RESEARCH PAPER

Species-specific responses of herbivores to within-plant and environmentally mediated between-plant variability in plant chemistry

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Abstract Allocation of resources to growth and defense against herbivores crucially affects plant competitiveness and survival, resulting in a specific distribution of assimilates and defense compounds within plant individuals. Additionally, plants rarely experience stable environmental conditions, and adaptations to abiotic and biotic stresses may involve shifts in resistance to herbivores. We studied the allocation of phytochemicals in *Brassica oleracea* (Brussels sprouts) due to leaf age, drought stress and herbivore damage and assessed effects on two lepidopteran herbivores differing in diet breadth: the generalist *Spodoptera littoralis* and the specialist *Pieris brassicae*. Glucosinolates as secondary defense compounds and total nitrogen and carbon were quantified and linked to plant palatability, i.e., herbivore feeding preference. Herbivore responses were highly species-specific and partially related to changes in phytochemicals. *Spodoptera littoralis* preferred middle-aged leaves with intermediate levels of glucosinolates and nitrogen over young, glucosinolate and nitrogen rich leaves, as well as over old leaves, poor in glucosinolates and nitrogen. In contrast, *P. brassicae* preferred young leaves. Both species preferred severely drought-stressed plants to the well-watered control, although analyzed glucosinolate concentrations did not differ. Both *S. littoralis* and *P. brassicae* feeding induced an increase of indole glucosinolate levels, which may

explain a reduced consumption of damaged plants detected for *S. littoralis* but not for *P. brassicae*. By revealing distinct, sometimes contrasting responses of two insect herbivores to within-plant and stress-mediated intraspecific variation in phytochemistry of *B. oleracea*, this study emphasizes the need to consider specific herbivore responses to understand and predict the interactions between herbivores and variable plants.

Introduction

Plants are immobile and therefore unable to physically escape unfavorable abiotic conditions or herbivore attack. However, plants possess alternative strategies to cope and withstand these disadvantageous conditions. Plant resistance to abiotic and biotic stress involves an optimized allocation of resources as well as direct protection of plant tissues by secondary metabolites (Frost and Hunter 2008; Stamp 2003; van der Meijden 1996). Generally, plants possess constitutive adaptations to their prevailing environment and further can rapidly adjust to extreme stress situations by inducing an increase in phytochemical resistance (Heil 2010; Karban and Baldwin 1997). These constitutive and induced aspects of plant chemistry comprise a combination of plant traits that are beneficial (nutritional quality) and detrimental (defense compounds) to herbivores, and strongly determine plant–herbivore interactions (Behmer et al. 2002; Bennett and Wallsgrove 1994). Understanding chemical changes in plants to abiotic and biotic stresses and their ecological implications for plant–herbivore interactions is becoming

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increasingly important in the face of growing environmental instability due to global climate change (Tylianakis et al. 2008).

Plant resistance to insect herbivores involves complex interactions, and hence, resistance responses may vary between different plant–insect combinations (Halitschke et al. 2011; Inbar et al. 2001; Stoeckli et al. 2009) and strongly depend on plant and herbivore species-specific characteristics (Agrawal 2000; Underwood 1999). Within-plant variations of resistance occur between different plant tissues and organs as well as between different development stages (Barton and Koricheva 2010; Brown et al. 2003; Gutbrodt et al. 2011b). Furthermore, responses of plants to stressful environmental conditions may affect their ability to resist insect herbivores, either limiting or enhancing plant defense depending on type and intensity of abiotic stress (Mody et al. 2009), plant genotype (Ballhorn et al. 2011) and affected insect herbivore species (Hale et al. 2005). The two most important herbivore characteristics recognized to cause variations in the effectiveness of plant resistance are insect feeding guild (Huberty and Denno 2004; Root 1973) and specialization to host plants (Gutbrodt et al. 2011a; Kessler and Halitschke 2007; for feeding guild and specialization see Bidart-Bouzat and Kliebenstein 2011). The latter characteristic, herbivore specialization or diet breadth, is particularly relevant as it often determines whether potential defensive plant metabolites contribute to plant resistance (against generalists), or rather increase attractiveness and suitability of a plant for herbivores (specialists) (Schoonhoven et al. 2005). Besides physiological adaptations to phytochemicals by specialists (Ballhorn et al. 2010; Piskorski and Dorn 2011), herbivores of different diet breadth are known to evade detrimental effects of plant defense by switching between host plants (Mody et al. 2007; Unsicker et al. 2008).

The myrosinase-glucosinolate defense system, characteristic for Brassicales including *Brassica oleracea* L., is a well-studied system involving toxic, growth inhibitory and deterrence properties to a wide range of herbivores (Halkier and Gershenzon 2006). Glucosinolates themselves show little biological activity, but upon hydrolysis by myrosinases they are transformed to bioactive products responsible for toxicity and deterrence, such as isothiocyanates, thiocyanates, nitriles and epithionitriles (Hopkins et al. 2009; Scascighini et al. 2005). In plants, myrosinases and glucosinolates are stored in separate cellular compartments, whereby tissue damage by herbivore feeding causes hydrolysis and activation of glucosinolates (Bones and Rossiter 1996; Winde and Wittstock 2011). In wild cabbage (*B. oleracea*), glucosinolate profiles strongly determine herbivore communities mediating distribution and abundances in the field due to consistent but species-specific preferences of herbivores for certain phenotypes (Newton

et al. 2010). In *B. oleracea* cultivars, glucosinolate levels are generally reduced compared to wild plants and their ability to up-regulate resistance, for example as a response to herbivore damage, has been questioned (Coleman et al. 1996; Gols et al. 2008). Understanding how abiotic and biotic environmental factors influence concentrations and profiles of glucosinolates in *Brassica* crops and assessing consequences for plant defense against different insect herbivores may contribute to optimizing sustainable pest control. A recent review on environmentally mediated variations in phytochemicals of Brassicaceae (Björkman et al. 2011) calls for more studies under controlled conditions, which address the multiple effects of abiotic and biotic factors separately.

In this study, we assessed the effects of leaf age, drought stress and herbivore damage on resistance of *B. oleracea* plants (Brussels sprouts; *B. oleracea* var. *gemmifera*; Brassicaceae) to two lepidopteran larval herbivores differing in their degree of host specialization: the generalist *Spodoptera littoralis* Boisduval (Noctuidae) and the specialist *Pieris brassicae* L. (Pieridae). We determined the extent to which secondary compounds and nutrients varied within plant individuals alone and among plant individuals exposed to abiotic (drought stress) and biotic (herbivore damage) environmental stress, and we related this within-plant and between-plant variability in plant chemistry to herbivore feeding responses. We expected differences in causes and consequences of plant resistance for the tested herbivores due to their host specificity and adaptations to plant-specific chemical defenses.

Materials and methods

Study organisms and experimental setup

Brassica oleracea plants were grown from seeds and transplanted individually in pots ($9 \times 9 \times 9.5$ cm) filled with commercial potting soil (Klasmann Substrat 2, containing 200 mg/L nitrogen, 320 mg/L phosphate, 360 mg/L potassium, 100 mg/L magnesium, Klasmann-Deilmann, Geeste, Germany) when they reached the 4-leaf-stage. Plants were subjected to different treatments according to the factor tested (experiment) and used in bioassays and for chemical analysis when they were 4-weeks old. The plants were initially reared in climate chambers. For the experiments, two fully equipped closed greenhouse chambers with internal air circulation were used. The controlled climatic conditions were similar in both chambers, with minor differences in day:night conditions, greenhouse 1: 24:16°C, 60% RH, L16h:D8h; greenhouse 2: 22:19°C, 40–60% RH, L14h:D10h.

Leaf-chewing larvae (caterpillars) of the two lepidopteran species *S. littoralis* and *P. brassicae* were used as

test herbivores. Whereas *S. littoralis* is a generalist herbivore and feeds on a broad host range (Salama et al. 1971). *Pieris brassicae* exclusively feeds on plants of a limited number of families within the order Brassicales such as Brassicaceae and represents a major pest of *Brassica* crops (Chew and Renwick 1995). *Spodoptera littoralis* larvae were obtained from Syngenta Crop Protection AG (Stein, Switzerland), and *P. brassicae* larvae were reared as described by Mattiacci et al. (2001a). Before feeding tests were initiated, larvae of the two test species were allowed to acclimatize to the experimental conditions for 24 h. They were fed leaves of well-watered undamaged *B. oleracea* plants and maintained at greenhouse conditions described above.

Three experiments (consisting of one to three plant series) were conducted to assess the effects of leaf age, drought stress and herbivore damage on the resistance of *B. oleracea* to the two test herbivores. Plant series consisted of a comparable set of *B. oleracea* plants grown under consistent greenhouse conditions. Experimental manipulation of the plants (treatments), and the herbivore feeding tests were conducted under greenhouse conditions.

Leaf age

Three plant series (I, II and III) each consisting of 20 plants were grown, resulting in three replicates of the experiment. Plants were reared in a growth chamber during 4 weeks and were then transferred to a common greenhouse (greenhouse 1). After an adaptation period of three days, plants were used in feeding choice tests. From each plant two leaf discs were excised from the blade of the youngest fully expanded leaf, a middle-aged leaf (3rd or 4th youngest) and an old but still green leaf (6th or 7th youngest). Leaf discs of the three leaf ages were then offered to both *S. littoralis* and *P. brassicae* larvae in triple-choice feeding tests ($n = 20$ per insect species and plant series). Chemical plant traits were quantified using the remaining leaf material of plants used in the feeding tests of plant series III ($n = 20$).

Drought stress

The plants were grown in two plant series (IV and V) each consisting of 60 plants in greenhouse 1 (IV) and greenhouse 2 (V). When plants consisted of four true leaves, they were subjected to one out of three pulsed drought stress treatments: no drought stress (i.e., well-watered control), moderate drought stress (i.e., low stress) or severe drought stress (i.e., high stress). Plants of a specific treatment simultaneously received a defined amount of water. Well-watered control plants received water every 2–3 days ensuring constantly moistened soil throughout the experiment. Low stress plants were watered when leaves started drooping and received on average $57.5 \pm 7.5\%$ of the water supplied to well-watered control plants during the experiment. High stress plants received water every 4–6 days just before necrosis occurred, which corresponded to $30 \pm 5\%$ of the amount of water supplied to well-watered control plants. After 30 days and a minimum of four drought cycles for high stress plants, the treatment was terminated and all plants were rehydrated for 24 h to achieve fully turgescent plants. Leaf discs excised from a fully expanded young leaf of each drought stress treatment were offered to *S. littoralis* and *P. brassicae* larvae in triple-choice feeding tests ($n = 20$ per insect species and plant series). Chemical analyses were conducted using leaf material of the plant series V ($n = 20$).

Herbivore damage

Four-week-old *B. oleracea* plants were subjected to a controlled herbivore damage treatment in a single series (VI) of 54 plants in greenhouse 1. Two middle-aged (3rd–5th youngest) leaves of each plant were covered with a perforated plastic bag, each containing either no insect (i.e., control treatment), five *P. brassicae* larvae (L2) (*Pieris*-damaged treatment), or ten *S. littoralis* larvae (L2) (*Spodoptera*-damaged treatment). The number of damaging herbivores per treatment was adjusted according to previous observations of species-specific feeding behaviors to ensure a consistent damage of 70–90% consumed leaf area within the damaging period. Larvae of both species started feeding immediately. After a previously defined effective induction period of 4 days (Mattiacci et al. 2001a), the plastic bags including all damaging herbivores were removed from the test plants. Leaf blade discs were excised from undamaged, fully expanded young leaves (systemic effects of herbivore damage) and offered to a new set of test insects in dual-choice feeding tests according to the following cross-comparison setup: control versus *Spodoptera*-damaged; control versus *Pieris*-damaged; *Spodoptera*-damaged versus *Pieris*-damaged ($n = 18$ per insect species and combination). Chemical analyses were conducted using remaining leaf blade material of leaves used in the feeding tests ($n = 18$; series VI).

Plant traits

Glucosinolate concentrations, total leaf nitrogen and carbon as well as leaf mass per area were quantified for leaves used in herbivore feeding tests for plants of each treatment in single plant series of each experiment. Additionally, carbon stable isotope values were quantified for plants in the drought stress experiment, as increased $\delta^{13}C$ values indicate decreased photosynthetic activity due to stomatal closure and can hence be used as a measure of plant drought stress (Farquhar et al. 1989).

For glucosinolate analysis, 20–25 mg finely ground lyophilized leaf material was extracted with 80% methanol (1.2 ml) containing 50 μM 4-hydroxybenzylglucosinolate as internal standard. After centrifugation (2,500g 10 min) the supernatants were loaded on columns containing 10% suspension of DEAE Sephadex A25 in H_2O (0.4 ml). Columns were washed with 80% methanol (1 ml), water (2 ml), and 0.02 M MES buffer (1 ml pH 5.2), before sulfatase solution (50 μl) was applied. After overnight incubation, desulfated glucosinolates were eluted with water (1 ml) and analyzed by HPLC on an Agilent HP 1100 Series instrument (Agilent, Waldbronn, Germany) equipped with diode-array detector and a LiChrospher RP18ec column (250 × 4.6 mm, 5 μm, Merck, Darmstadt, Germany). A gradient of water (solvent A) and acetonitrile (solvent B) was used: 5–20% B (10 min), 20–100% (0.1 min), 100% B (1.9 min), 100–5% B (0.1 min), and 5% B (3.9 min). Spectra were acquired from 190–360 nm and compounds quantified at 229 nm (Burow et al. 2006).

Total leaf nitrogen and carbon as well as carbon isotope values were determined by CHN elementary analysis using a Flash EA 1112 Series elemental analyzer (Thermo Italy, Rhodano, Italy) coupled to a Finnigan MAT Delta plus XP isotope ratio mass spectrometer (Finnigan, Bremen, Germany) as described in Plath et al. (2011). Ratios of carbon to nitrogen (C:N) were calculated for each test leaf.

Leaf mass per area as a measure for leaf density and thickness was calculated for remaining leaf discs used in herbivore feeding tests, based on the dry mass and unit area of remaining leaf discs (Poorter et al. 2009). Values of the two leaf discs excised per test leaf (or four in case of the herbivore damage experiment) were averaged.

Herbivore feeding tests

Test leaves were cut at petiole level, and from each leaf blade two (or four in the herbivore damage experiment) leaf discs of 18 mm diameter were excised, of which one was offered to individual larvae of each insect species in feeding tests. The remaining leaf material was immediately flash frozen and used for chemical analysis. Leaf discs were offered to single (L2–L3) insect larvae in triple-choice or dual-choice feeding tests and followed the procedure described by Mody et al. (2009). Choice tests were conducted under standardized greenhouse conditions in plastic Petri dishes of 9 cm diameter, containing a moist filter paper on top of a 3-mm thick styrofoam layer. Petri dishes were randomly positioned and covered with a cloth to allow feeding in shaded conditions. Test insects were not starved prior to feeding tests and started feeding promptly. After 24 h (9 a.m.–9 a.m.), larvae were removed, digital photographs of leaf discs were taken and consumed leaf mass (CM) was quantified for each leaf disc based on the consumed area (CA), the dry mass of the remaining leaf disc (RM) and the remaining leaf disc area (RA): CM = CA × (RM/RA).

Statistical analyses

Data were transformed to meet the assumptions of normality and heteroscedasticity when necessary. Consumed dry mass values were $\log_{10}(x + 1)$ transformed, nitrogen values and C:N ratios were arcsin(x) transformed, and glucosinolate concentrations were $\log_{10}(x)$ transformed prior to analysis. Consumed dry mass values of triple-choice feeding tests in the leaf age and drought stress experiment were analyzed by repeated measures mixed model ANOVAs with 'leaf age' and 'drought stress treatment', respectively, as repeated fixed factor, and 'plant series' as random factor. Differences between factors were assessed by *LSD* post hoc tests and covariance estimates were used to assess effects of the random factor. In the induction experiment consumed dry masses of dual-choice feeding tests were analyzed using paired samples *t* tests. Plants traits such as glucosinolate concentrations, total leaf nitrogen, C:N ratios, $\delta^{13}C$ values and leaf mass per area were analyzed by means of one-way ANOVAs, followed by *LSD* post hoc tests. All statistical analyses were conducted using PASWStatistics 18.0.
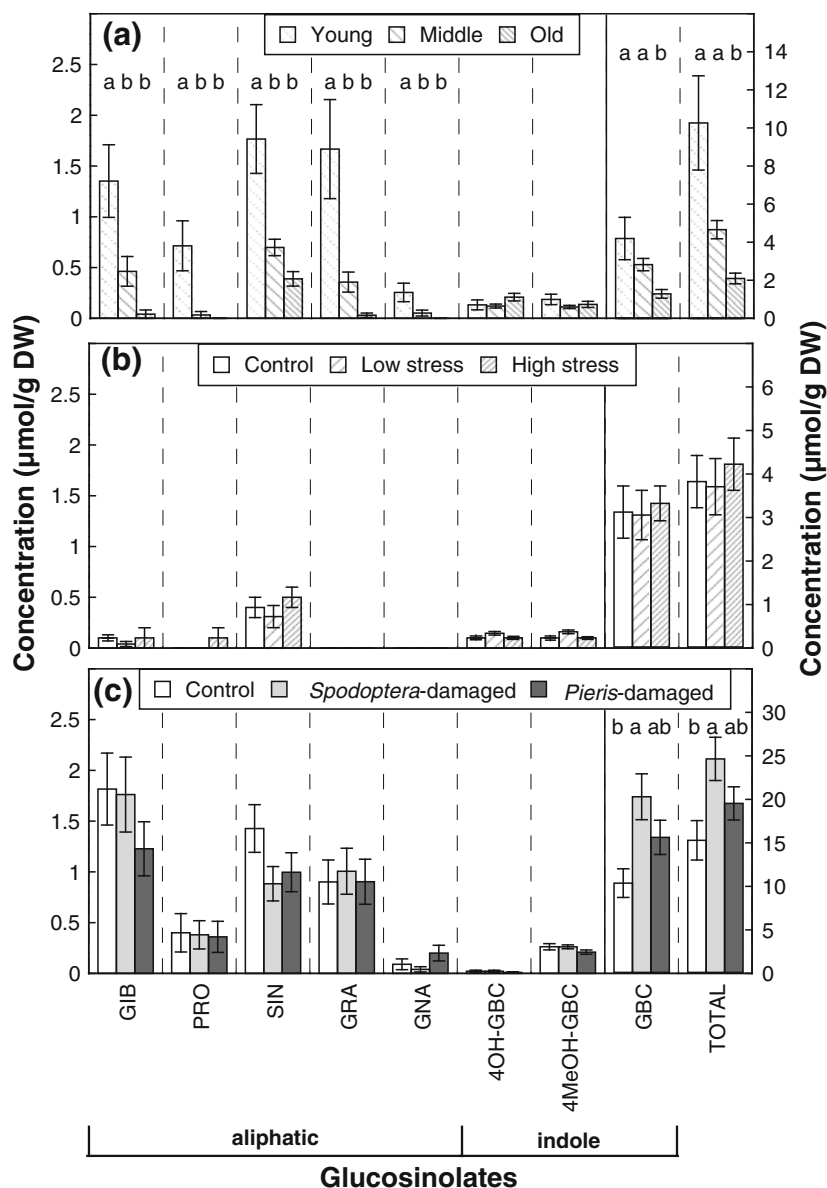
Results

Leaf age

Leaf age had a significant effect on the concentration of six out of eight detected glucosinolate compounds, of which glucobrassicin showed the highest concentration (Fig. 1a). Total glucosinolate concentration as well as the concentrations of the six age-affected compounds, were significantly lower in old leaves compared to young leaves. The levels of two compounds, the indole glucosinolates hydroxyglucobrassicin and 4-methoxyglucobrassicin, were not significantly affected by leaf ages. Total leaf nitrogen was lower in old leaves compared to young leaves (Table 1). Total leaf carbon was comparably reduced in old leaves leading to a stable C:N ratio across the three tested leaf ages. Leaf mass per area was not different between leaf ages.

Spodoptera littoralis consumed middle-aged leaves most, intermediate amounts of young leaves and old leaves least (mixed model; $F_{2,52} = 57.2$, $P < 0.001$; Fig. 2a). *Pieris brassicae* consumed highest amounts of young and middle-aged leaves and lowest amounts of the old leaves (mixed model; $F_{2,51} = 83.8$, $P < 0.001$; Fig. 2b). No significant effect of the random factor 'plant series' was detected for either insect species (covariance estimate; *S. littoralis*: $P = 0.7$, *P. brassicae*: $P = 0.4$).

Drought stress

Drought stress had no significant effect on the concentration of total glucosinolates and of the individual compounds in the plants (Fig. 1b). A total of six glucosinolates were identified, with highest levels of glucobrassicin (Fig. 1b). Aliphatic compounds were found in low concentrations, and two compounds observed in the 'leaf age' and the 'herbivore damage' studies (glucoraphanin and gluconapin) were not detectable. Total leaf nitrogen was unaffected by drought but significant differences were found for C:N ratios with lower values in well-watered control compared to stressed plants (Table 1). Differences in $\delta^{13}C$ values were significant between all three watering schemes, with lowest $\delta^{13}C$ values in well-watered control and highest values in high stress plants. Leaf mass per area was unaffected by drought treatment.

Spodoptera littoralis consumed significantly more leaf disc mass of high stress plants compared to low stress and control plants (mixed model; $F_{2,29} = 17.9$, $P < 0.001$; Fig. 3a). Similarly, *P. brassicae* consumed significantly more of high stress plants compared to control plants, while low stress plants were not significantly different from other treatments (mixed model; $F_{2,28} = 3.7$, $P < 0.05$; Fig. 3b). No significant effect of the random factor 'plant series' was detected for either insect species (covariance estimate; *S. littoralis*, $P = 0.7$; *P. brassicae*, $P = 0.7$).

Herbivore damage

In *B. oleracea* plants subjected to herbivore damage treatments, eight glucosinolate compounds identical to compounds detected in the 'leaf age experiment' were

Table 1 Leaf traits (mean ± SE) of *Brassica oleracea* var. *gemmifera* for the different experiments and treatments

Experiment	Treatment	Plant trait			
		N (%)	C:N	$\delta\ ^{13}C$ (‰)	LMA (mg/cm^2)
Leaf age	Young	**6.2 ± 0.3[a]**	7.1 ± 0.4	–	2.4 ± 0.1
	Middle-aged	**5.1 ± 0.3[a, b]**	8.5 ± 0.7	–	2.6 ± 0.2
	Old	**4.3 ± 0.3[b]**	8.7 ± 0.8	–	2.3 ± 0.1
Drought stress	Control	5.5 ± 0.2	**6.9 ± 0.3[b]**	**−36.7 ± 0.1[c]**	1.6 ± 0.1
	Low stress	4.6 ± 0.4	**9.5 ± 0.6[a]**	**−36.1 ± 0.1[b]**	1.7 ± 0.1
	High stress	4.8 ± 0.3	**8.7 ± 0.3[a]**	**−35.7 ± 0.2[a]**	1.9 ± 0.1
Herbivore damage	Control	4.3 ± 0.2	10.0 ± 0.6	–	3.2 ± 0.2
	Spodoptera-damaged	4.2 ± 0.2	10.4 ± 0.7	–	3.1 ± 0.1
	Pieris-damaged	4.3 ± 0.2	10.0 ± 0.6	–	3.1 ± 0.1

N total leaf nitrogen, *C:N* ratio of total carbon to total nitrogen, $\delta\ ^{13}C$ measure of carbon isotope ratios, *LMA* leaf mass per area

Different letters indicate significant differences between treatments within an experiment (values shown in bold) (one-way ANOVA, *LSD* post hoc test)
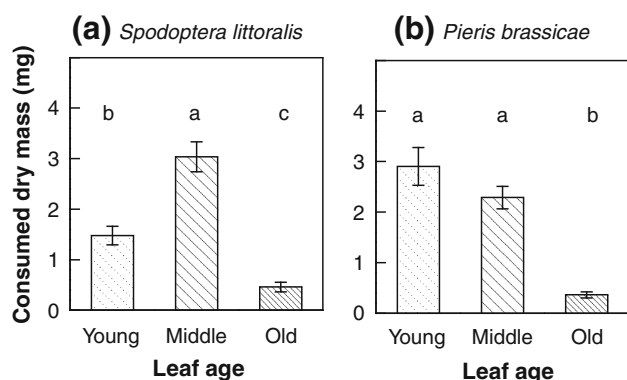

Fig. 2 Leaf dry mass (mean ± SE) consumed of differently aged *Brassica oleracea* leaves by **a** *Spodoptera littoralis* and **b** *Pieris brassicae*. Different letters above *bars* indicate significant differences in triple-choice tests (three plant series; repeated measures mixed model, *LSD* post hoc test)
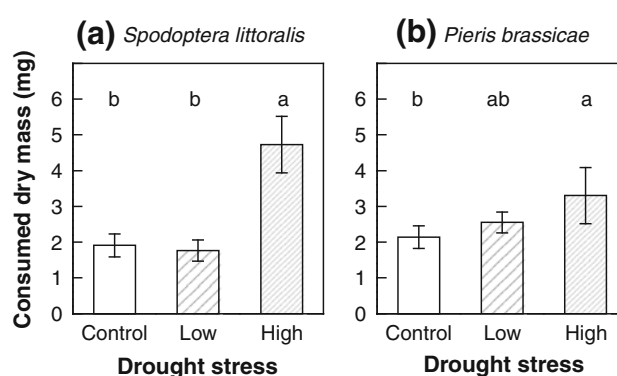
Fig. 3 Leaf dry mass (mean ± SE) consumed of *Brassica oleracea* plants grown under well-watered control or drought stress treatments by **a** *Spodoptera littoralis* and **b** *Pieris brassicae*. Different letters above *bars* indicate significant differences in triple-choice tests (two plant series; repeated measures mixed model, *LSD* post hoc test)

found, with highest levels of glucobrassicin (Fig. 1c). Glucobrassicin concentrations significantly increased in *Spodoptera*-damaged plants compared to the undamaged control. In *Pieris*-damaged plants mean glucobrassicin levels were also elevated compared to control plants; however, the levels were not statistically different and did not reach the levels found in *Spodoptera*-damaged plants (Fig. 1c). On average, control plants in this experiment showed elevated glucosinolate concentrations, including glucobrassicin, in comparison to plants of the leaf age and drought stress experiment. Herbivore damage had no effect on total nitrogen, C:N ratio or leaf mass per area (Table 1).

Spodoptera littoralis consumed significantly more leaf disc mass of undamaged control plants than of *Spodoptera*-damaged (paired samples *t* test; *t* = 2.9, *P* < 0.01, *n* = 18) and *Pieris*-damaged plants (paired samples *t* test; *t* = 3.6, *P* < 0.01, *n* = 18; Fig. 4a). In dual-choice tests comparing

Spodoptera-damaged with *Pieris*-damaged plants, no significant difference was found in the feeding preference of *S. littoralis* (paired samples *t* test; *t* = −1.2, *P* = 0.3, *n* = 17). The feeding choice of *P. brassicae* was not affected by previous damage of either insect species (paired samples *t* test; undamaged vs. *Spodoptera*-damaged: *t* = −0.2, *P* = 0.9, *n* = 18, undamaged vs. *Pieris*-damaged: *t* = 1.7, *P* = 0.1, *n* = 18, *Spodoptera*-damaged vs. *Pieris*-damaged: *t* = 0.1, *P* = 0.9, *n* = 18; Fig. 4b).

Discussion

The results of our study suggest that *B. oleracea* plants express significantly within-plant, leaf age-related variation of resistance to insect herbivores and in plant chemistry. Furthermore, we found that the abiotic environmental
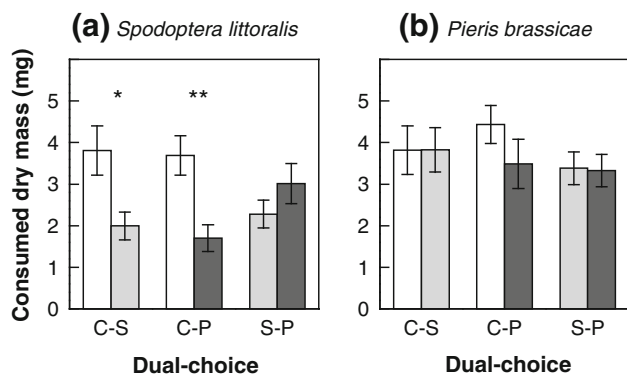
Fig. 4 Leaf dry mass (mean ± SE) consumed of systemically induced *Brassica oleracea* plants by **a** *Spodoptera littoralis* and **b** *Pieris brassicae*. Dual-choice feeding tests: undamaged control versus *Spodoptera*-damaged (C–S); undamaged control versus *Pieris*-damaged (C–P); and *Spodoptera*-damaged versus *Pieris*-damaged (S–P). *Asterisks* indicate significant differences between bars in dual-choice tests (*t* test): $*P < 0.05$, $**P < 0.01$

factor 'drought stress' as well as the biotic environmental factor 'herbivore damage' evoked significant variation in *B. oleracea* resistance. The variation of the analyzed defense compounds largely corresponded with the detected herbivore feeding preferences, although differences in the effects of glucosinolate concentrations on the generalist and the specialist were apparent. The generalist *S. littoralis* and the specialist *P. brassicae* showed diverging feeding responses to different leaf ages and to herbivore-damaged plants. This finding supports the general notion that plant resistance strongly depends on herbivore species identity and that the contribution of particular defense traits to a plant's overall resistance may strongly vary with the specific herbivores actually attacking a plant.

We found an overall decrease in leaf glucosinolate and nitrogen concentrations from young to old leaves as well as diverging herbivore feeding preferences for different leaf ages. The generalist *S. littoralis* preferred feeding on middle-aged leaves with intermediate glucosinolate and intermediate nitrogen concentrations, while the specialist *P. brassicae* preferred feeding on young leaves that were characterized by higher glucosinolate and nitrogen concentrations in comparison. Reduced levels of defense compounds as well as carbon and nitrogen in old leaves have previously been described for several Brassicales, including *B. oleracea* (Gols et al. 2007; Travers-Martin and Müller 2008). Higher levels of defense compounds in younger, more valuable leaves (Harper 1989), are expected according to the optimal defense hypothesis, which states that plant defenses are adjusted to the risk of damage, the cost of a module and its value to a plant (McCall and Fordyce 2010; Rhoades 1979). However, specialist herbivores, such as *P. brassicae*, often rely on these compounds as host recognition cues and may be attracted to high levels

of specific defense compounds in young leaves, resulting in an inverse effect to the optimal defense hypothesis (Lankau 2007; Renwick and Lopez 1999). Furthermore, *P. brassicae* as a specialist herbivore is known to tolerate high concentrations of glucosinolates due to successful detoxification mechanisms (Agerbirk et al. 2006) and may even gain a nutritional benefit when feeding on diets of higher glucosinolate concentrations (Smallegange et al. 2007). Hence, our findings are in line with previous conclusions of studies reporting a phytochemically mediated diverging feeding preference for leaf age of specialists and generalists, with young leaves being attractive for specialists but avoided by generalists (Blüthgen and Metzner 2007; Lambdon and Hassall 2005). Interestingly, however, although our findings confirm that generalists may avoid strongly protected glucosinolate-rich leaves (young leaves) (Arany et al. 2008; Gols et al. 2008; Gutbrodt et al. 2011a), in the current study *S. littoralis* did not simply choose the lowest glucosinolate concentration available (old leaves), which may indicate that a certain threshold level defines deterrence properties of glucosinolates for this generalist species. In conclusion, both generalists and specialists may perceive leaf age-related variations in defense compounds, possibly resulting in feeding preferences for a specific leaf age of host plants.

Drought stress clearly affected certain plant properties and resistance to insect herbivores, with a significant increase in δ ^{13}C values and in C:N ratios, and increased feeding by herbivores compared to well-watered conditions, but did not affect glucosinolate concentrations. Many hypotheses address drought-induced alterations of plant chemistry (Gutbrodt et al. 2011a) and suggest stressed plants to be nutrient-richer (White 1984), but also to show an accumulation in defense compounds (Huberty and Denno 2004; Rhoades 1979), especially at intermediate levels of drought stress (Herms and Mattson 1992). Hence, consequences of drought stress for insect herbivores are difficult to predict and generally depend on complex interactions of numerous factors (Gutbrodt et al. 2011a; Mody et al. 2009). In this study, changes in δ ^{13}C values indicated drought treatments to impair photosynthetic activity in stressed plants. Total nitrogen was highest and C:N ratio lowest in well-watered control plants, which contradicts the plant stress hypothesis (White 1984). A significant shift in the C:N ratio may indicate the importance of primary metabolites in mediating feeding preferences for stressed plants, as certain sugar compounds are known to increase under drought conditions (Gutbrodt et al. 2012). Furthermore, concentrations of main secondary defense compounds, glucosinolates, were unaffected by drought stress. These findings contrast general theory and previous studies reporting increased (Schreiner et al. 2009; Zhang et al. 2008) or decreased (Gutbrodt et al. 2011a;

Khan et al. 2011) glucosinolate levels in drought-stressed Brassicales, including Brussels sprouts (Ciska et al. 2000; Heaney et al. 1983). Nevertheless, both tested herbivore species in our study showed a similar feeding preference for severely drought-stressed plants. Hence, plant resistance was reduced in plants suffering severe stress but not increased in intermediately stressed plants as proposed by the growth-differentiation balance hypothesis (Herms and Mattson 1992). Our results suggest other drought-sensitive compounds to additionally mediate feeding preferences in the *B. oleracea*-herbivore system, such as, for example, flavonoids or sugars (Gutbrodt et al. 2012), which have previously been identified as feeding stimulants for herbivores of *B. oleracea* (Bartlet et al. 1994; van Loon et al. 2002). Additionally, water availability can considerably affect leaf surface waxes (Björkman et al. 2011), which may pose an important form of defense against both specialist and generalist insect herbivores (Hariprasad and van Emden 2010). Irrespective of the responsible underlying mechanisms, the finding of a drought-mediated feeding preference for both the specialist and generalist insect herbivore may indicate possible future shifts in plant susceptibility in case of increased and prolonged drought events.

Herbivore damage resulted in increased concentrations of the main indole glucosinolate glucobrassicin, while the levels of the other glucosinolates and of nitrogen and carbon remained unaffected. Glucobrassicin was elevated in plants following damage by both the generalist and the specialist herbivore. However, in quantitative terms, the increase in glucobrassicin concentration in damaged plants compared to undamaged control was significant only following damage by *S. littoralis* with intermediate concentrations in plants damaged by *P. brassicae*. Although herbivore damage to *B. oleraceae* seems to trigger a rather general induction pathway of the myrosinase-glucosinolate defense system, leading to increased levels of indole glucosinolates independent of herbivore specialization (Poelman et al. 2008; Textor and Gershenzon 2009), generalist and specialist herbivores may exert oppositional selection pressures on the expression of induced resistance (Lankau 2007). Considering that larvae of *S. littoralis* as generalist herbivores are strongly repelled by indole glucosinolates, including glucobrassicin, while larvae of *P. brassicae* as specialist herbivores are attracted to these compounds (Müller et al. 2010; Schlaeppi et al. 2008), it may be beneficial for a plant to respond more strongly to damage by generalist herbivores. Nevertheless, in our study herbivore responses indicated previous damage by both conspecifics and heterospecifics to enhance the resistance of *B. oleracea* to the generalist *S. littoralis*. Moreover, the feeding preference of the specialist *P. brassicae* was not affected by herbivore damage, indicating the increase in glucobrassicin to have neither attractant

nor deterrent effects. This finding contrasts with former studies that report pierid species to be attracted to previously damaged plants, promoting oviposition as well as larval feeding on induced, glucosinolate-enriched plants compared to undamaged plants (Mattiacci et al. 2001b; Poelman et al. 2010). However, we cannot exclude that induced resistance in *B. oleracea* may negatively affect pierid performance in the long run, for example by prolonged development on damaged plants (Agrawal and Kurashige 2003; Mattiacci et al. 2001b; Qiu et al. 2009). According to our study, glucobrassicin seems to be a likely candidate compound responsible for induced resistance in *B. oleracea* at least to generalist insect herbivores.

In conclusion, the direct comparison of specialist and generalist herbivore feeding behavior on plants varying in their chemistry due to different abiotic and biotic factors provides new insights into the individual effects of some of the multiple interacting factors that may influence plant–herbivore interactions. Our study highlights that even domesticated *B. oleracea* plants may show strong within-plant and stress-mediated between-plant variation in resistance to different insect herbivores and in defense-related plant chemistry, and that they show marked phenotypic plasticity, as indicated by the large amount of variation in glucosinolate concentrations observed across the different experiments even under controlled growth conditions. Variation in glucosinolates was also partially related to herbivore feeding preferences, and the glucosinolate glucobrassicin was found to be a potential key compound in conveying induced resistance to lepidopteran larvae. The observation that contrasting feeding responses of the specialist and generalist herbivore were only found when the glucosinolate levels of tested leaves were high, points to a possible threshold level up to which generalist herbivores can tolerate defense compounds. The study emphasizes the need to consider specific herbivore and plant characteristics to understand and predict responses of herbivores to plants affected by variable abiotic and biotic stressors.

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