ORIGINAL ARTICLE

# Within-plant distribution of induced resistance in apple seedlings: rapid acropetal and delayed basipetal responses

Bettina Gutbrodt · Karsten Mody · Raphaël Wittwer · Silvia Dorn

**Abstract** Induction of plant resistance by herbivory is a complex process, which follows a temporal dynamic and varies spatially at the within-plant scale. This study aimed at improving the understanding of the induction process in terms of time scale and within-plant allocation, using apple tree seedlings (*Malus × domestica*) as plant model. Feeding preferences of a leaf-chewing insect (*Spodoptera littoralis*) for previously damaged and undamaged plants were assessed for six different time intervals with respect to the herbivore damage treatment and for three leaf positions. In addition, main secondary defense compounds were quantified and linked to herbivore feeding preferences. Significant herbivore preference for undamaged plants (induced resistance) was first observed 3 days after herbivore damage in the most apical leaf. Responses were delayed in the other leaf positions, and induced resistance decreased within 10 days after herbivore damage simultaneously in all tested leaf positions. Chemical analysis revealed higher concentrations of the flavonoid phloridzin in damaged plants as compared to undamaged plants. This indicates that herbivore preference for undamaged apple plants may be linked to phloridzin, which is the main secondary metabolite of apple leaves. The observed time course and distribution of resistance responses within plants contribute to the understanding of induction processes and patterns, and support the optimal defense theory stating young tissue to be prioritized. Moreover, induced resistance responses occurred also basipetally in leaves below the damage site, which suggests that signaling pathways involved in resistance responses are not unidirectional.

**Keywords** Apple (*Malus × domestica*) · Induced defense · Phloretin · Phloridzin · Plant resistance · *Spodoptera littoralis*

**Abbreviations**
| | |
|---|---|
| RH | Relative humidity |
| L | Larval instar |
| CM | Consumed dry mass |
| CA | Consumed area |
| RA | Remaining leaf disc area |
| RM | Remaining leaf disc dry mass |
| CMu | Consumed dry mass undamaged plant |
| CMd | Consumed dry mass damaged plant |
| PI | Preference index |
| SE | Standard error |

## Introduction

Plants are continuously threatened by insect herbivory and have evolved costly resistance responses to defend themselves, for example with secondary metabolites that have deleterious effects on insects. These defense compounds are present constitutively and induced systemically as a result of prior attack by herbivores (Baldwin and Preston 1999; Howe and Jander 2008). Generally, resistance to insects is greater in plants that have been previously damaged by herbivores as compared to undamaged plants (Stout et al. 2009). However, induced responses to herbivores are complex and depend on many factors, including

B. Gutbrodt · K. Mody (✉) · R. Wittwer · S. Dorn
ETH Zurich, Institute of Plant, Animal and Agroecosystem Sciences, Applied Entomology, Schmelzbergstrasse 9/LFO, 8092 Zurich, Switzerland
e-mail: karsten.mody@ipw.agrl.ethz.ch

plant species (Hui and Jin 2004), plant genotype (McGuire and Johnson 2006), attacking insect herbivore characteristics (Agrawal 2000; Mattiacci et al. 2001; Zong and Wang 2007), as well as abiotic parameters, such as nitrogen and water availability (Olson et al. 2009). Furthermore, induced resistance is known to vary in its timing and in within-plant allocation, two aspects that are fundamental for understanding the process of induced resistance in an ecological and evolutionary context (Baldwin and Preston 1999; Kaplan et al. 2008a).

Induced resistance is a gradual process, which follows a plant and herbivore species-specific time course (Karban and Baldwin 1997). The conceptual model suggests that responses to herbivore damage are commonly delayed in time, with an increase in resistance to a certain maximum followed by decay, if no additional damage occurs (Underwood 1998; Metlen et al. 2009). Several studies on the timing of induced resistance provide support for this suggested pattern, for example in soybean *Glycine max* (Underwood 1998), cotton *Gossypium hirsutum* (Anderson et al. 2001), alfalfa *Medicago sativa* (Agrell et al. 2003) and white clover *Trifolium repens* (Gomez et al. 2010). The time lag between herbivore damage and plant response resulting in increased resistance strongly depends on the study system and may take longer in long-living woody plants as compared to annual herbaceous plants (Zvereva et al. 1997; Zhao et al. 2010). In contrast to herbaceous plants, information on the time course of induced resistance responses in perennial woody plant species is remarkably scarce.

The optimal defense theory (Rhoades 1979) suggests that plants should prioritize defense investments to valuable parts, such as young or reproductive tissues (McCall and Fordyce 2010). Indeed, different studies found induced resistance to markedly depend on leaf age, with stronger and more persistent effects in young top leaves as compared to mature leaves, for example in cotton *Gossypium hirsutum* (Anderson and Agrell 2005), tobacco *Nicotiana sylvestris* (Ohnmeiss and Baldwin 2000), lima bean *Phaseolus lunatus* (Ballhorn et al. 2008) and rice *Oryza sativa* (Stout et al. 2009). Furthermore, leaf position relative to the damage site and not merely leaf age can be of importance in the effectiveness of induced resistance responses. First, insect herbivores may show species-specific feeding site preferences (Bingham and Agrawal 2010). Second, plants may be restricted in their induced resistance responses to specific locations due to physiological and anatomical constraints (Orians 2005; Kaplan et al. 2008b). These two factors further emphasize the need for an optimal allocation of costly defenses within a plant. To date, studies on the timing of induced resistance have rarely considered leaf position effects, although they may be crucial in understanding complex induced resistance responses.

Secondary compounds are key mediators in plant resistance against insect herbivores, and detailed analysis of their changing levels with time and their within-plant distribution may offer solid insight into the process of induced resistance (Ohnmeiss and Baldwin 2000; Howe and Jander 2008). Phenolic compounds dominate the secondary chemistry of apple plants, consisting mainly of the two dihydrochalcones phloretin (Fig. 1) and its glucoside phloridzin (Gosch et al. 2009; Szankowski et al. 2009). These two flavonoid compounds are related with phloretin being the hydrolysis product of phloridzin (Gosch et al. 2009). Phloretin is known to negatively affect insects at high concentrations (Mitchell et al. 1993). Phloridzin is the more abundant of the two dihydrochalcones in apple. It acts as feeding deterrent against adult Japanese beetles (*Popillia japonica*) (Fulcher et al. 1998), and it is reportedly involved in resistance to apple pathogens (Leser and Treutter 2005). Picinelli et al. (1995) described the inhibitory ability of phloridzin towards apple scab fungus (*Venturia aequalis*) and observed an increase in phloridzin after fungus infection, suggesting a possible induced resistance response. Petkovsek et al. (2008) further confirmed this observation, reporting a 1.6 to 3.4-fold increase in phloridzin in apple scab infected leaves. However, phloridzin as inducible broad spectrum defense compound against insect herbivores has not been reported so far. Flavonoids are suggested to have multiple functions in plants, comprising both the protection from harmful UV radiation as well as constitutive and inducible defense against herbivory (Agrell et al. 2003; Treutter 2005). The distribution and accumulation of flavonoids within plants may thus depend on complex interactions of their various functions. Hence, the significance of flavonoids, in particular that of the predominating compound phloridzin, in the process of induced resistance in apple remains to be elucidated.

This study aimed at investigating the temporal and spatial distribution of induced resistance in apple tree seedlings. The main objective of this study was to determine the time frame in which induced resistance occurs
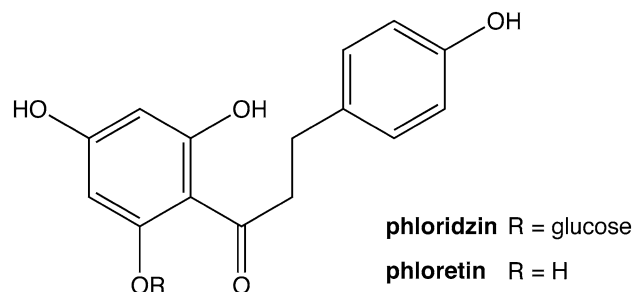


**Fig. 1** The two major flavonoid compounds in apple leaves: phloridzin and its aglycone phloretin

and how it is affected by leaf age and position in relation to damage site. Finally, main secondary plant metabolites in apple leaves were analyzed to assess their role in induced resistance.

## Materials and methods

### Study organisms

Apple plants (*Malus* × *domestica* Borkh., var. Golden Delicious, open-pollinated) were grown from pre-germinated 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, 2200 mg/l calcium, 32 mg/l magnesium, trace elements and chelates) and a 5-mm layer of quartz sand. The plants were watered regularly and grown in a greenhouse at day:night conditions of 22:18°C, L16 h:D8 h photoperiod and 65% RH.

Larvae of *Spodoptera littoralis* Boisduval (Noctuidae) were used as insect herbivores. *S. littoralis* is a leaf-chewing caterpillar with a broad host range and is reported to eagerly feed on apple leaves (Mody et al. 2009). *S. littoralis* larvae reared on artificial diet were obtained from Syngenta Crop Protection AG, Stein, Switzerland. Before feeding tests were initiated, *S. littoralis* larvae were fed with fresh apple leaves and exposed to greenhouse conditions (as above) for at least 24 h.

### Herbivore damage

Apple seedlings (8-week-old) were randomly assigned to two treatments each containing 118 plant individuals. On an average, plants were $18.2 \pm 0.3$ cm tall and consisted of 10 fully developed leaves. They were either subjected to herbivore damage inflicted over a total of 3 days, or served as undamaged control. Irrespective of treatment, one round clip cage (4-cm diameter) was first mounted to the fifth youngest leaf at 9 a.m. for 36 h and then to the fourth youngest leaf at 9 p.m. for another 36 h on all tested plants simultaneously. The fourth and fifth youngest leaf was positioned on opposite sides of the stem (90°–180° angle), and are termed damage site hereafter. According to rules of orthostichy and plant phyllotaxy (3/8 for apple), a vascular connection between the damage site and leaves positioned above and below the damage site can be presumed (Roach 1939; Kaplan et al. 2008b). Each clip cage mounted to plants belonging to the herbivore damage treatment harbored 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 (36 h) that resulted in an estimated 80–90% consumption of leaf area available in the clip cage. Plants were watered regularly during the treatment and after removal of the clip cages, and were maintained at the greenhouse conditions (as above).

### Herbivore feeding preference

Dual-choice feeding tests were conducted to determine feeding preferences of *S. littoralis* larvae for damaged and undamaged apple plants. Preferences were tested at different time intervals after the damage period (that lasted 3 days, and was initiated for all plants at the same time): immediately after removal of clip cages (i.e. 3 days after damage treatment was initiated, $n = 20$ plant pairs), as well as 4 ($n = 20$), 6 ($n = 20$), 8 ($n = 20$), 10 ($n = 19$) and 15 ($n = 19$) days after onset of herbivore damage. For every time interval since onset of damage, the preference for damaged and undamaged plants was tested for three different leaf positions in relation to the damage site: the youngest fully expanded top leaf, and the leaves directly above and directly below the damage site. Preference tests were initiated at 9 a.m. of the specific test days simultaneously for all the three leaf positions per plant pair and lasted 24 h.

Feeding tests were conducted using excised leaf discs, which previously evoked concern about the reliability of assessed herbivore responses in some systems (Jones and Coleman 1988). In our study system, however, we followed an established procedure that additionally provided comparable results to bioassays using intact plant-connected leaves (Mody et al. 2009). Leaves were cut at petiole level and standardized discs (18-mm diameter) were excised from leaf centers. The excised leaf discs were fixed with a pin in plastic Petri dishes of 9-cm diameter, inlaid with a 3-mm thick Styrofoam layer and a moist filter paper that prevented desiccation of the larvae. Single (L2) *S. littoralis* larvae were released in the center of each Petri dish and started feeding promptly. Feeding tests were conducted in shaded conditions at greenhouse conditions (as above).

After the feeding tests, digital photographs were taken and the consumed leaf area was quantified using a reference leaf disc and Adobe Photoshop CS3 (Mody et al. 2009). The consumed dry mass (CM) was calculated for each leaf disc based on the consumed area (CA), remaining leaf disc area (RA) and dry weight of the remaining leaf disc (RM): $CM = (RM/RA) \times CA$. The consumed dry masses of leaf discs from the undamaged plant (CMu) and the damaged plant (CMd) were used to calculate a preference index [$PI = CMu/(CMu + CMd)$] for each tested

Petri dish, whereby a PI of 0.5 indicates no difference in feeding between undamaged and damaged plants (Underwood 1998).

## Leaf phenolics

The main apple leaf phenolics, phloridzin and phloretin (Gosch et al. 2009) were quantified in a subsample of top leaves used in the feeding tests 4, 6, 8 and 10 days after herbivore damage of damaged and undamaged plants each. After excision of leaf discs for herbivore preference tests, the remaining leaf material was immediately flash frozen in liquid nitrogen and stored at −80°C.

Samples were freeze-dried, weighed and extracted (Escarpa and Gonzalez 1998; Kindt et al. 2007). Dried 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 −20°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 system (Agilent 1200 Series, Santa Clara, CA, USA) equipped with a G1315D diode array detector (Schieber et al. 2001). Separation was achieved on an RP $C_{18}$ column (Aqua, 125 Å, 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). The gradient was as follows: 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, which were obtained from Sigma (Buchs, Switzerland).

## Statistical analysis

Preference indices (PI) of herbivore feeding tests were arcsin transformed ($x' = \arcsin \sqrt{x}$) and leaf phenolic concentrations were log-transformed to ensure a normal distribution. By calculating a PI, we took into account the dependency of leaf masses consumed in the dual-choice feeding tests. The PIs were evaluated by two-way ANOVA with 'days to herbivore damage' and 'leaf position' as fixed factors. One-sample $t$ tests were performed to determine significant differences from the test value PI = 0.5 (i.e. no preference) for each test day and leaf position separately.

Independent sample $t$ tests were used to test for differences in leaf phenolic compounds of top leaves between damaged and undamaged plants for each test day. In addition, differences in concentrations of phloridzin and phloretin in top leaves between test days were assessed in two-way ANOVAs with 'damage treatment' and 'test day' as fixed factors, followed by LSD post hoc tests. ANCOVAs were calculated to assess the effects of the difference in top leaf chemistry $\Delta C$ (difference in leaf phloridzin and phloretin contents of damaged and undamaged plants; $\Delta C$ as continuous explanatory variable) and test day (as fixed categorical explanatory variable) on the difference in herbivore preference $\Delta P$ (difference in ($\log_{10} x + 1$)-transformed consumed mass of top leaves of damaged and undamaged plants). All statistical analyses were conducted with SPSS 16.0 for Mac OSX (2007; SPSS, Chicago, USA).

## Results

Induced resistance was defined as being present in apple seedlings subjected to herbivore damage, when *S. littoralis* showed a preference for undamaged plants over damaged plants in dual-choice feeding tests. Feeding preference for undamaged leaves, and hence occurrence of induced resistance, changed over time and significantly depended on the tested leaf position (Table 1). It decayed 10 days after herbivore damage for all leaf positions (Fig. 2).

Leaf position had a strong effect on the magnitude of induction responses. In the top leaf, induced resistance occurred rapidly, with a significant feeding preference of *S. littoralis* for undamaged over damaged leaves 3 days after herbivore damage, and persisted for 6 days (Fig. 2a). Delayed by 1 day (4 days after onset of herbivore damage), induced resistance was also detected in the leaf directly above and directly below the damage site (DS). In the leaf directly above DS, significant effects persisted for 5 days (Fig. 2b). In the leaf directly below DS, herbivore preference for undamaged plants was weaker. Preference became apparent 4 days after herbivore damage ($P = 0.05$), fell

**Table 1** Influence of time since herbivore damage and of leaf position on feeding preference (preference index PI) of *Spodoptera littoralis* caterpillars for leaves of herbivore damaged and undamaged apple plants

| Source of variation | df | F | P |
|---|---|---|---|
| Time to damage | 5 | 6.5 | *<0.001* |
| Leaf position | 2 | 9.0 | *<0.001* |
| Time × leaf position | 10 | 0.6 | 0.8 |
| Error | 326 | | |

Significant effects are marked in *italics*

Two-way ANOVA, with time to damage and leaf position as fixed factors
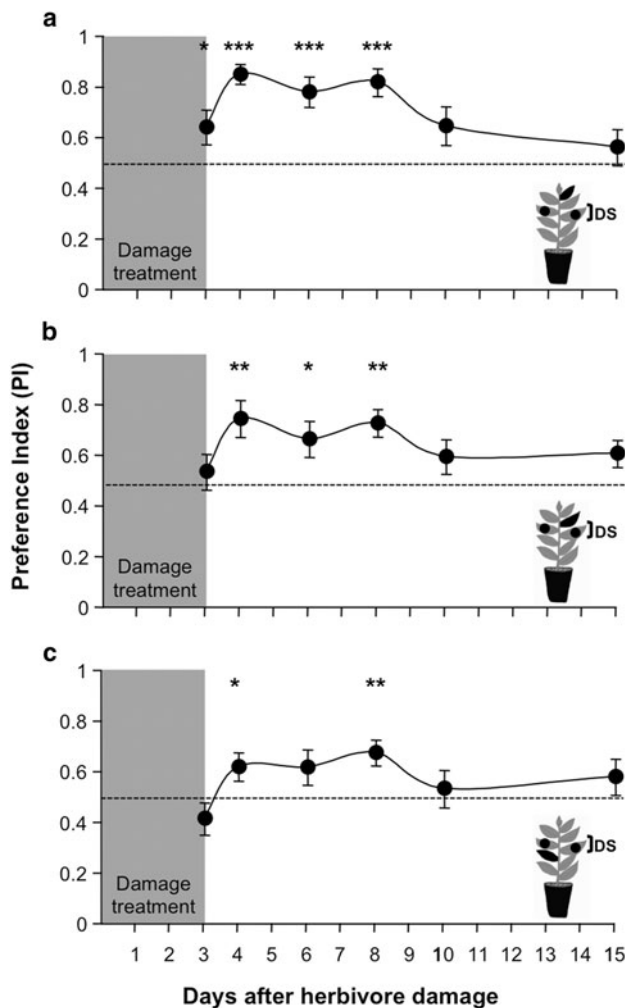
**Fig. 2** Feeding preference of *Spodoptera littoralis* for damaged and undamaged plants in dual-choice feeding tests for 3, 4, 6, 8, 10 and 15 days after herbivore damage. Three leaf positions were tested. **a** Youngest top leaf, **b** leaf directly above damage site (DS) and **c** leaf directly below damage site. Each point shows the average (±SE) preference index (PI) based on the consumed dry masses of the two leaf discs in dual-choice tests. A value of 0.5 indicates no preference. Significant preferences for damaged (PI < 0.5) or undamaged (PI > 0.5) plants are indicated with *asterisks*: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, one-sample *t* test ($n = 17$–20)

short of significance 6 days after herbivore damage ($P = 0.07$), and was again significant 8 days after herbivore damage. Hence, weakly or marginally significant induced resistance responses were observed basipetally to damage over a 5-day period (Fig. 2c).

Chemical analysis of top leaves revealed significant differences in phenolic compounds between leaves of damaged and undamaged apple plants. The total amount of the dihydrochalcones phloridzin and its aglycone phloretin was approximately 20% higher in leaves of damaged plants as compared to undamaged plants 4 and 6 days after herbivore damage. Mean values of phloridzin were higher in

damaged plants on both days as well, but the difference was significant only on test day 4 (Table 2). The levels of phloretin were not significantly different between the damage treatments (Table 2). Phloridzin contents were generally decreasing over time ('test day': $F_{3,66} = 13.3$, $P < 0.001$), with significantly higher contents on test days 4 and 6 compared with contents on days 8 and 10 independent of damage treatment ('test day' × 'damage treatment': $F_{3,66} = 1.6$, $P = 0.2$; LSD post hoc test results not shown). Significantly higher total dihydrochalcone contents in damaged as compared to undamaged plants corresponded to herbivore preference for undamaged plants on test days 4 and 6. On test day 8, total dihydrochalcone contents were not significantly different between damage treatments, although feeding preference indicated that induced resistance was still prevalent in damaged plants. The difference ($\Delta C$) in total dihydrochalcone and phloridzin content affected the difference in feeding preference ($\Delta P$) for damaged and undamaged plants, whereas differences in phloretin content had no significant effect on differential feeding preference (Table 3).

## Discussion

This study revealed marked changes in apple leaf acceptability to insect herbivores and in contents of leaf phenolic compounds after feeding damage. It thereby provides the first record of systemically induced direct resistance responses to insect herbivores for apple plants and contributes to completing the picture of factors affecting direct apple resistance to insect herbivores (Stoeckli et al. 2008; Mody et al. 2009; Stoeckli et al. 2009). Induced resistance showed a clear temporal pattern and strong within-plant variation. The findings, hence, allow comparing models on the time course and within-plant allocation of induced resistance based on findings for herbaceous plants with induced resistance responses of tree seedlings.

Temporal aspect of induced resistance

The time course of induced resistance presented in this study fits the conceptual model of increasing resistance after a certain time lag (3–4 days after damage for leaves positioned directly above and below the damage site) with a subsequent decline (between 8 and 10 days after damage in all tested leaf positions) as long as no additional damage occurs (Metlen et al. 2009). Furthermore, these findings may indicate that induced resistance responses in tree seedlings may occur more rapidly than in mature trees (Boege and Marquis 2005; Barton and Koricheva 2010). This makes sense, as tree seedlings are more vulnerable and show reduced regeneration ability compared with

**Table 2** Phloridzin and phloretin content (mean ± SE in mg/g dry weight) found in the top leaves of undamaged and damaged apple plants for selected days after herbivore damage

| Test day | Compound | Undamaged | Damaged | Statistics |
|---|---|---|---|---|
| *Day 4 (n = 8)* | Total (phloridzin and phloretin) | 210.1 ± 13.8 | 261.0 ± 11.9 | * |
| | Phloridzin | 184.7 ± 13.4 | 244.1 ± 10.3 | ** |
| | Phloretin | 25.4 ± 4.6 | 16.9 ± 4.0 | *n.s.* |
| *Day 6 (n = 10)* | Total (phloridzin and phloretin) | 220.4 ± 7.4 | 261.0 ± 13.0 | * |
| | Phloridzin | 204.5 ± 8.2 | 230.4 ± 10.4 | *n.s.* |
| | Phloretin | 15.9 ± 2.6 | 30.4 ± 7.1 | *n.s.* |
| *Day 8 (n = 9)* | Total (phloridzin and phloretin) | 147.7 ± 19.3 | 186.7 ± 19.8 | *n.s.* |
| | Phloridzin | 127.3 ± 18.0 | 168.8 ± 18.7 | *n.s.* |
| | Phloretin | 20.4 ± 4.2 | 17.8 ± 4.7 | *n.s.* |
| Day 10 (n = 10) | Total (phloridzin and phloretin) | 175.7 ± 7.3 | 178.7 ± 14.3 | *n.s.* |
| | Phloridzin | 156.9 ± 5.2 | 160.1 ± 14.5 | *n.s.* |
| | Phloretin | 18.8 ± 4.8 | 18.6 ± 3.1 | *n.s.* |

Test days on which *S. littoralis* significantly preferred undamaged over damaged leaf discs (and induced resistance is assumed) are marked in italics

Significant differences between contents in leaves of damaged and undamaged plants are indicated with asterisks: ** $P < 0.01$, * $P < 0.05$, *n.s.* not significant, independent-sample *t* test

**Table 3** Effects of the chemical difference between damaged and undamaged top leaves ($\Delta C$) and of test day on difference in feeding preference ($\Delta P$) of *Spodoptera littoralis* for top leaves of damaged and undamaged apple plants

| Compound | Source | df | F | P |
|---|---|---|---|---|
| *Total dihydrochalcones* | $\Delta C$ phloridzin and phloretin | 1 | 7.8 | *<0.01* |
| | Test day | 3 | 3.0 | 0.05 |
| | Error | 29 | | |
| *Phloridzin* | $\Delta C$ phloridzin | 1 | 8.7 | *<0.01* |
| | Test day | 3 | 2.6 | 0.07 |
| | Error | 29 | | |
| *Phloretin* | $\Delta C$ phloretin | 1 | 0.2 | 0.7 |
| | Test day | 3 | 4.0 | *<0.05* |
| | Error | 29 | | |

Significant effects are marked in *italics*

ANCOVA, with $\Delta C$ as continuous and test day as fixed categorical variable

mature trees (Boege et al. 2007; Eichhorn et al. 2010). Time lags in systemically induced resistance result from complex plant responses based on the detection of damage, production and allocation of signals (e.g. phytohormones) that activate gene expression, and other cellular processes resulting in increased plant defense (Schaller and Ryan 1996; Howe 2004; Hudgins et al. 2006). Minimizing time lags after herbivore damage to an optimal level ensures that plants do not suffer irreversible damage, and a relaxation of induced resistance renders this form of resistance cost-effective (Metlen et al. 2009). Nevertheless, induced resistance is generally considered to be costly (Strauss et al. 2002), which may result in a reduction in resistance to

below the constitutive level ('induced susceptibility', see Underwood 1998). Increased susceptibility in apple was previously shown for herbivore-induced volatiles that attract ovipositing females to already infested fruit at the end of the growing season (Hern and Dorn 2001, 2002). Although no increased susceptibility following induced resistance was found in our study, prolonged testing periods may reveal susceptibility effects, as in a study on soybeans, a herbivore preferred damaged over intact plants 15–20 days after herbivore damage (Underwood 1998). The costs of induced resistance and especially a reduction in growth and fruit production could be economically important and need to be considered for this high quality crop (Eyles et al. 2010).

Spatial aspect of induced resistance

Systemic-induced resistance relies on the assumption that a signal is locally generated at the damage site and then transported to other plant parts, where it causes a change in plant defense (Howe 2004; Heil and Ton 2008). Although we found a similar time course of induced resistance in all tested leaf positions, effects were strongest and more rapid in the youngest top leaf. This finding is consistent with the optimal defense theory, which suggests young developing tissue to represent more valuable plant tissue and suggests these parts to be prioritized in defense (Radhika et al. 2008; McCall and Fordyce 2010). Surprisingly, induced resistance responses were observed even in leaves below the damage site. The occurrence of systemic-induced resistance basipetally to the damage site is a rarely described phenomenon (Jones et al. 1993; Schittko and Baldwin

2003). This may reflect a lack of studies testing occurrence of induced resistance in basipetal leaf positions, rather than a general inability of plants to express basipetal induced resistance. A basipetal induction of resistance is indicated by studies that include responses of belowground plant parts to foliar herbivore damage and that report changes in gene transcription and accumulation of secondary chemicals in roots (Kaplan et al. 2008b; Erb et al. 2009). The findings of induced changes belowground hence suggest signals to be directed downward and below damage site plant organs to be involved in induced plant resistance, rendering a basipetal induced resistance response and bidirectional responses up and down the shoot feasible.

The transport pathways of these signals within plants are presumed to be largely phloem based and linked to the assimilate flow from source to sink leaves (Wardlaw 1990; Arnold et al. 2004). However, uncertainty about this assumption is still large, and research in possible translocation of defense signals is still ongoing (Heil and Ton 2008). Herbivore damage, for example, not only alters plant defense, but also strongly affects primary metabolism with alterations in the transport and partitioning of assimilates and within-plant distribution of nutrients (Babst et al. 2005; Frost and Hunter 2008). This could cause dynamic shifts in the direction of the phloem flow as sink-source relationships change rapidly after herbivore attack and result in bidirectional signal transportation (Babst et al. 2005; Schwachtje et al. 2006). Another point to consider is that physical proximity to the damage site may be misleading, as a physically more distant leaf may actually be the phyllotactically nearest one due to the vascular connectivity (Arnold et al. 2004; Viswanathan and Thaler 2004; Orians 2005). Therefore, top leaves could be structurally closest to the damage site and receive signals before leaves directly above the damage site, as was recently observed in poplar *Populus trichocarpa × delitopdes* (Philippe et al. 2010). Schittko and Baldwin (2003) suggested that a bidirectional signal translocation to both acropetal and basipetal leaves contradicts the theory of assimilate-linked-induced resistance and further suggest the direction of phloem flow to be of minor importance. Our findings support the bidirectional nature of signal translocation, although a delayed basipetal effect may point to constraints involving either flow direction or plant phyllotaxis.

Chemical aspect of induced resistance in apple

Phloridzin is an important apple-specific phenolic compound associated with deterrence and resistance properties, yet its role in induced direct resistance to insect herbivores remains inconclusive. The covariance of phloridzin with feeding preferences by a generalist insect herbivore not closely associated with apple plants indicates a certain general role of this compound in apple defense. Phloretin, on the other hand, did not affect feeding preferences and seemed uninvolved in apple defense, possibly due to the low concentrations in leaves. As differences in phloridzin concentration only partially conformed to the observed feeding preferences, phloridzin concentrations cannot fully explain the observed induced resistance responses in apple seedlings. Phloridzin may, therefore, be considered as a defense compound that possibly acts in combination with further factors mediating induced apple resistance, such as other secondary metabolites (including other phenolic compounds) or defense-related proteins (Howe and Jander 2008; Eyles et al. 2010). Similar conclusions have been made by previous studies on induced tree resistance responses to herbivores, where phenolics represent major defense compounds, but failed to explain induced resistance responses (Lempa et al. 2004; Mumm and Hilker 2006). Ruuhola et al. (2008) found polyphenoloxidases to increase in previously damaged plants and suggest the pro-oxidant activity of phenolic compounds rather than the compounds themselves to mediate insect responses to induced plant resistance.

## Conclusions

This study demonstrates the occurrence of rapid induced resistance responses in apple tree seedlings following insect herbivore damage. The presented results confirm the conceptual general model on the distribution of induced resistance over time. Furthermore, the spatial distribution of induced resistance followed the predictions of optimal defense theory. The discovery of a delayed basipetal-induced resistance offers novel insight into general defense theories and emphasizes the importance of understanding the timing of induced resistance in concert with the connectivity and directionality of involved signaling pathways.

## References

Agrawal AA (2000) Specificity of induced resistance in wild radish: causes and consequences for two specialist and two generalist caterpillars. Oikos 89:493–500

Agrell J, Oleszek W, Stochmal A, Olsen M, Anderson P (2003) Herbivore-induced responses in alfalfa (*Medicago sativa*). J Chem Ecol 29:303–320

Anderson P, Agrell J (2005) Within-plant variation in induced defence in developing leaves of cotton plants. Oecologia 144:427–434

Anderson P, Jonsson M, Morte U (2001) Variation in damage to cotton affecting larval feeding preference of *Spodoptera littoralis*. Entomol Exp Appl 101:191–198

Arnold T, Appel H, Patel V, Stocum E, Kavalier A, Schultz J (2004) Carbohydrate translocation determines the phenolic content of *Populus* foliage: a test of the sink-source model of plant defense. New Phytol 164:157–164

Babst BA, Ferrieri RA, Gray DW, Lerdau M, Schlyer DJ, Schueller M, Thorpe MR, Orians CM (2005) Jasmonic acid induces rapid changes in carbon transport and partitioning in *Populus*. New Phytol 167:63–72

Baldwin IT, Preston CA (1999) The eco-physiological complexity of plant responses to insect herbivores. Planta 208:137–145

Ballhorn DJ, Schiwy S, Jensen M, Heil M (2008) Quantitative variability of direct chemical defense in primary and secondary leaves of lima bean (*Phaseolus lunatus*) and consequences for a natural herbivore. J Chem Ecol 34:1298–1301

Barton KE, Koricheva J (2010) The ontogeny of plant defense and herbivory: characterizing general patterns using meta-analysis. Am Nat 175:481–493

Bingham RA, Agrawal AA (2010) Specificity and trade-offs in the induced plant defence of common milkweed *Asclepias syriaca* to two lepidopteran herbivores. J Ecol 98:1014–1022

Boege K, Marquis RJ (2005) Facing herbivory as you grow up: the ontogeny of resistance in plants. Trends Ecol Evol 20:441–448

Boege K, Dirzo R, Siemens D, Brown P (2007) Ontogenetic switches from plant resistance to tolerance: minimizing costs with age? Ecol Lett 10:177–187

Eichhorn MP, Nilus R, Compton SG, Hartley SE, Burslem D (2010) Herbivory of tropical rain forest tree seedlings correlates with future mortality. Ecology 91:1092–1101

Erb M, Lenk C, Degenhardt J, Turlings TCJ (2009) The underestimated role of roots in defense against leaf attackers. Trends Plant Sci 14:653–659

Escarpa A, Gonzalez MC (1998) High-performance liquid chromatography with diode-array detection for the determination of phenolic compounds in peel and pulp from different apple varieties. J Chromatogr A 823:331–337

Eyles A, Bonello P, Ganley R, Mohammed C (2010) Induced resistance to pests and pathogens in trees. New Phytol 185:893–908

Frost CJ, Hunter MD (2008) Herbivore-induced shifts in carbon and nitrogen allocation in red oak seedlings. New Phytol 178:835–845

Fulcher AF, Ranney TG, Burton JD, Walgenbach JF, Danehower DA (1998) Role of foliar phenolics in host plant resistance of *Malus* taxa to adult Japanese beetles. Hortscience 33:862–865

Gomez S, van Dijk W, Stuefer JF (2010) Timing of induced resistance in a clonal plant network. Plant Biol 12:512–517

Gosch C, Halbwirth H, Kuhn J, Miosic S, Stich K (2009) Biosynthesis of phloridzin in apple (*Malus domestica* Borkh.). Plant Sci 176:223–231

Heil M, Ton J (2008) Long-distance signalling in plant defence. Trends Plant Sci 13:264–272

Hern A, Dorn S (2001) Induced emissions of apple fruit volatiles by the codling moth: changing patterns with different time periods after infestation and different larval instars. Phytochemistry 57:409–416

Hern A, Dorn S (2002) Induction of volatile emissions from ripening apple fruits infested with *Cydia pomonella* and the attraction of adult females. Entomol Exp Appl 102:145–151

Howe GA (2004) Jasmonates as signals in the wound response. J Plant Growth Regul 23:223–237

Howe GA, Jander G (2008) Plant immunity to insect herbivores. Annu Rev Plant Biol 59:41–66

Hudgins JW, Ralph SG, Franceschi VR, Bohlmann J (2006) Ethylene in induced conifer defense: cDNA cloning, protein expression, and cellular and subcellular localization of 1-aminocyclopropane-1-carboxylate oxidase in resin duct and phenolic parenchyma cells. Planta 224:865–877

Hui X, Jin C (2004) Interspecific variation of plant traits associated with resistance to herbivory among four species of *Ficus* (Moraceae). Ann Bot 94:377–384

Jones CG, Coleman JS (1988) Leaf disk size and insect feeding preference: implications for assays and studies on induction of plant defense. Entomol Exp Appl 47:167–172

Jones CG, Hopper RF, Coleman JS, Krischik VA (1993) Control of systemically induced herbivore resistance by plant vascular architecture. Oecologia 93:452–456

Kaplan I, Halitschke R, Kessler A, Sardanelli S, Denno RF (2008a) Constitutive and induced defenses to herbivory in above- and belowground plant tissues. Ecology 89:392–406

Kaplan I, Halitschke R, Kessler A, Sardanelli S, Denno RF (2008b) Effects of plant vascular architecture on aboveground-belowground-induced responses to foliar and root herbivores on *Nicotiana tabacum*. J Chem Ecol 34:1349–1359

Karban R, Baldwin IT (1997) Induced responses to herbivory. The University of Chicago Press, Chicago

Kindt M, Orsini MC, Costantini B (2007) Improved high-performance liquid chromatography-diode array detection method for the determination of phenolic compounds in leaves and peels from different apple varieties. J Chromatogr Sci 45:507–514

Lempa K, Agrawal AA, Salminen JP, Turunen T, Ossipov V, Ossipova S, Haukioja E, Pihlaja K (2004) Rapid herbivore-induced changes in mountain birch phenolics and nutritive compounds and their effects on performance of the major defoliator, *Epirrita autumnata*. J Chem Ecol 30:303–321

Leser C, Treutter D (2005) Effects of nitrogen supply on growth, contents of phenolic compounds and pathogen (scab) resistance of apple trees. Physiol Plant 123:49–56

Mattiacci L, Rudelli S, Rocca BA, Genini S, Dorn S (2001) Systemically-induced response of cabbage plants against a specialist herbivore, *Pieris brassicae*. Chemoecology 11:167–173

McCall AC, Fordyce JA (2010) Can optimal defence theory be used to predict the distribution of plant chemical defences? J Ecol 98:985–992

McGuire RJ, Johnson MTJ (2006) Plant genotype and induced responses affect resistance to herbivores on evening primrose (*Oenothera biennis*). Ecol Entomol 31:20–31

Metlen KL, Aschehoug ET, Callaway RM (2009) Plant behavioural ecology: dynamic plasticity in secondary metabolites. Plant Cell Environ 32:641–653

Mitchell MJ, Keogh DP, Crooks JR, Smith SL (1993) Effects of plant flavonoids and other allelochemicals on insect cytochrome P-450 dependent steroid hydroxylase-activity. Insect Biochem Mol Biol 23:65–71

Mody K, Eichenberger D, Dorn S (2009) Stress magnitude matters: different intensities of pulsed water stress produce non-mono-tonic resistance responses of host plants to insect herbivores. Ecol Entomol 34:133–143

Mumm R, Hilker M (2006) Direct and indirect chemical defence of pine against folivorous insects. Trends Plant Sci 11:351–358

Ohnmeiss TE, Baldwin IT (2000) Optimal defense theory predicts the ontogeny of an induced nicotine defense. Ecology 81:1765–1783

Olson DM, Cortesero AM, Rains GC, Potter T, Lewis WJ (2009) Nitrogen and water affect direct and indirect plant systemic induced defense in cotton. Biol Control 49:239–244

Orians C (2005) Herbivores, vascular pathways, and systemic induction: facts and artifacts. J Chem Ecol 31:2231–2242

Petkovsek MM, Stampar E, Veberic R (2008) Increased phenolic content in apple leaves infected with the apple scab pathogen. J Plant Pathol 90:49–55

Philippe RN, Ralph SG, Mansfield SD, Bohlmann J (2010) Transcriptome profiles of hybrid poplar (*Populus trichocarpa × deltoides*) reveal rapid changes in undamaged, systemic sink leaves after simulated feeding by forest tent caterpillar (*Malacosoma disstria*). New Phytol 188:787–802

Picinelli A, Dapena E, Mangas JJ (1995) Polyphenolic pattern in apple tree leaves in relation to scab resistance: a preliminary study. J Agric Food Chem 43:2273–2278

Radhika V, Kost C, Bartram S, Heil M, Boland W (2008) Testing the optimal defence hypothesis for two indirect defences: extrafloral nectar and volatile organic compounds. Planta 228:449–457

Rhoades DF (1979) Evolution of plant chemical defense against herbivores. In: Rosenthal GA, Janzen DH (eds) Herbivores: their interaction with secondary plant metabolites. Academic Press, New York, pp 3–54

Roach WA (1939) Plant injection as a physiological method. Ann Bot 3:155–226

Ruuhola T, Yang SY, Ossipov V, Haukioja E (2008) Foliar oxidases as mediators of the rapidly induced resistance of mountain birch against *Epirrita autumnata*. Oecologia 154:725–730

Schaller A, Ryan CA (1996) Systemin: a polypeptide defense signal in plants. Bioessays 18:27–33

Schieber A, Keller P, Carle R (2001) Determination of phenolic acids and flavonoids of apple and pear by high-performance liquid chromatography. J Chromatogr A 910:265–273

Schittko U, Baldwin IT (2003) Constraints to herbivore-induced systemic responses: bidirectional signaling along orthostichies in *Nicotiana attenuata*. J Chem Ecol 29:763–770

Schwachtje J, Minchin PEH, Jahnke S, van Dongen JT, Schittko U, Baldwin IT (2006) SNF1-related kinases allow plants to tolerate herbivory by allocating carbon to roots. Proc Natl Acad Sci USA 103:12935–12940

Stoeckli S, Mody K, Gessler C, Patocchi A, Jermini M, Dorn S (2008) QTL analysis for aphid resistance and growth traits in apple. Tree Genet Genomes 4:833–847

Stoeckli S, Mody K, Gessler C, Christen D, Dorn S (2009) Quantitative trait locus mapping of resistance in apple to *Cydia pomonella* and *Lyonetia clerkella* and of two selected fruit traits. Ann Appl Biol 154:377–387

Stout MJ, Riggio MR, Yang Y (2009) Direct induced resistance in *Oryza sativa* to *Spodoptera frugiperda*. Environ Entomol 38:1174–1181

Strauss SY, Rudgers JA, Lau JA, Irwin RE (2002) Direct and ecological costs of resistance to herbivory. Trends Ecol Evol 17:278–285

Szankowski I, Flachowsky H, Li H, Halbwirth H, Treutter D, Regos I, Hanke MV, Stich K, Fischer TC (2009) Shift in polyphenol profile and sublethal phenotype caused by silencing of anthocyanidin synthase in apple (*Malus* sp.). Planta 229:681–692

Treutter D (2005) Significance of flavonoids in plant resistance and enhancement of their biosynthesis. Plant Biol 7:581–591

Underwood NC (1998) The timing of induced resistance and induced susceptibility in the soybean Mexican bean beetle system. Oecologia 114:376–381

Viswanathan DV, Thaler JS (2004) Plant vascular architecture and within-plant spatial patterns in resource quality following herbivory. J Chem Ecol 30:531–543

Wardlaw IF (1990) The control of carbon partitioning in plants. New Phytol 116:341–381

Zhao T, Krokene P, Bjorklund N, Langstrom B, Solheim H, Christiansen E, Borg-Karlson AK (2010) The influence of *Ceratocystis polonica* inoculation and methyl jasmonate application on terpene chemistry of Norway spruce, *Picea abies*. Phytochemistry 71:1332–1341

Zong N, Wang CZ (2007) Larval feeding induced defensive responses in tobacco: comparison of two sibling species of *Helicoverpa* with different diet breadths. Planta 226:215–224

Zvereva EL, Kozlov MV, Niemela P, Haukioja E (1997) Delayed induced resistance and increase in leaf fluctuating asymmetry as responses of *Salix borealis* to insect herbivory. Oecologia 109:368–373