 nm, and violet 420 nm). UV-A 365 nm, UV-A 385 nm, and violet 420 nm were supplied by LEDs. UV-B 310 nm was supplied by UV-B light tubes. Control lighting was supplied by standard fluorescent tubes (400 to 700 nm). Capital Letters indicate significant effects of aphid infestations averaged over the level of variant. Lower case letters indicate significant differences of light treatments averaged over the level of infestation (GLMM and Tukey *post hoc* tests, $P < 0.001$, $N = 8$ biological replicates)

Aliphatic glucosinolates

Two aliphatic methylsulfinylalkyl glucosinolates (3-methylsulfinylpropyl and 4-methylsulfinylbutyl) were quantified in the broccoli leaves in all treatments (Fig. 8). The predominant aliphatic glucosinolate was 4-methylsulfinylbutyl. 4-methylthiobutyl glucosinolate was found only in non-infested control plants (*data not shown*). Concentrations of 3-methylsulfinylpropyl glucosinolate and 4-methylsulfinylbutyl glucosinolate were about 2-fold higher in *M. persicae*-infested plants than in *B. brassicae*-infested plants or non-infested plants. Regardless of aphid infestation, the concentrations of both methylsulfinylalkyl glucosinolates and the corresponding precursor of 4-methylsulfinylbutyl glucosinolate, 4-methylthiobutyl glucosinolate, tended to be higher in UV-A 385 nm-treated plants than in control plants (Fig. 8).

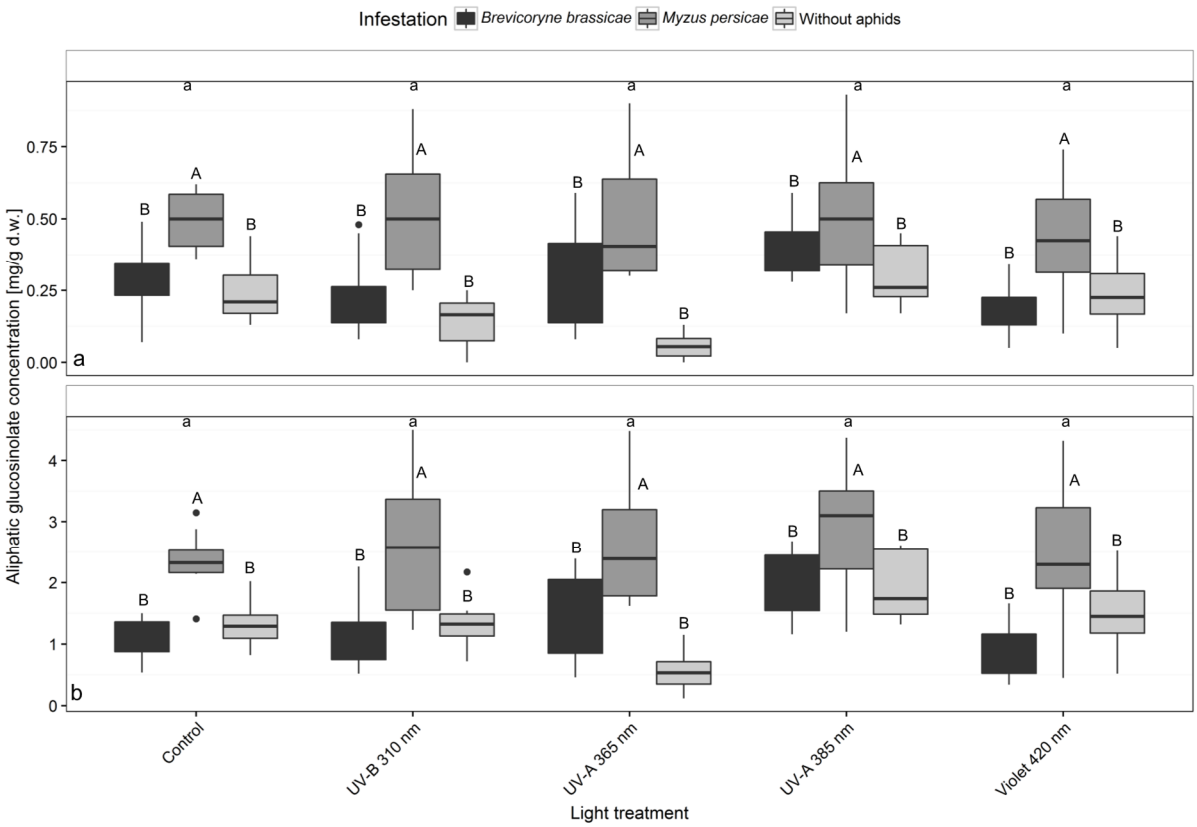

Fig. 8 Concentrations of aliphatic glucosinolates, 3-methylsulfinylpropyl (a) and 4-methylsulfinylbutyl (b) in broccoli plants (infested with *Brevicoryne brassicae*, infested with *Myzus persicae*, or non-infested) grown in a climate chamber and exposed to control lighting with or without one of four light treatments (UV-B 310 nm, UV-A 365 nm, UV-A 385 nm, and violet 420 nm). UV-A 365 nm, UV-A 385 nm, and violet 420 nm were supplied by LEDs. UV-B

310 nm was supplied by UV-B light tubes. Control lighting was supplied by standard fluorescent tubes (400 to 700 nm). Capital Letters indicate significant effects of aphid infestations averaged over the level of variant. Lower case letters indicate significant differences of light treatments averaged over the level of infestation (GLMM and Tukey *post hoc* tests, $P < 0.001$, $N = 8$ biological replicates)

Indole glucosinolates

Four indole glucosinolates (3-indolylmethyl, 4-hydroxy-3-indolylmethyl, 4-methoxy-3-indolylmethyl, and 1-methoxy-3-indolylmethyl) were quantified in the broccoli leaves in all treatments (Fig. 9). Concentrations of 4-hydroxy-3-indolylmethyl glucosinolate were increased by infestation of either aphid species compared to non-infested control plants. Concentrations of the indole glucosinolate 3-indolylmethyl were significantly increased by the UV-B 310 nm treatment but significantly decreased by *B. brassicae* infestation. Concentrations of 3-indolylmethyl were higher in UV-B 310 nm-treated plants than in violet 420 nm-treated plants. The concentration of its methoxylated form, 4-methoxy-3-indolylmethyl glucosinolate, was promoted by UV-B 310 nm and UV-A 365 nm treatments. Regardless of aphid infestation, concentrations of 4-methoxy-3-indolylmethyl glucosinolate were 2-fold higher in UV-B 310 nm- and UV-A 365 nm-treated plants than in control plants, while concentrations of 1-methoxy-3-indolylmethyl glucosinolate tended to be increased by UV-B 310 nm and UV-A 365 nm treatment (Fig. 9).

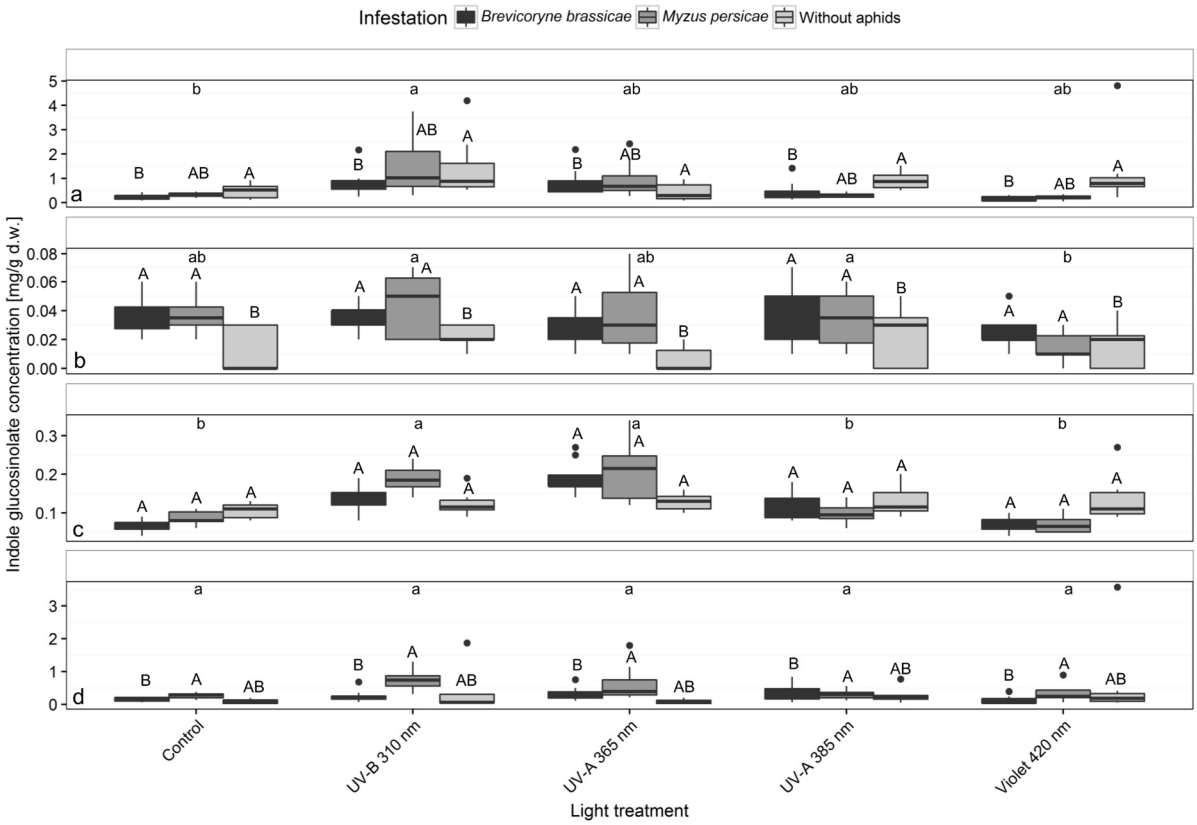

Fig. 9 Concentrations of indole glucosinolates, 3-indolylmethyl (a), 4-hydroxy-3-indolylmethyl (b), 4-methoxy-3-indolylmethyl (c), and 1-methoxy-3-indolylmethyl (d) in broccoli plants (infested with *Brevicoryne brassicae*, infested with *Myzus persicae*, or non-infested) grown in a climate chamber and exposed to control lighting with or without one of four light treatments (UV-B 310 nm, UV-A 365 nm, UV-A 385 nm, and violet 420 nm). UV-A 365 nm, UV-A 385 nm, and violet 420 nm were supplied by LEDs. UV-B 310 nm was supplied by UV-B light tubes. Control lighting was supplied by standard fluorescent tubes (400 to 700 nm). Capital Letters indicate significant effects of aphid infestations averaged over the level of variant. Lower case letters indicate significant differences of light treatments averaged over the level of infestation (GLMM and Tukey *post hoc* tests, $P < 0.001$, $N = 8$ biological replicates)

Discussion

The experimental setup in this study enabled a precise separation between the effects of UV-B, UV-A, and low wavelength visible light (violet) on the growth and secondary metabolites of broccoli plants and on the performance of *B. brassicae* and *M. persicae* on the treated plants. Regarding the effects of the light treatments on the aphids, because the clip cages that were used did not transmit UV radiation, the direct effects of UV-B and UV-A treatments on aphids were eliminated. Therefore, the discussion only focus on indirect, plant-mediated effects of

the light treatments on the aphids. The light treatments did not affect the developmental time of either aphid species. However, *B. brassicae* adult weight and fecundity were greatly reduced on UV-B 310 nm-treated plants (Fig. 3). In contrast, *M. persicae* adult weight and fecundity were increased on UV-B 310 nm- and UV-A 365 nm-treated plants (Fig. 4). Fecundity greatly affects aphid population dynamics and is a sensitive indicator of antifeedant or antibiotic properties induced in the host plants, i.e., the results suggest that UV-B induced resistance against *B. brassicae*. These findings corroborate other studies (Kuhlmann & Müller 2011; Rechner & Poehling 2014). The improved performance of *M. persicae* on plants treated with UV-B and UV-A indicates a kind of systemically induced susceptibility. Dader et al. (2014) also reported an increase in the growth of *M. persicae* populations on eggplant and pepper plants treated with UV-A radiation.

To our knowledge, this is the first narrow-band light study to describe the changes in concentrations of specific hydroxycinnamic acids, quercetin- and kaempferol-glycosides, and glucosinolates induced by generalist and specialist aphids. Aphid infestation can lead to altered concentrations of flavonol glycosides and glucosinolates, and we found that specific secondary plant metabolites reacted differently to aphids that differ in their degree of host specialization (Figs. 5-9). Mewis et al. (2012b) also reported that changes in concentrations of flavonol aglycones and glucosinolates after aphid attack differed depending on the aphid species, i.e., *M. persicae* feeding reduced the concentrations of flavonoids and glucosinolates while *B. brassicae* feeding increased the concentration of 4-hydroxy-3-indolylmethyl glucosinolate. In our study, single compounds reacted differently to the two aphid species. The results indicated that the increase in sinapic acid-containing hydroxycinnamic acids and kaempferol glycosides (non-acylated di- and triglycosides and diacylated pentaglycosides) as a defense reaction in broccoli plants is much stronger against the generalist *M. persicae* than against the specialist *B. brassicae*.

The concentrations of secondary plant metabolites (glucosinolates and flavonol glycosides) in our study were different in plants grown under the different light treatments. Most kaempferol glycosides and quercetin-3-*O*-sophoroside-7-*O*-glucoside increased in response to UV-B 310 nm treatment (Figs. 6 and 7). The indole glucosinolate 4-methoxy-3-indolylmethyl showed light-dependent effects, and its concentrations were 2-fold increased after UV-B 310 nm and UV-A 365 nm treatments. Concentrations of 3-indolylmethyl glucosinolate were also

increased after UV-B treatment (Fig. 9). Mewis et al. (2012a) also found that UV-B enhances the concentrations of glucosinolates in broccoli sprouts but that greater doses of UV-B and multiple exposure times did not result in greater increases in the accumulation of aliphatic glucosinolates. In contrast to our study, Wang et al. (2011) reported decreased contents of indole glucosinolates like 4-methoxy-3-indolylmethyl and lower expression of glucosinolate metabolism-related genes after continued exposure of UV-B radiation at 1.55 W/m² (66 kJ m^{-2} d^{-1}).

The light-induced alterations in the concentrations of secondary plant metabolites (especially non-acylated diglycoside, the monoacylated triglycosides and quercetin-3-*O*-sophoroside-7-*O*-glucoside) might partially explains the reduced performance of *B. brassicae* (Kuhlmann & Müller 2010) or the induced susceptibility via phagostimulants for *M. persicae* under UV-B treatment (Chacon-Fuentes et al. 2015; Zavala et al. 2015).

The mechanisms by which UV-B alters the concentrations of secondary plant metabolites were not determined in our study. However, it is known from other studies that UV-B can induce wound-triggered response pathways (salicylic-, jasmonic acid or ethylene) or can control a specific pathway operating over the UVR8 receptor protein, which is not analyzed in detail yet (Christie et al. 2012; Mewis et al. 2012a). These pathways could partially overlap and cross-talk leading to defense reactions against herbivores (Ballare 2014; Demkura et al. 2010; Izaguirre et al. 2003; 2007). The changes in aphid performance caused by treatment of broccoli with UV-B might be explained by UV-B-induced biosynthesis of some secondary plant metabolites, particularly kaempferol-3-*O*-caffeoyl-sophoroside-7-*O*-glucoside, kaempferol-3-*O*-sinapoyl-sophoroside-7-*O*-glucoside, kaempferol-3-*O*-feruloyl-sophoroside-7-*O*-glucoside, and glucosinolate 4-methoxy-3-indolylmethyl.

Several studies have described an enrichment of flavonol glycosides by UV-B (Neugart et al. 2012; 2014), and the UV-B-dependent induction of kaempferol glycoside biosynthesis was confirmed in our study. Concentrations of kaempferol glycosides (kaempferol glycosides were kaempferol-3-*O*-caffeoyl-sophoroside-7-*O*-glucoside, kaempferol-3-*O*-sinapoyl-sophoroside-7-*O*-glucoside, kaempferol-3-*O*-feruloyl-sophoroside-7-*O*-glucoside, kaempferol-3-*O*-coumaroyl-sophoroside-7-*O*-glucoside, kaempferol-3-*O*-sophoroside-7-*O*-glucoside, and kaempferol-3,7-*O*-diglucoside) were greatly increased by UV-B 310 nm treatment in our study and could be responsible for the reduced fecundity of *B. brassicae*. In other studies,

kaempferols are generally reported as strong inhibitors of herbivorous insects (Schreiner et al. 2012; Onkokesung et al. 2014). Concentrations of the quercetin 3-*O*-sophoroside-7-*O*-glucoside were also prominently elevated in plants treated with UV-B radiation and can be considered as a potential additional factor responsible for the low fecundity of *B. brassicae* on these plants. Because they can scavenge free radicals, most of the quercetin glycosides and also the specific kaempferol glycosides could have been synthesized by the plant to reduce the concentrations of reactive oxygen species produced after increased UV-B and UV-A radiation (Harborne & Williams 2000; Schreiner et al. 2012). It is not clear whether these specific reactive oxygen scavenging metabolites like kaempferol- and quercetin glycosides are responsible for the enhanced performance of *M. persicae* on UV-B 310 nm- and UV-A 365 nm-treated plants. Perhaps *M. persicae* can metabolize and neutralize kaempferol- and quercetin glycosides better than *B. brassicae* and consequently profit more from reduced reactive oxygen species.

In this study, concentrations of glucosinolates and especially of the indole glucosinolate 4-methoxy-3-indolylmethyl increased in UV-B- and UV-A-treated plants. These findings are in agreement with other studies (Björkman et al. 2011; Mewis et al. 2012a). Glucosinolates are the characteristic secondary plant metabolites of the Brassicaceae and are thought to be linked to the plant defense against herbivores. For instance, Mewis et al. (2012a) reported that the UV-B-triggered induction of 4-methylsulfinylbutyl glucosinolate and 4-methoxy-3-indolylmethyl glucosinolate was related to reduced numbers of *M. persicae*. In our study, however, the increase in 4-methoxy-3-indolylmethylglucosinolate concentration was associated with improved performance of *M. persicae*, i.e., the fecundity and adult weight were higher when 4-methoxy-3-indolylmethylglucosinolate concentrations increased. Perhaps our clone of *M. persicae*, which was collected outdoors on broccoli plants and which was reared on Brassicaceae for several generations, had adapted to this specific nutritional source and could therefore tolerate the high concentrations of indole glucosinolates. Adaptation could also be shown for the phloem-feeding whitefly *Bemisia tabaci* to nicotine and indolic glucosinolates depending on enhanced gene expression of detoxifying genes (Elbaz et al. 2012; Kliot et al. 2014).

The reduced fecundity of *B. brassicae* on UV-B-treated plants cannot be explained by increased concentrations of 4-methoxy-3-indolylmethyl glucosinolate because concentrations

of 4-methoxy-3-indolylmethyl glucosinolate were increased in both UV-B- and UV-A-treated plants but *B. brassicae* fecundity was not reduced on UV-A-treated plants.

Because the interactions between plants and herbivores are complex, it is probably unwise to attribute a reduced or enhanced performance of aphids on plants subjected to different light treatments to one secondary plant compound. Our examination of a wide range of secondary metabolites influenced by light treatments suggests that aphid reactions may only be explained by considering multiple secondary compounds and by considering the feeding characteristics of the aphids.

In conclusion, this study has demonstrated that narrow-bandwidth light treatments in addition to PAR on similar intensities can alter growth and induce metabolic changes in broccoli plants. Generalized aphids growing on these plants reacted with increased performance, while specialized aphids showed decreased performance parameters. The induced changes in concentrations of secondary plant metabolites depend on light quality and are highly specific, even for single compounds of the same class. In general, UV-B radiation had the strongest indirect effect on aphids, i.e., UV-B radiation of broccoli plants substantially reduced the fecundity of *B. brassicae*. UV-A treatments, in addition to normal background illumination, changed the concentrations of secondary metabolites in broccoli and enhanced the performance of *M. persicae*.

From a basic point of view and future look, more studies are needed on the antibiotic or antifeedant properties of the light induced secondary plant metabolites as single compound or blends. For instance, this research might benefit from artificial feeding systems for insects and a deeper examination of the involved signal transduction pathways.

Regarding a possible implementation of additional illumination with narrow bandwidth light sources such as LEDs for plant protection purposes, it is necessary to consider the species specificity of induced effects critically. Other plant or pest species and different light qualities should be investigated in detail, before drawing more general conclusions. Another relevant aspect concerning practical use of light induced metabolic changes is the noticed influence of important yield parameters of the produce such as reduced growth of broccoli plants under UV-B radiation with high intensities, which should be avoided in plant cultivation (Kuhlmann & Müller 2011; Jansen et al. 2008). Here more studies optimizing the induction technology

(inter crop lighting or combination of different wavelengths) and the intensity threshold for inducing a certain kind of metabolic changes without reducing the plant growths are desirable.

Acknowledgement

This research project was funded by the German Research Foundation DFG, grant Po 207/39-1.

Supplementary Material

Tab. 1 Concentrations [mg g^{-1} d.w.; mean (± SE)] of hydroxycinnamic acids in broccoli plants (non-infested, infested with *Brevicoryne brassicae*, or infested with *Myzus persicae*) grown in a climate chamber and exposed to control lighting plus one of four light treatments (UV-B 310 nm, UV-A 365 nm, UV-A 385 nm, and violet 420 nm) or to control lighting without additional illumination. UV-A 365 nm, UV-A 385 nm, and violet 420 nm were supplied by LEDs. UV-B 310 nm was supplied by UV-B light tubes. Control lighting was supplied by standard fluorescent tubes (400 to 700 nm)

Hydroxycinnamic acids	**Light treatment and concentration of hydroxycinnamic acids**				
Without aphid infestation	**Control**	**UV-B 310 nm**	**UV-A 365 nm**	**UV-A 385 nm**	**Violet 420 nm**
Sin-Fer-Triglc$^{(B)}$	0.08 ± 0.01$^{(ab)}$	0.08 ± 0.01$^{(c)}$	0.05 ± 0.01$^{(d)}$	0.09 ± 0.01$^{(bc)}$	0.09 ± 0.01$^{(a)}$
Sin-Fer-Gent$^{(B)}$	1.06 ± 0.27$^{(ab)}$	0.65 ± 0.10$^{(c)}$	0.40 ± 0.08$^{(c)}$	1.17 ± 0.20$^{(b)}$	1.14 ± 0.38$^{(a)}$
Disin-Gent$^{(B)}$	0.37 ± 0.12$^{(a)}$	0.24 ± 0.06$^{(b)}$	0.19 ± 0.05$^{(b)}$	0.42 ± 0.13$^{(a)}$	0.34 ± 0.15$^{(a)}$
Disin-Fer-Gent$^{(C)}$	0.31 ± 0.09$^{(a)}$	0.24 ± 0.04$^{(b)}$	0.14 ± 0.03$^{(b)}$	0.37 ± 0.08$^{(a)}$	0.34 ± 0.14$^{(a)}$
Difer-Gent$^{(AB)}$	0.14 ± 0.03$^{(b)}$	0.09 ± 0.01$^{(cd)}$	0.06 ± 0.01$^{(d)}$	0.13 ± 0.02$^{(bc)}$	0.22 ± 0.13$^{(a)}$
Trisin-Gent$^{(C)}$	0.14 ± 0.05$^{(a)}$	0.10 ± 0.02$^{(b)}$	0.08 ± 0.02$^{(b)}$	0.17 ± 0.04$^{(a)}$	0.13 ± 0.05$^{(a)}$
With *B. brassicae* infestation					
Sin-Fer-Triglc$^{(B)}$	0.09 ± 0.01$^{(ab)}$	0.07 ± 0.01$^{(c)}$	0.04 ± 0.01$^{(d)}$	0.07 ± 0.01$^{(bc)}$	0.10 ± 0.01$^{(a)}$
Sin-Fer-Gent$^{(B)}$	1.28 ± 0.19$^{(ab)}$	0.42 ± 0.14$^{(c)}$	0.31 ± 0.14$^{(c)}$	0.95 ± 0.11$^{(b)}$	1.40 ± 0.14$^{(a)}$
Disin-Gent$^{(B)}$	0.46 ± 0.07$^{(a)}$	0.16 ± 0.07$^{(b)}$	0.17 ± 0.07$^{(b)}$	0.39 ± 0.10$^{(a)}$	0.46 ± 0.06$^{(a)}$
Disin-Fer-Gent$^{(B)}$	0.49 ± 0.07$^{(a)}$	0.21 ± 0.04$^{(b)}$	0.15 ± 0.06$^{(b)}$	0.44 ± 0.06$^{(a)}$	0.56 ± 0.09$^{(a)}$
Difer-Gent$^{(B)}$	0.14 ± 0.02$^{(b)}$	0.07 ± 0.01$^{(cd)}$	0.05 ± 0.01$^{(d)}$	0.11 ± 0.02$^{(bc)}$	0.20 ± 0.05$^{(a)}$
Trisin-Gent$^{(B)}$	0.22 ± 0.03$^{(a)}$	0.09 ± 0.02$^{(b)}$	0.09 ± 0.03$^{(b)}$	0.19 ± 0.05$^{(a)}$	0.22 ± 0.03$^{(a)}$
With *M. persicae* infestation					
Sin-Fer-Triglc$^{(A)}$	0.11 ± 0.03$^{(ab)}$	0.07 ± 0.01$^{(c)}$	0.06 ± 0.01$^{(d)}$	0.10 ± 0.03$^{(bc)}$	0.13 ± 0.04$^{(a)}$
Sin-Fer-Gent$^{(A)}$	1.55 ± 0.52$^{(ab)}$	0.61 ± 0.29$^{(c)}$	0.55 ± 0.25$^{(c)}$	1.25 ± 0.50$^{(b)}$	1.82 ± 0.73$^{(a)}$
Disin-Gent$^{(A)}$	0.53 ± 0.23$^{(a)}$	0.28 ± 0.16$^{(b)}$	0.22 ± 0.11$^{(b)}$	0.52 ± 0.29$^{(a)}$	0.58 ± 0.23$^{(a)}$
Disin-Fer-Gent$^{(A)}$	0.63 ± 0.21$^{(a)}$	0.30 ± 0.16$^{(b)}$	0.22 ± 0.09$^{(b)}$	0.55 ± 0.26$^{(a)}$	0.73 ± 0.28$^{(a)}$
Difer-Gent$^{(A)}$	0.19 ± 0.05$^{(b)}$	0.07 ± 0.02$^{(cd)}$	0.06 ± 0.02$^{(d)}$	0.12 ± 0.03$^{(bc)}$	0.27 ± 0.11$^{(a)}$
Trisin-Gent$^{(A)}$	0.28 ± 0.11$^{(a)}$	0.15 ± 0.09$^{(b)}$	0.12 ± 0.06$^{(b)}$	0.27 ± 0.16$^{(a)}$	0.28 ± 0.11$^{(a)}$

Capital Letters indicate significant effects of aphid infestations averaged over the level of variant. Lower case letters indicate significant differences of light treatments averaged over the level of infestation (GLMM and Tukey *post hoc* tests, $P < 0.001$, $N = 8$ biological replicates). Sin-Fer-Triglc: sinapoyl-feruloyl-

triglucoside, Sin-Fer-Gent: sinapoyl-feruloyl-gentiobiose, Disin-Gent: disinapoyl-gentiobiose, Disin-Fer-Gent: disinapoyl-feruloyl-gentiobiose, Difer-Gent: diferuloyl-gentiobiose, Trisin-Gent: trisinapoyl-gentiobiose.

Tab. 2 Concentrations [mg g^{-1} d.w.; mean (± SE)] of quercetin glycosides in broccoli plants (non-infested, infested with *Brevicoryne brassicae*, or infested with *Myzus persicae*) grown in a climate chamber and exposed to control lighting plus one of four light treatments (UV-B 310 nm, UV-A 365 nm, UV-A 385 nm, and violet 420 nm) or to control lighting without additional illumination. UV-A 365 nm, UV-A 385 nm, and violet 420 nm were supplied by LEDs. UV-B 310 nm was supplied by UV-B light tubes. Control lighting was supplied by standard fluorescent tubes (400 to 700 nm)

Quercetin glycosides	**Light treatment and concentration of quercetin glycosides**				
Without aphid infestation	**Control**	**UV-B 310 nm**	**UV-A 365 nm**	**UV-A 385 nm**	**Violet 420 nm**
Q-3-hfer-sin-triglc-7-diglc$^{(A)}$	1.08 ± 0.32$^{(a)}$	1.30 ± 0.24$^{(a)}$	0.57 ± 0.13$^{(b)}$	1.16 ± 0.31$^{(a)}$	0.98 ± 0.46$^{(a)}$
Q-3-soph-7-glc$^{(B)}$	0.03 ± 0.01$^{(bc)}$	0.17 ± 0.04$^{(a)}$	0.02 ± 0.00$^{(c)}$	0.04 ± 0.01$^{(bc)}$	0.03 ± 0.01$^{(b)}$
With *B. brassicae* infestation					
Q-3-hfer-sin-triglc-7-diglc$^{(B)}$	0.93 ± 0.14$^{(a)}$	0.92 ± 0.23$^{(a)}$	0.31 ± 0.13$^{(b)}$	0.81 ± 0.28$^{(a)}$	1.03 ± 0.14$^{(a)}$
Q-3-soph-7-glc$^{(AB)}$	0.03 ± 0.01$^{(bc)}$	0.19 ± 0.06$^{(a)}$	0.02 ± 0.01$^{(c)}$	0.04 ± 0.02$^{(bc)}$	0.06 ± 0.02$^{(b)}$
With *M. persicae* infestation					
Q-3-hfer-sin-triglc-7-diglc$^{(AB)}$	0.88 ± 0.33$^{(a)}$	1.13 ± 0.61$^{(a)}$	0.33 ± 0.18$^{(a)}$	0.93 ± 0.39$^{(a)}$	1.18 ± 0.62$^{(a)}$
Q-3-soph-7-glc$^{(A)}$	0.05 ± 0.02$^{(bc)}$	0.18 ± 0.06$^{(a)}$	0.03 ± 0.01$^{(c)}$	0.06 ± 0.02$^{(bc)}$	0.08 ± 0.04$^{(b)}$

Capital Letters indicate significant effects of aphid infestations averaged over the level of variant. Lower case letters indicate significant differences of light treatments averaged over the level of infestation (GLMM and Tukey *post hoc* tests, $P < 0.001$, $N = 8$ biological replicates). Q-3-hfer-sin-triglc-7-diglc: quercetin-3-*O*-hydroxy-ferul-oyl-sinapoyl-triglucoside-7-*O*-diglucoside, Q-3-soph-7-glc: quercetin-3-*O*-sophoroside-7-*O*-glucoside.

Tab. 3 Concentrations [mg g^{-1} d.w.; mean (± SE)] of kaempferol glycosides in broccoli plants (non-infested, infested with *Brevicoryne brassicae*, or infested with *Myzus persicae*) grown in a climate chamber and exposed to control lighting plus one of four light treatments (UV-B 310 nm, UV-A 365 nm, UV-A 385 nm, and violet 420 nm) or to control lighting without additional illumination. UV-A 365 nm, UV-A 385 nm, and violet 420 nm were supplied by LEDs. UV-B 310 nm was supplied by UV-B light tubes. Control lighting was supplied by standard fluorescent tubes (400 to 700 nm)

Kaempferol glycosides	**Light treatment and concentrations of kaempferol glycosides**				
Without aphid infestation	**Control**	**UV-B 310 nm**	**UV-A 365 nm**	**UV-A 385 nm**	**Violet 420 nm**
K-3-sin-soph-7-glc$^{(A)}$	0.66 ± 0.10$^{(c)}$	1.67 ± 0.24$^{(a)}$	0.35 ± 0.06$^{(d)}$	0.57 ± 0.06$^{(cd)}$	0.76 ± 0.18$^{(b)}$
K-3-fer-soph-7-glc$^{(A)}$	0.21 ± 0.04$^{(c)}$	1.38 ± 0.14$^{(a)}$	0.17 ± 0.03$^{(c)}$	0.18 ± 0.03$^{(c)}$	0.30 ± 0.12$^{(b)}$
K-3-cou-soph-7-glc$^{(A)}$	0.03 ± 0.01$^{(b)}$	0.63 ± 0.14$^{(a)}$	0.03 ± 0.01$^{(b)}$	0.03 ± 0.01$^{(b)}$	0.05 ± 0.02$^{(b)}$
K-3-sin-hfer-triglc-7-diglc$^{(B)}$	0.15 ± 0.08$^{(a)}$	0.08 ± 0.02$^{(b)}$	0.05 ± 0.03$^{(b)}$	0.20 ± 0.06$^{(a)}$	0.16 ± 0.08$^{(a)}$
K-3-sin-caf-triglc-7-diglc$^{(B)}$	0.32 ± 0.17$^{(b)}$	0.37 ± 0.09$^{(b)}$	0.10 ± 0.03$^{(c)}$	0.42 ± 0.13$^{(b)}$	0.41 ± 0.21$^{(a)}$
K-3-soph-7-glc$^{(B)}$	0.11 ± 0.01$^{(c)}$	0.22 ± 0.02$^{(a)}$	0.08 ± 0.01$^{(c)}$	0.13 ± 0.01$^{(b)}$	0.14 ± 0.02$^{(ab)}$
K-3-7-diglc$^{(B)}$	0.06 ± 0.01$^{(bc)}$	0.32 ± 0.05$^{(a)}$	0.04 ± 0.01$^{(c)}$	0.08 ± 0.01$^{(bc)}$	0.08 ± 0.02$^{(b)}$
K-3-caf-soph-7-glc$^{(A)}$	1.57 ± 0.30$^{(c)}$	4.10 ± 0.36$^{(a)}$	0.78 ± 0.19$^{(d)}$	1.67 ± 0.17$^{(c)}$	1.98 ± 0.59$^{(b)}$
With *B. brassicae* infestation					
K-3-sin-soph-7-glc$^{(A)}$	0.71 ± 0.08$^{(c)}$	1.34 ± 0.42$^{(a)}$	0.29 ± 0.12$^{(d)}$	0.56 ± 0.20$^{(cd)}$	1.10 ± 0.11$^{(b)}$
K-3-fer-soph-7-glc$^{(B)}$	0.15 ± 0.03$^{(c)}$	1.18 ± 0.33$^{(a)}$	0.10 ± 0.04$^{(c)}$	0.15 ± 0.06$^{(c)}$	0.35 ± 0.03$^{(b)}$
K-3-cou-soph-7-glc$^{(A)}$	0.02 ± 0.01$^{(b)}$	0.57 ± 0.17$^{(a)}$	0.02 ± 0.01$^{(b)}$	0.03 ± 0.01$^{(b)}$	0.04 ± 0.01$^{(b)}$
K-3-sin-hfer-triglc-7-diglc$^{(B)}$	0.23 ± 0.04$^{(a)}$	0.05 ± 0.02$^{(b)}$	0.08 ± 0.04$^{(b)}$	0.18 ± 0.03$^{(a)}$	0.22 ± 0.04$^{(a)}$
K-3-sin-caf-triglc-7-diglc$^{(B)}$	0.46 ± 0.09$^{(b)}$	0.33 ± 0.07$^{(b)}$	0.10 ± 0.05$^{(c)}$	0.40 ± 0.05$^{(b)}$	0.65 ± 0.15$^{(a)}$
K-3-soph-7-glc$^{(B)}$	0.11 ± 0.01$^{(c)}$	0.17 ± 0.04$^{(a)}$	0.07 ± 0.02$^{(c)}$	0.14 ± 0.02$^{(b)}$	0.17 ± 0.02$^{(ab)}$
K-3-7-diglc$^{(B)}$	0.06 ± 0.01$^{(bc)}$	0.33 ± 0.11$^{(a)}$	0.04 ± 0.01$^{(c)}$	0.07 ± 0.02$^{(bc)}$	0.12 ± 0.02$^{(b)}$
K-3-caf-soph-7-glc$^{(A)}$	1.62 ± 0.17$^{(c)}$	3.01 ± 0.76$^{(a)}$	0.67 ± 0.29$^{(d)}$	1.28 ± 0.44$^{(c)}$	2.52 ± 0.31$^{(b)}$
With *M. persicae* infestation					
K-3-sin-soph-7-glc$^{(A)}$	0.66 ± 0.20$^{(c)}$	1.68 ± 0.67$^{(a)}$	0.36 ± 0.09$^{(d)}$	0.62 ± 0.15$^{(cd)}$	1.08 ± 0.43$^{(b)}$
K-3-fer-soph-7-glc$^{(B)}$	0.12 ± 0.04$^{(c)}$	1.15 ± 0.15$^{(a)}$	0.08 ± 0.01$^{(c)}$	0.14 ± 0.02$^{(c)}$	0.27 ± 0.12$^{(b)}$
K-3-cou-soph-7-glc$^{(B)}$	0.01 ± 0.00$^{(b)}$	0.45 ± 0.08$^{(a)}$	0.01 ± 0.00$^{(b)}$	0.02 ± 0.01$^{(b)}$	0.03 ± 0.02$^{(b)}$
K-3-sin-hfer-triglc-7-diglc$^{(A)}$	0.28 ± 0.11$^{(a)}$	0.06 ± 0.04$^{(b)}$	0.11 ± 0.06$^{(b)}$	0.23 ± 0.14$^{(a)}$	0.26 ± 0.08$^{(a)}$
K-3-sin-caf-triglc-7-diglc$^{(A)}$	0.66 ± 0.19$^{(b)}$	0.34 ± 0.22$^{(b)}$	0.18 ± 0.10$^{(c)}$	0.56 ± 0.30$^{(b)}$	0.85 ± 0.28$^{(a)}$
K-3-soph-7-glc$^{(A)}$	0.13 ± 0.03$^{(c)}$	0.18 ± 0.04$^{(a)}$	0.11 ± 0.02$^{(c)}$	0.18 ± 0.05$^{(b)}$	0.20 ± 0.05$^{(ab)}$
K-3-7-diglc$^{(A)}$	0.07 ± 0.04$^{(bc)}$	0.36 ± 0.15$^{(a)}$	0.06 ± 0.01$^{(c)}$	0.10 ± 0.03$^{(bc)}$	0.17 ± 0.07$^{(b)}$
K-3-caf-soph-7-glc$^{(A)}$	1.30 ± 0.43$^{(c)}$	2.91 ± 0.86$^{(a)}$	0.78 ± 0.21$^{(d)}$	1.57 ± 0.36$^{(c)}$	2.46 ± 0.89$^{(b)}$

Capital Letters indicate significant effects of aphid infestations averaged over the level of variant. Lower case letters indicate significant differences of light treatments averaged over the level of infestation (GLMM and Tukey *post hoc* tests, $P < 0.001$, $N = 8$ biological replicates). K-3-sin-soph-7-glc: kaempferol-3-*O*-sinapoyl-sophoro-side-7-*O*-glucoside, K-3-fer-soph-7-glc: kaempferol-3-*O*-feruloyl-sophoroside-7-*O*-glucoside, K-3-cou-soph-7-glc: kaempferol-3-*O*-coumaroyl-sophoroside-7-*O*-glucoside, K-3-sin-hfer-triglc-7-diglc: kaempferol-3-*O*-sinapoyl-

hydroxyferuloyl-triglucoside-7-*O*-diglucoside, K-3-sin-caf-triglc-7-diglc: kaempferol-3-*O*-sinapoyl-caffeoyl-triglucoside-7-*O*-diglucoside, K-3-soph-7-glc: kaempferol-3-*O*-sophoroside-7-*O*-glucoside, K-3-7-diglc: kaempferol-3,7-*O*-diglucoside, K-3-caf-soph-7-glc: kaempferol-3-*O*-caffeoyl-sophoroside-7-*O*-glucoside.

Tab. 4 Concentrations [mg g^{-1} d.w.; mean (± SE)] of aliphatic glucosinolates in broccoli plants (non-infested, infested with *Brevicoryne brassicae*, or infested with *Myzus persicae*) grown in a climate chamber and exposed to control lighting plus one of four light treatments (UV-B 310 nm, UV-A 365 nm, UV-A 385 nm, and violet 420 nm) or to control lighting without additional illumination. UV-A 365 nm, UV-A 385 nm, and violet 420 nm were supplied by LEDs. UV-B 310 nm was supplied by UV-B light tubes. Control lighting was supplied by standard fluorescent tubes (400 to 700 nm)

Aliphatic glucosinolates	**Light treatment and concentrations of aliphatic glucosinolates**				
Without aphid infestation	**Control**	**UV-B 310 nm**	**UV-A 365 nm**	**UV-A 385 nm**	**Violet 420 nm**
3-methylsulfinylpropyl$^{(B)}$	0.24 ± 0.10$^{(a)}$	0.14 ± 0.08$^{(a)}$	0.06 ± 0.05$^{(a)}$	0.30 ± 0.11$^{(a)}$	0.24 ± 0.13$^{(a)}$
4-methylsulfinylbutyl$^{(B)}$	1.33 ± 0.37$^{(a)}$	1.35 ± 0.42$^{(a)}$	0.57 ± 0.37$^{(a)}$	1.93 ± 0.56$^{(a)}$	1.55 ± 0.67$^{(a)}$
With *B. brassicae* infestation					
3-methylsulfinylpropyl$^{(B)}$	0.29 ± 0.12$^{(a)}$	0.23 ± 0.15$^{(a)}$	0.27 ± 0.19$^{(a)}$	0.40 ± 0.10$^{(a)}$	0.18 ± 0.09$^{(a)}$
4-methylsulfinylbutyl$^{(B)}$	1.01 ± 0.34$^{(a)}$	1.18 ± 0.68$^{(a)}$	1.34 ± 0.75$^{(a)}$	1.97 ± 0.59$^{(a)}$	0.85 ± 0.45$^{(a)}$
With *M. persicae* infestation					
3-methylsulfinylpropyl$^{(A)}$	0.50 ± 0.10$^{(a)}$	0.52 ± 0.24$^{(a)}$	0.50 ± 0.24$^{(a)}$	0.50 ± 0.24$^{(a)}$	0.43 ± 0.21$^{(a)}$
4-methylsulfinylbutyl$^{(A)}$	2.35 ± 0.52$^{(a)}$	2.64 ± 1.19$^{(a)}$	2.69 ± 1.15$^{(a)}$	2.88 ± 1.09$^{(a)}$	2.47 ± 1.22$^{(a)}$

Capital Letters indicate significant effects of aphid infestations averaged over the level of variant. Lower case letters indicate significant differences of light treatments averaged over the level of infestation (GLMM and Tukey *post hoc* tests, $P < 0.001$, $N = 8$ biological replicates).

Tab. 5 Concentrations [mg g^{-1} d.w.; mean (± SE)] of indole glucosinolates in broccoli plants (non-infested, infested with *Brevicoryne brassicae*, or infested with *Myzus persicae*) grown in a climate chamber and exposed to control lighting plus one of four light treatments (UV-B 310 nm, UV-A 365 nm, UV-A 385 nm, and violet 420 nm) or to control lighting without additional illumination. UV-A 365 nm, UV-A 385 nm, and violet 420 nm were supplied by LEDs. UV-B 310 nm was supplied by UV-B light tubes. Control lighting was supplied by standard fluorescent tubes (400 to 700 nm)

Indole glucosinolates	**Light treatment and concentrations of indole glucosinolates**				
Without aphid infestation	**Control**	**UV-B 310 nm**	**UV-A 365 nm**	**UV-A 385 nm**	**Violet 420 nm**
3-indolylmethyl$^{(A)}$	0.48 ± 0.29$^{(b)}$	1.44 ± 1.27$^{(a)}$	0.43 ± 0.35$^{(ab)}$	0.91 ± 0.37$^{(ab)}$	1.24 ± 1.17$^{(ab)}$
4-hydroxy-3-indolylmethyl$^{(B)}$	0.01 ± 0.01$^{(ab)}$	0.02 ± 0.00$^{(a)}$	0.01 ± 0.00$^{(ab)}$	0.02 ± 0.02$^{(a)}$	0.02 ± 0.02$^{(b)}$
4-methoxy-3-indolylmethyl$^{(A)}$	0.11 ± 0.02$^{(b)}$	0.12 ± 0.03$^{(a)}$	0.13 ± 0.02$^{(a)}$	0.13 ± 0.04$^{(b)}$	0.14 ± 0.06$^{(b)}$
1-methoxy-3-indolylmethyl$^{(AB)}$	0.09 ± 0.06$^{(a)}$	0.34 ± 0.24$^{(a)}$	0.08 ± 0.06$^{(a)}$	0.25 ± 0.22$^{(a)}$	0.61 ± 0.20$^{(a)}$
With *B. brassicae* infestation					
3-indolylmethyl$^{(B)}$	0.23 ± 0.12$^{(b)}$	0.85 ± 0.58$^{(a)}$	0.82 ± 0.62$^{(ab)}$	0.46 ± 0.44$^{(ab)}$	0.15 ± 0.11$^{(ab)}$
4-hydroxy-3-indolylmethyl$^{(A)}$	0.04 ± 0.01$^{(ab)}$	0.04 ± 0.01$^{(a)}$	0.03 ± 0.01$^{(ab)}$	0.04 ± 0.02$^{(a)}$	0.02 ± 0.02$^{(b)}$
4-methoxy-3-indolylmethyl$^{(A)}$	0.07 ± 0.02$^{(b)}$	0.13 ± 0.04$^{(a)}$	0.19 ± 0.05$^{(a)}$	0.11 ± 0.04$^{(b)}$	0.07 ± 0.02$^{(b)}$
1-methoxy-3-indolylmethyl$^{(B)}$	0.14 ± 0.07$^{(a)}$	0.25 ± 0.19$^{(a)}$	0.32 ± 0.21$^{(a)}$	0.34 ± 0.27$^{(a)}$	0.13 ± 0.12$^{(a)}$
With *M. persicae* infestation					
3-indolylmethyl$^{(AB)}$	0.32 ± 0.09$^{(b)}$	1.52 ± 1.22$^{(a)}$	0.95 ± 0.76$^{(ab)}$	0.29 ± 0.09$^{(ab)}$	0.21 ± 0.09$^{(ab)}$
4-hydroxy-3-indolylmethyl$^{(A)}$	0.04 ± 0.01$^{(ab)}$	0.05 ± 0.02$^{(a)}$	0.04 ± 0.03$^{(ab)}$	0.03 ± 0.02$^{(a)}$	0.02 ± 0.01$^{(b)}$
4-methoxy-3-indolylmethyl$^{(A)}$	0.09 ± 0.02$^{(b)}$	0.19 ± 0.03$^{(a)}$	0.21 ± 0.08$^{(a)}$	0.10 ± 0.03$^{(b)}$	0.07 ± 0.02$^{(b)}$
1-methoxy-3-indolylmethyl$^{(A)}$	0.27 ± 0.09$^{(a)}$	0.75 ± 0.32$^{(a)}$	0.64 ± 0.56$^{(a)}$	0.31 ± 0.17$^{(a)}$	0.34 ± 0.26$^{(a)}$

Capital Letters indicate significant effects of aphid infestations averaged over the level of variant. Lower case letters indicate significant differences of light treatments averaged over the level of infestation (GLMM and Tukey *post hoc* tests, $P < 0.001$, $N = 8$ biological replicates

Chapter 2

Can Longer Wavelengths of Narrow-Bandwidth Light Alter Secondary Plant Metabolism and Increase Plant Defenses Against Aphids?

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Submitted to PLoS ONE (10 May 2017)

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Abstract

Light of different wavelengths is essential for plant growth and development. Short-wavelength radiation such as UV can shift the composition of flavonoids, glucosinolates, and other plant metabolites responsible for enhanced defense against certain herbivorous insects. The intensity of light-induced, metabolite-based resistance is plant- and insect species-specific and depends on herbivore feeding guild and specialization. The increasing use of light-emitting diodes (LEDs) in horticultural plant production systems in protected environments enables the creation of tailor-made light scenarios for improved plant cultivation and induced defense against herbivorous insects. In this study, broccoli (*Brassica oleracea* var. *italica*) plants were grown in a climate chamber under broad spectra photosynthetic active radiation (PAR) and were additionally treated with the following narrow-bandwidth light generated with LEDs: UV-A (365 nm), violet (420 nm), blue (470 nm), or green (515 nm). We determined the influence of narrow-bandwidth light on broccoli plant growth, secondary plant metabolism, and plant-mediated light effects on the performance and behavior of the specialized cabbage aphid *Brevicoryne brassicae*. Green light increased plant height more than UV-A, violet, or blue LED treatments. Among flavonol glycosides, specific quercetin and kaempferol glycosides were increased under violet light, while aliphatic and indole glucosinolates were increased under UV-A light. The concentration of 3-indolylmethyl glucosinolate in plants was increased by UV-A treatment. *B. brassicae* performance was not influenced by the different light qualities, but in host-choice tests, *B. brassicae* preferred blue-illuminated plants (but not UV-A-, violet-, or green-illuminated plants) over control plants.

Key Words

Brevicoryne brassicae, Light-emitting diodes, Glucosinolates, Flavonoids, Host-plant choice, Broccoli

Introduction

Arthropod pests like aphids (Hemiptera: Aphididae) can damage horticultural plants by removing assimilates (phloem-feeding), producing honeydew, and transmitting viruses (Blackman & Eastop 1994). The effect of aphids and other arthropod pests on plants can be affected by light quality (Ballare 2014). UV-B radiation, for example, increases the biosynthesis of protective phenolic compounds like kaempferol and quercetin glycosides in plants (Neugart et al. 2012; 2014). Furthermore, the concentration of specific glucosinolates in *Brassica oleracea* var. *italica* P. (Brassicaceae; broccoli) can be increased by treatment with UV-B or UV-A radiation (Mewis et al. 2012a; Rechner et al. 2016). This light-induced increase in plant metabolites results from the stimulation of specific photoreceptors followed by the activation of a signal transduction chain and the triggering of transcription factors and genes involved in secondary metabolite biosynthesis (Ballare 2014). Expression of these genes shifts the composition of metabolites in the plant, and changes in specific secondary plant metabolites can enhance or decrease the susceptibility to certain herbivorous insects (Ballare 2014; Schreiner et al. 2012). In addition to UV-B and UV-A, other light qualities, e.g., blue, green, and red, may also induce the biosynthesis of certain plant metabolites such as flavonoids and glucosinolates and alter the resistance of the plant (Dader et al. 2014; Dhakal et al. 2015; Quian et al. 2015; Kim et al. 2015; Abe et al. 2015; Yang et al. 2015).

With the increasing development of LED technology and its use in horticultural production systems in protected environments, plant producers are now able to create specific light scenarios for influencing plant growth and quality and also plant metabolites so as to protect plants against herbivory (Singh et al. 2015; Vänninen et al. 2010). The application of LED-generated narrow-bandwidth light of different quality is a promising approach for enhancing the production of secondary metabolites in plants (Ahmad et al. 2016).

The effect of LED light treatments on plants is receiving increasing attention from researchers. Additional green light generated with LEDs did not influence the growth of *Cucumis sativus* L. (Cucurbitaceae; cucumber seedlings) (Hernandez & Kubota 2016). In contrast, green LED light promoted growth of *Lactuca sativa* L. (Asteraceae; lettuce) (Johkan et al. 2012). Increases in blue light intensity enhanced the chlorophyll content per leaf area and photosynthetic rate in cucumber, resulting in improved primary plant metabolism and growth (Hernandez & Kubota 2016; Huche-Thelier et al. 2016).

LED lighting can also affect concentration of secondary plant metabolites. Artificial LED lighting enriched with blue light enhanced the growth and increased the total phenolic content of *Ocimum basilicum* L. (Lamiaceae; basil) compared to broad spectra fluorescent light (Bantis et al. 2016). Treatment of *Brassica rapa* ssp. *pekinensis* (Brassicaceae; Chinese cabbage) with blue LED light also increased the biosynthesis of phenylpropanoids including quercetin and kaempferol glycosides (Kim et al. 2015). Furthermore, *Cardamine fauriei* Maxim. (Brassicaceae; Ezo-wasabi in Japanese) contained increased concentrations of aliphatic glucosinolates after irradiation with blue and red LED light (Abe et al. 2015). Dader et al. (2014) irradiated two plant species with artificial UV-A and induced flavonoids in *Capsicum annuum* L. (Solanaceae; pepper) but not in *Solanum melongena* L. (Solanaceae; eggplant), indicating species-specific reactions to various light treatments. Green and yellow lights enhanced production of total phenolics and total flavonoids in callus cultures of *Prunella vulgaris* L. (Lamiaceae; self-heal) (Fazal et al. 2016).

By altering plant metabolites, light can affect the behavior, performance, and development of herbivorous insects (Ballare 2014; Kuhlmann & Müller 2010; Schreiner et al. 2012). Treatment with UV-B increased the concentrations of kaempferol glycosides and specific glucosinolates in broccoli plants, and feeding on these plants reduced the fecundity of the specialist aphid *Brevicoryne brassicae* Linnaeus but improved the performance of the generalist aphid *Myzus persicae* Sulzer (both Hemiptera: Aphididae) (Rechner et al. 2016). Moreover, *B. brassicae* preferred to colonize broccoli plants grown under ambient UV conditions than under low-UV conditions in open, plastic tunnels equipped with UV-blocking *vs.* UV-transmitting films but *B. brassicae* population growth was reduced on plants grown under high-UV conditions (Kuhlmann & Müller 2009; 2010; Gulidov & Poehling 2013; Rechner & Poehling 2014). This indicates that insect reactions to light treatments can be insect-specific.

To our knowledge, no study has compared the effects of short-wavelength light (such as UV-A) and longer wavelength light (violet to green in the visible spectrum) on plant growth, plant metabolic composition, and specialized herbivorous insects in protected horticultural production systems. In this study, we tested the hypothesis that different LED-generated light qualities (ranging from UV-A to green) can alter the growth as well as the metabolic composition (flavonoids and glucosinolates) of *Brassica oleracea* var. *italic* (broccoli) plants

and indirectly influence (via plant metabolites) the choice of host plant and performance of the cabbage aphid *B. brassicae*.

Materials and Methods

Rearing of insects

Cabbage aphids (*B. brassicae*) were collected outdoors from broccoli plants at the Leibniz University Hannover, Institute of Horticultural Production Systems, Section of Phytomedicine, Hannover (N 52° 23`39.22``, E 9° 42`18.86``). The aphids were reared on 4-week-old broccoli plants in a gauze cage with a wooden frame (85 cm × 60 cm × 60 cm); the cages were kept in a climate chamber (20 ± 2°C, relative humidity 65 ± 10%, photoperiod 16:8 h L:D). Every week, half of the plants in each cage were replaced to continuously provide a high quality food source. Adult aphids used in experiments were randomly collected from these cages.

Plant material and growth conditions

Broccoli plants [*B. oleracea* var. *italica*, cv Monopoly; F1 Hybrid; Syngenta Enkhuizen, Netherlands] were grown under specific light conditions (see Experimental layout and light treatments) from seeds in pots (12 cm diameter, 9 cm height, one seedling per pot) containing fertilized soil (Fruhstorfer Erde Type P, Hawita Gruppe, Vechta, Germany) for 4 weeks. Four-week-old broccoli plants were used for the experiments with aphids.

Experimental layout and light treatments

The experiments were conducted in a climate chamber (Viessmann, 4 m x 3 m x 2.40 m, Allendorf, Germany) with the following conditions: temperature 20 ± 2°C, relative humidity 70 ± 10%, and photoperiod 16:8 h L:D. The climate chamber contained five metal tables that were covered with black mulch film (PP-Gewebe, supplied by Raiffeisen GmbH, Bad Zwischenahn, Germany). On the tables in the chamber, 20 compartments (0.75 m x 0.3 m x 1 m) were separated by wooden frames covered with reflective mulch film (full metal on black film, supplied by Sunup Reflective Films/Star Metal Plating, Escondido, California USA) to prevent light interference from neighboring areas; the tops of the compartments were not closed or covered. The chamber was illuminated with 50 fluorescent tubes (Osram Lumilux Interna, L 58 W / T8, 840, 5200 lm, 4000 K, Munich, Germany), which were mounted 1 m above the tables to provide equal photon flux densities of photosynthetic active radiation (PAR).

Additional light treatments with specific narrow-bandwidth wavelengths were generated with hexagonal 1-W high-power single-chip LED emitters. For each LED illuminated compartment, two small aluminum plates (25 cm x 5 cm) were each equipped with three high-power LEDs. The aluminum plates were separated by 5 cm to ensure minimal shading for PAR radiation. The intensities of the high-power LEDs were regulated with rotary potentiometers by high-power LED drivers (LED-Slave, PWM Dimmer Onboard, PCB Components, Hildesheim, Germany). The LED panels were located 12 cm above the plants. The peak wavelengths of the LEDs were UV-A 365 nm (H2A1-H365-E), violet 420 nm (H2A1-H420), blue 470 nm (H2A1-H470), and green 515 nm (H2A1-H515). The LEDs were supplied by Roithner Laser Technik GmbH, Vienna, Austria. The radiation spectra of the LEDs and the light tubes were measured with a UV/VIS fiber and a compatible fiber optic spectrometer (AvaSpec 2048-2, supplied by AVANTES, Appeldoorn, The Netherlands) (Fig. 1). PAR was measured with a Licor LI-250-A light meter (Lincoln, Nebraska USA) and was adjusted to 100 ± 10 µmol m^{-2} s^{-1} in all treatments such that 50% was generated by violet, blue, or green LEDs (Table 1). The UV-A intensities were measured in W/m² and µW/cm², respectively, with an ALMEMO 2390-5 spectra radiometer (Ahlborn Mess- und Regelungstechnik GmbH, Holzkirchen, Germany) and light intensities were comparable among all treatments. Intensities were converted to photon flux density (µmol m^{-2} s^{-1}) based on the spectrum, Planck's constant, and Avogadro's number. For the UV-A treatment, the energy of the light source (kJ m^{-2} s^{-1}) was also determined so that the results could be compared with those of other studies (Table 1). Each light compartment contained six broccoli plants, and each light treatment was represented by four replicate light compartments. Thus, the climate chamber contained 120 plants (5 treatments including the control x 4 replicate compartments per treatment x 6 plants per compartment).

Tab. 1 Light intensities provided by the four light treatments with LEDs and the control. The background and control illumination was provided by Osram 840 fluorescent tubes

	Measured light intensities			
Light treatment	**PAR Osram 840 (400 – 700 nm) [µmol m^{-2} s^{-1}]**	**PAR LEDs (400 – 700 nm) [µmol m^{-2} s^{-1}]**	**UV-A (315 – 380 nm) [µmol m^{-2} s^{-1}]**	**UV-A (315 – 380 nm) [kJ m^{-2} d^{-1}]**
Control	100 ± 10	0	0	0
UV-A 365 nm	100 ± 10	0	**61 ± 3**	**11520**
Violet 420 nm	50 ± 5	50 ± 5	0	0
Blue 470 nm	50 ± 5	50 ± 5	0	0
Green 515 nm	50 ± 5	50 ± 5	0	0

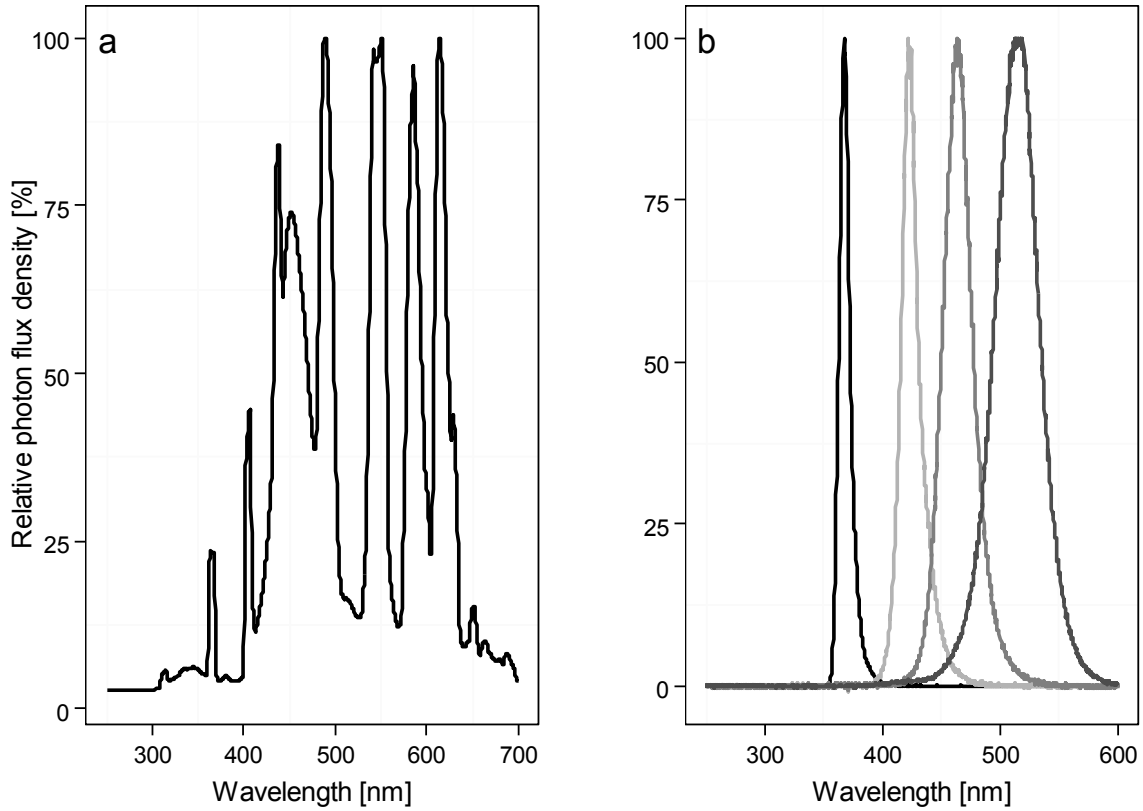

Fig. 1 Wavelength [nm] spectra and corresponding photon flux density [%] for (a) Osram 840 fluorescent tubes (Lumilux Interna L 58 W / T8, 840, 5200 lm, 4000 K) and for (b) hexagonal 1-W high-power single-chip LED emitters (H2A1-H365-E, H2A1-H420, H2A1-H470, H2A1-H515) used in the climate chamber. The spectra were measured from 200 to 800 nm in the dark with a UV/VIS fiber and a compatible fiber optic spectrometer (AvaSpec 2048-2, supplied by AVANTES, Appeldoorn, The Netherlands)

B. brassicae performance experiment

For determination of aphid performance, a brush was used to carefully place 12 *B. brassicae* adults on the underside of the second leaf of two of the six plants per light compartment. The leaf with aphids was enclosed in a 3-cm-diameter clip cage attached to the underside of the leaf. The adult aphids were allowed to deposit larvae for 24 h before they were removed. Twelve larvae were permitted to develop per plant (per clip cage). The number of days required for the larvae to develop into adults was recorded (developmental time), and the final weights of 10 adults per clip cage were measured with a microbalance (Type MC 5 Sartorius, Goettingen, Germany). Two adults per plant were kept separately in clip cages on the same plants (second and third leaf), and their offspring were counted every second day to measure fecundity [Md].

B. brassicae host selection experiment

Choice experiments were designed to investigate the behavioral response of *B. brassicae* to plants grown with the five light treatments. Plants were grown for 4 weeks under fluorescent tubes (Osram Lumilux Interna L 58 W / T8, 840, 5200 lm, 4000 K, control conditions) in the climate chamber and were additionally treated with UV-A 365 nm, violet 420 nm, blue 470 nm, or green 515 nm or received no additional light treatment. These plants were used for the host selection experiment, which was carried out under usual broad spectra illumination in the climate chamber and not under the specific light treatments to avoid direct and visual effects of the light treatments on the aphids' behavior. The experiment used three arenas. One release arena for *B. brassicae* was located in the middle, one arena for a plant leaf was located on the left side of the release arena, and one arena for another plant leaf was located on the right side of the release arena. All three arenas were connected by holes so that *B. brassicae* was able to walk between the leaves. The leaves were not detached from the plants. Twenty synchronized *B. brassicae* adults were released in the middle arena and always had the choice between the leaf of one light-induced and one control plant; these clipped leaves were randomly located in the left or the right arena. The choice experiment was run for 20 h so that sufficient numbers of aphids moved to the left or right arena (insufficient numbers had moved after 12 h). Each comparison of control leaf *vs.* light-induced leaf was represented by 10 replicate assays in each of two arena systems, resulting in the testing of a total of 200 *B. brassicae* per treatment.

Effects of light treatments on plant leaf number, height, and weight without aphid infestation

To determine how the five light treatments affected plant morphology, additional plants were grown without aphids for 4 weeks under the same conditions described above. A total of 16 4-week-old broccoli plants for each light treatment (four from each replicate compartment) were randomly selected and destructively sampled for biomass analysis. Leaves were counted, and plant height was measured from the main stem base to the top of the plants. After the harvested plants were kept at 65 °C for 5 days, their dry weights were determined with an electronic balance (Type BP 3100 P, Sartorius, Goettingen, Germany).

Sample preparation for metabolite analysis

For each light treatment, leaves were collected from eight 6-week-old broccoli plants (two per replicate compartment) that were infested or not infested with *B. brassicae*. Each infested plant had two infested leaves in clip cages as described earlier. The non-infested leaves were obtained from separate plants, i.e., plants without infestation. A mixed sample of all leaves per plant (excluding stems and midribs) was placed in liquid nitrogen, freeze-dried for 5 days (using a Christ Alpha 1-4 LSC freeze drier), and subsequently ground to a powder (≤ 0.25 mm).

Flavonoid analysis

Flavonoids were analyzed according to Schmidt et al. (2010) with modification. Lyophilized broccoli tissue (0.02 g) was extracted with 600 µl of 60% aqueous methanol on a magnetic stirrer plate for 40 min at 20°C. The extract was centrifuged at 4500 rpm for 10 min at the same temperature, and the supernatant was collected in a reaction tube. This process was repeated twice with 300 µl of 60% aqueous methanol for 20 min and 10 min, respectively; the three supernatants per sample were combined. The extract was subsequently evaporated until it was dry and was then suspended in 200 µl of 10% aqueous methanol. The extract was centrifuged at 3000 rpm for 5 min at 20°C through a Corning® Costar® Spin-X® plastic centrifuge tube filter (Sigma Aldrich Chemical Co., St. Louis, MO, USA) for HPLC analysis. Each extraction was carried out in duplicate.

Flavonoid composition (including hydroxycinnamic acid derivatives and glycosides of flavonols) and concentrations were determined using a series 1100 HPLC (Agilent Technologies, Waldbronn, Germany) equipped with a degaser, binary pump, autosampler,

column oven, and photodiode array detector. An Ascentis® Express F5 column (150 mm × 4.6 mm, 5 µm, Supelco) was used to separate the compounds at 25°C. Eluent A was 0.5% acetic acid, and eluent B was 100% acetonitrile. The gradient used for eluent B was 5-12% (0-3 min), 12-25% (3-46 min), 25-90% (46-49.5 min), 90% isocratic (49.5-52 min), 90-5% (52-52.7 min), and 5% isocratic (52.7-59 min). The determination was conducted at a flow rate of 0.85 ml min^{-1} and a wavelength of 320 nm, 330 nm, and 370 nm for hydroxycinnamic acid derivates, acylated flavonol glycosides, and non-acylated flavonol glycosides, respectively. The hydroxycinnamic acid derivatives and glycosides of flavonols were identified as deprotonated molecular ions and characteristic mass fragment ions according to Schmidt et al. (2010) by HPLC-DAD-ESI-MS^n using an Agilent series 1100 ion trap mass spectrometer in negative ionization mode. Nitrogen was used as the dry gas (10 L min^{-1}, 325°C) and the nebulizer gas (40 psi) with a capillary voltage of -3500 V. Helium was used as the collision gas in the ion trap. The mass optimization for the ion optics of the mass spectrometer for quercetin was performed at *m/z* 301 or arbitrarily at *m/z* 1000. The MS^n experiments were performed in auto up to HPLC-DAD-ESI-MS^3 in a scan from *m/z* 200-2000. Standards (chlorogenic acid, quercertin 3-glucoside, and kaempferol 3-glucoside; Roth, Karlsruhe, Germany) were used for external calibration curves. Results are presented as µg g^{-1} dry weight. Flavonol glycoside and hydroxycinnamic acid derivative concentrations were determined for four replicate light compartments per treatment with two broccoli plants per replicate compartment; each replicate sample was measured in duplicate.

Glucosinolate analysis

Glucosinolate concentration was determined as desulfo-glucosinolates using a modified method according to Wiesner et al. (2013). A 20.0-mg quantity of powdered sample plus 100 µl of 0.1 mM 2-propenyl glucosinolate (BCR-367R, Community Bureau of Reference, Brussels, Belgium) as the internal standard was extracted with 750 µl of 70% (v/v) methanol at 70°C. The preparation was boiled for 10 min and then centrifuged (2250 g) for 5 min at room temperature. The supernatant was decanted, and the residue was re-extracted twice with 500 µl of hot 70% methanol each time. The pooled extracts were loaded onto a mini column containing 500 µl of DEAD-Sephadex A-25 that had been conditioned with 2 M acetic acid and washed with 6 M imidazole formate. After loading, the column was washed with 0.02 M sodium acetate buffer. Finally, 75 µl of an aryl sulfatase solution (Sigma-Aldrich, Steinheim,

Germany) was added, and the preparation was incubated overnight. Desulfo-glucosinolates were eluted with water and analyzed by HPLC using a Merck HPLC system (Merck-Hitachi, Darmstadt, Germany) with a Spherisorb ODS2 column (Bischoff, Leonberg Germany; particle size 5 µm, 250 mm x 4 mm). HPLC conditions were as follows: solvent A, MilliQ water; solvent B, 20% v/v acetonitrile in MilliQ water; solvent C, 100% acetonitrile. The 60-min run consisted of 1% (v/v) B (2 min), 1% to 20% (v/v) B (34 min), a 6-min hold at 20% (v/v) B, 20% B to 100% (v/v) C (2 min), a 5-min hold at 100% (v/v) C, 100% (v/v) C to 1% (v/v) B (2 min), and finally a 10-min hold at 1% (v/v) B. Determination was conducted at a flow rate of 0.7 ml min^{-1} and a wavelength of 229 nm. Desulfo-glucosinolates were identified based on comparison of retention times and UV absorption spectra with those of known standards. Additionally, desulfo-glucosinolates were previously identified in other *Brassica* species by HPLC-ESI–MS^2 using Agilent 1100 series (Agilent Technologies, Waldbronn, Germany) in positive ionization mode (Krumbein et al. 2005; Zimmermann et al. 2007). Glucosinolate concentration was calculated using 2-propenyl glucosinolate as an internal standard and the response factor of each compound relative to 2-propenyl glucosinolate (Brown et al. 2003). Results are presented as µg g^{-1} dry weight. Glucosinolate concentration was determined in four replicate light compartments per treatment with two broccoli plants per replicate compartment; each replicate sample was measured in duplicate.

Statistical analysis

The data were analyzed in R 2.15.2 (R Development Core Team 2008). Graphs were made with the package ggplot2 (Wickham 2009). The effects of the light treatments on plant height, plant dry weight, and aphid adult weight were analyzed using generalized linear mixed models (GLMM) followed by Tukey *post hoc* tests (Pinheiro et al. 2012).

The effects of aphid infestation and light treatments on plant secondary metabolites (concentrations of flavonoids and glucosinolates) were also analyzed using GLMM and the package lsmeans by estimating least-squares means and differences of contrast. Differences between single light treatments and the control were subsequently determined with a Tukey *post hoc* test. Effects of light treatments were averaged over the two levels of infestation, and effects of infestation were averaged over the levels of variant. The total numbers of offspring and the developmental time of aphids as well as the leaf number per plant were analyzed by generalized linear models (GLM) using a log-link together with a quasi-Poisson distribution.

The effects of light treatments on aphid fecundity, aphid developmental time, and leaf number were assessed by Tukey *post hoc* tests.

The total numbers of *B. brassicae* that selected a control leaf *vs.* a narrow-bandwidth-treated leaf were analyzed by GLM with quasibinomial distribution. Pair-wise comparisons with control plants were carried out for each of the four narrow-bandwidth light treatments.

Results

Leaf number, plant height, and weight (without aphid infestation)

Leaf number per broccoli plant without aphid infestation was not significantly affected by the light treatments (Fig. 2a). Plant height was higher ($P < 0.01$) for plants treated with green light (515 nm) than for plants treated with UV-A light (365 nm), violet light (420 nm), or blue light (470 nm) (Fig. 2b). The dry weight of broccoli plants was not affected by the light treatments (Fig. 2c).

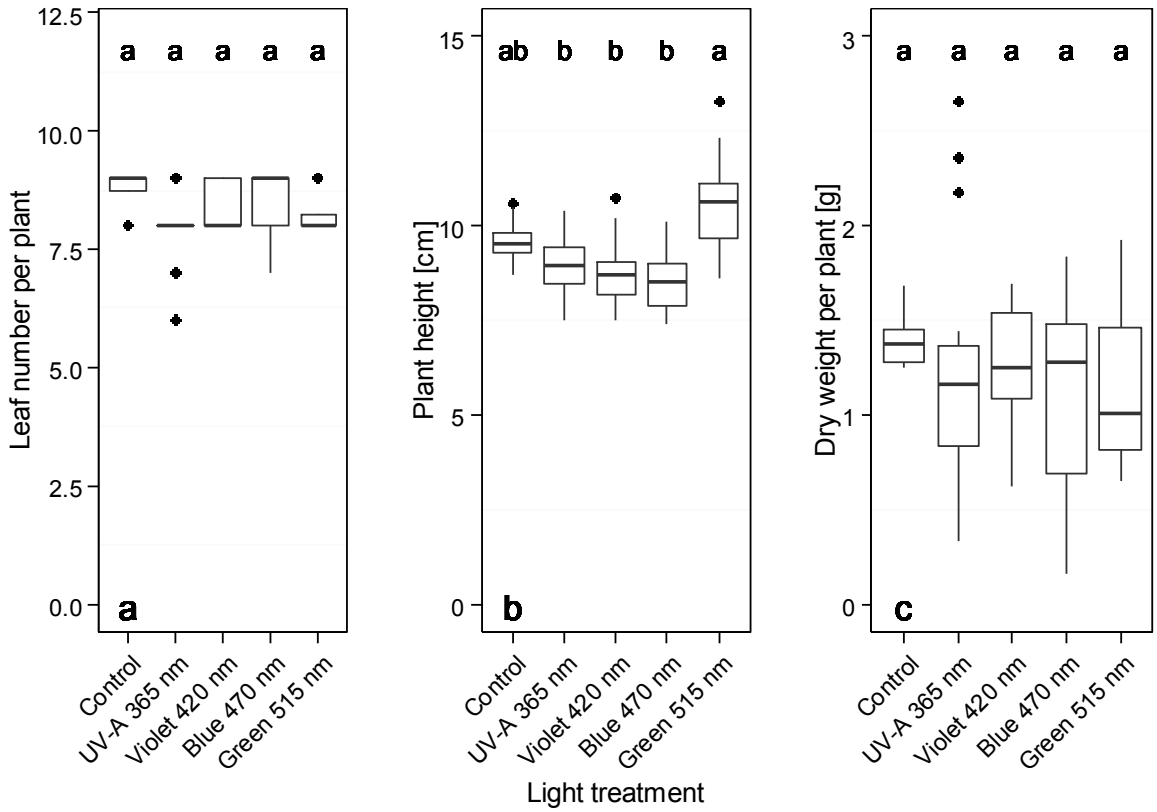

Fig. 2 Leaf number (a), plant height, (b) and plant dry weight (c) of 4-week-old broccoli plants that were grown in a climate chamber and exposed to control lighting plus one of four light treatments (UV-A 365 nm, violet 420 nm, blue 470 nm, or green 515 nm) or to control lighting without additional illumination. Additional illuminations were supplied by LEDs. Control

lighting was supplied by Osram Lumilux Interna L 58 W / T8, 840, 5200 lm, 4000 K fluorescent tubes (400 to 700 nm). Different letters indicate significant differences (GLM (Fig. 2a), GLMM (Fig. 2b, 2c), and Tukey *post hoc* tests at $P < 0.05$; $N = 16$ biological replicates)

Performance and Behavior of *B. brassicae*

Adult weight, fecundity, and developmental time of *B. brassicae* were not significantly affected by the light treatments (Fig. 3a–c). The selection of host plant by *B. brassicae* was influenced by the light treatments. Significantly more *B. brassicae* selected blue 470 nm-treated plants than UV-A 365 nm-treated plants ($P < 0.01$) or violet 420 nm-treated plants ($P < 0.01$) (Fig. 4). The green 515 nm-treated plants tended to be more attractive than control plants but the difference was not significant ($P < 0.11$).

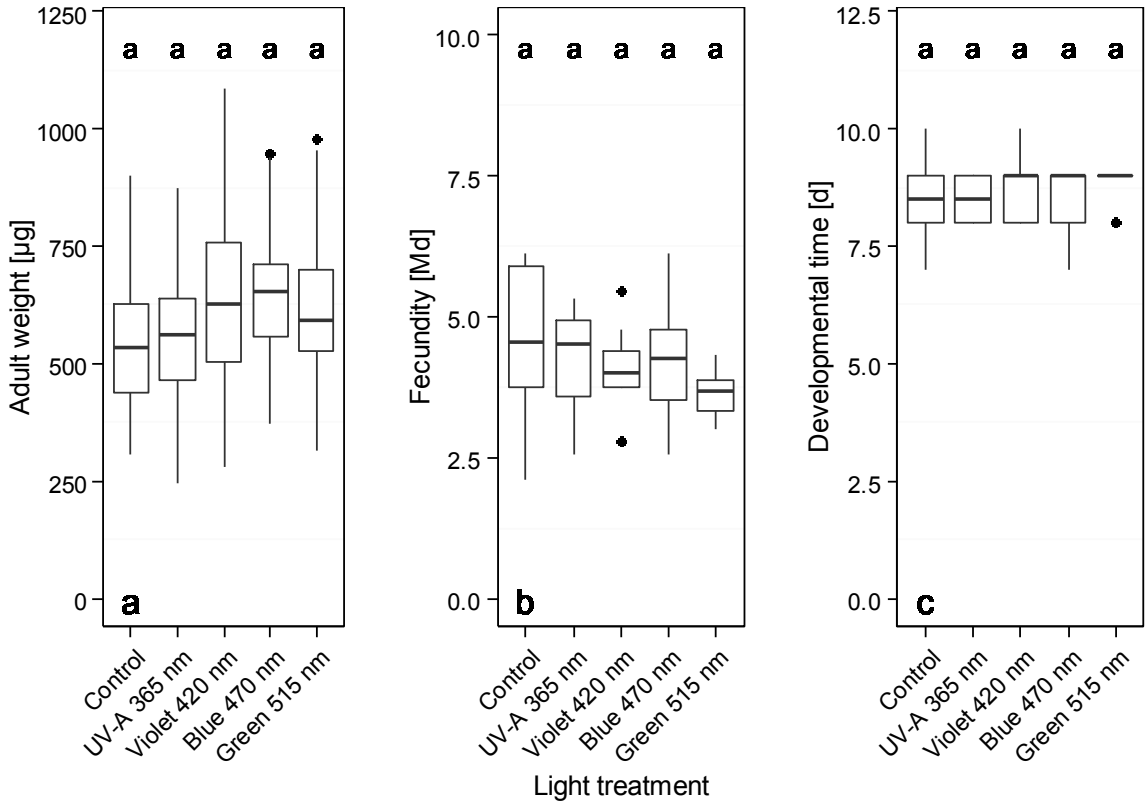

Fig. 3 Adult weight (a), fecundity, (b) and developmental time (c) of *B. brassicae* kept on broccoli plants that were grown in a climate chamber and exposed to control lighting plus one of four light treatments (UV-A 365 nm, violet 420 nm, blue 470 nm, or green 515 nm) or to control lighting without additional illumination. Additional illuminations were supplied by LEDs. Control lighting was supplied by Osram Lumilux Interna L 58 W / T8, 840, 5200 lm, 4000

K fluorescent tubes (400 to 700 nm). The aphids were in clip cages and were not directly exposed to the light treatments. Different letters indicate significant differences (GLMM (Fig. 3a), GLM (Fig. 3b, 3c), and Tukey *post hoc* tests, $P < 0.05$; $N = 8$ biological replicates)

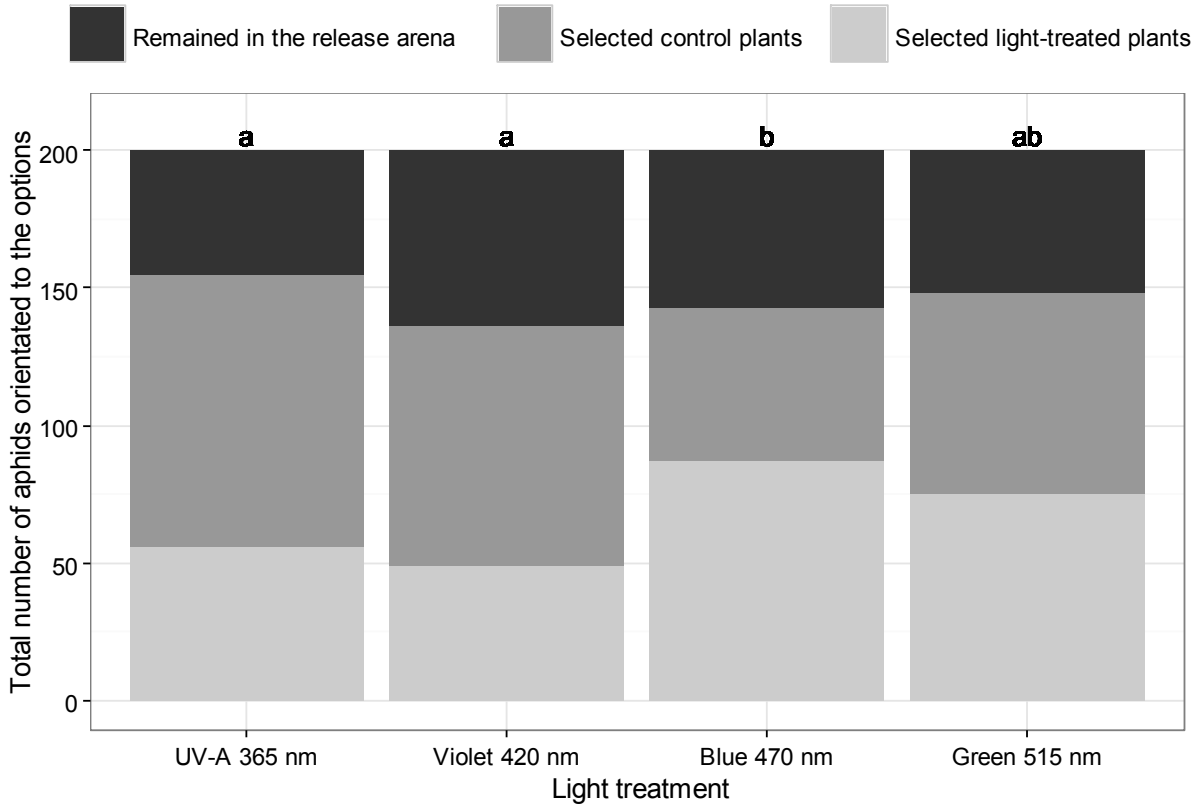

Fig. 4 Total number of *B. brassicae* adults preferring plants grown under different additional narrow-bandwidth LED treatments (UV-A 365 nm, violet 420 nm, blue 470 nm, or green 515 nm) or control plants that received only PAR light from Osram Lumilux Interna L 58 W / T8, 840, 5200 lm, 4000 K fluorescent tubes (400 to 700 nm). Different letters indicate significant differences between light treatments (GLM with quasibinomial distribution and pair-wise comparison of different light treatments, $P < 0.01$; $N = 20$ biological replicates)

Hydroxycinnamic acids

The following 12 hydroxycinnamic acid derivatives were detected in broccoli plants: caffeoylquinic acid (3-clorogenic acid), caffeoyl-glucoside, sinapoyl-gentiobiose, feruloyl-glucoside, sinapoyl-glucoside, sinapoyl-feruloyl-triglucoside, sinapoyl-feruloyl-gentiobiose (isomer), disinapoyl-gentiobiose, sinapoyl-feruloyl-gentiobiose, diferuloyl-gentiobiose, trisinapoyl-gentiobiose, and disinapoyl-feruloyl-gentiobiose. Among these, the six considered most relevant to the study are listed in Fig. 5 and Supplementary Table 1. Plants infested with

B. brassicae generally contained increased levels of the monosinaopyl sinapoyl-feruloyl-gentiobiose and of the polysinapoyls disinapoyl-gentiobiose, disinapoyl-feruloyl-gentiobiose, and trisinapoyl-gentiobiose.

The concentration of hydroxycinnamic acids was lowest when broccoli plants were exposed to UV-A light (365 nm) independent of *B. brassicae* infestation. Furthermore, quantities of the monosinapoyl sinapoyl-feruloyl-gentiobiose and of the polysinapoyls disinapoyl-gentiobiose, disinapoyl-feruloyl-gentiobiose, and trisinapoyl-gentiobiose did not differ among the control, violet 420 nm, blue 470, and green 515 nm treatments regardless of *B. brassicae* infestation (Fig. 5 and Supplementary Table 1).

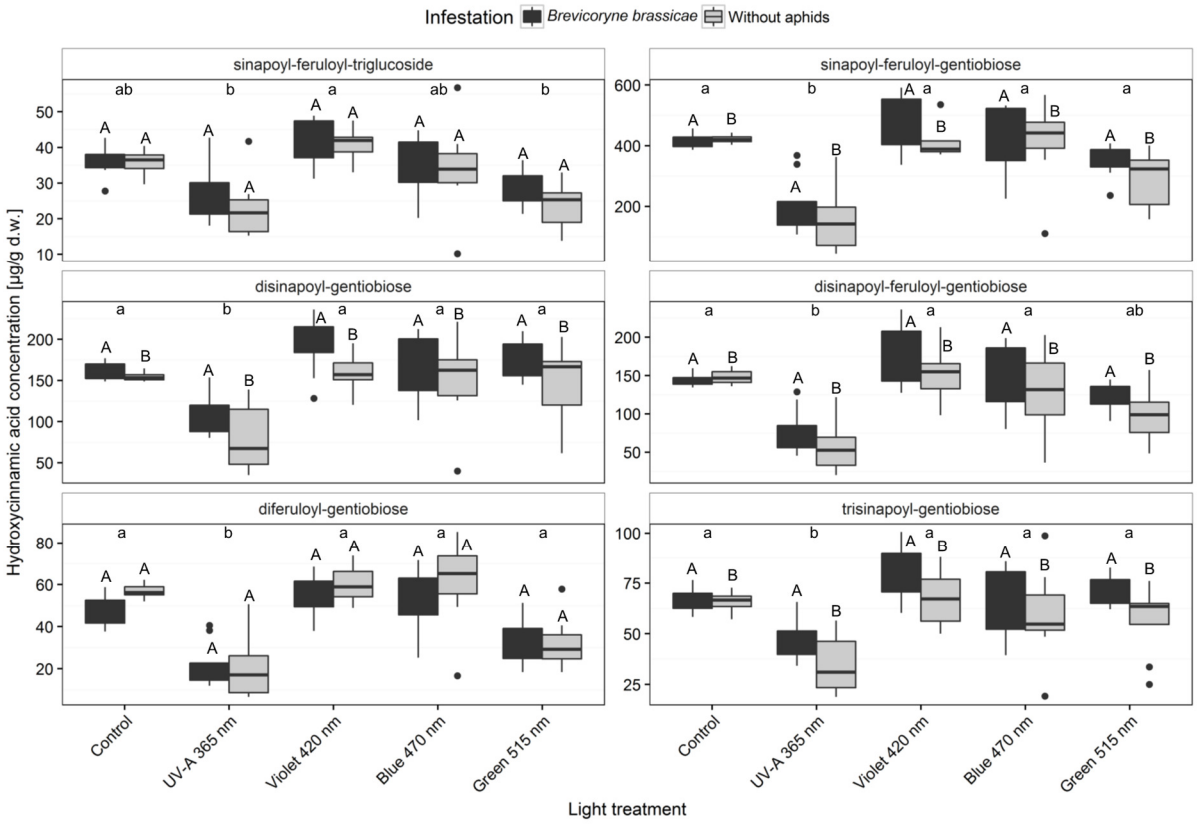

Fig. 5 Concentrations of the hydroxycinnamic acids sinapoyl-feruloyl-triglucoside, sinapoyl-feruloyl-gentiobiose, disinapoyl-gentiobiose, disinapoyl-feruloyl-gentiobiose, diferuloyl-gentiobiose, and trisinapoyl-gentiobiose in broccoli plants (infested or non-infested with *Brevicoryne brassicae*) grown in a climate chamber and exposed to control lighting with one of four light treatments (UV-A 365 nm, violet 420 nm, blue 470 nm, or green 515 nm) or to control lighting without additional illumination. UV-A 365 nm, violet 420 nm, blue 470 nm, and green 515 nm were supplied by LEDs. Control lighting was supplied by standard fluorescent tubes (400 to 700 nm). Uppercase letters indicate significant effects of aphid infestation within each light treatment averaged over the level of variant. Lowercase letters

indicate significant differences among light treatments averaged across infestation level (GLMM and Tukey *post hoc* tests, $P < 0.001$, $N = 8$ biological replicates)

Quercetin glycosides

The less complex non-acylated quercetin-3-*O*-sophoroside-7-*O*-glucoside and the complex diacylated quercetin-3-*O*-hydroxyferuloyl-sinapoyl-triglucoside-7-*O*-diglucoside were detected in the broccoli plants (Fig. 6 and Supplementary Table 2). *B. brassicae* infestation did not affect the concentrations of quercetin glycosides. The concentrations of quercetin glycosides were lowest in broccoli plants treated with UV-A 365 nm and green 515 nm. Concentrations of quercetin-3-*O*-hydroxyferuloyl-sinapoyl-triglucoside-7-*O*-diglucoside were significantly increased in plants treated with violet 420 nm regardless of *B. brassicae* infestation (Fig. 6 and Supplementary Table 2).

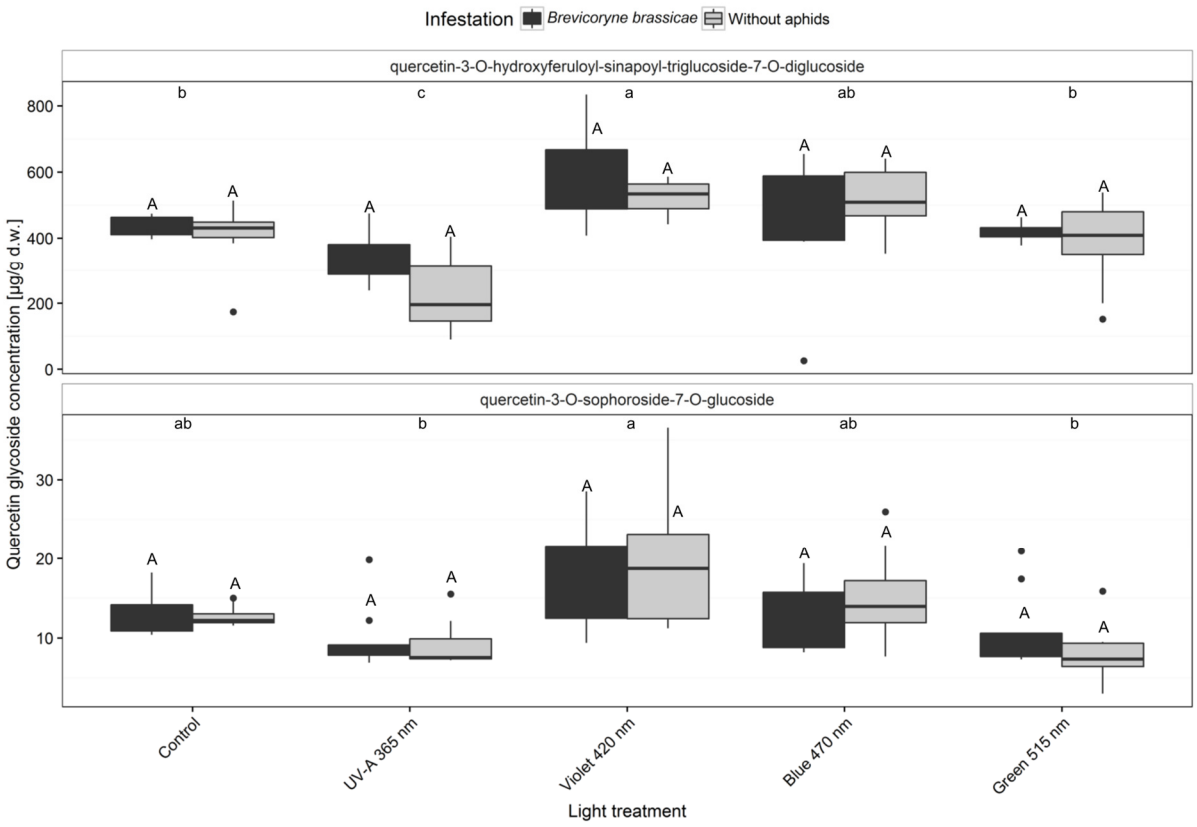

Fig. 6 Concentrations of the quercetin glycosides quercetin-3-*O*-hydroxyferuloyl-sinapoyl-triglucoside-7-*O*-diglucoside and quercetin-3-*O*-sophoroside-7-*O*-glucoside in broccoli plants (infested or non-infested with *Brevicoryne brassicae*) grown in a climate chamber and exposed to control lighting with one of four light treatments (UV-A 365 nm, violet 420 nm, blue 470 nm, or green 515 nm) or to control lighting without additional illumination. UV-A 365 nm,

violet 420 nm, blue 470 nm, and green 515 nm were supplied by LEDs. Control lighting was supplied by standard fluorescent tubes (400 to 700 nm). Uppercase letters indicate significant effects of aphid infestations averaged over the level of variant. Lowercase letters indicate significant differences among light treatments averaged over the level of infestation (GLMM and Tukey *post hoc* tests, $P < 0.001$, $N = 8$ biological replicates)

Kaempferol glycosides

The following 14 kaempferol glycosides were detected in broccoli plants: kaempferol-3-*O*-hydroxyferuloyl-sophoroside-7-*O*-glucoside, kaempferol-3-*O*-caffeoyl-sophoroside-7-*O*-glucoside, kaempferol-3-*O*-sinapoyl-sophoroside-7-*O*-glucoside, kaempferol-3-*O*-sinapoyl-sophoroside-7-*O*-diglucoside, kaempferol-3-*O*-feruloyl-sophoroside-7-*O*-glucoside, kaempferol-3-*O*-coumaroyl-sophoroside-7-*O*-glucoside, kaempferol-3-*O*-caffeoyl-sophoroside-7-*O*-glucoside (isomer), kaempferol-3-*O*-sinapoyl-hydroxyferuloyl-triglucoside-7-*O*-diglucoside, kaempferol-3-*O*-sinapoyl-caffeoyl-triglucoside-7-*O*-diglucoside, kaempferol-3-*O*-sinapoyl-feruloyl-triglucoside-7-*O*-diglucoside, kaempferol-3-*O*-sinapoyl-feruloyl-triglucoside-7-*O*-diglucoside (isomer), kaempferol-3-*O*-sophoroside-7-*O*-glucoside, kaempferol-3,7-*O*-diglucoside, and kaempferol-3-*O*-glucoside-7-*O*-diglucoside. Among these, eight structurally different compounds were considered most relevant to the study (Fig. 7 and Supplementary Table 3). *B. brassicae* infestation had no effect on the concentration of kaempferol glycosides, which are the main flavonoid glycosides in broccoli. The concentrations of kaempferol glycosides were lowest in UV-A 365 nm- and green 515 nm-treated plants regardless of *B. brassicae* infestation. The concentrations of the monoacylated triglycosides kaempferol-3-*O*-sinapoyl-sophoroside-7-*O*-glucoside, kaempferol-3-*O*-feruloyl-sophoroside-7-*O*-glucoside, kaempferol-3-*O*-caffeoyl-sophoroside-7-*O*-glucoside, and kaempferol-3-*O*-coumaroyl-sophoroside-7-*O*-glucoside were significantly increased in broccoli plants treated with violet 420 nm light. Treatment with blue 470 nm light significantly increased concentrations of kaempferol-3-*O*-coumaroyl-sophoroside-7-*O*-glucoside compared with plants grown under control, UV-A 365 nm, or green 515 nm light conditions regardless of *B. brassicae* infestation. Concentrations of kaempferol-3-*O*-feruloyl-sophoroside-7-*O*-glucoside and kaempferol-3-*O*-caffeoyl-sophoroside-7-*O*-glucoside were higher in broccoli plants treated with additional blue 470 nm light (Fig. 7 and Supplementary Table 3).

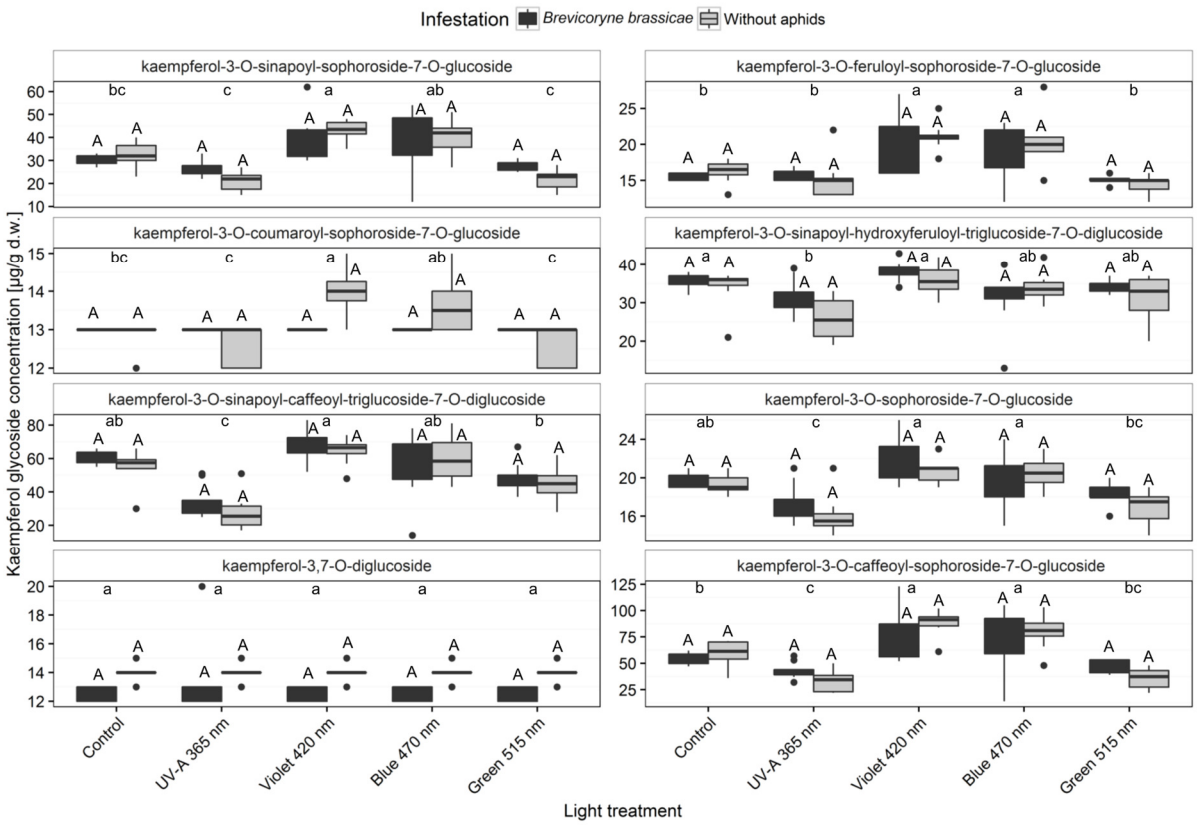

Fig. 7 Concentrations of the kaempferol glycosides kaempferol-3-*O*-sinapoyl-sophoroside-7-*O*-glucoside, kaempferol-3-*O*-feruloyl-sophoroside-7-*O*-glucoside, kaempferol-3-*O*-coumaroyl-sophoroside-7-*O*-glucoside, kaempferol-3-*O*-sinapoyl-hydroxyferuloyl-triglucoside-7-*O*-diglucoside, kaempferol-3-*O*-sinapoyl-caffeoyl-triglucoside-7-*O*-diglucoside, kaempferol-3-*O*-sophoroside-7-*O*-glucoside, kaempferol-3,7-*O*-diglucoside, and kaempferol-3-*O*-caffeoyl-sophoroside-7-*O*-glucoside in broccoli plants (infested or non-infested with *Brevicoryne brassicae*) grown in a climate chamber and exposed to control lighting with one of four light treatments (UV-A 365 nm, violet 420 nm, blue 470 nm, or green 515 nm) or to control lighting without additional illumination. UV-A 365 nm, violet 420 nm, blue 470 nm, and green 515 nm were supplied by LEDs. Control lighting was supplied by standard fluorescent tubes (400 to 700 nm). Uppercase letters indicate significant effects of aphid infestations averaged over the level of variant. Lowercase letters indicate significant differences among light treatments averaged over infestation level (GLMM and Tukey *post hoc* tests, $P < 0.001$, $N = 8$ biological replicates)

Aliphatic glucosinolates

Three aliphatic glucosinolates (4-methylthiobutyl, 3-methylsulfinylpropyl, and 4-methylsulfinylbutyl) were quantified in the broccoli leaves in all treatments (Fig. 8 and Supplementary Table 4). The predominant aliphatic glucosinolate was 4-methylsulfinylbutyl.

Concentrations of all aliphatic glucosinolates were higher in *B. brassicae*-infested plants than in non-infested plants. Regardless of *B. brassicae* infestation, the concentration of 4-methylthiobutyl glucosinolate was increased by violet 420 nm light. Concentrations of the methylsulfinylalkyl glucosinolates 3-methylsulfinylpropyl and 4-methylsulfinylbutyl were also increased by blue light both without and with *B. brassicae* infestation, but the values were not significantly different from those of the control. UV-A 365 nm treatment together with *B. brassicae* infestation caused the largest increase in the concentration of methylthiobutyl glucosinolate (Fig. 8 and Supplementary Table 4).

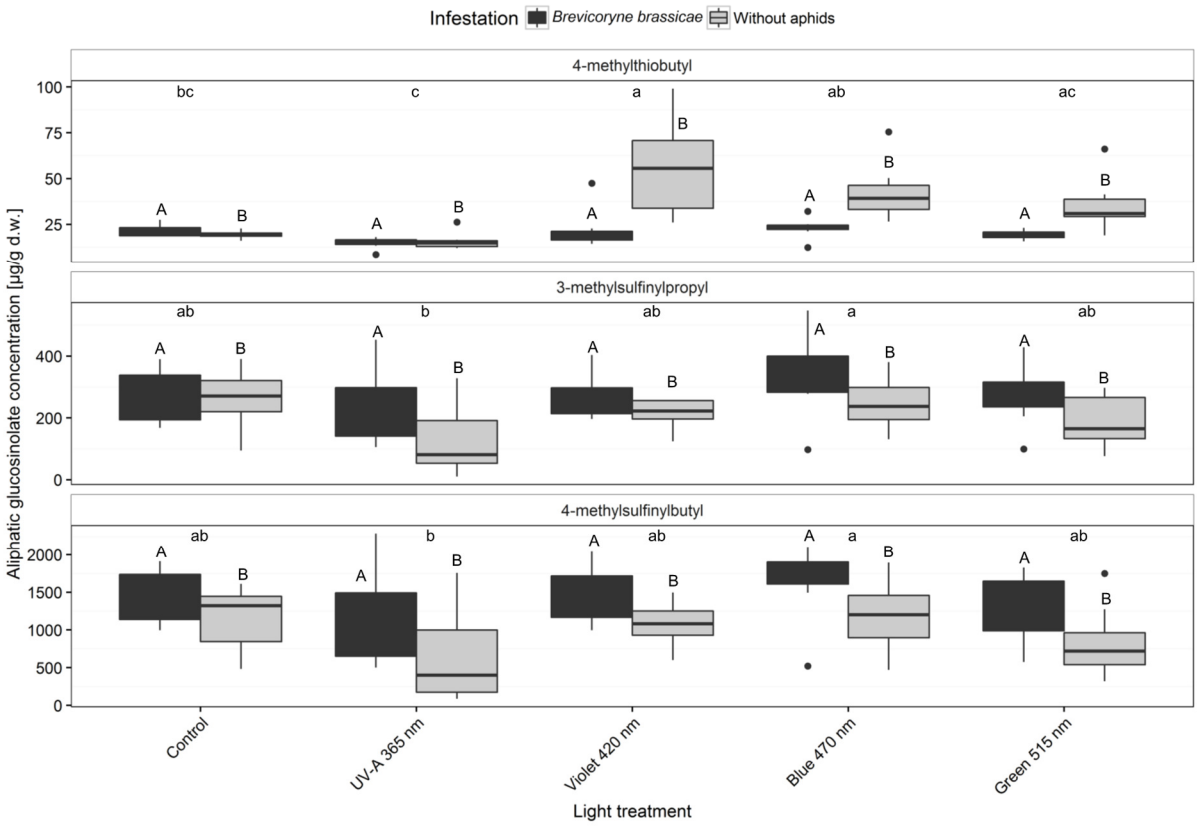

Fig. 8 Concentrations of the aliphatic glucosinolates 4-methylthiobutyl, 3-methylsulfinylpropyl, and 4-methylsulfinylbutyl in broccoli plants (infested or non-infested with *Brevicoryne brassicae*) grown in a climate chamber and exposed to control lighting with one of four light treatments (UV-A 365 nm, violet 420 nm, blue 470 nm, or green 515 nm) or to control lighting without additional illumination. UV-A 365 nm, violet 420 nm, blue 470 nm, and green 515 nm were supplied by LEDs. Control lighting was supplied by standard fluorescent tubes (400 to 700 nm). Uppercase letters indicate significant effects of aphid infestations averaged over the level of variant. Lowercase letters indicate significant differences among light treatments averaged over infestation level (GLMM and Tukey *post hoc* tests, $P < 0.001$, $N = 8$ biological replicates)

Indole glucosinolates

Four indole glucosinolates (3-indolylmethyl, 4-hydroxy-3-indolylmethyl, 4-methoxy-3-indolylmethyl, and 1-methoxy-3-indolylmethyl) were quantified in the broccoli leaves in all treatments (Fig. 9 and Supplementary Table 5). Regardless of light treatment, *B. brassicae* infestation increased concentrations of all indole glucosinolates. Concentrations of the 3-indolylmethyl glucosinolate were significantly increased by the UV-A 365 nm treatment, particularly with *B. brassica* infestation. The concentration of its methoxylated forms, 4-methoxy-3-indolylmethyl glucosinolate and 1-methoxy-3-indolylmethyl glucosinolate, tended to be increased by UV-A 365 nm treatment. The concentration of 4-hydroxy-3-indolylmethyl glucosinolate was significantly increased by violet 420 nm treatment (Fig. 9 and Supplementary Table 5).

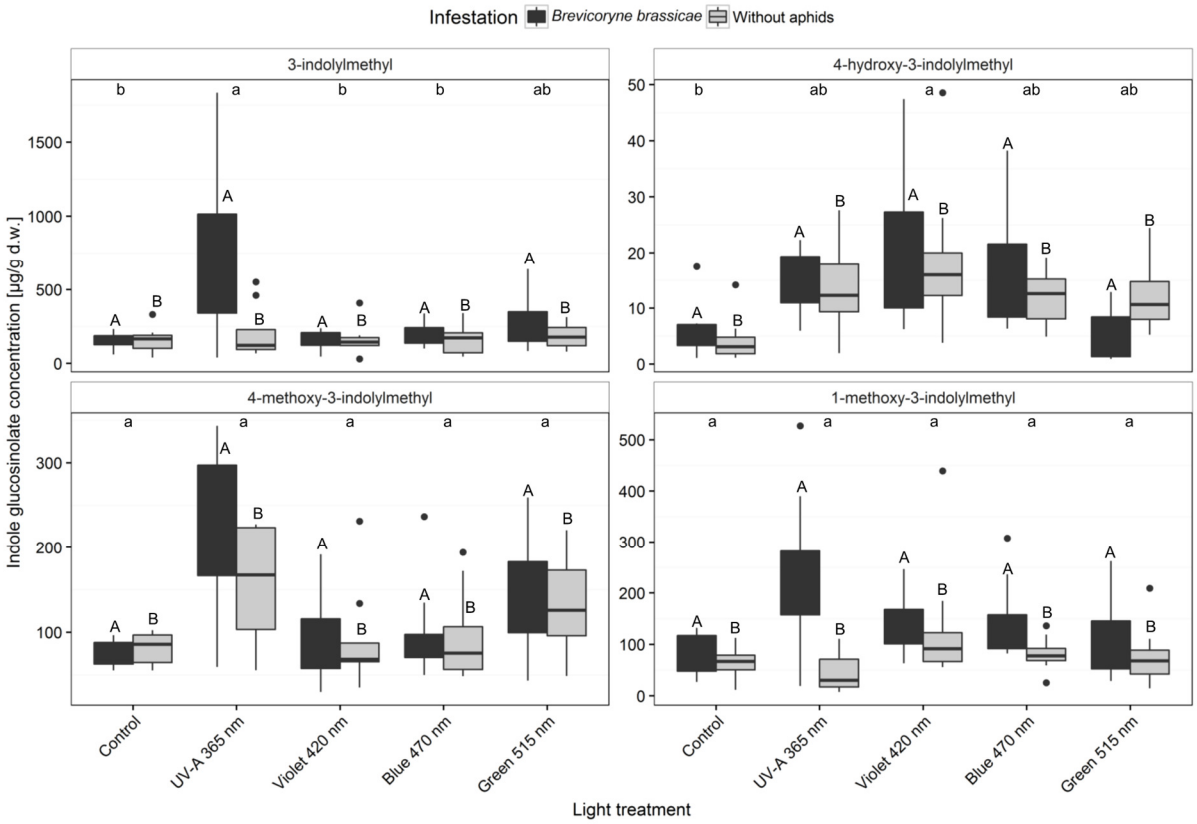

Fig. 9 Concentrations of the indole glucosinolates 3-indolylmethyl, 4-hydroxy-3-indolylmethyl, 4-methoxy-3-indolylmethyl, and 1-methoxy-3-indolylmethyl in broccoli plants (infested or non-infested with *Brevicoryne brassicae*) grown in a climate chamber and exposed to control lighting with one of four light treatments (UV-A 365 nm, violet 420 nm, blue 470 nm, or green 515 nm) or to control lighting without additional illumination. UV-A 365 nm, violet 420 nm, blue 470 nm, and green 515 nm were supplied by LEDs. Control lighting was supplied by

standard fluorescent tubes (400 to 700 nm). Uppercase letters indicate significant effects of aphid infestation averaged over the level of variant. Lowercase letters indicate significant differences among light treatments averaged over infestation level (GLMM and Tukey *post hoc* tests, $P < 0.001$, $N = 8$ biological replicates)

Discussion

The present study investigated the effects of different narrow-bandwidths of light on the growth of broccoli plants, on the concentrations of glucosinolates and flavonol glycosides in the plants, and on the interaction between the plants and the aphid *B. brassicae*, which is a specialized herbivore of *Brassica* spp. We were particularly interested in comparing the effects of short-wavelength light (UV-A) with longer PAR wavelength light (violet, blue, and green).

Broccoli plants grown under additional green light (50 µmol m^{-2} s^{-1}) in our study were significantly taller than plants grown under UV-A, violet, or blue light treatments (Fig. 2). Plant responses to green light are typically low-light responses that may help plants grow when under foliage or when near other plants. From a plant perspective, it makes sense to grow taller so as to avoid shade in areas with higher green light intensities (Wang & Folta 2013). Johkan et al. (2012) reported that lettuce plant growth was increased under additional high-intensity green LED light (300 µmol m^{-2} s^{-1}) with a peak wavelength of 510 nm. In the current study, broccoli plant leaf number and dry weight were unaffected by the light treatments (Fig. 2). Fan et al. (2013), in contrast, found that Chinese cabbage plants weighed more and were shorter when treated with blue 460 nm LED light than with green 520 nm LED light with intensities of 150 µmol m^{-2} s^{-1}. These differences between studies demonstrate that the effect of light of different wavelengths can be species-specific.

The LED light treatments had no indirect effect via secondary metabolite composition of the broccoli plant on the performance (adult weight, fecundity, and developmental time) of *B. brassicae* (Fig. 3). However, shorter wavelengths with a higher amount of energy (such as UV-B treatments) have been previously shown to increase the concentrations of kaempferol glycosides and indole glucosinolates (3-indolylmethyl and 4-methoxy-3-indolylmethyl) and to reduce the fecundity of *B. brassicae* on broccoli plants (Rechner & Poehling 2014; Rechner et al. 2016; Kuhlmann & Müller 2010). In another study, UV-A treatments reduced the reproduction of soybean aphids (Burdick et al. 2015). Illumination of Brussels sprout plants

with additional LED-generated UV-A radiation (259 kJ m^{-2} d^{-1}) in a greenhouse increased the concentrations of 3-indolylmethyl glucosinolate in the plants and decreased the fecundity of *B. brassicae* relative to blue light-treated plants (Acharya et al. 2016). In the latter study, 3-indolylmethyl glucosinolate concentrations were as high as 2304 µg g^{-1} d.w. in UV-A-treated Brussels sprout plants. The concentrations in the latter study were clearly higher than those in the broccoli plants (207 µg g^{-1} d.w.) that were treated with additional UV-A radiation in a climate-chamber in the present study (Fig. 9). This difference might be explained by a plant species-specific difference in sensitivity to UV-A treatments or to a dose-response reaction.

Although some defense compounds increased, i.e., quercetin-3-*O*-hydroxyferuloyl-sinapoyl-triglucoside-7-*O*-diglucoside and mono-acylated triglycosides of kaempferol, were increased by violet light, the concentrations in the present study were quite low and did not significantly affect aphid performance (Figs. 6 and 7). Broccoli plants in a previous study that were grown under UV-B treatments contained up to 4100 µg g^{-1} d.w. of single kaempferol glycosides such as kaempferol-3-*O*-caffeoyl-sophoroside-7-*O*-glucoside (Rechner et al. 2016), while broccoli plants in the present study contained low concentrations (< 100 µg g^{-1} d.w.) of all specific kaempferol glycosides even though the illumination times, light intensities, and plant stages were the same in both studies (Fig. 7). In experiments with Chinese cabbage, Kim et al. (2015) also detected very low concentrations of quercetin and kaempferol glycosides (< 10 µg g^{-1} d.w.) after 12 days of illumination with blue, red, or white LEDs.

Plant choice by aphids was indirectly affected by the light treatments in the present study, i.e., significantly more aphids selected plants that had been grown with additional blue light rather than with control light (Fig. 4). There was also a non-statistically significant tendency for aphids to prefer plants that had been grown with additional green light rather than with control light, but the aphids showed no preference for plants that had been grown with additional UV-A or violet light (Fig. 4). This behavior could only be partly explained by increasing amounts of secondary plant metabolites, because the only enhanced compound was 3-indolylmethyl glucosinolate, which had significantly higher concentrations in plants exposed to UV-A than in plants exposed to blue and violet light treatments. The green peach aphid *Myzus persicae*, on the other hand, preferred Chinese cabbage plants with reduced concentrations of glucosinolates, indicating that secondary plant metabolites could affect host selection by

aphids (Cao et al. 2016). The indirect effects of light quality on host selection by aphids warrants additional study.

In the choice experiment in the current study, the aphids were able to switch between the two plants after unsuccessful probing on one plant or after determining that one plant was a better nutrient source than the other. Probing by the aphid *Sitobion avenae* was reduced on plants that were treated with enhanced UV-B irradiation as indicated by less phloem ingestion and fewer aphids reaching the sustained phloem ingestion phase (Hu et al. 2013). Host selection by aphids could also be influenced by host volatiles (Stam et al. 2014) or by visual cues (Döring 2014). To separate between visual or olfactory cues and probing behavior-induced differences in host selection, it would be helpful to conduct olfactory experiments in which aphids did not directly contact the plant.

Blue light can increase the chlorophyll content per leaf area and the photosynthetic rate, resulting in better primary plant metabolism (Hernandez & Kubota 2016; Huche-Thelier et al. 2016). This could cause host plants to be more attractive to aphids, at least after the initial probing by the aphids. Future studies on host choice by aphids should include the alteration of primary metabolites in the phloem sap.

The light quality of the background spectra can also modify the metabolic composition of a plant, and high PAR intensities with a high amount of blue light can improve photosynthetic performance and acclimatization to and recovery from UV irradiation (Hofmann et al. 2015; Huche-Thelier et al. 2016). The background spectrum contained more blue light but less red light in the current study (Fig. 1) than in our previous study (Rechner et al. 2016), although the light intensity was the same (100 µmol m^{-2} s^{-1} PAR) in both studies. The induction of secondary metabolites by PAR may provide a basic level of UV protection that is optimized and increased by UV-B and UV-A radiation (Götz et al. 2010). Concentrations of secondary plant metabolites (such as glucosinolates and flavonol glycosides) in broccoli plants grown in a climate chamber with specific UV-B, UV-A, and violet light treatments were greater with more red light in the background spectrum (as in Rechner et al. 2016) than with more blue light in the background spectrum (as in the current study). This could partially explain the differences in aphid performance between these two studies, but the effect of the background spectrum was not investigated in detail in either study. Shorter wavelength (UV-A) light as well as longer PAR wavelength (violet to green) light in combination with a blue background spectrum were

unable to sufficiently alter the concentrations of glucosinolates and flavonol glycosides so as to reduce the performance of *B. brassicae* on broccoli plants in the present study. Future studies should carefully consider the effect of differences in background light quality and quantity.

In conclusion, this study has demonstrated that similar intensities of narrow-bandwidth light treatments in addition to PAR can alter the concentration of specific secondary metabolites in broccoli plants. The concentrations of flavonol glycosides and glucosinolates in this study were quite low and did not affect the performance of the specialized aphid *B. brassicae*. Host choice by *B. brassicae* was indirectly influenced by the narrow-bandwidth light treatments in that the aphid preferred blue light-illuminated plants (but not UV-A-, violet-, or green-illuminated plants) to control plants.

In future studies, insect feeding assays should be used to determine the concentrations of primary or secondary plant metabolites necessary to influence host choice and population increase of target herbivores. Future studies should also investigate whether higher LED intensities, optimal illumination times, and combinations of light qualities can increase the concentrations of secondary plant metabolites so as to protect greenhouse cultured plants against insect herbivores.

Acknowledgments

This research project was funded by the German Research Foundation DFG, grant Po 207/39-1.

Supplementary Material

Tab. 1 Concentrations [µg g^{-1} d.w.; mean (± SE)] of hydroxycinnamic acids in broccoli plants (non-infested, or infested with *B. brassicae*) grown in a climate chamber and exposed to control lighting plus one of four light treatments (UV-A 365 nm, violet 420 nm, blue 470 nm and green 515 nm) or to control lighting without additional illumination. UV-A 365 nm, violet 420 nm, blue 470 nm and green 515 nm were supplied by LEDs. Control lighting was supplied by standard fluorescent tubes (400 to 700 nm), Osram Lumilux Interna L 58 W / T8, 840, 5200 lm, 4000 K tubes

Hydroxycinnamic acids	**Light treatment and concentration of hydroxycinnamic acids**				
Without aphid infestation	**Control**	**UV-A 365 nm**	**Violet 420 nm**	**Blue 470 nm**	**Green 515 nm**
Sin-Fer-Triglc$^{(A)}$	36 ± 4$^{(ab)}$	23 ± 9$^{(b)}$	41 ± 4$^{(a)}$	34 ± 13$^{(ab)}$	24 ± 6$^{(b)}$
Sin-Fer-Gent$^{(B)}$	422 ± 15$^{(a)}$	155 ± 108$^{(b)}$	409 ± 54$^{(a)}$	410 ± 54$^{(a)}$	292 ± 93$^{(a)}$
Disin-Gent$^{(B)}$	155 ± 5$^{(a)}$	80 ± 42$^{(b)}$	161 ± 24$^{(a)}$	150 ± 53$^{(a)}$	145 ± 51$^{(a)}$
Disin-Fer-Gent$^{(B)}$	148 ± 10$^{(a)}$	57 ± 33$^{(b)}$	151 ± 36$^{(a)}$	130 ± 55$^{(a)}$	100 ± 35$^{(ab)}$
Difer-Gent$^{(A)}$	57 ± 3$^{(a)}$	20 ± 14$^{(b)}$	60 ± 9$^{(a)}$	61 ± 21$^{(a)}$	32 ± 12$^{(a)}$
Trisin-Gent$^{(B)}$	66 ± 6$^{(a)}$	35 ± 15$^{(b)}$	68 ± 15$^{(a)}$	59 ± 23$^{(a)}$	57 ± 18$^{(a)}$
With *B. brassicae* infestation					
Sin-Fer-Triglc$^{(A)}$	36 ± 5$^{(ab)}$	27 ± 9$^{(b)}$	42 ± 6$^{(a)}$	36 ± 8$^{(ab)}$	29 ± 6$^{(b)}$
Sin-Fer-Gent$^{(A)}$	415 ± 26$^{(a)}$	196 ± 100$^{(b)}$	468 ± 92$^{(a)}$	435 ± 113$^{(a)}$	352 ± 57$^{(a)}$
Disin-Gent$^{(A)}$	160 ± 12$^{(a)}$	108 ± 25$^{(b)}$	192 ± 36$^{(a)}$	171 ± 41$^{(a)}$	174 ± 26$^{(a)}$
Disin-Fer-Gent$^{(A)}$	144 ± 8$^{(a)}$	76 ± 31$^{(b)}$	174 ± 40$^{(a)}$	146 ± 47$^{(a)}$	123 ± 18$^{(ab)}$
Difer-Gent$^{(A)}$	48 ± 7$^{(a)}$	21 ± 11$^{(b)}$	55 ± 11$^{(a)}$	54 ± 15$^{(a)}$	32 ± 11$^{(a)}$
Trisin-Gent$^{(A)}$	67 ± 6$^{(a)}$	46 ± 10$^{(b)}$	78 ± 14$^{(a)}$	65 ± 18$^{(a)}$	71 ± 8$^{(a)}$

Uppercase letters indicate significant effects of aphid infestation averaged over the level of variant. Lowercase letters indicate significant differences of light treatments averaged over the level of infestation (GLMM and Tukey *post hoc* tests, $P < 0.001$, $N = 8$ biological replicates). Sin-Fer-Triglc: sinapoyl-feruloyl-triglucoside, Sin-Fer-Gent: sinapoyl-feruloyl-gentiobiose, Disin-Gent: disinapoyl-gentiobiose, Disin-Fer-Gent: disinapoyl-feruloyl-gentiobiose, Difer-Gent: diferuloyl-gentiobiose, Trisin-Gent: trisinapoyl-gentiobiose.

Tab. 2 Concentrations [µg g^{-1} d.w.; mean (± SE)] of quercetin glycosides in broccoli plants (non-infested, or infested with *B. brassicae*) grown in a climate chamber and exposed to control lighting plus one of four light treatments (UV-A 365 nm, violet 420 nm, blue 470 nm and green 515 nm) or to control lighting without additional illumination. UV-A 365 nm, violet 420 nm, blue 470 nm and green 515 nm were supplied by LEDs. Control lighting was supplied by standard fluorescent tubes (400 to 700 nm), Osram Lumilux Interna L 58 W / T8, 840, 5200 lm, 4000 K tubes

Quercetin glycosides	**Light treatment and concentration of quercetin glycosides**				
Without aphid infestation	**Control**	**UV-A 365 nm**	**Violet 420 nm**	**Blue 470 nm**	**Green 515 nm**
Q-3-hfer-sin-triglc-7-diglc$^{(A)}$	407 ± 102$^{(b)}$	225 ± 118$^{(c)}$	525 ± 51$^{(a)}$	517 ± 96$^{(ab)}$	387 ± 141$^{(b)}$
Q-3-soph-7-glc$^{(A)}$	13 ± 1$^{(ab)}$	9 ± 3$^{(b)}$	20 ± 9$^{(a)}$	15 ± 6$^{(ab)}$	8 ± 3$^{(b)}$
With *B. brassicae* infestation					
Q-3-hfer-sin-triglc-7-diglc$^{(A)}$	438 ± 30$^{(b)}$	340 ± 76$^{(c)}$	576 ± 147$^{(a)}$	471 ± 203$^{(ab)}$	419 ± 27$^{(b)}$
Q-3-soph-7-glc$^{(A)}$	13 ± 3$^{(ab)}$	10 ± 4$^{(b)}$	17 ± 7$^{(a)}$	13 ± 5$^{(ab)}$	11 ± 5$^{(b)}$

Uppercase letters indicate significant effects of aphid infestations averaged over the level of variant. Lowercase letters indicate significant differences of light treatments averaged over the level of infestation (GLMM and Tukey *post hoc* tests, $P < 0.001$, $N = 8$ biological replicates). Q-3-hfer-sin-triglc-7-diglc: quercetin-3-*O*-hydroxyferuloyl-sinapoyl-triglucoside-7-*O*-diglucoside, Q-3-soph-7-glc: quercetin-3-*O*-sophoroside-7-*O*-glucoside.

Tab. 3 Concentrations [µg g^{-1} d.w.; mean (± SE)] of kaempferol glycosides in broccoli plants (non-infested, or infested with *B. brassicae*) grown in a climate chamber and exposed to control lighting plus one of four light treatments (UV-A 365 nm, violet 420 nm, blue 470 nm and green 515 nm) or to control lighting without additional illumination. UV-A 365 nm, violet 420 nm, blue 470 nm and green 515 nm were supplied by LEDs. Control lighting was supplied by standard fluorescent tubes (400 to 700 nm), Osram Lumilux Interna L 58 W / T8, 840, 5200 lm, 4000 K tubes

Kaempferol glycosides	**Light treatment and concentrations of kaempferol glycosides**				
Without aphid infestation	**Control**	**UV-A 365 nm**	**Violet 420 nm**	**Blue 470 nm**	**Green 515 nm**
K-3-sin-soph-7-glc$^{(A)}$	33 ± 5$^{(bc)}$	21 ± 4$^{(c)}$	43 ± 4$^{(a)}$	40 ± 8$^{(ab)}$	22 ± 4$^{(c)}$
K-3-fer-soph-7-glc$^{(A)}$	16 ± 2$^{(b)}$	15 ± 3$^{(b)}$	21 ± 2$^{(a)}$	20 ± 4$^{(a)}$	14 ± 1$^{(b)}$
K-3-cou-soph-7-glc$^{(A)}$	13 ± 1$^{(bc)}$	13 ± 1$^{(c)}$	14 ± 1$^{(a)}$	14 ± 1$^{(ab)}$	13 ± 1$^{(c)}$
K-3-sin-hfer-triglc-7-diglc$^{(A)}$	34 ± 5$^{(a)}$	26 ± 6$^{(b)}$	36 ± 4$^{(a)}$	34 ± 4$^{(ab)}$	31 ± 7$^{(ab)}$
K-3-sin-caf-triglc-7-diglc$^{(A)}$	55 ± 11$^{(ab)}$	28 ± 11$^{(c)}$	64 ± 8$^{(a)}$	60 ± 13$^{(ab)}$	45 ± 10$^{(b)}$
K-3-soph-7-glc$^{(A)}$	19 ± 1$^{(ab)}$	16 ± 2$^{(c)}$	21 ± 1$^{(a)}$	21 ± 2$^{(a)}$	17 ± 2$^{(bc)}$
K-3-7-diglc$^{(A)}$	14 ± 1$^{(a)}$	15 ± 1$^{(a)}$	14 ± 1$^{(a)}$	15 ± 1$^{(a)}$	16 ± 1$^{(a)}$
K-3-caf-soph-7-glc$^{(A)}$	33 ± 5$^{(b)}$	21 ± 4$^{(c)}$	43 ± 4$^{(a)}$	40 ± 8$^{(a)}$	21 ± 4$^{(bc)}$
With *B. brassicae* infestation					
K-3-sin-soph-7-glc$^{(A)}$	30 ± 2$^{(bc)}$	26 ± 4$^{(c)}$	41 ± 10$^{(a)}$	38 ± 14$^{(ab)}$	28 ± 2$^{(c)}$
K-3-fer-soph-7-glc$^{(A)}$	16 ± 1$^{(b)}$	16 ± 1$^{(b)}$	2 ± 4$^{(a)}$	19 ± 4$^{(a)}$	15 ± 1$^{(b)}$
K-3-cou-soph-7-glc$^{(A)}$	13 ± 1$^{(bc)}$	13 ± 1$^{(c)}$	14 ± 1$^{(a)}$	14 ± 1$^{(ab)}$	13 ± 1$^{(c)}$
K-3-sin-hfer-triglc-7-diglc$^{(A)}$	36 ± 2$^{(a)}$	31 ± 5$^{(b)}$	38 ± 3$^{(a)}$	31 ± 8$^{(ab)}$	34 ± 2$^{(ab)}$
K-3-sin-caf-triglc-7-diglc$^{(A)}$	61 ± 4$^{(ab)}$	34 ± 11$^{(c)}$	68 ± 10$^{(a)}$	57 ± 21$^{(ab)}$	48 ± 9$^{(b)}$
K-3-soph-7-glc$^{(A)}$	20 ± 1$^{(ab)}$	17 ± 2$^{(c)}$	22 ± 3$^{(a)}$	20 ± 3$^{(a)}$	18 ± 1$^{(bc)}$
K-3-7-diglc$^{(A)}$	13 ± 1$^{(a)}$	13 ± 1$^{(a)}$	13 ± 1$^{(a)}$	14 ± 1$^{(a)}$	14 ± 1$^{(a)}$
K-3-caf-soph-7-glc$^{(A)}$	30 ± 2$^{(b)}$	26 ± 3$^{(c)}$	41 ± 10$^{(a)}$	37 ± 13$^{(a)}$	27 ± 2$^{(bc)}$

Uppercase letters indicate significant effects of aphid infestations averaged over the level of variant. Lowercase letters indicate significant differences of light treatments averaged over the level of infestation (GLMM and Tukey *post hoc* tests, $P < 0.001$, $N = 8$ biological replicates). K-3-sin-soph-7-glc: kaempferol-3-*O*-sinapoyl-sophoroside-7-*O*-glucoside, K-3-fer-soph-7-glc: kaempferol-3-*O*-feruloyl-sophoroside-7-*O*-glucoside, K-3-cou-soph-7-glc: kaempferol-3-*O*-coumaroyl-sophoroside-7-*O* glucoside, K-3-sin-hfer-triglc-7-diglc: kaempferol-3-*O*-sinapoyl-hydroxyferuloyl-triglucoside-7-*O*-diglucoside, K-3-sin-caf-triglc-7-diglc: kaempferol-3-*O*-sinapoyl-caffeoyl-triglucoside-7-*O*-diglucoside, K-3-soph-7-glc: kaempferol-3-*O*-sophoroside-7-*O*-glucoside, K-3-7-diglc: kaempferol-3,7-*O*-diglucoside, kaempferol-3-*O*-sophoroside-7-*O*-glucoside, K-3-caf-soph-7-glc: kaempferol-3-*O*-caffeoyl-sophoroside-7-*O*-glucoside.

Tab. 4 Concentrations [µg g^{-1} d.w.; mean (± SE)] of aliphatic glucosinolates in broccoli plants (non-infested, or infested with *B. brassicae*) grown in a climate chamber and exposed to control lighting plus one of four light treatments (UV-A 365 nm, violet 420 nm, blue 470 nm and green 515 nm) or to control lighting without additional illumination. UV-A 365 nm, violet 420 nm, blue 470 nm and green 515 nm were supplied by LEDs. Control lighting was supplied by standard fluorescent tubes (400 to 700 nm), Osram Lumilux Interna L 58 W / T8, 840, 5200 lm, 4000 K tubes

Aliphatic glucosinolates	**Light treatment and concentrations of aliphatic glucosinolates**				
Without aphid infestation	**Control**	**UV-A 365 nm**	**Violet 420 nm**	**Blue 470 nm**	**Green 515 nm**
4-methylthiobutyl$^{(B)}$	62 ± 34$^{(bc)}$	47 ± 41$^{(c)}$	137 ± 29$^{(a)}$	81 ± 34$^{(ab)}$	77 ± 62$^{(ac)}$
3-methylsulfinylpropyl$^{(B)}$	257 ± 98$^{(ab)}$	128 ± 117$^{(b)}$	213 ± 51$^{(ab)}$	244 ± 87$^{(a)}$	187 ± 83$^{(ab)}$
4-methylsulfinylbutyl$^{(B)}$	1140 ± 431$^{(ab)}$	669 ± 65$^{(b)}$	1095 ± 297$^{(ab)}$	1168 ± 477$^{(a)}$	837 ± 465$^{(ab)}$
With *B. brassicae* infestation					
4-methylthiobutyl$^{(A)}$	85 ± 41$^{(bc)}$	229 ± 159$^{(c)}$	141 ± 62$^{(a)}$	142 ± 84$^{(ab)}$	111 ± 89$^{(ac)}$
3-methylsulfinylpropyl$^{(A)}$	280 ± 86$^{(ab)}$	222 ± 121$^{(b)}$	274 ± 74$^{(ab)}$	340 ± 135$^{(a)}$	278 ± 105$^{(ab)}$
4-methylsulfinylbutyl$^{(A)}$	1459 ± 359$^{(ab)}$	1099 ± 629$^{(b)}$	1501 ± 384$^{(ab)}$	1638 ± 493$^{(a)}$	1295 ± 457$^{(ab)}$

Uppercase letters indicate significant effects of aphid infestations averaged over the level of variant. Lowercase letters indicate significant differences of light treatments averaged over the level of infestation (GLMM and Tukey *post hoc* tests, $P < 0.001$, $N = 8$ biological replicates).

Tab. 5 Concentrations [µg g^{-1} d.w.; mean (± SE)] of indole glucosinolates in broccoli plants (non-infested, or infested with *B. brassicae*) grown in a climate chamber and exposed to control lighting plus one of four light treatments (UV-A 365 nm, violet 420 nm, blue 470 nm and green 515 nm) or to control lighting without additional illumination. UV-A 365 nm, violet 420 nm, blue 470 nm and green 515 nm were supplied by LEDs. Control lighting was supplied by standard fluorescent tubes (400 to 700 nm), Osram Lumilux Interna L 58 W / T8, 840, 5200 lm, 4000 K tubes

Indole glucosinolates	**Light treatment and concentrations of indole glucosinolates**				
Without aphid infestation	**Control**	**UV-A 365 nm**	**Violet 420 nm**	**Blue 470 nm**	**Green 515 nm**
3-indolylmethyl$^{(B)}$	158 ± 93$^{(b)}$	207 ± 188$^{(a)}$	166 ± 109$^{(b)}$	162 ± 99$^{(b)}$	185 ± 86$^{(ab)}$
4-hydroxy-3-indolylmethyl$^{(B)}$	5 ± 2$^{(b)}$	13 ± 8$^{(ab)}$	19 ± 14$^{(a)}$	12 ± 5$^{(ab)}$	12 ± 6$^{(ab)}$
4-methoxy-3-indolylmethyl$^{(B)}$	81 ± 19$^{(a)}$	159 ± 72$^{(a)}$	92 ± 63$^{(a)}$	95 ± 56$^{(a)}$	133 ± 64$^{(a)}$
1-methoxy-3-indolylmethyl$^{(B)}$	62 ± 34$^{(a)}$	47 ± 40$^{(a)}$	137 ± 128$^{(a)}$	81 ± 34$^{(a)}$	77 ± 62$^{(a)}$
With *B. brassicae* infestation					
3-indolylmethyl$^{(A)}$	151 ± 64$^{(b)}$	735 ± 618$^{(a)}$	164 ± 65$^{(b)}$	191 ± 79$^{(b)}$	282 ± 183$^{(ab)}$
4-hydroxy-3-indolylmethyl$^{(A)}$	6 ± 3$^{(b)}$	15 ± 6$^{(ab)}$	22 ± 14$^{(a)}$	17± 11$^{(ab)}$	12 ± 6$^{(ab)}$
4-methoxy-3-indolylmethyl$^{(A)}$	76 ± 15$^{(a)}$	229 ± 107$^{(a)}$	96 ± 56$^{(a)}$	100 ± 60$^{(a)}$	142 ± 72$^{(a)}$
1-methoxy-3-indolylmethyl$^{(A)}$	85 ± 41$^{(a)}$	229 ± 159$^{(a)}$	141 ± 62$^{(a)}$	142 ± 83$^{(a)}$	111 ± 89$^{(a)}$

Uppercase letters indicate significant effects of aphid infestations averaged over the level of variant. Lowercase letters indicate significant differences of light treatments averaged over the level of infestation (GLMM and Tukey *post hoc* tests, $P < 0.001$, $N = 8$ biological replicates).

Chapter 3

Impact of Different Light Qualities on the Performance of Specialized and Generalized Lepidopteran Pests and on the Flavonol Absorbance and Chlorophyll Fluorescence in Broccoli

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Abstract

Light quality and quantity is essential for plant growth and development. Different light qualities can activate various photoreceptors and induce signal cascades that are responsible for different metabolic reactions in plants. Secondary plant metabolites such as phenolic substances (flavonol glycosides) could be differently affected by various light qualities. These metabolites have potential to influence herbivorous insects positively or negatively in their performance or behavior. The effects are depending on insect species and feeding-guild. In this study, a usual broad spectra illuminated climate chamber was equipped with additional light qualities: UV-B 310 nm, UV-A 365 nm, and violet 420 nm generated with UV-B tubes or LEDs. Broccoli plants were grown for two or four weeks under usual climate chamber illumination and afterwards treated for two weeks with the specific light qualities. Aim of this study was to investigate the performance of the specialized lepidopteran pests: the cabbage butterfly *Pieris brassicae* and the diamondback moth *Plutella xylostella* as well as the generalized pests: the cotton bollworm *Helicoverpa armigera* and the fall armyworm *Spodoptera frugiperda* on various light-treated plants serving as host plants for these insects. Furthermore, the effects of different light qualities on the relative overall flavonol absorbance and the relative chlorophyll fluorescence in broccoli were investigated. The specialized cabbage butterfly *P. brassicae* did not differ in larvae weight feeding on plants in the different light treatments but showed decreased pupa weight if reared on violet 420 nm plants compared to UV-B 310 nm- and UV-A 365 nm-treated plants. The developmental time of *P. brassicae* was not influenced by various light treatments. The specialized diamondback moth *P. xylostella* reacted with decreased pupa weight on UV-B-treated plants, but the developmental time was not influenced. The generalized cotton bollworm *H. armigera* and the fall armyworm *S. frugiperda* did not react on plant-mediated effects of various light treatments when regarding the parameters of larvae and pupae weight or developmental time. The relative overall flavonol absorbance showed significant increased values in plants treated with UV-B or violet light, while the relative chlorophyll fluorescence did not differ between the light treatments.

Key Words

Biting-Chewing Insects, LEDs, Broccoli, Induced Resistance, Flavonol Absorbance, Chlorophyll Fluorescence

Introduction

Light is one of the main abiotic factors driving photosynthesis and plant growth. Different light qualities are able to distinctly influence the primary and secondary metabolism of plants (Ballare 2014; Huche-Thelier et al. 2016). From an herbivorous insects' point of view, these alterations in the chemical composition of a host plant can be positive or negative. Furthermore, they can have antibiotic or antixenotic effects on the performance or behavior (host plant choice) of herbivorous insects as shown in chapter one and two of this study and can therefore also be positive or negative for plant growers (Mewis et al. 2012a; Rechner & Poehling 2014; Kuhlmann & Müller 2010; Singh et al. 2015; Vänninen et al. 2010).

Different narrow-band light ranges alter plant secondary metabolism and plant defense response to aphids. This could be shown for broccoli as well as Brussels sprouts as host plants for the associated specialized cabbage aphid *Brevicoryne brassicae* L. (Hemiptera: Aphididae). The investigated secondary plant metabolites in former studies were mainly flavonol glycosides and glucosinolates, which concentrations altered after additional UV-B or UV-A radiation in climate or greenhouse chambers (Rechner et al. 2016; Acharya et al. 2016).

How could be the reaction of plant biting-chewing insects (such as lepidopteran pests) with different degrees of specialization, feeding on various light-treated plants and thus exposed to different concentrations of light-induced secondary plant metabolites? Flavonoids (such as kaempferol and quercetin glycosides) have been described to have negative impacts on larval growth, development and survival of the specialized lepidopteran species *Pieris brassicae* L. (Lepidoptera: Pieridae) as well as the generalist *Helicoverpa armigera* H. (Lepidoptera: Noctuidae) if supplied in artificial diets (Onkokesung et al. 2014; Liu et al. 2012). High glucosinolate concentrations, i.e., sinigrin also showed detrimental effects for larvae development of the specialist *P. xylostella* L. (Lepidoptera: Plutellidae) but had the potential to attract adult moth towards their host plants (Kaur et al. 2016; Caputo et al. 2006). Contrasting results are reported for the generalized lepidopteran species *Chilesia rudis* B. (Lepidoptera: Arctiidae) feeding on murtilla plants with different kaempferol, quercetin and rutin concentrations. Larvae of this insect species preferred leaves with higher concentrations of flavonols, ate more leaf material with increasing concentrations and performed better on these plants, indicating that these phenolic compounds stimulate feeding of *C. rudis* (Chacon-Fuentes et al. 2015). Considering light effects, the cabbage specialist *P. xylostella* showed

different reactions on certain secondary metabolites. *Plutella xylostella* infestation was more severe on arabidopsis plants grown in the field under attenuated UV-B radiation. Adult moth laid more eggs on UV-B attenuated plants, but larvae did not respond to changes in plant quality (Caputo et al. 2006). The effects of secondary plant metabolites, either naturally different in host plants or induced by light treatments, on leaf biting-chewing insects in literature seem to be variable and species specific (like for phloem-sucking insects). The former studies analyzed quantitatively concentrations of single flavonol glycosides and glucosinolates destructively with HPLC at the end of the experiments, but relative overall flavonols can also be measured and quantified non-destructively during experiments via the relative overall flavonol absorbance in the leaf measured using a Dualex Scientific device (Cerovic et al. 2002). Up to now, it could be shown that phloem-sucking insects react differently on various light treatments depending on their degree of specialization (Kuhlmann & Müller 2010; Mewis et al. 2012a; Rechner et al. 2016).

This study aimed to investigate the specialized cabbage butterfly *P. brassicae*, the specialized diamondback moth *P. xylostella* as well as the generalized cotton bollworm *H. armigera* and the generalized fall armyworm *S. frugiperda* on various light-treated broccoli plants. Aim was to characterize the light-induced alterations in the broccoli plant on the performance of these herbivorous insects. Furthermore, the effects of different narrow-band light treatments (UV-B – violet) on the relative overall flavonol absorbance and the relative chlorophyll fluorescence in broccoli plants were measured to show associations between the performance of insects and the plants secondary metabolism.

Materials and Methods

Rearing of insects

Pieris brassicae was obtained from the Laboratory of Entomology, Wageningen University (Wageningen, The Netherlands) and reared in wood-framed cages (85 cm × 60 cm × 60 cm) covered with gauze in a climate chamber (temperature 20–22°C, humidity 65–75%, 16:8 h L:D) on 4-week-old broccoli plants. Once a week half of the plants were replaced with new plants to ensure sufficient availability of food sources for the insects. Adults of *P. brassicae* were additionally adequately supplied with a 20% sugar-water-solution on cotton wool in a petri dish (ø 9 cm), which was renewed every two days. Adults were allowed to lay their eggs

directly on the broccoli plant. Adults and larvae were in separate cages and pupae were collected from the larvae cage and transferred to the adult cage.

Adults of *Plutella xylostella* were collected outdoors from broccoli plants at the Leibniz University Hannover, Institute of Horticultural Production Systems, Section of Phytomedicine (N 52° 23`39.22``, E 9° 42`18.86``) in Hannover. The rearing was similar to the rearing of *P. brassicae*, except that adults, larvae and pupae were in one cage and a 20% honey-water-solution instead of 20% sugar-water-solution was provided for the adults.

Helicoverpa armigera and *Spodoptera frugiperda* were obtained from the stock culture of Bayer CropScience AG (Mohnheim, Germany). Larvae were reared on an artificial diet in a climate cabinet (temperature 23–25°C, humidity 65–75%, 16:8 h L:D). The components of the diet were water (4 liters), agar (106 g), maize meal (760 g), yeast (200 g), wheat germ (184 g), ascorbic acid (28 g), benzoic acid (12 g), methyl *p*-hydroxybenzoate (9.2 g), Vanderzant vitamin mixture for insects (0.2 g), and Wesson salt mixture (30 g), prepared as described by Smith (1966). Newly hatched larvae were reared in groups of 100-200 in 1000-ml plastic shells of which the ground was covered with artificial diet. The larvae were separated into individual petri dishes (Ø 9 cm) to prevent cannibalism in the second instars. The larvae completed their development to pupae in the petri dishes and were transferred into 1000-ml plastic shells of which the ground was covered with paper towel to avoid crushing of the pupae. On emergence, 20 adults (randomly mixed sexes) were released in plastic cylinders (Ø 18 cm; height 30 cm) with two gaze ventilation windows (5 cm x 5 cm) on the sides and covered with a green paper napkin to provide the oviposition substrate for adults. The adults were provided with 10% sucrose solution on cotton wool in a petri dish. The green paper napkins were removed from the cage daily and replaced with fresh napkins. Napkins removed from cages were cut into smaller pieces, and pieces containing eggs were transferred into plastic shells with artificial diet and covered until the second instar larvae were placed into single petri dishes again.

Plant material, growth conditions, experimental layout and light treatments

The experiments were conducted in a climate chamber (Viessmann, 4 m x 3 m x 2.40 m, Allendorf, Germany) with the following conditions: temperature 22 ± 2°C, relative humidity 70 ± 10%, and photoperiod 16:8 h L:D. Broccoli plants [*B. oleracea* var. *italica*, cv Monopoly; F1 Hybrid; Syngenta Enkhuizen, Netherlands] were grown from seeds in fertilized soil

(Fruhstorfer Erde Type P, Hawita Gruppe, Vechta, Germany) in individual pots (diameter: 12 cm, height: 9 cm). Additional light treatments with specific narrow-bandwidth wavelengths were generated with narrowband UV-B tubes with a peak wavelength of 310 nm (Philips TL 20W/01 RS SLV UV-B Narrowband G13, Hamburg, Germany) or with hexagonal 1 W high-power single-chip LED emitters. The peak wavelengths of the LEDs were UV-A 365 nm (H2A1-H365-E), and violet 420 nm (H2A1-H420). For each LED-illuminated compartment, two small aluminum plates (50 cm x 3 cm) were each equipped with six high-power LEDs to reach a larger area for plant illumination as described in chapter one and two, because more plants were needed for the experiments with leaf biting-chewing lepidopteran larvae compared to the experiments with aphids. The light intensities were the same as described in chapter one. Plants were illuminated for 16 h a day for two weeks with the specific light qualities when they were two- or four-weeks-old and grown under usual broad spectra climate chamber conditions before (Osram Lumilux Interna, L 58 W / T8, 827, 5200 lm, 2700 K fluorescence tubes, 400 to 700 nm). Four-weeks-old or six-weeks-old light-treated broccoli plants were placed individually in small insect proof gauze cages (25 cm × 25 cm × 35 cm) that were covered with a plastic-film. The additional light treatments with UV-B tubes or LEDs did not persist during the experiments with insects. The experiments took place under usual climate chamber conditions to avoid direct effects of light treatments on the insects.

For the exact climate chamber set-up, light intensities, spectra and measurements please see the material and method section in chapter one page 15-17.

Developmental parameters *Pieris brassicae, Plutella xylostella*, *Helicoverpa armigera* and *Spodoptera frugiperda*

For *P. brassicae* and *P. xylostella* experiments two four-week-old broccoli plants (non-light-treated control plants) were placed for 18 h in the main breeding cage to collect synchronized eggs. Afterwards for *P. xylostella* ten eggs were transferred with a wet brush to the second leave of four-week-old broccoli plants (two-week-light-treated). Eight plants were used per treatment as biological repeats. The development of the larvae was checked daily and the developmental time [d] (larvae to pupa stage) and the pupa weight [mg] were recorded. The first six fully hardened pupae were weighed within 24 h with a microbalance (Type MC5, Sartorius, Goettingen, Germany) to determine the pupa weight. For *P. brassicae* two L1-larvae (18 h old) were transferred with a wet brush to the second leave of six-week-old broccoli

plants (two-week-light-treated). Twelve plants were used per treatment as biological repeats. The larvae were weighed after six days of feeding. The development of the larvae was checked daily and the developmental time [d] (larvae to pupa stage) and the pupa weight [mg] were recorded.

For *H. armigera* or S. *spodoptera* three newly hatched L1-larvae were transferred with a wet brush to the second leave of six-week-old broccoli plants (two-week-light-treated). The larvae were weight every five days to determine the increase of larvae weights [mg]. The development of the larvae was checked daily and the developmental time [d] (larvae to pupa stage) and the pupa weight [mg] were recorded. Eight plants were used per treatment as biological repeats for each insect species.

Measurements of relative overall flavonol absorbance and relative chlorophyll fluorescence

Relative chlorophyll fluorescence and relative overall epidermal flavonol absorbance were measured non-destructively with the hand-held optical sensor Dualex ® Scientific 4 (FORCE-A, Orsay, France) over time on plants without insect infestation. Measurements were started when broccoli plants were two weeks old one day before and one day after the light treatments started. The measurements were repeated every fifth day during the illumination time under the specific light treatments. Plants were treated similar than plants used for the experiments with lepidopterans. Measurements took place in the middle on the left half of the third leaf next to the midrib, and three measurements were taken on each plant and repeated every time on eight plants per treatment. The relative overall flavonol concentration was calculated with the decadic logarithm of the red to UV excitation ratio of far-red chlorophyll fluorescence (flavonol absorbance). This value is proportional to the flavonol content of the leaves (Cerovic et al. 2002). Plants infested with lepidopteran larvae could not be measured on the same leaf over time during the experiments because the larvae removed at least parts of the leaf due to their biting-chewing feeding activity.

Statistical analysis

The data were analyzed in R 2.15.2 (R Development Core Team 2008). Graphs were made with the package ggplot2 (Wickham 2009). The effects of the light treatments on relative overall flavonol absorbance, chlorophyll fluorescence, larvae and pupae weight of all lepidopteran species were analyzed using generalized linear mixed models (GLMM) followed by Tukey *post*

hoc tests (Pinheiro et al. 2012). The developmental time of all lepidopteran species was analyzed by generalized linear models (GLM) using a log-link together with a quasi-Poisson distribution.

Results

Developmental parameters *Pieris brassicae*

The larvae weight and the developmental time of *P. brassicae* were not significantly different between various light treatments. The final pupa weight of *P. brassicae* was significantly lower if larvae were reared on violet 420 nm-treated plants (reduced by 6-7%) compared to UV-B 310 nm and UV-A 365 nm-treated plants ($P < 0.001$) (Fig. 1).

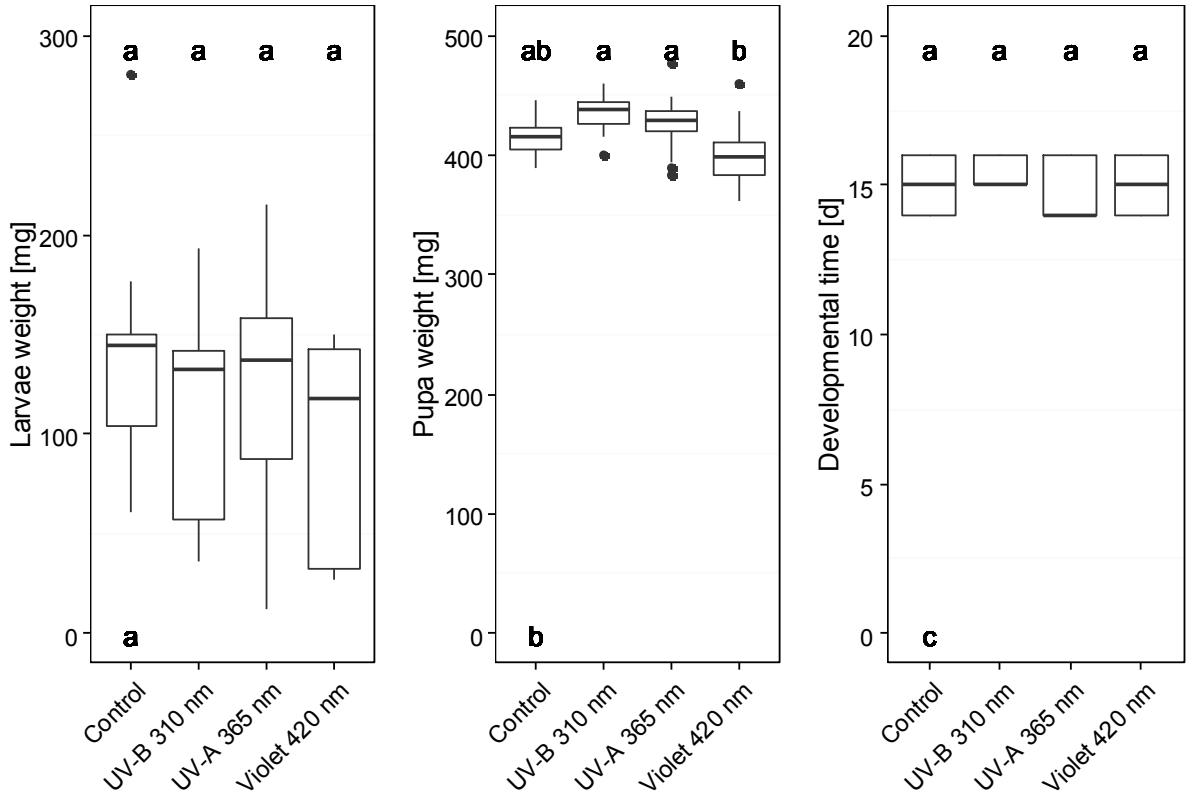

Fig. 1 Larvae weight [mg] (a), pupa weight [mg] (b), and developmental time (c) of *P. brassicae* kept on broccoli plants that were grown in a climate chamber and exposed to control lighting plus one of three light treatments (UV-B 310 nm, UV-A 365 nm, and violet 420 nm) or to control lighting without additional illumination. Additional illuminations were supplied by UV-B tubes (310 nm) and LEDs (UV-A 365 nm and violet 420 nm). Control lighting was supplied by Osram Lumilux Interna, L 58 W / T8, 827, 5200 lm, 2700 K fluorescence tubes (400 to 700 nm).

Different letters indicate significant differences (GLMM (Fig. 3a) and GLM (Fig. 3b), and Tukey *post hoc* tests, $P < 0.05$; $N = 12$ biological replicates)

Developmental parameters *Plutella xylostella*

The final pupa weight of *P. xylostella* was significant lower and reduced by 12-13%, but only if larvae reared on UV-B 310 nm-treated plants were compared with larvae reared on control- and UV-A 365 nm-treated plants ($P < 0.01$). Other treatments did not differ between each other. The developmental time of larvae from egg to pupa stage was not different in all treatments (Fig. 2).

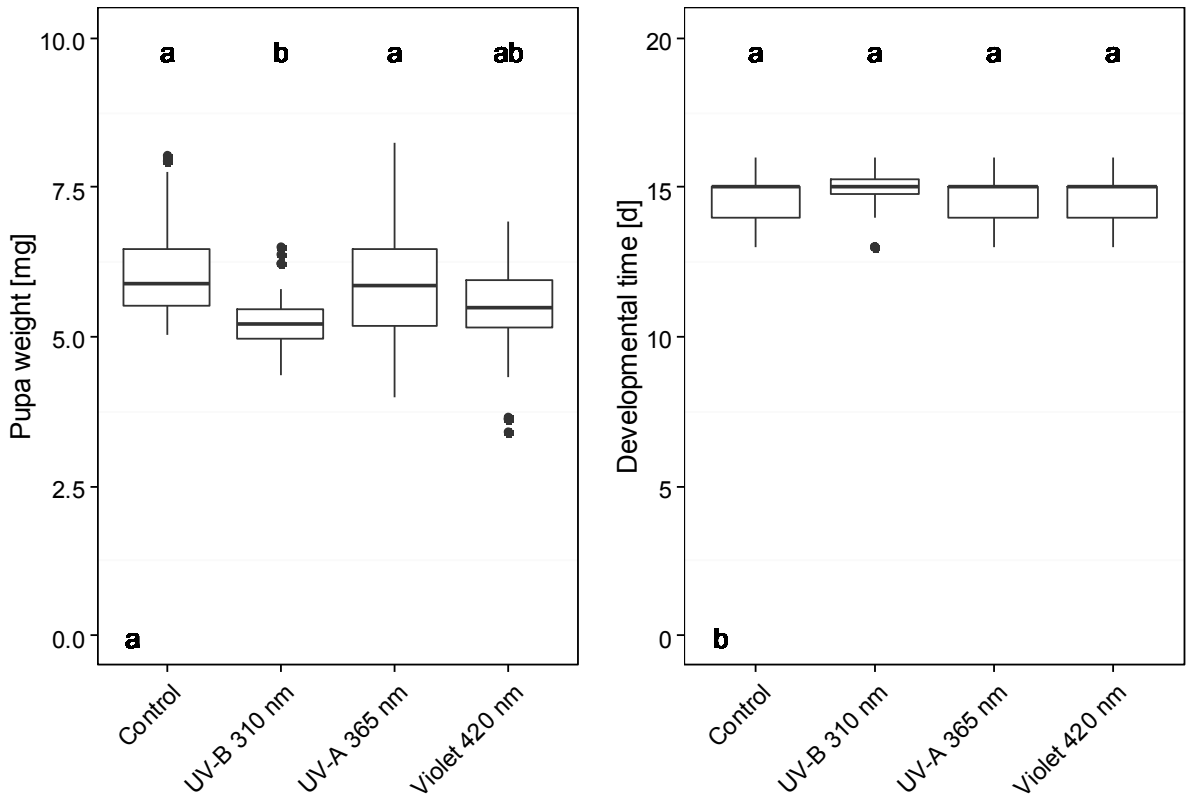

Fig. 2 Pupa weight [mg] (a) and developmental time (b) of *P. xylostella* kept on broccoli plants that were grown in a climate chamber and exposed to control lighting plus one of three light treatments (UV-B 310 nm, UV-A 365 nm, and violet 420 nm) or to control lighting without additional illumination. Additional illuminations were supplied by UV-B tubes (310 nm) and LEDs (UV-A 365 nm and violet 420 nm). Control lighting was supplied by Osram Lumilux Interna, L 58 W / T8, 827, 5200 lm, 2700 K fluorescence tubes (400 to 700 nm). Different letters indicate significant differences (GLMM (Fig. 3a) and GLM (Fig. 3b), and Tukey *post hoc* tests, $P < 0.05$; $N = 8$ biological replicates)

Developmental parameters *Helicoverpa armigera*

Helicoverpa armigera larvae weights (L1-L3-larvae) and pupa weight were not significantly different between various light treatments ($P < 0.05$) (Fig. 3). Developmental times did also not differ between the treatments and were average over all variants 21 ± 1.6 days (*data not shown*).

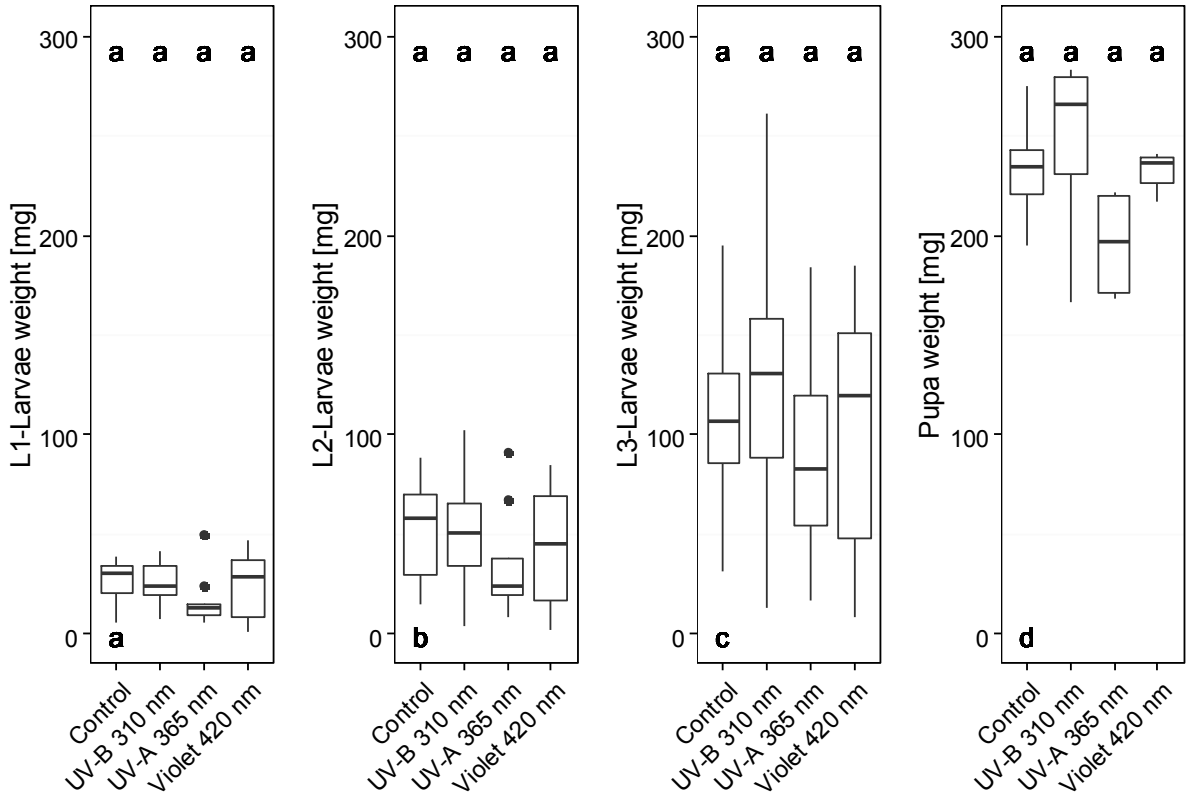

Fig. 3 L1-larvae weight (a), L2-larvae weight (b), L3-larvae weight (c) and pupa weight (d) of *H. armigera* kept on broccoli plants that were grown in a climate chamber and exposed to control lighting plus one of three light treatments (UV-B 310 nm, UV-A 365 nm, and violet 420 nm) or to control lighting without additional illumination. Additional illuminations were supplied by UV-B tubes (310 nm) and LEDs (UV-A 365 nm and violet 420 nm). Control lighting was supplied by Osram Lumilux Interna, L 58 W / T8, 827, 5200 lm, 2700 K fluorescence tubes (400 to 700 nm). Different letters indicate significant differences (GLMM and Tukey *post hoc* tests, $P < 0.05$; $N = 8$ biological replicates)

Developmental parameters *Spodoptera frugiperda*

Spodoptera frugiperda larvae weights (L1-L3 larvae) and pupa weight were not significantly different between various light treatments ($P < 0.05$) (Fig. 4). Developmental times did also

not differ between the treatments and amounted on average over all variants 23 ± 2.4 days (*data not shown*).

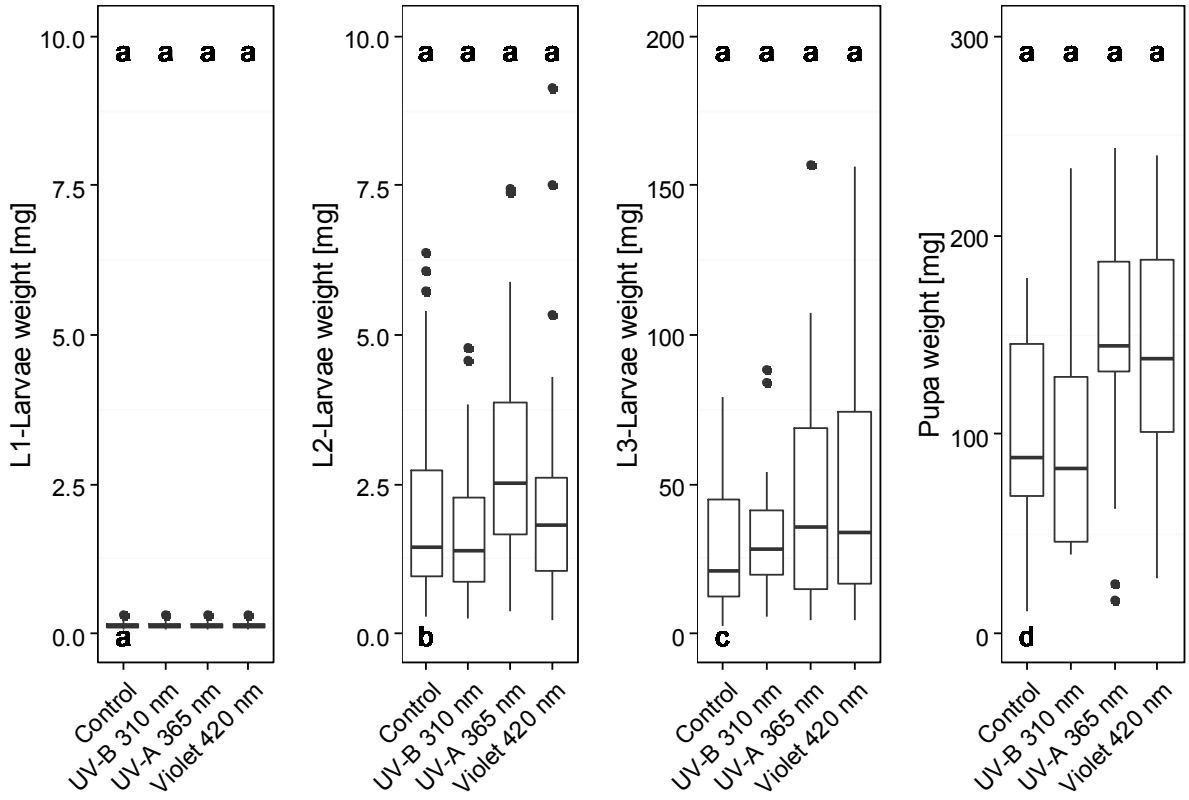

Fig. 4 L1-larvae weight (a), L2-larvae weight (b), L3-larvae weight (c) and pupa weight (d) of *S. frugiperda* kept on broccoli plants that were grown in a climate chamber and exposed to control lighting plus one of three light treatments (UV-B 310 nm, UV-A 365 nm, and violet 420 nm) or to control lighting without additional illumination. Additional illuminations were supplied by UV-B tubes (310 nm) and LEDs (UV-A 365 nm and violet 420 nm). Control lighting was supplied by Osram Lumilux Interna, L 58 W / T8, 827, 5200 lm, 2700 K fluorescence tubes (400 to 700 nm). Different letters indicate significant differences (GLMM and Tukey *post hoc* tests, $P < 0.05$; $N = 8$ biological replicates)

Relative overall flavonol absorbance

Mean relative overall flavonol absorbance of control plants was significantly lower compared to plants grown under UV-B 310 nm and violet 420 nm light treatments on all measurement dates ($P < 0.001$, $P < 0.05$). UV-A 365 nm-treated plants showed significantly decreased relative flavonol absorbance compared to plants grown under UV-B 310 nm ($P < 0.001$),

control ($P < 0.01$) or violet 420 nm ($P < 0.001$) light conditions on all measurement dates (Fig. 5).

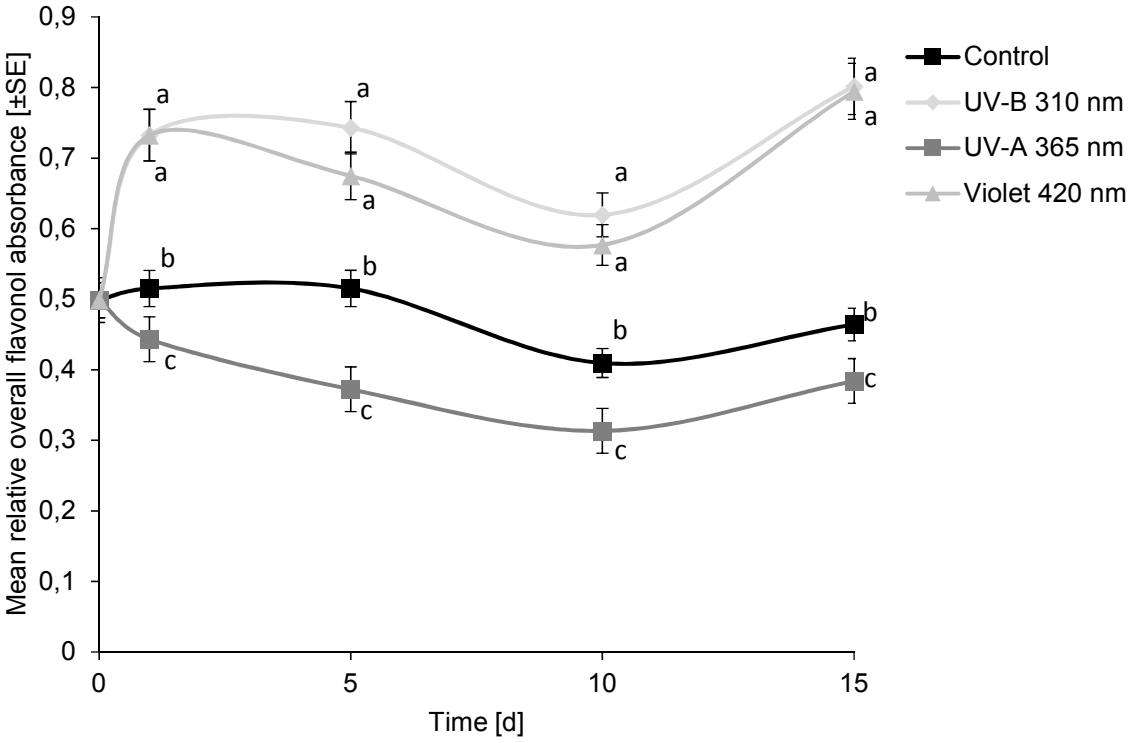

Fig. 5 Mean relative overall flavonol absorbance [± SE] measured with a Dualex scientific over time on the third leaf of broccoli plants without insect infestation. Plants were grown in a climate chamber and exposed to control lighting plus one of three light treatments (UV-B 310 nm, UV-A 365 nm, and violet 420 nm) or to control lighting without additional illumination. Additional illuminations were supplied by UV-B tubes (310 nm) and LEDs (UV-A 365 nm and violet 420 nm). Control lighting was supplied by Osram Lumilux Interna, L 58 W / T8, 827, 5200 lm, 2700 K fluorescence tubes (400 to 700 nm). Different letters indicate significant differences (GLMM and Tukey *post hoc* tests, $P < 0.05$; $N = 8$ biological replicates)

Relative chlorophyll fluorescence

Mean relative chlorophyll fluorescence was not significant different between the light treatments and varied between 24 ± 3 and 36 ± 5 (Fig. 6).

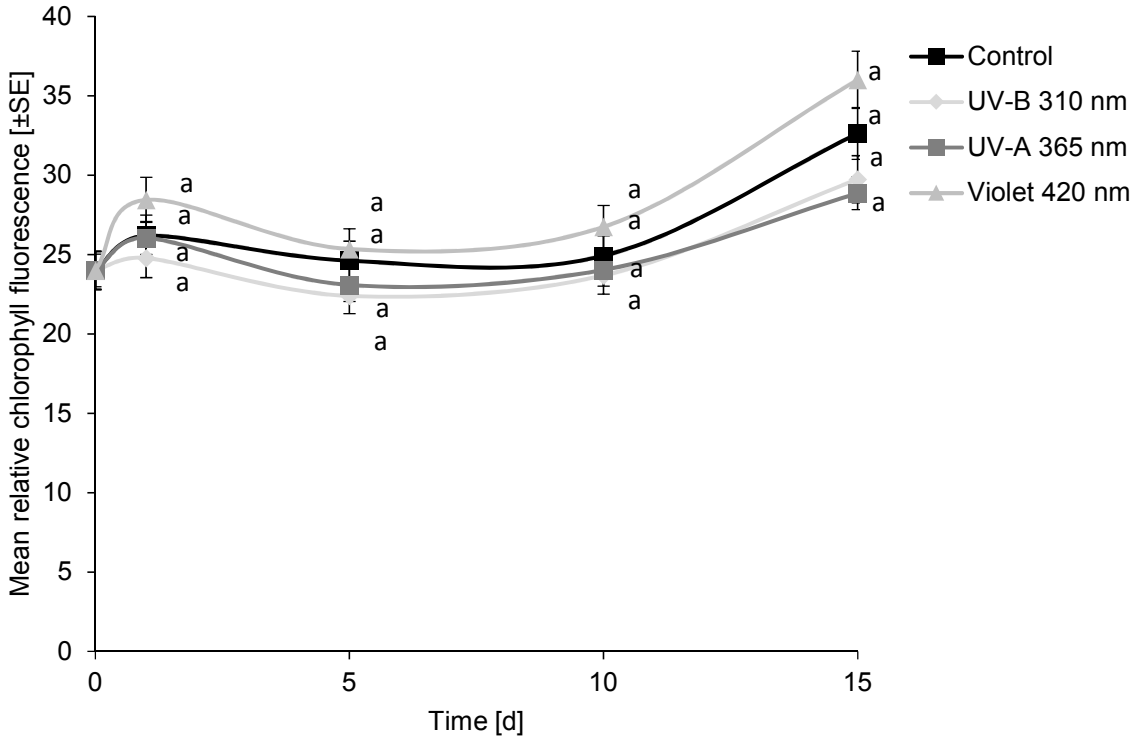

Fig. 6 Mean relative chlorophyll fluorescence [± SE] measured with a Dualex scientific over time on the third leaf of broccoli plants without insect infestation. Plants were grown in a climate chamber and exposed to control lighting plus one of three light treatments (UV-B 310 nm, UV-A 365 nm, and violet 420 nm) or to control lighting without additional illumination. Additional illuminations were supplied by UV-B tubes (310 nm) and LEDs (UV-A 365 nm and violet 420 nm). Control lighting was supplied by Osram Lumilux Interna, L 58 W / T8, 827, 5200 lm, 2700 K fluorescence tubes (400 to 700 nm). Different letters indicate significant differences (GLMM and Tukey *post hoc* tests, $P < 0.05$; $N = 8$ biological replicates)

Discussion

The experiments in this part of the study investigated the effects of UV-B, UV-A, and low wavelength visible light (violet) on the performance of the specialized lepidopteran pests *P. brassicae* and *P. xylostella* and the generalized lepidopteran pests *H. armigera* and *S. frugiperda* feeding on various light-treated broccoli plants. Furthermore, the light-induction of the relative overall flavonol absorbance and the relative chlorophyll fluorescence were analyzed in broccoli plants without insect infestation. Direct effects of various light treatments on the lepidopteran larvae were eliminated by switching off the LEDs during the experiments. The additional plant illumination with LEDs to alter the metabolic composition of the host plants took only place during plant growth before the experiments with herbivorous insects.

Therefore, regarding the effects of light treatments on the lepidopteran larvae, the discussion will only focus on indirect, plant-mediated effects of the light treatments. According to literature, this is the first narrow-band light study to describe the changes in relative overall flavonol absorbance and relative chlorophyll fluorescence in broccoli induced by various light treatments and investigate the indirect, plant-mediated effects on the performance of generalized as well as specialized lepidopteran pest insects.

The light treatments did not affect the developmental time of the different lepidopteran species and did also not influence the performance of the generalists *H. armigera* and *S. frugiperda* (Figs. 3-4). The specialist *P. xylostella* showed decreased pupa weight by 12-13% if reared on broccoli plants treated with UV-B 310 nm compared to larvae feeding on plants grown under UV-A 365 nm or control conditions. In contrast, *P. brassicae* pupa weight was decreased by 6-7% if reared on violet 420 nm-treated plants compared to larvae that fed on plants grown under UV-B 310 nm, but the larvae weight did not differ significantly between the different light treatments. For comparison of reactions the pupa weight seems to be a convenient parameter, since it includes possible compensations of feeding intensity over larval time (Rechner & Poehling 2014). Furthermore, the pupa biomass greatly affects later egg laying capacity and fecundity of adult moth, so that it could serve as an indicator of antifeedant or antibiotic properties induced in the host plants. The results show that only the UV-B treatment induced stronger resistance and only against the specialist *P. xylostella* if compared to control plants.

It could be shown in previous parts of this study with aphids, that some flavonols especially non-acylated diglycoside, the monoacylated triglycosides, and quercetin-3-*O*-sophoroside-7-*O*-glucoside heavily increased with UV-B treatments in broccoli (Rechner et al. 2016). Most studies describe, especially kaempferol glycosides but also quercetin glycosides as strong growth inhibitors against specialist (*P. brassicae*) as well as generalist (*H. armigera and S. frugiperda*) lepidopteran pest insects (Onkokesung et al. 2014; Liu et al. 2015; Silva et al. 2016). Most of these studies were done with lepidopteran larvae on artificial diet and not on living plants. For instance, an increased susceptibility to *P. brassicae* larvae feeding on arabidopsis plants with decreased kaempferol-3,7-dirhamnoside content could be shown. Larvae performed less on an artificial diet containing kaempferol-3,7-dirhamnoside or on arabidopsis plants that were exogenously treated (solution was pipetted over a leaf and

evenly distributed using a brush) with this compound, indicating its involvement in plant defense against *P. brassicae* (Onkokesung et al. 2014). Quercetin had significant negative effects on the growth, development and survival of *H. armigera* when added to artificial diet (Liu et al. 2015). The flavonoid rutin added to artificial diet increased the larval development time and reduced the weight of larvae of *S. frugiperda*, indicating negative effects of this compound (Silva et al. 2016). Liu et al. (2015) could find strong negative effects of quercetin on the performance of the cotton bollworm *H. armigera* after feeding of 3 and 10 mg g^{-1} quercetin to larvae in artificial diet, but no significant toxic effect was found at 0.1 mg g^{-1} quercetin treatment. The latter study indicates that very high concentrations of quercetin are necessary to induce significant detrimental effects against the cotton bollworm. In chapter one of this study with comparable light treatments the UV-B triggered concentrations of quercetin ranged only between 0.2 and 1.5 mg g^{-1} dry weight (Rechner et al. 2016). Same light reaction effects should occur in this part of the study (with the same experimental light setup, illumination time and plant species), but probably too low amounts of this inhibitory compound were induced to show measurable effects on *H. armigera* or *S. frugiperda* biology parameters (Rechner et al. 2016). War et al. (2013) identified compounds such as chlorogenic acid and caffeic acid added in artificial diet as potential growth inhibitors against *H. armigera*, but concentrations of hydroxycinnamic acids were not induced with various light treatments in the previous study with aphids in chapter one (Rechner et al. 2016). However, Caasi-Lit et al. (2007) could show that the flavonoid isovitexin-2'-*O*-β-[6-*O*-E-*p*-coumaroyl-glucopyranoside] reduced the fertility of *H. armigera* compared with insects reared on control diet, but other flavonoids had no effect on insects performance. In conclusion and according to literature, specific flavonoids in high concentrations can alter the performance of lepidopteran larvae negatively and inhibit the population growth. UV-B 310 nm and violet 420 nm light treatments significantly increased the relative overall flavonol absorbance in broccoli plants (Fig. 5). The UV-B induced increase of relative overall flavonol concentrations in broccoli flower buds could also be shown in other studies (Rybarczyk-Plonska et al. 2016). Due to the non-destructive analytic technology with the Dualex scientific measurement of relative overall flavonols it is not possible to conclude on exact concentrations and specific types of these compounds. The concentrations were probably not induced high enough in the host plants or the flavonol glycosides that were induced were not responsible for resistance against the specific lepidopteran pest insects that were used. Only for the specialized lepidopteran pest

P. xylostella a UV-B induced resistance with higher concentrations of leaf flavonols could be found. Hence the relative overall flavonol absorbance is in general not a reliable indicator of light-induced resistance induction against lepidopteran pests in broccoli plants.

The chlorophyll fluorescence in broccoli was not influenced by the different light treatments in this study (Fig. 6). In another study, two different plant species reacted differently. *Vitis vinifera* showed reduced chlorophyll fluorescence, while *Arbutus unedo* did not react with altered chlorophyll fluorescence on UV-B and UV-A treatments comparable to broccoli in this study, indicating species specific reactions of chlorophyll fluorescence on UV-A or UV-B treatments (Grifoni et al. 2016).

Another class of secondary plant defense metabolites are glucosinolates, which were not included in the measurement of the relative overall flavonol absorbance and not measured in this particular part but in previous parts of this study (Chapter one and two) (Björkman et al. 2011; Schreiner et al. 2012). In chapter one, the indole glucosinolate 4-methoxy-3-indolylmethyl increased its concentrations 2-fold in broccoli plants after UV-B 310 nm and UV-A 365 nm treatments. Also concentrations of 3-indolylmethyl glucosinolate were increased after UV-B treatment in chapter one and higher concentrations of glucosinolates could also be responsible for enhanced plant resistance against aphids (Rechner et al. 2016). Adults of *P. xylostella* moths visit and prefer plants with high sinigrin concentrations for oviposition. Conversely, leaves with high sinigrin concentrations were not preferred by larvae and decreased the performance of *P. xylostella* larvae (Kaur et al. 2016). Besides glucosinolates, saponins act as feeding deterrents for *P. xylostella* larvae. *Plutella xylostella* laid more eggs on leaves with higher concentrations of glucosinolates and saponins compared to leaves with lower concentrations of glucosinolates and saponins (Badanes-Perez et al. 2014). High contents of aliphatic glucosinolates protected plants more against the lepidopteran pest insects *Pieris rapae* and *Mamestra brassicae*, whereas indole glucosinolates were not effective. Plants with lower glucosinolate concentrations were more consumed and larvae gained more weight (Santolamazza-Carbone et al. 2016). Specialist and generalist lepidopterans showed no differences in the latter study. For instance, Mewis et al. (2012a) could show that *P. brassicae* decreased its performance on UV-B-treated plants that had higher concentrations of 4-methylsulfinylbutyl glucosinolate and 4-methoxy-3-indolylmethyl glucosinolate. Severely drought stressed collard plants had less fiber and glucosinolate

contents, but they showed higher total nitrogen and lipid concentrations. Larvae performance (weight and survival) were lower when the leaf biting-chewing larvae of *P. xylostella* were reared on severely drought stressed collards (Volim et al. 2016). In this study, mainly indole glucosinolates were increased and the only candidate for resistance responsibility and the reduced pupa weight of *P. xylostella* in the UV-B treatment could be 3-indolylmethyl glucosinolate in the UV-B treatment (Rechner et al. 2016). Further experiments with feeding trials in artificial diet should be performed to verify this hypothesis, because the reactions of lepidopteran pests on specific secondary plant metabolites in literature are conflictive and diverse and are highly depending on compound class and metabolite concentration mostly fed in artificial diet.

Altogether the light treatments in this part of the study could only reduce the pupa weight of the specialist *P. xylostella* significantly compared to control plants. How big the influence of reduced pupa weight by 12-13% on the egg laying capacity or the fertility of the next generation could be, requires further investigation of following insect generations. An important methodical conclusion is that the relative overall flavonol absorbance is not a good indicator for the proof of light dependent resistance induction in broccoli, because the overall flavonol absorbance was significantly increased in broccoli plants under UV-B 310 nm or violet 420 nm treatments but could not be correlated with the insects' performance parameters, except the UV-B treatment of broccoli and the reduced pupa weight of *P. xylostella* feeding on this plants. A better way is to analyze the specific compounds with HPLC analysis, like it was performed in chapter one and two of this study.

The question why one specialist (*P. xylostella*) reacted differently than the other (*P. brassicae*) remains open in this chapter of the study. Further studies in the future should consider feeding and biomass conversion rates of the larvae and oviposition preference of adults on various light-treated plants. Furthermore, the analysis of proteinase inhibitors and responsible resistance genes could be helpful to identify the responsible plant mechanism behind the induced resistance against *P. xylostella*.

Acknowledgments

This research project was funded by the German Research Foundation DFG, grant Po 207/39-1.

Chapter 4

Plant Mediated Effects of Single LEDs with Low Intensities on Different Herbivorous Insects Feeding on Broccoli

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Abstract

In this additional chapter, the effects of broccoli plant illumination with lower light intensities generated with single LEDs (UV-B 300 nm, UV-A 365 nm and violet 420 nm) were investigated. The LEDs were installed on variable panels and had four times lower intensities than the high-power LED-panels described in Chapter one, two and three. The aim was to reduce potential negative effects of short wavelength radiation (UV-B and UV-A) on broccoli plant growth, and try to find the intensity treshold for inducing certain kinds of resistance against *B. brassicae* and *P. xylostella*. Furthermore, less LEDs and less intensity results in lower electricity consumption of plant illumination and can reduce the costs for the production of horticultural plants in protected environments. Due to overlapping informations in literature with the previous chapters there is no additional introduction provided for this chapter. The same plant-insect-models, that showed light-depended effects in the previous chapters, were used to investigate the effects of low intensity light treatments with single LEDs: Broccoli infested with *Brevicoryne brassicae*, *Myzus persicae* or *Plutella xylostella*. Broccoli plants were illuminated from germination onward with single high-power LEDs. The mean relative overall flavonol absorbance in broccoli plants treated with UV-B 300 nm or violet 420 nm light was significant increased compared to plants treated with UV-A 365 nm or control conditions after ten and fifteen days of illumination. Broccoli plants grown under UV-B 300 nm and UV-A 365 nm treatments showed reduced plant heights, while the dry weights were only by trend reduced with UV-B 300 nm treatments. The specialized cabbage aphid *B. brassicae* did not react with differences in performance on various low-intensity light treatments generated with single LEDs. The generalized green peach aphid *M. persicae* did also not show significant performance differences, only the fecundity could be by trend increased in UV-B 300 nm- and UV-A 365 nm-treated plants but this difference was not statistically significant. The diamondback moth *P. xylostella* did not react with differences in larvae weight after one week of feeding on broccoli plants grown under various light treatments, but showed reduced pupa weight on broccoli plants exposed to UV-B 300 nm compared with pupa that fed on plants treated with UV-A 365 nm or violet 420 nm light.

Key Words

Resistance, *Brevicoryne brassicae*, *Myzus persicae*, *Plutella xylostella*, UV-B LEDs, Glucosinolates, Flavonoids, *Brassica oleracea,* Low Intensity Light

Materials and Methods

The experiments were conducted in a climate chamber (Johnson Controls, 3 m x 2 m x 2.40 m, Milwaukee, Wisconsin, Vereinigte Staaten) with the following conditions: temperature 20 ± 2°C, relative humidity 70 ± 10%, and photoperiod 16:8 h L:D. Broccoli plants [*B. oleracea* var. *italica*, cv Monopoly; F1 Hybrid; Syngenta Enkhuizen, Netherlands] were grown from seeds in fertilized soil (Fruhstorfer Erde Type P, Hawita Gruppe, Vechta, Germany) in individual pots (diameter: 12 cm, height: 9 cm). Additional light treatments with specific narrow-bandwidth wavelengths were generated with one hexagonal 1 W high-power single-chip LED emitters on variable metal panels (please see genral introduction page eight Fig. 2 b + c). The peak wavelengths of the LEDs were UV-B 300 nm (UVLUX295-3), UV-A 365 nm (H2A1-H365-E), and violet 420 nm (H2A1-H420). The intensities used are shown in Table 1. Spectra were identical to the high-power LEDs described in chapter five Fig. 1 for UV-B 300 nm or chapter one Fig. 1 for UV-A 365 nm and violet 420 nm. The measurement of the relative overall flavonol absorbance is described in the materials and methods section of chapter three page 79. For determination of insects parameters and insects rearing conditions please refer to the materials and methods section in chapter one page 15-18 for the aphids and in chapter three page 77-79 for *P. xylostella*. Additionally, after one week of *P. xylostella* larvae feeding the larvae weight was determined with a microbalance (Type MC5, Sartorius, Goettingen, Germany). Measurements of morphological plant parameters, insect parameters and statistical analysis were carried out in exactly the same way as described in chapter one page 15-21 and chapter three page 76-79.

Tab. 1 Light intensities provided by the four light treatments with single high-power LEDs and the control. The background and control illumination was provided by Osram 827 fluorescent tubes

Light treatment	**PAR Osram 827 (400 – 700 nm) [µmol m^{-2} s^{-1}]**	**UV-A (315 – 380 nm) [µmol m^{-2} s^{-1}]**	**UV-A (315 – 380 nm) [kJ m^{-2} d^{-1}]**	**UV-B (290 – 315 nm) [µmol m^{-2} s^{-1}]**	**UV-B (290 – 315 nm) [kJ m^{-2} d^{-1}]**
Control	100 ± 10	0	0	0	0
UV-B 300 nm	100 ± 10	1 ± 0.75	20	0.03 ± 0.001	**0.72**
UV-A 365 nm	100 ± 10	15 ± 0.75	**288**	0.02 ± 0.001	0.007
Violet 420 nm	100 ± 10	0	0	0	0

Results

Relative overall flavonol absorbance

After ten and fifteen days the mean relative overall flavonol absorbance of UV-A 365 nm- and control-treated plants was significantly lower compared to plants grown under UV-B 300 nm and violet 420 nm light treatments ($P < 0.05$) (Fig. 1). All other comparisons (light treatments) did not show any differences.

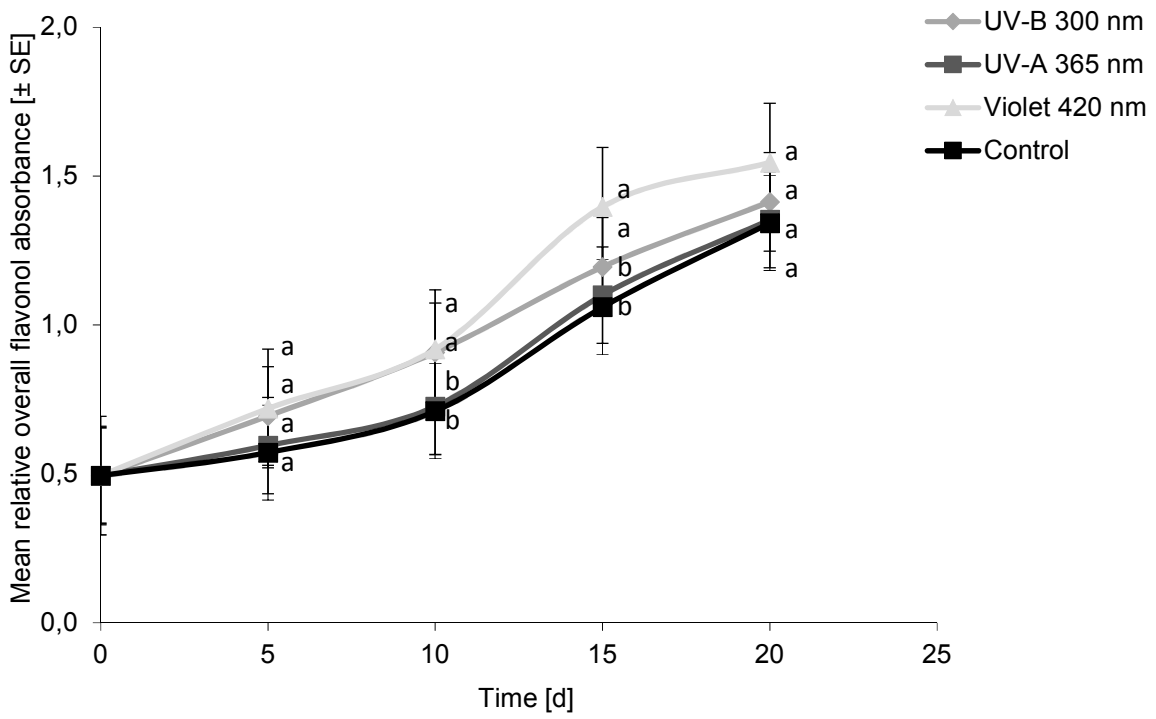

Fig. 1 Mean relative overall flavonol absorbance [± SE] measured with a Dualex scientific over time on the third leaf of broccoli plants without insect infestation. Plants were grown in a climate chamber and exposed to control lighting plus one of three light treatments (UV-B 300 nm, UV-A 365 nm, and violet 420 nm) or to control lighting without additional illumination. UV-B 300 nm, UV-A 365 nm, and violet 420 nm were supplied by single LEDs. Control lighting was supplied by Osram Lumilux Interna, L 58 W / T8, 827, 5200 lm, 2700 K fluorescence tubes (400 to 700 nm). Different letters indicate significant differences (GLMM, and Tukey *post hoc* tests at $P < 0.05$; $N = 8$ biological replicates)

Plant parameters

Leaf number per broccoli plant without insect infestation was not significantly affected by the light treatments with single high-power LEDs (Fig. 2a). Plant height was lower ($P < 0.01$) for

plants treated with UV-B 300 nm or plants treated with UV-A 365 nm than for control plants (Fig. 2b). Dry weight of broccoli plants was not significantly affected between the treatments, but tended to be lowest in the UV-B treatment (Fig. 2c).

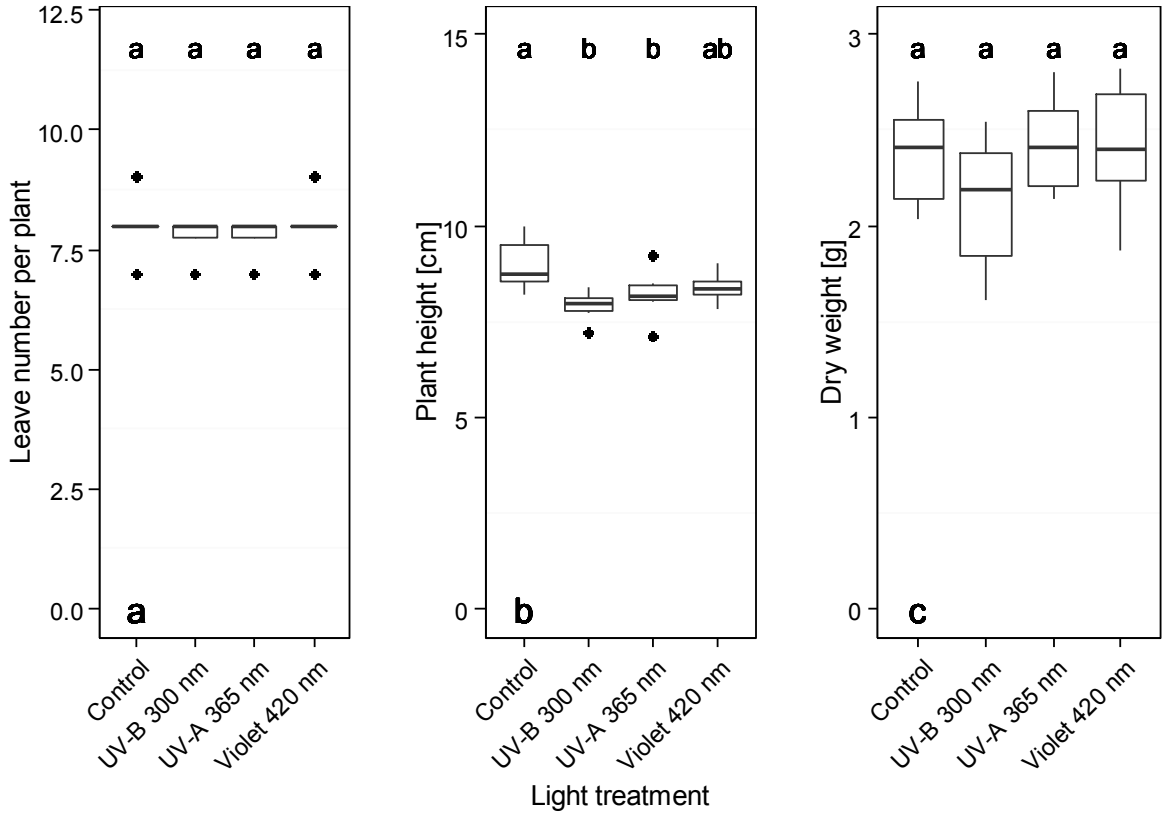

Fig. 2 Leaf number (a), plant height, (b) and plant dry weight (c) of 4-week-old broccoli plants grown in a climate chamber and exposed to control lighting plus one of three light treatments (UV-B 300 nm, UV-A 365 nm, and violet 420 nm) or to control lighting without additional illumination. UV-B 300 nm, UV-A 365 nm, and violet 420 nm were supplied by single high-power LEDs. Control lighting was supplied by Osram Lumilux Interna, L 58 W / T8, 827, 5200 lm, 2700 K fluorescence tubes (400 to 700 nm). Different letters indicate significant differences (GLM (Fig. 2a), GLMM (Fig. 2b, 2c), and Tukey *post hoc* tests at $P < 0.05$; $N = 8$ biological replicates)

Developmental parameters *Brevicoryne brassicae*

Performance parameters such as adult weight, fecundity and developmental time of *B. brassicae* were not significantly affected by different light treatments (Fig. 3a, 3b, 3c).

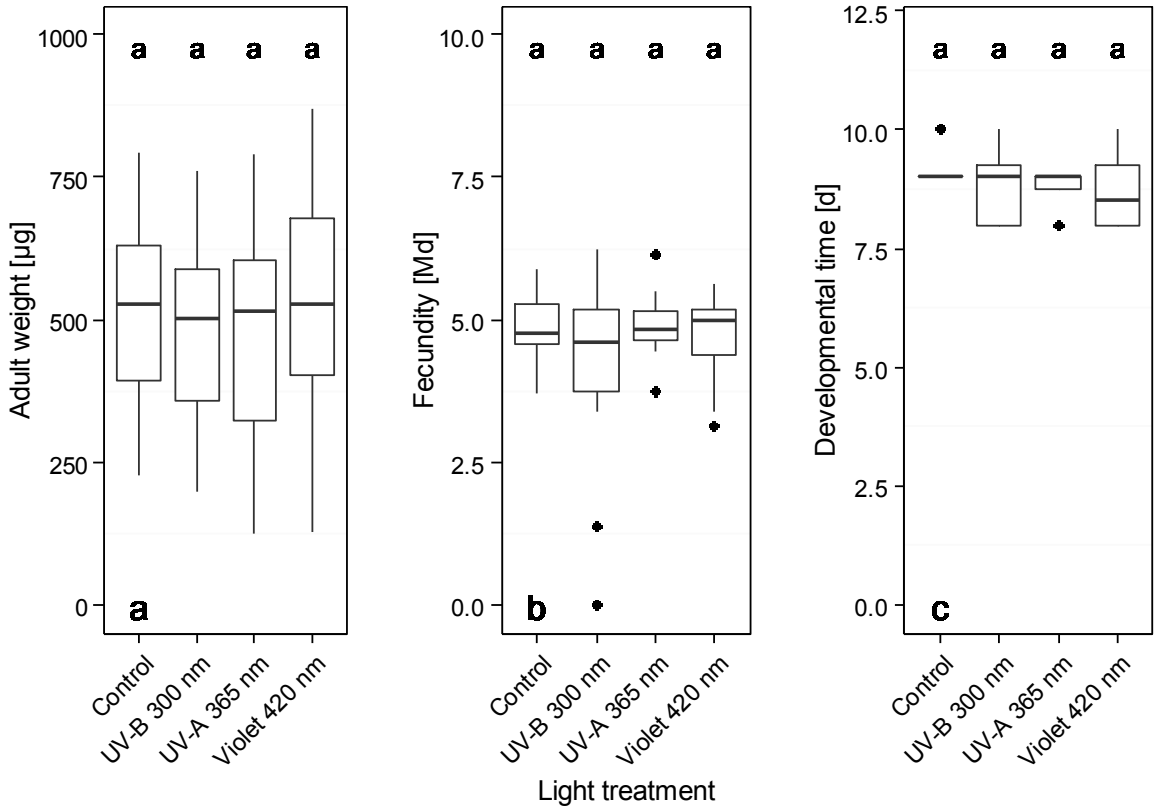

Fig. 3 Adult weight (a), fecundity (b), and developmental time (c) of *B. brassicae* kept on broccoli plants that were grown in a climate chamber and exposed to control lighting plus one of three light treatments (UV-B 300 nm, UV-A 365 nm, and violet 420 nm) or to control lighting without additional illumination. UV-B 300 nm, UV-A 365 nm, and violet 420 nm were supplied by single high-power LEDs. Control lighting was supplied by Osram Lumilux Interna, L 58 W / T8, 827, 5200 lm, 2700 K fluorescence tubes (400 to 700 nm). The aphids were in clip cages and were not directly exposed to the light treatments. Different letters indicate significant differences (GLMM (Fig. 3a), GLM (Fig. 3b, 3c), and Tukey *post hoc* tests, $P < 0.05$; $N = 8$ biological replicates)

Developmental parameters *Myzus persicae*

Performance parameters such as adult weight, fecundity and developmental time of *M. persicae* were not significantly affected by different light treatments (Fig. 4a, 4b, 4c). A slightly trend could be observed for the fecundity to increase with UV-B 300 nm ($P < 0.16$) and UV-A 365 nm treatments ($P < 0.47$), but the difference was not significant.

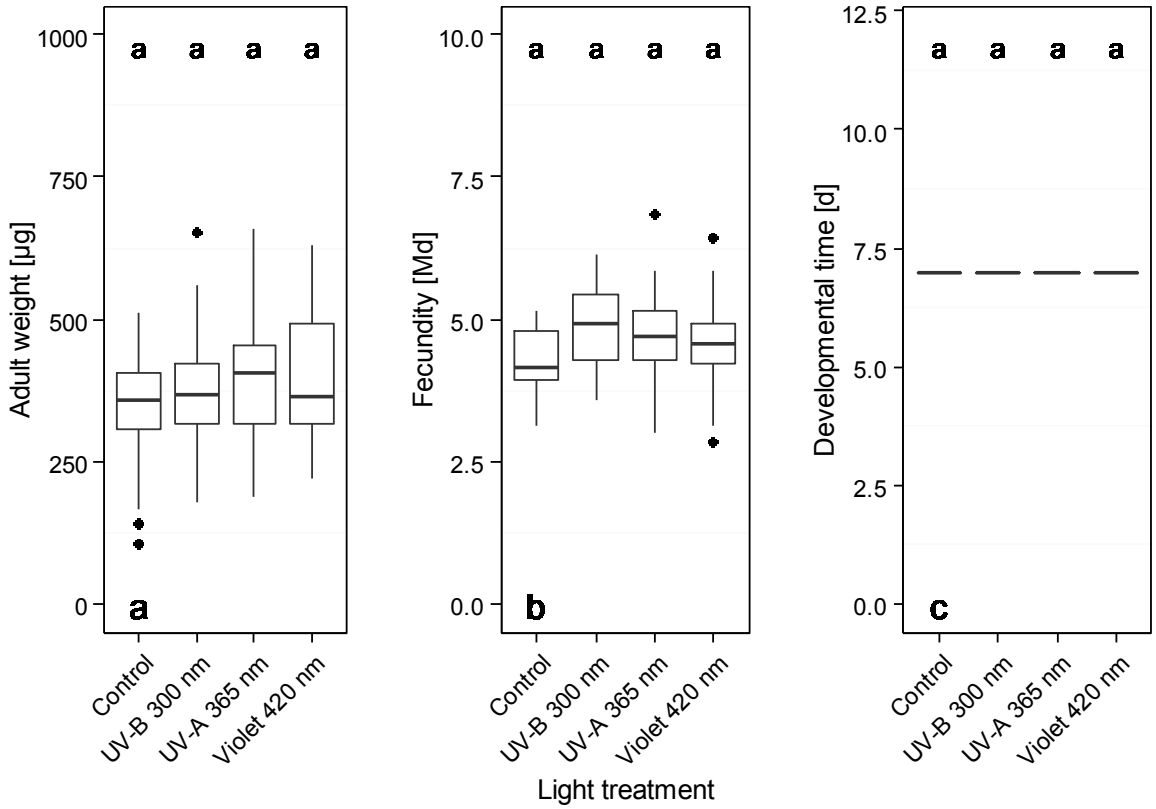

Fig. 4 Adult weight (a), fecundity (b), and developmental time (c) of *M. persicae* kept on broccoli plants that were grown in a climate chamber and exposed to control lighting plus one of three light treatments (UV-B 300 nm, UV-A 365 nm, and violet 420 nm) or to control lighting without additional illumination. UV-B 300 nm, UV-A 365 nm, and violet 420 nm were supplied by single high-power LEDs. Control lighting was supplied by Osram Lumilux Interna, L 58 W / T8, 827, 5200 lm, 2700 K fluorescence tubes (400 to 700 nm). The aphids were in clip cages and were not directly exposed to the light treatments. Different letters indicate significant differences (GLMM (Fig. 3a), GLM (Fig. 3b, 3c), and Tukey *post hoc* tests, $P < 0.05$; $N = 8$ biological replicates)

Developmental parameters *Plutella xylostella*

Larvae weight of *P. xylostella* after one week of feeding on light-treated broccoli plants was not significantly affected by different light treatments (Fig. 5a). The developmental time from egg to pupa stage did not differ significantly (*data not shown*). Final pupa weight was lowest with UV-B 300 nm treatment (reduced by 16-16.5%) and differed significantly to UV-A 365 nm and violet 420 nm light treatments ($P < 0.05$) but not to control treatments (Fig. 5b).

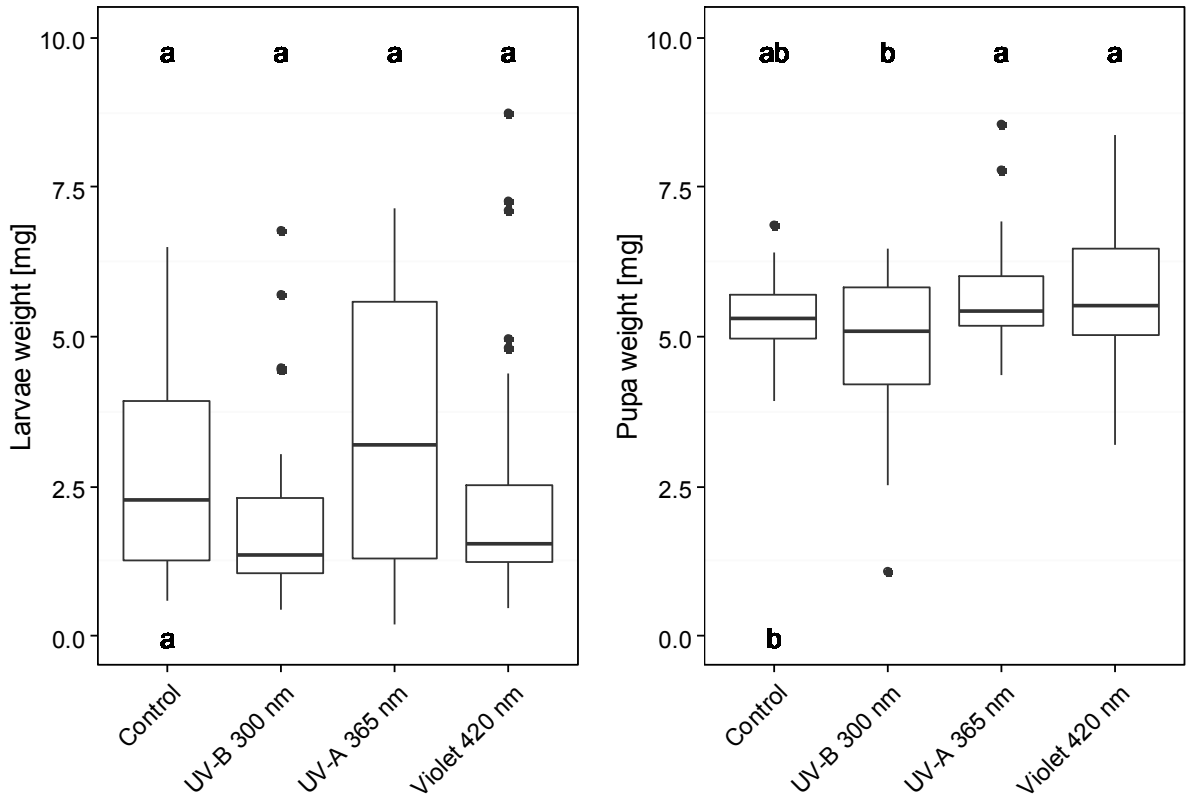

Fig. 5 Larvae weight after one week of larvae development (a) and pupa weight (b) of *P. xylostella* kept on broccoli plants that were grown in a climate chamber and exposed to control lighting plus one of three light treatments (UV-B 300 nm, UV-A 365 nm, and violet 420 nm) or to control lighting without additional illumination. UV-B 300 nm, UV-A 365 nm, and violet 420 nm were supplied by single high-power LEDs. Control lighting was supplied by Osram Lumilux Interna, L 58 W / T8, 827, 5200 lm, 2700 K fluorescence tubes (400 to 700 nm). Different letters indicate significant differences (GLMM, and Tukey *post hoc* tests, $P < 0.05$; $N = 8$ biological replicates)

Discussion

This chapter of the study investigated the influence of broccoli plant illumination with low light intensities generated with single LEDs, and the interaction with herbivorous insects which showed light-induced, plant-mediated reactions in performance or behavior in chapter one, two or three. The research aim of this chapter was to reduce the light intensity that was used in the previous chapters with high-power LEDs or UV-B tubes and to decrease the detrimental effects of high-intensity UV-B radiation on plant growth. Furthermore, the use of single LEDs with low intensities can save on consumption of electricity and reduce the production costs for plant producers. In previous parts of this study it could be shown, that *B. brassicae* reacted

with reduced reproduction potential on UV-B 310 nm-treated plants (UV-B tubes) with high intensities of 2.9 kJ m^{-2} d^{-1} UV-B and 80 kJ m^{-2} d^{-1} UV-A radiation. *Myzus persicae* increased its fecundity and adult weights on plants treated with UV-B 310 nm or UV-A 365 nm radiation (Rechner et al. 2016). In this single LED illumination approach the intensities (0.72 kJ m^{-2} d^{-1} UV-B and 20 kJ m^{-2} d^{-1} UV-A radiation for the UV-B LEDs and 288 kJ m^{-2} d^{-1} UV-A radiation for the UV-A LEDs) provided by the single LEDs were able to reduce the plant height of broccoli in UV-B 300 nm and UV-A 365 nm treatments, indicating a low threshold for plant growth depression with low-intensity UV-B and UV-A treatments. The two aphid species did not react with altered performance parameters (like in chapter one). *Myzus persicae* increased its fecundity by trend on UV-B 300 nm- and UV-A 365 nm-treated plants, but the difference was not significant. Probably, because the intensities were four times lower in this experiment than in the other experiments in chapter one, two and three of this study. Also the relative overall flavonol absorbance was increased in UV-B 300 nm- and violet 420 nm-treated plants, at least on two measurement dates. Interestingly, the pupa weight of *P. xylostella* was significantly reduced by 16-16.5% if treated with single low-intensity UV-B 300 nm LEDs compared to UV-A 365 nm and violet 420 nm treatments, but not compared to the control treatment. Single compounds of flavonol glycosides and specific glucosinolates react quite differently under various light treatments (Rechner et al. 2016) and the relative overall flavonol absorbance only gives a broad overview about the total concentrations of flavonols in the leaf and is not considering specific single compounds. Next to this, the reaction of glucosinolates was not measured in this part of the study. Mewis et al. (2012a) could show, that specific aliphatic and indole glucosinolates increased with UV-B treatment and that this light induction of glucosinolates was not depended on the UV-B intensity. The question if glucosinolates increased in the low intensity UV-B treatment remains open in this part of the study. Light treatment with violet 420 nm resulted in increased relative overall flavonol absorbance, but the insects were not reacting with altered performance on this metabolic changes in the broccoli plants. This was the same result as found in chapter three. It indicates that only single specific secondary plant metabolites could be responsible for certain kinds of induced resistance against herbivorous insects in broccoli and that the relative overall flavonol absorbance measured non-destructively using Dualex is not able to detect these differences in the plants metabolism. Another reason for differences in the results, next to the four times lower light intensities used in this part of the study, could be the fact that the UV-B tubes used

in chapter one and three had a peak wavelengths of 310 nm and a bit broader spectra, also emitting some UV-A radiation. The UV-B 300 nm LEDs used in this part of the study were really narrow-bandwidth with the peak wavelength at 300 nm, perhaps not ideal to hit the UVR8 receptor and increase the defense potential of broccoli plants grown under these low intensity light sources. The UVR8 receptor has its peak absorbance at 282 nm and a characteristic shoulder at 290 nm (Yang et al. 2015). Furthermore, the UV-B intensity could be too low to induce usual wounding pathways and increase the plant resistance in broccoli with single UV-B LEDs. This fact will be cleared, if studies are able to use new UV-B high power LEDs with intensities comparable to UV-B tubes or UV-B LEDs mixed with different broader wavelengths, i.e., 280 nm to 310 nm to cover more of the UV-B spectrum. In addition, the light intensity threshold for inducing responsible metabolites, which are able to alter the defense answer of the plant, seems to be higher than the threshold for inducing growths depression in the plants with UV-B or UV-A treatments.

Overall for gently conclusion, it could be shown that plants answered to low-intensity UV-B 300 nm treatment with reduced plant heights and increased relative overall flavonol absorbance. Unfortunately, no indirect, plant-mediated effects on the insects could be observed, except the UV-B resistance effect of *P. xylostella* pupa weight, if compared to UV-A 365 nm- or violet 420 nm-treated plants but not to control plants. This indicates that the plant first induced a basic reaction by producing relatively low amounts of photoprotective flavonols which lead to a certain growth depression as metabolic costs. However, the induction of herbivore resistance with protective flavonols needs obviously higher concentrations of secondary plant metabolites responsible for enhanced resistance in broccoli.

Acknowledgments

This research project was funded by the German Research Foundation DFG, grant Po 207/39-1.

Chapter 5

Effects of Light-Emitting Diode Treatments on *Brevicoryne brassicae* Performance Mediated by Secondary Metabolites in Brussels Sprouts

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Journal of Plant Diseases and Protection (2016) 123:321–330.

DOI 10.1007/s41348-016-0029-9

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Abstract

Although short-wavelength light and especially UV radiation can induce resistance in plants against herbivorous insects, the optimal wavelengths, light intensities (photon flux rates), and illumination schedules for inducing resistance have not been previously determined. Light-emitting diode (LED) illumination enables researchers to subject insects and plants to narrow-banded light treatments. In this study, we assessed how the metabolite-based defense response in Brussels sprout plants (*Brassica oleracea* var. *gemmifera*) against the cabbage aphid, *Brevicoryne brassicae* (Hemiptera: Aphididae), was affected by the following LED-generated wavelengths of light: 300 nm (UV-B), 365 nm (UV-A), 470 nm (blue light), and 660 nm (red light). Ambient greenhouse light supplemented with light from sodium vapor lamps served as the control treatment. The results showed a significant reduction in *B. brassicae* performance in the UV-A treatment compared to the blue light treatment; the reduction in the UV-A treatment was accompanied by a significant increase in glucosinolate concentrations. The UV-B treatment induced significant changes in plant flavonoid concentrations but did not affect *B. brassicae* performance.

Key Words

UV, Glucosinolates, Flavonoids, Resistance, LED, Cabbage Aphids

Introduction

Plants perceive light with photoreceptors used for photosynthesis or for other specific plant processes (Ballare 2014). Photoreceptors for blue light have been well characterized and are phototropins, cryptochromes, and members of the Zeitlupe family (Demarsy & Fankhauser 2009). Moreover, UV-B plays a key role in regulating plant morphology, growth and defense potential (Ballare 2014). In terms of plant defense against herbivores high UV-B intensities induce unspecific reactions comparable to the reactions induced by wounding (Jenkins 2009). However, researchers recently demonstrated that dimers of the UVR8 protein can perceive even low doses of ambient UV-B (Rizzini et al. 2011; Heijde & Ulm 2013), which can not only fine tune plant growth and development but also trigger defense reactions, mainly based on stimulating metabolism of numerous secondary plant metabolites (Kuhlmann & Müller 2009a; 2009b; 2011; Schreiner et al. 2012; Ballare 2014). The light triggered induction of resistance in plants could influence insect development immediately before feeding (repellent or antixenosis effects) or after feeding (antibiosis effects) as a consequence. Flavonoids are ubiquitous but their specific composition is species-specific (Harborne & Williams 2000). Mewis et al. (2012a) observed an increased accumulation of the flavonoids kaempferol and quercetin in broccoli sprouts after 24 h of UV-B treatment, and flavonoid-deficient mutants of *Arabidopsis thaliana* were highly sensitive to UV-B radiation (Ormrod et al. 1995). Similarly change in ratio of kaempferol and quercetin glycosides due to UV exposure in Brassicaceae are described by Reifenrath & Müller (2007). Flavonoids function as feeding and oviposition deterrents against many herbivorous insects, and some specialist insects use these compounds even as cues for oviposition and host selection (Harborne & Williams 2000).

Furthermore, specific UV-B-mediated induction of glucosinolates was reported in Brassicaceae. For instance, the UV triggered accumulation of defensive glucosinolate metabolites in broccoli was accompanied by increased expression of genes associated with the salicylic- and jasmonic acid signaling defense pathways (Mewis et al. 2012a; Textor & Gershenzon 2009). Some glucosinolates in the Brassicaceae negatively affect generalist insect herbivores. Glucosinolate effects are also species specific in that these compounds can stimulate the feeding and oviposition by some specialized herbivores, i.e., there is no simple relationship between glucosinolate content and insect response (Halkier & Gershenzon 2006; Rohr et al. 2011). Researchers have speculated that aphids are more exposed to intact glucosinolates rather than to toxic hydrolysis products because these phloem-feeding insects

only come in contact with the phloem and cause slight tissue damage relative to leaf-chewing insects (Kim et al. 2008; Tjallingii 2006).

Moreover, it has been shown that glucosinolate profiles of plants can be differently triggered by different light qualities in particular blue *vs.* red (Abe et al. 2015). Red light and the ratio between red and far red light, received by phytochromes primarily triggers growth reactions (red/far red "shade avoidance syndrome") and could interfere with reactions controlled by the UVR8 receptor ("trade off" between plant investment in growth *vs.* resistance) in arabidopsis (Mazza & Ballare 2015). Recently, also the induction of pathogen and nematode resistance in soybean and tomato by plant illumination with red light-emitting diode (LED) could be discovered (Dhakal et al. 2015; Yang et al. 2015). Until now, regarding the induction of resistance to herbivores by different light spectra, studies exploring the resistance mechanism and specificity always used light in the UV range generated by light sources emitting broad wavelength spectra. In particular, a differentiation of UV-B and UV-A triggered effects is still missing (Ballare 2014). LED technology now offers improved tools to fill up this gap.

Herewith we address the hypotheses that different narrow-bandwidths light qualities in addition to broad spectra light sources have the potential to influence Brussels sprout plants growths, increase its secondary metabolite composition (glucosinolates and flavonoids) and its plant defense potential against the cabbage aphid *Brevicoryne brassicae* (Hemiptera: Aphididae). Altogether this study assesses the effects of narrow-bandwidth UV-LED light (UV-B 310 nm, UV-A 365 nm) compared to longer wavelength of blue 470 nm and red 660 nm on Brussels sprout plants growths and for affecting the performance of the cabbage aphid (*B. brassicae*) in relation to the combined light and aphid induced modulation of selected glucosinolates and flavonoids.

Materials and Methods

Plant material and rearing of insects

Brussels sprout plants (*Brassica oleracea* var. *gemmifera* DC, obtained from HILD Samen GmbH, Germany) were grown from seeds in individual pots (diameter: 12 cm, height: 9 cm) containing fertilized soil (Fruhstorfer Erde Type P, supplied by Archut GmbH, Lauterbach-Wallenrod, Germany). Pots with seeds were directly exposed to different light qualities

generated by LEDs for the whole period of germination and following experiment with aphid infestation under greenhouse conditions (22-25°C, humidity 65-75%, photoperiod 16:8 h L:D). The experiment was repeated once over time, swapping the different light environments to another compartment to avoid microclimatic gradients between the different compartments in the greenhouse. Plants were irrigated once each day and fertilized (Wuxal Top N, supplied by Aglukon Spezialduenger GmbH and Co. KG, Düsseldorf, Germany) once each week.

B. brassicae was reared on 4-week-old Brussels sprout plants in a gauze cage with a wooden frame (85 cm × 60 cm × 60 cm); the cages were kept in a climate chamber (18-22°C, humidity 65-75%, photoperiod 16:8 h L:D). Every week, half of the plants were replaced so as to continuously provide a high quality food source. For experiments, adult aphids were randomly collected from these cages.

Experimental setup and light treatments

The experiment was conducted in a greenhouse chamber that was completely isolated from external UV radiation by covering the bench (6 m x 1.5 m) that supported the plants with UV-absorbing plastic film (FVG Sun 5 Pro, supplied by FVG Folien-Vertriebs GmbH, Dernbach, Germany); the film was located 2 m above the bench. The side walls of the greenhouse chamber were covered with reflective mulch film (Full metal on black film, supplied by Sunup Reflective Films/Star Metal Plating, Escondido, CA, USA); thus, UV-filtered global radiation could reach inside only from the top. To provide optimal light conditions for growth, three sodium vapor lamps (Philips Son-T Agro 400) located over the bench below the UV-absorbing plastic film illuminated the whole bench for 16 h/day. Five compartments that were isolated from each other by frames coated with reflective mulch film were constructed. The frames were placed at 1-m intervals, which created a row of small light-isolated compartments where plants were subjected to different LED treatments. Each compartment with LED illumination contained an aluminum frame variable adjustable 25 cm above the plants and equipped with a panel containing four high-power LEDs (supplied by Roithner Laser Technik GmbH, Vienna, Austria). Panels with the following LED light qualities were constructed: UV-B 300 nm (UVTOP 295-FW-TO18), UV-A 365 nm (H2A1-H365-E), blue 470 nm (H2A1-H470), and red 660 nm (H2A1-H660). A control compartment was lighted by the sodium vapor lamps only. The intensities of light emitted by the LED panels were measured with a LI 250 Light Meter with Sensor LI 190 Quantum Sensor (supplied by LI-COR Biosciences, Lincoln, Nebraska, USA),

which measured photosynthetic active radiation (PAR) (400-700 nm), and a data logger ALMEMO 2390-5 (supplied by Ahlborn Mess- und Regelungstechnik GmbH, Holzkirchen, Germany) with sensor FLA 623 UV-A with a measurement range from 310 to 400 nm, which measured UV-A radiation, and sensor FLA 623 UV-B with a measurement range from 265 to 315 nm, which measured UV-B-radiation. The intensity of the light emitted by each LED panel was measured in a dark room and at 25 cm below the panel to determine the maximum intensity (at the center of the compartment) and the minimum intensity (at the edge of the compartment) (Table 1). The radiation spectra of the LEDs, which were measured with a UV/VIS fiber and a compatible fiber optic spectrometer (AvaSpec 2048-2, supplied by AVANTES, Appeldoorn, The Netherlands) are shown in Fig. 1. Six Brussels sprout plants per compartment were grown under these light settings in each experiment; the positions of the plants under the LED panel were changed three times per week so that all of the plants were equally illuminated.

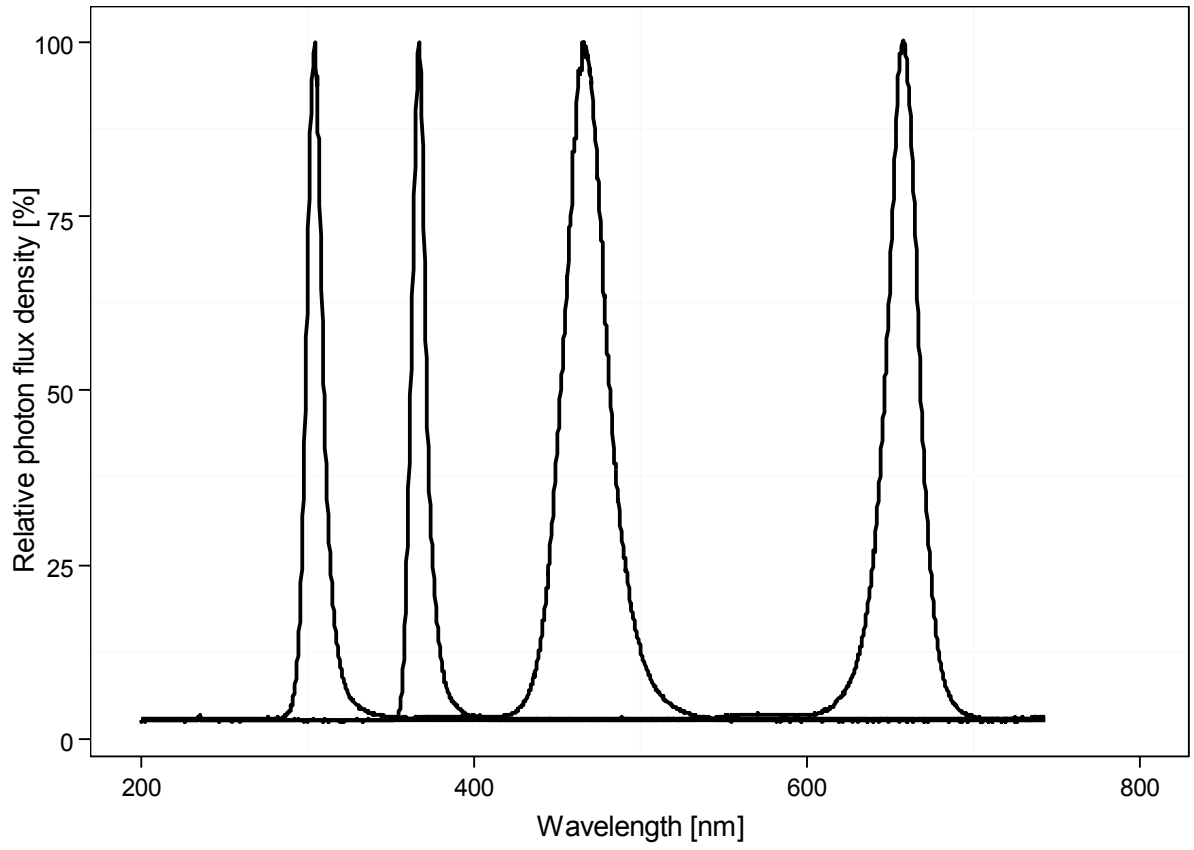

Fig. 1 Spectra showing the wavelengths [nm] and the corresponding photon flux density [%] for UV-B 300 nm (UVTOP 295-FW-TO18), UV-A 365 nm (H2A1-H365-E), blue 470 nm (H2A1-H470), and red 660 nm (H2A1-H660) lights (LEDs supplied by Roithner Laser Technik GmbH, Vienna, Austria). The spectra were measured in the range of 200 to 800 nm in the dark with a

UV/VIS fiber and a compatible fiber optic spectrometer (AvaSpec 2048-2, supplied by AVANTES, Appeldoorn, The Netherlands)

Tab. 1 Mean intensities of narrow bandwidth lights (UV-B 300 nm, UV-A 365 nm, blue 470 nm, and red 660 nm) generated by four high-power LEDs. These light treatments were applied to Brussels sprout plants for 16 h per day in a greenhouse. The plants also received lighting from sodium vapor lamps and from UV-filtered sunlight

Light treatment	**Intensity [µmol m^{-2} s^{-1}]**
Control and background illumination with sodium vapor lamps	100 ± 10
UV-B (peak 300 nm)	0.02 ± 0.001 (0.03 kJ m^{-2} d^{-1})
UV-A (peak 365 nm)	13.84 ± 10.18 (259 kJ m^{-2} d^{-1})
Blue (peak 470 nm)	11.04 ± 6.03
Red (peak 660 nm)	2.09 ± 1.29

Development of *B. brassicae*

On six 4-week-old Brussels sprout plants per light treatment, 12 adults of *B. brassicae* were carefully placed on the underside of the third or fourth leaf (a brush was used) and not directly illuminated by the LED treatments. Moreover, aphids were confined inside a plastic leaf cage (diameter: 3 cm) impermeable for UV radiation and clipped onto the underside of the leaf, to avoid any detrimental direct effects of light treatments to the aphids. The adult aphids were allowed to deposit larvae for 24 h before they were removed. Twelve larvae were permitted to develop per plant (per clip cage) in each experiment. The number of days required for the larvae to develop into adults was recorded, and the final weights of 10 adults per clip cage were measured with a microbalance (Type MC 5 Sartorius, Goettingen). Two adults per plant were kept separately in clip cages on the same plants, and their offspring counted every second day to measure fecundity [Md]. When these plants were 7-week-old, they were used for the determination of plant dry mass and secondary metabolite content.

Dry matter of Brussels sprout plants

After the two aphids had stopped reproducing in the clip cages (see the previous section), the dry matter [DM] was determined for six (three per experiment) 7-week-old plants per light treatment. The aboveground parts of each plant were cut, dried for 5 days at 70°C, and weighed with an electronic balance (Type BP 3100 P, Sartorius, Goettingen).

Analysis of secondary metabolites in Brussels sprout plants

Sample preparation

For each light treatment, leaves were collected from four (two per experiment) 7-week-old Brussels sprout plants infested with *B. brassicae*, i.e., plants with one infested leaf in a clip cage as described earlier. A mixed sample of all leaves per plant (excluding stems and midribs) was placed in liquid nitrogen and was then freeze-dried for 5 days (using a Christ Alpha 1-4 LSC freeze drier) and subsequently ground to a powder (≤ 0.25 mm).

Flavonoid analysis

Flavonoids were analyzed according to Schmidt et al. (2010) with modification. Lyophilized broccoli sprout tissue (0.02 g) was extracted with 600 µl of 60% aqueous methanol on a magnetic stirrer plate for 40 min at 20°C. The extract was centrifuged at 4500 rpm for 10 min at the same temperature, and the supernatant was collected in a reaction tube. This process was repeated twice with 300 µl of 60% aqueous methanol for 20 min and 10 min, respectively; the three supernatants per sample were combined. The extract was subsequently evaporated until it was dry and was then suspended in 200 µl of 10% aqueous methanol. The extract was centrifuged at 3000 rpm for 5 min at 20°C through a Corning® Costar® Spin-X® plastic centrifuge tube filter (Sigma Aldrich Chemical Co., St. Louis, MO, USA) for the HPLC analysis. Each extraction was carried out in duplicate.

Flavonoid composition (including hydroxycinnamic acid derivatives and glycosides of flavonols) and concentrations were determined using a series 1100 HPLC (Agilent Technologies, Waldbronn, Germany) equipped with a degaser, binary pump, autosampler, column oven, and photodiode array detector. An Ascentis® Express F5 column (150 mm × 4.6 mm, 5 µm, Supelco) was used to separate the compounds at a temperature of 25°C. Eluent A was 0.5% acetic acid, and eluent B was 100% acetonitrile. The gradient used for eluent B was 5-12% (0-3 min), 12-25% (3-46 min), 25-90% (46-49.5 min), 90% isocratic (49.5-52 min), 90-5%

(52-52.7 min), and 5% isocratic (52.7-59 min). The determination was conducted at a flow rate of 0.85 ml min^{-1} and a wavelength of 320 nm, 330 nm, and 370 nm for hydroxycinnamic acid derivates, acylated flavonol glycosides, and non-acylated flavonol glycosides, respectively. The hydroxycinnamic acid derivatives and glycosides of flavonols were identified as deprotonated molecular ions and characteristic mass fragment ions according to Schmidt et al. (2010) by HPLC-DAD-ESI-MS^n using an Agilent series 1100 ion trap mass spectrometer in negative ionisation mode. Nitrogen was used as the dry gas (10 L min^{-1}, 325°C) and the nebulizer gas (40 psi) with a capillary voltage of -3500 V. Helium was used as the collision gas in the ion trap. The mass optimization for the ion optics of the mass spectrometer for quercetin was performed at *m/z* 301 or arbitrarily at *m/z* 1000. The MS^n experiments were performed in auto up to HPLC-DAD-ESI-MS^3 in a scan from *m/z* 200-2000. Standards (chlorogenic acid, quercertin 3-glucoside, and kaempferol 3-glucoside; Roth, Karlsruhe, Germany) were used for external calibration curves. Results are presented as µg g^{-1} dry weight. Flavonol glycoside concentration was determined for two broccoli plants per experiment, resulting in four plants per treatment in total; each replicate sample was measured in duplicate.

Glucosinolate analysis

Glucosinolate concentration was determined as desulfo-glucosinolates using a modified method according to DIN EN ISO 9167-1 and Wiesner et al. (2013). A 20.0 mg quantity of powdered sample plus 100 µl of 0.1 mM 2-propenyl glucosinolate (BCR-367R, Community Bureau of Reference, Brussels, Belgium) as the internal standard was extracted with 750 µl of 70% (v/v) methanol at 70°C. The preparation was boiled for 10 min and then centrifuged (2250 g) for 5 min at room temperature. The supernatant was decanted, and the residue was re-extracted twice with 500 µl of hot 70% methanol each time. The pooled extracts were loaded onto a mini column containing 500 µl of DEAD-Sephadex A-25 ion-exchanger (acetic acid-activated, Sigma–Aldrich Chemie GmbH, Taufkirchen, Germany) that had been conditioned with 2 M acetic acid and washed with 6 M imidazole formate. After loading, the column was washed with 0.02 M sodium acetate buffer. Finally, 75 µl of an aryl sulfatase solution (Sigma-Aldrich, Steinheim, Germany) was added, and the preparation was incubated overnight. Desulfo-glucosinolates were eluted with water and analyzed by HPLC using a Merck HPLC system (Merck-Hitachi, Darmstadt, Germany) with a Spherisorb ODS2 column (Bischoff, Leonberg Germany; particle size 5 µm, 250 mm x 4 mm). HPLC conditions were as follows: solvent A, MilliQ water; solvent B, 20% v/v acetonitrile in MilliQ water; solvent C, 100%

acetonitrile. The 60-min run consisted of 1% (v/v) B (0-2 min), 1% to 20% (v/v) B (2-36 min), 20% (v/v) B (36-41 min), 20% B to 100% (v/v) C (41-43 min), 100% (v/v) C (43-48 min), 100% (v/v) C to 1% (v/v) B (48-50 min), and finally a 10-min hold at 1% (v/v) B (50-60 min). The remaining percentage was applied by solvent A. Determination was conducted at a flow rate of 0.7 ml min^{-1} and a wavelength of 229 nm. Desulfo-glucosinolates were identified based on comparison of retention times and UV absorption spectra with those of known standards. Additionally, desulfo-glucosinolates were previously identified in other *Brassica* species by HPLC-ESI–MS^2 using Agilent 1100 series (Agilent Technologies, Waldbronn, Germany) in positive ionization mode (Krumbein et al. 2005; Zimmermann et al. 2007). Glucosinolate concentration was calculated using 2-propenyl glucosinolate as an internal standard and using the response factor of each individual glucosinolate was calculated relative to 2-propenyl glucosinolate (Brown et al. 2003; Buchner 1987). Results are presented as µg g^{-1} dry weight. Glucosinolate concentration was determined for two broccoli plants per experiment, resulting in four plants per treatment in total; each replicate sample was measured in duplicate.

Statistical analysis

The data were analyzed in R 3.1.1 (R Develoment Core Team 2008), and package ggplot2 was used for graph construction (Wickam 2009). To ensure normal distribution of the data qqplots in R were performed. In addition, Levene-tests were carried out to check for variance homogeneity. Normal distribution and variance homogeneity were given in our data set. Furthermore, counted data (fecundity and developmental time) were analyzed with generalized linear models using a log-link together with quasi-Poisson distribution. Differences between the light treatments for developmental time were compared using ANOVA chi-square test. The effects of narrow-band light treatments on aphid developmental parameters and plant parameters were tested by ANOVAs followed by Tukey's HSD tests. Data of two similar experiments were combined considering experiments as a factor in the analysis. The experiments showed the same significant results and are finally shown together to compensate the low number of repeats in each experiment. Two plants per experiment infested with cabbage aphids were chosen randomly from each treatment, and mixed samples of all leaves were analyzed for glucosinolate and flavonoid contents. The samples were run twice for HPLC, and means were taken for ANOVA.

Results

Developmental parameters of *B. brassicae*

Aphid adult weight

The LED treatments significantly affected the weight of adult aphids ($F_{(4, 12)} = 26.05$, $P < 0.001$) (Fig. 2). Aphid adult weight were lower on plants illuminated with UV (UV-B 300 nm and UV-A 365 nm) than on plants illuminated with blue 470 nm ($P < 0.001$) or red 660 nm light ($P < 0.05$ with UV-B and $P < 0.001$ with UV-A). Aphid adult weight were significantly higher in the blue and red light treatments than in the control group ($P < 0.001$). Aphid adult weight tended to be lower in the UV-A treatment than in the control group ($P = 0.65$) but the difference was not significant (Fig. 2).

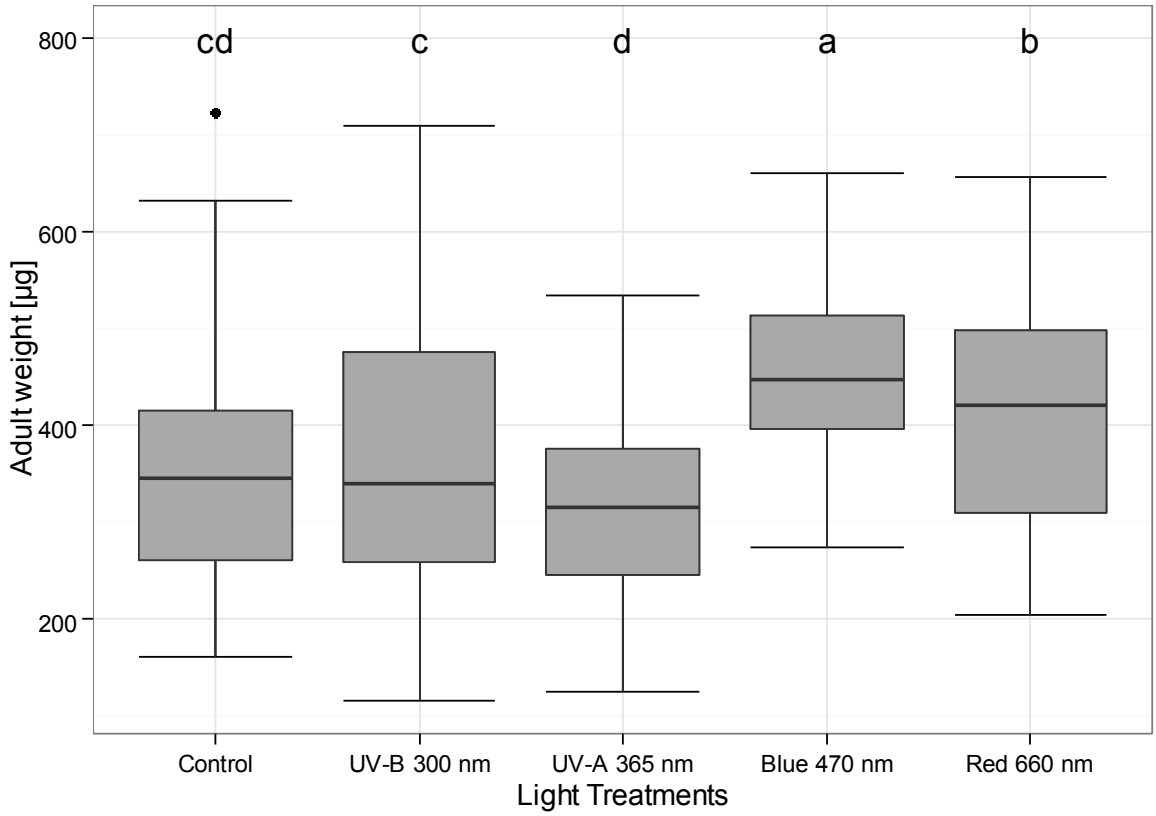

Fig. 2 Adult weight [µg] per *B. brassicae* adult that fed on Brussels sprout plants grown in a greenhouse under sodium vapor lamps only (Control) or under sodium vapor lamps plus LED illumination: UV-B 300 nm, UV-A 365 nm, blue 470 nm, or red 660 nm. All plants were also illuminated by UV-filtered sunlight. Different letters indicate significant differences (ANOVA and Tukey *post hoc* test, $P < 0.05$, $N = 12$)

Developmental time

Aphid developmental time tended to be shortest for aphids in the blue 470 nm (P = 0.45) and red 660 nm light treatments (P = 0.47) (8.10 ± 0.03 d and 8.30 ± 0.05 d, respectively), followed by the control (8.40 ± 0.05 d) and the UV-B 300 nm (P = 0.83) and UV-A 365 nm (P = 0.73) treatments (8.49 ± 0.05 d and 8.55 ± 0.07 d, respectively). However, these differences were not significant (ANOVA $\chi^2_{(4, 12)} = 1.5$, $P = 0.82$).

Fecundity

Brevicoryne brassicae fecundity was significantly affected by light treatment ($F_{(4, 12)} = 3.13$, $P < 0.05$) (Fig. 3). Fecundity was significantly lower with UV-A 365 nm light than with blue 470 nm light ($P < 0.05$). Fecundities were intermediate and did not significantly differ with UV-B 300 nm ($P = 1$), UV-A 365 nm ($P = 0.57$) and red 660 nm ($P = 0.9$) compared to control light treatments (Fig. 3).

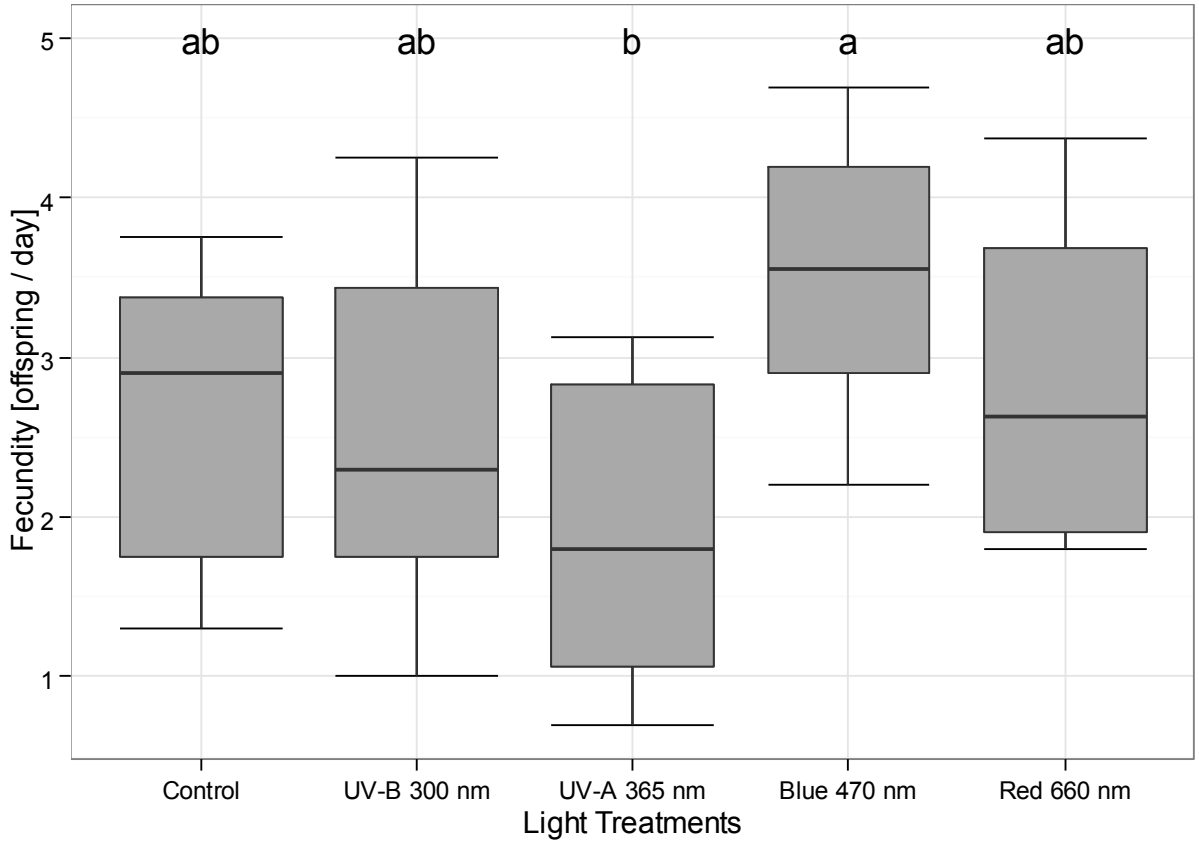

Fig. 3 Fecundity [offspring/day] of *B. brassicae* feeding on Brussels sprout plants grown in a greenhouse under sodium vapor lamps only (Control) or under sodium vapor lamps plus LED illumination: UV-B 300 nm, UV-A 365 nm, blue 470 nm, or red 660 nm. All plants were also illuminated by UV-filtered sunlight. Different letters indicate significant differences (ANOVA and Tukey *post hoc* test, $P < 0.05$, $N = 12$)

Plant parameters

Dry matter

The dry matter of Brussels sprout plants was not significantly affected ($F_{(4, 12)} = 1.66$, $P = 0.17$) by the light treatments but tended to lower with UV-B ($P = 0.06$) and UV-A ($P = 0.9$) (*data not shown*).

Plant secondary metabolites

Glucosinolates

The concentrations in the leaves of the predominant aliphatic glucosinolates, 3-methylsulfinylpropyl glucosinolate and 4-methylsulfinylbutyl glucosinolate, did not significantly differ among the light treatments but tended to be greater in the blue and red treatments than in the control (Table 2). Lower concentrations of the less prevalent, aliphatic (hydroxy) alkenyl 2-propenyl glucosinolate, 3-butenyl glucosinolate and 2-hydroxy-3-butenyl glucosinolate as well as of the 4-methylthiobutyl glucosinolate, were assessed in the control leaves and remained unchanged under the various LED treatments. In addition, 3-indolylmethyl glucosinolate and its derivatives (4-hydroxy-3-indolylmethyl glucosinolate, 4-methoxy-3-indolylmethyl glucosinolate, and 1-methoxy-3-indolylmethyl glucosinolate) were measured in the leaves. The 3-indolylmethyl glucosinolate was the major indole glucosinolate marked by a distinct elicitation at UV-A treatment (Table 2).

Tab. 2 Mean concentrations of specific glucosinolates [± SE] in aphid-infested Brussels sprout plants grown in a greenhouse under sodium vapor lamps only (Control) or under sodium vapor lamps plus LED illumination: UV-B 300 nm, UV-A 365 nm, blue 470 nm, or red 660 nm. All plants were also illuminated by UV-filtered sunlight. Means in a row followed by different letters are significantly different (ANOVA and Tukey *post hoc* test, $P < 0.05$, $N = 4$)

	Light treatments and glucosinolate concentrations [µg g^{-1} d.w.]				
Glucosinolates (GS)	**Control**	**UV-B 300 nm**	**UV-A 365 nm**	**Blue 470 nm**	**Red 660 nm**
Aliphatic GS	741.3 ± 242.1$^{(a)}$	1246.8 ± 466.2$^{(a)}$	1491.0 ± 281.3$^{(a)}$	1244.9 ± 170.1$^{(a)}$	1930.2 ± 412.9$^{(a)}$
4-methylthiobutyl GS	4.3 ± 1.7$^{(c)}$	8.3 ± 1.5$^{(bc)}$	8.3 ± 0.4$^{(bc)}$	13.4 ± 1.4$^{(a)}$	9.4 ± 0.6$^{(ab)}$
3-methylsulfinylpropyl GS	248.8 ± 78.5$^{(a)}$	401.0 ± 142.9$^{(a)}$	510.4 ± 89.1$^{(a)}$	409.5 ± 49.7$^{(a)}$	696.6 ± 139.3$^{(a)}$
4-methylsulfinylbutyl GS	335.6 ± 30.4$^{(a)}$	575.0 ± 227.8$^{(a)}$	638.5 ± 134.1$^{(a)}$	550.3 ± 85.8$^{(a)}$	1002.8 ± 237.1$^{(a)}$
2-propenyl GS	78.4 ± 21.2$^{(b)}$	127.7 ± 43.9$^{(ab)}$	238.4 ± 44.3$^{(a)}$	178.2 ± 38.3$^{(ab)}$	133.1 ± 24.6$^{(ab)}$
3-butenyl GS	30.8 ± 8.2$^{(a)}$	52.2 ± 27.9$^{(a)}$	25.6 ± 4.9$^{(a)}$	47.7 ± 4.3$^{(a)}$	32.0 ± 8.2$^{(a)}$
2-hydroxy-3-butenyl GS	43.3 ± 5.9$^{(a)}$	82.5 ± 28.6$^{(a)}$	69.8 ± 13.1$^{(a)}$	45.8 ± 4.7$^{(a)}$	56.3 ± 12.3$^{(a)}$
Indole GS	821.1 ± 229.5$^{(b)}$	551.5 ± 89.1$^{(b)}$	2432.0 ± 466.8$^{(a)}$	803.7 ± 171.3$^{(b)}$	686.9 ± 201.3$^{(b)}$
3-indolylmethyl GS	759.9 ± 212.3$^{(b)}$	482.1 ± 100.4$^{(b)}$	2304.7 ± 452.0$^{(a)}$	767.5 ± 163.1$^{(b)}$	653.3 ± 200.2$^{(b)}$
4-hydroxy-3-indolylmethyl GS	7.5 ± 3.1$^{(b)}$	5.8 ± 1.4$^{(b)}$	40.3 ± 2.8$^{(a)}$	4.2 ± 3.3$^{(b)}$	2.1 ± 1.1$^{(b)}$
4-methoxy-3-indolylmethyl GS	49.3 ± 12.5$^{(ab)}$	62.1 ± 15.3$^{(ab)}$	77.2 ± 16.4$^{(a)}$	24.5 ± 4.9$^{(b)}$	30.4 ± 6.7$^{(ab)}$
1-methoxy-3-indolylmethyl GS	4.4 ± 1.9$^{(a)}$	1.4 ± 0.4$^{(a)}$	9.7 ± 2.7$^{(a)}$	7.5 ± 3.3$^{(a)}$	1.2 ± 0.4$^{(a)}$

Flavonoids

Of the 17 flavonol glycosides and 13 hydroxycinnamic acid derivatives identified in the Brussels sprout plants, four main flavonol glycosides and two main hydroxycinnamic acid derivatives were considered most relevant to the current study (Table 3). Quercetin-3-*O*-sinapoyl-sophoroside-7-glucoside concentration was not significantly affected by the treatments but was highest with UV-B treatment. The concentrations of sinapic acid acylated kaempferol tri- and tetraglycosides were decreased with UV-A light, whereas the concentration of kaempferol-3-*O*-sinapoyl-triglucoside-7-diglucoside was increased by all of the LED treatments relative to the control and was highest with UV-B and red light. The concentrations of the hydroxycinnamic acid derivatives disinapoyl-gentiobiose and trisinapoyl-gentiobiose did not significantly differ among the light treatments (Table 3).

Tab. 3 Mean concentrations of specific flavonol glycosides of quercetin and kaempferol and of hydroxycinnamic acid glycosides [± SE] in aphid-infested Brussels sprout plants grown in a greenhouse under sodium vapor lamps only (Control) or under sodium vapor lamps plus LED illumination: UV-B 300 nm, UV-A 365 nm, blue 470 nm, or red 660 nm. All plants were also illuminated by UV-filtered sunlight. Means in a row followed by different letters are significantly different (ANOVA and Tukey *post hoc* test, $P < 0.05$, $N = 4$)

	Light treatment and flavonoid concentration [µg g^{-1} d.w.]				
Flavonoids	**Control**	**UV-B 300 nm**	**UV-A 365 nm**	**Blue 470 nm**	**Red 660 nm**
Quercetin-3-*O*-sinapoyl-sophoroside-7-*O*-glucoside	5.2 ± 1.8$^{(a)}$	25.9 ± 12.1$^{(a)}$	13.9 ± 3.8$^{(a)}$	21.8 ± 7.9$^{(a)}$	13.0 ± 0.9$^{(a)}$
Kaempferol-3-*O*-sinapoyl-sophoroside-7-*O*-glucoside	74.0 ± 14.3$^{(a)}$	48.7 ± 14.2$^{(ab)}$	18.8 ± 5.2$^{(b)}$	46.7 ± 15.4$^{(ab)}$	28.6 ± 5.0$^{(ab)}$
Kaempferol-3-*O*-sinapoyl-sophoroside-7-*O*-diglucoside	91.7 ± 15.5$^{(a)}$	71.9 ± 17.0$^{(ab)}$	27.2 ± 7.7$^{(b)}$	73.5 ± 17.6$^{(ab)}$	48.3 ± 6.1$^{(ab)}$
Kaempferol-3-*O*-disinapoyl-triglucoside-7-*O*-diglucoside	8.1 ± 1.8$^{(c)}$	102.7 ± 11.4$^{(a)}$	55.6 ± 11.4$^{(b)}$	88.5 ± 9.8$^{(ab)}$	99.4 ± 10.7$^{(a)}$
Disinapoyl-gentiobiose	102.5 ± 7.9$^{(a)}$	182.1 ± 25.2$^{(a)}$	136.6 ± 21.8$^{(a)}$	160.2 ± 32.1$^{(a)}$	183.8 ± 43.5$^{(a)}$
Trisinapoyl-gentiobiose	85.6 ± 9.9$^{(a)}$	112.7 ± 23.2$^{(a)}$	73.7 ± 19.1$^{(a)}$	91.5 ± 16.3$^{(a)}$	95.6 ± 10.8$^{(a)}$

Discussion

Among the LED light treatments in this study, the UV-A (365 nm) and blue (470 nm) light treatments most affected the performance of *B. brassicae* on Brussels sprout plants. Aphid fecundity and adult weight on Brussels sprout plants were lowest under UV-A light and highest under blue light (Fig. 2 and 3). Red light (660 nm) treatment only increased aphid adult weight but did not affect reproduction potential. Hence we will focus the discussion on the low wavelength comparison and combined aphid and light induced effects on glucosinolates and flavonol glycosides.

Reductions in *B. brassicae* fecundity were also reported on broccoli plants that were treated with broad-spectrum UV sources or that were kept under UV-transmitting plastic film (Rechner & Poehling 2014). Kuhlmann & Müller (2010) also reported reduced numbers of *B. brassicae* on broccoli plants grown under UV conditions, but the current study is the first to separate the effects of narrow bandwidths of the UV range in comparison to the lower blue and red range of the light spectrum on aphid performance. Exposure of Brussels sprout plants to different narrow bandwidths of light not only affected aphid performance but also affected the contents of secondary metabolites in the plants together with the aphid infestation (Table 2 and 3). Under the influence of UV radiation, plants produce flavonoids and phenolic compounds as a protective mechanism (Harborne & Williams 2000; Schreiner et al. 2012). Other metabolites that have no direct role in UV protection, such as glucosinolates, are also reported to be increased by UV-B (Mewis et al. 2012a). The production of these metabolites generates costs, and plants must undergo trade-offs in resource allocation that limit their growth (Kuhlmann & Müller 2011). In our study, the dry matter of aphid infested Brussels sprout plants tended to be lower under UV-B and UV-A lighting than under the control lighting, which generally agreed with the results obtained by Kuhlmann & Müller (2010) for broccoli plants grown under ambient UV conditions (combined UV-B and UV-A) with average intensities of 19 kJ m^{-2} d^{-1} for UV-B and 1.49 kJ m^{-2} d^{-1} for UV-A. However, the decrease in plant dry matter was significant in Kuhlmann & Müller (2010) but was not significant in our study. Probably the UV-B intensities (0.03 kJ m^{-2} d^{-1}) generated with the UV-B LEDs were too low.

It could be shown that the level of glucosinolates in plants was significantly higher under LED-generated UV-A radiation in combination with *B. brassicae* infestation than under the other LED treatments (Table 2). However, no comparable data are available to corroborate our

findings regarding the glucosinolate increase under UV-A. Mewis et al. (2012a) reported an increase in the concentration of glucosinolates, especially the aliphatic 3-methylsulfinylpropyl and 4-methylsulfinylbutyl glucosinolates as well as the indole 4-methoxy-3-indolylmethyl glucosinolate in broccoli plants under UV-B tubes that simulated ambient UV-radiation intensity. The important difference between the two studies was the intensity of the UV-B irradiation. UV-B exposure intensity was high (0.3-0.6 kJ m^{-2} d^{-1}) in Mewis et al. (2012a) but was very low in the current study (0.03 kJ m^{-2} d^{-1}) because only UV-B-LEDs were used. This LEDs do not allow generation of high photon fluxes (Table 1). Contrary to our findings, glucosinolate concentrations did not significantly differ in broccoli plants grown under UV and control conditions in a previous study (Kuhlmann & Müller 2009a). In our study, concentrations of methylsulfinylalkyl glucosinolates did not increase in the LED-generated UV-B treatment but did increase in the blue (470 nm) and red (660 nm) LED treatments. Increasing glucosinolate contents were also described in *Cardamine fauriei* Maxim. (Brassicaceae; Ezo-wasabi in Japanese) exposed to additional blue and red LED lighting with higher intensities of 75 µmol m^{-2} s^{-1} than the intensities we used in our study with 11 µmol m^{-2} s^{-1} for blue and 2 µmol m^{-2} s^{-1} for red LED light (Abe et al. 2015).

In the current study, the concentration of some of the selected flavonoid glycosides and hydroxycinnamic acids were higher in plants grown under UV-B and blue light than under UV-A light (Table 3). Treatment of kale with a moderate UV-B radiation intensity of 0.22-0.88 kJ m^{-2} d^{-1} also increased the concentration of quercetin-3-*O*-sinapoyl-sophoroside-7-glucoside but reduced the concentration of kaempferol-3-*O*-sinapoyl-sophoroside-7-glucoside and did not affect the concentration of disinapoyl-gentiobiose (Neugart et al. 2014), showing specific reaction of single flavonol glycosides and hydroxycinnamic acid derivatives (Neugart et al. 2012). This confirms the present findings. The significant induction of flavonoid glycosides and hydroxycinnamic acid derivatives in our low-intensity UV-B treatment suggests that the Brussels sprout plants are highly sensitive to UV-B and corroborated reports that UVR8 is very sensitive to low UV-B doses and strongly triggering flavonoid metabolism (Ballare 2014).

We found that aphid fecundity tended to increase with an increase in the concentration of 4-methylthiobutyl glucosinolate under blue light (470 nm) but to decrease with an increase in the indole glucosinolate concentration under UV-A (365 nm) radiation. Different studies support the idea that glucosinolates can act as feeding stimulants for specialized herbivores (Miles et al. 2005; Halkier & Gershenzon 2006; Landosky & Karowe 2014). We hypothesize

that the increase in 4-methylthiobutyl glucosinolate in blue-illuminated Brussels sprout plants acts as such a feeding stimulant for the specialist *B. brassicae* and improves aphid performance on convenient high value diets. This is reasonable because blue light increases photosynthetic activity and protein metabolism, resulting in higher sugar and protein contents in leaves (Fukuda et al. 2008). Some glucosinolates in our study, such as 2-propenyl and 3-indolylmethyl glucosinolates increased in plants infested with *B. brassicae* under UV-A radiation, while 4-methylthiobutyl increased under blue light. Consistent with our findings, *B. brassicae* fecundity on *Brassica nigra* increased as glucosinolate concentrations increased under natural light conditions (Chaplin-Kramer et al. 2011), suggesting that *B. brassicae* performance is favored by increasing glucosinolate concentrations under ambient light. On the other hand, the intrinsic rate of increase of *B. brassicae* was lower on plants with lower concentrations of 2-hydroxy-butenyl and 2-propenyl glucosinolates (Cole 1997). It is obviously very important which specific glucosinolate concentrations are enhanced.

When Brussels sprout plants and *B. brassicae* were subjected to the LED treatments in the current study, flavonoid responses and effects differed from those of glucosinolates. Relative to UV-A treated Brussels sprout plants, UV-B-treated plants contained higher concentrations of quercetin-3-*O*-sinapoyl-sophoroside-7-glucoside and kaempferol-3-*O*-disinapoyl-triglucoside-7-diglucoside and supported normal aphid performance, suggesting that flavonoids function primarily in UV-B protection rather than in defense against *B. brassicae* in Brussel sprout plants. However, the flavonoid concentrations in our study were very low while the hydroxycinnamic acid concentration of hydroxycinnamic acid derivatives was comparable to that of kale (Neugart et al. 2014). The higher concentrations of flavonoid glycosides and hydroxycinnamic acid derivatives in plants under blue light along with the significant increase in *B. brassicae* performance indicate that this specialist benefits more from the increase in glucosinolate-triggered feeding intensity than it suffers from the slightly increased phenolic contents. However, toxic plant secondary metabolites, such as glucosinolates and phenolic compounds (flavonoids, flavonols, anthocyans and hydroxinammic acids), are one factor influencing the nutrition and performance of herbivores (Kuhlmann & Müller 2010; Schreiner et al. 2012). Other important factors can be the C/N ratio of plants and specific compounds inhibiting an efficient use of important nutritional compounds, like proteins, if proteolytic enzymes are inhibited by proteinase inhibitors (PIs) but these compounds were not measured in our study (Harborne & Williams 2000; Kuhlmann & Müller 2009a; 2009b; Treutter 2005).

In conclusion, this study demonstrated that specific secondary plant metabolites can be changed by exposure to *B. brassicae* and narrow-banded UV-A radiation generated by LEDs. Moreover, there is still a need for further detailed study of changes in primary and secondary plant metabolites and aphid performance under differentiated light treatments (in terms of wavelength and intensity) in the UV range. Also light effects on studied plant metabolic compounds were always a combination of light effects and aphid treatment. So in addition to assess plant properties, these studies should provide detailed analysis of the aphid–plant interaction and plant-light interaction separately. Further studies with insects should also investigate host plant choice and feeding intensity.

Acknowledgements

We acknowledge the technical assistance of Niklas Stukenberg in the installation LEDs. This research project was funded by the German Research Foundation DFG, grant Po 207/39-1.

General Discussion

Insecticides have harmful side effects on the environment and human health. Therefore, in times of intensive use of insecticides in protected horticultural plant production systems, it is necessary to develop alternative biological, technical or physical protection methods against herbivorous pest insects. Furthermore, pest insects develop massive resistance against insecticides, making chemical compounds ineffective in control (Gibbons et al. 2015; Lopez et al. 2016). The main aim of this research study was to increase the concentrations of certain secondary plant metabolites in broccoli and Brussels sprouts plants by tailored narrow-bandwidth supplementary light treatments, to enhance the intrinsic plants defense capability and indirectly reduce or inhibit the population development of different herbivorous pest insects. This approach was executed by using different narrow-bandwidth light treatments generated with high-power LEDs or UV-B tubes in addition to PAR illumination for plant cultivation in protected environments. This study could be seen as a first screening for effective narrow-bandwidth light wavelengths, which have potential to affect plant-insect-interactions by light-induced plant responses (indirect effect). Broccoli and Brussels sprouts were used as model plants, based on previous research with these plant species regarding light manipulation and treatments (UV), as well as already existing knowledge on metabolite composition (Kuhlmann & Müller 2010; 2011; Gulidov & Poehling 2013; Rechner & Poehling 2014). Additionally, young broccoli or Brussels sprout plants are regularly grown in protected UV-deficient environments (greenhouses or plastic-film tunnels) before they are transferred into the field as plantings and could therefore be primed from germination onwards by various light qualities during this vulnerable phase of seedling growing. Priming can result in increased resistance against certain biotic and abiotic stressors (Pastor et al. 2014) and especially UV-B radiation is known to induce secondary plant metabolites which are able to inhibit the development of pest insects on cultivated plants (Kuhlmann & Müller 2010; Schreiner et al. 2012; Rechner & Poehling 2014; Caputo et al. 2006; Mewis et al. 2012a).

The first initial step of this research study was to explore effective light treatments, in particular, convenient wavelength ranges for resistance induction against phloem-sucking aphids in broccoli. These wavelengths should be able to trigger alterations of the plant metabolism leading to a decrease of herbivorous insect performance. Optimally, these light treatments were also expected to promote plant growth and development next to the

metabolite alterations necessary for inducing certain kinds of resistance in plants (Vänninen et al. 2010). In the first part of this study, UV-B and UV-A radiation were identified as the most potential wavelengths to alter secondary metabolism of plants by up as well as down regulating them. The influence of these wavelengths on the performance of phloem-sucking aphids with different degrees of specialization were investigated. Obviously, shorter wavelengths (UV-B and UV-A) have more potential to induce metabolic changes than longer wavelength (such as violet light) at the edge of the PAR spectrum (Confirmation of hypothesis I in chapter one, please see general introduction page nine). The induction of metabolic changes in plants with UV-B and UV-A have also been shown in other studies (Dader et al. 2014; Kuhlmann & Müller 2010; Mewis et al. 2012a). Interestingly, the generalized green peach aphid *M. persicae* reacted by increasing performance on broccoli plants treated with UV-B or UV-A radiation; this has also been confirmed in other studies with UV-A irradiation of sweet pepper and eggplants (Dader et al. 2014). However, the specialized cabbage aphid *B. brassicae* feeding on broccoli plants, reduced its reproduction potential, and the resistance in broccoli against this phloem-sucking specialist could be successfully induced by using UV-B radiation generated with UV-B tubes in the climate chamber. On the other hand, UV-A radiation generated with LEDs had no effect on cabbage aphid (Confirmation of hypothesis II in chapter one, please see general introduction page nine). Studies by Kuhlmann & Müller (2010) and Rechner & Poehling (2014) reported decreased performance of *B. brassicae* on broccoli plants exposed to broad spectra UV-mix containing UV-B as well as UV-A, generated using UV-lamps in the greenhouse, or by manipulating UV intensity by using UV-absorbing plastic-films under more field like conditions (plastic-film tunnels). Interestingly, aphids with different degrees of specialization showed different performance reactions on host plants grown under various light treatments, the aphids were also able to alter the concentrations of different secondary plant metabolites depending on their specialization (Confirmation of hypotheses III and IV in chapter one, please see general introduction page nine). The reason for the observed differences in performance reactions, could be linked to the alterations in secondary plant metabolites (such as glucosinolates and flavonol glycosides), which were quantified with HPLC at the end of the experiments. Furthermore, earlier studies have shown that flavonoids and glucosinolates could act as inhibiting as well as stimulating metabolites for different herbivorous insects (Caputo et al. 2006; Onkokesung et al. 2014; Chacon-Fuentes et al. 2015; Zavala et al. 2015). From the hereby presented results, it can be speculated that

certain secondary plant metabolites are responsible for alterations of aphids' performance, but a causal allocation or ascription of single molecules is not possible yet. This could be due to the complex blend of changing metabolites with light treatments. Identification of responsible compounds or compound mixtures will either need a detachment from the plant by using artificial feeding systems for insects, or by considering multiple primary and secondary compounds (complete metabolome analysis of the plant) and performance responses of the aphids by using sophisticated statistical methods such as principal component analysis of metadata sets (Paula & Andow 2016). Feeding assays using artificial diet enriched with specific light-induced secondary plant metabolites could be the way to really identify resistance responsible molecules. Furthermore, the exact effects on aphid feeding or digestion could be characterized. The possible alterations in the primary plant metabolism, the thickness and the composition of the wax layer were not analyzed in this study. It cannot be completely excluded that such more physical alterations had influence on the performance of the phloem-sucking herbivore insects on various light-treated plants. At least the thickness of the wax layer could be increased or decreased with higher UV-B radiation, depending on plant species (the wax layer of broccoli is decreased with higher UV-B radiation intensities) and can influence the feeding behavior and intensity of aphids (Kuhlmann & Müller 2011).

Another interesting aspect, not considered in this study, is the basic signal transduction pathway responsible for the heavily increased concentrations of, i.e., flavonol glycosides or indole glucosinolates in plants after UV-B or UV-A treatments. The first possible way is the UV-B specific pathway, operating over the UVR8 receptor protein and suggested to work already at very low intensities of UV-B radiation (Christie et al. 2012; Heijde & Ulm 2012). This first UV-B specific way is also able to catalyze the flavonol metabolism even by very low UV-B intensities (Demkura et al. 2012). In chapter four of this study, it could be shown that the relative overall flavonol absorbance could be induced with very low intensities of UV-B (four times lower than previously shown in chapter one), but the herbivorous insects were not reacting on these small alterations in flavonol metabolites. The second UV-B signal transduction possibility in the investigated plant-insect interactions, which could also be responsible for increased flavonol glycoside or glucosinolate concentrations in broccoli, could be of usual hormonal defense pathways like jasmonic and salicylic acid, or ethylene usually operating against herbivorous insects and pathogens (Demkura et al. 2010; Schreiner et al.

2012; Izaguirre et al. 2003; 2007). Here it can only be speculated if usual wounding hormone pathways are responsible for the differences in insect performance with high illumination intensities in chapter one. It would be very interesting for future studies to investigate the responsible mechanism behind the metabolic changes in the plant after treatment with UV-B or UV-A radiation in cases of phytohormones, signal transduction pathways and transcriptomics.

In this study, it could also be shown that secondary plant metabolites (such as single glucosinolates and flavonol glycosides) were reacting differently on the various light treatments. Flavonol glycosides were heavily increased with UV-B treatment, so it can be assumed, that the photoprotective and antioxidant mechanisms of kaempferol and quercetin glycosides are in the foreground of the induction, and the side effects on the insects are a byproduct (Kuhlmann & Müller 2011; Schreiner et al. 2012). Indole glucosinolates were induced with UV-B and UV-A radiation treatment over an unknown mechanism. Probably they were activated over usual hormonal defense pathways, because glucosinolates are not involved in photoprotection, but are able to deploy their positive or negative effects on insect performance after treatment with UV-B radiation (Schreiner et al. 2012). However, the plants treated with UV-B radiation suffered from decreased growth and performance, resulting in decreased plant height and reduced dry weights, which is not very satisfactory from a plant producers' point of view. This fact has also been proven in earlier studies (Kuhlmann & Müller 2010), which makes the transfer of these findings into practical horticultural production systems difficult. Another interesting question would be: How big is the influence of reduced aphid performance on a larger scale in horticultural plants production systems, with reduced harvest yields due to UV-B treatment? If an adult aphid produces 50% less progeny per day over its lifespan because it lives on UV-B-treated plants, could the effect throughout the plant-growing period until harvest of ripe broccoli or Brussels sprouts be strong enough to compensate for the 40% decrease in dry weights through UV-B radiation? This could be the case, but the positive effect of 10% is small for the grower, and unfortunately not comparable with the effect of chemical insecticides (80-90% insect mortality), but other plant-insect-light-combinations have to be investigated in more detail, before drawing general conclusions. Furthermore, the combination of different biological control methods (release of predators, parasitoids or nematodes in greenhouses or climate chambers) and light-induced resistance could result in antagonistic or synergistic effects and have to be evaluated carefully in plant

production systems (Otieno et al. 2016). This could allow the possibility of developing a strong tool by combining biological and technical plant protection methods. Additionally, the investigation of the intensity threshold for inducing certain kind of UV-B resistance could be helpful to reduce the harmful effects on plant growth and to find the optimal treatments for plant protection. To investigate three different UV-B intensities, there is need to have at least twelve independent UV-B compartments in the climate chamber to get enough repeats (four light treatments per intensity) for the research, which unfortunately was not possible in this study. Moreover, other light qualities (longer wavelengths) should also be investigated in this study. Most interesting would be, the combination of different wavelength, i.e., UV-B as a resistance inducer and blue or red for additional plant growth. Perhaps it is possible to preserve the resistance effect and reduce the growth inhibition induced with UV-B treatments at the same time.

The second step of this research was to address the question: “Can longer wavelengths of narrow-bandwidth light alter secondary plant metabolism and increase plant defenses against aphids?” If so, this would allow the development of a plant growth promoting and resistance inducing illumination system. In this part of the study, the specialized cabbage aphid *B. brassicae* was used because it was the only organism that responded with reduced performance against the light-induced metabolic changes in broccoli in chapter one so far, and the aim of this research study was to induce resistance in the plants and not susceptibility such as for *M. persicae* in chapter one. Another difference to chapter one of this study was the broad spectra background illumination in the climate chamber. The tubes used in chapter two of this study contained a high amount of blue light compared to the tubes used in chapter one which had a high amount of red light in the background spectra. High amounts of blue light in the background spectra are supposed to promote better plant acclimatization to UV-radiation (Hoffmann et al. 2015). Additional green LED light increased plant height in broccoli compared to those grown under UV-A, violet or blue light (partly confirming hypothesis I in chapter two, please see general introduction page nine). Unfortunately, the light treatments only slightly increased the concentrations of secondary plant metabolites (flavonol glycosides and glucosinolates) in this part of the study (partly confirming hypothesis II in chapter two, please see general introduction page nine and ten), and did not affect the performance of *B. brassicae* on broccoli plants. Therefore, this hypothesis was rejected (rejection of hypothesis III (a) in chapter two, please see general introduction page ten). The concentrations of

secondary plant metabolites were below 100 µg g^{-1} d.w. and probably not able to influence the herbivorous insects in their food-uptake behavior or digestion. Kim et al. (2015) also detected very low concentrations of quercetin and kaempferol glycosides (< 10 µg g^{-1} d.w.) in Chinese cabbage after 12 days of illumination with blue, red, or white LEDs, corroborating the findings in the present study. Whether the low secondary metabolite concentrations depend on background light quality in this study needs further investigation with focus on different background light qualities or intensities. Nevertheless, the plant choice behavior of aphids was indirectly affected by the light treatments in chapter two (confirmation of hypothesis III (b) in chapter two, please see general introduction page ten). Significantly more aphids selected plants that had been grown under additional blue light rather than under control light conditions. UV-A-treated plants contained higher concentrations of 3-indolylmethyl glucosinolate compared to blue- or violet-treated plants, and it has been shown that some aphid species prefer plants with reduced glucosinolate concentrations (Cao et al. 2016). Blue light could also lead to stronger primary plant metabolism and therefore increase the host plants nutritional quality for aphids (Hernandez & Kubota 2016; Huche-Thelier et al. 2016). Future studies should investigate primary as well as secondary plant metabolism, and also consider the separation of olfactory and visual aspects in host-choice experiments. Furthermore, the impact of the fact that more aphids settled down on blue-treated plants needs to be investigated in detail regarding the final outcome of the aphid population increase.

Another arising question concerning the aphids' performance is: Why were the additional longer wavelengths (such as violet, blue and green) not working to take influence via plant-mediated effects on the performance of *B. brassicae* like shorter wavelength (UV-B radiation) in chapter one of this study? One possibility could be that these additional generated wavelengths with LEDs are already present in the broad spectra light tubes, i.e., violet, blue, green, emitted by the Osram fluorescent tubes, used as background illumination. Additional triggering of the already activated photoreceptors, may not result in higher concentrations of secondary plant metabolites, except for short wavelengths with very high-energy potential (such as UV-B). UV-B radiation was not present in the background light and could activate the specific UVR8 receptor or the unspecific UV-B pathway via wounding. Another possibility could be the light intensity of additional treatments. The light treatments with additional PAR wavelength of 50 µmol m^{-2} s^{-1} intensity generated using LEDs were quite low and equal to the

intensity produced by the broad spectra tubes in the background to exclude an effect through different PAR intensities between the treatments. Future studies should consider treatments with higher graded intensities of longer wavelengths generated using LEDs in the PAR region.

The third chapter of this research study aimed to investigate the plant-mediated reaction of biting-chewing lepidopteran larvae feeding on plants grown under various light treatments. Therefore, the most efficient shorter wavelengths (such as UV-B, UV-A and violet from the first two chapters) were selected. Experiments were performed using two specialized and two generalized lepidopteran pest insects. Successful induction of resistance in broccoli with additional UV-B treatment was only possible against the specialized lepidopteran insect *P. xylostella* (partly confirming hypothesis I in chapter three, please see general introduction page ten). These light treatments were performed before the larvae could feed on their host-plants to exclude direct effects of the light treatments on the insects. Concerning literature, most studies showed detrimental effects of flavonoids on the performance of specialized as well as generalized lepidopteran larvae, but these studies were mostly performed on artificial diets with quiet high concentrations of flavonoids (Silva et al. 2016; Liu et al. 2015; Onkokesung et al. 2014; War et al. 2013). Only very few studies reported neutral or positive effects of flavonoids on lepidopteran performance (Chacon-Fuentes et al. 2015; Caasi-Lit et al. 2007). Plants containing high concentrations of glucosinolates had mostly inhibiting effects on larvae performance and growth (Kaur et al. 2016; Santolamazza-Carbone et al. 2016; Badanes-Perez et al. 2014). In chapter three of this study, only *P. xylostella* reacted with significantly reduced pupa weight on UV-B-treated plants compared to larvae that had been feeding on control plants. The only glucosinolate significantly increased in the UV-B treatment was 3-indolylmethyl in the earlier HPLC analysis (chapter one). The generalized lepidopteran pests (*H. armigera* and *S. frugiperda*) were not reacting to the different light treatments (partly rejection of hypothesis I in chapter three, please see general introduction page ten). The induced concentrations of flavonol glycosides or glucosinolates in the light-treated plants were probably too low to trigger detrimental effects on lepidopteran larvae development. Mixing graded concentrations of interesting secondary plant metabolites in artificial diet, or using host plants varying heavily in their concentrations of secondary plant metabolites and feeding of the lepidopteran larvae, could create clarity about concentration thresholds and the inhibiting activity of single secondary plant compounds. The relative overall flavonol absorbance measured non-destructively using the Dualex scientific, is obviously not a reliable

indicator of induced resistance effects. The relative overall flavonol absorbance was significantly induced in violet and UV-B treated plants (confirmation of hypothesis II in chapter three, please see general introduction page ten). However, from the overall absorption of UV-A in the epidermal flavonols, it is not possible to conclude on the effects of single substances on herbivorous insects. Future studies should consider growing more plants under different illumination times or intensities, and measuring of specific secondary plant metabolites destructively using HPLC. This is quite more effort, but worth trying to identify the specific compounds, which can later be used for feeding experiments in artificial diet. The chlorophyll fluorescence in broccoli was not influenced by various light treatments (partly rejecting hypothesis II in chapter three, please see general introduction page ten), and could not be used for the explanation of *P. xylostella* performance differences. Another problem could have been the plant age in the experiments with biting-chewing lepidopteran larvae. Older plants of the same species and variety are usually more resistant than younger plants and also contain higher concentrations of secondary plant metabolites (Kellner et al. 2010). In the experiments with aphids, four-weeks-old plants were used. These plants were illuminated from germination onward with the additional light qualities. The biting-chewing lepidopteran larvae consumed a lot of leaf material during their development and therefore more and bigger plants under the specific light treatments were needed. The plants were grown for two to four weeks under usual control conditions (because UV-B reduces dry weight) to produce enough leaf material, and afterward treated with specific additional light qualities. Older control plants can show stronger resistance, and if the effect of induced resistance visible in differences for larvae and pupae weight is small, the effect of induced resistance could get lost if older plants are used. For future studies, it would be better to illuminate the plants from germination onward, as younger plants show at least stronger reactions to UV-treatments (Kuhlmann & Müller 2009a). The use of more and younger plants, instead of less and older plants for the experiments with lepidopteran larvae, could result in clearer effects between the light treatments and the control. The size of the resistance effects and the corresponding plant age should be considered carefully in future experiments. Another interesting point could be that the different light treatments were switched off when the experiments with lepidopteran larvae started. The experiments with aphids (chapter one and two) took place under the LED-light treatments, because aphids were enclosed in plastic clip-cages on the underside of the leaf, so that direct light effects on the aphids could be excluded. The use of

these clip cages was not possible for the experiments with lepidopteran larvae, because of their biting-chewing and leaf removing feeding activity. Therefore, the lepidopteran larvae had to stay on the whole plant for feeding. The only possibility to exclude direct effects was to switch off the LEDs during the experiments with lepidopteran larvae. Future studies should consider measuring the concentrations of secondary plant metabolites using HPLC after switching off the light treatments to exclude decreasing concentrations in the experimental plants. The light induced increase of the relative overall flavonol absorbance was not reversible after the LED-light treatments stopped, as older plants contain higher amounts of flavonols also in the control treatment, hence the flavonol absorbance could not be seen as a good indicator of single secondary plant metabolites responsible for resistance.

Another important aspect of light-triggered plant reactions, alongside light quality in cases of wavelengths, is light intensity. This was investigated in the additional chapter four. Moreover, low light intensities could also be economically important in practical application of additional light systems, because of the reduction in electricity costs. Hence, the fourth chapter aimed to specify the plant-insect reactions observed in the previous chapters, in relation to light intensity and gained light qualities with single LEDs. The LED intensities used in chapter four were four times lower (full power of the available single LEDs) than the intensities used for plant illumination with high-power LED-panels and UV-B tubes in the first three chapters. The negative influence of these lower light intensities on broccoli plant growth (dry weight) was less severe than with high-light intensities in chapter one (confirmation of hypothesis I in chapter four, please see general introduction page ten). Even though the flavonol absorbance in broccoli plants treated with single LEDs of UV-B 300 nm and violet 420 nm was significantly different after ten and fifteen days of illumination compared to plants treated with UV-A 365 nm or under control conditions, they reached even higher levels than the flavonol absorbance measured in chapter three with high light intensities (rejection of hypothesis II in chapter four, please see general introduction page ten). The reason for this remains unclear, because plant growth conditions as well as background light quality and intensity were the same, except that the low intensity LED-illumination approach was set up in a different climate chamber (location difference). None of the two aphid species (*B. brassicae* and *M. persicae*) reacted to the treatments. Following this result, lower light intensities have less potential to indirectly influence herbivorous insects on their host plants (confirmation of hypothesis III in chapter four, please see general introduction page ten). Again, the relative overall flavonol absorbance

measured non-destructively during the experiments seems not to be the appropriate method to identify flavonol based resistance properties in the plants. *Plutella xylostella* did also not react with differences in larval weight after one week of feeding on broccoli plants grown under various low light treatments, but showed reduced pupa weight on broccoli plants exposed to UV-B 300 nm compared to the ones that fed on plants treated with UV-A 365 nm or violet 420 nm. This difference was not significant compared to larvae that had been fed on control plants, but the trend of reduced pupa weight indicated an intensity depended resistance reaction compared to the high light intensities against lepidopteran pests in chapter three, that is not correlating with the relative overall flavonol absorbance. Obviously, *P. xylostella* seems to be more sensitive in its reaction to very specific and small amounts of light-induced metabolites compared to *B. brassicae*. It could be speculated that such metabolites are more accumulated in the leaf tissue which is consumed by the lepidopteran larvae compared to the phloem sap taken up by the aphids only. Moreover, the very destructive feeding properties with additional wounding effects could be responsible for this more sensitive reaction. It would be very interesting to run an intensity graded experiment, if the intensities of new high-power UV-B LEDs are strong enough to induce resistance in the model systems broccoli with *B. brassicae* and *P. xylostella*. Regarding the above mentioned economical background, in this kind of intensity graded experiments, it is important to rate the cost of different light treatments. The costs of the ten single high-power UV-B LEDs used in this experiment amounted already to 3000 €. Thus, gaining higher intensities with LED-panels such as those used in the first experiments are uneconomical. However, due to an expected decline of prices and the increasing power for LEDs, it will be possible for future research studies and implementation efforts to get stronger and cheaper high-power UV-B LEDs, and either run experiments with different graded intensities or implement even LED-panels in crops.

The fifth chapter, working with the Brussels sprouts - cabbage aphid system, focused on the light-induced reactions in a different plant species, and possible modifications of the response (plants, herbivores) in a greenhouse set-up with sodium vapor lamps as PAR light sources in the background. Also the intensities used in this part of the study were quite low. Nevertheless, the concentrations of secondary plant metabolites could be influenced in Brussels sprouts. Low power UV-B LEDs induced the specific kaempferol-3-*O*-disinapoyl-triglucoside-7-*O*-diglucoside, but no influence on the cabbage aphid could be observed.

Kaempferol-3-*O*-disinapoyl-triglucoside-7-*O*-diglucoside was the only kaempferol glycoside that increased with such low dose UV-B LED treatment in Brussels sprouts, probably because the intensities of the UV-B LEDs were too low or the narrow-band wavelength with its peak at 300 nm did not hit the UVR8 receptor (peak absorbance at 282 nm) in the ideal position compared to the more broader spectra UV-B tubes. The same situation occurred in the experiments with single LEDs in chapter four. The low UV-B induced kaempferol glycoside function obviously primarily as a sunscreen and are not responsible, or concentrated enough in the phloem to alter cabbage aphid performance in Brussels sprouts (Kuhlmann & Müller 2010). Indole glucosinolates increased in plants treated with UV-A radiation. The specialized cabbage aphid *B. brassicae* feeding on Brussels sprout showed a decreased performance on plants grown under UV-A radiation and an increased performance on plants grown under blue light treatments. Specifically, 3-indolylmethyl and 4-hydroxy-3-indolylmethyl could be responsible for the reduced fecundity of *B. brassicae* between UV-A and blue light-treated Brussels sprouts plants. This could also be confirmed in chapter one and two for broccoli plants (Rechner et al. 2016). A weak point in this part of the study was that the illumination area in the greenhouse with only four high-power LEDs was not as big as in the climate chamber, and therefore only a limited amount of Brussels sprout plants could be illuminated. The HPLC analysis at the end of the experiments with aphids could only analyze Brussels sprout plants treated with different light qualities and simultaneously infested with cabbage aphids, so that the effects of light and aphid infestation on secondary metabolite concentrations are always in combination and not separated like in chapter one and two for broccoli plants. For future studies it would be better to construct a bigger approach in the greenhouse which allows separating the corresponding light and aphid effects on the concentrations of secondary plant metabolites. Furthermore, using higher numbers or more powerful LEDs could increase the intensity which can be used for plant illumination. This could be a very interesting approach, especially for plant growth promoting longer wavelength out of the PAR spectrum (red and blue), because there is an increasing use of red and blue LEDs as above panels or intercrop lighting in horticultural plant production to regulate plant growth processes (Ahmad et al. 2016; Hoffmann et al. 2015; Vänninen et al. 2010).

The investigated plant-insect-interactions and the corresponding light effects were highly plant and insect species specific in all the experiments. Resistance in broccoli could only be induced with UV-B radiation against one specialized aphid (*B. brassicae*) and one specialized

lepidopteran pest (*P. xylostella*), but plants grown under UV-B treatments had reduced plant growth. In Brussels sprout plants, UV-A was effective in inducing resistance against *B. brassicae* in a greenhouse setup. The effects of the reduced aphid fecundity and the reduced lepidopteran pupa weight on the following generations of pest insects, and their negative influence on plant growth in the climate chamber or the greenhouse need to be investigated in the future, to estimate the potential for plant protection concepts and calculate the positive effect of various light treatments. Especially the combination with other biological protection methods could be effective in reducing the pest density in crops. The light-induced resistance research is still in a basic research stage in an academic environment and not in use in commercial horticultural plant production systems. The indirect light effects on herbivorous insects are reduced by the growth depression of the plants, and thus the implementation of the induction technology in protected horticultural production systems will be difficult in the near future. This fact has three reasons, firstly, the reduced growth of broccoli plants treated with UV-B radiation if resistance against the specialized cabbage aphid *B. brassicae* was induced. Secondly, the species specificity in the investigated reactions (the fact that the generalist *M. persicae* performed better on UV-B and UV-A treated plants and *B. brassicae* preferred blue-treated plants), and thirdly the costs of additional LED-based illumination systems, especially in the UV-A and UV-B range of the light spectrum. Furthermore, data on the performance and behavior of important greenhouse pests such as whiteflies, thrips and spider mites are still missing and future studies should also consider investigating these pests. Nevertheless, from an academic research point of view, it is a new and very exciting approach and this research study provided some first answers, but also a lot of powerful new questions. Future studies should entangle the molecular (transcriptomics and proteomics) and the exact metabolic mechanism (complete primary and secondary plant metabolism) in the plant, and explain more possible reasons behind the alterations in herbivores food quality. Additional studies should focus on the behavior and performance of the next trophic level, for example parasitoids and predators of aphids and lepidopteran larvae. Furthermore, other relevant plants also produced in climate chambers or greenhouses (tomato, sweet pepper, cucumber, lettuce or herbs), and their corresponding pest insects should be screened for their reactions on various light treatments (qualities, intensities, and illumination times). Maybe one day it might be possible to develop a plant growth promoting, insect repelling and inhibiting illumination system for protected horticultural production systems, if all details and especially

the high plant and insect species specificity are considered carefully. Furthermore, the research could be expanded against fungal, bacterial and viral plant pathogens or nematodes. Some studies have indicated first results against bacteria, nematodes and viruses (Dhakal et al. 2015; Yang et al. 2015; Matsuura & Ishikura 2014). Specific supplementary tailored light scenarios for plant illumination in combination with other biological (release of beneficial parasitoids and predators) or technical approaches (such as LED-based insect traps), could be able to protect cultured plants from destructive insect pests and plant pathogens in the future without affecting human health and the environment negatively.

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Acknowledgements

I owe my deepest gratitude to my supervisor Prof. Dr. Hans-Michael Poehling. I appreciate our fruitful discussions, your amplifying comments and also the responsibility and the freedom you committed me during this work. Our discussions on the research had a big impact on the quality of the study and our publications. I would further like to thank you for making this great research project, my travelling to the International Congress of Entomology 2016 in Orlando, Florida, and our publications of my work possible.

I would also like to deeply thank Prof. Dr. Monika Schreiner and Dr. Susanne Neugart, Department of Quality Leibniz-Institute of Vegetable and Ornamental Crops Großbeeren/ Erfurt e.V. for laboratory analysis of secondary plant metabolites, the supervision of the M. Sc. Student Sasa Wu in your laboratory and the promoting comments on our publications. Your comments on the manuscripts had a big impact on the quality of our publications. Thank you very much for the great cooperation.

I would also like to deeply thank Prof. Dr. Edgar Maiss for doing the Co-referee of my work and Prof. Dr. Hartmut Stützel for doing the chair of the examination board.

Big thanks to Niklas Stukenberg, Dr. Elias Böckmann, Mirko Rakoski, Dr. Jacinter Atieno Otieno for being such awesome office mates during this nice time. Furthermore, I would like to deeply thank Niklas Stukenberg for technical support during the first LED installations, the fruitful discussions about our research and the rest of the nice time we joined as friends together. I am also grateful to Mirko Rakoski for continuing the light-induced resistance research project and for very good ideas and exciting discussions about the topic and the off-topic.

I would like to thank Prof. Dr. Edgar Maiss, Dr. Rainer Meyhöfer, Dr. Peter Hondelmann, Dr. Konstanze Gebauer for their supporting comments and discussions after my talks and for being such good colleagues.

I am deeply grateful to the other current and former PhD students Sebastian Laurenz, Dr. Pamela Akoth Ogada, Anton Sartisohn, Dr. Monica Zamora, Sina Rogge, Jan-Uwe Niemann, Dr. Martin Ludwig, Sergej Gulidov, Dr. Josephine Muthoni Karanja, Jackline Mworia, Dr. Niklas Bald, Carolin Popp, Dr. Johanna Knopp, Dr. Dominik Klinkenbuß, Hamza Mohammad and Hanna Rose for being such good colleagues and the nice time we spend together at our institute. It was my pleasure to be with you all.

Big thanks also go to the M. Sc and B. Sc. Students Sasa Wu, Florian Wulf, Miguel Zehntner, Jeewan Acharya for your studies and your ambitious work on my research topic. It was my pleasure to supervise your experiments and your theses.

I would like to thank Seraphine Hermann, Hans-Joachim Seelbinder, Johannes Specht and the other gardeners for plant care and for your understanding of special issues.

Big thanks to Timo Michel, Dr. Andreas Olsowski und Birgit Milde for plant and insect care, laboratory and technical assistance during my studies and for being such nice colleagues.

Furthermore, I would like to thank Dr. Gökhan Akyazi, Andreas Meyer, Werner Hock and Norbert Grisat for material and constructions of LED-panels when our research approach got bigger and we wanted to use much more space for our light experiments in climate chambers and greenhouses. Without Dr. Gökhan Akyazi the constructions of this large technical and electrical equipment would not have been possible.

Thanks to Dr. Daniel Gerhard and Dr. Frank Schaarschmidt for supporting the statistical evaluation of datasets.

Thanks to Melanie Rothenhäuser and Ute Meisel for financial project coordination and secretary work.

Thanks to Bayer CropScience AG (Mohnheim, Germany) for providing *H. armigera* and *S. spodoptera* eggs and giving rearing suggestions, and thanks to the Laboratory of Entomology, Wageningen University (Wageningen, The Netherlands) for providing *P. brassicae* eggs and giving rearing suggestions.

I am very grateful to my parents Gabriele and Stefan Rechner, my grandparents Hilde and Heiko Neuner and my sister Birte Rechner. Furthermore, I am also very grateful to all my friends. Without all your support during my studies, I would not have been able to walk up all the steps to start and finish this research project.

Special thanks go to Deutsche Forschungsgemeinschaft (DFG) for making this research project possible with financial support of the research project grant Po 207/39-1.

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Employment History

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April 2009 – April 2013	Different Student Research & Teaching Assistant Jobs at the Leibniz University Hannover, Institute of Horticultural Production Systems, Section Phytomedicine
August 2009 – October 2009	Internship at the E-Nema GmbH, Schwentinental, Germany

School Education & University

October 2009 – April 2013	Study at the Leibniz University Hannover (M. Sc. Horticultural Science) Main Focus: Plant Breeding & Entomology
October 2006 – October 2009	Study at the Leibniz University Hannover (B. Sc. Horticultural Science) Main Focus: Plant Breeding & Entomology
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Publications

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