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

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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 cynogenesis 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). For example, toxic compounds such as cyanide are thought to defend a plant in two principle ways: directly by reducing insect oviposition or feeding (Ballhorn *et al.* 2007) or indirectly, through slowing down the rate of herbivore development and thereby increasing their susceptibility to natural enemies (the 'slow-growth-high-mortality hypothesis', see Lill & Marquis 2001).

However, co-expression of multiple defences might be costly for a plant (Mole 1994; Mauricio 1998), since investment in defensive traits is assumed to reduce the resource availability for growth and reproduction (Herms & Mattson 1992; Bergelson & Purington 1996; Koricheva 2002; Strauss *et al.* 2002). Constraints on simultaneous resource allocation to multiple defensive strategies are consequently thought to result in negative associations (trade-offs) among different types of defences (Heil & Bostock 2002; Koricheva *et al.* 2004).

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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 (Schreiner *et al*. 1984). Indeed, many specialist herbivores have evolved physiological mechanisms to tolerate cyanide (e.g. Feeny 1976; Provenza *et al*. 1992).

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 & Lieberei 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 disruption and VOC release in response to jasmonic acid (JA) treatment as a measure of maximum expression of these two defences, we studied the genetic relationships among our accessions using amplified fragment length polymorphism (AFLP) analyses, and we compared different ontogenetic leaf stages of individual plants. Our results provide new insights into the ecology and the evolutionary flexibility of direct and indirect defences of lima bean.

Methods

PLANT MATERIAL AND CULTIVATION

We used 12 cultivars and four wild-types of lima bean (Fabaceae: *Phaseolus lunatus* L.). Seeds were provided by the Institute of Plant Genetics and Crop Plant Research (IPK) in Gatersleben, Germany; the Centro Internacional de Agricultura Tropical (CIAT), Recta Cali-Palmira, Colombia; the MPI for Chemical Ecology, Jena, Germany; Betty Benrey from the University of Neuchâtel, Switzerland; and Marco Gross from the University of Hamburg, Germany. Additional seeds were directly collected from native populations of lima bean growing in different parts of the state of Oaxaca, Mexico (see Table S1 in Supplementary material for detailed information on the accessions used).

Plants used for quantification of their defences were cultivated under greenhouse conditions (16 : 8 L : D with a photon flux density of 350–450 μmol s^{-1} m^{-2} at the plant container and 800–950 μmol s^{-1} m^{-2} on the top of the plants, depending on natural radiation). Additional light was provided by 400 watt high-pressure sodium lamps (Son-Targo 400, Philips®). To avoid effects by hot spots under the lamps, plants were moved every 3 days. Day/night temperature and ambient relative air humidity were set to 25 °C : 20 °C and 60–70%, respectively. Plants were fertilized with nitrogen–phosphate fertilizer (Blaukorn®-Nitrophoska®-Perfekt, Compo GmbH) twice a week (3 mg pot^{-1}) and cultivated in plant-containers of 18 cm in diameter in a 1 : 1 mixture of standard substrate (TKS®-1-Instant, Floragard®) and sand (grain size 0.5–2.0 mm). Thirty plants per accession were cultured (full sibs). Plants used for the experiments were 5 weeks old and had developed five to seven trifoliate secondary leaves.

CYANOGENIC FEATURES OF PLANTS

We analysed the cyanogenic potential (HCNp; concentration of cyanogenic precursors) of primary and secondary leaves. Leaves of individual plants were pooled for each leaf developmental stage (Fig. 1). For analysis, leaves were removed with a razor blade, weighed to the nearest 0.001 g and ground with liquid nitrogen in a cooled mortar and pestle at 4 °C. Five (for primary leaves) or 25 mL (secondary leaves) ice-cold Na_2HPO_4 buffer (67 mmol L^{-1}) were added. The samples were quantitatively analysed for their HCNp by complete enzymatic hydrolysis of cyanogenic precursors with specific β-glucosidase isolated from rubber tree (Euphorbiaceae: *Hevea brasiliensis*). We used closed glass vessels (Thunberg vessels) for incubation of leaf extracts together with enzyme solution adjusted to an activity of 20 nkat. Quantitative detection of the released HCN was carried out spectrophotometrically at 585 nm using the Spectroquant® cyanide test (Merck, Darmstadt, Germany; method described in detail in Ballhorn *et al*. 2005).

VOLATILE ORGANIC COMPOUNDS (VOCs)

Plants were induced for volatile production by spraying 10 mL of a 1-mmol L^{-1} aqueous solution of jasmonic acid (JA) per plant at 09:00 AM. Control plants were sprayed with 10 mL of water. Plants were sprayed until runoff, subsequently allowed to dry (*c*. 30 min), before the procedure was repeated. After drying, the plant part bearing the secondary leaves was placed in a PET bag ('Bratschlauch', Toppits®, Minden, Germany; this material does not emit detectable amounts of volatiles even after exposure to temperatures up to 150 °C; Fig. 1). Both ends of the bag were tied avoiding shoot damage. Primary leaves of individual plants were placed in separate

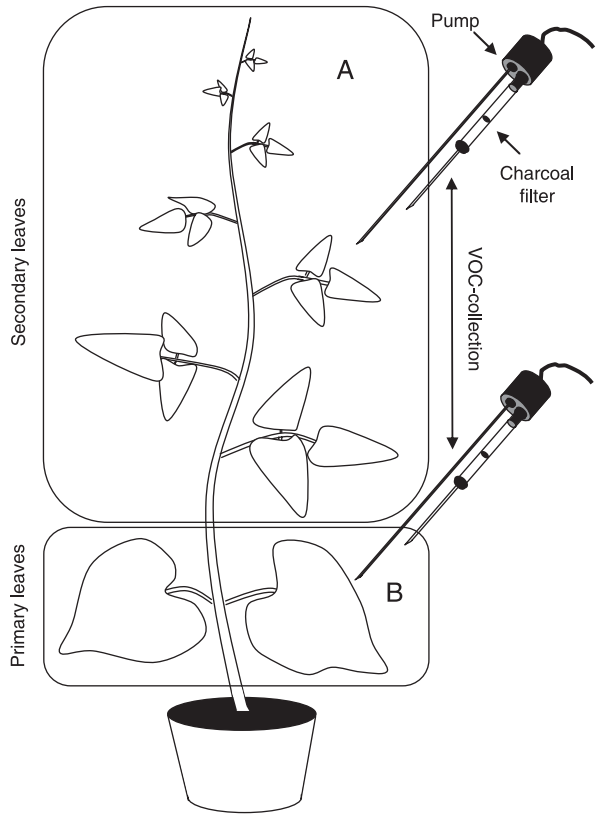

Fig. 1. Experimental setup for analysis of VOCs and HCNp. Plants were induced with JA, and secondary (A) and primary (B) leaves were then separately bagged in PET foil to collect VOCs simultaneously from both stages using pumps containing charcoal filters. After 24 h, the leaves of individual plants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

PET bags (Fig. 1). The six highest and five lowest cyanogenic accessions were analysed for volatile emission from primary leaves. Bagged plants remained in their respective pot and were placed in the greenhouse under the same ambient conditions as for plant cultivation.

Volatiles were collected continuously over 24 h on charcoal filters (1.5 mg charcoal, CLSA-Filters, Le Ruissaeu de Montbrun, France) using air circulation in closed loop stripping as described previously (Donath & Boland 1995). After 24 h, volatiles were eluted from the carbon filter with dichloromethane (40 µL) containing 1-bromodecane (200 ng μL^{-1}) as internal standard (IS). Samples were analysed on a GC-Trace mass spectrometer (Trace GC Ultra DSQ; Thermo Electron, Austin, TX). The program for separation [Rtx5-Ms column (Restek, Philadelphia, PA), 15 m × 0.25 mm; 0.25 µm coating] was 40 °C initial temperature (2 min), 10 °C min^{-1} to 200 °C, then 30 °C min^{-1} to 280 °C with He (constant flow 1.5 mL min^{-1}) as carrier gas. Compounds were identified by comparison to standard substances (Fluka Seelze, Germany) and with the Nist 05 library (Xcalibur 1.4 software; Thermo Electron Corp., Austin, TX). Individual compounds (peak areas) were quantified with respect to the peak area of the IS (1-bromodecane), and quantities are presented as a percentage of the IS area. Only compounds for which reference substances were available were included in quantitative analysis (*cis*-3-hexenyl acetate, 2-ethylhexan-1-ol, *cis*-β-ocimene, linalool, *cis*-3-hexanol butyrate, methyl salicylate, *cis*-3-hexenyl isovalerate, indole, *cis*-jasmone, β-caryophyllene, methyl jasmonate). The quantitative data on volatile production were divided by the leaf fresh weight of the respective plant in order to calculate total volatile mass released per gram fresh mass.

AFLP ANALYSIS

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 Mse1, 5 U *Eco*RI, 1 U T4 DNA ligase, 1 µL *Mse*I adapter, 1 µL *Eco*R1 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 *Eco*RI end was: 5′-GACTGCGTACCAATTCA-3′ (*Eco*RI-A). The MseI preselective primer sequence was: 5′-GATGAGTCCTGAGTAAC (*Mse*I-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 $MgCl_2$, 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). *Eco*RI primers (Applied Biosystems, Forster City, CA) were labelled with fluorescing dye. The PCR reaction mixture consisted of 1× PCR buffer (Roche), 1.5 mM $MgCl_2$, 1× BSA, 0.16 mM of each dNTP, 0.3 µm of the *Mse*I-C++ primer, 0.1 µm of the labelled *Eco*RI-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 Peakscanner 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 $S = 2a/(2a + b + c)$, 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

Table 1. Level of polymorphism found in a group of wild *Phaseolus* as well as wild and cultivated *Phaseolus lunatus* accessions and genotypes by means of AFLPs as indicated by primer combination

		Number of bands				
Primer combination	Dye	Analysed	Polymorph	Per accession	Polymorph for *P. lunatus*	Per *P. lunatus* acc.
EcoRI-CAA/MseIAAG	NED	93	93	5–58	72	36–58
*Eco*RI-CTC/*Mse*I-ACC	6-FAM	73	73	1–26	46	15–38
*Eco*RI-CTC/*Mse*IAAG	NED	95	93	6–50	75	20–50
*Eco*RI-CTT/*Mse*I-AGC	VIC	46	46	3–22	34	7–22
*Eco*RI-CCG/*Mse*I-ACG	VIC	60	60	2–26	54	9–26
*Eco*RI-CCG/*Mse*I-ACC	6-FAM	37	37	1–16	28	2–14
*Eco*RI-CCG/*Mse*I-AAG	NED	56	56	8–26	36	10–26
*Eco*RI-CCG/*Mse*I-AGA	PET	49	49	12–26	39	17–26
All		509	507	114–207	384	104–216

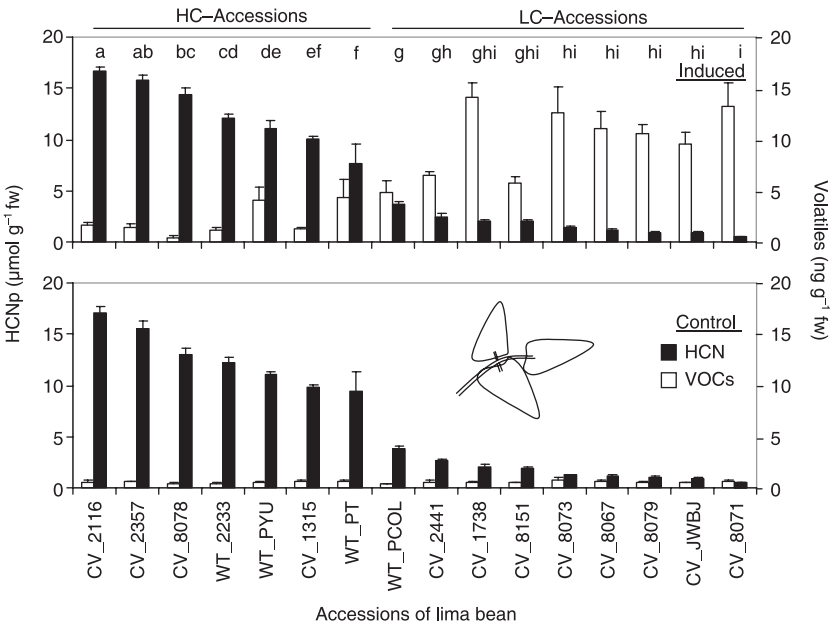

Fig. 2. Variability of HCNp and VOC-emission among lima bean accessions. Secondary leaves of cultivated (CV) and wild-type (WT) lima beans were analysed for cyanogenic potential (HCNp) and total emission of volatiles ($N = 5$ induced plants and five control plants per accession; values shown are means + SD). Data are presented separately for JA-induced (upper panel) and control plants (lower panel). Lima bean accessions marked with different letters in the upper panel differ significantly in their HCNp [according to *post-hoc* test after one-way ANOVA (Tukey's HSD; $P < 0.05$)].

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

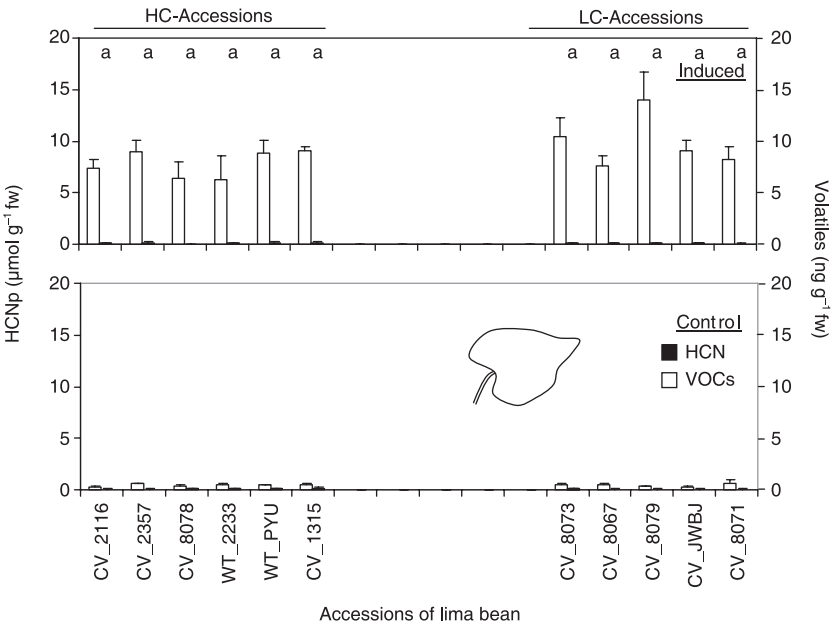

Fig. 3. VOC-dominated defence of primary leaves. Accessions selected for highest and lowest HCNp in secondary leaves were analysed for HCNp and total emission of VOCs from primary leaves. Means + SD obtained from the same individual plants as for analyses of secondary leaves (Fig. 2) are presented separately for JA-induced (upper panel) and control plants (lower panel), using the same scales as in Fig. 2 ($N = 5$ induced plants and five control plants per accession). No significant differences in the HCNp of primary leaves were found among accessions [all *post-hoc* tests after one-way ANOVA (Tukey's HSD): $P < 0.05$)].

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 using values of the primary and the secondary leaves of each individual plant as pairs, $P < 0.001$).

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 ($F_{1,78} = 2.236$, $P = 0.137$).

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_JWBJ 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

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

Source	Dependent variable	*SS*	d.f.	*F*	*P*
Accession	VOCs	721.132	15	9.987	< 0.001
	HCNp	2543.157	15	161.163	< 0.001
Treatment	VOCs	3085.856	1	641.056	< 0.001
	HCNp	0.136	1	0.129	0.719
Accession × Treatment	VOCs	691.288	15	9.574	< 0.001
	HCNp	10.423	15	0.661	0.821
Leaf stage (Accession × Treatment)	VOCs	713.781	22	6.740	< 0.001
	HCNp	5427.293	22	234.500	< 0.001
Error	VOCs	1039.760	216		
	HCNp	227.233	216		

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.

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?

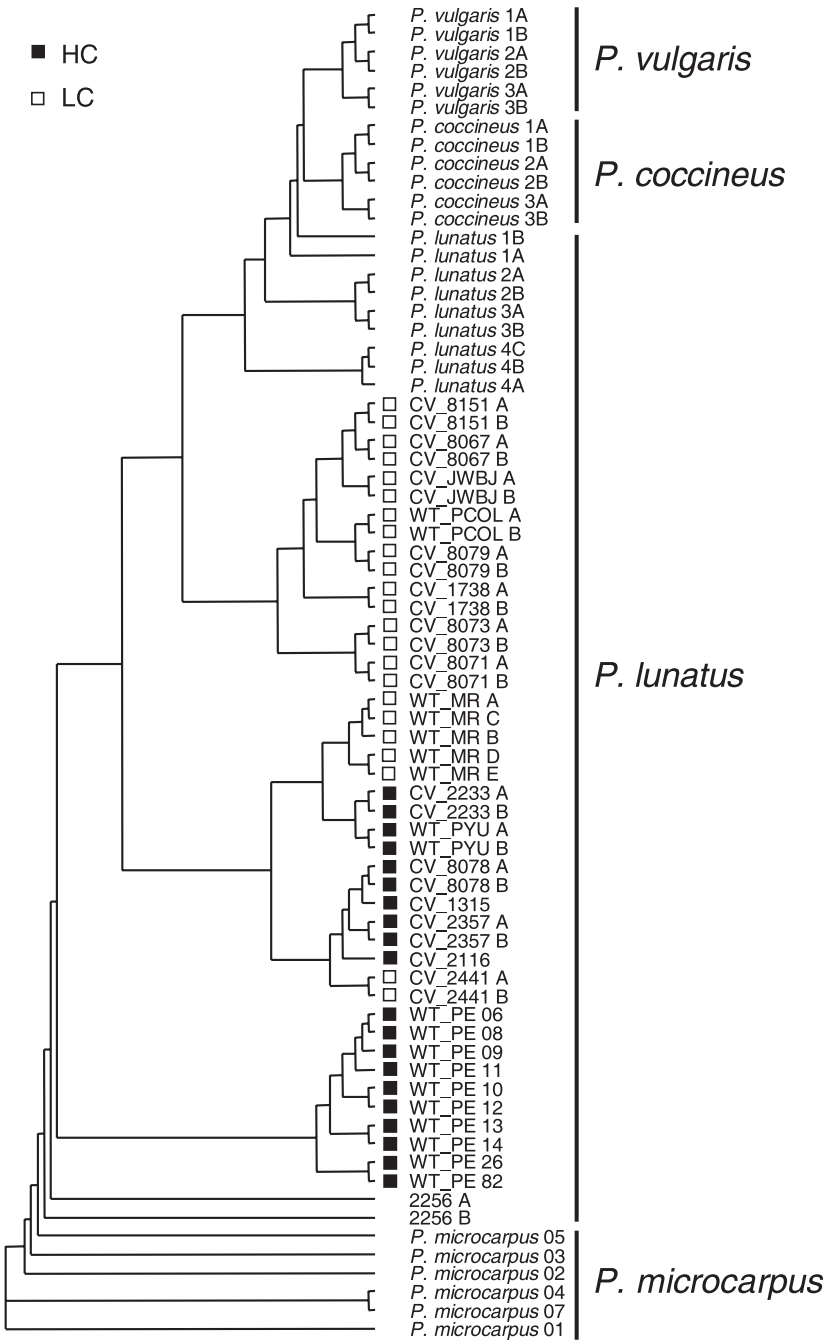

Fig. 4. Genetic relationships among lima bean accessions and closely related species as revealed by AFLP analysis. The dendrogram was developed using the Neighbour Joining method of Nei–Li similarity values for all eight primer combinations. High cyanogenic (HC) and low cyanogenic (LC) accessions as 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.

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

VOC-dominated to cyanogenesis-dominated, demonstrating a high phenotypic plasticity (Schlicting & 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).

ONTOGENETIC CHANGES

Qualitative and/or quantitative ontogenetic changes are known for both indirect defence by VOCs (Anderson & Agrell 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 cyanogensis 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; Thaler *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; Thaler *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 (Møller & 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 defence syndromes and on the restrictions trade-offs such as that described place on the evolution of plant defences.

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References

Adewusi, S.R.A. (1990) Turnover of dhurrin in green sorghum seedlings. *Plant Physiology*, **94**, 1219–1224.

Agrawal, A.A. (2006) Macroevolution of plant defense strategies. *Trends in Ecology and Evolution*, **22**, 103–109.

Agrawal, A.A. & Fishbein, M. (2006) Plant defense syndromes. *Ecology*, **87**, 132–149.

Agrawal, A.A., Janssen, A., Bruin, J, Posthumus, A. & Sabelis, M.W. (2002) An ecological cost of plant defense: attractiveness of bitter cucumber plants to natural enemies of herbivores. *Ecology Letters*, **5**, 377–385.

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

Arimura, G.I., Kost, C. & Boland, W. (2005) Herbivore-induced, indirect plant defences. *Biochimica et Biophysica Acta*, **1734**, 91–111.

Arimura, G.I., Ozawa, R., Nishioka, T., Boland, W., Koch, T., Kühnemann, F. & Takabayashi, J. (2002) Herbivore-induced volatiles induce the emission of ethylene in neighboring lima bean plants. *Plant Journal*, **29**, 87–98.

Ballhorn, D.J. & Lieberei, R. (2006) Oviposition choice of Mexican bean beetle (*Epilachna varivestis*) depends on host plants cyanogenic capacity. *Journal of Chemical Ecology*, **32**, 1861–1865.

Ballhorn, D.J., Heil, M. & Lieberei, R. (2006) Phenotypic plasticity of cyanogenesis in lima bean *Phaseolus lunatus* – activity and activation of β-glucosidase. *Journal of Chemical Ecology*, **32**, 261–275.

Ballhorn, D.J., Heil, M., Pietrowski, A. & Lieberei, R. (2007) Quantitative effects of cyanogenesis on an adapted herbivore. *Journal of Chemical Ecology*, **33**, 2195–2208.

Ballhorn, D.J., Lieberei, R. & Ganzhorn, J.U. (2005) Plant cyanogenesis of *Phaseolus lunatus* and its relevance for herbivore–plant interaction: the

importance of quantitative data. *Journal of Chemical Ecology*, **31**, 1445–1473.

Becerra, J.X., Venable, D.L., Evans, P.H. & Bower, W.S. (2001) Interactions between chemical and mechanical defenses in the plant genus *Bursera* and their implications for herbivores. *American Zoologist*, **41**, 865–876.

Benrey, B., Callejas, A., Rios, L., Oyama, K. & Denno, R.F. (1998) The effects of domestication of *Brassica* and *Phaseolus* on the interaction between phytophagous insects and parasitoids. *Biological Control*, **11**, 130–140.

Bergelson, J. & Purington, C.B. (1996) Surveying patterns in the cost of resistance in plants. *The American Naturalist*, **148**, 536–558.

Bernays, E.A., Chapman, E.M., Leather, E.M. & McCaffery, A.R. (1977) The relationship of *Zonocerus variegatus* (L.) (Acridoidea: Prygomorphidae) with Cassava (*Manihot esculenta*). *Bulletin of Entomological Research*, **67**, 391–404.

Biere, A., Marak, H.B. & van Damme, J.M.M. (2004) Plant chemical defense against herbivores and pathogens: generalized defense or trade-offs? *Oecologia*, **140**, 430–441.

Clegg, D.O., Conn, E.E. & Janzen, D.H. (1979) Developmental fate of the cyanogenic glycoside linamarin in Costa Rica wild lima bean seeds. *Nature*, **278**, 343–344.

Coley, P.D. (1980) Effects of leaf age and plant life history patterns on herbivory. *Nature*, **284**, 545–546.

Coley, P.D. (1988) Effects of plant growth rate and leaf lifetime on the amount and type of anti-herbivore defense. *Oecologia*, **74**, 531–536.

Cork, S.J. (1996) Optimal digestive strategies for arboreal herbivorous mammals in contrasting forest types: why Koalas and Colobines are different. *Australian Journal of Ecology*, **21**, 10–20.

de Moraes, C.M., Mescher, M.C. & Tumlinson, J.H. (2001) Caterpillar induced nocturnal plant volatiles repel nonspecific females. *Nature*, **410**, 577–580.

Dicke, M. (1994) Local and systemic production of volatile herbivore-induced terpenoids: their role in plant-carnivore mutualism. *Journal of Plant Physiology*, **143**, 465–472.

Dicke, M. (1999a) Are herbivore-induced plant volatiles reliable indicators of herbivore identity to foraging carnivorous arthropods? *Entomologia Experimentalis et Applicata*, **91**, 131–142.

Dicke, M. (1999b) Specifity of herbivore-induced plant defences. *Insect–plant Interactions and Induced Plant Defence* (eds D.J. Chadwick & J.A. Goode), pp. 43–54. John Wiley & Sons, Chichester, UK.

Dicke, M., van Beek, T.A., Posthumus, M.A., Ben Dom, N., van Bokoven, H. & e Groot, A.E. (1990) Isolation and identification of volatile kairomones that affects acarine predator–prey interactions. Involvement of host plants in its production. *Journal of Chemical Ecology*, **16**, 381–396.

Donath, J. & Boland, W. (1995) Biosynthesis of acyclic homoterpenes: enzyme selectivity and absolute configuration of the nerolidol precursor. *Phytochemistry*, **39**, 785–790.

Dyer, L.A., Dodson, C.D., Beihoffer, J. & Letourneau, D.K. (2001) Trade-offs in anti-herbivore defenses in *Piper cenocladum*: ant mutualists versus plant secondary metabolites. *Journal of Chemical Ecology*, **27**, 581–592.

Ehlers, C.L., Garcia-Andrade, C., Wall, T.L., Cloutier, D., Philips, E., Harvey, J.A., Jervis, M.A., Gols, R., Jiang, N. & Vet, L.E.M. (1999) Development of the parasitoid, *Cotesia rubecula* (Hymenoptera: Braconidae) in *Pieris rapae* and *Pieris brassicae* (Lepidoptera: Pieridae): evidence for host regulation. *Journal of Insect Physiology*, **45**, 173–182.

Feeny, P. (1976) Plant apparency and chemical defense. *Recent Advances in Phytochemistry*, **10**, 1–40.

Felton, G.W. & Korth, K.L. (2000) Trade-offs between pathogen and herbivore resistance. *Current Opinion in Plant Biology*, **3**, 309–314.

Frehner, M. & Conn, E.E. (1987) The linamarin β-glucosidase in Costa Rica wild bean (*Phaseolus lunatus* L.) is apoplastic. *Plant Physiology*, **84**, 1296–1300.

Fritz, R.S. (1990) Effects of genetic and environmental variation on resistance of willow to sawflies. *Oecologia*, **82**, 325–332.

Frost, C.J, Appel, H.M, Carlson, J.E., De Moraes, C.M., Mark, C., Mescher, M.C. & Schultz, J.C. (2007) Within-plant signalling via volatiles overcomes vascular constraints on systemic signalling and primes responses against herbivores. *Ecology Letters*, **10**, 490–498.

Gouinguené, S., Degen, T. & Turlings, T.C.J. (2001) Variability in herbivore-induced odour emissions among maize cultivars and their wild ancestors (teosinte). *Chemoecology*, **11**, 9–16.

Hare, J.D., Elle, E. & van Dam, N.M. (2003) Costs of glandular trichomes in *Datura wrightii*: a 3-year study. *Evolution*, **57**, 793–805.

Hartmann, T. & Dietrich, B. (1998) Chemical diversity and variation of pyrrolizidine alkaloids of the senecionine type: biological need or coincidence? *Planta*, **206**, 443–451.

Heil, M. (2004) Direct defense or ecological costs: responses of herbivorous beetles to volatiles released by wild lima bean (*Phaseolus lunatus*). *Journal of Chemical Ecology*, **30**, 1289–1295.

Heil, M. (2008) Indirect defence via tritrophic interactions. *New Phytologist*, **178**, 41–61.

Heil, M. & Bostock, R.M. (2002) Induced systemic resistance (ISR) against pathogens in the context of induced plant defences. *Annals of Botany*, **89**, 503–512.

Heil, M. & Silva Bueno, J.C. (2007) Within-plant signalling by volatiles leads to induction and priming of an indirect plant defense in nature. *Proceedings of the National Academy of Science of the United States of America*, **104**, 5467–5472.

Heil, M., Delsinne, T., Hilpert, A., Schürkens, S., Andary, C., Linsenmair, K.E., Sousa, M.S. & McKey, D. (2002) Reduced chemical defence in ant-plants? A critical re-evaluation of a widely accepted hypothesis. *Oikos*, **99**, 457–468.

Herms, D.A. & Mattson, W.J. (1992) The dilemma of plants: to grow or defend. *Quarterly Review of Biology*, **67**, 283–335.

Joshi, A. & Thompson, J.N. (1995) Trade-offs and the evolution of host specialization. *Evolutionary Ecology*, **9**, 82–92.

Karban, R. & Baldwin, I.T. (1997) *Induced Responses to Herbivory*. University of Chicago Press, Chicago and London.

Karban, R. & Myers, J.H. (1989) Induced plant responses to herbivory. *Annual Review of Ecology and Systematics*, **20**, 331–348.

Karban, R. & Thaler, J.S. (1999) Plant phase change and resistance to herbivory. *Ecology*, **80**, 510–517.

Karban, R., Shiojiri, K., Huntzinger, M. & McCall, A.C. (2006) Damage-induced resistance in sagebrush: volatiles are key to intra- and interplant communication. *Ecology*, **87**, 922–930.

Koricheva, J. (2002) Meta-Analysis of sources of variation in fitness costs of plant antiherbivore defences. *Ecology*, **83**, 176–190.

Koricheva, J., Nykänen, H. & Gianoli, E. (2004) Meta-analysis of trade-offs among plant antiherbivore defences: are plants jacks-of-all-trades, masters of all? *The American Naturalist*, **163**, 64–75.

Letourneau, D.K. & Barbosa, P. (1999) Ants, stem borers, and pubescence in *Endospermum* in Papua New Guinea. *Biotropica*, **31**, 295–302.

Lieberei, R. (1988) Relationship of cyanogenic capacity (HCN-c) of the rubber tree *Hevea brasiliensis* to susceptibility to *Microcyclus ulei*, the agent causing South American leaf blight. *Journal of Phytopathology*, **122**, 54–67.

Lieberei, R., Biehl, B., Giesemann, A. & Junqueira, N.T.V. (1989) Cyanogenesis inhibits active defence reactions in plants. *Plant Physiology*, **90**, 33–36.

Lieberei, R., Fock, H.P. & Biehl, B. (1996) Cyanogenesis inhibits active defense in plants: inhibition by gaseous HCN of photosynthetic CO_2 fixation and respiration in intact leaves. *Angewandte Botanik*, **70**, 230–238.

Lill, J.T. & Marquis, R.J. (2001) The effects of leaf quality on herbivore performance and attack from natural enemies. *Oecologia*, **126**, 418–428.

Linhart, Y.B. (1991) Disease, parasitism and herbivory: multidimensional challenges in plant evolution. *Trends in Ecology and Evolution*, **6**, 392–396.

Marak, H.B., Biere, A. & van Damme, J.M.M. (2002a) Systemic, genotype-specific induction of two herbivore-deterrent iridoid glycosides in *Plantago lanceolata* L. in response to fungal infection by *Diaporthe adunca* (Rob.) niessel. *Journal of Chemical Ecology*, **28**, 2429–2448.

Marak, H.B., Biere, A. & van Damme, J.M.M. (2002b) Two herbivore-deterrent iridoid glycosides reduce the *in-vitro* growth of a specialist but not of a generalist pathogenic fungus of *Plantago lanceolata* L. *Chemoecology*, **12**, 185–192.

Marquis, R.J. (1990) Genotypic variation in leaf damage in *Piper arieanum* (Piperaceae) by a multispecies assemblage of herbivores. *Evolution*, **44**, 104–120.

Marquis, R.J. (1992) The selective impact of herbivores. *Plant Resistance to Herbivores and Pathogens* (eds R.S. Fritz & E.L. Simms), pp. 301–325. University of Chicago Press, Chicago.

Mattson, W.J., Lawrence, R.K., Haack, R.A, Herms, D.A. & Charles, P.-J. (1988) Defensive strategies of woody plants against different insect-feeding guilds in relation to plant ecological strategies and intimacy of association with insects. *Mechanisms of Woody Plant Defences against Insects* (eds W.J. Mattson, J. Levieux & C. Bernard-Dagan), pp. 3–38. Springer, New York.

Mauricio, R. (1998) Costs of resistance to natural enemies in field populations of the annual plant *Arabidopsis thaliana*. *The American Naturalist*, **151**, 20–28.

Mole, S. (1994) Trade-offs and constraints in plant-herbivore defence theory: a life-history perspective. *Oikos*, **71**, 3–12.

Møller, B.L. & Seigler, T.S. (1999) Biosynthesis of cyanogenic glucosides, cyanolipids, and related compounds. *Plant Amino Acids, Biochemistry and Biotechnology* (ed. B.K. Singh), pp. 563–609. Marcel Dekker, New York.

Morris, W.F., Traw, M.B. & Bergelson, J. (2006) On testing for a tradeoff between constitutive and induced resistance. *Oikos*, **112**, 102–110.

Nei, M. & Li, W. (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences of the United States of America*, **76**, 5269–5273.

Page, R.D.M. (1996) Treeview: an application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences*, **12**, 357–358.
Paré, P.W. & Tumlinson, J.H. (1999) Plant volatiles as a defence against insect herbivores. *Plant Physiology*, **121**, 325–331.
Paul, N.D., Hatcher, P.E. & Taylor, J.E. (2000) Coping with multiple enemies: an integration of molecular and ecological perspectives. *Trends in Plant Science*, **5**, 220–225.
Paul, V.J. & Hay, M.E. (1986) Seaweed susceptibility to herbivory: chemical and morphological correlations. *Marine Ecology Progress Series*, **33**, 255–264.
Pichersky, E. & Gershenzon, J. (2002) The formation and function of plant volatiles: perfumes for pollinator attraction and defence. *Current Opinion in Plant Biology*, **5**, 237–243.
Pray, L.A. (2004) Epigenetics: genome, meet your environment. *The Scientist*, **18**, 22–23.
Provenza, F.D., Pfister, J.A. & Cheney, C.D. (1992) Mechanisms of learning diet selection with reference to phytotoxicosis in herbivores. *Journal of Range Management*, **45**, 36–45.
Rausher, M.D. (1996) Genetic analysis of coevolution between plants and their natural enemies. *Trends in Genetics*, **12**, 212–217.
Romeo, J.T. (1998) Functional multiplicity among non-protein amino acids in Mimosoid legumes: a case against redundancy. *Ecoscience*, **5**, 287–294.
Romeo, J.T., Saunders, J.A. & Barbosa, P. (1996) *Phytochemical Diversity and Redundancy in Ecological Interactions*. Plenum Press, New York.
Rostás, M. & Eggert, K. (2008) Ontogenetic and spatio-temporal patterns of induced volatiles in *Glycine max* in the light of the optimal defence hypothesis. *Chemoecology*, **18**, 29–38
Schlicting, C.D. & Pigliucci, M. (1998) *Phenotypic Evolution: A Reaction Norm Perspective*. Sinauer, Sunderland.
Schreiner, I., Nafus, D. & Pimentel, D. (1984) Effects of cyanogenesis in bracken fern (*Pteridium aquilinum*) on associated insects. *Ecological Entomology*, **9**, 69–70.
Selmar, D. (1993) Transport of cyanogenic glucosides: linustatin uptake by *Hevea* cotyledons. *Planta*, **191**, 191–199.
Selmar, D., Lieberei, R., Conn, E.E. & Biehl, B. (1989) α-Hydroxynitrile lyase in *Hevea brasiliensis* and its significance for rapid cyanogenesis. *Physiologia Plantarum*, **75**, 97–101.
Simms, E.L. (1992) Costs of plant resistance to herbivory. *Plant Resistance to Herbivores and Pathogens: Ecology, Evolution and Genetics* (eds R.S. Fritz & E.L. Simms), pp. 392–425. University of Chicago Press, Chicago.
Siritunga, D., Arias-Garzon, D., White, W. & Sayre, R.T. (2002) Over-expression of hydroxynitrile lyase in transgenic cassava roots accelerates cyanogenesis and food detoxification. *Plant Biotechnology Journal*, **2**, 37–43.
Stearns, S.C. (1992) *The Evolution of Life Histories*. Oxford University Press, Oxford, UK.
Steward, J.L. & Keeler, K.H. (1988) Are there trade-offs among antiherbivore defences in *Ipomea* (Convolvulaceae)? *Oikos*, **53**, 79–86.
Strauss, S.Y., Rudgers, J.A., Lau, J.A. & Irwin, R.E. (2002) Direct ecological costs of resistance to herbivory. *Trends in Ecology and Evolution*, **17**, 278–285.
Swofford, D.L. (2003) *PAUP**. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.
Takabayashi, J. & Dicke, M. (1996) Plant–carnivore mutualism through herbivore-induced carnivore attractants. *Trends in Plant Science*, **1**, 109–113.
Thaler, J.S., Fidantsef, A.L., Duffey, S.S. & Bostock, R.M. (1999) Trade-offs in plant defense against pathogens and herbivores: a field demonstration of chemical elicitors of induced resistance. *Journal of Chemical Ecology*, **25**, 1597–1609.
Thaler, J.S., Owen, B. & Higgins, V.J. (2004) The role of the jasmonate response in plant susceptibility to diverse pathogens with a range of lifestyles. *Plant Physiology*, **135**, 530–538.
Tiffin, P. & Rausher, M.D. (1999) Genetic constraints and selection acting on tolerance to herbivory in the common morning glory *Ipomea purpurea*. *American Naturalist*, **154**, 700–716.
Turlings, T.C.J., Tumlinson, J.H. & Lewis, W.J. (1990) Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science*, **250**, 1251–1253.
van Noordwijk, A.J. & de Jong, G. (1986) Acquisition and allocation of resources: their influence on variation in life-history tactics. *American Naturalist*, **128**, 137–142.
Vaughan, B. & Eichorst, T (2006) Thoughts on species and speciation – phenoplasticity. *American Conchologist*, **34**, 26–29.
Walling, L.L. (2000) The myriad plant responses to herbivores. *Journal of Plant Growth Regulation*, **19**, 195–216.
Williams, G.C. (1957) Pleiotropy, natural selection, and the evolution of senescence. *Evolution*, **11**, 398–411.

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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
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