

Testing the optimal defence hypothesis for two indirect defences: extrafloral nectar and volatile organic compounds

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Abstract Many plants respond to herbivory with an increased production of extrafloral nectar (EFN) and/or volatile organic compounds (VOCs) to attract predatory arthropods as an indirect defensive strategy. In this study, we tested whether these two indirect defences fit the optimal defence hypothesis (ODH), which predicts the within-plant allocation of anti-herbivore defences according to trade-offs between growth and defence. Using jasmonic acid-induced plants of *Phaseolus lunatus* and *Ricinus communis*, we tested whether the within-plant distribution pattern of these two indirect defences reflects the fitness value of the respective plant parts. Furthermore, we quantified photosynthetic rates and followed the within-plant transport of assimilates with ^{13}C labelling experiments. EFN secretion and VOC emission were highest in younger leaves. Moreover, the photosynthetic rate increased with leaf age, and pulse-labelling experiments suggested transport of carbon to younger leaves. Our results demonstrate that the ODH can explain the within-plant allocation pattern of both indirect defences studied.

Keywords Extrafloral nectar · Indirect defence · Ontogeny · Optimal defence hypothesis · Volatile organic compounds

Abbreviations

DMNT	(<i>E</i>)-4,8-dimethylnona-3,5,7-triene
EFN	Extrafloral nectar
IAEA	International Atomic Energy Agency
IRMS	Isotope ratio mass spectrometry
LSD	Least significant difference
JA	Jasmonic acid
MeSA	Methyl salicylate
ODH	Optimal defence hypothesis
PAR	Photosynthetic active radiation
PET	Polyethyleneterephthalate
TMTT	(<i>E,E</i>)-4,8,12-trimethyltrideca-1,3,7,11-tetraene
VBDB	Vienna Peedee belemnite
VOC	Volatile organic compounds

Introduction

Herbivores exert an immense selection pressure on plants, and the resulting arms-race has led to the evolution of an enormous variety of plant defences against herbivores (Walling 2000; Kessler and Baldwin 2002). Defences, which directly target the performance or survival of the herbivore, are generally referred to as ‘*direct*’ defences. In contrast, plant traits that do not directly affect the herbivore but rather function via the attraction, nourishment or housing of predatory organisms, thereby increasing the predation pressure on herbivores, are termed ‘*indirect*’ defences (Heil 2008). These plant defences, albeit often significantly contributing to the plant’s ecological success, do however, not come without fitness costs (Gulmon and Mooney 1986).

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From an evolutionary perspective, any organism should respond to the resulting trade-offs in a way that maximises its reproductive output and minimises any investment in non-reproductive traits—even if they are essential for its survival. One example for such an evolutionary optimisation response that is generally regarded as a cost-saving strategy are herbivore-induced plant defences, which are activated only in case of an herbivore attack (Karban and Baldwin 1997; Dicke and Hilker 2003). The drawback of inducible defences, however, is the lag-time, which is the time required for the induction of the defence after the first contact with the herbivore, during which the plant remains vulnerable (Heil and Baldwin 2002; Zangerl 2003). Since most plant defences are neither consistently expressed throughout a plant's life nor evenly distributed within a plant (Zangerl and Rutledge 1996), several hypotheses have been suggested to predict their phenotypic variation depending on environmental or genetic factors (Karban and Baldwin 1997; Herms and Mattson 1992; Stamp 2003).

The optimal defence hypothesis (ODH) states that organisms evolved to allocate their defences in a way that maximises fitness (McKey 1974, 1979; Rhoades 1979). The underlying assumption is that defence is costly and thus, the spatio-temporal patterns of an adaptive defence allocation among plant parts should reflect the fitness-value of these organs (McKey 1974, 1979). In other words, the theoretical expectations of the ODH are that within a plant, young, still developing leaves should be better defended than older leaves.

However, physiological constraints may operate on plants, thereby causing them to deviate from these theoretical predictions. Empirical tests of the ODH are therefore required and many validating reports of this theory are indeed known for direct defences (Zangerl and Rutledge 1996; Ohnmeiss and Baldwin 2000; Barto and Cipollini 2005). Very little information, however, is available on the allocation pattern of indirect defensive strategies of plants.

This study aims at testing the predictions made by the ODH for two particularly widespread indirect defence traits: extrafloral nectar (EFN) and volatile organic compounds (VOCs), which are both involved in mediating the interaction between herbivore-damaged plants and members of the third trophic level (Arimura et al. 2005; Heil 2008). By offering EFN as a carbohydrate-rich reward (Bentley 1977; Koptur 1992) or by emitting VOCs that indicate the increased presence of potential prey to predators and parasitoids (Turlings et al. 1990; Pare and Tumlinson 1997), plants defend themselves indirectly against herbivores. Both EFN and VOCs are inducible traits, i.e. their production rate increases in response to herbivory or mechanical damage and this response is known to be regulated by the octadecanoid pathway, in which the phytohormone jasmonic acid (JA) plays a key role (Hopke et al.

1994; Heil et al. 2001). Exogenous treatment of plants with JA results in increased production rates of both EFN and VOCs, which closely resemble the plant's response induced by herbivore feeding in terms of quality and quantity (Dicke et al. 1999; Heil 2004).

We used lima bean (*Phaseolus lunatus* L., Fabaceae) and castor (*Ricinus communis* L., Euphorbiaceae) as experimental systems. Both plants bear extrafloral nectaries at the petioles of their leaves. In addition, lima bean releases VOCs after herbivory or when treated with JA that attract, e.g. carnivorous mites or parasitoid wasps under laboratory conditions (Dicke et al. 1999). At its natural growing site, JA-mediated EFN secretion has been shown to benefit the plant (Heil 2004; Kost and Heil 2005, 2008). In *R. communis*, herbivore or mechanical damage is known to increase EFN production (Wäckers et al. 2001). In the present investigation, we used JA to induce the production of EFN (both species) and VOCs (lima bean only) and tested the following predictions, which are derived from the ODH:

1. Both constitutive (i.e. untreated) and induced levels of EFN secretion and VOC emission are higher in younger leaves.
2. The ontogenetic pattern of indirect defence production (both EFN and VOCs) cannot be explained solely by the photosynthetic rate of the respective leaves.
3. Allocation of these defences to younger leaves is mediated by transporting newly assimilated carbohydrates from older source to younger sink leaves.

Materials and methods

Plant material and growth conditions

Plants of *P. lunatus* L. (lima bean) were cultivated from seeds derived from a native population growing in the coastal area near Puerto Escondido in the state of Oaxaca, Mexico. The parental plants have been used previously in field experiments on indirect plant defences (Heil 2004; Kost and Heil 2005, 2008). *Ricinus communis* L. plants (castor oil plant) were grown from seeds (Weber Seeds, Römhild, Germany) harvested from greenhouse-grown plants. Growing conditions were 20–22°C, 30–55% humidity during a 16 h photoperiod. Experiments were performed with 4-week-old plants (i.e. 5–6 leaf stage for *P. lunatus* and 4 leaf stage for *R. communis*). To study the ontogenetic pattern, both plants were grown in Klasmann clay substrate (Klasmann-Deilmann, Geeste, Germany). All experiments were performed in the greenhouse.

Numbering of leaves was based on their age as assessed by their insertion order into the main shoot. In *P. lunatus*, leaf 1 was the youngest, still unfolding leaf, leaves 2 and 3

were mostly unfolded, and leaves 4 and 5 were slightly to completely hardened leaves, respectively (Fig. 1a). The four leaves of *R. communis* were numbered accordingly (Fig. 1d).

Measurement of EFN secretion rates

To ensure that no nectar was present at the onset of the experiment, extrafloral nectaries were rinsed thoroughly with tap water and allowed to dry. EFN secretion was quantified one day after spraying either tap water (control treatment) or an aqueous solution of 1 mM JA (JA treatment) on all the leaves until runoff. Plants were treated twice at an interval of 30 min and after that leaves were allowed to dry for 1 h before plants were placed back into the greenhouse. The EFN produced after 24 h was quantified as the amount of secreted soluble solids (i.e. sugars and amino acids) using a temperature-compensated refractometer (ATAGO N-10E refractometer, Leo Kübler GmbH, Karlsruhe,

Germany) as described by Heil et al. (2000, 2001). EFN was quantified as amount of soluble solids per dry weight of the secreting leaf material per 24 h.

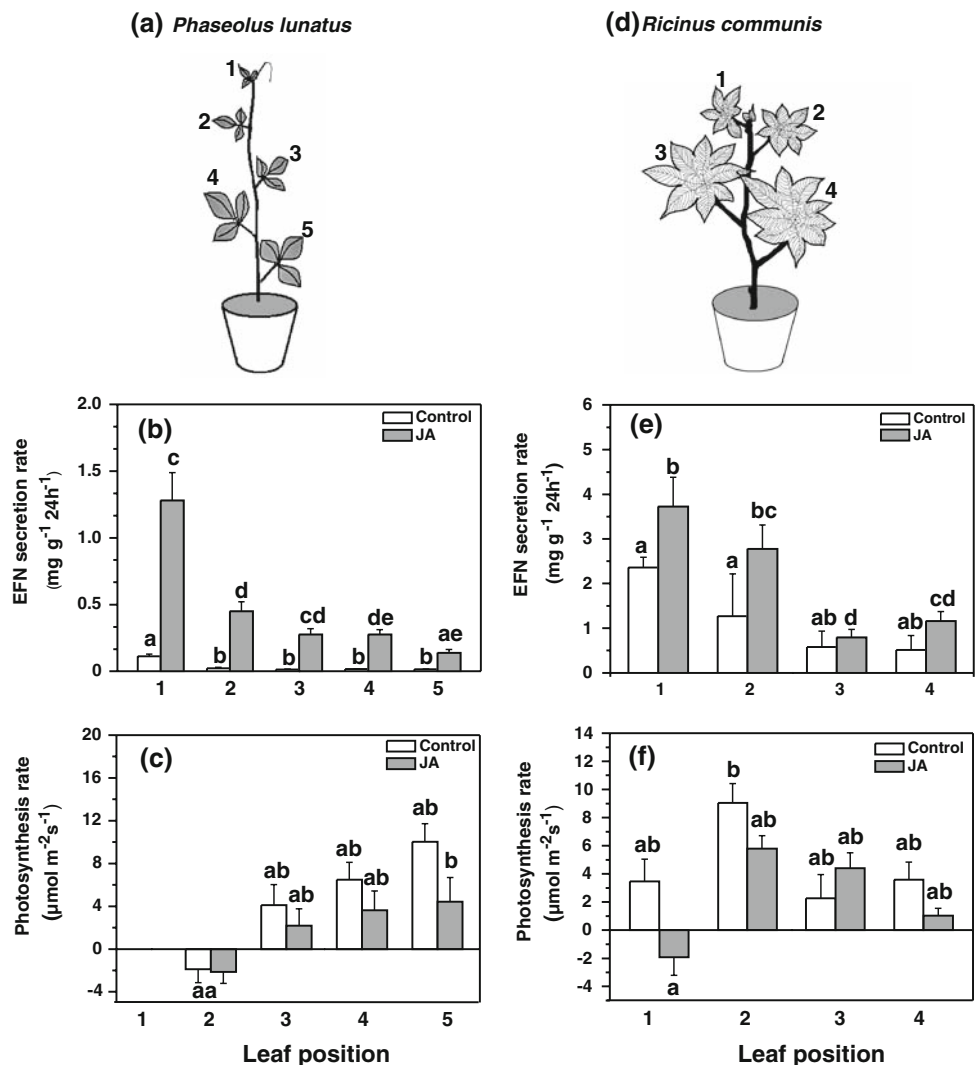
Measurement of photosynthetic rate

The photosynthetic rate was measured with a portable open-mode photosynthesis system LI-6400 (LI-COR, Lincoln, NE, USA) using the 6400-15 *Arabidopsis* chamber on leaves attached to the plant. Measurements were taken between 9:00 a.m. and 2:00 p.m. using $360 \mu\text{l l}^{-1} \text{CO}_2$ in the reference stream under approximately $900 \mu\text{M m}^{-2} \text{s}^{-1}$ PAR.

Collection and analysis of VOCs

The VOC emission as a function of leaf age in lima bean plants was measured by bagging all leaves individually in PET hoses (Toppits® 'Bratschlauch', Melitta, Minden,

Fig. 1 **a** Numbering of differentially aged leaves, **b** ontogenetic variation of EFN secretion rate ($n = 9$), and **c** photosynthetic rate ($n = 6$), of untreated and jasmonic acid (JA)-induced *Phaseolus lunatus* plants. **d** Numbering of differentially aged leaves, **e** EFN secretion rate ($n = 7$), and **f** photosynthetic rate ($n = 7$) of untreated and JA-induced *R. communis* plants. EFN secretion rate is given in milligrams of soluble solids per g leaf dry weight per 24 h. The net photosynthetic rate is given as rates of CO_2 uptake in $\mu\text{mol m}^{-2} \text{s}^{-1}$. Due to the small size, the photosynthetic rate of the youngest leaf could not be measured in *P. lunatus*. Different letters denote significant differences between groups (global LSD post hoc for all factor combinations between leaf position and treatment after univariate ANOVA, $P < 0.05$). Data are presented as mean \pm 95% CI



Germany) that do not emit detectable volatiles by themselves. VOCs emitted from each individual leaf were collected continuously for 24 h on charcoal traps (1.5 mg charcoal, Gränicher and Quartero, Daumazan sur Arize, France) by pulling air at about 500 ml min⁻¹ using a 12 V vacuum pump (Gast Manufacturing, Benton Harbor, MI, USA). The traps were eluted with 2 × 20 µl of dichloromethane containing 200 ng µl⁻¹ of 1-bromodecane as an internal standard. The leaves were dried for dry weight determination. VOC samples were analysed on a Thermo Finnigan Trace GC-MS (Thermo, Bremen, Germany) equipped with a fused silica Alltech EC5 column (15 m × 0.25 mm internal diameter × 0.25 µm film thickness) using 1.5 ml min⁻¹ helium as carrier gas. Separation was achieved under programmed conditions (45°C for 2 min, 10°C min⁻¹ to 200°C, then 30°C min⁻¹ to 280°C for 1 min; injector temperature: 220°C). MS analysis was performed on a TraceMS in electron impact full-scan mode at 70 eV with source temperature at 200°C and GC interface temperature at 280°C. Individual compounds were quantified with respect to the peak area of the internal standard and related to the dry weight of the leaf. The ten most dominantly emitted compounds, namely (*Z*)-3-hexenyl-acetate, (*E*)-β-ocimene, (*R*)-(-)-linalool, (*E*)-4,8-dimethylnona-1,3,7-triene (DMNT), (*E*)-2,6-dimethyloctatetraene (C₁₀H₁₄), methyl salicylate (MeSA), 2,6-dimethyl-3,5,7-octatriene-2-ol (C₁₀H₁₆O), *cis*-jasmone, (*E*)-β-caryophyllene, and (*E,E*)-4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT), were summed up to test for a putative effect of leaf position on total VOC emission.

Labelling experiment

In order to follow the internal transport of newly assimilated carbon, experiments were performed using synthetically premixed air containing ¹³CO₂ instead of ¹²CO₂ at a natural concentration of 380 ppm. In all cases, the air with ¹³CO₂ was purged for 24 h after induction with 1 mM JA solution. For each plant, each of five leaves were bagged individually in a PET hose (i.e. 'Bratschlauch', see above) and in each case, one of the five leaves was purged with labelled air, while all the other four leaves were purged with normal air. After 24 h, the ¹³C content in the tissue of all five leaves as well as in the EFN secreted from this leaf was quantified using an isotope ratio mass spectrometer (IRMS). This procedure was applied to a total of eight replicates of four plants each, with one of the five leaves having experienced the ¹³CO₂-treatment until each leaf position within the four-plant group had received the ¹³C treatment once. Due to technical reasons, we focussed this analysis on leaves in positions 1–3 and 5.

For IRMS measurements of EFN, nectar samples were filled in small 0.04 ml tin capsules for liquid samples (d:

3.5 mm, l: 5.5 mm; part. No. 184.9915.26, Lüdi AG, Flawil, Switzerland), dried in a desiccator filled with P₂O₅ as drying agent, and weighed before further analysis. For the solid leaf sample measurements, dried and powdered leaf material was weighed in 0.07 ml tin capsules (d: 4.0 mm, l: 6.0 mm; part. No. 176.1305.53, Lüdi AG). Capsules were sealed and combusted (oxidation at 1,020°C, reduction at 650°C) in a constant helium stream (80 ml min⁻¹) quantitatively to CO₂, N₂, and H₂O using an elemental analyzer (EuroEA CN2 dual, HEKAtech, Wegberg, Germany). After passing a water trap (MgClO₄), the gases were separated chromatographically at 85°C and transferred via an open split to a coupled isotope ratio mass spectrometer (IsoPrime, Micromass, Manchester, UK). Our laboratory working standard (acetanilide) has been calibrated on the VPDB scale using IAEA reference material, NBS 22, with a δ ¹³C value of -29.78‰ (Werner and Brand 2001). All isotope ratios are given as δ ¹³C values: δ ¹³C (‰) = [(R_{sample}/R_{standard}) - 1] × 10³, where *R* corresponds to the ¹³C/¹²C ratio of the sample and the standard.

Statistical analysis

All experiments were analysed with linear mixed-effect models with 'treatment' as fixed and 'plant individual' as random factor. Values of EFN secretion and total VOC emission have been log-transformed to meet the test assumptions of normality and homogeneity of variances. Global LSD post hoc tests were applied to the measured values for EFN secretion, VOC emission, and photosynthetic rates to test for between-group differences between all factor combinations of leaf position and treatment. All statistical analyses were performed using SPSS 13.0 (SPSS Inc., Chicago, IL, USA). To control for multiple testing in comparing qualitative differences in the VOCs blend with leaf age, false-discovery rate (FDR) procedure was used.

Results

Ontogenetic pattern of EFN production

In both, *P. lunatus* and *R. communis*, the youngest leaf (i.e. leaf position 1) secreted the highest amount of EFN in undamaged controls as well as in JA-treated plants and the EFN secretion rate significantly decreased with leaf age (Fig. 1b, e, LSD post hoc test after univariate ANOVA: *P* < 0.01, *n* = 9 and 7, respectively). In case of lima bean, the mean amount of EFN secreted from the youngest leaf exceeded that of the oldest leaf about fivefold. In this species, the two youngest leaves secreted on average two times more EFN than the two older leaves. An analysis of the inducibility with respect to leaf age indicated that in both

plant species the youngest leaf did not only show the highest level of constitutive EFN secretion, but was also inducible to higher defence levels than the older leaves (LSD post hoc test after univariate ANOVA, $P < 0.05$, $n = 9$ in lima bean and $n = 7$ in *R. communis*).

Ontogenetic pattern of photosynthetic rate

Quantification of the photosynthetic rate indicated that younger leaves showed a lower gas exchange capacity than older ones in both control and JA-treated plants (Fig. 1c, f, LSD post-hoc test after univariate ANOVA: $P < 0.02$, $n = 6$ and 7 , respectively), thus indicating a negative relation between EFN production and photosynthetic capacity. JA treatment did not significantly alter the photosynthetic rate in both plant species investigated (univariate ANOVA, $P > 0.05$, $n = 7$ in lima bean and $n = 6$ in *R. communis*).

Labelling experiment

Due to the absence of a positive relation between photosynthetic rate and EFN secretion (Fig. 1), we hypothesized that there should be a flow of photosynthates within the plant from older source to younger sink leaves. In labelling experiments with lima bean plants, in which one of five leaves was purged with artificial air containing $^{13}\text{CO}_2$ at 380 ppm while the other leaves were treated with natural air for 24 h, we measured the $^{13}\text{C}/^{12}\text{C}$ -ratios of the EFN from each leaf (Fig. 2a) and the corresponding leaf tissue (Fig. 2b). After labelling leaf 1, no increased ^{13}C concentration in the tissues as well as EFN of the untreated leaves was observed. Treatment of leaves 2 and 3 showed for some replicates a clear, but for others only a slightly increased incorporation of ^{13}C into the younger leaves 1 or 1 and 2, respectively. In no case was a downstream transport, i.e., from the younger (1–3) to older (4–5) leaves, observed. The $\delta^{13}\text{C}$ values of downstream leaves were in all experiments close to the natural abundance level (–20 to –30‰; i.e. values of control plants). Labelling of leaf 5 led to a strong incorporation of ^{13}C in the tissues and the EFN of leaves 3, 2 and 1. The incorporation of ^{13}C in the leaf material was strongest in the most distal leaf number 1 and decreased continuously with increasing leaf age (i.e. the level of incorporation followed the leaf order $5^* \gg 1 > 2 > 3 > 4$, $*$ = labelled leaf).

No increase of ^{13}C in the tissue and the EFN of leaf 4 could be detected, not even after treatment of leaf 5. In all experiments, the $\delta^{13}\text{C}$ values of leaf 4 were in the range of the natural abundance level. The amount of ^{13}C incorporated into EFN was on average about ninefold higher than that observed for the leaf tissue. Taken together, this experiment revealed a unidirectional transport of photosynthates from older source to younger sink leaves. Furthermore, no

photosynthetic products were transported to leaf 4 and no transport occurred downstream to older leaves.

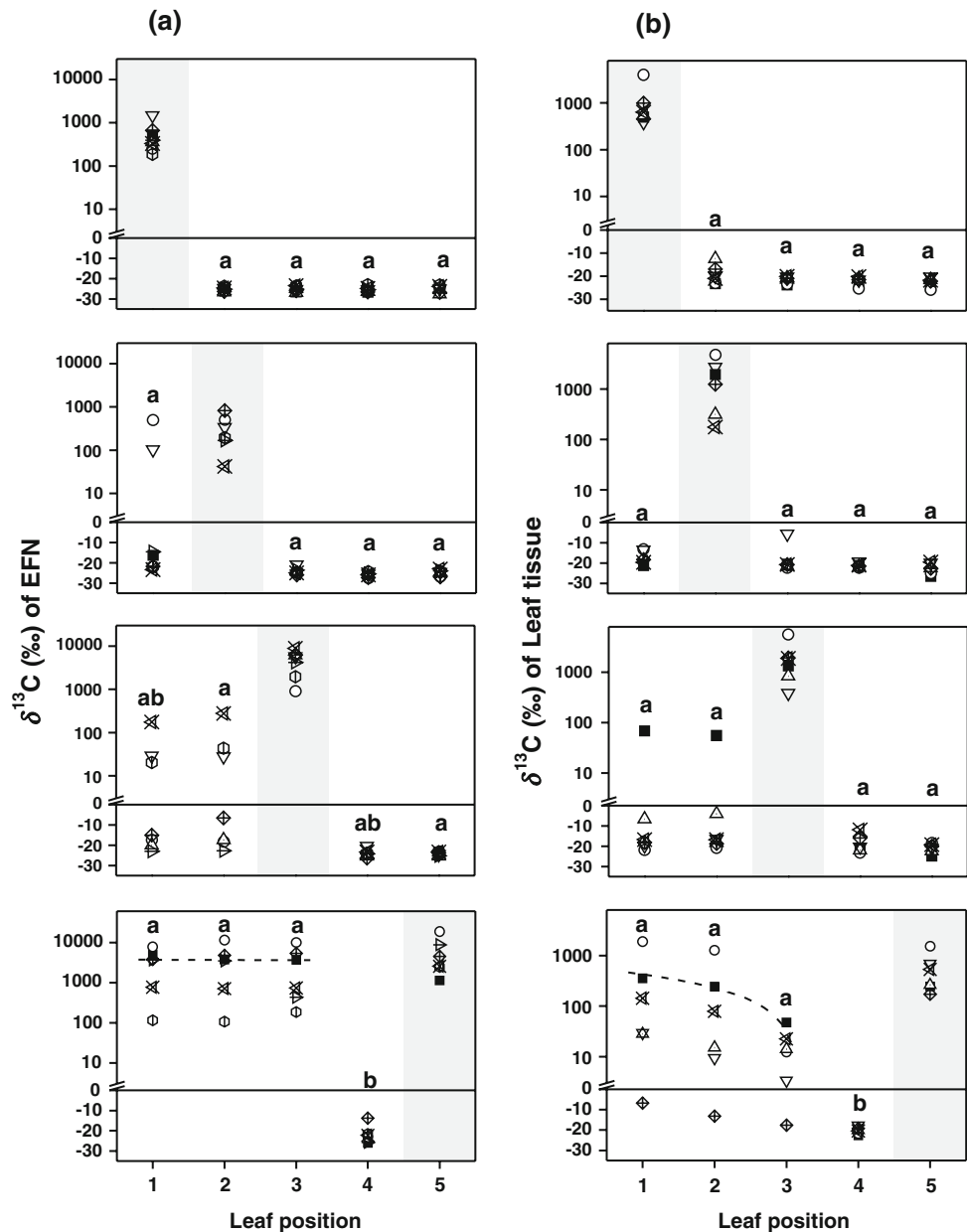
Ontogenetic pattern of VOC emission

Volatile organic compounds emitted from individual leaves were collected from uninduced controls and JA-treated lima bean plants and compared among leaf positions (Fig. 3). The total amount of VOCs released after induction from young leaves was significantly higher than the amounts emitted from older leaves (LSD post hoc test after univariate ANOVA, $P < 0.01$, $n = 8$). Constitutive VOC emission levels, however, were extremely low in leaf number 1 and virtually absent in all other leaves (Fig. 3). JA induction significantly increased the total VOC emission of leaves 1 and 2 over that of the older leaves 3, 4, and 5 (univariate ANOVA, $P < 0.01$, $n = 8$). Similar to our observation for the EFN secretion, the youngest lima bean leaf showed both the highest level of constitutive VOC emission and was inducible to higher levels than all the older leaves. Qualitative changes among differently aged leaves were observed in some of the main constituents of the emitted VOC blend (Fig. 3b, FDR-corrected univariate ANOVA: $P \leq 0.03$, $n = 8$). No significant difference was observed in levels of (*Z*)-*cis*-3-hexenyl acetate, MeSA, β -caryophyllene and TMTT emitted with leaf positions. In general, younger leaves (leaf positions 1–3) emitted more volatiles than the older leaves (leaf positions 4–5).

Discussion

The ODH predicts that the spatial allocation of defensive traits within a plant should favour more valuable and vulnerable plant parts (McKey 1974, 1979; Rhoades 1979). In line with these predictions, the young leaves of both lima bean and castor showed the highest level of the two indirect defences, EFN secretion and VOC emission (Figs. 1b, e, 3). Young leaves are generally important for future plant fitness since they already have caused high construction costs without having contributed very much yet to the plant's pool of photo-assimilates. Consequently, they have the highest future life span and can therefore be expected to contribute bulk to the prospective photosynthetic assimilation. Moreover, very young leaves usually still lack effective mechanical defences (Harper 1989) and indeed it has been shown for several plant species that young leaves, which are more nutritious (Slansky 1993), suffer more from herbivory than older ones within the same plant (Kursar and Coley 1991; Boege and Marquis 2006). Our results show that the young leaves are defended more, both before and after induction (Fig. 1). This observation is in line with the interpretation that EFN and VOCs are allocated based

Fig. 2 Accumulation of ^{13}C in **a** EFN of lima bean plants when leaf position 1 ($n = 8$), 2, 3 ($n = 7$), and 5 ($n = 6$) were purged with air containing $^{13}\text{CO}_2$ (**a**), and leaf tissue ($n = 6$) when leaf positions 1, 2, 3 and 5 were purged with air containing $^{13}\text{CO}_2$ (**b**). Values are given as $\delta^{13}\text{C}$ (‰) with different symbols representing individual replicates. The grey box denotes the leaf position purged with air containing $^{13}\text{CO}_2$. The dashed line indicates the trend of the mean values of all replicates. Different letters indicate significant differences among leaf positions (LSD post hoc after univariate ANOVA, $P < 0.05$)



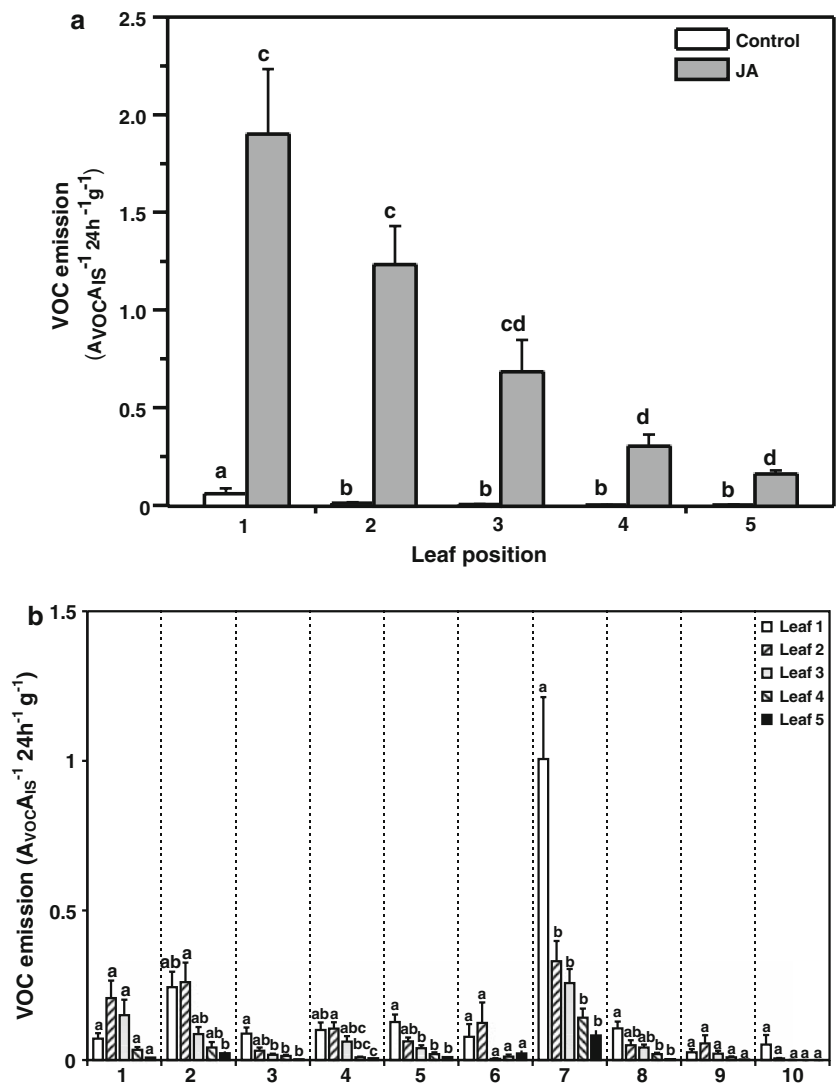
on the value and probability of attack of the leaves, as predicted by the ODH (Anderson and Agrell 2005). Also in the lima bean, which is a cyanogenic plant species, similar patterns have already been demonstrated for its direct defence, as young leaves were characterised by increased amounts of cyanide-containing precursors and higher capacities to release HCN per time unit than mature leaves (Ballhorn et al. 2005).

Our study lends support to previous findings where more valuable plant parts showed increased defence levels upon herbivore feeding (Heil et al. 2000; Wäckers and Bonifay 2004; Rostas and Eggert 2008). Furthermore, it is known that in myrmecophytes, ants preferably patrol and defend young leaves (Heil et al. 2001). In obligate ant-plants, however, this

pattern is not necessarily caused by the spatial distribution of ant rewards (i.e. food bodies and EFN; Heil et al. 1997), but could also be achieved by special behavioural adaptations of the ants (Heil et al. 2004). In contrast, optimal distributions of defenders in facultative interactions with unspecialised animals require that the plants distribute the attractive traits accordingly (Downhower 1975; Heil et al. 2000).

Indeed, the overall emission rate of VOCs increased from young to older leaves, while the qualitative composition of the emitted blend changed only slightly. Both the quantitative and qualitative emission of VOCs have been shown to be highly variable depending on several interacting factors such as plant and herbivore species, type of damage (chewing vs. piercing-sucking) and abiotic factors

Fig. 3 **a** Ontogenetic variation of the total VOC emission (mean \pm 95% CI) of lima bean plants ($n = 8$). The amount of emitted VOCs is given as peak area (A_{voc}) relative to the peak area of an internal standard (A_{IS}) per 24 h per g leaf dry weight. Different letters denote significant differences between groups (global LSD post hoc for all factor combinations between leaf position and treatment after univariate ANOVA, $P < 0.05$). **b** Mean (\pm 95% CI) relative amounts of volatiles emitted by JA-treated plants as determined by the ratio of peak area of the particular compound (A_{voc}) to the peak area of the internal standard (A_{IS}) per 24 h per g dry weight. Different letters denote significant differences among leaf positions as determined with a FDR-corrected univariate ANOVA: $P \leq 0.03$, $n = 8$. *Tr* trace quantities (i.e. ≤ 0.005). Ten most dominant peaks of the TIC were chosen: 1 (3*Z*)-hexenyl acetate, 2 ocimene, 3 (*R*)-linalool, 4 DMNT, 5 $\text{C}_{10}\text{H}_{14}$, 6 methyl salicylate, 7 $\text{C}_{10}\text{H}_{16}\text{O}$, 8 *cis*-jasmone, 9 β -caryophyllene, 10 TMTT



like rainfall and light intensity (for review, see Arimura et al. 2005). Our finding that VOCs are emitted more from younger leaves could be interpreted as a strategy of a directional attraction of parasitoids or other arthropod predators to younger leaves (Hazarika et al. 2007)—a hypothesis that remains to be tested in future studies.

The consistent release patterns of EFN and VOCs give rise to the question whether both traits contribute equally to the plant's protection. Since it was recently shown for lima bean that VOCs act as airborne signals and induce EFN secretion in undamaged plant parts (Kost and Heil 2006; Heil and Silva Bueno 2007), an alternative scenario to the defense hypothesis could be that the primary function of the emitted VOCs is to induce EFN. In this case, parasitoids and other insect predators learning to associate increased VOC levels with an increased presence of herbivores could be a secondary function of the emitted VOCs.

Furthermore, the cost of these two indirect defences remains elusive, though VOCs have been estimated to

cause low costs in corn plants (Hoballah et al. 2004). VOCs and EFN are carbon-based defences and thus might even compete for a common pool of metabolites. The amount of VOCs emitted ranges orders of magnitudes below the amount of carbohydrates that is secreted as EFN. In case of the lima bean for example, a young leaf emits only 1.9 ng/24 h g $^{-1}$ dry weight of mainly carbon-based VOCs, while the same leaf secretes 1.3 mg EFN/24 h g $^{-1}$ dry weight as sugars. It is thus likely that EFN accounts for higher metabolic costs than VOCs. However, further investigation is needed to fully understand the partitioning of plant metabolites for these two indirect defences and future studies must be directed to assess these costs and benefits of both VOC emission and EFN secretion under different herbivore pressures and inductive situations.

Despite being shaped by evolution as an adaptive response, the spatio-temporal distribution of defence traits within plants has to obey limitations in organ-wide or plant-wide resource availabilities. EFN and VOCs are primarily

carbon-based defences, and differences in photosynthetic C-assimilation among organs may thus also cause different production rates of these defensive traits. However, patterns in C-assimilation did not entirely match those observed for EFN and VOCs production, as older leaves were generally characterised by higher photosynthetic rates than younger leaves. On average, younger leaves showed a negative photosynthesis (Fig. 1c, f), i.e. respiration rate was higher than the rate of C-assimilation.

Leaf photosynthesis is the main source for the sugars secreted as EFN (Wardlaw 1990). Young, still developing leaves were characterised by low photosynthetic rates (Fig. 1c, f) and presumably had very low reserves for producing defensive compounds (Larson and Gordon 1969). Thus, they act as physiological sinks and import nutrients until they become competent enough to synthesize defence compounds on their own (Lalonde et al. 2004). Indeed, our ¹³C labelling experiment in lima bean plants indicated a net transport of C assimilated by leaf 5 to younger leaves (1–3; Fig. 2) when all leaves were treated with JA. This result illustrates the transport of photosynthates within the plant from mature to young leaves, where protection is most essential. This finding is in line with previous studies showing that plants can metabolically reorganize in response to herbivory by reallocating resources to growing plant parts (Strauss and Agrawal 1999; Hui et al. 2003) as well as by making younger leaves stronger sinks for defensive metabolites (Arnold and Schultz 2002).

Transport of photosynthates depends on the vascular architecture, and studies have shown that the systemic induction of plant defences can depend on the way the leaves are connected by the vascular system (Davis et al. 1991; Orians et al. 2000; Schittko and Baldwin 2003; Orians 2005; Gomez and Stuefer 2006). In our study, we mimicked herbivory on a plant-wide level by spraying JA on all leaves. In this inductive situation, all the observed pattern could be explained with the ODH.

In summary, we have tested the predictions made by the ODH for two of the most widely distributed indirect plant defences, secretion of EFN and emission of VOCs. We have shown that the plant's induced defensive strategy involves channelling resources in a way that maximises the protection of its most valuable parts. This result is consistent with the ODH in that the youngest leaf, which is a greater contributor towards future plant fitness, enjoys higher defence levels by importing carbohydrates from older leaves. To our knowledge, this is the first report verifying that the within-plant distribution pattern of these two indirect defences does not simply reflect patterns of carbon assimilation, but actually represents an optimal defence strategy.

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