Trade-offs between direct and indirect defences of lima bean (*Phaseolus lunatus*)

Daniel J. Ballhorn*, Stefanie Kautz¹, Ulrich Lion¹ and Martin Heil¹²

¹Department of General Botany, Plant Ecology, University of Duisburg-Essen, Universitätsstr. 5, 45117 Essen, Germany; and ²Departamento de Ingeniería Genética, CINVESTAV, Irapuato Km. 9.6 Libramiento Norte, Carretera Irapuato-León, 36821 Irapuato, Guanajuato, Mexico

**Summary**

1. Plant defence theory predicts trade-offs among defence traits as a result of resource limitation or pleiotropic effects. Although theoretically widely accepted, empirical demonstrations of such trade-offs are surprisingly scarce and mechanistic explanations are usually lacking.

2. We quantified cyanogenesis (the release of hydrogen cyanide (HCN)) as a direct defence and the emission of volatile organic compounds (VOCs) as an indirect defence against herbivores. To elucidate whether the trade-offs occur at the genetic or phenotypic level we investigated cultivated and wild-type accessions of lima bean (Fabaceae: *Phaseolus lunatus* L.) and compared different leaf developmental stages. Genetic relationships among the accessions were studied using amplified fragment length polymorphism (AFLP) analysis.

3. Cyanogenesis and the release of VOCs differed significantly among the accessions and were negatively correlated: high cyanogenic accessions released low amounts of VOCs and vice versa. The same remained true for the ontogenetic stages, since primary leaves of all accessions hardly ever produced HCN at all, yet regularly showed high release rates of VOCs.

4. Low and high cyanogenic accessions of lima bean formed distinct clades in an AFLP-based dendrogram, while wild-types and cultivars did not separate. The first pattern indicates that the underlying defensive syndromes are genetically conserved, while the latter is likely to be caused by a multiple origin of cultivated lima beans or an extensive gene flow among cultivated and wild plants.

5. **Synthesis.** Trade-offs between cyanogenesis and VOC release were obvious both between accessions and at the ontogenetic level, and thus cannot be explained by pleiotropy. We contend that allocation restrictions and/or adaptations to different enemy pressures are most likely to explain why lima bean can invest into cyanogenesis or VOCs, but not both.

**Key-words:** AFLP analysis, allocation costs, cyanogenesis, defence syndrome, direct defence, HCN, lima bean, *Phaseolus lunatus* L., pleiotropy, VOCs

**Introduction**

Plants express different types of defence against herbivores, ranging from the constitutive and inducible synthesis of many chemical compounds to the production of structural traits (Karban & Baldwin 1997; Becerra *et al.* 2001; Hare *et al.* 2003). In general, plants do not rely on a single defence mechanism, but rather express multiple defences (Paul & Hay 1986; Hartmann & Dietrich 1998; Romeo 1998; Paul *et al.* 2000; Walling 2000). The combination of different traits often leads to the evolution of ‘defence syndromes’, since the association with specific ecological interactions results in covariation of defensive traits (Romeo *et al.* 1996; Agrawal & Fishbein 2006).

*Correspondence author. E-mail: daniel.ballhorn@uni-due.de*

© 2008 The Authors. Journal compilation © 2008 British Ecological Society
In the present study, we quantified a direct defence mechanism (cyanogenesis) and an indirect defence (the emission of volatile organic compounds, VOCs) of lima bean. Both traits are mainly directed against herbivores, but are likely to target different guilds and have only partly overlapping functions. While traits such as cyanogenesis directly affect the herbivores, indirect defences generally act via the third trophic level (Paré & Tumlinson 1999; Heil 2008). Cyanogenesis is the enzymatically accelerated release of toxic hydrogen cyanide (HCN) from cyanide-containing precursors in response to cell damage (Frehner & Conn 1987; Selmar et al. 1989) and is generally considered as a constitutive plant defence (but see Ballhorn et al. 2006). Since HCN release is directly correlated with the amount of tissue damaged, cyanogenesis is most effective against chewing herbivores (Cork 1996; Ballhorn et al. 2005). Piercing-sucking arthropods, in contrast, generally cause minimal tissue disruption and thus avoid activation of this defence process (Schréiner et al. 1984). Indeed, many specialist herbivores have evolved physiological mechanisms to tolerate cyanide (e.g. Feeny 1976; Provenza et al. 1989).

While cyanogenesis thus functions mainly against generalist chewing herbivores, the release of VOCs is induced by chewing insects as well as by the relatively minor injuries caused by small sucking herbivores such as spider mites (e.g. Dicke et al. 1990). Due to the involvement of the third trophic level, VOC-mediated indirect defence appears largely unaffected by the degree of specialization of the herbivores (e.g. Ehlers et al. 1999). Moreover, the ecological function of VOCs is diverse and besides their function as host-location cues for carnivores (Turlings et al. 1990; Takabayashi & Dicke 1996; Dicke 1994, 1999a,b; for review see Pichersky & Gershenzon 2002) — may comprise the direct repellence of herbivores (de Moraes et al. 2001; Heil 2004), plant–plant signalling (e.g. Arimura et al. 2002) and a role as plant internal signals (Karban et al. 2006; Frost et al. 2007; Heil & Silva Bueno 2007).

We compared 16 accessions of cultivated and wild-type lima beans originating from Mexico, Cuba and Colombia, whose cyanogenic features are well characterized (Ballhorn et al. 2005, 2006, 2007; Ballhorn & Lieberek 2006). The aims of our study were (i) to investigate whether trade-offs between direct and indirect defence occur, (ii) to estimate the overall degree of genetic fixation of the traits investigated, and (iii) to elucidate whether a putative trade-off is caused by pleiotropy. Pleiotropy (a single gene affecting multiple phenotypic traits) is often used to explain trade-offs between different traits (Williams 1957; Joshi & Thompson 1995) and would become obvious by a complete lack of ontogenetic variability in the relationships among the traits in question. In order to meet these goals we quantified HCN release after complete tissue damage. Primary leaves of individual plants were pooled for each leaf developmental stage (Fig. 1). Both ends of the bag were tied avoiding shoot damage. Primary leaves of individual plants were placed in separate

Trade-off between cyanogenesis and VOC-emission

All accessions investigated for their cyanogenic features and their VOC emission were included in an AFLP analysis and combined with a larger set of wild-types of *Phaseolus lunatus* L., *Phaseolus coccineus* L., *Phaseolus microcarpus* Martius and *Phaseolus vulgaris* L.. Two individuals per accession were analysed, resulting in a total of 72 specimens (see Table S1). Total genomic DNA was extracted from finely ground (Qiagen Tissue Lyser) plant material using the Qiagen Plant Mini Kit (Qiagen, Santa Clarita, CA) and following the manual’s instructions. The restriction and ligation mix with a total volume of 20 μL contained 1× T4 buffer, 0.1 mm NaCl, 1 mm BSA, 1 U MseI, 5 U EcoRI, 1 U T4 DNA ligase, 1 μL MseI adapter, 1 μL EcoRI adapter and 15 μL of genomic DNA. Incubation was 3 h at 37 °C followed by 10 min at 72 °C to stop the reaction. Consecutively, samples were diluted 1 : 9 with 180 μL TE buffer (0.1 mm EDTA).

Preselective PCR was conducted with primers complementary to the adapter sequences and having one additional nucleotide on their 3’ end. The preselective primer sequence complementary to the EcoRI end was: 5’-GACTGCGTACCAATTTCA-3’ (EcoRI-A). The MseI preselective primer sequence was: 5’-GATGAGTCCTGAG-TAACT (MseI-C). Underlined letters correspond to the first selective nucleotide. The preselective PCR was performed in a 25 μL reaction containing 1× PCR buffer (Roche, Basel, Switzerland), 1.5 mm MgCl₂, 1× BSA, 0.16 mm of each dNTP, 0.3 μm of each primer, 1 U of *Taq* DNA Polymerase (Roche) and 5 μL of the restricted and ligated DNA (after dilution). Samples were amplified on a MJ Research DYAD with the following PCR profile: initial denaturation for 2 min at 94 °C followed by 26 cycles of 94 °C for 1 min, 56 °C for 1 min (primer annealing), 72 °C for 1 min and a final elongation step of 10 min at 72 °C; holding temperature was set at 4 °C. Amplification products were diluted with 175 μL TE buffer (0.1 mm EDTA).

Eight primer combinations were used for the selective PCR of the DNA restriction fragments (Table 1). EcoRI primers (Applied Biosystems, Forster City, CA) were labelled with fluorescing dye. The PCR reaction mixture consisted of 1× PCR buffer (Roche), 1.5 mm MgCl₂, 1× BSA, 0.16 mm of each dNTP, 0.3 μm of the MseI-C+ primer, 0.1 μm of the labelled EcoRI-A+ primer, 0.6 U of *Taq* DNA Polymerase (Roche) and 2.5 μL of the PCR product derived from the preselective PCR in a total reaction volume of 10 μL. Thermal cycling parameters were: initial denaturation of 2 min at 94 °C followed by 12 cycles of 30 s at 94 °C, 30 s at 65 °C (–0.7 °C per cycle), and 2 min at 72 °C, 25 cycles of 30 s at 94 °C, 30 s at 56 °C and 2 min at 72 °C, and a final elongation step of 30 min at 72 °C; holding temperature was set at 4 °C.

Samples were scored on an ABI 3730 with 9.0 μL HiDi formamide and 0.3 μL LIZ 500 ladder (Applied Biosystems), and 0.7 μL of the product. Fragments were analysed using the Peaksscanner 1.0 software (Applied Biosystems).

A binary matrix reflecting the presence (1) or absence (0) of each AFLP band was generated for each specimen. Only bands between 100–500 bp were included in the analysis. Genetic similarities among all accessions were calculated using the program PAUP* (Swofford 2003) after the Nei–Li model (Nei & Li 1979), which is \[ S = 2ab / (a + b + c) \], where \( a \) bands shared by both individuals; \( b \), bands present in individual (1) but not in (2), and \( c \), bands present in individual (2) but not (1). Dendrograms were visualized using TREEview (Page 1996). For each primer combination one dendrogram was

**Fig. 1.** Experimental setup for analysis of VOCs and HCNp. Plants were induced with JA, and secondary (A) and primary (B) leaves were then separately bagged in PET foil to collect VOCs simultaneously from both stages using pumps containing charcoal filters. After 24 h, the leaves of individual plants were pooled depending on leaf stage (A, B) to be analysed for HCNp (N = 5 induced plants and five control plants per accession).

calculated (data not shown) and one was calculated for all primer combinations together. The correlation coefficient between similarity matrices for each primer was 0.99, indicating that each primer combination provided similar information about this group of accessions.

### Results

#### PLANT DEVELOPMENT

Wild-types of lima bean had developed higher numbers of (smaller) secondary leaves as compared to the cultivars after 5 weeks of cultivation (for statistical analysis on plant development see Table S2 in Supplementary material). Total leaf biomass accumulation, in contrast, was heterogeneous among the accessions. While no clear sorting of biomass accumulation of secondary leaves was observed depending on domestication, biomass accumulation of primary leaves was significantly lower for the wild-types than for the cultivars (see Table S2).

#### CYANOGENIC POTENTIAL

The cyanogenic potential (HCNp) of secondary leaves differed significantly among the 16 accessions of lima bean ($F_{15,44} = 211.81, P < 0.001$ according to one-way ANOVA; $N = 10$ plants per accession). Overall, the plants showed cyanogenic potentials from 0.47 ± 0.08 to 16.88 ± 1.05 μmol HCN g⁻¹ fw (mean ± SD). The wild-types exhibited values of cyanogenic potential concentration between 3.78 ± 0.43 and 12.18 ± 0.96 μmol HCN g⁻¹ fw and ranged in the upper half of the total range of values observed. However, the eight lowest and the three highest cyanogenic accessions were cultivars (Fig. 2), and no obvious sorting of wild-types and cultivated accessions occurred according to the cyanogenic potential (Fig. 2).

The concentration of cyanide-containing compounds in the primary leaves was generally very low (0.03 ± 0.05 to 0.08 ± 0.08 μmol HCN g fresh wt⁻¹; Fig. 3). No significant
differences could be observed between the lines ($F_{10,99} = 0.422$, $P = 0.932$), although the same accessions differed significantly in cyanide concentration of their secondary leaves ($F_{10,99} = 603.986$, $P < 0.001$). The comparison of the two leaf developmental stages showed strong ontogenetic variability in HCNp concentrations. Primary leaves of each accession and each individual plant showed significantly lower concentrations of cyanogenic precursors than secondary leaves of the respective plants (according to Wilcoxon Signed Rank test for two dependent variables, $F_{10,99} = 0.422$, $P = 0.004$, $CV_{2357}$: $P = 0.024$, $CV_{8079}$: $P < 0.001$), but not for accessions characterized by high release of VOCs from secondary leaves ($CV_{8073}$, $CV_{8067}$, $CV_{8079}$, $CV_{1315}$) and among leaves of both developmental stages ($F_{15,64} = 0.543$, $P = 0.849$), untreated secondary leaves ($F_{15,64} = 0.611$, $P = 0.855$) and among leaves of both developmental stages ($F_{15,12} = 0.709$, $P = 0.771$).

VOLATILE ORGANIC COMPOUNDS (VOCs)

Treatment with JA led to a substantial increase in volatile emission from leaves of all accessions (Figs 2 and 3), as treated leaves of both developmental stages showed significantly higher VOC emission than the corresponding untreated leaves (Wilcoxon Signed Rank test, $P < 0.001$; $N = 10$ plants per accession). However, the quantitative analysis of the 11 most prominent VOCs emitted by JA-treated secondary leaves demonstrated highly significant differences among accessions ($F_{15,64} = 14.445$, $P < 0.001$ according to one-way ANOVA; $N = 5$ induced plants per accession). Total amounts of volatiles ranged from 1.70 ± 0.42 to 14.15 ± 3.23 ng volatiles g⁻¹ fw 24 h⁻¹ (Fig. 2). No sorting of wild-types and cultivated accessions occurred according to the emission of total volatiles by induced secondary leaves ($F_{15,64} = 2.236$, $P = 0.137$).

Primary leaves of all accessions tested ($N = 11$ accessions) showed a strong release of volatiles (Fig. 3), even in those accessions that were characterized by extremely low VOC-emission from secondary leaves (Fig. 2). The total amounts of volatiles emitted by primary leaves in response to JA-treatment ranged from 6.27 ± 5.26 to 13.98 ± 6.12 ng volatiles g⁻¹ fw 24 h⁻¹ and were not significantly different between lines ($F_{10,44} = 1.944$, $P = 0.064$). The quantitative difference in VOC emission between induced primary and secondary leaves was significant only for accessions that were characterized by low emission of volatiles by secondary leaves (according to Wilcoxon Signed Rank test for two dependent variables, $CV_{2116}$: $P = 0.004$, $CV_{2357}$: $P = 0.004$, $CV_{8078}$: $P = 0.023$, $WT_2233$: $P = 0.010$, $WT_PYU$: $P = 0.024$, $CV_{1315}$: $P < 0.001$), but not for accessions characterized by high release of VOCs from secondary leaves ($CV_{8073}$, $CV_{8067}$, $CV_{8079}$, $CV_{1857}$ and $CV_{8071}$).

Untreated (control) leaves generally showed low release of volatiles (VOCs). For all accessions we found no significant differences among untreated primary leaves ($F_{10,44} = 0.543$, $P = 0.849$), untreated secondary leaves ($F_{15,64} = 0.611$, $P = 0.855$) and among leaves of both developmental stages ($F_{15,12} = 0.709$, $P = 0.771$).

CYANOGENESIS VS. EMISSION OF VOLATILES

The quantitative emission of volatiles and the concentration of cyanogenic potential (HCNp) in secondary leaves of the different accessions were significantly negatively correlated (two-tailed Pearson correlation: $r = -0.858$, $P < 0.001$; $N = 16$), since secondary leaves of all accessions that were characterized by a high HCNp released small amounts of volatiles in response to JA treatment, and vice versa (Fig. 2). Accessions selected for comparative analyses of both leaf developmental stages (Fig. 3) consequently also showed a clear negative correlation of HCNp and VOC-emission among secondary leaves ($r = -0.946$, $P < 0.001$; $N = 11$). Among primary leaves, in contrast, HCNp concentration and quantitative emission of volatiles were not correlated ($r = 0.149$, $P = 0.661$; $N = 11$), since primary leaves of all accessions showed very low concentration of cyanide, but substantial emission of volatiles.
following induction (Fig. 3). A GLM-model for analysis of variance after multivariate design confirmed highly significant effects of accession and leaf stage on both HCNp and VOCs (Table 2). Due to the constitutive character of cyanogenesis, ‘treatment’ exclusively had a significant effect on inducible VOCs. All statistical analyses were carried out using spss 13.0 (SPSS for Windows, SPSS, Chicago, IL).

AFLP ANALYSIS

The eight AFLP primer combinations produced a total of 509 variable bands (Table 1). The number of bands amplified per primer combination varied from 1 to 56. A total of 99.6% of all bands was polymorphic across the entire sample while 75.4% were polymorphic for *P. lunatus*.

Two major clusters could be distinguished. One cluster comprised all specimens of *P. lunatus*, while the second was formed by two subclades, one with *P. vulgaris*, the other with *P. coccineus* and *P. microcarpus* (Fig. 4). Within lima bean, accessions were separated into five subclusters: One comprised several wild-types as well as cultivars, all of which were low cyanogenic genotypes with corresponding high emission of volatiles. The second and third clade comprised wild-types that were collected close to Puerto Escondido (Pacific coast) or at a site c. 40 km west from there. One further group consisted of both wild-type and cultivated forms and included most of the high cyanogenic cultivars. Finally, wild lima beans that were collected close to Matias Romero in the Isthmus of Tehuantepec fell into a fifth clade. In summary, genotypes with high HCNp and corresponding low emission of volatiles from secondary leaves were generally genetically separate from the accessions with low HCNp and high emission of VOCs.

**Table 2.** Effects of accession, treatment and leaf stage on VOCs and HCNp. Results obtained using the GLM for analysis of variance after a multivariate design with VOCs and HCNp as variables. The term ‘Leaf Stage (Accession × Treatment)’ is nested, because each measure for plant part comes from only one combination of the accession and treatment.

<table>
<thead>
<tr>
<th>Source</th>
<th>Dependent variable</th>
<th>SS</th>
<th>d.f.</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accession</td>
<td>VOCs</td>
<td>721.132</td>
<td>15</td>
<td>9.987</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>HCNp</td>
<td>2543.157</td>
<td>15</td>
<td>161.163</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Treatment</td>
<td>VOCs</td>
<td>3085.856</td>
<td>1</td>
<td>641.056</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>HCNp</td>
<td>0.136</td>
<td>1</td>
<td>0.129</td>
<td>0.719</td>
</tr>
<tr>
<td>Accession × Treatment</td>
<td>VOCs</td>
<td>691.288</td>
<td>15</td>
<td>9.574</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>HCNp</td>
<td>10.423</td>
<td>15</td>
<td>0.661</td>
<td>0.821</td>
</tr>
<tr>
<td>Leaf stage (Accession × Treatment)</td>
<td>VOCs</td>
<td>713.781</td>
<td>22</td>
<td>6.740</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>HCNp</td>
<td>5427.293</td>
<td>22</td>
<td>234.500</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Error</td>
<td>VOCs</td>
<td>1039.760</td>
<td>216</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HCNp</td>
<td>227.233</td>
<td>216</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discussion

We compared two leaf developmental stages and 16 different cultivated and wild accessions of lima bean to study trade-offs among cyanogenesis as a direct and the emission of VOCs as an indirect resistance trait. We found a substantial variation among accessions for both cyanogenesis and VOCs emission by secondary leaves, and the two traits were significantly negatively correlated with each other (Fig. 2). Hence, we found a clear quantitative trade-off between a direct and an indirect defence against herbivores.

Interestingly, though wild-types and cultivars were characterized by different morphological and developmental parameters (see Table S2), no clear association of cyanogenic precursor concentration (HCNp) with status of cultivation was found, since the cultivated accessions comprised the highest as well as the lowest cyanogenic accessions (Fig. 2). Correspondingly, patterns of VOC emission did not clearly correlate with the status of cultivation (Fig. 2). A similar lack of a clear effect of domestication on overall VOCs release has been reported in earlier studies for other crop plants (Benrey et al. 1998; Gouinguené et al. 2001).

Trade-offs between different types of defence have repeatedly been reported (Heil & Bostock 2002; Marak et al. 2002a,b; Biere et al. 2004) to occur between chemical and mechanical defences (Steward & Keeler 1988) or between constitutive and induced defences (Mattson et al. 1988; Karban & Myers 1989; Agrawal et al. 2002). Morris et al. (2006) discussed difficulties in finding clear trade-offs between constitutive and induced defences that can arise from sampling variation, measurement error and induced susceptibility (lower resistance in damaged than in undamaged plants). However, although being principally an induced defence, only maximum release rates of VOCs were evaluated in our study and these showed a significant negative correlation with cyanogenesis. We therefore found a clear trade-off between a direct and an indirect defence.

Although this is a widespread assumption, several studies failed to find trade-offs between direct and indirect defences (Steward & Keeler 1988; Letourneau & Barbosa 1999; Dyer et al. 2001; Heil et al. 2002). Such discrepancies are difficult to explain as long as we lack a mechanistic understanding of how trade-offs arise (Simms 1992; Rausher 1996; Strauss et al. 2002; reviewed in Agrawal 2006). Is the strong negative correlation between cyanogenesis and VOC-emission caused by pleiotropic effects? Is it caused by physiological trade-offs (i.e. resource limitations), or does it represent an adaptation to changing selective pressures during ontogenetic development?
PLEIOTROPY OR PHENOTYPIC PLASTICITY

Single genes can affect multiple phenotypic traits (pleiotropy) and then cause invariant co-occurrence of particular traits that represent a consequence of genetic constraints rather than adaptive strategies (Fritz 1990). Consequently, clear patterns of genetic correlations should be apparent and predict how these traits will jointly respond to selection (Simms 1992; Stearns 1992). To understand whether the trade-offs observed in our study can be caused by such genetic constraints we investigated the relatedness among the lima bean associations studied here, including additional Phaseolus species, and we studied whether the patterns observed are subject to phenotypic plasticity.

AFLP analysis revealed mostly exclusive clades comprising either high or low cyanogenic accessions (= low or high VOC emitting accessions, see Fig. 4). This indicates a comparably high level of genetic conservation of these traits. Interestingly, no clustering of cultivated and wild accessions became obvious, a pattern that can be explained by a multiple origin of cultivated lima bean or intense gene flow among wild and domesticated forms. Anyway, cyanogenesis and emission of VOCs apparently do not depend on the status of domestication.

While this pattern could indeed hint to genetic constraints on the expression of either intensive cyanogenesis or VOCs release, the primary leaves of all accessions were characterized by very low HCNp but released high amounts of VOCs. Thus, several lines change their defensive strategy from being

---

Fig. 4. Genetic relationships among lima bean accessions and closely related species as revealed by AFLP analysis. The dendrogram was developed using the Neighbour Joining method of Nei–Li similarity values for all eight primer combinations. High cyanogenic (HC) and low cyanogenic (LC) accessions as defined from HCNp values of secondary leaves (Fig. 2) are indicated with black and white squares. See Table S1 for further information on Phaseolus specimens included in this analysis.
VOC-dominated to cyanogenesis-dominated, demonstrating a high phenotypic plasticity (Schlichting & Pigliucci 1998; Pray 2004; Vaughan & Eichorst 2006) of both traits that significantly depends on ontogeny (Table 2). This observation rejects the assumption of pleiotropy causing the observed patterns: the trade-offs between direct and indirect defence apparently were caused at the ontogenetic and/or physiological level (Tiffin & Rausher 1999).

ONTGENETIC CHANGES

Qualitative and/or quantitative ontogenetic changes are known for both indirect defence by VOCs (Anderson & Agrawal 2005; Rostás & Eggert 2008) and direct defence via cyanogenesis (Bernays et al. 1977, Ballhorn et al. 2005). In the present study we found that all accessions investigated defend their primary leaves mainly indirectly via VOCs (Fig. 3), while some, but not all, accessions then shift to a preferred direct defence by cyanogenesis in the secondary leaves.

Which factors cause these changes, and why do the accessions behave differently? Leaf age often has strong effects on leaf biochemistry (Coley 1980, 1988; van Noordwijk & de Jong 1986), and many secondary compounds – including cyanogenic precursors – are mobilized and transported from senescing to growing tissues (Clegg et al. 1979; Adewusi 1990; Selmar 1993; Siritunga et al. 2002). However, senescence of primary leaves can be excluded for our experimental conditions, since primary leaves of lima bean remain viable much longer than our experiment lasted (D.J. Ballhorn, pers. obs.). Similarly, all accessions experienced the same growing conditions, and putative restrictions resulting from mere allocation costs thus cannot explain the differences among the accessions that we observed.

The observed patterns thus more likely represent changing defensive strategies that evolved in adaptation to specific environmental conditions. Plants face multiple enemies both in natural and in agronomic ecosystems (Linhart 1991; Walling 2000) and different developmental stages suffer from different enemy pressures. For example, besides herbivores, fungal pathogens are almost ubiquitous (Felton & Korth 2000). Trade-offs between cyanogenesis and resistance to fungal pathogens have been reported (Lieberei 1988; Lieberei et al. 1989, 1996; Thalet et al. 1999; Felton & Korth 2000), and primary and secondary leaves of lima bean are likely to differ in the pressure they experience from pathogens and herbivores. Different accessions almost certainly have evolved under very different ecological conditions. Hence, it is tempting to speculate that the different defensive strategies of primary vs. secondary leaves that we found and the different degrees to which the plants change this strategy during their ontogeny represent adaptations to changing enemy pressures (Marquis 1990, 1992; Karban & Thaler 1999; Thalet et al. 2004).

Conclusions

We report a negative correlation between a direct and an indirect defence against herbivores. Such trade-offs can severely compromise a plant’s ability to combine different traits for the achievement of an optimal protection. Toxic compounds are assumed to slow down the developmental rates of herbivores and thereby increase their exposition to natural enemies. The trade-off we found is not consistent with this expectation, since plants that invest much in growth rate-decreasing HCN lack the VOCs to attract natural enemies of their herbivores and vice-versa. However, direct defence by HCN and indirect defence via VOCs appear targeted against different types of herbivores, and the comparison of primary with secondary leaves demonstrates that the relative dominance of direct vs. indirect defence can change during plant ontogeny. Adaptations to varying enemy pressures thus are the most likely explanation of the observed phenomenon. More than 10% of higher plants are cyanogenic (Moller & Seigler 1999) and many, if not all, higher plants emit volatiles in response to herbivore feeding (Dicke 1999b; Pichersky & Gershenzon 2002; Arimura et al. 2005; Heil 2008). Both traits are thus likely to co-occur in many species. Future studies should consider the interaction of cyanogenesis and VOC emissions to gain a better understanding of plant defense syndromes and on the restrictions trade-offs such as that described place on the evolution of plant defences.

Acknowledgements

We are grateful to Monika Fillippek for plant cultivation, to Susann Schiwy for quantification of volatile compounds, and to Michael Neugebauer for technical support (all Essen). Alfonso Delgado Salinas (UNAM, Mexico) kindly helped with species determination, and Kevin Feldheim, Thorsten Lumbsch and Ole Shelton (all Field Museum, Chicago) are thanked for advice concerning the AFLP analysis. This work was financially supported by the DFG (grant He 31694-2) and a start up fund by the University of Duisburg-Essen to MH. The molecular part of this work was conducted in the Prizker Laboratory of Molecular Systematics and Evolution at the Field Museum of Natural History in Chicago.

References


Trade-off between cyanogenesis and VOC-emission


Received 22 March 2008; accepted 23 April 2008

Handling Editor: Joop Ouborg

**Supplementary material**

The following supplemental material is available for this article:

**Table S1** *Phaseolus* specimens included in the AFLP analysis

**Table S2** Domestication and biomass accumulation of different lima bean accessions

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/full/10.1111/ j.1365-2745.2008.01404.x

(This link will take you to the article abstract).

Please note: Blackwell Publishing is not responsible for the content or functionality of any supplementary materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.