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Drought stress affects constitutive but not induced herbivore resistance in apple plants

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Abstract Plant–herbivore interactions are influenced by chemical plant traits, which can vary depending on the plants' abiotic and biotic environment. Drought events, which are predicted to become more frequent and prolonged due to climate change, may affect primary and secondary plant metabolites contributing to constitutive resistance. Furthermore, the ability of plants to respond to herbivore attack in terms of induced resistance may be altered under drought conditions. We assessed the effects of drought stress on constitutive and induced apple plant resistance to a generalist insect herbivore by quantifying plant and herbivore responses in concert. Plants were exposed to different drought stress intensities (constitutive resistance) and subsequently to herbivore damage treatments that included different damage durations (induced resistance). As drought stress intensified, plant growth and concentrations of the leaf phenolic phloridzin decreased, whereas leaf glucose concentrations increased. Changes in fructose concentrations and in herbivore feeding preferences indicated a non-monotonic shift in constitutive resistance. Moderately stressed plants showed reduced fructose concentrations and were consumed least, while severely stressed plants were fructose-enriched and consumed most compared to well-watered control plants showing intermediate fructose concentrations and palatability. We found no evidence for effects of drought stress on induced resistance, as herbivore feeding preferences for undamaged over damaged plants were independent of

drought intensity. Our results suggest a strong role of primary metabolites for drought-dependent variation in constitutive plant resistance and offer novel experimental insights into the effects of drought stress on induced plant resistance across a gradient of water deprivation.

Keywords Climate change · Constitutive and induced defense · Growth-differentiation balance hypothesis · *Malus domestica* · *Spodoptera littoralis*

Introduction

Understanding the effects of global climate change on plant–insect interactions remains a challenging and urgent quest. In the scope of climate change, drought occurrences are predicted to steadily increase in the future (Christensen et al. 2007; Easterling et al. 2000), most likely causing shifts and alterations in chemically mediated interactions between drought-stressed host plants and their associated herbivores (McDowell et al. 2008; Tylianakis et al. 2008). Plant responses to drought involve adaptations in morphology, physiology and biochemistry (Chaves et al. 2003), which may affect plant resistance to insect herbivores in a complex way (Kanaga et al. 2009; Massad and Dyer 2010).

Different hypotheses in plant defense theory predict the potential effects of changing abiotic conditions on plant resistance mediated by nutritional quality and secondary defense compounds. The plant stress hypothesis (White 1969) and the optimal defense hypothesis (Rhoades 1979) assume a decreased resistance in stressed plants due to an increased availability of nutrients and a limitation of stressed plants to invest in costly defense. The plant vigor hypothesis (Price 1991) suggests stressed plants to have an

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increased resistance compared to well-watered fast-growing plants. These seemingly oppositional hypotheses are now accepted as complementary views with strong emphasis on the characteristics of the involved herbivores (Gutbrodt et al. 2011a; Huberty and Denno 2004; White 2009). Moreover, plants experience stress along a certain continuum rather than as a present-absent state, and plant resistance can increase or decrease along a stress gradient (Mody et al. 2009). The most important hypothesis considering plant responses on a scale of low to severe stress is the growth-differentiation balance hypothesis (Herms and Mattson 1992). According to this hypothesis, plant resistance is presumed to be largest at an intermediate level of stress, predicting a parabolic response of plant resistance to drought stress (Herms and Mattson 1992). At intermediate levels of stress, plant growth is restricted before photosynthesis is affected, resulting in higher concentrations of secondary defense compounds in plants exposed to intermediate stress levels compared to non-stressed fast-growing plants, whereas in severely stressed plants, both growth and secondary metabolism are impaired (Herms and Mattson 1992).

Plant resistance to insect herbivores acts on multiple levels involving complex plant-plant (Agrawal et al. 2006; Plath et al. 2011), plant-herbivore (Plath et al. 2012; Wu and Baldwin 2010) and plant-herbivore-antagonist (Kessler and Heil 2011; Mody et al. 2011) interactions. Plant properties conveying resistance limit host recognition of herbivores (escape), reduce herbivore feeding (antixenosis) and negatively affect herbivore performance (antibiosis) (Frei et al. 2003; Stoeckli et al. 2008). Chemical plant traits involved in changing plant resistance comprise attractants and feeding stimulants as well as toxic compounds (Howe and Jander 2008; Smith and Clement 2012). Primary plant metabolites such as sugar compounds may act as feeding attractants and stimulants (Isono and Morita 2010), whereas secondary plant metabolites may stimulate feeding, but can also act as deterrents or toxic compounds (Schoonhoven et al. 2005). Plant resistance may occur independently of herbivore presence (constitutive resistance), and it may also be up-regulated or newly developed following herbivore damage (induced resistance) (Karban and Baldwin 1997; Stout 2007). General signaling pathways, involving the phytohormone jasmonic acid and its derivatives, have been identified to play the central role in induced plant responses to herbivore damage (Bodenhausen and Reymond 2007; Koo and Howe 2009). However, the effect of induced resistance on herbivores strongly depends on the time interval between herbivore damage and plant response (Nykänen and Koricheva 2004) as defenses cannot be up-regulated immediately (Metlen et al. 2009). Considering the frequent occurrence of induced resistance in plants and the additional requirement of plants to actively respond to changing environmental conditions, it

is important to include this aspect of plant resistance when assessing effects of abiotic stress on plant-herbivore interactions.

The growth-differentiation balance hypothesis explicitly addresses induced resistance separately from constitutive resistance and suggests the capacity of plants to induce defenses to become increasingly limited as stress increases due to physiological constraints (Herms and Mattson 1992). Such constraints may comprise a lack in resources to synthesize new compounds (Strauss et al. 2002) as well as restricted signaling pathways in slow-growing and photosynthetically inactive plants (André et al. 2009; Heil and Ton 2008). Additionally, stressed plants may show delayed induced resistance requiring longer periods between herbivore damage and plant response compared to well-watered plants (Gutbrodt et al. 2011b). Drought-mediated changes in induced resistance may thus counterbalance but also enhance drought effects on constitutive resistance, which emphasizes the need to consider the interplay of abiotic and biotic plant stress to better understand herbivore-plant interactions. However, experimental studies testing the effects of drought stress on plant resistance and the growth-differentiation balance hypothesis so far have either only included one drought stress modality (Hale et al. 2005; Halpern et al. 2010; Haugen et al. 2008; Olson et al. 2009) or have not specifically addressed the aspect of induced resistance (Ballhorn et al. 2011; Daane and Williams 2003; English-Loeb et al. 1997; Mody et al. 2009; Showler and Moran 2003).

The main objectives of this study were (1) to determine the effects of drought stress simultaneously for constitutive and induced plant resistance to a generalist insect herbivore by focusing on antixenosis effects and (2) to characterize stress-related changes in primary and secondary plant compounds in leaves of apple seedlings, which were used as a model study system. As a measure of plant resistance in terms of antixenosis, feeding preferences of the insect herbivore were assessed for plants grown under different watering treatments and experiencing herbivore damage over two different time periods. Plant growth and plant chemistry were quantified to characterize plant responses to drought and to explain observed resistance patterns.

Materials and methods

Study organisms

Apple plants (*Malus x domestica* Borkh., var. Golden Delicious, open-pollinated) were grown from pregerminated seeds in 200-ml pots filled with 3 cm Perlite, 6 cm of soil ('Optima Einheitserde', Optima-Werke, Arlesheim, Switzerland: a peat substitute with a pH-value between 5.5

and 6.2, containing following nutrients: 400 mg/l total nitrogen, 200 mg/l phosphorous, 370 mg/l potassium, 2,200 mg/l calcium, 32 mg/l magnesium, trace elements and chelates) and a 5-mm layer of quartz sand. The plants were grown in a common greenhouse under day:night conditions of 22:18°C, 65% RH and a L16:D8 light cycle.

Larvae of the noctuid moth *Spodoptera littoralis* Boisduval were used as herbivores in this study. Being generalist leaf-chewing herbivores, *S. littoralis* larvae have a broad host range that includes apple (Salama et al. 1970). *S. littoralis* larvae were obtained weekly from Syngenta Crop Protection AG, Stein, Switzerland on artificial diet. Prior to herbivore damage treatments and feeding tests, *S. littoralis* larvae were fed with fresh apple leaves for at least 24 h and held in insect rearing cages exposed to greenhouse conditions (see above).

Experimental set-up

When apple seedlings were 4 weeks old, they were randomly assigned to one of three drought stress levels (well-watered control, moderate drought stress or severe drought stress) and then subsequently subjected to one of two herbivore damage treatments (undamaged control or herbivore damaged). Two different durations of herbivore damage were tested according to this set-up (48 and 72 h). The experiments in which herbivore damage treatments lasted 48 h were replicated three times (replicates 1, 2 and 3; $n = 42$ plants per drought stress level and herbivore damage treatment combined over the three replicates), and the herbivore damage treatment lasting 72 h was analyzed in a single replicate ($n = 20$ plants per drought stress level and herbivore damage treatment). The impact of drought stress on primary and secondary apple leaf chemistry was assessed by quantification of soluble sugars and of the two main apple phenolics, phloretin and its glucoside phloridzin (Gosch et al. 2009), in undamaged plants. As a measure of plant resistance, herbivore feeding preferences were quantified in choice-bioassays using undamaged plants (constitutive resistance), or by comparing undamaged and damaged plants (induced resistance).

Drought stress treatment

Apple seedlings (4 weeks old; average leaf number: 8.2 ± 0.1 ; shoot height: 12.0 ± 0.2 cm) were randomly subjected to one of three drought stress levels: no drought stress (i.e. well-watered control), moderate drought stress (i.e. low stress) or severe drought stress (i.e. high stress). To simulate different natural stress conditions with alternating dry and wet periods (Huberty and Denno 2004), drought treatments were performed as pulsed (intermittent) drought stress treatments (Mody et al. 2009), which means

that a certain drought period was followed by rehydration of the plants. Plants of a specific drought level were all watered simultaneously and received the same amount of water. Well-watered control plants received water every 1–2 days to ensure constantly moistened soil throughout the experiment. Low stress plants were watered every 2–3 days when leaves started drooping and received 40–50% 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, resulting in 15–20% the amount of water supplied to well-watered control plants. After approximately 4 weeks and a minimum of three drought cycles for high stress plants, the drought stress treatment was terminated. All plants were rehydrated overnight (12 h) and subsequently subjected to the herbivore damage treatment. Rehydration of plants was conducted to ensure that herbivore responses reflected plant responses to the different stress treatments and not merely differences in leaf water content (vigorous and wilting plants).

Herbivore damage treatment

Apple plants of each drought stress level were randomly assigned to either an undamaged control treatment or an herbivore damage treatment, with treatments lasting 48 or 72 h. Irrespective of treatment, one round clip cage (4 cm diameter) was mounted to the fifth youngest leaf for the first half of the damage duration and then to the fourth youngest leaf for the remaining duration of the herbivore damage treatment. Clip cages mounted to plants belonging to the herbivore damage treatment contained two (L2-L3) *S. littoralis* larvae, while the clip cages of plants assigned to the undamaged control remained empty. Herbivores started feeding immediately and caused consistent damage throughout the damage period. After termination of the herbivore damage treatment, clip cages were removed and feeding tests were conducted.

Plant responses to drought stress

The effects of drought stress on plant growth and leaf primary and secondary chemistry were assessed in undamaged plants. The production of new leaves and shoot growth during the drought stress treatment was recorded for every plant individual. Additionally, the content in soluble sugars as well as the concentrations of the main secondary metabolites phloridzin and phloretin was quantified in the second youngest leaf of a random subsample of undamaged plants used in feeding tests. Sugar content was quantified in plants of each drought stress level in replicate 2 ($n = 13$), and leaf phenolics were quantified in a subsample of plants of each drought stress level in replicate 3

($n = 7$). For chemical analyses, the leaf blade material remaining after excision of leaf disks for herbivore feeding tests was immediately flash frozen in liquid nitrogen, freeze-dried and stored at -60°C until analysis.

Individual soluble sugar compounds were extracted and quantified in 60-mg dried apple leaf samples according to established methods (Cheng et al. 2005; Li and Li 2005). Dried apple leaves were ground to a fine powder and extracted three times with 10 ml of 80% ethanol at 80°C for 30 min, followed by centrifugation (650 g, 10 min). The supernatants were combined and evaporated to dryness at 55°C , redissolved in 10 ml water and filtered (0.45 μm). Sorbitol, glucose, fructose and sucrose concentrations were quantified by HPLC (Dionex GS50 Series, Sunnyvale, CA, USA) equipped with an ED50 electrochemical detector. The compounds were separated on a 2×250 mm CarboPac PA-1 column (Dionex, Sunnyvale, CA, USA) maintained at 30°C using a gradient of water (solvent A) and 20 mM sodium hydroxide (solvent B): 4.5–25% B (16 min), 25–50% B (6 min), 50–4.5% B (6 min), 4.5% B (7 min). The individual sugar compounds were identified at appropriate dilutions and quantified based on peak area and the calibration curve derived from corresponding pure sugar standards (Sigma-Aldrich, Buchs, Switzerland).

Apple phenolics phloridzin and phloretin were analyzed as in Gutbrodt et al. (2011b). Dried leaf material was homogenized with methanol (10 ml) containing 1% 2,6-di-tert-butyl-4-methylphenol and extracted for 1 h while shaking. The extract was centrifuged (650 g, 10 min), and the solid residue was extracted a second and third time with pure methanol (10 and 5 ml) for 30 min each and centrifuged. The extracts were combined and evaporated to dryness. The samples were stored under argon at -60°C until analysis. For determination of phloridzin and phloretin, samples were redissolved in 10 ml of 1% aqueous acetic acid:acetonitrile (3:2, v/v) and analyzed with an HPLC–DAD system (Merck-Hitachi ‘La Chrome’, Darmstadt, Germany) equipped with a diode array detector (L-7450A). Separation was achieved on an C18 column (Aqua, 250×4.6 mm, 5 μm , Phenomenex, Torrance, CA, USA) using a gradient of 2% aqueous acetic acid (solvent A) and 0.5% aqueous acetic acid:acetonitrile (1:1, solvent B): 20–55% B (10 min), 55–100% B (5 min), 100% B (5 min). The wavelength for detection of analyzed compounds was 280 nm, and spectra were acquired from 190 to 450 nm. Identification of phloridzin and phloretin was achieved by comparing their spectra and retention times with those of authentic compounds (Sigma-Aldrich, Buchs, Switzerland).

Herbivore feeding preference

Choice tests were conducted to quantify herbivore feeding preferences as a measure of resistance of plants exposed to

different drought stress and herbivore damage treatments. Choice tests included triple-choice (constitutive resistance: direct comparison of the three drought stress levels of undamaged plants) and dual-choice (induced resistance: direct comparison of undamaged and damaged plants at the same drought stress level) feeding tests. Triple-choice feeding tests were conducted with the second youngest fully developed leaf in replicates 1–3, and dual-choice feeding tests were conducted with the youngest fully developed leaf for the 48-hour damage treatment in replicates 2 and 3 and the 72-hour damage treatment.

Generally, choice tests followed the procedure described by Mody et al. (2009). Leaves were cut at petiole level, and standardized disks (18 mm) were excised from leaf centers. The leaf disks were equidistantly (approx. 1 cm) placed in the center of plastic Petri dishes (9 cm diameter) inlaid with a moist filter paper to prevent desiccation of the larvae on a Styrofoam layer (3 mm). A single *S. littoralis* larva (L2) was placed in the center of each Petri dish and allowed to feed freely for 12 h. After the feeding tests, digital photographs were taken, and the consumed dry mass (CM) was calculated for each leaf disk based on the consumed area (CA), remaining leaf disk area (RA) and dry weight of the remaining leaf disk (RM): $\text{CM} = (\text{RM}/\text{RA}) \times \text{CA}$.

Statistical analysis

Data were transformed to meet the assumptions of normality and heteroscedasticity when necessary. Effects of drought stress on the number of new leaves (square root-transformed) and shoot growth (\log_{10} -transformed) were analyzed by full-factorial two-way ANOVAs with ‘drought’ as fixed factor and ‘replicate’ as random factor, followed by *LSD* post hoc tests. Plants of the 72-hour damage treatment were considered as a fourth replicate in this analysis. Concentrations of the individual sugar compounds sorbitol, glucose, fructose and sucrose were \log_{10} or $\log_{10}(x + 1)$ -transformed, and effects of drought stress were analyzed using a one-way ANOVA with ‘drought’ as fixed factor, followed by *LSD* post hoc tests. The effect of drought stress on the individual leaf phenolics, phloridzin and phloretin was analyzed using \log_{10} -transformed data and one-way ANOVAs with ‘drought’ as fixed factor, followed by *LSD* post hoc tests. Consumed dry masses of leaf disks in herbivore feeding tests were $\log_{10}(x + 1)$ -transformed and analyzed as Petri dish-dependent variables in mixed model analysis, followed by *LSD* post hoc tests. For constitutive resistance assessed in triple-choice tests, ‘drought’ was the repeated within-subject fixed factor and ‘replicate’ the random factor. Covariance estimates were used to assess effects of the random factor in mixed model analyses. For induced resistance assessed in dual-choice tests, a mixed model with ‘damage’ as repeated fixed factor and ‘drought’ as between-

subject fixed factor was used. Additionally, ‘replicate’ as fixed factor was used to analyze consumed dry masses in the combined analysis of replicates 2 and 3. All statistical analyses were conducted using SPSS Statistics 19 for MacOSX (2010; IBM, Armonk, NY, USA).

Results

Plant responses to drought stress

Increasing drought stress resulted in a significant, monotonic reduction of plant growth (‘drought’; Table 1). The number of leaves as well as the length of shoots newly produced during the drought treatments was highest in well-watered control plants, intermediate in low stress plants and lowest in high stress plants (Table 1). This pattern was observed across all independent replicates covering plants of both the 48- and 72-hour damage treatments (‘drought’ × ‘replicate’; Table 1), although plant growth differed significantly between the replicates (‘replicate’; Table 1).

Individual soluble sugar compounds in apple leaves were differently affected by drought stress intensity (Fig. 1). Glucose concentrations increased monotonically from well-watered control to low stress plants and again from low stress to high stress conditions. Fructose concentrations varied in a non-monotonic way: they were highest in high stress plants and lowest in low stress plants and showed an intermediate level in the well-watered control plants (Fig. 1). Sorbitol and sucrose concentrations were not significantly affected by different drought stress intensities experienced by plants, although mean values of sorbitol increased along the stress gradient (Fig. 1).

Concentrations of the main apple phenolic phloridzin were also affected by changing drought conditions (one-way ANOVA; $F_{2,18} = 8.4$, $P < 0.001$), while phloretin remained unaffected ($F_{2,18} = 0.6$, $P = 0.6$). Similar to plant growth and contrary to leaf glucose, phloridzin levels decreased with increasing drought stress with significantly reduced levels in high stress plants compared to the well-watered control (Fig. 2).

Constitutive resistance and drought stress

Leaf consumption by *S. littoralis* larvae was significantly different for apple plants exposed to different drought stress levels (‘drought’, mixed model: $F_{2,33} = 16.6$, $P < 0.001$), and no differences between replicates were recorded (covariance estimate ‘replicate’: $P = 0.4$). In line with non-monotonic changes in fructose concentrations and in contrast to plant growth, glucose concentrations and secondary chemistry, preference patterns changed in a non-monotonic way. Herbivores consumed highest amounts of leaf disk material of high stress plants, intermediate amounts of well-watered control and lowest amounts of low stress plants (Fig. 3).

Induced resistance and drought stress

Herbivore damage caused significant differences in herbivore preferences for damaged and undamaged plants, however only after a time lag exceeding 48 h (‘damage’; Table 2). Herbivore damage subjected to plants during 72 h significantly enhanced apple resistance (induced resistance) as herbivores preferred undamaged over damaged plants (Table 2; mean consumed dry mass ± SE

Table 1 Effect of drought stress on plant growth (mean ± SE) measured as (a) number of new leaves and (b) shoot growth (length in cm)

	Drought stress treatment			Statistics*
	Control a	Low stress b	High stress c	
a) No. new leaves				
Replicate 1	7.4 ± 0.4	6.4 ± 0.2	4.7 ± 0.3	Drought: $F_{2,371} = 74.9$, $P < 0.001$
Replicate 2	6.9 ± 0.2	5.5 ± 0.3	3.6 ± 0.2	Replicate: $F_{3,371} = 31.9$, $P < 0.001$
Replicate 3	6.4 ± 0.2	4.9 ± 0.2	4.1 ± 0.2	Drought × Replicate: $F_{6,371} = 1.7$, $P = 0.1$
Replicate 4	5.8 ± 0.3	4.7 ± 0.2	3.4 ± 0.2	
b) Shoot growth				
Replicate 1	11.8 ± 0.8	8.0 ± 0.5	6.1 ± 0.4	Drought $F_{2,371} = 44.6$, $P < 0.001$
Replicate 2	11.1 ± 0.7	9.4 ± 0.7	5.8 ± 0.4	Replicate $F_{3,371} = 39.5$, $P < 0.001$
Replicate 3	11.6 ± 0.8	8.1 ± 0.4	6.7 ± 0.3	Drought × Replicate: $F_{6,371} = 1.3$, $P = 0.2$
Replicate 4	9.2 ± 0.8	7.3 ± 0.5	5.4 ± 0.4	

* Statistical analyses were conducted with ‘drought stress’ as fixed factor and ‘replicate’ as random factor in two-way ANOVAs (significant effects in *bold*)

Different letters a, b, c indicate significant differences ($P < 0.05$) between drought stress treatments across all replicates (two-way ANOVAs, *LSD* post hoc test)

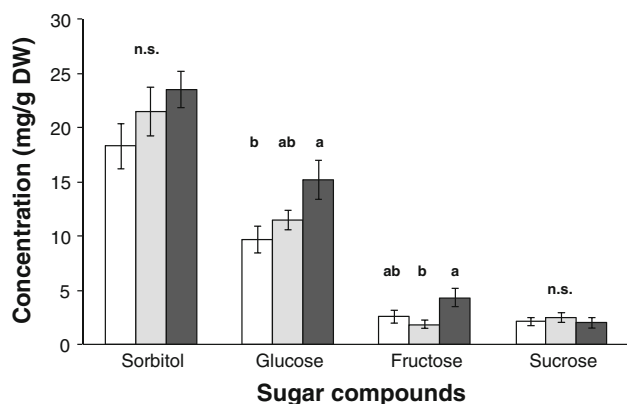


Fig. 1 Concentrations of individual soluble sugar compounds (mean \pm SE) in undamaged apple plants experiencing different levels of drought stress: well-watered control (white bars), low stress (gray bars) and high stress plants (dark bars). Different letters indicate significant differences ($P < 0.05$) between drought treatments (one-way ANOVAs, LSD post hoc test)

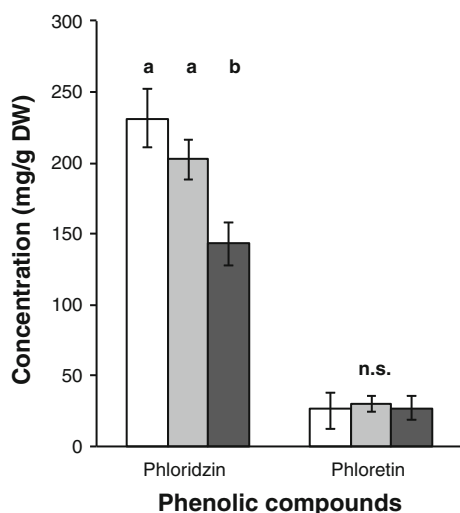


Fig. 2 Phloridzin and phloretin concentrations (mean \pm SE) in undamaged apple plants experiencing different levels of drought stress: well-watered control (white bars), low stress (gray bars) and high stress plants (dark bars). Different letters indicate significant differences ($P < 0.05$) between drought treatments (one-way ANOVAs, LSD post hoc test)

undamaged: 2.5 ± 0.3 mg; damaged: 1.5 ± 0.2 mg). The expression of induced resistance was not affected by drought stress ('damage' \times 'drought'; Table 2), and the total amount of consumed dry mass was not significantly different between drought levels ('drought'; Table 2).

Discussion

Our study demonstrated that drought stress may have differing effects on constitutive and induced plant resistance to insect herbivores. Whereas differing drought stress

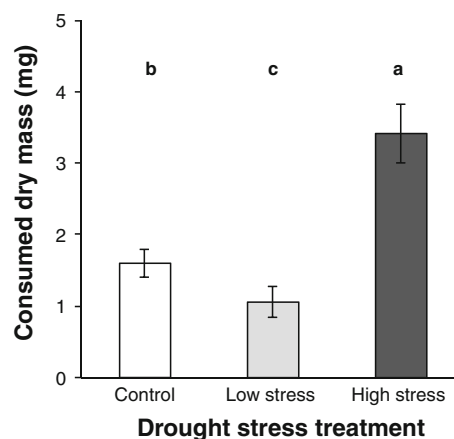


Fig. 3 Dry mass (mean \pm SE) consumed by *Spodoptera littoralis* from apple leaf disks when offered undamaged plants of different drought stress intensities in triple-choice feeding tests. Different letters indicate significant differences ($P < 0.05$) between drought stress treatments across damage treatments and replicates (mixed model, LSD post hoc test)

Table 2 Effects of damage treatment on herbivore feeding preferences under different drought stress conditions measured as dry mass (mean \pm SE in mg dry mass) consumed by *Spodoptera littoralis* in dual-choice feeding tests with damage treatments lasting 48 and 72 h (linear mixed models)

Damage period	Factor	df	F	P
48 h	Damage	1	0.6	0.4
	Drought	2	0.4	0.7
	Damage \times Drought	2	0.2	0.9
	Replicate	1	0.1	0.8
	Error	59		
72 h	Damage	1	10.5	< 0.01
	Drought	2	1.4	0.3
	Damage \times Drought	2	0.6	0.6
	Error	53		

intensities resulted in a non-monotonic expression of constitutive plant resistance, induced resistance responses were surprisingly independent of drought stress. Furthermore, fructose concentration in leaves was the only observed plant trait to correspond to non-monotonic herbivore responses, suggesting this primary compound to mediate feeding preferences of *S. littoralis* for differently drought-stressed plants.

The non-monotonic expression of constitutive resistance in the studied apple plants, with an increased feeding preference of *S. littoralis* for severely drought-stressed plants and a strongly reduced acceptability of moderately stressed plants, was a consistent finding across several independent trial replicates. It is also in line with a previous study in the same system, which equally reported non-monotonic patterns in constitutive resistance both in terms

of herbivore feeding preference (antixenosis) and herbivore performance (antibiosis) (Mody et al. 2009). Indications for non-monotonic responses in plant resistance in terms of antibiosis to drought stress were also revealed by field tests with bush beans (*Phaseolus vulgaris*) and experimental studies with tomato plants (*Solanum lycopersicum*) (English-Loeb 1990, 1997). The finding of non-monotonic resistance responses of plants corresponds to the predictions of the growth-differentiation balance hypothesis, which suggests a parabolic response in resistance with increasing resource deprivation. However, the growth-differentiation balance hypothesis states that this resistance response is due to corresponding non-monotonic changes in secondary defense compounds (Herms and Mattson 1992).

In our study, however, we observed a monotonic decrease of phloridzin, the main secondary compound of apple leaves previously suggested to affect *S. littoralis* feeding behavior (Fulcher et al. 1998; Gutbrodt et al. 2011b), with increasing drought stress. Although this finding may offer some explanation for an increased feeding preference of *S. littoralis* for high stress plants containing reduced concentrations of phloridzin compared to the well-watered control, it fails to explain why low stress plants were consumed least. To our knowledge, past findings on changes in secondary metabolites under increasing drought stress show similar discrepancy from predictions of the growth-differentiation balance hypothesis, with compounds increasing or decreasing in a monotonic fashion along a stress gradient (Ballhorn et al. 2011; Turtola et al. 2003). In our study, changes in fructose concentrations along the stress gradient closely followed herbivore response patterns, suggesting feeding preferences to be strongly linked to this primary compound. Non-monotonic responses of primary compounds to drought stress have previously been observed for the total content of soluble carbohydrates in tomato (*S. lycopersicum*) (English-Loeb et al. 1997), contrary to a lack of these findings in literature in case of secondary metabolites. In apple, previous studies do not report non-monotonic responses of primary compounds as observed for fructose in our study, but found an increase in sugar compounds with increasing drought stress intensity, similar to our findings for sorbitol and glucose (Li and Li 2005; Sircelj et al. 2005). Nevertheless, altered herbivore responses under drought conditions seem closely associated to changes in fructose levels, and secondary metabolites seem to play a minor role in drought-mediated shifts in the constitutive resistance of apple (see Berenbaum (1995) for the potential role of primary compounds for plant resistance). This finding strongly emphasizes the need to consider effects of drought stress on both primary and secondary phytochemicals to better understand plant–herbivore interactions in a changing environment.

In our study, induced plant resistance to the feeding insect larvae was not impaired by drought stress. This contradicts the growth-differentiation balance hypothesis (Herms and Mattson 1992), which suggests induced resistance to decline as plants suffer increasing intensities of drought stress. Previous studies comparing induced resistance in drought-stressed to well-watered plants report ambiguous effects of drought stress on induced resistance. Few studies actually found a decrease of chemically mediated induced resistance in stressed plants, such as for cotton (*Gossypium hirsutum*) plants, where feeding preferences of *Spodoptera exigua* for undamaged over *Spodoptera*-damaged plants disappeared when plants were drought-stressed (Olson et al. 2009). Furthermore, in wild tobacco (*Nicotina quadrivalvis*), herbivore damage by the tobacco hornworm (*Manduca sexta*) caused the induction of floral nectar only in well-watered plants (Halpern et al. 2010) and in loblolly pine (*Pinus taeda*), mechanically induced resin flow was highest in well-watered conditions (Lombardero et al. 2000). However, findings similar to our results of a drought stress-independent induced resistance were also made. In Drummond's rockcress (*Boechea stricta*), concentrations of defense compounds increased upon herbivore damage by diamondback moth larvae (*Plutella xylostella*) in both well-watered and drought-stressed plants (Haugen et al. 2008), and in wild tobacco, leaf chemicals remained unaffected by combined effects of drought stress and herbivore damage by *M. sexta* (Halpern et al. 2010). Furthermore, in black poplar (*Populus nigra*), induced resistance to gypsy moth (*Lymantria dispar*) was independent of water availability (Hale et al. 2005). To our knowledge, no studies report enhanced induced resistance to insect herbivores in drought-stressed plants, although studies assessing herbivore performance as measure for plant resistance probably cannot exclude combined effects of constitutive and induced resistance effects.

Under natural conditions, both constitutive and induced direct plant resistance act in concert, rendering the consideration of combined effects important to predict plant–herbivore interactions. Induced resistance is becoming increasingly interesting for sustainable pest control in agricultural systems (Gurr and Kvedaras 2010), and understanding its response to environmental conditions seems crucial. Although our study revealed unaltered induced resistance under drought conditions, we did not show that the underlying mechanisms remain the same in stressed and in well-watered plants. In the absence of drought stress, increased phloridzin concentrations following herbivore damage paralleled induced resistance in apple to *S. littoralis* (Gutbrodt et al. 2011b). It remains to be elucidated whether comparable factors convey induced resistance also in drought-stressed plants. Furthermore, other aspects of plant resistance may be altered in plants

subjected to drought conditions that affect plant–herbivore interactions, such as herbivore host location, herbivore performance and oviposition behavior (Showler and Moran 2003). Altered volatile emissions (Ebel et al. 1995; Vallat et al. 2005) may contribute to such behavioral changes (Vallat and Dorn 2005), and they may also cause differences in predator and parasitoid activity between stressed and well-watered plants (Yule et al. 2011). Hence, plant–herbivore interactions are determined by the combination of all resistance aspects (Agrawal 2011), which means that it is crucial to consider these when generating predictions on how drought stress may change plant–herbivore interactions.

In conclusion, this study presents experimental support for the predictions of the growth-differentiation balance hypothesis regarding constitutive plant resistance responses to drought stress. However, the hypothesis was not supported for induced resistance responses. Therefore, induced resistance to some extent may compensate reduced constitutive resistance to insect herbivores in severely drought-stressed plants. This study hence emphasizes the need to consider constitutive and induced resistance in combination when assessing effects of abiotic stress on plant–herbivore interactions, and it further underlines the importance of studying different modalities of abiotic stress. Finally, it remains to be elucidated to which extent the growth-differentiation balance hypothesis can be employed for other aspects of stress-mediated plant resistance, such as indirect resistance conveyed by natural antagonists of herbivores.

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