The influence of ultraviolet radiation on plant-insect interactions

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Synopsis

Abiotic and biotic environmental conditions determine development, physiology and life history of plants. The phenotypic plasticity enables plants to respond, adjust and acclimatise to a changing environment. Thereby plants are capable to react with short and long term plastic morphological and chemical responses (Lichtenthaler, 1998; Sultan, 2000). Consequently, specific signal perception and transduction mechanisms need to be highly developed. UV induced changes in plants potentially influence the next trophic levels such as herbivores and parasitoids and may have the ability to shift plant-insect interactions.

1.1 Sunlight and ultraviolet (UV) radiation – Plant responses to UV radiation

Plants need to capture sunlight (Fig. 1.1.1) for photosynthesis. Therefore sunlight is an essential and unavoidable environmental factor in plants' life. The most energetic fraction of solar radiation reaching the biosphere is UV-B (280-315 nm) which is primarily absorbed by the stratospheric ozone layer. Other factors affecting UV-B radiation intensities on earth are the angle of sun rays, cloud cover, season, aerosols, altitude, surface reflectance, shading and plant canopies (Madronich *et al.*, 1998; McKenzie *et al.*, 2003; Paul and Gwynn-Jones, 2003; Jenkins and Brown, 2007).

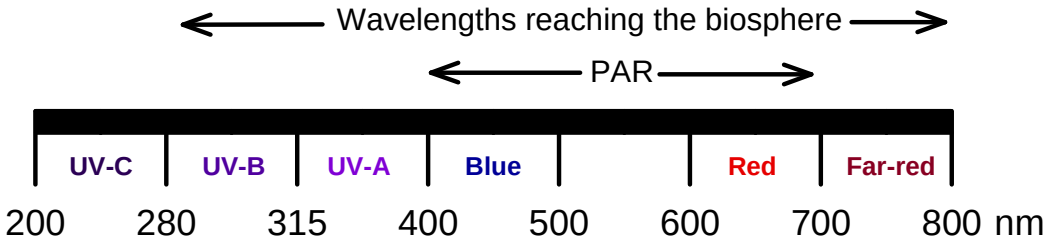

Fig. 1.1.1 Fractions of sunlight that reach the earth's surface are ultraviolet-B (UV-B, 280-315 nm), ultraviolet-A (UV-A, 315-400 nm), photosynthetic active radiation (PAR; 400-700 nm) and infrared (700 nm-1 mm) radiation (Frohnmeyer and Staiger, 2003; Paul and Gwynn-Jones, 2003).

UV-B radiation can cause damage to DNA, proteins and lipids, generate reactive oxygen species (ROS) and alter hormone levels. Therefore rather effective mechanisms for UV-protection and repair, including accumulation of protective phenolic compounds and activation of repairing enzymes like DNA photolyases as well as the free-radical scavenging system have evolved (Rozema *et al.*, 1997; Jansen *et al.*, 1998; Frohnmeyer and Staiger, 2003). The magnitude of stress for the individual plant might depend on the

ecological context of the species, on its developmental stage and on the level of acclimation to and on the quantity of UV-B. In natural environments symptoms of UV-damage are rare. Therefore plants must have a highly elaborate system of UV perception and signal transduction that enables plants to adjust to their surrounding radiation challenges, even though UV-B receptors still have not been identified yet (Frohnmeyer and Staiger, 2003; Jenkins and Brown, 2007; Brown and Jenkins, 2008). It is presumed that two fluence rate dependent non-specific (stress) and UV-B specific (photomorphogenic) signalling pathways might exist (Frohnmeyer and Staiger, 2003; Ulm and Nagy, 2005; Jenkins and Brown, 2007; Brown and Jenkins, 2008), whereas UV-B specific responses do not result from DNA damage or stress (Brown and Jenkins, 2008; Safrany *et al.*, 2008). The chalcone synthase is the key enzyme for the biosynthesis of phenylpropanoids and is believed to be the terminal step of a UV-B signalling pathway (Safrany *et al.*, 2008).

UV-A radiation (315-400nm) is not absorbed by the ozone layer and is present at much higher intensities in sunlight than UV-B radiation. UV-A can impact plant morphology and pigment formation as well (Paul and Gwynn-Jones, 2003). So far, only a few studies investigated the interactions between UV-A, UV-B and PAR. In New red fire lettuce (*Lactuca sativa* L., Asteraceae), for example, only UV-B radiation affected UV-B absorbing flavonoid concentrations, though anthocyanins were also influenced by UV-A (Krizek *et al.*, 1998).

Flavonoids are responsible for the coloration and pigmentation of plant flowers, fruits and seeds (Shirley, 1996) and they play essential roles in development, fertility, defence and UV protection (absorption in the 280-320 nm region (Harborne and Williams, 2000)) of plants (Peer and Murphy, 2007). The three most important and widespread flavonoid classes are anthocyanins, flavones and flavonols (Harborne, 1991). The three-ringed structure of flavonoids consists of two aromatic and one O-heterocyclic ring, the two aromatic rings can be substituted by one or more hydroxyl groups. In living plant cells flavonoids mostly occur in a combination with sugar as flavonoid glycosides, which provide solubility and protection from enzymatic or light degradation (Harborne, 1991). Flavonoids are derived from the aromatic amino acid phenylalanine that is deaminised by the phenylalanine ammonium lyase (PAL) to cinnamic acid. Different hydroxycinnamic acids are formed by several hydroxylation and methylation steps. Esterification by coenzyme A (CoA) results in a wide range of intermediates of phenylpropanoid derivates, e.g. coumarines, stilbenes, lignins and flavonoids (Heller and Forkmann, 1994; Weisshaar and Jenkins, 1998). The flavonoid skeleton is formed by the key enzyme chalcone synthase (CHS), which catalyses the condensation of three acetate units from malonyl-CoA with hydroxycinnamic acid to a chalcone. The cyclisation of the chalcone is catalysed by the chalcone isomerase (CHI) (Fig. 1.1.2). Flavanones are the direct precursors for a large class of flavonoids. The enormous diversity of flavonoid metabolites derives from enzymes catalysing hydroxylation, methylation, glycosylation, acylation and various other reactions (Heller and Forkmann, 1994). Flavonol glycosides of kaempferol, quercetin and myricetin are present almost

always in vacuoles of leaf epidermal cells. The flavonols belong to the most numerous structures among the 14 flavonoid classes (Harborne, 1991).

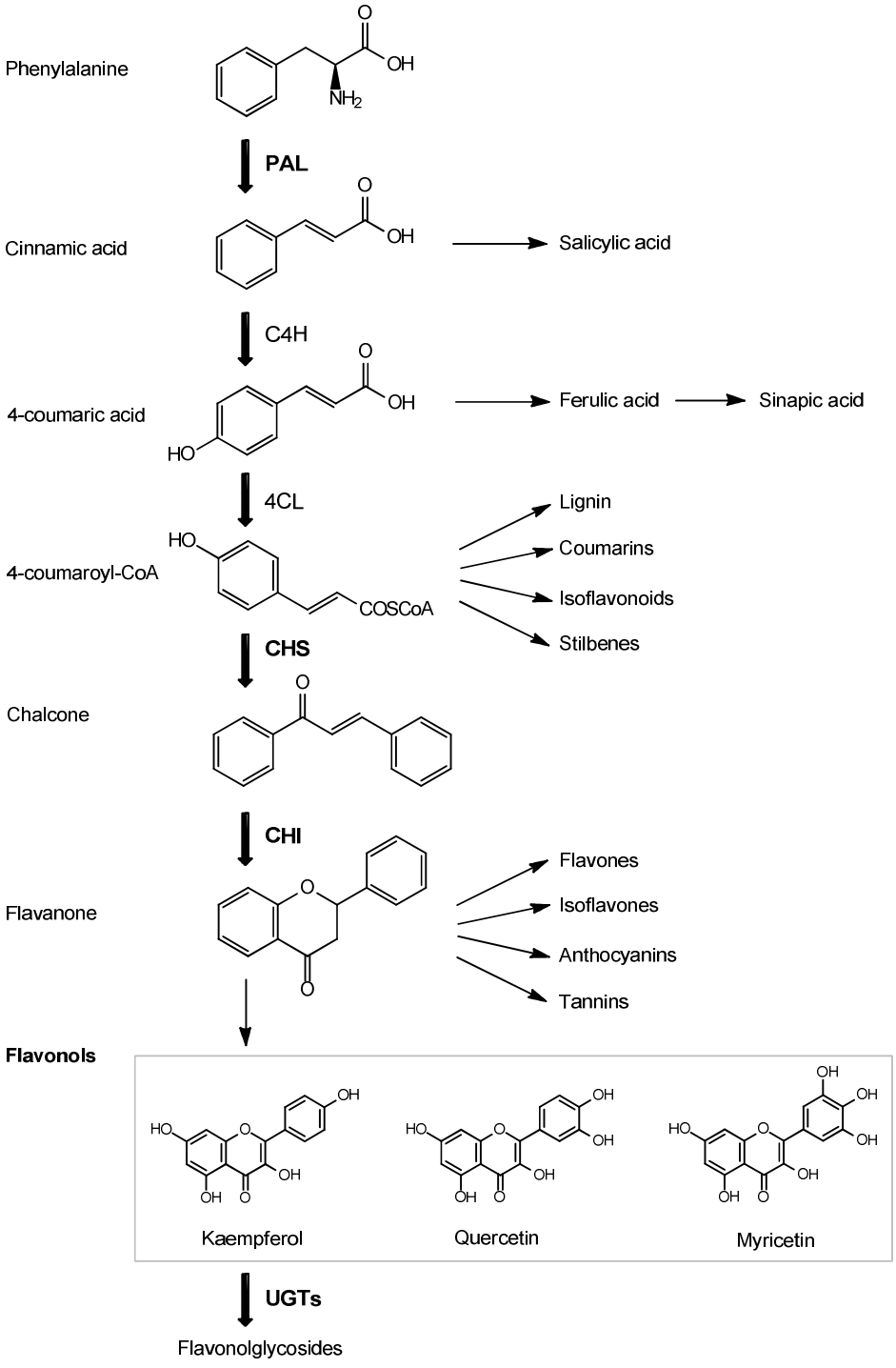

Fig. 1.1.2 Major steps of the flavonoid biosynthesis and schematic view of major branches of the phenylpropanoid metabolism. PAL, phenylalanine ammonialyase; C4H, cinnamate 4-hydroxylase; 4CL, 4-coumaroyl-CoA ligase; CHS, chalcone synthase; CHI, chalcone isomerase; UGTs, UDP- dependent sugar glycosyl transferases. According to (Heller and Forkmann, 1994; Shirley, 1996; Weisshaar and Jenkins, 1998).

Sugar linked hydroxycinnamyl acylated flavonol glycosides belong to the most frequently cited flavonoids ascribed as being sunscreens (Harborne, 1991; Harborne and Williams, 2000). UV-B radiation exposure induced higher increases in quercetin glycoside compared to kaempferol glycoside concentrations in several plant species (Markham *et al.*, 1998; Olsson *et al.*, 1998; Hofmann *et al.*, 2003; Reifenrath and Müller, 2007; Winter and Rostás, 2008; Kuhlmann and Müller, in press, **Chapter II**). It is presumed that quercetin flavonols have more favourable attributes for free radical scavenging than kaempferol flavonols (Harborne and Williams, 2000). Hydroxycinnamic acid esters are also important UV-B protectants, as, for example, described for young unrolled leaves of rye (*Secale cereale* L. cv. Kustro, Poaceae). During acclimation and leaf development of this plant species, flavonoids become more important (Burchard *et al.*, 2000). It is known that flavonoid aglycones (e.g. kaempferol, quercetin, apigenin) can modulate and inhibit the transport of the phytohormone auxin (indole-3-acetic acid, IAA) in plants and therefore affect plant architecture (Brown *et al.*, 2001; Jansen, 2002; Peer and Murphy, 2007). It has not yet been proven whether UV induced flavonoid glycoside increases also can influence auxin transport processes (Jansen, 2002). Free auxin levels can be controlled and reduced by phenol-oxidizing peroxidases. Further the peroxidase activity is related to UV-tolerance of plants (Jansen *et al.*, 2001; Jansen, 2002). Typical phenotypic acclimation processes of plants to UV-B are reduced growth and accumulation of phenolic compounds (Caldwell *et al.*, 2007; Kuhlmann and Müller, 2009, **Chapter I**; in press, **Chapter II**; submitted, **Chapter III**). It is likely that the above described substances are a part of the UV regulation in plants.

While UV-acclimation plants face a trade-off for resource allocation either to growth or to protection. This trade-off is more pronounced in young developing plants (Kuhlmann and Müller, 2009, **Chapter I**). UV-exposure leads to far-reaching consequences in plants' metabolism, which includes changes in wax coverage (Gonzalez *et al.*, 1996; Fukuda *et al.*, 2008; Kuhlmann and Müller, submitted, **Chapter III**) and in phloem sap amino acid constitution (Kuhlmann and Müller, submitted, **Chapter III**). The latter had not been considered before.

Different approaches were used to examine the influence of UV-radiation on plants and plant-insect interactions. With the assistance of UV-lamps experiments were conducted in climate chambers (Hatcher and Paul, 1994; Grant-Petersson and Renwick, 1996; Lindroth *et al.*, 2000; Tegelberg and Julkunen-Tiitto, 2001; Foggo *et al.*, 2007), greenhouses (McCloud and Berenbaum, 1994, 1999; Caasi-Lit, 1998; Lavola *et al.*, 1998; Izaguirre *et al.*, 2003) or under field conditions (Björn *et al.*, 1997; Salt *et al.*, 1998; Buck and Callaghan, 1999; Gwynn-Jones, 1999; Veteli *et al.*, 2003) to simulate stratospheric ozone depletion. In order to test plant responses under more realistic solar radiation conditions ambient radiation levels were selectively excluded (low UV(-B)) or transmitted (high UV(-B)) by using filter materials in the field (Caputo *et al.*, 2006; Winter and Rostás, 2008; Kuhlmann and Müller, 2009, **Chapter I**; Reifenrath and Müller, 2009). We also conducted experiments in greenhouses covered with innovative materials, which transmit more UV-B than conventional greenhouse glass (Fig. 1.1.3,

Fig. 1.1.4). Herbivorous insects could be excluded or included on purpose to investigate specifically questions concerning the influence of UV-B radiation conditions on plant-insect interactions (Kuhlmann and Müller, in press, **Chapter II**; submitted, **Chapter III**).

Fig. 1.1.3 Different experimental designs. Twelve filter tents (left) covered with either UV-B and UV-A including (+UV, Teflon foil) or excluding (-UV, Lee 226 foil) filter foils and three greenhouses (right) covered with innovative materials, transmitting either high (80%, ETFE foil), medium (23%, MM glass) or low (4%, float glass) levels of UV-B (for filter qualities see Fig. 1.1.4).

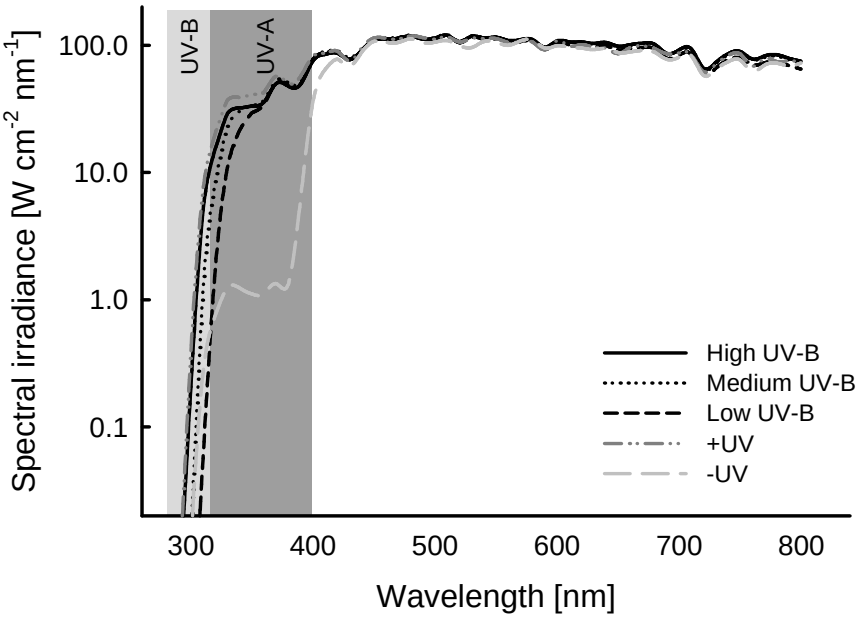

Fig. 1.1.4 Spectral irradiance of greenhouse (high UV-B, ETFE foil, black solid line; medium UV-B, MM micro-structured solar glass, black dotted line; low UV-B, float glass, black medium dashed line) and filter tent materials (+UV, Teflon foil, dark grey dash-dot-dot line; -UV, Lee 226 foil, light grey long dashed line). UV-B (280-315 nm) and UV-A (315-400 nm) regions are highlighted in grey scales. Note the logarithmic y-axis.

Pronounced changes of plant morphology and chemistry due to UV-radiation quality and quantity raise the question: what are the effects on the next trophic level?

1.2 UV radiation and insect feeding

UV radiation can modulate interactions between plants and their natural consumers. This has been shown by several experiments with UV attenuating filters. The intensity of herbivory is generally associated with UV-induced changes in plant tissue characteristics. However, insects are also capable to respond directly to different UV conditions (Mazza *et al.*, 1999; Mazza *et al.*, 2002). We found that naturally occurring whiteflies (Aleyrodidae) and aphids (Aphididae) favoured high ambient UV-conditions over low UV-conditions, whereas thrips (Thripidae) avoided high UV-conditions (Kuhlmann and Müller, 2009, **Chapter I**). A preference of whiteflies and aphids for high UV radiation conditions has also been detected by Antignus *et al.* (1996); Costa and Robb (1999); Costa *et al.* (2002); Chyzik *et al.* (2003) and Díaz *et al.* (2006). However, contrasting results have been found for thrips behaviour in response to different UV-conditions (Antignus *et al.*, 1996; Costa and Robb, 1999; Costa *et al.*, 2002; Díaz *et al.*, 2007). Mazza *et al.* (1999; 2002) have proven that thrips are able to perceive UV-B radiation and to respond with avoidance behaviour, whereas UV-A triggers attraction.

Due to overlapping gene expression patterns induced by UV-B and insect damage it is presumed that UV-B radiation can protect plants against herbivorous insects (Stratmann, 2003). This assumption is supported by various studies examining the influence of UV-B radiation on plant-insect interactions, which showed that UV-B exposed plants were to lesser extent damaged by herbivorous insects compared to non UV-B irradiated plants (Ballaré *et al.*, 1996; Rousseaux *et al.*, 1998, 2004; Zavala *et al.*, 2001; Caputo *et al.*, 2006). In this regard almost only leaf-chewing insects were investigated. However, also phloem feeding psyllid (*Strophingia ericae* (Curtis), Sternorrhyncha, former Homoptera) populations were reduced by direct or plant-mediated influences of enhanced UV-B (Salt *et al.*, 1998). We found that the reproduction of cabbage aphids (*Brevicoryne brassicae* (L.), Aphididae, Sternorrhyncha) on broccoli (*Brassica oleracea* L. convar. *botrytis*, Brassicaceae) was reduced under high UV-B conditions compared to low UV-B conditions whereas no differences between UV-B treatments could be found for reproduction of the generalised green peach aphid (*Myzus persicae* (Sulzer), Aphididae, Sternorrhyncha) (Kuhlmann and Müller, submitted, **Chapter III**), which indicates species-specific responses to UV-B radiation (McCloud and Berenbaum, 1999). However, when differently UV-B pre-treated broccoli plants were exposed to homogenous radiation conditions in the field no clear choice behaviour of thrips, whiteflies and aphids could be recorded (Kuhlmann and Müller, in press, **Chapter II**) (Fig. 1.2.1).

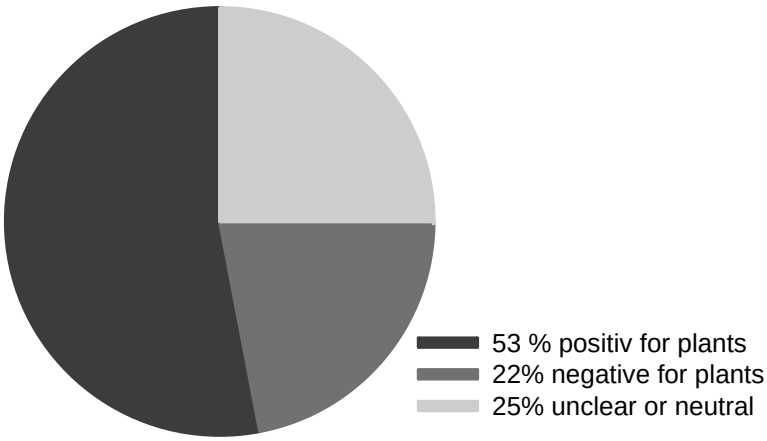

Fig. 1.2.1 Literature evaluation of UV-radiation impacts on plant-insect interactions. Results of 36 studies were examined. Positive (less herbivory, bad insect performance), negative (more herbivory, good insect performance) or unclear/neutral findings relating to the influence of (high) UV-radiation on plants and their herbivorous opponents are given in percent (%).

It has been suggested that UV radiation influences herbivorous insects through changes in plant chemistry, particularly phenolic compounds (Bergvinson *et al.*, 1994; Grant-Petersson and Renwick, 1996; Zavala *et al.*, 2001; Rousseaux *et al.*, 2004; Caputo *et al.*, 2006), which concentrations almost always increase with higher UV irradiance (Caldwell *et al.*, 2007; Jenkins and Brown, 2007). Phenolic compounds are described to have feeding deterrent effects (Lattanzio *et al.*, 2000; Treutter, 2005). However, flavonoid concentrations were not influenced by massive insect attacks in the field. Therefore it can be reasoned that the primary function of flavonoids is UV protection and the deterrence against insects may be a side effect (Close and McArthur, 2002; Kuhlmann and Müller, in press, **Chapter II**). The nutritional value of plants' tissue can be well described by the carbon / nitrogen ratio (Walling, 2000). No clear pattern of UV effects on the C/N ratio could be seen in several species of Brassicaceae, Fabaceae and Ericaceae (Hatcher and Paul, 1994; Salt *et al.*, 1998; Lindroth *et al.*, 2000; Zavala *et al.*, 2001; Reifenrath and Müller, 2007, 2009; Kuhlmann and Müller, 2009; **Chapter I**; in press, **Chapter II**). Therefore it can be concluded that this parameter is not appropriate to describe changes in plant-insect relationships influenced by UV-radiation. Furthermore, protein digestion is essential for insect survival. Plants have evolved specific proteins (proteinase inhibitors) that tightly bind proteolytic enzymes and thus inhibit dietary protein digestion of herbivores (Kehr, 2006). The increased resistance to insect feeding of UV-irradiated plants might be caused among other factors by increased levels of proteinase inhibitors. In *Nicotiana longiflora* Cav. (Solanaceae) an insect-responsive proteinase inhibitor gene is down-regulated, whereas in *Nicotiana attenuata* Torr. Ex W., UV treatments induce proteinase inhibitor gene expression (Izaguirre *et al.*, 2003). Proteinase inhibitor levels do not differ in *Lycopersicon esculentum* Mill. (Solanaceae) just irradiated with UV-B (Stratmann *et al.*, 2000). Synergistic effects of UV-B radiation and wounding are detected in tobacco (*N. attenuata*) and tomato (*L.*

esculentum) but not in *N. longiflora*. UV-B exposed *N. attenuata* and *L. esculentum* have enhanced proteinase inhibitor levels compared to non-UV-B exposed plants in response to wounding (Stratmann *et al.*, 2000; Izaguirre *et al.*, 2003). Different UV-exposure conditions of broccoli did not result in changes of proteinase inhibitor concentrations (Kuhlmann and Müller, 2009, **Chapter I**). Moreover, insect-defence related specific secondary metabolites like compounds of the glucosinolate-myrosinase system (see below) may change due to UV-exposure. However, broccoli plants without herbivore contact did not show any differences in glucosinolate accumulation in response to UV-B treatment (Kuhlmann and Müller, in press, **Chapter II**).

1.3 The characteristic secondary metabolites of Brassicaceae – Glucosinolates

Plants not only need to hedge against abiotic environmental factors, they also have to fight against pathogens and herbivores. The glucosinolate-myrosinase defence system of Brassicaceae has antifungal (Bednarek *et al.*, 2009), antimicrobial (Clay *et al.*, 2009) and insect-deterring functions (Hopkins *et al.*, 2009). However, specialists can circumvent or even take advantage of these safeguards (Hopkins *et al.*, 2009). Since thousands of years cruciferous vegetables are important ingredients for human nutrition because of their taste and flavour. Furthermore anti-tumorigenic activities and a role in crop protection of glucosinolates and their hydrolysis products are discussed (Halkier and Gershenzon, 2006). Glucosinolates are hydrophilic compounds sequestered in vacuoles of plant cells (Grubb and Abel, 2006). The approximately 120 structures of glucosinolates are grouped in indolyl, aliphatic, and aromatic glucosinolates due to their precursor amino acids, which are tryptophan for indolyl glucosinolates and seven additional protein amino acids like methionine, alanine, valine, leucine, isoleucine for aliphatic glucosinolates and phenylalanine and tyrosine for aromatic glucosinolates (Fahey *et al.*, 2001). The glucosinolate biosynthesis encompasses three major steps: first side chain elongation of amino acids, secondly glucone formation and thirdly side chain modifications (Grubb and Abel, 2006). The chemical structure of glucosinolates comprises a ß-D-glucopyranose residue attached via sulfur to a (Z)-*N*-hydroximinosulfate ester and a variable side chain (Fahey *et al.*, 2001; Halkier and Gershenzon, 2006). Plants attacked by herbivores and pathogens activate myrosinases (ß-thioglucosidases) localised in myrosin cells, which hydrolyse glucosinolates and thus generate various side chain structure related bioactive degradation products (Grubb and Abel, 2006; Bednarek *et al.*, 2009; Clay *et al.*, 2009) (Fig. 1.3.1). The hydrolysis products of the glucosinolate-myrosinase system intensify the repelling or attracting effect of those secondary metabolites (Travers-Martin *et al.*, 2008). Nevertheless, the post-ingestive breakdown of indolyl glucosinolates independent of myrosinases is capable of defending plants against herbivores that avoid enzymatic glucosinolate hydrolysis such as aphids (Barth and Jander, 2006; Kim and Jander, 2007; Kim *et al.*, 2008).

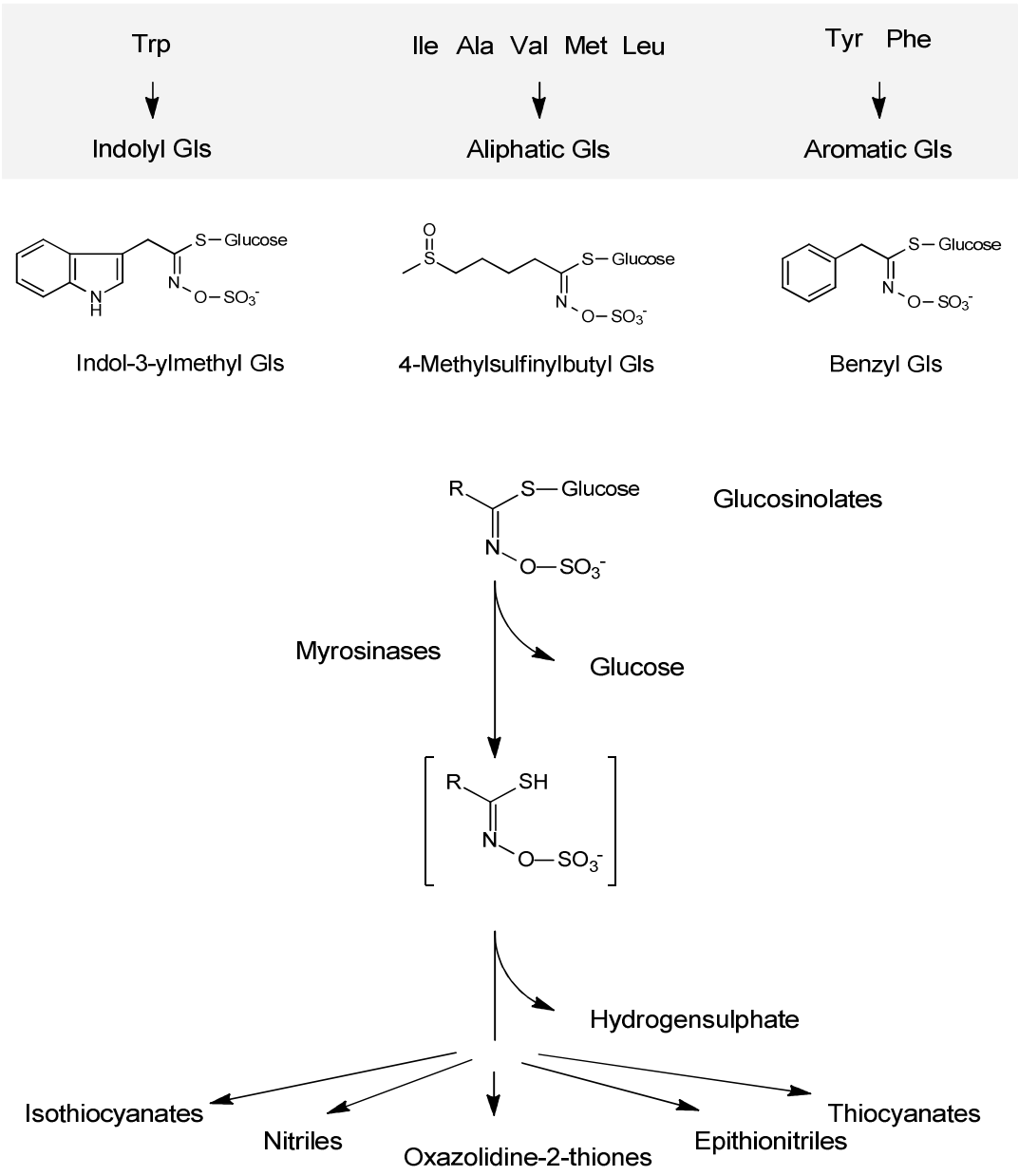

Fig. 1.3.1 Amino acid precursors of major glucosinolate classes and enzymatic degradation of glucosinolates by myrosinases. Gls, glucosinolate; R, variable side chain (Fahey *et al.*, 2001; Wittstock and Halkier, 2002).

1.4 Glucosinolates and insect feeding

Glucosinolates are constitutive defence metabolites but their concentrations can be influenced by insect feeding (Textor and Gershenzon, 2009). The feeding strategy of the herbivore attacker determines perceptive and defensive responses of plants (Hopkins *et al.*, 1998; Walling, 2000; Thompson and Goggin, 2006; Goggin, 2007; Textor and Gershenzon, 2009). Once the insect attack is perceived the metabolic reprogramming of the host plant is regulated by the immunity-related key phytohormones salicylic acid,

jasmonic acid and ethylene (Thompson and Goggin, 2006). In order to adjust expression profiles of defence-related genes both synergistic and antagonistic interactions between plant hormones take place. This crosstalk between these pathways provides the capability to regulate defence responses elicitor-specifically (Taylor *et al.*, 2004; Thompson and Goggin, 2006). Jasmonate signalling plays a major role in shoot induction of especially indolyl glucosinolates (Agerbirk *et al.*, 2009; Textor and Gershenzon, 2009; van Dam *et al.*, 2009). Generally, the artificial application of salicylic acid to shoots induced no or far smaller increases of indolyl glucosinolates, for example, in *Brassica oleracea* L. and *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae) (van Dam *et al.*, 2009). The expression of genes in *Arabidopsis thaliana* was regulated by the jasmonic acid pathway after attack by leaf-chewing and cell-content feeding insects, whereas phloem-sucking insects induced salicylic-acid-regulated genes (de Vos *et al.*, 2007; Kempema *et al.*, 2007; Zarate *et al.*, 2007; Abe *et al.*, 2008; Hopkins *et al.*, 2009). Jasmonic acid-induced defences are able to reduce aphid infestation more effectively than salicylic acid-mediated defences (Thompson and Goggin, 2006; de Vos *et al.*, 2007). Studies examining the potential influence of glucosinolates in artificial diets (Kim and Jander, 2007) as well as in *Arabidopsis* (Pfalz *et al.*, 2009) on aphid performance showed that 4-methoxy-indol-3-ylmethyl (4MOI3M) is the strongest inhibitor of aphid growth. 4MOI3M plays a major role in pathogen resistance and triggers callose deposition at infection sites (Bednarek *et al.*, 2009; Clay *et al.*, 2009). Artificial increases of glucosinolates in *Arabidopsis* via mutations made this plant less attractive for the green peach aphid (Levy *et al.*, 2005).

It is hypothesised that aphids as well as whiteflies can manipulate plant defence responses by inducing salicylic acid defences and repressing the potentially more effective jasmonic acid signalling (Thompson and Goggin, 2006; Kempema *et al.*, 2007; Zarate *et al.*, 2007). This is in accordance with findings in broccoli, infested with high numbers of cabbage aphids, which led to decreased indolyl glucosinolate concentrations (Kuhlmann and Müller, submitted, **Chapter III**) as well as in brussels sprouts (*Brassica oleracea* var. *gemmifera*) infested with high numbers of cabbage aphids that had the lowest concentrations of total glucosinolates (Yusuf and Collins, 1998). Low numbers of green peach aphids did not induce glucosinolate changes in broccoli (Kuhlmann and Müller, submitted, **Chapter III**). The infestation of *Arabidopsis thaliana* (L*er*, Col-0) by cabbage aphids or green peach aphids led to lower amounts of aliphatic glucosinolates whereas indolyl glucosinolates did not change (Kim and Jander, 2007; Kuśnierczyk *et al.*, 2008). However, a slight increase of aliphatic glucosinolates and no changes in indolyl glucosinolate concentrations or no changes at all were reported in two other studies examining *Arabidopsis thaliana* (Col-0) infested by cabbage aphids or green peach aphids (Mewis *et al.*, 2005, 2006). Differences between these studies might be due to timing, numbers of aphids, aphid species and plant ecotype. Generally, aphid induced plant changes are characterised by very low increases if not decreases of total glucosinolate levels or only changes in specific glucosinolate classes (see also **Appendix**). These findings support speculations whether aphids are able to circumvent plant defences for their own benefit. Moreover, the

question arises whether plants are able to recognise infestation by either generalist or specialist aphids. Plants may need an exceeding of a certain infestation threshold to respond to aphid infestation with pronounced changes in plant chemistry.

Under field conditions plants face multiple enemies with different feeding strategies. Therefore they need to have a sophisticated perception and defence system. Broccoli plants, which were freely accessible to insects for a period of 72 hours in the field, were strongly infested by thrips, whiteflies and aphids and showed threefold increased glucosinolate concentrations. It seems to be likely that the high abundance of herbivorous insects induced strong increases of these metabolites (Kuhlmann and Müller, in press, **Chapter II**).

1.5 Different feeding strategies of insects affect plant-insect-interactions differently

Insects' diets are divers but the nutritional requirements of insects are rather homogenous. They have to protect themselves against consumer defending compounds and need to utilize nutritionally unbalanced food sources. Toxic secondary metabolites can be eliminated, detoxified and sequestered. The feeding strategy and the level of specialisation influence the acquisition and processing of food plants (Dadd, 1973; Douglas, 2009). Therefore, for example, the generalised green peach aphid (*Myzus persicae*) can use a broader spectrum of plant species than the cabbage aphid (*Brevicoryne brassicae*) which is specialised on Brassicaceae. In contrast to leaf chewing and cell content feeding insects, phloem-feeding insects only cause slight tissue damage and only get in contact with plant compounds transferred by phloem sap (sampling method Fig. 1.5.1).

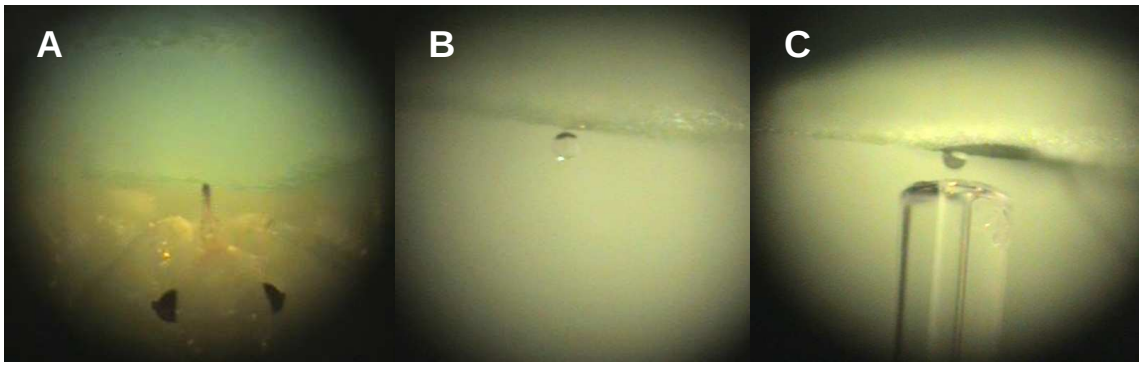

Fig. 1.5.1 Sampling of phloem sap via stylectomy. A: head and stylet of *M. persicae* on the lower leaf surface of broccoli, B: phloem droplet after cutting the stylet by means of a laser beam, C: phloem sap uptake with a glass capillary.

However, also phloem feeding insects are faced with various plant components like sugars, organic acids, phytohormones, proteins and defence related metabolites like proteinase inhibitors and glucosinolates (Chen *et al.*, 2001; Kehr, 2006). The phloem sap is a low-nitrogen diet, but aphids can overcome this restriction by an alliance with microorganisms, which provide them with essential amino acids (Douglas, 2006, 2009;

Gündüz and Douglas, 2009). The relationship between aphids and their mutualistic endosymbiotic bacteria can be influenced by the quality of phloem amino acids and thus can affect aphid development (Chandler *et al.*, 2008). UV-B radiation mediated phloem sap changes and plant quality may influence aphid reproduction and viability (Kuhlmann and Müller, submitted, **Chapter III**). During plant infestation, aphids first get in contact with the upper leaf surface and therefore with the wax composition of the host plant (Powell *et al.*, 1999; Müller and Riederer, 2005; Powell *et al.*, 2006). Specialist cabbage aphids do not colonise non-waxy cabbage plants (Thompson, 1963). We found that broccoli grown with high ambient UV-B irradiation had reduced wax coverage and cabbage aphid performance (Kuhlmann and Müller, submitted, **Chapter III**). Aphids identify a suitable host plant by short intercellular probes (Powell *et al.*, 2006). Aphids' settling, feeding and reproduction on the lower leaf surface will not take place when the appropriate cues are lacking (Thompson, 1963; Powell *et al.*, 2006). For example, specialist cabbage aphids use glucosinolates as feeding stimulants (Wensler, 1962; Moon, 1967; Gabrys and Tjallingii, 2002) and they are able to sequester host plant glucosinolates (Rossiter *et al.*, 2003). Upon tissue damage by enemies, aphid-specific myrosinases hydrolyse these sequestered glucosinolates (Bridges *et al.*, 2002). The degradation products of this glucosinolate-myrosinase system protect these aphids against natural enemies (Francis *et al.*, 2000; Kazana *et al.*, 2007; Pratt *et al.*, 2008). It may be concluded that specialist cabbage aphids depend on the defensive metabolites of Brassicaceae, specific glucosinolates may be of particular importance. Plants may circumvent this exploitation of their own defense by reducing their total glucosinolate concentrations when infestation by cabbage aphids exceeded a defined infestation threshold (Yusuf and Collins, 1998; Kuhlmann and Müller, submitted, **Chapter III**). It is questionable, whether a decrease of glucosinolates is capable to retard growth of specialist cabbage aphids. In contrast to cabbage aphids, generalist green peach aphids excrete ingested glucosinolates (Müller, 2009) but glucosinolates are also used as feeding stimulants (Klingauf *et al.*, 1972). It would be advantageous for plants to recognise their insect enemies and to defend themselves according to the stimulus of the herbivore. The aphid performance-pattern seems to be influenced by the genus of the plant species. *Arabidopsis* Col-0 had the highest glucosinolate concentrations compared to the glucosinolate concentrations of three cabbage varieties but was less suitable for the specialist cabbage aphid, whereas the generalist green peach aphid performed best on *Arabidopsis*. This may be caused by the different glucosinolate compositions of the host plants or other defensive mechanisms (see **Appendix**). Furthermore, in *Arabidopsis* high amounts of green peach aphids also induced reductions of some aliphatic glucosinolates. It can be concluded that plant responses to aphid infestation as well as aphid responses to plants are highly dependent on the species composition of both counterparts. Furthermore, the coevolutionary imprint of these interactions may also play a role. Next to glucosinolates, it has been reported that flavonoids can inhibit aphid performance (Lattanzio *et al.*, 2000). Quercetin and kaempferol glycosides were found in petiole exudates thought to be phloem sap of cassava (*Manihot esculenta*, Crantz, Euphorbiaceae) (Calatayud *et al.*, 1994). Upon stylet penetration aphids

overcome plant wound-induced plugging of sieve plates by protein clogging and callose sealing through watery saliva (Will and van Bel, 2006; Will *et al.*, 2007). Further, cell wall modifications can enhance the mechanical barrier to stylet insertion (Goggin, 2007). Thus, next to chemical changes, UV-B radiation induced morphological plant changes may be responsible for reduced cabbage aphid performance on broccoli (Kuhlmann and Müller, submitted, **Chapter III**). It has been reported that UV-B radiation (Mert-Turk *et al.*, 2003) as well as cabbage aphids induce camalexin accumulation in *Arabidopsis* WT plants and it has been proven that increased camalexin concentrations reduce aphid fitness (Kuśnierczyk *et al.*, 2008). It still needs to be proven, which phloem sap components are responsible to retard aphid growth and improve plant resistance to aphids.

Whiteflies and aphids are phloem-feeding herbivores, whereas thrips are cell content feeders; they pierce plant cells and suck out the contents (Abe *et al.*, 2008). All of them are serious crop pests, which inhibit plant growth and decrease crop productivity. Insects are vectors for viruses, which can additionally impair plant growth. Alternative pest control strategies are required to reduce the application of toxic insecticides and pesticides (Antignus, 2000). Visual cues and plants' status can influence insect behaviour. Different UV (-B) levels can serve as instruments to manipulate plant-insect interactions during crop plant cultivation, which may reduce the use of chemicals and increase plant health and quality.

1.6 Aims of the study

Insufficient qualities and quantities of sunlight (specifically the lack of UV-B and reduced PAR) in conventional greenhouses can generate plants, which are morphological instable, more vulnerable to insects and endangered to get "sunburned" when exposed in the field. In this study, chemical and morphological changes of the crop species broccoli (*Brassica oleracea* L. convar. *botrytis*, Brassicaceae) in response to different UV radiation- and/ or herbivore-exposure were investigated. Furthermore, behavioural responses of herbivores (aphids (Aphididae), whiteflies (Aleyrodidae) and thrips (Thripidae)) to direct UV radiation and to indirect UV-mediated plant changes were examined. The study aimed to understand plant-insect relationships in dependence of an abiotic modulating factor by means of a multi-level approach. Using innovative greenhouse covering materials, it was possible to investigate the unknown effect of UV-B on plants and of UV-B mediated plant changes on phloem-feeding generalist and specialist aphids. Furthermore, the chemical compositions of phloem sap amino acid concentrations in response to UV-B radiation as well as various other chemical plant components were examined.

Filter tent constructions, which were covered with either UV-transmitting (+UV) or UV-blocking (-UV) filter foils (Fig. 1.1.3; Fig. 1.1.4), were utilized to examine UV-A / UV-B effects on two different developmental stages of broccoli. Furthermore, behavioural decision-making of naturally occurring herbivorous insects was investigated, when given free host plant choice. It was expected that broccoli plants of

different developmental stages and different growth conditions should respond differently to UV-inclusion or exclusion, which could result in distinct infestation patterns of herbivorous insects. Plants were either grown for 27 days from germination onwards under the two different UV conditions or were first kept in a climate chamber for 22 days under low irradiation and subsequently transferred for 19 days to the filter tents. Plants' leaf area, shoot length, fresh weight, water content, carbon / nitrogen (C/N) ratio, trypsin inhibitor activity, flavonoid and glucosinolate concentrations were analysed as well as plant infestation and visual orientation by herbivorous insects monitored. Plants in the field of both developmental stages and independently to the growing conditions were protected by increased concentrations of phenolic compounds, e.g. flavonoids, in response to UV-radiation. Glucosinolate and proteinase inhibitor concentrations remained unaffected. Morphological differences were only detected when broccoli germinated already under different UV-conditions. A shaping effect is therefore more pronounced in young developing plants. Whiteflies and aphids were more abundant on +UV plants, whereas thrips avoided +UV-conditions. The behavioural responses of the cabbage whitefly *Aleyrodes proletella* L. (Aleyrodidae) were navigated by light quality directly rather than indirectly by the host plant metabolite composition. Host plant infestation by insects is driven by direct and indirect plant-mediated responses to UV-conditions (Kuhlmann and Müller, 2009, **Chapter I**).

Experiments in three different greenhouses were conducted to examine the impact of UV-B radiation on plant-insect interactions in particular. Two greenhouses were covered with innovative materials. High (80 %, ETFE foil), medium (23 %, MM micro-structured solar glass) and low (4 %, conventional float glass) levels of UV-B radiation (Fig. 1.1.3; Fig. 1.1.4) were transmitted into the greenhouses. According to commercial applications, experiments were designed to investigate the effect of ambient solar radiation conditions on different UV-B pre-treated broccoli plants and their interactions with herbivorous insects in the field. Broccoli plants were first grown in these greenhouses and then one group of plants remained in the greenhouses, whereas the other was transferred in the field with ambient light conditions and herbivore access over a period of three days. Plants were probed after zero, 24 hours and 72 hours of field exposure. Biomass, C/N ratio, flavonoid and glucosinolate concentrations of all plant treatments, as well as insect infestation of field-exposed plants were measured. It is believed that plant responses to UV-B radiation and insect herbivory overlap (see 1.2). Therefore, it was expected that different UV-B pre-treated broccoli plants should have different morphological and chemical features, which should influence host choice behaviour of naturally occurring herbivores after transfer in the field. It was supposed that plants with higher concentrations of secondary metabolites should exhibit a better protection against herbivores. Higher UV-B irradiation induced increases in flavonoid concentrations and decreases in biomass accumulation, whereas glucosinolate concentrations remained unaffected. Despite UV-B induced plant changes, no differences in infestation patterns were observed as the broccoli plants were equally infested with thrips, whiteflies and aphids. This plant infestation most likely led to a threefold increase of indolyl glucosinolates after 72 hours of field exposure independent

of UV-B pre-treatment. A different host plant morphology and chemistry induced by UV-B radiation is not necessarily mirrored by different insect infestation patterns. Plants are able to perceive and to respond specifically to distinct abiotic and biotic cues (Kuhlmann and Müller, in press, **Chapter II**).

The impact of UV-B mediated plant changes on phloem-feeding insects was investigated with broccoli grown in greenhouses either under high (80 %) or low (4 %) UV-B conditions. The effect of UV-B mediated plant changes on generalist and specialist phloem-feeding aphids under controlled experimental conditions has not yet been examined. Also the UV-B impact on amino acid concentrations in the phloem-sap has never been analysed before. Therefore, three-week-old plants were infested with 10 individuals of either the specialist cabbage aphid *Brevicoryne brassicae* or of the generalist green peach aphid *Myzus persicae* (Fig. 1.6.1). Analyses of biomass, leaf area, flavonoid and glucosinolate concentrations were carried out to investigate UV-B-induced and aphid-induced plant changes. Aphid population growth was determined by counting the numbers of aphids per plant after five days. In addition wax coverage and amino acid concentrations (sampling Fig. 1.5.1) of broccoli plants grown under the two UV-B regimes were analysed. Species-specific differences in aphid population growth and different plant responses to the aphid species were expected, whereby high UV-B irradiated plants should be better protected against them. Broccoli plants grown under high UV-B conditions were smaller and had higher flavonoid concentrations. In addition, high UV-B irradiation reduced the cuticular wax coverage, whereas amino acid concentrations were only slightly reduced, with significant lower concentrations of the amino acid proline. The population growth of cabbage aphids was lowered on plants grown in greenhouses with high UV-B radiation, but performed much better on both plants in comparison to green peach aphids, which reproduced equally little on both plants. Only a high infestation with cabbage aphids induced decreases in indolyl glucosinolate concentrations, whereas the low number of green peach aphids did not affect glucosinolate concentrations. The responses of aphid species to UV-B modulated plants are species-specific. Several factors like the aphid species, its degree of specialisation, the exceeding of an infestation threshold or the distinct perception and processing mechanisms in the plant may influence plant responses (Kuhlmann and Müller, submitted, **Chapter III**).

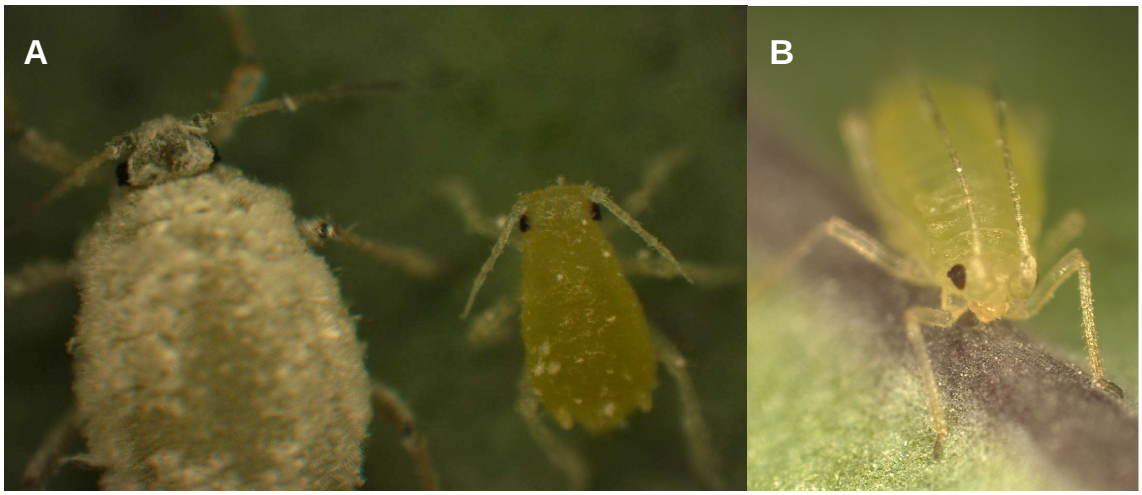

Fig. 1.6.1 Adult and juvenile specialist cabbage aphid *B. brassicae* (A) and generalist green peach aphid *M. persicae* (B).

Three different varieties of cabbage (white cabbage (*Brassica oleracea* L. convar. capitata (L.) Alef. var. alba DC.), red cabbage (*Brassica oleracea* L. convar. capitata (L.) Alef. var. rubra DC.), broccoli (*Brassica oleracea* L. convar. botrytis (L.) Alef. var. cymosa Duch.)) and *Arabidopsis thaliana* Col-0 were grown in a climate chamber for 28 days. Cabbage plants were infested with 5 aphids and *Arabidopsis* plants with 3 aphids of either cabbage aphids *Brevicoryne brassicae* or of green peach aphids *Myzus persicae* (Fig. 1.6.1). Their reproduction was counted 7 days after aphid infestation. The aim of this study was to explore the interactions between the different plant and aphid species under constant light conditions and low radiation. Thus, effects of different Brassicaceae species on aphid performance and the impact of aphid feeding on the plants' dry weight, glucosinolate and flavonoid concentration (only for the cabbages) were investigated. *Arabidopsis* had the highest concentrations and the most diverse glucosinolate composition. The glucosinolate profiles of white and red cabbage were more similar in comparison to broccoli, but all of them differed highly from the profile in *Arabidopsis*. Aphids, especially high numbers of *M. persicae* on *Arabidopsis*, induced a decrease in particular glucosinolates, whereas total glucosinolate concentrations did not change. Regarding the flavonoids, kaempferols were higher concentrated than quercetins in all cabbage varieties, but were present always only in low amounts. Aphid performance was species-dependent; cabbage aphid performed on cabbage varieties generally better than on *Arabidopsis*, whereas the performance of the green peach aphid was more pronounced on *Arabidopsis*. The plant glucosinolate profile and the degree of aphid specialisation determine plant-aphid interactions (**Appendix**).

1.7 Future prospects

UV-B radiation influences the metabolites and the architecture of plants, but it is currently not known, which structures are capable to perceive this UV-B radiation and only little is known about the processes that regulate the acclimation of plants to UV-B.

Therefore, methods that include molecular, analytical and ecological approaches must be combined to understand plants' regulation and interactions with their abiotic and biotic environment. This could provide basic approaches for an ecologically sound pest control. Although photoreceptors for UV-A are well characterised, in particular more detailed ecological studies of UV-A and UV-B influences are needed.

Metabolomics or at least metabolite profiling can be a useful tool to disentangle metabolite changes caused by different abiotic and biotic environmental cues. At present, only some responses of target plant components are known, but the interplay between different metabolites in plants, especially between flavonoids, glucosinolates and phytohormones in Brassicaceae, and their biosynthetic pathways in response to different environmental stimuli are unknown (Fig. 1.7.1).

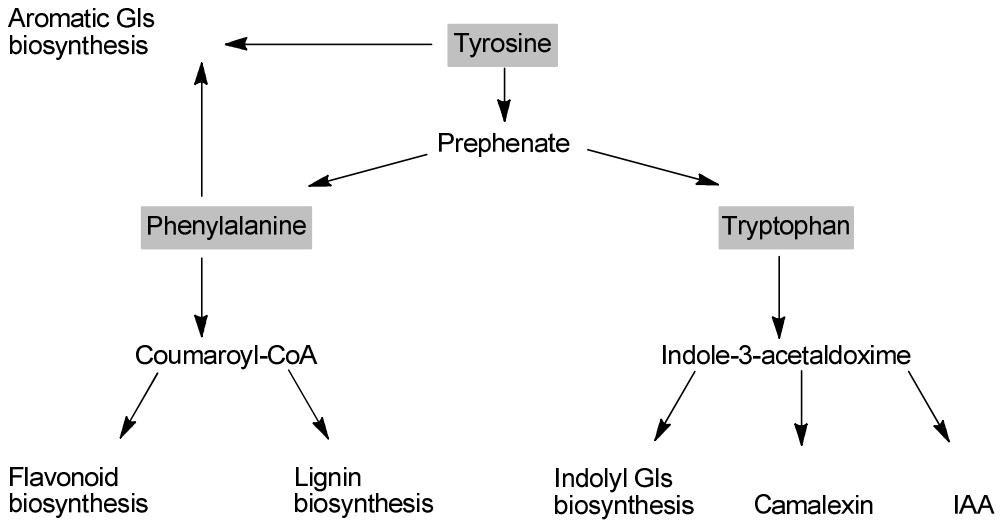

Fig. 1.7.1 Overview of known junctions between flavonoid and glucosinolate biosynthesis. Gls, glucosinolates, IAA, indole-3-acetic acid (Fahey *et al.*, 2001; Kuśnierczyk *et al.*, 2008; Bender and Celenza, 2009).

Phloem-feeding insects come in contact with different dietary plant compounds than leaf chewing insects and they induce distinct defensive responses in plants. It is not entirely clarified, which metabolites influence and deter phloem-feeders. Especially, almost nothing is known about the presence and composition of flavonoids in phloem sap. Therefore, the stylectomy is a useful method to get a better understanding of phloem sap metabolites that may have the ability to retard the performance of phloem-feeding herbivores.

The considerable responses of broccoli plants towards ambient UV-B radiation conditions can have an impact on the next trophic level. However, UV-B mediated plant changes were intermingled with direct influences of UV radiation on piercing-sucking herbivores. It remains to be proven whether aphids and whiteflies are able to perceive UV-B radiation as thrips are. Further, it will be interesting to understand why species

with different feeding modes show different behavioural responses and which message is behind the UV (-B) signal that navigates these insects.

Plants interact with various other organisms like, for example, mycorrhiza, endophytic fungi, epiphytic bacteria and parasitoids and it would be interesting to reveal, how the network of distinct trophic levels and life forms is influenced by different UV-B radiation regimes under ambient light conditions in an ecological context.

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Chapter I

Development-dependent effects of UV radiation exposure on broccoli plants and interactions with herbivorous insects

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Abstract

The responses of plants to stress can highly depend on their developmental stage and furthermore influence biotic interactions. Effects of outdoor exposure to different ambient radiation conditions including (+UV) or excluding (-UV) solar ultraviolet radiation were investigated in broccoli plants (*Brassica oleracea* L. convar. *botrytis*) at two developmental stages. Plants either germinated directly under these different outdoor UV conditions, or were first kept for three weeks in a climate chamber under low radiation before outside exposure at +UV and -UV. Access of herbivores to the plants was possible under the outdoor conditions. Plants of both groups protected their tissue against destructive UV by increasing concentrations of phenolic compounds (flavonoids and hydroxycinnamic acids) after +UV exposure. But only plants that germinated under +UV conditions kept smaller than plants grown under –UV conditions, indicating certain costs for production of phenolics or for other potential metabolic processes specifically in young, growing plants. In contrast, growth of plants transferred at a later stage did not differ under both UV conditions. Thus, plants responded much more sensitive to the environment they experienced at first growth. Glucosinolates, the characteristic secondary compounds of Brassicaceae, as well as proteinase inhibitors, remained unaffected by UV in all plants, demonstrating independent regulation pathways for different metabolites. Plant infestation by phloem-feeding insects, Aleyrodidae and Aphididae, was more pronounced on +UV exposed plants, whereas cell content feeders, like Thripidae were more abundant on plants under the -UV condition. Choice experiments with the cabbage whitefly *Aleyrodes proletella* L. (Aleyrodidae), commonly found on *Brassica* spp., revealed that the key environmental cue navigating their behaviour seems to be the radiation composition, rather than plant quality itself. In conclusion, stress mediated changes of plant chemistry and morphology depend on the plant life cycle stage and are not necessarily mirrored in the behavioural responses of herbivorous insects.

Keywords: Brassicaceae; Flavonoids; Glucosinolates; Growth parameters; Host-finding behaviour; Induction

2.1 Introduction

Plants are exposed to various abiotic and biotic stress factors throughout their life time. As organisms with a high phenotypic plasticity, they can adapt to changing environmental natural and agricultural conditions by different morphological, physiological and biochemical means (Lichtenthaler, 1998; Walling, 2000; Díaz et al., 2007). Thereby, the responses will depend on the developmental stage of the individual plant. The particular plant traits can influence in turn the attractiveness and susceptibility of the plants to herbivorous insects (Lavola et al., 1998; Zavala et al., 2001; Rousseaux et al., 2004).

Solar ultraviolet (UV) radiation is a highly dynamic abiotic environmental factor of major importance, which serves as an essential cue for growth and differentiation processes in plants. UV-B (280-315 nm) is the most energetic radiation reaching the earth's surface (Paul and Gwynn-Jones, 2003). When plants are not acclimatised or are irradiated with UV-B levels above the current ambient radiation, this radiation can have detrimental effects on lipids, proteins and nucleic acids, and specifically affect the photosystem II by damaging its membranes and decreasing enzyme activities (Rozema et al., 1997; Kolb et al., 2001; Bassman, 2004). UV-B leads also to an inhibition of cell expansion by reducing levels of indole-3-acetic acid (IAA), thereby affecting plant morphology (Rozema et al., 1997; Jansen et al., 1998). Plants have evolved various ways to cope with UV-B radiation, mainly by incorporating UV-absorbing flavonoids and hydroxycinnamic acids in the epidermis (Caldwell et al., 1983; Kolb et al., 2001). These phenolic compounds shield the photosystem against harmful radiation, serve as antioxidants, and change the optical properties of the plant (Treutter, 2005; Pfündel et al., 2006). They are also known to be involved in defence against herbivorous insects and pathogens (Treutter, 2005; Caputo et al., 2006) and UV-induced changes in plant chemistry can even effect members of the third trophic level, such as parasitoids (Foggo et al., 2007). Less is known about effects of UV-A (315-400 nm), however, also UV-A can induce the production of phenolics (Krizek et al., 1997; 1998). In general, signalling hormones are involved in the stress responses to UV. These hormones can mediate various other plant growth and defence responses (Mackerness, 2000; Stratmann, 2003). Furthermore, stress responses of plants to UV and herbivory overlap in gene expression. However, plants are also able to react in a stress-specific way (Mackerness, 2000; Stratmann, 2003; Pandey and Baldwin, 2008).

Different approaches were followed to test responses of plants to changed UV-radiation regimes under controlled conditions. Effects of increased UV-radiation were tested by using UV-lamps either in climate chambers (Lindroth et al., 2000; Tegelberg and Julkunen-Tiitto, 2001; Hofmann et al., 2003), greenhouses (Lavola et al., 1998; Wang et al., 2007) or under field conditions (Björn et al., 1997; Veteli et al., 2003). However, in these experimental set-ups partly unrealistic relative and absolute levels of photosynthetically active radiation (PAR, 400-700 nm) and UV radiation might be obtained (Rozema et al., 1997). In another approach, plants can be exposed to ambient

outdoor radiation levels from which selectively predetermined wavelengths of the sunlight are excluded by the use of filter material (Hunt and McNeil, 1999; Mazza et al., 1999a; Kolb et al., 2001; Caputo et al., 2006; Reifenrath and Müller, 2007). With such filters plant responses can be tested under more ecophysiologically relevant conditions.

The formative imprint of an environmental cue highly depends on the stage of the plant life cycle and on the species to which the stress is applied, as well as on the duration of the treatment (Grammatikopoulos et al., 1998; Mazza et al., 1999a; Sultan, 2000; Reifenrath and Müller, 2007). Plants usually face a trade-off for resource allocation either to growth or to defence (Matyssek et al., 2005). However, this trade-off might differ in its extent for a young, developing *versus* a mature plant. A germinating plant has to build up an efficient protection system against abiotic and biotic harms rather rapidly to be able to produce photosynthetically active tissue for maturation. Thereby, a seedling or young plant might be exposed to a much stronger trade-off. For a mature plant, the resource distribution might be more flexible because of its stock of reserves. As it possesses already a substantial amount of photosynthetic active tissue, a mature plant might be able to invest more in the induction of chemical defence without measurable consequences in tissue growth.

A lot of domesticated crop plants like broccoli (*Brassica oleracea* L. convar. *botrytis*, Brassicaceae) are grown from seeds under attenuated ambient radiation conditions in greenhouses and are planted outside at an age of two to three weeks. In the field they have to adapt to the ambient radiation, and they have to cope and interact with various herbivorous insect species. Host plant quality is an important parameter that affects the performance of herbivorous insects (Awmack and Leather, 2002). Several insects are able to detect qualitative differences between individual plants and respond to different combinations of secondary metabolites in plants rather specifically (e.g., Reifenrath and Müller, 2008). But host plant chemistry is not the only information that triggers host choice behaviour of insects. For example, Mazza et al. (2002) demonstrated that thrips can perceive ambient UV-B radiation and prefer environments with low UV-B levels. Thus, visual cues are also of high importance for host seeking insects.

The aim of this study was to investigate the effects of different environmental irradiation conditions on growth and physical and chemical characteristics of broccoli in dependence of the plants' developmental stage and to measure the impact on natural insect infestation in a comprehensive approach. Cabbage whiteflies (*Aleyrodes proletella*, Aleyrodidae) were used as model to examine the cues navigating their behaviour in relation to the experimental design, as they were commonly found on the broccoli plants.

2.2 Methods and materials

2.2.1 Plant material and growth conditions

Broccoli plants [*Brassica oleracea* L. convar. *botrytis* (L.) Alef. var. *cymosa* Duch. Monopoly; F1 Hybrid; Syngenta Enkhuizen, Netherlands] were grown from seeds in fertilised soil (ED 73, pH 6) in individual pots (diameter: 12 cm, height: 9 cm). Plants used for the *"late stress experiment"* (see below) were first kept in a climate chamber (20 °C, 16: 8 h L: D, 70 % r.h.) and after three weeks transferred outdoors in two types of filter tents including or excluding ultraviolet radiation (see below). Irradiance spectra in the climate chamber lacked UV-B, while low levels of UV-A were detected with 8 W/m². PAR was 371 µmol $m^{-2}s^{-1}$. Irradiance spectra were measured with an $X1_2$ Optometer (Gigahertz Optik, Puchheim, Germany). Pots with seeds used for the *"early stress experiment"* (see below) were placed directly outside in the filter tents (ambient climatic conditions: temperature 6-30 °C, humidity 40-98 %, 12:12 h L:D, mostly cloudless sky).

For plant exposure, twelve filter tents were built outdoors in the Botanical Garden of Würzburg directly before the start of the experiments. Spectrometer measurements (UNICAM UV4, ATI Unicam) of the filters were conducted regularly to control for alterations in filter transmission (Winter and Rostás, 2008). Tents consisted of wooden frames (1.20 × 1 m ground area, 2.5 cm beam width) with the longer axis aligned in an east–west direction. The roof sloped from 1.3 m (north) to 0.9 m (south) height. Roofs and walls were covered with foil filters, except the northern wall. This wall was kept open to allow air circulation and insect entrance. At the roof and at the east and west side, the filter material overlapped the wooden frames for 10 cm. Plants were positioned close to the southern front in the tents, to limit the level of scattered radiation reaching the plants. Pots were placed in a distance of approximately 10 cm from each other. Six tents were covered by a teflon foil (Nowofol, Siegsdorf, Germany) transmitting the complete visible light spectrum and the ambient ultraviolet radiation ("+UV") (Fig. 2.2.1). The six other tents were covered by a Lee 226 UV foil (FFL-Rieger, Munich, Germany), which transmitted the complete visible light but filtered the entire UV-B range and most of UV-A ("-UV") (Fig. 2.2.1). Radiation-measurements were conducted in the early afternoon under cloudless sky with a portable high accuracy UV–visible spectroradiometer with 0.25 mm entrance configuration and exit slits resulting in a half bandwidth of ≤ 2 nm (OL 754, Optronic Laboratories, Orlando, USA). Tents of both filter types were located in alternating order 2 m apart from each other in order to avoid shading. Surrounding radiation parameters (UV-A and UV-B-radiation, PAR, global radiation) were recorded by a meteorological station (Thies Clima, Göttingen, Germany) in 15 m distance from the tents. On average a UV-A radiation of 985 kJ $m^{-2}d^{-1}$, UV-B radiation of 14 kJ $m^{-2}d^{-1}$, PAR of 4896 kJ $m^{-2}d^{-1}$ and global radiation of 13600 kJ $m^{-2}d^{-1}$ were recorded during the plant exposure periods. Temperature and relative humidity in all tents were comparable with a mean deviation between tents of 0.06 °C

and 0.8 %, respectively (measured with Tiny Tag Ultra, Gemini data loggers, UK). Mean tent temperature was 1.5 °C higher than in the field.

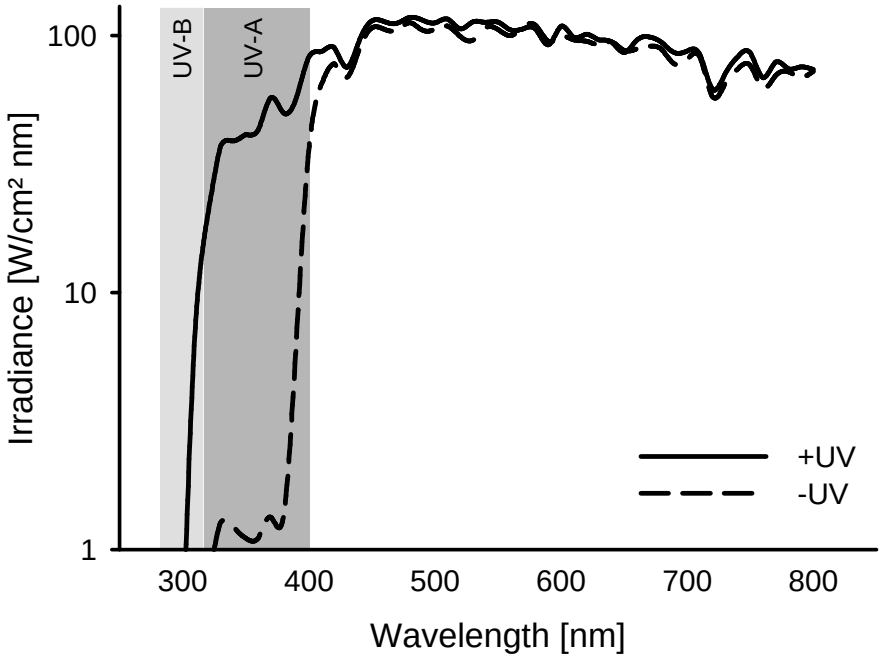

Fig. 2.2.1 Spectral irradiances under the filters (+UV: teflon foil, solid line; –UV: Lee 226 foil, dashed line) used in the experiments. Measurements took place in the early afternoon under cloudless sky. Areas of UV-B (280-315 nm) and UV-A (315-400 nm) wavelengths are highlighted in grey scales. Note the logarithmic y-axis.

In the *early stress experiment*, six plant individuals were grown from germination onwards in each of the twelve tents ("*early stress* plants"). At an age of 27 days (4-5 leaf stage) all plants were surveyed and harvested, to investigate the continuous influence of different exposure conditions on growth parameters, UV shield, plant chemistry and insect infestation levels.

In the *late stress experiment* ("*late stress* plants") 120 plants were transferred at an age of 22 days (4–5 leaf stage) from the climate chamber to the filter tents, to investigate the stress response and adaptability to changing radiation conditions at a later developmental stage (comparable to the stage at harvest of the early stress plants) as well as resulting susceptibility to insects. Ten plants were kept in each tent for further 19 days. Insect infestation was monitored on all plants. Five plants per tent were used for measurements of growth and UV shield (see below). The four youngest leaves of each of the other five plants per tent were sampled at the end of the UV acclimatisation period for chemical analyses (see below). Pest infestation, growth and UV shields were surveyed after 2, 4, 8, 12 and 19 days of exposure. Experiments were performed in September 2006.

2.2.2 Physical and morphological plant parameters and insect infestation

Leaf epidermal UV-A screening capacity (UV shield) was measured with a UV-A-PAM chlorophyll fluorometer (Gademann Meßinstrumente, Würzburg, Germany) at the adaxial leaf sides of individual leaves. Light-emitting diodes generate a quasi-simultaneous excitation of chlorophyll fluorescence at 375 nm [F(UV-A)] and 470 nm [blue- green light, F(BL)] in the UV-A-PAM, and the UV shield (%) is then calculated from 100×[1 – F(UV-A)/F(BL)]. The measured fluorescence excitation ratio relates to the pigment content of phenolic compounds like flavonoids and hydroxycinnamic acids and can be used for a fast, non-destructive assessment of the UV screening capacity of plants (Bilger et al., 1997; 2001). In *early stress* plants UV shield was measured in the oldest leaves at the day of harvest. In *late stress* plants, the second youngest unfolded leaves were labelled at their petioles with small sticky stripes at the beginning of the outdoor exposure, and UV shield was measured from these leaves subsequently after 2, 4, 8, 12 and 19 days.

As plant growth parameters, shoot lengths (from the first to the last nodium) of *early stress* and *late stress* plants were measured with a digital calliper rule (Mitutoyo, Digimatic, Japan) at day of harvest. Leaf width and length at the widest diameter of the youngest unfolded leaves at day of exposure were measured regularly during the exposure period in *late stress* plants to follow changes in relative growth over time under both UV environments (-UV, +UV).

To determine insect infestation levels of plants, numbers of plants infested by insects of different families (Aphididae, Aleyrodidae and Thripidae) were counted per radiation treatment. For thrips, additionally the degree of infestation was ranked using four categories, from 0 (uninfested plants) to 3 (high infestation), because almost every plant was infested with thrips but to various extents.

2.2.3 Chemical analyses of plants

For chemical analyses, above-ground plant material was harvested, frozen in liquid nitrogen and homogenised (mixer mill 301, Retsch, Haan, Germany). All samples were stored at –80 °C until analyses.

To analyse water content, frozen homogenised leaf material was weighed, lyophilised, weighed dry, and water content determined. Carbon and nitrogen content of lyophilised plant samples were measured by quantitative decomposition of substances by oxidative combustion (CHN-O-Rapid, Elementar, Hanau, Germany). As indicator for digestibility reducing compounds for herbivores proteinase (trypsin) inhibitor concentrations were analysed from frozen, homogenised plant material by using a radial diffusion assay following the protocol of Jongsma et al. (1994) and Cipollini and Bergelson (2000).

For determination of flavonoid contents, aliquots of dried plant material were extracted in aqueous 80 % methanol with kaempferol (Extrasynthèse, Genay, France) as internal standard. Chlorophyll was removed by adding petrolether (Fluka, Taufkirchen, Germany) to the extracts and discarding the resulting upper phases. The purified

extracts were analysed by HPLC (1100 Series, Hewlett-Packard, Waldbronn, Germany) with a quaternary pump and a 1040M diode array detector. Gradient separation of flavonoids was achieved on a Supelco C-18 column (Supelcosil LC- 18, 250×4.6 mm, 5 µm, Supelco) with an eluent gradient (solvent A: 0.1 % formic acid in aqua bidest, solvent B: acetonitrile, Rotisol >99.9 %, Roth, Karlsruhe, Germany) of 5–8 % B (10 min), 8–15 % B (5 min), 15-16 % B (10 min), 16–17 % B (10 min), 17–18 % B (5 min), 18–22 % B (10 min), 22 % (5 min hold), 22-27 % (5 min), 27-90 % (7 min), followed by a cleaning cycle. Classification in glycosylated flavonols and hydroxycinnamic acids was done by comparison of retention time and UV spectra to those of purified standards. For quantification response factors were calculated by means of reference samples (1 for flavonoids and 2 for hydroxycinnamic acids). Concentrations of flavonoids and hydroxycinnamic acids were added for statistical analysis.

For determination of glucosinolate contents, the characteristic secondary plant defence compounds of Brassicaceae (Halkier and Gershenzon, 2006), aliquots of dried plant material were extracted in aqueous 80 % methanol with benzyl glucosinolate (Phytoplan, Heidelberg, Germany) as internal standard. Glucosinolates were converted to desulfoglucosinolates using purified sulfatase [E.C. 3.1.6.1, 'type H-1, from *Helix pomatia*, 15,100 units (gram solid)$^{-1}$; Sigma, Taufkirchen, Germany] (purification following Graser et al., 2001). The desulfoglucosinolates were analysed by HPLC and identified by comparison of retention times and UV spectra to those, which had been identified earlier (Müller and Wittstock, 2005; Gigolashvili et al., 2007). Quantification of desulfoglucosinolates was obtained by calculating the peak area at 229 nm (bandwidth 4 nm) relative to the area of the internal standard peak, corrected by the response factors as in Brown et al. (2003) and Müller and Martens (2005). Concentrations of individual glucosinolates were added for further analysis.

2.2.4 Flight behaviour of cabbage whiteflies

During the experimental period, the abundance of cabbage whiteflies (*Aleyrodes proletella* L., Aleyrodidae) was very high, and cabbage whiteflies were mostly attracted to the plants in the +UV exposure tents. Therefore, attraction of naturally occurring whiteflies to the filter tents in absence of any plant cues was investigated using five green sticky traps per tent. Sticky traps consisted of dark green cardboard (21 cm x 14.8 cm, Acco, Schorndorf, Germany) covered with insect glue (Temmen GmbH, Hattersheim, Germany). The cardboard was attached to bamboo sticks (length: 30 cm; Meyer, Rellingen, Germany) and sticks were pinned in pots (diameter: 12 cm, height: 9 cm) filled with floral arrangement mass and sand. Pots were placed in a distance of 15 cm from each other. The experiment was started at noon. After 24 h and 72 h, respectively, numbers of sticky traps with attached cabbage whiteflies were counted per treatment. Climate conditions during the experiment were 5-22 °C, 30-80 % r.h., and mostly cloudless sky.

To investigate the orientation behaviour of cabbage whiteflies to different radiation environments in a dual-choice assay, a further experiment was conducted. Cabbage

whiteflies were collected in the field (Botanical Garden of Würzburg) and reared on savoy (*Brassica oleracea* L. convar. *capitata* (L.) Alef. var. *sabauda* L., Sabrosa F1 Hybrid; Bejo Zaden, Warmenhuizen, Netherlands). The dual-choice arena consisted of two buckets (top diameter: 12.5 cm, bottom diameter: 10.5 cm, height: 13 cm) each covered with a removable filter cap (diameter: 10 cm) from one of the two filter types used also for the exposition filter tents (see above). The two buckets were connected with a white squared cardboard tunnel (length: 13 cm, height: 5 cm) attached at the lower third of the buckets. A hole in the middle of this tunnel served for entering a plastic tube covered with aluminium foil containing ten whiteflies. Five dual-choice arenas were located outside at cloudless sky and an average temperature of 18°C. Filter cap positions were randomised to eliminate effects induced by the position of the filters. The filters were facing directly the sunlight. Cabbage whiteflies were released in the tunnels at 1 pm and 2:15 pm, and after 20 minutes the number of cabbage whiteflies in each cup was counted. In total, 10 observations were carried out.

2.2.5 Statistical analyses

Morphological, physical and chemical parameters of plants of different UV treatments were compared with Student's *t*-tests for independent variables within each experiment. Therefore, data of two to six plants per tent were averaged and these data compared between both treatments with N = 6 per filter tent type. Data of the *early stress* and *late stress* experiments were not directly statistically compared with each other, as plants were of different age at harvest *(early stress* plants: 27 days old, *late stress* plants: 40 days old).

Temporal pattern of relative increase of UV shield, leaf area and shoot length (*late stress experiment*) were tested with repeated measurement ANOVA. Relative data were obtained by dividing the first measured value (straight before outdoor exposure) by the current measured values (at 2, 4, 8, 12 and 19 days after exposure) of each sample. Proportions of all plants and sticky traps of each tent type infested with insects were analysed by Chi^2 tests. Amounts of thrips (in categories) on each plant (*late stress* experiment) per tent type were analysed by a Mann-Whitney *U*-test. Numbers of cabbage whiteflies in the bucket dual-choice experiment were evaluated by a Wilcoxon matched pairs test. Data analysis was performed with Statistica 7.1 (StatSoft, Tulsa, USA).

2.3 Results

2.3.1 UV effects on growth, physical and chemical parameters of plants

UV radiation had significant effects on growth parameters of broccoli plants exposed in the tents from the onset of planting (*early stress*). Shoot length and fresh weight of +UV exposed plants were significantly lower than of –UV exposed plants at time of harvest (Table 2.3.1). In contrast, plants transferred to exposure tents at a later stage (*late stress*)

did not show significant UV treatment dependent differences of leaf area and shoot length at any time point (Table 2.3.1, Table 2.3.2, Fig. 2.3.1).

Plants that germinated in the +UV tents had significantly higher UV shields than plants grown in the –UV tents, whereas UV shields of plants exposed at a later stage to different UV conditions did not differ significantly at any given time point (Table 2.3.1, Table 2.3.2). The latter plants showed UV shields of about 88 % after two days of exposure and reached about 98 % UV shield after an overall shorter exposition time. Time significantly affected relative leaf and shoot growth and UV shield. For relative leaf growth and relative UV shield there was also a significant interaction of time and UV treatment detected (Table 2.3.2).

Overall, leaves of +UV exposed plants had significantly higher total flavonoid and hydroxycinnamic acid concentrations than plants grown under ambient radiation lacking UV, independent of the time of exposure (Table 2.3.1). Thereby, kaempferol glycosides accounted for most of the total concentration of phenolic compounds, followed by quercetin glycosides and hydroxycinnamic acids (data not shown). In contrast, different UV radiation exposure of broccoli plants had no significant effects on water content, carbon/nitrogen content, and trypsin inhibitor concentration, neither in the *early stress* experiment nor in the *late stress* experiment. Six different glucosinolates, two aliphatic and four indolic compounds, were identified in broccoli, with the main glucosinolate being indol-3-ylmethyl glucosinolate. Except for 4-hydroxyindol-3-ylmethyl glucosinolate, which was significantly higher concentrated in leaves of +UV exposed plants in the *early stress* experiment ($T_{(N=6)} = 3.84$, $P = 0.003$, *t*-test), concentrations of all other glucosinolates (data not shown) and total glucosinolate concentrations (Table 2.3.1) were not significantly affected by different UV treatments.

Table 2.3.1 Influence of exposure to different ambient radiation conditions including UV (+UV) or lacking UV (-UV) on morphological, physical and chemical parameters of broccoli shoot tissue (means and standard errors) as well as on insect infestation (percent of infested plants). Plants were either grown from seeds under those conditions and harvested after 27 d (early stress) or first kept under identical low radiation conditions for 22 d and afterwards exposed to different ambient radiation conditions for additional 19 days (late stress). Total glucosinolates (GS) are the sum of 3-methylsulfinylpropyl GS, 4-methylsulfinylbutyl GS, 4-hydroxyindol-3-ylmethyl GS, indol-3-ylmethyl GS, 4-methoxyindol-3-ylmethyl GS and 1-methoxyindol-3-ylmethyl GS; HCA - hydroxycinnamic acids; conc. - concentration. Statistical analyses of growth and analytical data were performed with N = 6 per filter tent type, averaging two to six broccoli plants of each tent. Data were analysed by Student's t-tests for independent variables. Statistical analysis of plant insect infestation was implemented with N = 29 to 32 (early stress) and N = 59 to 60 (late stress) plants per treatment, evaluating the proportion of infested plants per UV treatment with Chi²-tests. Significant P-values are highlighted in bold; a – significant P-values after Bonferroni correction carried out following Benjamini and Hochberg (1995).

Plant parameters	***early stress***						***late stress***					
	+UV		-UV		*t*-test		+UV		-UV		*t*-test	
	Mean	SE	Mean	SE	*T*	*P*	Mean	SE	Mean	SE	*T*	*P*
Shoot length (mm)	30.43	0.79	37.37	1.29	-4.59	**<0.001**[a]	98.77	3.16	96.15	2.01	0.70	0.500
Leaf area (mm²)	-	-	-	-	-	-	12330	438	12714	340	-0.70	0.504
Fresh weight (g)	4.19	0.26	5.33	0.18	-3.58	**0.005**[a]	-	-	-	-	-	-
Water content (%)	89.58	0.21	90.39	0.44	-1.65	0.129	81.22	0.45	81.04	0.53	0.26	0.800
Carbon content (%)	39.72	0.18	39.28	0.53	0.78	0.452	42.42	0.12	42.29	0.21	0.53	0.606
Nitrogen content (%)	3.78	0.14	4.40	0.39	-1.51	0.162	1.81	0.04	1.86	0.08	-0.53	0.602
C/N	11.27	0.28	9.43	0.84	-1.74	0.113	23.58	0.48	23.13	1.02	0.39	0.701
Trypsin inhibitor conc. (nmol/mg protein)	2.56	0.19	2.68	0.29	-0.32	0.754	6.95	0.81	6.13	0.50	0.86	0.408
UV shield (%)	83.46	1.16	71.31	2.26	4.78	**<0.001**[a]	98.34	1.05	97.85	1.36	0.28	0.783
Total flavonoid + HCA conc. (µmol/g DW)	39.07	1.69	25.30	2.10	5.12	**<0.001**[a]	44.80	1.12	37.32	0.66	5.74	**<0.001**[a]
Total glucosinolate conc. (µmol/g DW)	8.76	1.14	8.58	0.26	-0.27	0.795	15.86	0.35	17.01	0.53	-1.80	0.101
Insect infestation	%		%		Chi²	*P*	%		%		Chi²	*P*
Aleyrodidae	37.50		0		13.54	**<0.001**[a]	50.00		15.25		16.3	**<0.001**[a]
Aphididae	25.00		0		8.34	**0.004**[a]	36.66		8.47		13.48	**<0.001**[a]
Thripidae	62.50		68.75		1.27	0.260	91.66		93.22		0.1	0.749

Table 2.3.2 Temporal pattern of the impact of UV radiation on relative UV shield accumulation and relative leaf and shoot growth of broccoli plants during the late stress experiment. Plants were grown under identical low radiation regimes for 22 days, subsequently transferred to different ambient radiation regimes including or attenuating UV-A and B (factor UV treatment), and plant parameters measured after 2, 4, 8, 12 and 19 days (factor Time). Relative data were obtained by dividing the first measured value (straight before outdoor exposure) by the current measured values (at 2, 4, 8, 12 and 19 days after exposure) of each sample. To meet homogeneity of variances UV shield data were transformed ([-1/x]^3). Results of repeated measurement analysis of variance (rmANOVA); d.f.=degree of freedom, MS=mean square. Significant differences are highlighted in bold; treat. – treatment. Measurement data are shown in Fig. 2.3.1.

	UV shield				Relative leaf growth				Relative shoot growth			
	d.f.	MS	F	*P*	d.f.	MS	F	*P*	d.f.	MS	F	*P*
UV treatment	1	0.02	0.08	0.775	1	40.35	2.31	0.134	1	0.13	0.70	0.407
Error	57	0.19			57	17.43			57	0.18		
Time	4	0.41	19.94	**<0.001**	4	133.12	85.51	**<0.001**	4	4.97	711.06	**<0.001**
Time * UV treat.	4	0.11	5.48	**<0.001**	4	3.92	2.52	**0.042**	4	0.01	1.89	0.114
Error	228	0.02			228	1.56			228	0.01		

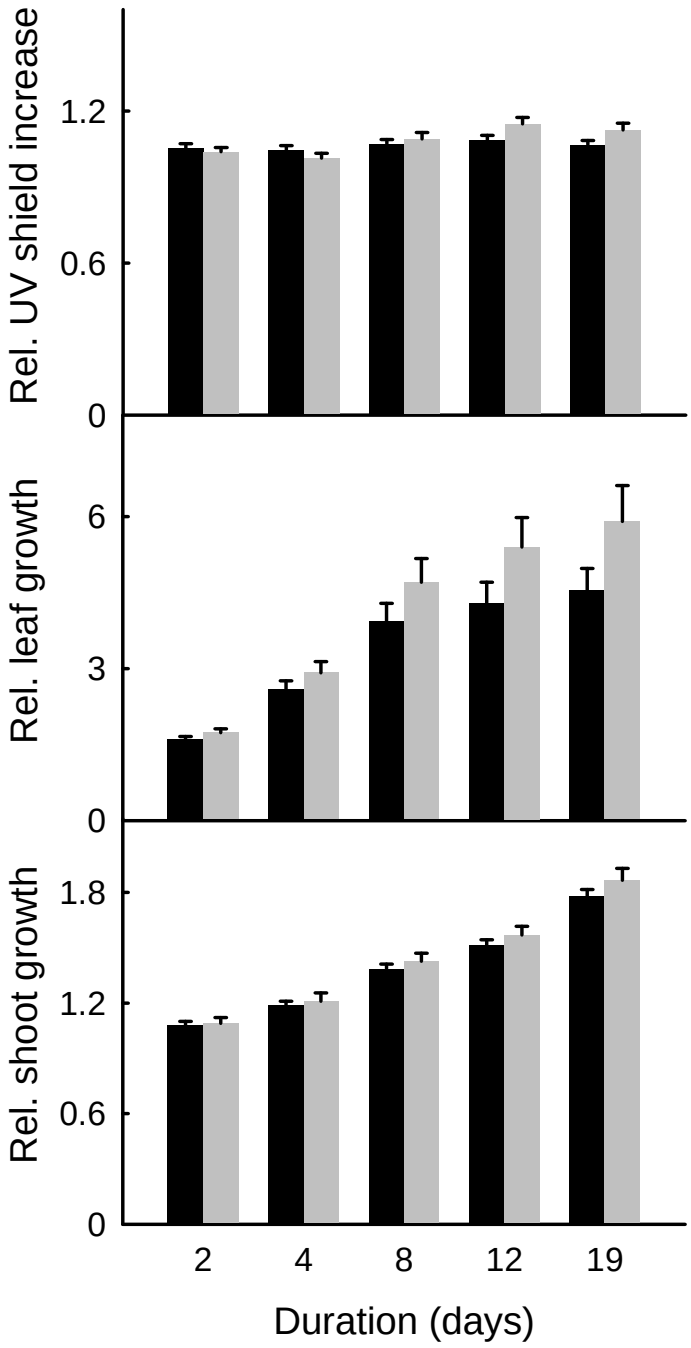

Fig. 2.3.1 Change (mean and standard error) of relative UV shield accumulation and relative leaf and shoot growth of broccoli plants during the late stress experiment. Plants were grown under identical low radiation regimes for 22 days, subsequently transferred to different ambient radiation regimes including UV (+UV, black bars, N=30 plants, pooled from 6 tents) or attenuating UV (-UV, grey bars, N=29), and plant parameters measured after 2, 4, 8, 12 and 19 days. Relative (Rel.) data were obtained by dividing the first measured value (straight before outdoor exposure) by the currently measured values (2, 4, 8, 12 and 19 days after exposure) of each sample. For statistical evaluation see (Table 2.3.2).

2.3.2 Indirect and direct UV effects on insects

The proportion of broccoli plants infested with Aleyrodidae and Aphididae was significantly higher in the +UV exposed plants than in the –UV exposed plants in both experimental approaches at the day of harvest (Table 2.3.1). Differences in infestation of *late stress* plants by these insect taxa were significant from the 12th day onwards and at the 19th day after exposure in +UV and –UV tents, respectively (Fig. 2.3.2). Thripidae did show the reciprocal infestation pattern, with plants of the –UV treatment being significantly more infested already after two days (Fig. 2.3.2). The percentage of thrip infested plants at day of harvest did not differ significantly between the UV treatments neither in the *early stress* nor the *late stress* experiment (Table 2.3.1). At the end of the *late stress* experiment every plant was infested with Thripidae, but significantly more thrips were found on the plants in the –UV tents (U-test, $U_{(N=59\ to\ 60)} = 1053.50$, $P < 0.001$).

Testing the preference response of naturally occurring whiteflies to different radiation conditions in the UV tents did result in a significantly higher catching rate on sticky traps in the +UV tents after one and three days compared to traps in –UV tents (24 hours: $Chi^2_{(N=30)} = 28.71$; $P < 0.001$; 72 hours: $Chi^2_{(N=30)} = 30.09$; $P < 0.001$). *Aleyrodes proletella* tested in the bucket dual-choice experiment also discriminated in favour of the more natural UV radiation and were found in much higher frequencies in the bucket covered with the teflon filter (+UV) (Wilcoxon matched pairs test, $Z_{(N=\ 10)} = 2.80$, $P = 0.005$).

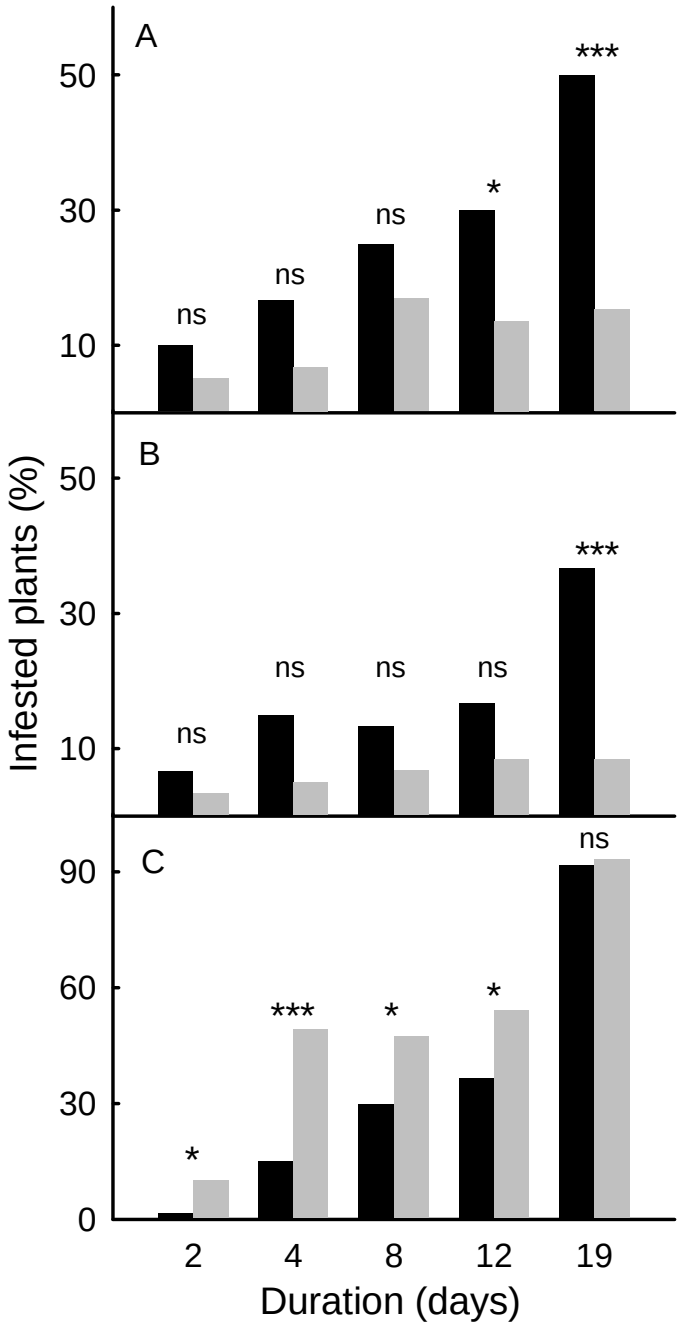

Fig. 2.3.2 Percentage of infested plants in dependence of time and UV treatment with Aleyrodidae (A), Aphididae (B) and Thripidae (C). Broccoli plants were grown under identical low radiation regimes for 22 days, subsequently transferred to different ambient radiation regimes including UV (+UV, black bars, N=60 plants, pooled from 6 tents) or attenuating UV (-UV, grey bars, N=59), and percentage of infested plants was determined at 2, 4, 8, 12 and 19 days after exposure. Infestation rates were compared by Chi^2 tests; * $P < 0.05$, *** $P < 0.001$, ns= not significant. After Bonferroni correction following Benjamini and Hochberg (1995) only $P \leq 0.001$ remain, whereas $P \leq 0.05$ are not significant any more.

2.4 Discussion

Effects of exposure to varying irradiation regimes on chemistry and morphology of broccoli plants highly depended on the developmental stage in which plants were confronted with the different environments (Table 2.3.1). To build up an efficient protection against UV, plants are well known to respond with an induction of flavonoids and hydroxycinnamic acids (Harborne and Williams, 2000; Kolb et al., 2001; Close and McArthur, 2002). However, the need of UV protection by formation of specific metabolites can affect the resource allocation pattern in plants. The growth differentiation hypothesis postulates trade-offs in allocation between growth and defence against herbivorous insects (Herms and Mattson, 1992; Matyssek et al., 2005). This hypothesis can be extended to plant responses to abiotic stressors like UV radiation and might be more applicable for young, growing plants than for mature plants. In broccoli plants, which germinated under ambient UV levels (*early stress*, Table 2.3.1), the increase of flavonoid and hydroxycinnamic acid concentrations was indeed coupled with a slower growth and biomass accumulation compared to plants grown under attenuated UV radiation. In contrast, plants germinated under constantly low radiation and transferred at a developmental stage, where early stress plants were harvested (four to five leaves developed), to different radiation regimes (*late stress*) increased the concentrations of phenolic compounds (measured by HPLC) only, without showing differences in growth pattern under ambient UV. The high UV shields measured with the UV-A-PAM in *late stress* plants even under attenuated UV conditions might be explained by a strong leaf age dependence in the capacity for the synthesis of epidermal UV-A screening compounds (Bilger et al., 2001). Therefore determining phenolic compounds by HPLC is a more accurate method than measuring UV shield with the UV-A-PAM, at least for older plants.

The environment during germination and early growth of plants obviously has a rather formative impact (Gedroc et al., 1996; Sultan, 2000), as became evident also for the broccoli experimental plants. The amount of UV radiation reaching the earth's surface can vary strongly and is highly dynamic (Paul and Gwynn-Jones, 2003), therefore the sensory perception of plants must be quite sensitive and well adapted to enable rapid changes of the phenotypic appearance by acclimatising to the current environment. Not acclimatised plants faced with a changed radiation environment need to react very fast to shield themselves from potentially damaging environmental influences. The need for survival forces both wild as well as crop plants to adapt to those constraints. The fastest response can be implemented by modifications of the chemical composition of the plant, whereas morphological variations of growing plant parts are much more ponderous. Furthermore mature plants have more reserves to compensate environmental constraints in comparison to seedlings. Distinct levels of radiation at the time of seed germination and seedling emergence have been shown to significantly affect plant morphology also in other species such as *Hordeum vulgare* L. (Poaceae), *Datura ferox* L. (Solanaceae), *Cucumis sativus* L. (Cucurbitaceae), and *Phaseolus vulgar*is L. (Fabaceae) (Ballaré et al., 1996; Krizek et al., 1997; Saile-Mark and Tevini, 1997;

Mazza et al., 1999a). In separate experiments with *Lycopersicon esculentum* Mill. (Solanaceae), *Ceratonia siliqua* L. (Fabaceae), *Laurus nobilis* L. (Lauraceae) and *Cistus creticus* L. (Cistaceae), no significant impacts on growth could be detected, when plants were raised under identical conditions and transferred after some weeks to distinct conditions (Grammatikopoulos et al., 1998; Stephanou and Manetas, 1998; Bacci et al., 1999). Our comparative investigations on broccoli prove that this pattern also holds within one plant species.

Little is known about the underlying mechanisms of these acclimation processes. Jansen (2002) postulated that the UV-B induced secondary metabolite induction results in morphogenetic changes as a consequence. Peroxidases might be responsible for formation of UV protection by phenolic compounds and for UV induced morphological changes by lowering IAA levels resulting in reduced cell elongation (Jansen, 2002). Experiments with *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae) revealed that plants change their morphology without showing stress symptoms when acclimatised to low dose rates of UV-B radiation (Hectors et al., 2007). Thus, the authors argued that UV-B responsible morphological changes may be functionally uncoupled from stress responses of plants. Our results show that there must be costs involved in acclimatisation processes of broccoli to UV because of the lower biomass accumulation of broccoli seedlings, when phenolic compounds such as flavonoids and hydroxycinammic acids were induced and maybe also other costly parameters, which we did not measure. But the regulatory effects of UV-B on plants are far-reaching and multiple signalling pathways are entangled (Mackerness, 2000; Stratmann, 2003; Jenkins and Brown, 2007).

It has been discussed that herbivory and UV protection might be induced by the same signalling pathways (Mackerness, 2000; Stratmann, 2003). Therefore plants, which are irradiated with UV should in theory be better defended against this abiotic factor but simultaneously also against herbivorous insects. However, glucosinolates, the characteristic secondary compounds of the Brassicaceae known as feeding deterrents against herbivorous insects (Halkier and Gershenzon, 2006), were not differently accumulated by different radiation exposure of plants. Similar results were also found in other species of Brassicaceae under identical growing conditions using filter tents (Reifenrath and Müller, 2007). Also, the levels of proteinase inhibitors, which interfere with the digestion of food in insects (Broadway and Colvin, 1992), were not affected by UV treatment in broccoli plants, independent of stress onset. The chemical and morphological plant responses we found in broccoli might not only be due to the different radiation regimes but could have been in part also induced by the different insect infestation rates. However, there is evidence that plants can distinguish and respond highly specific to different stresses. Recently it was shown that the non crop species *Nicotiana attenuata* can discriminate between stresses caused by UV-B and herbivorous insects, respectively. One important junction that diverge UV-B and herbivory responses of plants might be the RNA polymerase 2 (RdR2), being specifically involved in UV-response (Pandey and Baldwin, 2008).

Furthermore, the nutritional quality of broccoli for herbivores, which can be expressed by the relation between carbon and nitrogen content (Awmack and Leather, 2002), was not influenced by different environmental conditions. Broccoli plants of the +UV condition were not better protected against herbivorous insects compared to plants under attenuated radiation, although they had higher levels of phenolic compounds. In contrast, whiteflies and aphids from naturally occurring populations, which were able to practise a free host choice, strongly preferred plants grown in +UV tents, whereas thrips preferred the –UV conditions (Table 2.3.1, Fig. 2.3.2). UV avoidance behaviour of thrips was also reported by Mazza et al. (1999b; 2002). These authors were able to prove that Thripidae can perceive UV-B radiation, to which they responded by avoidance, whereas UV-A was attractive for thrips. Other studies have found contradictory results, when studying the behaviour of thrips with regard to UV radiation (Antignus et al., 1996; Costa and Robb, 1999; Díaz et al., 2006). These differences in results might have been caused by different transmission characteristics of the used filtering materials. Whiteflies obviously can distinguish between ambient and attenuated radiation conditions, as they showed an explicit preference for the spaces covered with +UV filters (tents and buckets), despite no host cue was available. The decision of cabbage whiteflies for ambient radiation conditions was rather fast and remained stable for three days. Similar preferences of whitefly behaviour had been observed earlier (Antignus et al., 1996; Costa and Robb, 1999). A preference of aphids for +UV conditions has also been reported (Antignus et al., 1996; Chyzik et al., 2003; Díaz et al., 2006). The attraction of by all three insect groups, Aphididae, Aleyrodidae and Thripidae, to the broccoli plants might have been driven primarily by the quality of the waveband spectrum rather than by plant chemistry.

2.5 Conclusion

The impact of UV radiation on plant growth parameters is highly dependent on the developmental stage of the plant, in which it is exposed to the stress, whereat the chemical composition of plants is more easily modified than plant morphology. The infestation of host plants by herbivores is mainly driven by the insects' visual perception of radiation quality and less by host plant quality. These experiments show that changes of UV radiation on the earth's surface might not only have direct impacts on plants but can also influence insect orientation and foraging behaviour. Such effects need to be considered when concluding from the outcome of laboratory experiments to the natural situation. The insect infestation of horticultural plant species can be strongly influenced by covering materials of greenhouses. Further research will be needed to examine the reasons for different behavioural responses by herbivores to UV radiation quality changes.

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Chapter II

Independent responses to ultraviolet radiation and herbivore attack in broccoli

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Abstract

Plant responses to ultraviolet-B radiation (UV-B) and insect herbivory are believed to be partially similar. In this study, responses to these factors were investigated in the crop species broccoli (*Brassica oleracea* L. convar. *botrytis*, Brassicaceae). Plants were first grown under three UV-B regimes (80%, 23% and 4% transmittance of ambient UV-B) in greenhouses covered with either innovative materials (high and medium transmittance) or conventional glass (low transmittance). Then half of the plants remained under these conditions, the other half were transferred to the field with ambient light and herbivore access for up to three days. Plant responses to distinct environmental conditions were examined by analysing morphological and chemical parameters of plants kept inside and plants exposed in the field. Furthermore, suitability of field-exposed plants to naturally occurring insects was investigated in relation to UV-B pre-treatment. High levels of UV-B radiation led to increased flavonoid concentrations, but to a lower biomass accumulation in broccoli. These patterns remained after outdoor exposure. However, UV-induced changes of plant traits did not alter attractiveness to herbivorous insects: thrips, whiteflies and aphids attacked plants independently of UV-B pre-treatment. A threefold increase of indolyl glucosinolate concentrations occurred in above-ground tissue of all plants most likely due to massive herbivore attack after three days of field exposure. The results show that plants respond with high specificity to different abiotic and biotic impacts, demonstrating separate perception and processing of stress factors.

Key words: Biomass, Brassicaceae, flavonoids, glucosinolates, herbivory, metabolite induction, plant-insect interactions, plant responses, UV-B radiation

3.1 Introduction

Plants are sessile organisms that are exposed to various abiotic and biotic environmental impacts. Plant responses to different environmental stresses such as ultraviolet UV-B (UV-B; 280-315 nm) and herbivory can overlap, measurable for example as gene expression pattern of signalling pathways (Izaguirre *et al.* 2003; Stratmann, 2003). However, plants should have stress-specific mechanisms to adjust to these multi-faceted environmental impacts (Lichtenthaler, 1998; Jansen *et al.*, 2008).

Sunlight is an important abiotic factor that influences various developmental processes in plants. A highly dynamic and energy-rich fraction of the solar spectrum that reaches the earth's surface is UV-B radiation (Paul and Gwynn-Jones, 2003). Depending on the physiological and developmental status of the plant and on the quality and duration of the UV-B exposure, this radiation can cause damage to macromolecules, generate reactive oxygen species, and act as an environmental stressor (Rozema *et al.*, 1997; Jansen *et al.*, 1998; Jenkins and Brown, 2007). UV-B can also function as a signal stimulating developmental processes of plants and promoting plant survival (Ulm and Nagy, 2005; Jenkins and Brown, 2007; Brown and Jenkins, 2008; Safrany *et al.*, 2008). Due to their high plasticity, plants respond with characteristic phenotypic acclimation processes to UV-B such as reduced growth and/or an increased accumulation of phenolic compounds, which act in epidermal cells as sunscreen (Caldwell *et al.*, 2007).

Little is known about UV-induced signalling processes. UV-B-mediated specific photomorphogenetic signalling is distinct from non-specific stress responses (Jenkins and Brown, 2007). However, UV-B-induced signalling pathways and wound-responsive signalling partly overlap (Izaguirre *et al*., 2003; Stratmann, 2003). In response to UV-B-mediated plant changes, reduced insect herbivory on UV-B-irradiated plants compared to non-UV-B-irradiated plants, has been observed in several plant-insect systems (Ballaré *et al.*, 1996; Rousseaux *et al.*, 1998; Rousseaux *et al.*, 2004; Caputo *et al.*, 2006). In addition to these plant-mediated effects, direct effects of UV radiation on insect behaviour can influence plant-insect interactions (Antignus *et al.*, 1996; Costa and Robb, 1999; Kuhlmann and Müller, 2009; Mazza *et al.*, 1999; 2002).

Apart from phenolic metabolites such as flavonoids, which serve as UV-protection, plants produce specific compounds to prevent damage by herbivorous insects. Glucosinolates are the characteristic defence-related secondary metabolites of Brassicaceae. Both flavonoid and glucosinolate induction depend partly on the same signalling pathways, which involve jasmonic acid (Mackerness *et al*., 1999; Textor and Gershenzon, 2009). Glucosinolates are nitrogen- and sulphur-containing metabolites, which are known for their deterrent effects on generalist herbivores, whereas they may stimulate feeding and oviposition by specialists (Halkier and Gershenzon, 2006). Upon insect feeding damage, Brassicaceae often respond with an increase in glucosinolate concentrations (Hopkins *et al.*, 2009; Textor and Gershenzon, 2009).

For several Brassicaceae species, responses to UV-exposure as well as influences on herbivores have been studied (Grant-Petersson and Renwick, 1996; Caputo *et al.*, 2006; Foggo *et al.*, 2007). Broccoli plants (*Brassica oleracea* L. convar. *botrytis*) exposed to ambient UV-A (315-400 nm) and UV-B levels, or grown under reduced levels of UV radiation, only show differences in biomass accumulation when plants experience the different environmental conditions during germination and early growth. Plants germinated under ambient UV radiation levels are smaller. In contrast, plants grown under low-UV and subsequently transferred to different UV-conditions are not affected in growth (Kuhlmann and Müller, 2009). Leaf flavonoid concentrations increase when UV-A and UV-B irradiance has been higher in all Brassicaceae species investigated to date (Reifenrath and Müller, 2007; Kuhlmann and Müller, 2009). In contrast, glucosinolate levels, as well as proteinase inhibitor activities, are unaffected by different irradiance (Reifenrath and Müller, 2007, 2008; Kuhlmann and Müller, 2009), indicating an independent regulation of different defence metabolites. However, plant responses to herbivory in relation to UV-B treatment conditions were not considered in these studies.

Vegetables such as broccoli usually germinate in greenhouses and are transplanted after a few weeks to the field. At this point plants are not adapted to ambient radiation conditions, because conventional greenhouse glasses have zero or low UV-B transmittance. In this study, the effects of UV-B treatment on young broccoli plants grown in greenhouses as well as plant responses after transfer to common field conditions with herbivore access were investigated. For germination and early growth, plants were placed in three differently covered greenhouses, of which two were covered with innovative materials. The cover materials transmitted high, medium (innovative materials) or low levels (conventional glass) of ambient UV-B radiation but had almost equally high levels of UV-A transmittance (Table 3.2.1). Plant biomass as well as defence metabolites (flavonoid and glucosinolate concentrations) and nutritional state (carbon:nitrogen ratio, C/N) were measured from greenhouse-kept plants and from plants that were transferred to the field after different UV-B pre-treatment for up to three days. Furthermore, the suitability of outdoor-transferred plants to naturally occurring herbivorous insects and the insects' impact on plant chemistry were examined in relation to UV-B pre-treatment. It was expected that the plants' defence response to UV and putative herbivores would depend on the radiation quality that plants experienced during the early growing period in the greenhouses. High-UV-B pre-treated plants may be less suitable for herbivores than plants that are exposed to only low UV-B at early growth due to overlapping defence responses that modify the abundance of secondary metabolites. Finally, effects on plant chemistry due to herbivore feeding were expected.

3.2 Material and methods

3.2.1 Plants and design of the experiment

Broccoli plants [*Brassica oleracea* L. convar. *botrytis* (L.) Alef. var. *cymosa* Duch. Monopoly F1 Hybrid; Syngenta Enkhuizen, Netherlands] (N=150) were grown from seeds in three differently covered greenhouses (*UV-B treatment*) from which insects were excluded (greenhouse construction see below; temperature on average 26 °C, humidity on average 60%). Plants were grown in fertilised soil (ED 73, pH 6) in individual pots (diameter: 12 cm, height: 9 cm).

Seventeen days after sowing (plants in four leaf-stage, *0 h*), the above-ground tissue of ten plants from each greenhouse was harvested for biomass determination and chemical analysis. Half of the remaining plants were kept in the greenhouses (N=60), the other half (N=60) were transferred outdoors (*exposure treatment*). Outdoors, plants were randomly positioned on a plane surface in the field, which was covered by a mulch film. The field was 10 m from the greenhouses. Plants were located in two rows with a distance of 30 cm between one another. To evaluate changes over time, ten plants of each condition (greenhouse-kept and outdoor plants of different UV-B radiation (pre)-treatments) were harvested for biomass determination as well as chemical analysis at day 18 (*24 h* after the first harvest and outdoor exposition, respectively) and at day 20 (*72 h* afterwards). Outdoor plants were inspected for insect infestation (see below). During the outdoor exposure time of broccoli, the mean temperature was 17 °C. During the entire growth and experimental period (16 May to 4 June 2007) ambient radiation levels averaged 20 kJ $m^{-2}d^{-1}$ UV-B, 1,390 kJ $m^{-2}d^{-1}$ UV-A, 6,250 kJ $m^{-2}d^{-1}$ PAR (photosynthetic active radiation) and 18,170 kJ $m^{-2}d^{-1}$ global radiation [recorded by a meteorological station (Thies Clima, Göttingen, Germany) located 25 m from the greenhouses]. During the outdoor exposure period radiation averaged 20 kJ $m^{-2}d^{-1}$ UV-B, 1,465 kJ $m^{-2}d^{-1}$ UV-A, 6,270 kJ $m^{-2}d^{-1}$ PAR and 18,500 kJ $m^{-2}d^{-1}$ global radiation.

3.2.2 Construction of greenhouses

Broccoli plants were grown in three experimental greenhouses in the Botanical Garden of Würzburg. These greenhouses had a ground area of 4.2 m × 3.0 m and were covered with different materials, which transmitted distinct ranges of UV-B radiation. Transmittance measurements of greenhouse materials were conducted under cloudless sky at noon with an $X1_2$ Optometer (Gigahertz Optik, Puchheim, Germany; Table 3.2.1). The longer axis of each greenhouse was aligned in a north-south direction. The roof was subdivided into three parts and sloped from 3.9 m in height (north) to 2.0 m in height (south). There were three inclinations of the roof from north to south of 14°, 21.8° and 28.8°. Plants were placed on U-shaped tables (85 cm height) joined to the east, south and west walls of the greenhouses (conception of greenhouses by Gerhard Reisinger, University of Bonn, Germany, construction by Siedenburger Gewächshausbau, Radhen, Germany). One greenhouse was covered with conventional float glass (low UV-B transmission; Siedenburger Gewächshausbau, Radhen,

Germany), the second with CENTROSOL MM solar glass, which was micro-structured on both sides (medium UV-B transmission; Centrosolar Glas, Fürth, Germany), and the third was covered with ethylene-tetrafluorethylene (ETFE) foil (high UV-B transmission; Asahi Glass Green-Tech, USA, China, South Korea, Japan) (Table 3.2.1). Only one greenhouse of each type could be built due to high costs. The greenhouses were closed systems to prevent insect entrance. Air circulation was provided by ventilators (Univent Ventilatoren, Villingen-Schwenningen, Germany). Evaporative cooling was achieved by showering two woven acryl fabric tubes (Schumann, Energieschirm und Schattierungstechnik, Kleinmaischeid, Germany) per house with water when the temperature of the houses exceeded a defined threshold of 23 °C. These were arranged under the east and west arms of the U-shaped tables.

Table 3.2.1 Transmittance (%) of greenhouse covering materials. Fractions of sunlight are classified to UV-B (280-315 nm), UV-A (315-400 nm) and PAR (photosynthetic active radiation, 400-700 nm).

Transmittance	UV-B treatment		
	High UV-B	Medium UV-B	Low UV-B
UV-B (%)	80	23	4
UV-A (%)	87	84	75
PAR (%)	97	95	92

3.2.3 Biomass determination and chemical analyses

The harvested above-ground broccoli plant material was immediately frozen in liquid nitrogen, stored at -80 °C and lyophilised to prevent any enzymatic degradation. Dry weight was determined. For chemical analyses, lyophilised material was homogenised (mixer mill 301, Retsch, Haan, Germany). Carbon and nitrogen content were measured by quantitative decomposition of substances by oxidative combustion (CHN-O-Rapid, Elementar, Hanau, Germany).

For determination of flavonoid aglycones, samples were hydrolysed according to protocols modified from Kolb *et al*. (2001) and Vallejo *et al.* (2004). Aliquots of dried plant material were extracted in aqueous 80 % methanol with the flavonol myricetin (Fluka, Seelze, Germany) as internal standard, and extracts were dried. Dried extracts were re-dissolved in aqueous 80 % methanol, and hydrolysed after addition of an equal volume of 2.5 M HCl for 30 minutes at 85 °C. Hydrolysis was stopped on ice and diethyl ether was added for phase separation. The upper diethyl ether fraction was taken, dried and dissolved in 80% aqueous methanol. These extracts were analysed by HPLC (1100 Series, Hewlett-Packard, Waldbronn, Germany) with a quaternary pump and a 1040M diode array detector. Gradient separation of flavonoid aglycones was achieved on an Agilent Zorbax Bonus RP column (250 mm x 4.6 mm x 5 µm) with an eluent gradient (solvent A: 0.5 % acetic acid in purified water, solvent B: acetonitrile) of 5–50 % B (5 min), 50 % B (5 min hold), 50-95 % B (5 min), 95 % B (5 min hold) followed by a cleaning cycle. Flavonol aglycones were identified by comparison of retention time and UV spectra to those of purified standards (standards from Phytoplan, Heidelberg, Germany and Extrasynthese, Genay, France). Quantification was achieved

by integration of the peak area at 360 nm (bandwidth 4 nm) relative to the area of the internal standard peak, corrected by response factors (0.79 for quercetin, 0.75 for kaempferol, determined by repeated injection of known concentrations of reference samples).

For determination of glucosinolate concentrations, dried plant material was extracted in aqueous 80 % methanol with benzyl glucosinolate (Phytoplan, Heidelberg, Germany) as internal standard. Glucosinolates were converted to desulfoglucosinolates using purified sulfatase [E.C. 3.1.6.1, 'type H-1, from *Helix pomatia*, 15,100 units (gram solid)$^{-1}$; Sigma, Taufkirchen, Germany; purified after Graser *et al.* (2001)]. The desulfoglucosinolates were analysed by HPLC, identified and quantified as had been previously described (Müller and Wittstock, 2005; Gigolashvili *et al.*, 2007).

3.2.4 Insect infestation of outdoor exposed plants

Infestation of plants by naturally occurring insects was recorded by counting the number of infested and uninfested plants (*24 h*) and by counting all insects per plant (*72 h*) on the entire above ground biomass of broccoli plants placed outdoors. At the second time point (*72 h*) all plants were already heavily infested by insects. Insects were determined to family level (Aleyrodidae, Aphididae and Thripidae).

3.2.5 Statistical analysis

Individual potted plants of the three greenhouses were considered as true replicates. Biomass and chemical parameters of 17 day old plants of the *UV-B treatment* group were compared using one-way ANOVA (*G 0 h*). Parameters of plants from the *exposure treatment* that remained either in the greenhouses (*G 72 h*) or were exposed outdoors for 72 hours (*F 72 h*) were compared by MANOVA. Plant parameters were transformed where necessary to reach homogeneity of variances. Proportion of insect-infested plants versus uninfested plants after one day exposure was evaluated by Pearsons Chi². Number of insects per plant and per gram fresh weight, respectively, were analysed after three days of exposure using Kruskal-Wallis analysis of ranks. To calculate the relationship of different chemical parameters and number of insects per plants after outdoor exposure (*72 h*) spearman rank correlations were performed. Data analysis was performed with Statistica 8.0 (StatSoft, Tulsa, USA).

3.3 Results

Broccoli plants grown under different UV-B regimes showed treatment dependent responses. Above-ground biomass accumulation was highest for plants grown under low UV-B conditions and lowest for plants grown under high UV-B irradiation (*UV-B treatment, 0 h*, Table 3.3.1, Fig. 3.3.1). This UV-B related differences in biomass accumulation persisted after 72 hours of common field exposure. Furthermore, biomass was significantly higher in greenhouse kept plants compared to field exposed plants

after 72 hours, probably due to warmer temperatures in the greenhouses (*exposure treatment*, *72 h*, Table 3.3.1, Fig. 3.3.1).

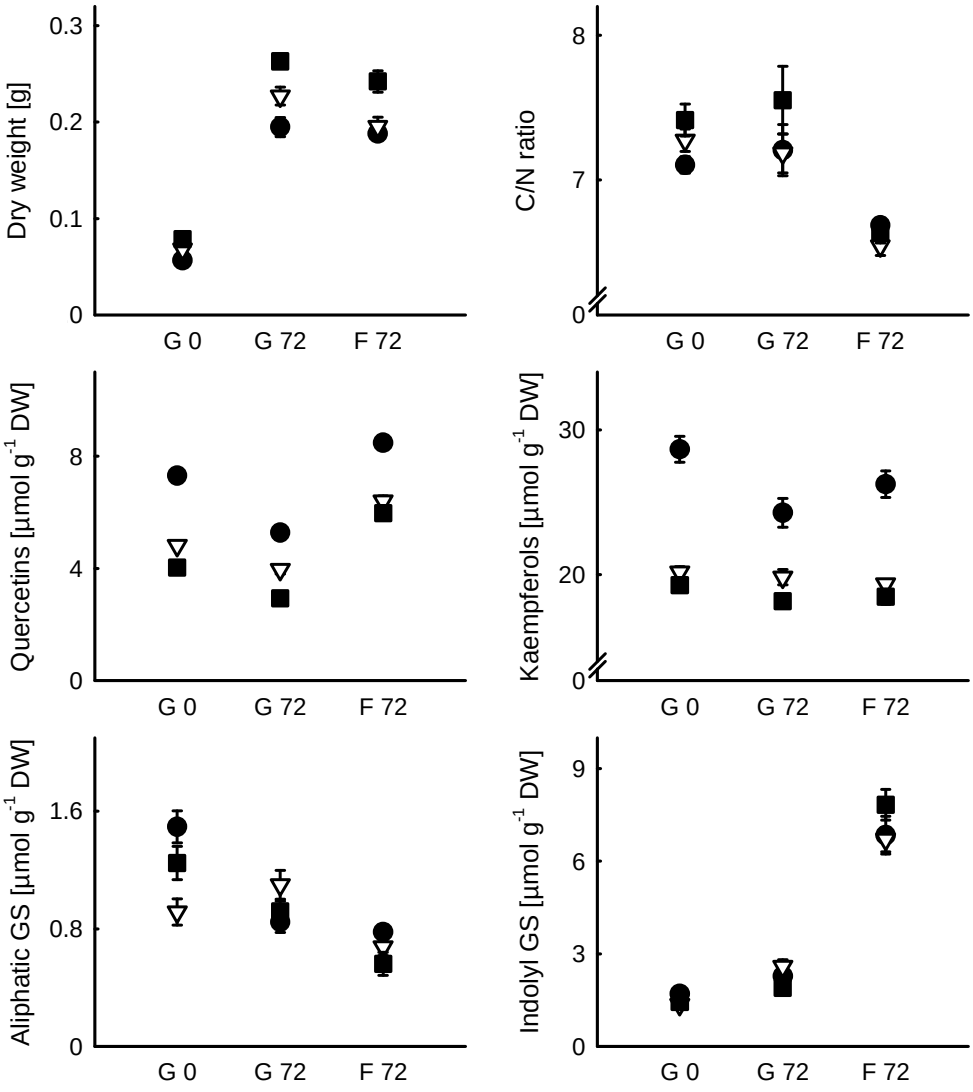

Fig. 3.3.1 Plant parameters (mean ± SE, N=10) of broccoli above-ground tissue grown under different UV-B irradiance and exposure conditions. Plants were grown in greenhouses for 17 days (G 0) with different levels of UV-B irradiation (80 %, 23 % and 4 % transmittance). After 17 days half of the plants from each condition were exposed outdoors for 72 hours (F 72), the other half remained in the greenhouses (G 72). For statistical analyses see Table 3.3.1. Please note the different scales of the y-axes. GS = glucosinolates; DW = dry weight. Filled circles = high UV-B, open triangles = medium UV-B, filled squares = low UV-B.

Table 3.3.1 Impact of *UV-B treatment* (0 h, 72 h) and *exposure treatment* (72 h) on growth and chemical parameters of above ground tissue of broccoli plants. Treatment effects were analysed by one-way ANOVA (0 h) and MANOVA (72 h). Plants were grown in greenhouses with different levels of UV-B irradiation (*UV-B treatment*, 80 %, 23 % and 4 % UV-B transmittance). After 17 days, half of the plants from each UV-B condition were kept in the greenhouses, whereas the other half were exposed outdoors for 72 hours (*exposure treatment*). GS = glucosinolates; DW = dry weight. Boldface indicates P<0.05. Asterisks denote significant P-values after Bonferroni correction carried out following Benjamini and Hochberg (1995) for each time of harvest. Some plant parameters were transformed to reach homogeneity of variances [*0 h*: quercetin ($x^{1/2}$); *72 h*: C/N ratio ($1\ x^{-5}$), indolyl GS ($x^{1/3}$), kaempferol (log x)]. Measured data are shown in Fig. 3.3.1.

Plant parameters	***0 h*** ANOVA		***72 h*** MANOVA					
	UV-B treatment		UV-B treatment		Exposure treatment		UV-B treatment x exposure treatment	
	$F_{(2;27)}$	*P*	$F_{(2;53)}$	*P*	$F_{(1;53)}$	*P*	$F_{(2;53)}$	*P*
Dry weight [g]	11.09	**<0.001***	24.02	**<0.001***	7.20	**0.010***	0.94	0.396
C/N ratio	3.38	**0.049**	1.15	0.324	50.23	**<0.001***	0.98	0.384
Quercetins [µmol g^{-1} DW]	184.48	**<0.001***	106.40	**<0.001***	438.60	**<0.001***	2.71	0.076
Kaempferols [µmol g^{-1} DW]	72.58	**<0.001***	63.17	**<0.001***	1.09	0.301	1.57	0.218
Aliphatic GS [µmol g^{-1} DW]	7.87	**0.002***	2.07	0.137	22.74	**<0.001***	3.34	**0.043**
Indolyl GS [µmol g^{-1} DW]	2.28	0.122	0.27	0.761	261.99	**<0.001***	3.27	**0.046**

The C/N ratio of plants grown under high UV-B was significantly lower than that of plants grown under low UV-B greenhouse conditions (*UV-B treatment, 0 h*, Table 3.3.1, Fig. 3.3.1). After 72 hours of field exposure, the C/N ratio of all outside exposed plants dropped significantly (*exposure treatment, 72 h*, Table 3.3.1, Fig. 3.3.1) due to a relative increase in nitrogen per dry weight compared to greenhouse kept plants. The total nitrogen content per plant was not significantly different between greenhouse kept and field exposed plants (*exposure treatment, 72 h*, MANOVA for total nitrogen content per plant: $F_{(2;51)} = 0.026$; $P = 0.873$).

Plants grown under high UV-B conditions had higher quercetin and kaempferol concentrations (*UV-B treatment, 0 h*, Table 3.3.1, Fig. 3.3.1) as well as a significantly higher quercetin/kaempferol ratio than medium and low UV-B exposed greenhouse plants (*UV-B treatment, 0 h*, one-way ANOVA for quercetin/kaempferol ratio: $F_{(2;27)} = 28.60$; $P < 0.001$). Differences in quercetin and kaempferol flavonoid concentration were still present after 72 hours field exposure, with plants taken from the high UV-B treatment showing significantly higher flavonoid concentrations than plants from the other two treatments (*UV-B treatment, 72 h*, Table 3.3.1, Fig. 3.3.1). All plants exposed outdoors had significantly increased quercetin concentrations compared to greenhouse-kept plants after 72 hours, whereas kaempferol concentrations did not change significantly (*exposure treatment, 72 h*, Table 3.3.1, Fig. 3.3.1). The differences in the quercetin/kaempferol ratio diminished after 72 hours of field exposure, probably due to a higher relative increase of quercetin flavonols in medium- and low-UV-B pre-treated plants compared to high-UV-B pre-treated plants (*UV-B treatment, F 72 h*, one-way ANOVA of outdoor-exposed plants from different pre-treatments: $F_{(2;27)} = 0.33$; $P = 0.725$). In general, kaempferol concentrations were over three times higher than quercetin concentrations in all plants (Fig. 3.3.1).

In this experiment, only one greenhouse of each type could be used, therefore one might consider the individual potted plants only as pseudoreplicates. However, in repeated experiments broccoli always responded in a similar fashion with reduced growth and increased flavonoid concentrations when grown under high UV-B conditions (Kuhlmann, unpublished).

The *UV-B treatment* had a significant effect on the concentration of aliphatic glucosinolates but was not directly related to the UV-B radiation levels plants received in the greenhouses (*0 h*, Table 3.3.1). Plants of the medium-UV-B treatment showed the lowest concentrations of aliphatic glucosinolates (Fig. 3.3.1). After outdoor exposure, no consistent effects could be detected for these metabolites. Overall, aliphatic glucosinolate concentrations were low (*72 h*, Fig. 3.3.1). Concentrations of indolyl glucosinolates were unaffected by *UV-B treatment* (*0 h*, Table 3.3.1, Fig. 3.3.1). In contrast, the *exposure treatment* had a significant effect on glucosinolate accumulation (*72 h*, Table 3.3.1). Field exposure of plants resulted in a threefold induction of indolyl glucosinolates after 72 hours, up to about 8 µmol g^{-1} dry weight (Fig. 3.3.1). After 24 h field exposure the total and indolyl glucosinolate concentrations were on average still

low with 2.6 and 1.8 µmol g^{-1} dry weight, respectively (compare with glucosinolate concentrations in Fig. 3.3.1).

Plant reactions to 72 hour outdoor exposure were independent of the pre-treatment in the different greenhouses, except for aliphatic and indolyl glucosinolates, for which a significant interaction between *UV-B treatment x exposure treatment* was detectable (*72 h*, Table 3.3.1, Fig. 3.3.1).

Plants grown under different UV-B regimes and exposed for 24 h or 72 h in the field were not significantly differently infested by insects (*24 h*, number of infested versus uninfested plants: Pearsons $Chi^2_{(N=10)} = 4.29$, $P = 0.117$; *72 h*, number of insects per plant and per unit biomass see Table 3.3.2). The plants were mainly infested by Thripidae.

After 72 hours of outdoor exposure, total glucosinolate amount per plant was significantly positively correlated with the number of insects found on each plant ($R_{(N=29)} = 0.45$; $P = 0.013$), but a relationship between total flavonoid amount and total insect infestation per plant was not observed ($R_{(N=29)} = 0.005$; $P = 0.979$). Total glucosinolate concentrations did not correlate with total flavonoid concentrations ($R_{(N=29)} = -0.24$; $P = 0.216$).

Table 3.3.2 Infestation of broccoli plants (mean ± SE number of insects per plant and per gram fresh weight, FW, N=10 plants) after 72 hours of field exposure by Aleyrodidae, Aphididae, and Thripidae. Plants were grown in greenhouses with different levels of UV-B transmittance (80 %, 23 % and 4 %) before field exposure. Statistical analysis was performed using Kruskal-Wallis analysis of ranks.

	Plant pre-treatment				
Insect families	High UV-B	Medium UV-B	Low UV-B	$H_{(2, N= 30)}$	P
Aleyrodidae $plant^{-1}$	3.3 ± 1.1	2.2 ± 0.7	2.5 ± 0.5	0.51	0.776
Aphididae $plant^{-1}$	0.2 ± 0.1	3.5 ± 2.0	2.5 ± 1.1	4.63	0.099
Thripidae $plant^{-1}$	22.0 ± 3.3	21.0 ± 2.5	22.5 ± 2.7	0.09	0.955
Aleyrodidae g^{-1} FW	2.2 ± 0.7	1.4 ± 0.5	1.3 ± 0.4	0.76	0.684
Aphididae g^{-1} FW	0.1 ± 0.1	2.6 ± 1.5	1.0 ± 0.6	2.67	0.264
Thripidae g^{-1} FW	14.7 ± 2.1	13.1 ± 1.2	12.4 ± 1.4	0.87	0.649

3.4 Discussion

Plants are able to recognise and respond to their surrounding environment with high specificity. Broccoli plants grown under different UV-B irradiance in greenhouses covered with innovative materials and later transferred to common field conditions with unrestricted herbivore access, showed increases in flavonoid concentrations and reduced growth, which were related to UV-B. Whereas these traits changed over time, the pattern (flavonoid concentration and growth differences between plants of different UV-B conditions) remained the same. In contrast, an increase of glucosinolate concentrations was induced most likely by herbivore attack in the field. UV-B pre-

treatment did not influence the plant susceptibility and attractiveness to naturally occurring insect herbivores.

A protection against potentially detrimental UV-B radiation is generally achieved by an induction of phenolic compounds (Caldwell *et al.*, 1983). In accordance with this, the concentration of quercetin and kaempferol flavonols in broccoli plants was positively related to the irradiance the plants faced in the differently covered greenhouses. However, the production of UV-B-screening pigments is likely to involve some constraints, as the accumulation of biomass was reduced in plants confronted with higher UV-B irradiance (Table 3.3.1, Fig. 3.3.1). A negative relationship between biomass and flavonoid concentration has also been observed in earlier experiments with broccoli that received different irradiances of UV-A and UV-B from germination onwards (Kuhlmann and Müller, 2009). From the current results, it can be concluded that the increased flavonoid concentration at reduced growth is predominantly caused by UV-B, whereas UV-A plays a subordinate role. Smaller plants may have experienced reduced cell expansion due to higher UV-B irradiance as has been proposed by previous studies (Rozema *et al.*, 1997; Jansen *et al.*, 1998; Jansen, 2002; Hectors *et al.*, 2007). It remains to be seen if flavonoid induction and biomass reduction are directly or indirectly linked, and whether those phenotypic changes in plants are typical responses to UV-B. Morphogenetic changes in *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae) exposed to low dose rates of UV-B radiation without stress were interpreted as redistribution rather than cessation in plants (Hectors *et al.*, 2007).

Kaempferol and quercetin flavonols responded differently in broccoli plants. Though kaempferol flavonol concentrations were higher in all plants, the quercetin/kaempferol ratio was highest in high UV-B exposed plants (Fig. 3.3.1). A relatively higher increase of quercetin compared to kaempferol flavonol concentrations in UV-exposed plants has also been found in earlier studies (Markham *et al.*, 1998; Olsson *et al.*, 1998; Hofmann *et al.*, 2003; Reifenrath and Müller, 2007; Winter and Rostás, 2008). It has been suggested that quercetin flavonols have a better ability for free radical scavenging than kaempferol flavonols (Harborne and Williams, 2000).

Besides the flavonoid concentration, the C/N ratio was influenced by UV-B treatment. However, after 72 hours of outdoor exposure differences in C/N ratio disappeared between differently pre-treated plants and field exposed plants had higher nitrogen concentrations than greenhouse-kept plants (Table 3.3.1, Fig. 3.3.1). This may be due to growth and allocation changes. Both, flavonoid levels and C/N ratio are known to be important factors influencing herbivore nutrition (Harborne and Williams, 2000; Awmack and Leather, 2002; Treutter, 2005). Despite these UV-B-induced changes of plant quality no significant differences in insect infestation of field-exposed plants could be observed (Table 3.3.2). Thus, flavonoids primarily acted as sunscreen but not as defence against herbivores (Close and McArthur, 2002; Izaguirre *et al.*, 2007). Broccoli plants attracted mainly thrips, which feed on cell content, followed by whiteflies and aphids, which are phloem-feeding herbivores. Whether phloem sap constitution varies between plants exposed to different levels of UV-B needs investigation. Leaf-chewing

insects were not abundant on the broccoli plants, but they might respond differently and be able to discriminate between plants of different UV-B pre-treatments. Contrasting results have been found for effects of plant UV-treatment on leaf chewers. Chewing herbivores are either deterred by high UV-exposed plants (Ballaré *et al.*, 1996; Caputo *et al.*, 2006; Foggo *et al.*, 2007) or not affected by UV treatment (Reifenrath and Müller, 2009).

The characteristic defence metabolites of Brassicaceae, glucosinolates, were mostly unaffected by UV-B. This has also been shown earlier for the combined effects of UV-A and UV-B on various species of Brassicaceae (Reifenrath and Müller, 2007; Kuhlmann and Müller, 2009). The low constitutive concentrations of glucosinolates in all greenhouse-grown broccoli plants may have been primarily responsible for the similar insect infestation patterns after outdoor exposure despite different UV-B pre-treatment. Most likely, insect infestation led to a strong induction of glucosinolates in all broccoli plants. Even though plants were already infested with insects after 24 hours, the threefold induction of indolyl glucosinolates could only be detected at 72 hours after field exposure and insect infestation (Table 3.3.1, Fig. 3.3.1). The total glucosinolate amounts per plant, mainly represented by indolyl glucosinolates, correlated with the number of attacking insect individuals per plant at the final harvest. Different abiotic factors, such as temperature or wind, may have additionally influenced the metabolite changes in field-exposed broccoli plants. A high induction of indolyl glucosinolates by herbivorous insects, i.e. leaf chewers, has already been described for several Brassicaceae (Textor and Gershenzon, 2009). Thrips were the most abundant herbivores on broccoli, therefore indolyl glucosinolate production was probably largely caused by these insects. However, the combination of multiple insect attacks may also have strong induction potential. Recently it was shown that thrip feeding induces an increase in jasmonate levels in *A. thaliana* (Abe *et al.*, 2008). Jasmonate is an important signalling hormone, also leading to the induction of indolyl glucosinolates in various Brassicaceae (Textor and Gershenzon, 2009; van Dam *et al.*, 2009). However, the effects of thrips on glucosinolate concentrations have never been directly investigated.

Flavonoid amounts per plant were not correlated with insect infestation and flavonoid concentrations were not correlated with glucosinolate concentrations. Broccoli thus responded specifically to UV-B with an induction of flavonoids and to insect infestation and outdoor-exposure with an induction of glucosinolates, showing separate stimulus-specific responses. Earlier studies reported an overlap in gene-expression due to UV-B and herbivore feeding (Izaguirre et al., 2003), but plants are obviously able to distinguish between impact by UV-B radiation and stress by herbivorous insects (Pandey and Baldwin, 2008). More studies are needed to disentangle plant responses to different environmental impacts on a molecular and a metabolite level.

Increases of defence metabolites might also increase the plant quality for human nutrition. Flavonoids, as well as aliphatic and indolyl glucosinolates and their hydrolysis products of broccoli, potentially have important benefits for human health due to their anti-inflammatory and anti-tumorigenic properties (Gomes *et al.*, 2008; Jeffery and

Araya, 2009). Growth of plants in innovative greenhouses transmitting higher UV-B radiation levels can increase the plant quality with regard to flavonoid concentrations, whereas these UV-B conditions will not improve glucosinolate quantities. Higher UV-B radiation during early plant growth did not forearm broccoli plants against the attack of various herbivorous insects when transferred to outdoor conditions. Longer field exposure of plants that received high UV-B at early growth may, however, reveal other values for these plants.

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Chapter III

UV-B impact on aphid performance mediated by plant quality and plant changes induced by aphids

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Abstract

Plants face various abiotic and biotic environmental factors and therefore need to adjust their phenotypic traits on several levels. UV-B radiation is believed to impact herbivorous insects via host plant changes. Plant responses to abiotic challenges (ultraviolet-B radiation) and their interaction with two aphid species were explored in a multifactor approach. Broccoli plants (*Brassica oleracea* L. convar. *botrytis* (L.), Brassicaceae) were grown in two differently covered greenhouses, transmitting either 80 % of ambient UV-B (*high UV-B*) or 4 % (*low UV-B*). Three-week-old plants were infested with either specialist cabbage aphids (*Brevicoryne brassicae* (L.), Aphididae, Sternorrhyncha) or generalist green peach aphids (*Myzus persicae* (Sulzer), Aphididae, Sternorrhyncha). Plants grown under high UV-B intensities were smaller and had higher flavonoid concentrations. Furthermore, these plants had reduced cuticular wax coverage, whereas the amino acid concentrations of the phloem sap were only little influenced by different UV-B intensities. Cabbage aphids reproduced less on plants grown under high UV-B than on plants grown under low UV-B, whereas green peach aphids reproduced on both plant sources equally little, demonstrating different sensitivities of species, which depend on their diet specialisation and feeding mode. Aphids also affected plant chemistry to some extent. The high infestation by cabbage aphids on low-UV-B plants led to decreased indolyl glucosinolate concentrations. The induced change of these glucosinolates might depend on a certain infestation threshold. UV-B radiation considerably impacts plant traits with effects on specialist phloem feeding aphids, whereas aphid growth forces broccoli plants to generate specific defence responses.

Keywords: Brassicaceae; cuticular waxes; flavonoids; glucosinolates; plant-aphid interactions; phloem-amino acids; UV-B radiation

4.1 Introduction

Sunlight provides the energy plants require for all metabolic processes. Ultraviolet-B radiation (UV-B, 280-315 nm) is the most energetic fraction of the sunlight, which is mainly absorbed by the ozone layer (Paul & Gwynn-Jones 2003; McKenzie *et al.* 2007). The intensity of UV-B that reaches the earth's surface depends on a variety of environmental factors. UV-B radiation can potentially destruct DNA, proteins, lipids and membranes of organisms and can cause reactive oxygen species generation. In response, plants have evolved various mechanisms for UV-protection and repair. Therefore UV-B radiation is also an environmental signal that influences plant development, morphology and chemical constitution (Rozema *et al.* 1997; Jansen *et al.* 1998; Jenkins & Brown 2007). The mechanisms of UV-B perception and signal transduction are poorly understood, but it is well known that plants respond to UV-B radiation with a reduced growth and an increased accumulation of phenolic compounds in the epidermal cells (Caldwell *et al.* 2007; Jenkins & Brown 2007). However, a multifactor investigation of UV-B-induced changes of various plant responses is lacking.

Plants are not only exposed to abiotic factors such as UV radiation but also to biotic impacts such as herbivorous insects. Several investigations of plant-insect relationships on differently UV-exposed plants recorded that UV-B irradiated plants were to a lesser extent damaged by insect herbivores than non UV-B irradiated plants (Ballaré *et al.* 1996; Zavala *et al.* 2001; Rousseaux *et al.* 2004). The surrounding environmental conditions provoke plants to build up a repertoire of efficient self-protection. The first interface boundary between plants and their environment is the cuticle, which is coated with a wax layer that protects plants against abiotic and biotic harms (Müller & Riederer 2005). The cuticular wax layer provides UV protection by absorptive and non-absorptive optical properties (Long *et al.* 2003; Pfündel *et al.* 2006) and can mediate resistance against herbivores (Müller 2008). Plant interior defence barriers are comprised of ubiquitous compounds such as flavonoids for UV and herbivore protection (Harborne & Williams 2000; Close & McArthur 2002; Treutter 2005), and of taxon-specific metabolites such as glucosinolates in Brassicaceae. The latter do not act as UV shield but are deterrent to generalist herbivorous insects and pathogens, whereas specialists are stimulated by these compounds (Hopkins *et al.* 2009). Herbivore damage and pathogen infestation provoke the enzymatic hydrolysis of glucosinolates by myrosinases, which enhances the repulsive or attracting effect of those secondary metabolites (Bednarek *et al.* 2009; Clay *et al.* 2009; Textor & Gershenzon 2009). UV radiation induces the accumulation of flavonoids in plants, whereas no UV-effects on glucosinolate concentrations could be found in Brassicaceae (Reifenrath & Müller 2007; Kuhlmann & Müller 2009).

Aphids are serious pest insects of many cultivated and wild plants, including Brassicaceae (Powell *et al.* 2006; Goggin 2007). It is likely that aphids are rather exposed to intact glucosinolates than to hydrolysis products, because they cause only

slight tissue damage (Kim *et al.* 2008). Glucosinolates can reduce fecundity of the generalist green peach aphid *Myzus persicae* to some degree (Kim & Jander 2007) but stimulate feeding of the specialised cabbage aphid *Brevicoryne brassicae* (Yusuf & Collins 1998), which derive a benefit from sequestering glucosinolates to protect themselves against enemies (Bridges *et al.* 2002; Kazana *et al.* 2007). Aphids contact the upper leaf surface first, when approaching a plant. The wax coverage, the texture and the chemical constitution of the plant, which is tested by brief intracellular probes, determine, whether the plant is a suitable host. If the plant is suitable, the aphids move to the lower leaf surface (Powell *et al.* 2006). Aphid growth and reproduction mainly depend on the total amount and quality of phloem-derived amino acids as nitrogen source (Douglas 2006). Additionally a cue-based control of aphid reproduction is discussed, because aphids will not settle, feed and reproduce, when appropriate cues are lacking (Thompson 1963; Powell *et al.* 2006). Nothing is known about plant mediated UV influences on aphids and possible effects of UV-B radiation intensities on amino acid composition and wax coverage of Brassicaceae host plants.

Next to influences of plant chemistry on aphids, aphid feeding can also induce changes in host plant quality. Aphids effect their host plants by injection of salvia and removal of assimilates (Goggin 2007) and can cause an induction or reduction of glucosinolate concentrations, as shown, for example, in *Arabidopsis thaliana* Heynh. (Mewis *et al.* 2005; 2006; Kim & Jander 2007; Kuśnierczyk *et al.* 2008).

This study aimed to analyse the effects of UV-B radiation on a Brassicaceae crop plant, broccoli (*Brassica oleracea*), in a multifactor approach and to study the consequences of plant chemistry changes on aphid performance as well as the consequences of aphid feeding to plants. Broccoli plants were grown in two differently covered greenhouses, transmitting either high or low levels of UV-B. Multiple plant traits and the reproduction of the generalist *M. persicae* and the specialist *B. brassicae* were measured after five days of aphid infestation in three-week-old plants. Moreover, effects of aphid feeding on plant secondary chemistry were considered. It was expected that UV-B influences aphid population growth by plant alterations in dependence of the species specialisation and that plant chemistry is modified differently by abiotic and biotic impacts.

4.2 Materials and Methods

4.2.1 Plant growth and aphid breeding

Broccoli seeds [*Brassica oleracea* L. convar. *botrytis* (L.) Alef. var. *cymosa* Duch. Monopoly F1 Hybrid; Syngenta Enkhuizen, Netherlands] germinated in fertilised soil (ED 73, pH 6) in individual pots (diameter: 12 cm, height: 9 cm) in two differently covered greenhouses. The greenhouses were located in the Botanical Garden of Würzburg (conception of greenhouses by Gerhard Reisinger, University of Bonn, Germany, construction by Siedenburger Gewächshausbau, Radhen, Germany) in a distance of 10.2 m (for details see also Kuhlmann & Müller in press). One greenhouse

was covered with conventional float glass (*low UV-B*; about 4 % UV-transmittance) (Siedenburger Gewächshausbau, Radhen, Germany) and the other with ethylene-tetrafluorethylene (ETFE) foil (*high UV-B*; about 80 % UV-transmittance) (Asahi Glass Green-Tech, USA, China, South Korea, Japan) (Fig. 4.2.1). The ground area of the greenhouses was 4.2 x 3.0 m with the longer axis aligned in north-south direction. The trisected roof sloped from 3.9 (north) to 2.0 m (south) and had three inclinations (from north to south: 14°; 21.8°; 28.8°). Plants were grown on U-shaped tables (85 cm height) that were adjoined to the east, south and west walls of the greenhouses. The greenhouses were closed systems to impede insect entrance. Air circulation was achieved by ventilators (Univent Ventilatoren, Villingen-Schwenningen, Germany). Two woven acryl fabric tubes per house (Schumann, Energieschirm und Schattierungstechnik, Kleinmaischeid, Germany), which were arranged under the U-shaped tables, were showered with water for cooling, when the greenhouse temperature exceeded a defined threshold of 23 °C. Plants were exposed to the natural day light cycle.

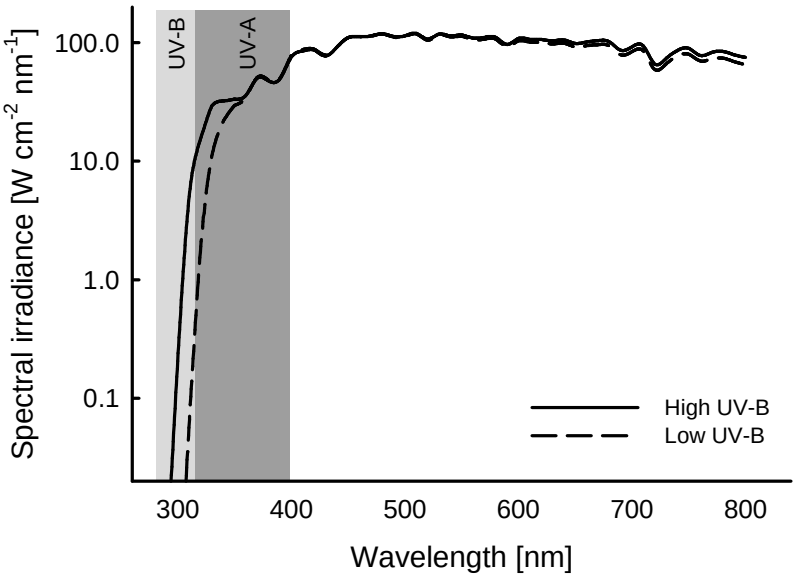

Fig. 4.2.1 Spectral irradiance in the two greenhouses (high UV-B: ETFE foil, solid line; low UV-B: float glass, dashed line). Radiation measurements were investigated in the early afternoon under cloudless sky with an UV-visible spectroradiometer (OL 754, Optronic Laboratories, Orlando, USA). Highlighted grey scales indicate UV-B (280- 315 nm) and UV-A (315- 400 nm) wave bands. Note the logarithmic y-axis.

Green peach aphids (*Myzus persicae* (Sulzer), Aphididae, Sternorrhyncha) and cabbage aphids (*Brevicoryne brassicae* (L.), Aphididae, Sternorrhyncha) were raised in the laboratory on savoy (*Brassica oleracea* L. convar. *capitata* (L.) Alef. Var. *sabauda* L., Sabrosa F1 Hybrid; Bejo Zaden, Warmenhuizen, Netherlands) at 21 °C and a 16:8 h light:dark cycle in bugdorms (60 x 60 x 60 cm, made of polyester netting and vinyl, MegaView Science Education Services Co., Taiwan).

4.2.2 Aphid infestation experiment

To investigate the interactions between plants and aphids under different UV-B conditions, plants (20 days old, five-leaf stage) in both greenhouses were each infested either with 10 individuals of *B. brassicae* (N=20 plants) or with 10 individuals of *M. persicae* (N=20) or were kept uninfested as control (N=10). Wingless (apterous) aphids (adults and fourth nymph stage) were placed with a soft paintbrush on the adaxial side of the second-oldest leaves. Within treatment, plants were placed at a distance of 15 cm from each other. Five days later, aphids were removed and numbers counted. The leaf area of the second-oldest leaf of all plants was measured with a digital calliper rule (Mitutoyo, Digimatic, Japan). The above-ground tissue of aphid-infested and uninfested control plants was harvested, immediately transferred in liquid nitrogen and stored at -80 °C. Ambient light conditions during the experimental time period (11 April to 5 May 2007) were on average 19 kJ m^{-2} d^{-1} UV-B; 1,490 kJ m^{-2} d^{-1} UV-A; 6,871 kJ m^{-2} d^{-1} PAR (photosynthetically active radiation) and 21,059 kJ m^{-2} d^{-1} global radiation [recorded in 25 m distance to the greenhouses by a meteorological station (Thies Clima, Göttingen, Germany)].

4.2.3 Biomass determination and chemical analyses

The harvested above-ground broccoli plant material was lyophilised and dry weight was determined. For chemical analyses dried material was homogenised (mixer mill 301, Retsch, Haan, Germany) and aliquots taken for investigation of flavonoid and glucosinolate concentrations.

4.2.4 Flavonoids

For determination of flavonoid aglycones samples were hydrolysed according to the protocol described by Kuhlmann & Müller (in press). Dried plant material was extracted in aqueous 80 % methanol with myricetin (Fluka, Seelze, Germany) as internal standard. Thereafter extracts were dried and re-dissolved in aqueous 80 % methanol. After addition of an equal volume of 2.5 M HCl hydrolysis was conducted for 30 minutes at 85°C and was stopped on ice. Diethyl ether was used for solvent extraction of hydrolysed samples. The upper diethyl ether fraction was separated, dried, and dissolved in 80% aqueous methanol. These extracts were analysed by HPLC (1100 Series, Hewlett-Packard, Waldbronn, Germany) with a quaternary pump and a 1040M diode array detector. Gradient separation of flavonoid aglycones was achieved on an Agilent Zorbax Bonus RP column (250 mm x 4.6 mm x 5 µm) with an eluent gradient (solvent A: 0.5 % acetic acid in bidest water, solvent B: acetonitrile) of 5–50 % B (5 min), 50 % B (5 min hold), 50-95 % B (5 min), 95 % B (5 min hold) followed by a cleaning cycle. Flavonol aglycones were identified by comparison of retention time and UV spectra to those of standards (kaempferol from Extrasynthese, Genay, France and quercetin from Phytoplan, Heidelberg, Germany). Quantification was obtained by integration of the peak area at 360 nm (bandwidth 4 nm) relative to the area of the

internal standard peak, corrected by the calculated response factors (0.79 for quercetin, 0.75 for kaempferol).

4.2.5 Glucosinolates

For determination of glucosinolate concentrations dried plant material was extracted in aqueous 80 % methanol with benzyl glucosinolate (Phytoplan, Heidelberg, Germany) as internal standard. Glucosinolates were converted to desulfoglucosinolates using purified sulfatase [E.C. 3.1.6.1, 'type H-1, from *Helix pomatia*, 15,100 units (gram solid)$^{-1}$; Sigma, Taufkirchen, Germany; purified after Graser *et al.* (2001)]. The desulfoglucosinolates were analysed by HPLC, identified and quantified as has been described previously (Müller & Wittstock 2005; Gigolashvili *et al.* 2007). Aliphatic and indolyl glucosinolates were each summed.

The following parameters (adaxial leaf wax and phloem composition) were investigated only in dependence of UV growth conditions from unifested plants.

4.2.6 Wax collection and analysis

To determine the composition of adaxial cuticular leaf waxes of uninfested plants in dependence of UV-conditions, plants (five-leaf stage) were taken from each greenhouse and waxes were extracted. Plant growth and wax collection were carried out during 23 April to 15 May 2008 with ambient light conditions averaging 20 kJ m^{-2} d^{-1} UV-B; 1,496 kJ m^{-2} d^{-1} UV-A; 7,248 PAR and 20,358 kJ m^{-2} d^{-1} global radiation. Plants were kept dark over night until wax extraction to guarantee stomatal closure. The second oldest leaves were placed on a flexible rubber mat and glass cylinders (diameter: 0.95 cm) were gently pressed onto the adaxial leaf sides. The cylinders were filled five times with 1 ml chloroform for 30 seconds. The solvent was agitated during these periods with a Pasteur pipette and then removed and collected in a glass vessel. Tetratriacontane (Fluka, Buchs, Switzerland) was added as internal standard. Chloroform was evaporated from the wax extracts under a gentle nitrogen stream. Hydroxyl-containing wax compounds were converted into the corresponding trimethylsilyl derivatives by reaction with N,O-bis-trimethylsilyl-trifluoroacetamide (BSTFA; Macherey-Nagel, Düren, Germany) in pyridine (30 min at 70 °C). Qualitative composition of wax compounds was identified with gas chromatography (GC: 6890N; Agilent Technologies, Santa Clara CA, USA) coupled with mass spectrometry (MS: 70 eV; m/z 50–750; 5973N; Agilent Technologies, Santa Clara CA). GC was carried out with on-column injection (DB-1, 30 m, 0.32 mm inner diameter, d.f. = 0.1 μm; J & W Scientific, Folsom, USA). Oven temperature was programmed as follows: 2 min at 50°C, 40°C/min to 200°C and 2 min hold, 3°C/min to 320°C and 30 min hold. Helium carrier gas inlet pressure was programmed as follows: 5 min at 50 kPa, 3.0 kPa/min to 150 kPa and 30 min hold. Quantitative composition of the wax compounds was determined by GC-FID (5890 II; Hewlett-Packard, Avondale PA, USA) under the same GC conditions as described

above, but with hydrogen as carrier gas. Wax compounds were quantified against the internal standard. Single compounds were subsumed into substance categories.

4.2.7 Phloem sap collection and amino acid analysis

To determine the amino acid composition of phloem sap in dependence of UV-conditions, phloem was collected from uninfested plants using stylectomy. Phloem sap collection from in total 9 plants per UV condition was performed over a longer period of time (9 April to 27 May 2008), because stylectomy is a rather labour intensive method. Ambient light conditions were averaging 16 kJ m^{-2} d^{-1} UV-B; 1,258 kJ m^{-2} d^{-1} UV-A; 5,762 kJ m^{-2} d^{-1} PAR and 16,124 kJ m^{-2} d^{-1} global radiation. Plants in the five-leaf stage were taken from each greenhouse and transferred to the laboratory. Second oldest broccoli leaves of intact plants were fixed on Plexiglas. Twenty aphids of *M. persicae* were caged on these leaves in Plexiglas cylinders (21 mm diameter) with foam rubber to prevent leaf damage on one side, whereas the other side was covered with gauze to prevent aphid escape. Cages were fixed with clamps. Aphids were kept on the leaves for a minimum time of two hours to allow settlement and start of feeding. Stylets were then disconnected by a laser beam (700-800 V, 1.06 nm, 0.5 Hz) of a laser microscope (constructed by Heinrich-Beck-Institute, Meiningen, Germany). After stylectomy, phloem exudates were collected with 0.5 µl capillaries (Hirschmann Laborgeräte, Eberstadt, Germany) for one hour using a micromanipulator. To prevent evaporation of phloem exudates the air was humidified. After sampling capillaries were immediately transferred into reaction vials, frozen in liquid nitrogen and stored at -80°C until amino acid analysis. Reaction vials with phloem-filled glass capillaries were vortexed with 15 µl norvaline (Phenomonex, Aschaffenburg, Germany) as internal standard and centrifuged (1 min, 7,500 r/min). Supernatants were transferred into sample processing glass vials (Phenomonex, Aschaffenburg, Germany). To increase the yield of phloem amino acids capillaries were washed three times with 5 µl 0.005M HCl by vortexing and centrifugation and supernatants were pooled. Further sample processing and analyses of phloem amino acids were conducted by the GC-MS EZ:faast free (physiological) amino acid analysis kit (Phenomenex, Aschaffenburg, Germany), whereby the solid phase extraction step was omitted.

Qualitative composition of the amino acids was identified with GC-MS. GC was carried out with split (1:15) injection on a Zebron ZB-AAA column (10 m, 0.25 mm inner diameter, Phenomenex, Aschaffenburg, Germany). Oven temperature was programmed for 1 min at 110°C, followed by a ramp of 30°C/min to 320°C. Helium carrier gas inlet pressure was 1.1 ml/min with constant flow. Quantitative composition of the amino acids was investigated using GC-FID (6850 II; Agilent Technologies, Santa Clara CA, USA) under the same GC conditions as described above, but with hydrogen as carrier gas. Amino acids were quantified by integrating their peak area relative to the area of the internal standard peak corrected by the calculated (by comparison to reference samples) response factors.

4.2.8 Statistical analysis

Data of morphological and chemical measurements of broccoli plants that were exposed to different UV-B and aphid infestation conditions were analysed by MANOVA. Aphid numbers on high and low-UV exposed plants as well as cuticular wax compounds of broccoli were compared with unpaired t-tests. Amino acid concentrations of broccoli phloem sap in dependence of UV-B treatment were analysed by Mann-Whitney U-tests. The analysis of data was performed with Statistica 8.0 (StatSoft, Tulsa, USA).

4.3 Results

4.3.1 Effects of UV-B and aphid infestation on growth and secondary metabolites

Broccoli plants grown in greenhouses with high ambient UV-B radiation were significantly smaller (smaller leaves, lower biomass; Fig. 4.3.1, Table 4.3.1) than plants grown under low UV-B radiation conditions, regardless of aphid infestation. Furthermore, they accumulated higher concentrations of UV-protective pigments, i.e. flavonoids (MANOVA for total flavonoids: UV-B treatment: $F_{(1;52)} = 30.50$; $P < 0.001$; Infestation treatment: $F_{(2;52)} = 1.45$; $P = 0.244$; UV-B x Infestation treatment: $F_{(2;52)} = 0.32$; $P = 0.731$), with kaempferol aglycones being higher concentrated than quercetins (Fig. 4.3.1). Total glucosinolate concentrations (MANOVA; UV-B treatment: $F_{(1;52)} = 1.67$; $P = 0.203$; Infestation treatment: $F_{(2;52)} = 1.43$; $P = 0.249$; UV-B x Infestation treatment: $F_{(2;52)} = 2.91$; $P = 0.064$) and aliphatic glucosinolate concentrations were not influenced by any treatment, whereas indolyl glucosinolate concentrations were significantly influenced by UV-B treatment x Infestation treatment (Table 4.3.1). Plants infested with *B. brassicae* had significantly reduced indolyl glucosinolate concentrations when grown under low UV-B radiation (Fig. 4.3.1).

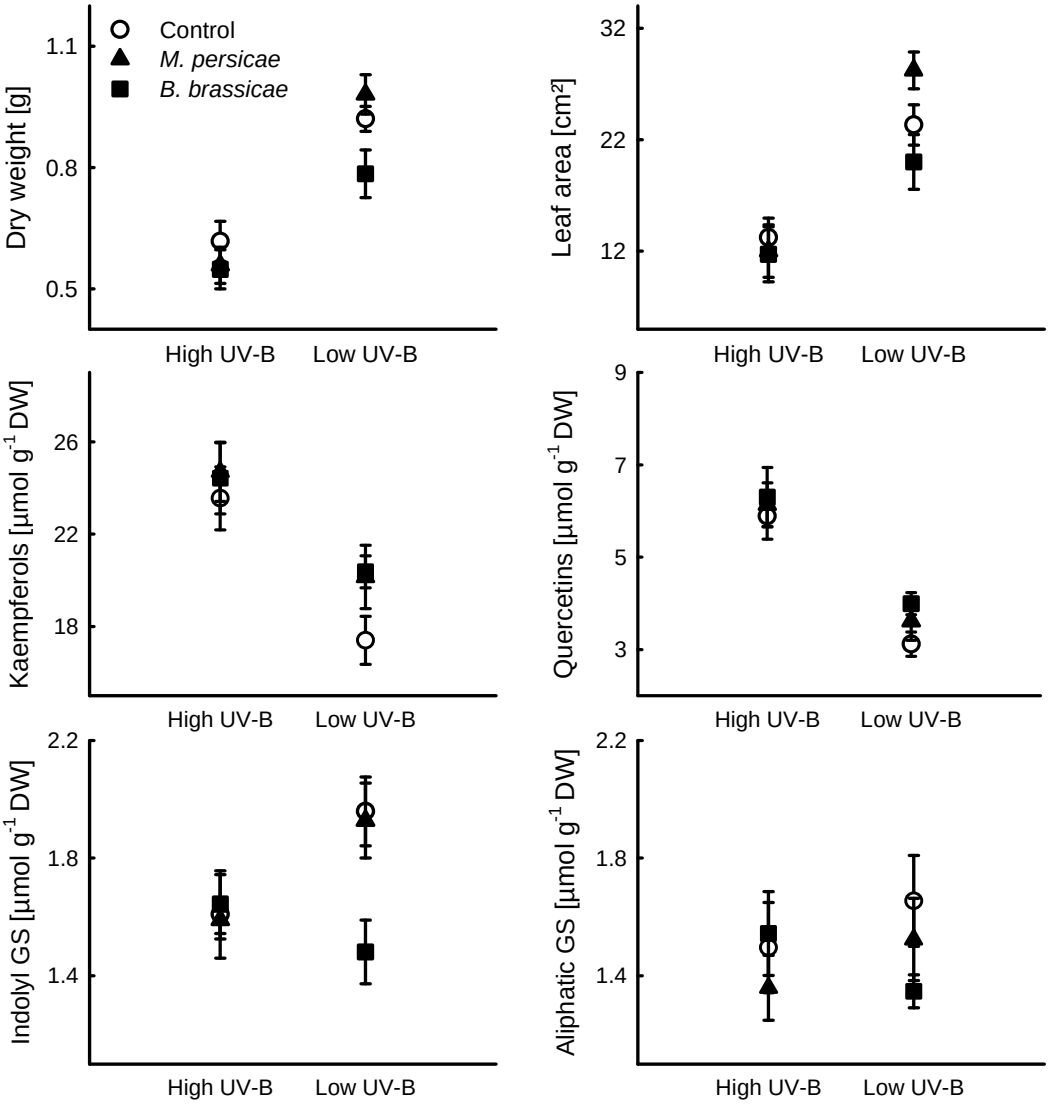

Fig. 4.3.1 Growth and chemical parameters (mean ± standard error) of plants grown under two intensities of UV-B radiation (either high UV-B: 80 % or low UV-B: 4% transmittance) with and without aphid infestation. Plants of both UV-B treatments were infested at the age of 20 days with ten aphid individuals of either *Myzus persicae* or *Brevicoryne brassicae*. Control plants were kept uninfested. Above-ground tissue was harvested five days after aphid infestation. GS = glucosinolates; DW = dry weight. Statistical analyses see Table 4.3.1.

Table 4.3.1 Influence of *UV-B treatment* and *Infestation treatment* on growth and chemical parameters of broccoli above ground tissue. MANOVA was used to analyse treatment effects. Broccoli plants were grown in two differently UV-B transmitting greenhouses (80% or 4% UV-B transmittance). At an age of 20 days plants were each infested with ten individuals of either the generalist aphid *Myzus persicae* or the specialist aphid *Brevicoryne brassicae*. Control plants were kept uninfested. Above-ground tissue was harvested five days after aphid infestation. Boldface indicates $P \leq 0.05$. GS – glucosinolates; DW = dry weight. [1] Significant P-values after Bonferroni correction carried out following Benjamini & Hochberg (1995). For presentation of descriptive data see Fig. 4.3.1.

Plant parameter	MANOVA					
	UV-B treatment		Infestation treatment		UV-B x Infestation treatment	
	$F_{(1;52)}$	*P*	$F_{(2;52)}$	*P*	$F_{(2;52)}$	*P*
Dry weight [g]	66.21	**<0.001**[1]	2.99	0.059	1.95	0.153
Leaf area [cm²]	44.69	**<0.001**[1]	2.05	0.139	1.97	0.150
Kaemperols [µmol g^{-1} DW]	23.43	**<0.001**[1]	1.59	0.213	0.38	0.685
Quercetins [µmol g^{-1} DW]	49.30	**<0.001**[1]	1.06	0.354	0.14	0.872
Indolyl GS [µmol g^{-1} DW]	3.50	0.067	2.21	0.120	3.22	**0.048**
Aliphatic GS [µmol g^{-1} DW]	0.16	0.693	0.68	0.511	1.26	0.293

4.3.2 Aphid population growth

The population growth of the specialist aphid *B. brassicae* was significantly reduced on broccoli grown under high UV-B radiation compared to low UV-B conditions (unpaired t-test; T = -2.07, P = 0.046), whereas the population growth of the generalist aphid *M. persicae* did not differ significantly on plants of both UV-B radiation conditions (unpaired t-test; T = 0.96, P = 0.345). In general, the specialist aphid *B. brassicae* was able to reproduce five to six times more than the generalist aphid *M. persicae* (Fig. 4.3.2).

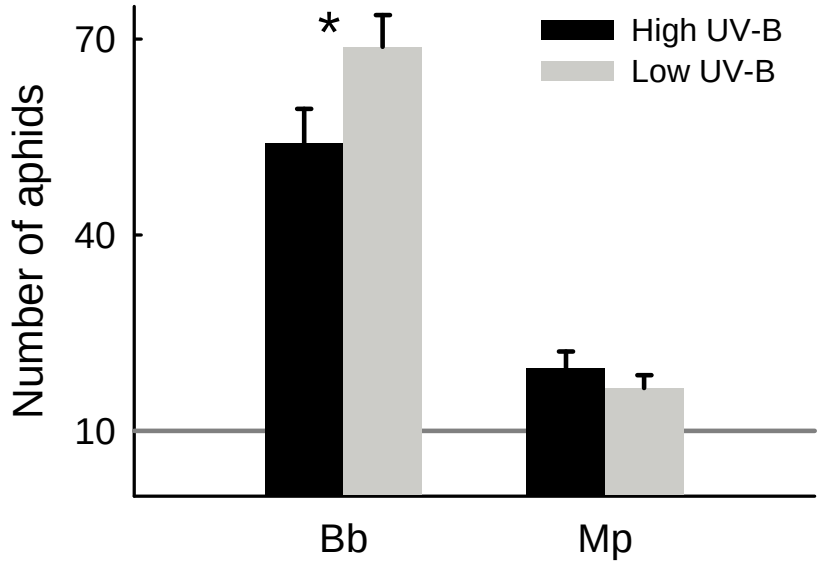

Fig. 4.3.2 Number of aphids (mean ± standard error) on broccoli plants five days after infestation. Plants were raised in two greenhouses transmitting high (80 %) and low UV-B (4%) radiation intensities, respectively. Plants had been infested with ten individuals (grey solid line) of either the specialist aphid *Brevicoryne brassicae* (Bb, N=19 and 20 plants) or the generalist aphid *Myzus persicae* (Mp, N=19). Statistics were performed with unpaired t-tests (*$P \leq 0.05$).

4.3.3 UV-B dependent effects on cuticular waxes and phloem amino acid contents

Plants grown under high UV-B radiation conditions had significantly lower total wax coverage on adaxial leaves than plants grown under low UV-B radiation (high UV-B 5.7 ± 0.3 µg cm^{-2}, low UV-B 6.8 ± 0.2 µg cm^{-2} [mean ± SE]; unpaired t-test; T = -2.81; P = 0.026; N = 4 and 5). The wax composition depended on UV-B treatment. Concentrations of aldehydes (T = -4.47, P = 0.003), alkanes (T = -2.49, P = 0.042) and a C-29 ketone (T = -2.88, P = 0.024) were significantly reduced in plants grown in greenhouses with high UV-B radiation, whereas alkenes (T = 3.69, P = 0.008) and one unidentified wax component (unpaired t-test; T = 2.93, P = 0.022) were significantly higher concentrated in these plants. Alkanes were the main components of the broccoli cuticular waxes (Fig. 4.3.3).

The total amino acid concentration of phloem exudates was on average lower on plants grown under high UV-B but did not differ significantly between plants of both radiation conditions (high UV-B 143 ± 25 nmol μl^{-1}, low UV-B 206 ± 45 nmol μl^{-1} [mean ± SE]; Mann-Whitney U-test; U = 29; P = 0.310; N = 9). Only the amino acid proline was significantly lower concentrated in phloem exudates of plants grown under high UV-B radiation conditions (U = 18, P = 0.047). Glutamic acid, aspartic acid and lysine were the three major amino acids in the phloem exudates of broccoli plants (Fig. 4.3.4).

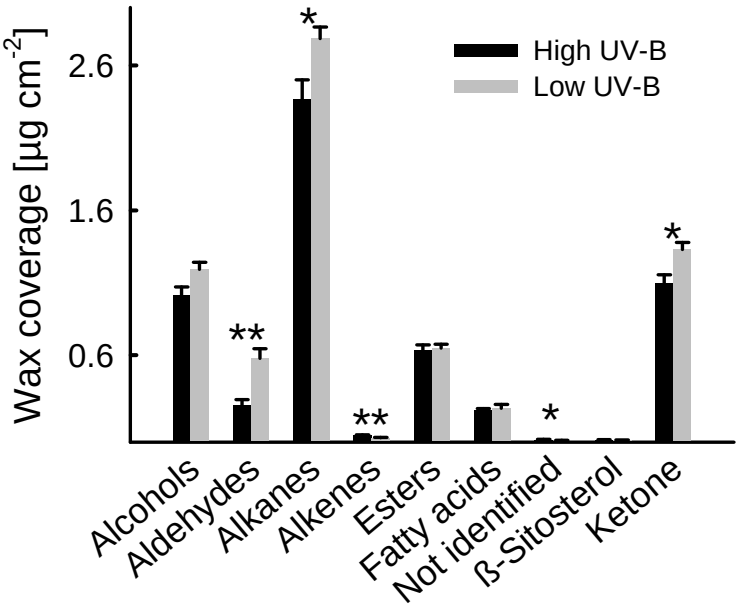

Fig. 4.3.3 Cuticular wax composition (mean ± standard error) of adaxial broccoli leaves (2nd oldest of plants with five leaves) grown either under high UV-B radiation (80 % transmittance, N=5) or low UV-B radiation conditions (4% transmittance, N= 4). Statistical analyses were performed by unpaired t-tests. Asterisks denote significant UV-B treatment differences (*$P \leq 0.05$; ** $P \leq 0.01$).

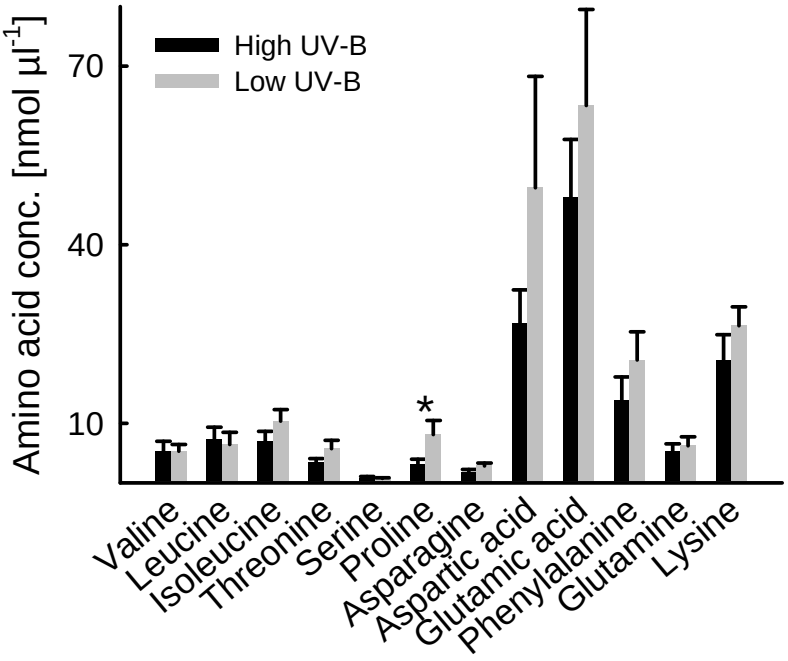

Fig. 4.3.4 Free amino acid concentrations (mean ± standard error) of phloem of broccoli plants grown under two different UV-B intensities (80 % or 4% transmittance, respectively). Probed plants had five leaves. Stylectomy for phloem sap collection was performed on Myzus persicae settled at the second oldest leaves. Conc. = concentration. Treatment effects were analysed with Mann-Whitney U-tests, asterisks indicate significant P-values (*P ≤ 0.05; N = 9).

4.4 Discussion

UV-B radiation forces plants to build up protective barriers and influences their growth and chemistry. Those changes of plant trait qualities can impact the next trophic levels. Various effects on broccoli plant traits were measured in plants grown under either high or low ambient UV-B conditions and interactions with specialist and generalist aphids were determined. Thereby plant traits influenced aphid performance of the specialist species, whereas aphid feeding had only little effects on plant chemistry.

4.4.1 UV-B effects on plants

In response to high ambient UV-B broccoli plants showed a reduced growth but increased flavonoid concentrations, as expected (Caldwell *et al.* 2007; Kuhlmann & Müller 2009; in press). Glucosinolate concentrations, which can serve as defence against insect herbivores, remained unaffected by UV-B, as has been also shown earlier in broccoli and other Brassicaceae (Reifenrath & Müller 2007; Kuhlmann & Müller 2009; in press) (Fig. 4.3.1, Table 4.3.1). For the first time, cuticular waxes of the adaxial leaf sides of broccoli were investigated in dependence of ambient or diminished UV-B radiation. Broccoli plants grown under high ambient UV-B conditions had lower wax coverage than plants grown under low UV-B (Fig. 4.3.3). This result is in contrast to earlier findings on pea and cucumber, in which artificial UV-B radiation led to an

increase of the cuticular wax coverage (Gonzalez *et al.* 1996; Fukuda *et al.* 2008). This discrepancy might be either species-specific or could be explained by the different UV-B exposure conditions (artificially increased *versus* ambient) of plants. Nothing was known about the influence of UV-radiation on the composition of the amino acids in the phloem sap, which might specifically influence phloem-feeding herbivores. Therefore, phloem samples were collected by stylectomy and analysed. The phloem composition was only little affected. Plants grown under high UV-radiation contained on average slightly lower concentrations of amino acids with a significant difference only found for proline (Fig. 4.3.4).

4.4.2 Plant mediated UV-B effects on aphids

UV-B induced changes in plant constitution did impact the population growth of specialist cabbage aphids, whereas the generalist green peach aphid in general performed poorer on plants of both growing conditions (Fig. 4.3.2). Kuśnierczyk *et al.* (2007) also found a higher sensitivity of the specialist *B. brassicae* to differences in host plant chemistry with regard to glucosinolate profiles of three *Arabidopsis thaliana* ecotypes compared to the generalist *M. persicae.* Moreover it has been shown that indolyl glucosinolates are an efficient defence against *M. persicae* (Kim & Jander 2007; Kim *et al.* 2008). Therefore it is likely that the indolyl glucosinolate concentrations of broccoli inhibited the reproduction of generalist *M. persicae*. The decreased performance of *B. brassicae* on 'high UV-B' broccoli plants might be either due to the plants' higher concentrations of flavonoids, which have been described to constrain aphid reproduction (Lattanzio *et al.* 2000) and/or due to UV-affected changes of plant tissue structures (Jansen 2002), which could act as mechanical barriers. Differences in the cuticular waxes between plants of both growing conditions could have also affected the aphid performance, as host plant selection and aphid performance are known to be influenced by the quality and quantity of the cuticular wax coverage (Thompson 1963; Powell *et al.* 1999). Amino acids are the major nitrogen source to aphids but the concentration of essential amino acids in the phloem is low (Douglas 2006). Symbiotic bacteria enable aphids to overcome the insufficient supply of essential amino acids. Thereby the amino acid composition can influence the relationship between aphids and their symbiotic bacteria and thus affect aphid development (Chandler *et al.* 2008). The slightly higher levels of amino acids in plants grown under low UV-B conditions could have improved the performance of *B. brassicae*. A direct effect of UV-B on aphids can be most likely excluded, because the aphids settled on the lower leaf sides. The oligophagous leaf chewer *Phaedon cochleariae* F. (Coleoptera: Chrysomelidae) was shown to be unaffected by UV-B induced differences in plant chemistry of Brassicaceae species (Reifenrath & Müller 2009). Similar results were also found for a specialist caterpillar feeding on UV-challenged leaves of *Plantago lanceolata* L. (Plantaginaceae), whereas the growth of a generalist caterpillar species was accelerated on leaves of *P. lanceolata* grown under elevated UV-B (McCloud & Berenbaum 1999). Different sensitivities of herbivores in response to UV-induced changes thus highly depend on the

feeding mode (phloem *versus* tissue feeding) and the degree of specialisation (specialist *versus* generalist) of the insect species.

4.4.3 Aphid effects on plants in interaction with UV-B

Plants did not only impact aphid performance but aphid feeding also influenced plant traits to some extent. Flavonoid concentrations of broccoli kept unaffected by aphid feeding. Similarly, in wild tobacco (*Nicotiana attenuata* Torr. and *Nicotiana longiflora* Cav., Solanaceae) flavonoids were only induced by UV-B radiation but not by simulated herbivory (Izaguirre *et al*. 2007). However, different phenolic compounds obviously respond differently as phenylpropanoid derivatives were induced by UV-B and artificial herbivory in wild tobacco (Izaguirre *et al.* 2007). However, in broccoli (data not shown) as well as in two other Brassicaceae (Reifenrath & Müller 2007) no UV dependent effects on hydroxycinnamic acids were found. A significant effect on glucosinolate induction was only apparent by the interaction of UV-B and aphid treatment on indolyl glucosinolates in broccoli. In control and *M. persicae*-infested plants the concentration of indolyl glucosinolates was slightly higher under low UV-B conditions compared to plants grown under high UV-B conditions (Fig. 4.3.1). These differences in concentrations might be explained by the different growth and thus developmental stages of these plants. In 'low UV-B' plants, feeding by *B. brassicae* led to a reduction of indolyl glucosinolate concentrations compared to control plants. The increased number of cabbage aphids on these plants could have exceeded a population density threshold in 'low UV-B' broccoli plants at which plants responded with a decrease of indolyl glucosinolates. *Brevicoryne brassicae*-infested 'low UV-B' plants also showed a slight reduction of biomass compared to control and *M. persicae*-infested plants, which indicates an induced inhibition of broccoli by cabbage aphids. Aliphatic glucosinolate concentrations remained uninfluenced by aphid feeding. Changes of glucosinolate concentrations due to aphid feeding are known for several Brassicaceae. Interestingly, Brussels sprouts (*Brassica oleracea* var. *gemmifera*, Brassicaceae) with very high numbers of cabbage aphids had the lowest concentrations of total glucosinolates and the highest concentrations of free thiocyanates compared to control plants (Yusuf & Collins 1998). *Arabidopsis thaliana* (L*er;* Col-0) plants infested with cabbage aphids or green peach aphids had lower amounts of aliphatic glucosinolates whereas indolyl glucosinolates did not change (Kim & Jander 2007; Kuśnierczyk *et al.* 2008). In contrast, a slight increase of aliphatic glucosinolates and no changes in indolyl glucosinolate concentrations or no changes at all were reported in two other studies investigating *Arabidopsis thaliana* Col-0 infested by *B. brassicae* and *M. persicae* (Mewis *et al.* 2005; Mewis *et al.* 2006). The low number of *M. persicae* aphids on broccoli in our study could have been below a necessary infestation threshold and therefore no changes in broccoli glucosinolate chemistry were detectable due to this aphid species. Furthermore, aphid infestation changed to a lesser extent and in a different manner the glucosinolate chemistry of broccoli than multiple herbivore attack did. A high infestation by thrips, white flies and aphids led to a threefold increase of indolyl glucosinolates within 72 h in broccoli (Kuhlmann & Müller in press). Overall

the different results show that plant responses are highly plant- and insect-species specific and furthermore could depend on the extent of insect infestation. A reduction of glucosinolate levels might reduce feeding by the specialist *B. brassicae*, whereas increased glucosinolate concentrations might prevent population growth of the generalist herbivore *M. persicae*. In future studies, infestation thresholds of plants, perception of aphid species by plants and plant species- and age-dependent influences of plant traits should be examined in more detail to gain a better understanding of plant-aphid interactions.

From a practical point of view, the new covering materials for greenhouses, which transmit more UV-B, not only improve plant quality by increasing flavonoid concentrations for human nutrition (Gomes *et al.* 2008; Schreiner *et al.* 2009) but also by inhibition of aphid population growth and therefore by reducing the application of unsustainable and expensive insecticides.

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Appendix

Controlled climate chamber experiments with three varieties of cabbage and one *Arabidopsis thaliana* ecotype (Col-0) (all Brassicaceae) were carried out in order to better distinguish between ultraviolet-B (UV-B)-induced and aphid-induced plant changes. Experiments were conducted in collaboration with Jacqueline Fuchs.

Seeds from white cabbage ((Kronos Hybrid, Seminis) *Brassica oleracea* L. convar. capitata (L.) Alef. var. alba DC.), red cabbage ((Autoro F1, Bejo) *Brassica oleracea* L. convar. capitata (L.) Alef. var. rubra DC.) and broccoli ((Monopoly, Syngenta) *Brassica oleracea* L. convar. botrytis (L.) Alef. var. cymosa Duch.) were grown in fertilised soil in individual pots (diameter: 9 cm, height: 7cm). *Arabidopsis thaliana* (L.) Heynh. ecotype Col-0 plants were grown from seeds in an autoclaved mixture of soil (Beetpflanzensubstrat Type RHP 19; Klasmann-Deilmann), vermiculite, and sand (10:0.5:0.5). All plants were grown in a climate chamber (24°C, 16:8 L:D; 70% r.h., light conditions: UV-B (280-315 nm) 0.014 W m^{-2}; UV-A (315-400 nm) 0.381 W m^{-2}, photosynthetic active radiation (400-700 nm) 71 µmol m^{-2} s^{-1}) for 28 days. Plants were either infested with specialist cabbage aphids (*Brevicoryne brassicae* (L.), Aphididae, Sternorrhyncha) or with generalist green peach aphids (*Myzus persicae* (Sulzer), Aphididae) or remained uninfested as control plants. Cabbage plants were infested with five wingless (apterous) aphid individuals, while *Arabidopsis* plants were infested with three aphid individuals per plant. At the age of 35 days aphid numbers were counted. The plants were harvested and immediately frozen in liquid nitrogen. Samples were stored at -80°C until sample processing. Whole plant samples were lyophilised, dry weight was determined and samples were homogenised. Aliquots were used for glucosinolate (all plants) and flavonoid (only cabbage) analyses (methods in **Chapter II** and **Chapter III**).

It was expected that aphids may grow differently on different cabbage varieties and *Arabidopsis* Col-0 due to diverging metabolite compositions. Furthermore, differing plant responses owing to aphid species and aphid proliferation were anticipated.

5.1 Plant chemistry

Table 5.1.1 Glucosinolate and flavonoid concentrations (mean ± standard deviation (SD), N = 7-10) in control plants of three different cabbage varieties and *Arabidopsis thaliana* ecotype Col-0. Abbreviations: GS, glucosinolate, -, not included, n.a., not analysed.

Plant compounds	**White cabbage**	**Red cabbage**	**Broccoli**	**Col-0**
[µmol g^{-1} DW]	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Aliphatic GS	2.64 ± 0.41	2.37 ± 0.56	0.52 ± 0.15	23.71 ± 5.32
Indolyl GS	1.62 ± 0.24	1.20 ± 0.17	3.46 ± 0.77	4.56 ± 0.95
Aromatic GS	0.38 ± 0.06	0.80 ± 0.11	-	0.38 ± 0.08
Total GS	4.64 ± 0.48	4.36 ± 0.75	3.98 ± 0.87	28.31 ± 6.08
Quercetins	0.08 ± 0.01	0.25 ± 0.08	0.08 ± 0.04	n.a.
Kaempferols	1.04 ± 0.27	1.80 ± 0.37	1.29 ± 0.52	n.a.
Total flavonoids	1.12 ± 0.28	2.05 ± 0.37	1.37 ± 0.52	n.a.

Aliphatic glucosinolates dominated in white and red cabbage, with sinigrin as main aliphatic glucosinolate (Table 5.1.1, Fig. 5.1.1). In broccoli indolyl glucosinolates (I3M) were most abundant, while aromatic glucosinolates were absent (Table 5.1.1, Fig. 5.1.1). *Arabidopsis* Col-0 had the most diverse glucosinolate composition and the highest total glucosinolate concentrations compared to cabbage plants. Aliphatic glucosinolates dominated in *Arabidopsis* Col-0 with 4MSOB as predominant component (Table 5.1.1, Fig. 5.1.2). Regarding the flavonoids, kaempferols were higher concentrated than quercetins in all cabbage varieties (Table 5.1.1). [Total flavonoid concentrations of climate chamber broccoli were very low compared to concentrations of outdoor exposed broccoli plants (**Chapter I, II, III**)].

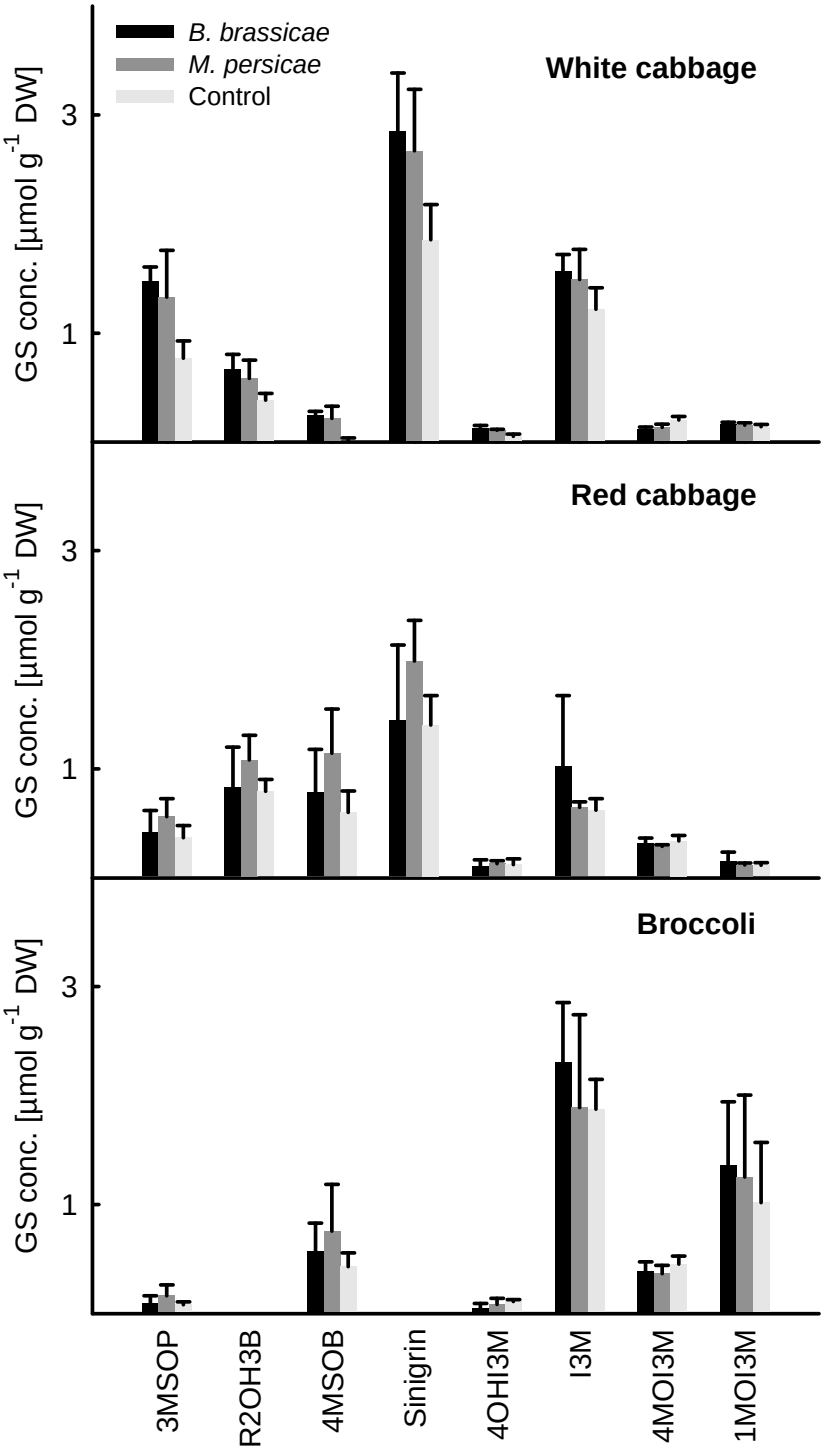

Fig. 5.1.1 Glucosinolate concentrations in three cabbage varieties, which were uninfested (control) or infested either with the specialist aphid *B. brassicae* or the generalist aphid *M. persicae*. GS, glucosinolates, conc., concentration, 3MSOP, 3-methylsulfinylpropyl, R2OH3B, R2-hydroxy-3-butenyl, 4MSOB, 4-methylsulfinylbutyl, sinigrin, 2-propenyl, 4OHI3M, 4-hydroxy-indol-3-methyl, I3M, indol-3-yl-methyl, 4MOI3M, 4-methoxy-indol-3-yl-methyl, 1MOI3M, 1-methoxy-indol-3-yl-methyl.

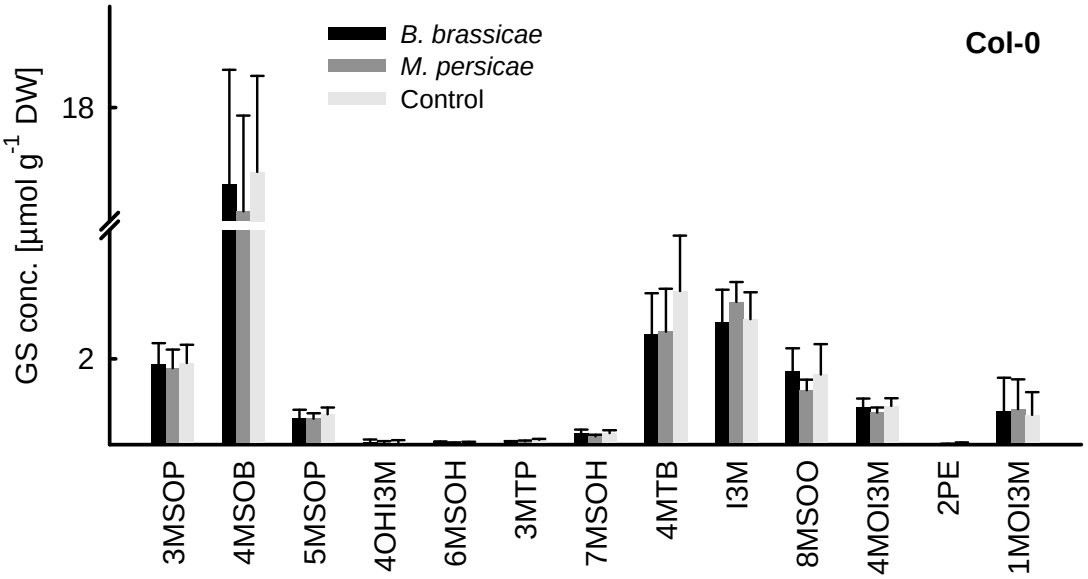

Fig. 5.1.2 Glucosinolate concentrations in *Arabidopsis thaliana* Col-0. GS, glucosinolates, conc., concentration, 3MSOP, 3-methylsulfinylpropyl, 4MSOB, 4-methylsulfinylbutyl, 5MSOP, 5-methylsulfinylpentyl, 4OHI3M, 4-hydroxy-indol-3-methyl, 6MSOH, 6-methylsulfinylhexyl, 3MTP, 3-methylthiopropyl, 7MSOH, 7-methylsulfinylheptyl, 4MTB, 4-methylthiobutyl, I3M, indol-3-yl-methyl, 8MSOO, 8-methylsufinyloctyl, 4MOI3M, 4-methoxy-indol-3-yl-methyl, 2PE, 2-phenylethyl, 1MOI3M, 1-methoxy-indol-3-yl-methyl.

Statistical evaluations with ANCOVA indicate only slight differences in plant chemistry induced by either specialist or generalist aphid species (Table 5.1.2). Significant differences after Bonferroni correction of glucosinolate concentrations were only found in *Arabidopsis* Col-0 plants. *Arabidopsis* plants infested by cabbage aphids did not contain 2-phenylethyl, plants infested by green peach aphids had only little amounts of 2-phenylethyl, whereas highest concentrations were present in control plants. The 3-methylthiopropyl glucosinolate showed the same pattern, but was abundant in *Arabidopsis* plants of all treatments. The slightest concentrations of 6MSOH glucosinolates in plants were found in *Arabidopsis* plants of the *M. persicae* treatment, while *B. brassicae* and control treatment 6MSOH concentrations were comparable. The same pattern can be seen for 7MSOH glucosinolate concentrations. Altogether, the strongest treatment differences were found for glucosinolates that were normally very low concentrated. Generally, the strong proliferation of green peach aphids on *Arabidopsis* induced reductions of specific glucosinolate concentrations compared to control plants, whereas cabbage aphid infestation did not change concentrations at all (Table 5.1.2, Fig. 5.2.1).

Table 5.1.2 Statistical results of analyses of covariance (ANCOVA) conducted for four plant species in order to reduce an unwanted source of variation. DW, dry weight, defined as covariate. Treatment plants remained uninfested (control) or were infested with either the specialist cabbage aphid *B. brassicae* or the generalist aphid *M. persicae*. Data were not transformed to achieve homogeneity of variances. [1] denote significant P-values after Bonferroni correction carried out for each plant species following Benjamini and Hochberg (1995).

Compounds	**White cabbage**				**Red cabbage**				**Broccoli**				**Col-0**			
[µmol g^{-1} DW]	DW [g]		Treatment		DW [g]		Treatment		DW [g]		Treatment		DW [g]		Treatment	
	$F_{(1;18)}$	*P*	$F_{(2;18)}$	*P*	$F_{(1;18)}$	*P*	$F_{(2;18)}$	*P*	$F_{(1;18)}$	*P*	$F_{(2;18)}$	*P*	$F_{(1;42)}$	*P*	$F_{(2;42)}$	*P*
3MSOP	9.69	**0.006**	0.56	0.581	7.81	**0.012**	1.81	0.192	0.04	0.839	1.98	0.162	17.05	**0.000**[1]	2.41	0.102
5MSOP	-	-	-	-	-	-	-	-	-	-	-	-	9.24	**0.004**	3.79	**0.031**
R2OH3B	1.24	0.281	1.38	0.277	3.05	0.098	2.65	0.098	-	-	-	-	-	-	-	-
4MSOB	10.80	**0.004**[1]	1.94	0.173	8.65	**0.009**	3.07	0.071	0.02	0.896	1.73	0.201	13.08	**0.001**[1]	2.75	0.075
Sinigrin	0.02	0.886	2.87	0.083	7.24	**0.015**	3.74	**0.044**	-	-	-	-	-	-	-	-
4OHI3M	0.12	0.731	6.59	**0.007**	0.21	0.651	0.05	0.950	0.05	0.821	3.33	0.055	3.09	0.086	1.51	0.234
6MSOH	-	-	-	-	-	-	-	-	-	-	-	-	8.72	**0.005**	11.84	**0.000**[1]
3MTP	-	-	-	-	-	-	-	-	-	-	-	-	4.98	**0.031**	12.21	**0.000**[1]
7MSOH	-	-	-	-	-	-	-	-	-	-	-	-	1.55	0.221	8.05	**0.001**
4MTB	-	-	-	-	-	-	-	-	-	-	-	-	3.99	0.052	1.85	0.170
I3M	1.00	0.330	0.46	0.641	6.47	**0.020**	5.47	**0.014**	0.06	0.806	1.13	0.342	9.47	**0.004**	2.20	0.123
8MSOO	-	-	-	-	-	-	-	-	-	-	-	-	0.29	0.591	4.26	**0.021**
4MOI3M	0.32	0.576	3.68	**0.046**	0.87	0.363	1.23	0.316	0.81	0.379	1.37	0.277	2.20	0.146	5.12	**0.010**
2PE	-	-	-	-	-	-	-	-	-	-	-	-	2.13	0.152	42.11	**0.000**[1]
1MOI3M	0.00	0.999	0.54	0.590	1.93	0.182	1.30	0.298	0.13	0.726	0.57	0.575	1.69	0.200	0.00	1.000
Aliphatic GS	1.53	0.233	2.28	0.131	8.26	**0.010**	3.24	0.063	0.00	0.946	1.78	0.192	7.08	**0.011**	3.08	**0.056**
Indolyl GS	0.75	0.396	0.46	0.640	5.61	**0.029**	4.43	**0.027**	0.02	0.899	0.61	0.555	8.81	**0.005**	0.36	0.699
Aromatic GS	1.24	0.281	1.38	0.277	3.05	0.098	2.65	0.098	-	-	-	-	2.13	0.152	42.11	**0.000**[1]
Total GS	1.96	0.179	2.46	0.114	2.01	0.173	2.39	0.120	0.02	0.900	0.59	0.564	8.52	**0.006**	2.56	0.089
Quercetins	0.38	0.543	2.43	0.117	3.99	0.060	3.88	**0.038**	0.16	0.691	0.04	0.961	n.a.	n.a.	n.a.	n.a.
Kaempferols	0.80	0.382	0.41	0.668	0.03	0.866	3.15	0.065	13.21	**0.002**	9.03	**0.002**	n.a.	n.a.	n.a.	n.a.
Total Flav	0.66	0.428	0.62	0.550	0.30	0.589	3.84	**0.039**	12.52	**0.002**	8.31	**0.003**	n.a.	n.a.	n.a.	n.a.

GS, glucosinolates, Flav, flavonoids, n.a., not analysed, 3MSOP, 3-methylsulfinylpropyl, 5MSOP, 5-methylsulfinylpentyl, R2OH3B, R2-hydroxy-3-butenyl, 4MSOB, 4-methylsulfinylbutyl, sinigrin, 2-propenyl, 4OHI3M, 4-hydroxy-indol-3-methyl, 6MSOH, 6-methylsulfinylhexyl, 3MTP, 3-methylthiopropyl, 7MSOH, 7-methylsulfinylheptyl, 4MTB, 4-methylthiobutyl, I3M, indol-3-yl-methyl, 8MSOO, 8-methylsufinyloctyl, 4MOI3M, 4-methoxy-indol-3-yl-methyl, 2PE, 2-phenylethyl, 1MOI3M, 1-methoxy-indol-3-yl-methyl.

5.2 Aphid proliferation

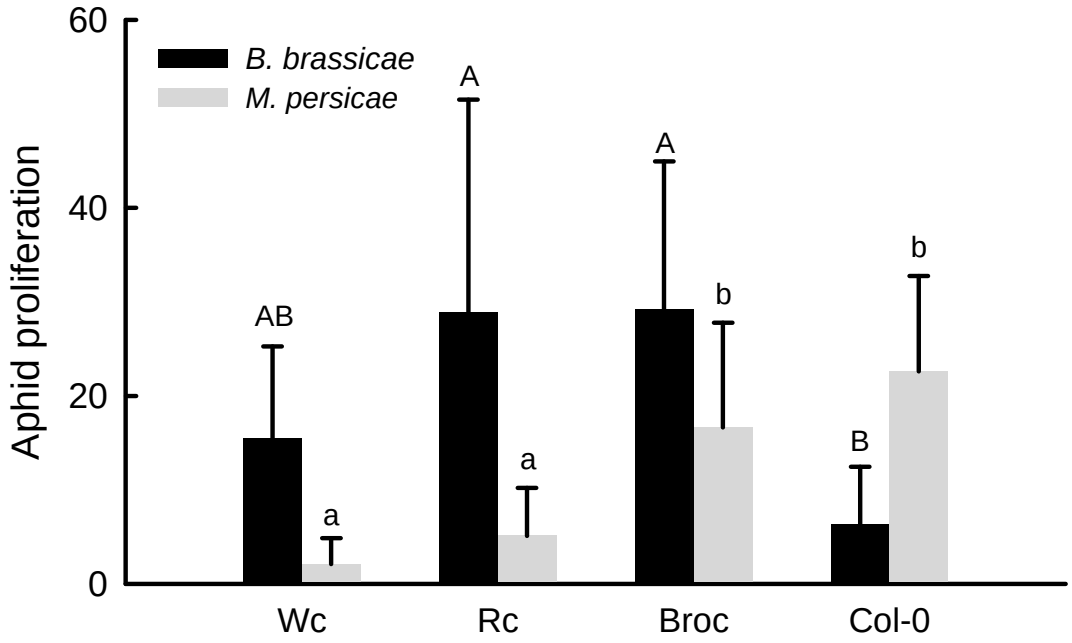

Fig. 5.2.1 Mean ± standard deviation (N = 20) of aphid increase after seven days of infestation with either the specialist cabbage aphid *B. brassicae* or the generalist green peach aphid *M. persicae*. Plants were infested with five (cabbage) or three (*Arabidopsis*) aphid individuals. Wc, White cabbage; Rc, Red cabbage; Broc, Broccoli; Col-0, *Arabidopsis thaliana* Col-0. To achieve homogeneity of variances data were transformed with log (x+10) for *B. brassicae* treatment and with (sqrt (x) for *M. persicae* treatment. Afterwards ANOVA were performed (ANOVA for *B. brassicae*: $F_{(3;76)} = 12.22$; $P < 0.001$, for *M. persicae*: $F_{(3;76)} = 33.30$; $P < 0.001$). Letters above bars indicate significant differences localised by Scheffé post-hoc tests. Upper case letters refer to *B. brassicae*, upper letters to *M. persicae* growth on different plant species.

B. brassicae performed significantly better on red cabbage and broccoli than on *Arabidopsis* Col-0. In contrast, *M. persicae* performed significantly better on *Arabidopsis* Col-0 and broccoli than on white cabbage and red cabbage. (Fig. 5.2.1).

Plants with different chemical profiles did influence the growth of two aphid species and aphids primarily induced reductions of glucosinolate concentrations particularly in *Arabidopsis*. The best performance of the generalist green peach aphid was on *Arabidopsis* with the highest and most diverse glucosinolate concentrations, whereas the specialist cabbage aphid reproduced worst compared to their performance on cabbage plants. Normally, one would expect the reverse, because the Brassicaceae specialist should have been better adapted to glucosinolate concentrations in host plants than the generalist green peach aphid. The same aphid-performance pattern of both aphid species on *Arabidopsis* has also been shown by Kuśnierczyk *et al.* (2007). Furthermore, specialist cabbage aphids as well as generalist green peach aphids use glucosinolates as

feeding stimulants (Wensler, 1962; Moon, 1967; Klingauf *et al.*, 1972; Gabrys *et al.*, 1997). *Arabidopsis* plants responded to higher amounts of the generalist green peach aphids with a reduction of some glucosinolates, which may indicate an exceeding of a certain infestation threshold.

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Summary

Plants must respond to multiple stimuli in a natural environment. Therefore they need the ability to rapidly reorganise and specifically build up appropriate metabolites to adapt to their environment. Abiotic cues, such as ambient solar radiation, influence the next trophic level directly, but also an altered plant composition triggered by these environmental cues can have an effect on the behaviour of herbivores. The aim of this study was to test effects of the important ultraviolet (UV) radiation on plants and on plant-insect interactions using multi-level investigations. The focus was on the conduction of controlled experiments with broccoli plants in highly engineered greenhouses covered with innovative materials, which only differed in their UV-B transmission. For the first time in this controlled environment the plant-mediated UV-B effects on phloem-feeding aphids were studied.

Broccoli plants (*Brassica oleracea* L. convar. *botrytis*, Brassicaceae) were under filter tents either exposed to (inclusion, +UV) or not exposed to (exclusion, -UV) UV-A / UV-B radiation. In greenhouses covered with new, innovative materials transmitting high (80%), medium (23%) or low (4%) levels of ambient solar UV-B radiation, in particular the influence of UV-B radiation on broccoli was examined. Different parameters of the above ground broccoli plant tissue were investigated: plant growth was measured, secondary plant metabolites (flavonoids, glucosinolates) analysed with high performance liquid chromatography (HPLC), cuticular waxes determined with gas chromatography coupled with mass spectrometry (GC-MS), proteinase inhibitor concentrations measured by radial diffusion assays and the combustion analysis allowed the calculation of the carbon / nitrogen ratio. By using a laser microscope (stylectomy) phloem sap was obtained from UV-B treated broccoli plants to analyse and quantify its amino acid constitution by GC-MS. Herbivore infestation and performance was investigated by counting the insect individuals or the number of infested and uninfested plants after a defined period of time.

The broccoli plants acclimatised to high UV-conditions by increasing the concentrations of phenolic compounds, e.g. flavonoids, and by reducing the accumulation of biomass. These responses to their UV-environment depended highly on the developmental stage of the plant. Young developing plants faced a much stronger trade-off between growth and defence than mature plants, as they may not have built up enough reserves yet. This was shown by exposing broccoli plants of two different developmental stages in filter tents including or excluding solar UV radiation. Broccoli plants either germinated under the filter tent constructions or were first grown in a climate chamber with low radiation and subsequently transferred under the filter tents in the field at an age of three weeks. Only broccoli plants, which already germinated in +UV filter tents, were smaller due to the UV-treatment. Thus, the environmental stimulus UV light is much more shaping at germination stage than it is for older plants. The concentration of Brassicaceae-specific

defensive compounds such as glucosinolates and also proteinase inhibitors remained unaffected in broccoli of both UV treatments. Innovative greenhouses were used to minimise disturbing factors and to measure UV-B induced plant changes selectively. Both experimental setups led to comparable plant responses (higher flavonoids, unchanged glucosinolates, smaller growth), which indicates that plant changes are mainly driven by high UV-B irradiation. The total cuticular wax coverage, which is the first protective barrier of plants, was significantly reduced in broccoli plants irradiated with high amounts of UV-B radiation. Total amino acid concentrations showed only slight reductions in high UV-B irradiated plants, although the amino acid proline was significantly reduced. No clear pattern was visible regarding the C/N ratio and therefore seemed not to be influenced by UV-radiation. Plant changes induced by ambient solar UV-B radiation are clearly measurable.

These plant responses caused by high UV-B radiation should result in discrimination behaviour of insect herbivores. Whiteflies (Aleyrodidae) and aphids (Aphididae) were attracted by +UV conditions, whereas thrips (Thripidae) avoided +UV conditions, which led to different infestation patterns of broccoli plants. The direct effect of UV radiation on behavioural choice responses of cabbage whiteflies (*Aleyrodes proletella* L. (Aleyrodidae)) was closer examined. Artificial plant dummy constructions made of green sticky traps were placed under both filter tents and only the dummies under +UV conditions were infested by cabbage whiteflies. On the contrary, different UV-B pre-treatments of broccoli plants in greenhouses did not lead to discrimination behaviour of whiteflies, aphids and thrips after plant transfer to the field. Despite known UV-B induced differences of plant metabolites, the insects did not respond to them. An artificial infestation of high and low UV-B irradiated greenhouse broccoli plants with the specialist cabbage aphid (*Brevicoryne brassicae* (L.), Aphididae) or the generalist green peach aphid (*Myzus persicae* (Sulzer), Aphididae) led to a reduced performance of cabbage aphids on high UV-B irradiated plants. Overall, the proliferation of the cabbage aphid was much higher on both UV-B treatments in comparison to the green peach aphid, which reproduced equally on plants of both treatments. This indicates species-specific behavioural responses of herbivorous insects to differences in UV radiation directly as well as to UV-B radiation induced host plant differences.

Insects can change the host plant chemistry, which was investigated after UV-B pre-treated broccoli plants were transferred from the three greenhouses to the field. A threefold increase of indolyl glucosinolate concentrations was only detectable due to a high infestation with thrips, whiteflies and aphids after 72 h in all plants independent of the UV-B pre-treatment. An artificial aphid infestation of broccoli plants affected the plant chemistry in a different manner. Although total glucosinolate concentrations did not change, only high numbers of cabbage aphids led to decreased indolyl glucosinolate concentrations of broccoli plants, whereas low reproducing green peach aphids did not change glucosinolate concentrations. Plants react specifically towards herbivore species and elicit an appropriate response only after an exceeding of an infestation threshold. Furthermore, it may also be possible that aphids manipulate plant responses for their own benefit.

In climate chambers with constant low light conditions three different varieties of cabbage (white cabbage (*Brassica oleracea* L. convar. capitata (L.) Alef. var. alba DC.), red cabbage (*Brassica oleracea* L. convar. capitata (L.) Alef. var. rubra DC.), broccoli (*Brassica oleracea* L. convar. botrytis (L.) Alef. var. cymosa Duch.)) and *Arabidopsis thaliana* (L.) Heynh. ecotype Col-0 were cultivated and infested with one aphid species (cabbage aphid or green peach aphid). Glucosinolate profiles were rather similar among the three cabbage varieties, but differed to the profile of *Arabidopsis* plants. *Arabidopsis* had the most diverse profile and accumulated highest glucosinolate concentrations. The performance-pattern of both aphid species mirrored this difference in glucosinolate composition and concentration; generally specialist cabbage aphids performed best on cabbage plants, whereas the generalist green peach aphids performed better on *Arabidopsis*. Thus, the generalist green peach aphid tolerated higher constitutive glucosinolate concentrations than the specialist cabbage aphid did. In all plants, both aphid species did again not change the total glucosinolate concentrations, but induced rather a decrease of particular glucosinolate compounds. Total flavonoid concentrations were and remained very low in all cabbage plants. Plant-aphid interactions are mainly driven by species-specific differences in plant chemistry within the family Brassicaceae. The two aphid species differed in their tolerance and optimum range towards distinct plant metabolites.

Plants respond highly specific to environmental stimuli such as UV-B radiation and herbivory. UV-B radiation has a strong impact on the plants' architecture and flavonoid contents, which can in turn influence plant-insect interactions. Phloem-feeding aphids can be negatively affected by UV-B mediated plant changes. However, a direct effect of UV radiation on the behaviour of herbivores is also evident. Mainly the number, composition and quality of herbivorous species as well as an exceeding of a certain infestation threshold determine the mode of plant changes.

In conclusion, UV-B radiation has the potential to harden plants against herbivores and simultaneously increases the concentrations of valuable secondary metabolites for human nutrition in important crop species such as broccoli.

Zusammenfassung

In ihrer natürlichen Umgebung sind Pflanzen verschiedensten und vor allem wechselnden Umwelteinflüssen ausgesetzt, auf die sie schnell und angemessen reagieren müssen. Das Insektenverhalten der nächsten trophischen Ebene wird direkt durch abiotische Umweltfaktoren, wie zum Beispiel Sonnenstrahlung, sowie durch daraus resultierende Veränderungen in Pflanzen gesteuert. Das Ziel dieser Untersuchung war es, herauszufinden, wie sich ultraviolette (UV) Strahlung auf Pflanzen und Pflanzen-Insekten Interaktionen auswirken kann. Dies wurde auf verschiedensten Ebenen untersucht. Mit Hilfe von speziell angefertigten Gewächshäusern konnten Brokkolipflanzen unter kontrollierten UV-B Bedingungen angezogen werden. Der Einfluss von UV-B Strahlung auf Brokkoli und von UV-B induzierten Effekten in Brokkoli auf phloem-fressende Blattläuse wurde erstmals untersucht.

Die Experimente wurden mit Brokkolipflanzen (*Brassica oleracea* L. convar. *botrytis*, Brassicaceae) durchgeführt, die in Folienzelten mit unterschiedlicher UV-Strahlungsdurchlässigkeit exponiert wurden. Die Eindeckungen der Folienzelte waren entweder UV-A / UV-B durchlässig (+UV) oder undurchlässig (-UV). Gewächshäuser mit innovativen Eindeckungsmaterialien, die speziell UV-B in hohen (80%), mittleren (23%) oder geringen (4%) Mengen transmittierten, wurden genutzt, um den alleinigen Effekt von UV-B Strahlung auf Pflanzen hervorzuheben. Verschiedene Parameter der oberirdischen Biomasse von Brokkolipflanzen wurden in diesem Zusammenhang erfasst: die Pflanzen wurden vermessen, sekundäre Pflanzeninhaltsstoffe (Flavonoide, Glucosinolate) mittels Flüssigkeitschromatographie (HPLC) analysiert, die cuticuläre Wachsauflagerung durch Gaschromatographie gekoppelt mit Massenspektrometrie (GC-MS) ermittelt, ein Enzymassay zur Bestimmung von Proteinaseinhibitoren durchgeführt und das Kohlenstoff / Stickstoffverhältnis (C/N) bestimmt. Mit Hilfe eines Lasermikroskops (Stylectomy) konnte pflanzlicher Phloemsaft zum ersten Mal von unterschiedlich UV-B behandelten Brokkolipflanzen gesammelt und dessen Aminosäuregehalt durch GC-MS bestimmt werden. Der Insektenbefall und das Insektenwachstum wurden durch Zählen der Herbivorenindividuen beziehungsweise der infizierten und nicht-infizierten Pflanzen innerhalb eines definierten Zeitraums ermittelt.

Die Pflanzen passten sich an hohe UV-Strahlungsbedingungen durch Anreicherung phenolischer Verbindungen, z.B. Flavonoide, und durch eine Reduzierung von Biomasse an. Dabei bestimmte der Entwicklungszustand der Pflanzen das Ausmaß der Reaktionen. Junge Pflanzen waren, vermutlich aufgrund geringerer Reserven, stärker von UV-Strahlung beeinflusst als ältere Pflanzen. Dies wurde gezeigt, indem Brokkolipflanzen zweier Entwicklungsstadien in Folienzelte mit UV-Ein- oder Ausschluss exponiert wurden. Die Pflanzen keimten und wuchsen entweder in diesen Folienzelten oder sie wurden zuerst in einer Klimakammer mit geringen

Strahlungsintensitäten aufgezogen und im Alter von drei Wochen ins Freiland in die Folienzelte transferiert. Nur Brokkolipflanzen, die bereits in den +UV Folienzelten keimten, waren aufgrund des UV-Einflusses kleiner. Somit prägt der Umweltfaktor UV-Licht die Pflanze während der Keimung stärker als in einem späteren Entwicklungsstadium. Glucosinolate als charakteristische Sekundärmetabolite der Brassicaceae und die Konzentrationen der Proteinaseinhibitoren wurden durch UV-Behandlung nicht beeinflusst. Innovative Gewächshäuser boten die Möglichkeit, Störfaktoren auszuschließen und speziell UV-B induzierte Pflanzenveränderungen und Herbivorenreaktionen auf diese Veränderungen zu untersuchen. Vergleichbare Ergebnisse (erhöhte Flavonoid-, unveränderte Glucosinolatgehalte, kleinerer Wuchs) aus beiden Versuchsansätzen lassen den Schluss zu, dass die pflanzlichen Veränderungen hauptsächlich durch hohe UV-B Strahlung verursacht werden. Die cuticuläre Wachsauflagerung stellt die erste Schutzbarriere der Pflanzen dar und war bei hoher UV-B Strahlung in Brokkolipflanzen signifikant reduziert. Die Gesamtaminosäurekonzentration im Phloemsaft war unter hoher UV-B Strahlung nur leicht vermindert, obwohl eine signifikante Reduktion des Prolingehaltes ermittelt werden konnte. Das C/N Verhältnis zeigte kein klares Muster und schien somit nicht durch UV-Strahlung beeinflusst zu sein. Durch UV-B Strahlung induzierte Pflanzenveränderungen sind jedoch eindeutig nachweisbar.

Diese durch UV-B Strahlung veränderten Pflanzen sollten das Wirtswahlverhalten von Herbivoren beeinflussen. Weiße Fliegen (Aleyrodidae) und Blattläuse (Aphididae) bevorzugten +UV Bedingungen, wohingegen Thripse (Thripidae) +UV Bedingungen mieden. Dies resultierte in unterschiedlichen Befallsmustern von Brokkolipflanzen. Der direkte Einfluss von UV-Strahlung auf die Kohlmottenschildlaus (*Aleyrodes proletella* L. (Aleyrodidae)) wurde genauer untersucht. Pflanzenattrappen bestehend aus grünen Klebetafeln wurden in den Folienzelten exponiert, wobei +UV Zelte von den Kohlmottenschildläusen eindeutig bevorzugt wurden. Im Gegensatz dazu zeigten Weiße Fliegen, Blattläuse und Thripse im Freiland keine Bevorzugung von Brokkolipflanzen, die in den Gewächshäusern verschiedenen Mengen an UV-B Licht ausgesetzt waren. Die Insekten reagierten somit nicht auf nachweisliche Unterschiede im Pflanzenmetabolitspektrum. Wurden Broccolipflanzen in Gewächshäusern mit hoher oder geringer UV-B Durchlässigkeit künstlich mit einer definierten Menge an spezialisierten Mehligen Kohlblattläusen (*Brevicoryne brassicae* (L.), Aphididae) oder generalistischen Grünen Pfirsichblattläusen (*Myzus persicae* (Sulzer), Aphididae) infiziert, wurde ein vermindertes Wachstum der spezialisierten Laus auf Pflanzen unter hohen UV-B Bedingungen festgestellt. Im Allgemeinen war die Vermehrungsrate der Mehligen Kohlblattlaus unter beiden UV-B Bedingungen sehr viel höher als die der Grünen Pfirsichblattlaus, die unter beiden Bedingungen gleich schlecht wuchs. Herbivore Insekten reagieren artspezifisch auf Unterschiede in ihrer direkten Strahlungsumwelt sowie auf UV-B vermittelte Wirtspflanzenunterschiede.

Durch herbivore Insekten hervorgerufene Änderungen in der Wirtspflanzenchemie wurden untersucht, indem UV-B vorbehandelte Brokkolipflanzen aus den drei Gewächshäusern ins Freiland exponiert wurden. Hohe Befallsdichten von Thripsen,

Weißen Fliegen und Blattläusen induzierten eine Verdreifachung der Indolylglucosinolatkonzentrationen nach 72 h in allen Pflanzen unabhängig von der UV-B Vorexposition. Das gezielte Infizieren von Brokkolipflanzen mit Blattläusen beeinflusste die Pflanzenchemie in anderer Weise. Obwohl sich die Gesamtglucosinolatkonzentrationen in Brokkoli nicht änderte, bewirkten erst große Mengen an Mehligen Kohlblattläusen eine Abnahme der Indolylglucosinolatkonzentrationen, wohingegen die sich schlecht entwickelnden Grünen Pfirsichblattläuse keine Veränderungen hervorriefen. Pflanzen reagieren demnach sehr spezifisch und erst nach dem Überschreiten einer Befallsschwelle auf ihre Fraßfeinde. Außerdem scheint es nicht ausgeschlossen, dass Blattläuse die Wirtspflanzenreaktionen zum eigenen Nutzen manipulieren können.

In Klimakammern mit konstant schwachen Lichtbedingungen wurden drei verschiedene Kohlarten (Weißkohl (*Brassica oleracea* L. convar. capitata (L.) Alef. var. alba DC.), Rotkohl (*Brassica oleracea* L. convar. capitata (L.) Alef. var. rubra DC.), Brokkoli (*Brassica oleracea* L. convar. botrytis (L.) Alef. var. cymosa Duch.)) und *Arabidopsis thaliana* (L.) Heynh. ecotype Col-0 angezogen und mit einer Blattlausart (Mehlige Kohlblattlaus oder Grüne Pfirsichblattlaus) infiziert. Die Glucosinolatprofile der Kohlpflanzen ähnelten sich untereinander aber unterschieden sich stark vom Profil der *Arabidopsis* Pflanzen. *Arabidopsis* hatte die meisten Glucosinolate und akkumulierte die höchsten Mengen. Diese Glucosinolatunterschiede der Wirtspflanzen spiegelten sich im Blattlausperformancemuster beider Arten wieder. Generell vermehrte sich die spezialisierte Mehlige Kohlblattlaus am besten auf Kohlpflanzen, wohingegen die generalistische Grüne Pfirsichblattlaus besser auf *Arabidopsis* wuchs. Die Grüne Pfirsichblattlaus tolerierte somit hohe Mengen an Glucosinolaten besser als die spezialisierte Mehlige Kohlblattlaus. In allen Pflanzen induzierten beide Blattläuse erneut keine Veränderungen der Gesamtglucosinolatkonzentrationen, sondern induzierten vielmehr eine Abnahme einzelner Glucosinolate. Die Gesamtflavonoidkonzentrationen waren und blieben in allen Kohlarten sehr gering. Pflanzen-Blattlaus-Interaktionen werden sowohl durch artspezifische Veränderungen der Pflanzenchemie als auch durch blattlausspezifische Unterschiede in der Toleranz gegenüber pflanzlichen Metaboliten bestimmt.

Pflanzen reagieren auf verschiedene Umweltreize wie zum Beispiel UV-B Strahlung und Herbivorie sehr zielgerichtet. UV-B Strahlung hat einen starken Einfluss auf das Pflanzenwachstum und die Flavonoidgehalte, was wiederum Pflanzen-Insekten Interaktionen artspezifisch steuern kann. Phloem-fressende Herbivoren können durch UV-B-induzierte Pflanzenveränderungen negativ beeinflusst werden. Ein direkter UV-Effekt auf das Verhalten von Herbivoren ist jedoch ebenfalls erwiesen. Sowohl die Anzahl, Zusammensetzung und Qualität von Herbivorenarten also auch das Überschreiten einer definierten Befallsschwelle bestimmen das Ausmaß der Pflanzenveränderungen.

Zusammenfassend ist zu sagen, dass UV-B Strahlung Pflanzen gegenüber Fraßfeinden abhärten und gleichzeitig die Konzentration wertvoller pflanzlicher Inhaltsstoffe für die menschliche Ernährung in Feldfrüchten erhöhen kann.

Publications, poster and oral presentations

Publications

Fiedler K., Kuhlmann F., Schlick-Steiner B.C., Steiner F.M., Gebauer G. (2007) Stable N-isotope signatures of central European ants – assessing positions in a trophic gradient. *Insectes Sociaux*, **54**, 393-402.

Travers-Martin N., Kuhlmann F., Müller C. (2008) Revised determination of free and complexed myrosinase activities in plant extracts. *Plant Physiology and Biochemistry*, **46**, 506-516.

Kuhlmann F., Müller C. (2009) Development-dependent effects of UV radiation exposure on broccoli plants and interactions with herbivorous insects. *Environmental and Experimental Botany*, **66**, 61-68.

Kuhlmann F., Müller C. (in press) Independent responses to ultraviolet radiation and herbivore attack in broccoli. *Journal of Experimental Botany*.

Kuhlmann F., Müller C. (submitted) UV-B impact on aphid performance mediated by plant quality and plant changes induced by aphids. *Plant Biology*.

Presented posters and oral presentations

Kuhlmann F., Müller C. (2007) Impact of UV radiation on crop plants and their pests. Poster presentation, 23rd annual meeting of the International Society of Chemical Ecology in Jena, Germany.

January 2008 “Anlockung herbivorer Insekten durch UV-Strahlung?” Meeting of the collaborative research centre, Theodor-Boveri-Institut for Biosciences, Würzburg, Germany

January 2008 “Impact of UV radiation on plants and herbivorous insects” Meeting of the joint project “Innovative greenhouses”, Bielefeld, Germany

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Erklärung

Hiermit erkläre ich ehrenwörtlich, dass ich die vorliegende Dissertation selbstständig angefertigt und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet habe. Ferner erkläre ich, dass die Dissertation weder in gleicher noch in ähnlicher Form bereits in einem anderen Prüfungsverfahren vorgelegen hat. Ich habe bisher noch keinen akademischen Grad erworben oder zu erwerben versucht.

Würzburg, den …… ..……

(Franziska Kuhlmann)