

Article Addendum

Qualitative variability of lima bean's VOC bouquets and its putative ecological consequences

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Key words: indirect defense, herbivore-induced plant volatiles, *Phaseolus lunatus*, trade-off, tritrophic interactions, plant-plant communication

Studies on direct and indirect defenses of lima bean (*Phaseolus lunatus* L.) revealed a quantitative trade-off between cyanogenesis and the total quantitative release of herbivore-induced volatile organic compounds (VOCs). In this addendum we focus on the qualitative variability in the VOC bouquets. We found intraspecific and ontogenetic variation. Five out of eleven lima bean accessions lacked particular VOCs in the bouquets released from secondary and/or primary leaves. These compounds (cis-3-hexenyl acetate, methyl salicylate and methyl jasmonate) can induce and prime indirect defenses in neighboring plants. Thus, the variability in VOC quality as described here might have substantial effects on plant-plant communication.

Plants can be attacked by multiple enemies and accordingly have evolved multiple defense strategies comprising direct and indirect mechanisms. Lima bean (Fabaceae: *Phaseolus lunatus* L.) represents a well established experimental plant for the analysis of three defenses: herbivore-induced volatile organic compounds (VOCs), extrafloral nectar (EFN) and plant cyanogenesis. Herbivore-induced VOCs have manifold functions associated to indirect plant defenses. For example, VOCs attract arthropod predators or parasitoids and thus can act as an indirect defense.¹⁻⁵ They also may be perceived by neighboring, yet-undamaged plant individuals (plant-plant signaling) or plant parts (within-plant signaling) and they prime or induce defensive responses.⁶ VOC-exposed plants may upregulate the secretion of EFN⁷⁻⁹ or the release of VOCs.¹⁰ In addition to these indirect defenses, lima bean shows cyanogenesis as a direct defense. Cyanogenesis means the release of toxic hydrogen cyanide (HCN) from preformed precursors in response

to cell damage¹¹ and is considered a constitutive direct defense against herbivores¹² (but see ref. 13).

Recently we demonstrated that cyanogenesis and total release of VOC in lima bean are negatively correlated to each other.¹⁴ Accessions characterized by strong cyanogenesis in secondary leaves released little amounts of VOCs in response to jasmonic acid (JA) treatment, whereas total VOC production in accessions with low cyanide concentrations was high. Interestingly, these findings also held true on the ontogenetic level, since primary leaves generally showed low concentrations of cyanide and released high amounts of VOCs. However, the question remained unanswered whether these differences are merely of quantitative or also of qualitative nature. We therefore selected eleven accessions from the larger set of lima beans that had been used in our previous study and searched for qualitative differences in their VOC bouquets.

Eight out of the eleven volatiles that were quantified in our study were consistently released from both primary and secondary leaves of all accessions. However, five accessions indeed lacked individual compounds in secondary or primary leaves. For example, we did not detect cis-3-hexenyl acetate in the headspace of secondary leaves of accessions CV_2357 and CV_8078. The latter accession also lacked methyl salicylate in all individual plants analyzed (N = 10 plants). In contrast to secondary leaves, primary leaves of these accessions showed the complete blend of eleven VOCs. While CV_2357 and CV_8078 showed qualitative variability in VOC blends depending on leaf developmental stage, the accessions WT_2233 and WT_PYU consistently lacked methyl jasmonate in both, secondary and primary leaves. Strikingly, accession CV_8073, which was characterized by high quantitative release of cis-3-hexenyl acetate and methyl salicylate from secondary and primary leaves, showed a very low release of methyl jasmonate from secondary leaves—and lacked this compound completely in the headspace of primary leaves (Fig. 1).

In addition to qualitative differences, the quantitative release of volatiles was significantly different among the accessions. This holds true for the three compounds we have focused on in this addendum (Fig. 1) as well as for all eleven volatiles that we have quantified in our previous study (data not shown). However, while quantitative release of total VOCs from secondary leaves was negatively correlated to their cyanide concentration,¹⁴ the qualitative differences in VOC composition were not that strictly correlated

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Submitted: 07/29/08; Accepted: 07/30/08

Previously published online as a *Plant Signaling & Behavior* E-publication: <http://www.landesbioscience.com/journals/psb/article/6693>

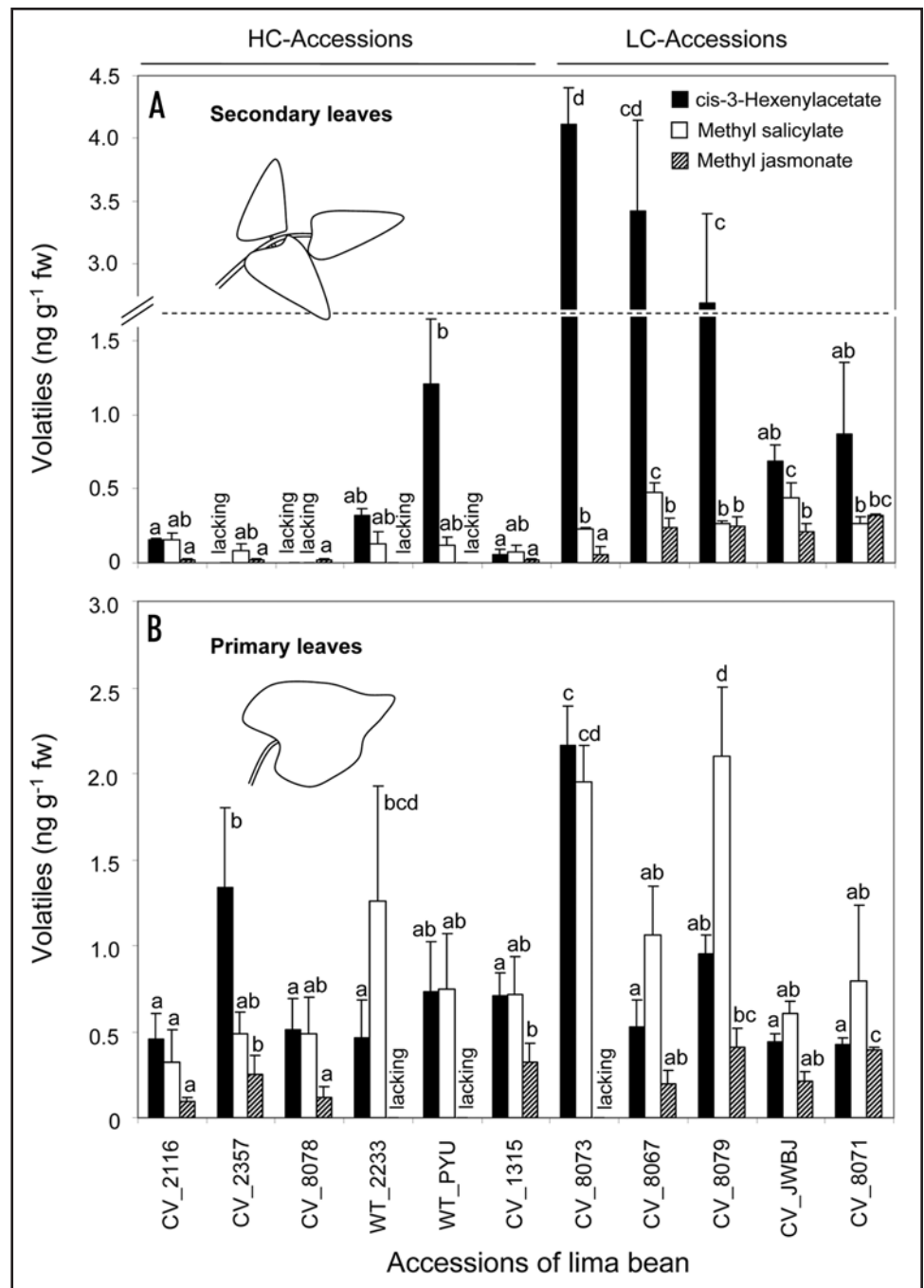
Addendum to: Ballhorn DJ, Kautz S, Lion U, Heil M. Trade-offs between direct and indirect defences of lima bean (*Phaseolus lunatus*). *J Ecol* 2008; 96:971–80; DOI: 10.1111/j.1365-2745.2008.01404.x.

Figure 1. Qualitative variation in VOC mixtures of lima bean. Secondary (A) and primary leaves (B) of cultivated (CV) and wildtype (WT) lima beans characterized by high (HC) or low (LC) concentration of cyanogenic precursors in secondary leaves were analysed for release of three selected VOC compounds. Volatiles were collected in a closed-loop stripping over an experimental period of exactly 24 hrs. Values shown represent the mean (\pm SE) of five plants per accession. Different letters on top of the columns indicate means that differ significantly (according LSD *post-hoc* analysis after one-way ANOVA. Statistical analyses were conducted separately for each compound and each leaf stage).

to cyanogenesis. Accessions CV_2357, CV_8078, WT_2233 and WT_PYU were characterized by high cyanogenic secondary leaves—and lacked at least one compound. However, also the generally low cyanogenic primary leaves did not always show the complete VOC blend (Fig. 1B). These findings demonstrate a high qualitative variability of VOC composition that did depend neither on cyanogenic leaf features nor on leaf ontogeny.

So far it remains elusive whether the observed intraspecific and ontogenetic variability is of relevance in natural systems, but ecological effects are highly likely. For some tritrophic systems an intriguing degree of sophistication in the communication between plants and the third trophic level has been demonstrated: bouquet compositions can provide specific information on the identity of the attacking herbivore and, hence, on its suitability for the prey-seeking carnivores.¹⁵ Variability in the composition of VOC compositions as observed for lima bean (and as also known for corn, cotton or cabbage¹⁶⁻²⁰) may compromise the reliability of herbivore-specific signals across a range of plant genotypes,²¹ although the ability of parasitoids to learn and associate successful foraging and egg-laying experience with the encountered odor pattern may help them in part overcome this problem.^{22,23}

In contrast to tritrophic interactions, the efficiency of volatiles in plant-plant signaling appears more restricted to specific compounds. Recent studies on lima bean demonstrated that *cis*-3-hexenyl acetate causes priming or induction of extrafloral nectar.^{7,24} In other plant species, the release of gaseous methyl jasmonate in response to herbivore attack has been demonstrated to induce the synthesis of proteinase-inhibitors, which represent an efficient defense against herbivores.^{6,25} Methyl salicylate has been reported to be an important elicitor of resistance responses directed towards pathogens and herbivores^{26,27} and as a carnivore-attractant.^{28,29} Thus, the quantitative variation or a complete lacking of single biological



active compounds of volatile blends may have dramatic effects on ecological interactions. We suggest that different defense strategies may be realized in these lima bean accessions: some genotypes have evolved strong cyanogenesis in secondary leaves and now ‘pay’ for this efficient direct defense with reduced or lost abilities for indirect defense and/or plant-plant communication, while others invest less in cyanogenesis and are more “communicative” concerning the third trophic level and conspecifics.

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