PLANT-MICROBE-ANIMAL INTERACTIONS - ORIGINAL RESEARCH

Chemical defense lowers plant competitiveness

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Abstract   Both plant competition and plant defense affect biodiversity and food web dynamics and are central themes in ecology research. The evolutionary pressures determining plant allocation toward defense or competition are not well understood. According to the growth–differentiation balance hypothesis (GDB), the relative importance of herbivory and competition have led to the evolution of plant allocation patterns, with herbivore pressure leading to increased differentiated tissues (defensive traits), and competition pressure leading to resource investment towards cellular division and elongation (growth-related traits). Here, we tested the GDB hypothesis by assessing the competitive response of lima bean (*Phaseolus lunatus*) plants with quantitatively different levels of cyanogenesis—a constitutive direct, nitrogen-based defense against herbivores. We used high (HC) and low cyanogenic (LC) genotypes in different competition treatments (intra-genotypic, intergenotypic, interspecific), and in the presence or absence of insect herbivores (Mexican bean beetle, *Epilachna varivestis*) to quantify vegetative and generative plant parameters (above and belowground biomass as well as seed

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production). Highly defended HC-plants had significantly lower aboveground biomass and seed production than LC-plants when grown in the absence of herbivores implying significant intrinsic costs of plant cyanogenesis. However, the reduced performance of HC- compared to LC-plants was mitigated in the presence of herbivores. The two plant genotypes exhibited fundamentally different responses to various stresses (competition, herbivory). Our study supports the GDB hypothesis by demonstrating that competition and herbivory affect different plant genotypes differentially and contributes to understanding the causes of variation in defense within a single plant species.

Introduction

Plants are unable to evade herbivore enemies through movement and show various defensive traits to protect themselves (Atsatt and O'Dowd 1976; Heil 2008; Agrawal et al. 2010). Great variation in plant defensive compounds and strategies of expression has developed over evolutionary time under the selection pressure of diverse herbivore antagonists (Ehrlich and Raven 1964; Bernays and Graham 1988). Given the importance of plants as primary producers in all terrestrial ecosystems, plant defense has the potential to vastly impact entire communities by directly affecting herbivores, and consequentially, higher trophic levels (Barton 2007; Viola et al. 2010). Surprisingly, despite the importance of plant defensive traits for complex food webs, plant defense-associated resource allocation is often not well understood. However, an improved understanding of the consequences of plant defense expression is a critical

task in ecological research (Herms and Mattson 1992; Siemens et al. 2002; Stamp 2003; Agrawal 2011; Moles et al. 2013).

Current ecological understanding of plant defense is divided among hypotheses based on resource allocation tradeoffs (Herms and Mattson 1992; Agrawal 2007; Agrawal et al. 2010; Kempel et al. 2011) or positive syndrome-forming correlations (Agrawal and Fishbein 2006; Viola et al. 2010; Ballhorn et al. 2013) such as the optimal defense, carbon–nutrient balance, growth rate, and growth–differentiation balance hypotheses (Stamp 2003; Ballhorn et al. 2008b, 2011a; Read et al. 2009; Kaplan et al. 2009; Agrawal 2011; Nomura et al. 2011; Dantas and Batalha 2012; Adler et al. 2012; Moles et al. 2013; Campbell and Kessler 2013). Overall trends are difficult to identify, as plant defensive strategies vary immensely across and within taxa, involving both chemical and mechanical traits (Moles et al. 2013). Available data are rarely entirely congruent with any one particular defense hypothesis, and often distinct discrepancies between theory and empirical data emerge in plant defense experiments (Barton 2007; Viola et al. 2010; Agrawal 2011; Thamer et al. 2011). In line with this, the predicted negative correlation between competitive ability and defense was not supported by a recent meta-analysis, which showed a slight positive correlation (Viola et al. 2010). In contrast, Kempel et al. (2011) found in a multi-species experiment that the competition–defense trade-off holds for constitutive resistance but is complemented by a positive relationship between competition and induced resistance. The necessity for empirical data on plant resource allocation to defense in different contexts is evident.

Defense compound production is considered costly to the plant, requiring resources which may otherwise be used for growth and reproduction, yet this cost is frequently invisible in empirical data (Siemens et al. 2002). The growth–differentiation balance (GDB) hypothesis uses the difference in resource requirements for cellular growth and photosynthetic net assimilation to explain patterns of plant investment in defense, assuming cellular growth requires more nutrient acquisition than photosynthesis. Defense expression is highest when cellular growth is limited, but photosynthesis is not (Stamp 2003; Barton 2007). The GDB hypothesis provides a theoretical framework in which to assess the cost of defense on a plant's fitness may be assessed, assuming tradeoffs between growth and defense (Herms and Mattson 1992; Kempel et al. 2011; Massad et al. 2012). Often, the high energetic cost of defense is shown as decreased biomass or reproductive performance, demonstrating the plant's lowered fitness, and only few studies show the costs of defense on plant fitness under nutrient or competition stress (Baldwin and Hamilton 2000; Siemens et al. 2002; Marak et al. 2003;

Barton 2007). An extension of the GDB hypothesis allows insight into the relative importance of herbivores or competitors in limiting plant fitness, resulting in plants with growth or differentiation-dominated life strategies, respectively (Stamp 2003). Often, plants form inducible rather than constitutive defenses in order to mitigate high cost of defense compounds (Ballhorn et al. 2008a, 2013a). Thus, plants enhance their level of defense by producing bioactive compounds only when needed, under herbivore attack (Ballhorn et al. 2008a; Kost and Heil 2008; Bennett et al. 2009; Cipollini and Heil 2010). Understanding metabolic costs of defense when plants are exposed to different stressors allows for an integrative approach to testing the GDB hypothesis (Agrawal 2011).

As plants are usually in permanent competition for multiple resources, plant–plant competition is likely a factor critically affecting plant performance in nature (Baldwin and Hamilton 2000; Chase et al. 2002; Siemens et al. 2002; Marak et al. 2003). In addition to competition between different plant species, intra-specific competition strongly affects plant fitness, in particular, as plants of the same species use similar resources and occupy similar spatial dimensions (Broz et al. 2010). Thus, plant genotypes of the same species expressing different defensive traits are ideal experimental systems to disentangle the concerted effects of defense and competition on plant fitness, serving as a control for background genetic variation (Ballhorn et al. 2011a). Moreover, they provide ideal systems to evaluate the importance of genetic diversity within plant species. Different genotypes of lima bean (*Phaseolus lunatus* L.) investing to different degrees into cyanogenesis (called cyanotypes hereafter), used in our study, represent such an experimental plant system.

Cyanogenesis is the enzymatically accelerated release of toxic hydrogen cyanide (HCN) from cyanide-containing precursors in response to cell damage (Frehner and Conn 1987; Selmar et al. 1989) and provides efficient plant defense against herbivores both in laboratory (Ballhorn et al. 2005, 2008b) and field experiments (Ballhorn et al. 2010, 2013b). Since HCN release is directly correlated with the amount of tissue damaged (Ballhorn et al. 2009), cyanogenesis is most effective against chewing herbivores (Cork 1996; Ballhorn et al. 2005). As the cyanide-containing precursors (in lima bean these are the cyanogenic glucosides linamarin and lotaustralin) contain nitrogen, cyanogenesis is considered more costly for a plant to produce and maintain than purely carbon-based defenses (Baldwin et al. 1990; Rask et al. 2000; Ballhorn et al. 2014). Intuitively, producing such a defense would limit the amount of resources available to growth and reproduction. Despite this cost, cyanogenesis occurs in about 10 % of vascular plant species, and has evolved independently in various families (Frehner and Conn 1987; Jones et al. 1999). The

ecological costs of cyanogenesis in the absence of enemies are only poorly understood, but negative effects on freezing tolerance and plant growth, reproduction, and competitive ability have been postulated (Kakes 1990; Noitsakis and Jacquard 1992; Hayden and Parker 2002).

The current study tests the assumption of the GDB hypothesis that the relative importance of competition and herbivory can shape plant resource allocation patterns. We test this by measuring plant performance under interspecific as well as inter- and intra-genotypic competition and herbivore stress, which has not previously been examined. We hypothesize that low cyanogenic genotypes are less affected by competition when grown under herbivore-free conditions than high cyanogenic genotypes. This may also lead to a competitive advantage over high cyanogenic plants without herbivores. The outcome of the competition between low and high cyanogenic genotypes should depend on the presence of herbivores, which should alleviate the competitive advantage of low cyanogenic genotypes.

Materials and methods

Plants

In this study, we used high (HC) and low (LC) cyanogenic lima bean (Fabaceae: *Phaseolus lunatus* L.) genotypes. These cyanotypes (HC_2116 and LC_8067) have been used in our previous studies (Ballhorn et al. 2005, 2007, 2008a, 2010) and showed very consistent cyanogenic potential (HCNp, amount of cyanide stored in form of cyanogenic precursors in plant tissues) over multiple generations (HC_2116: ~75 μmol HCN; LC_8067: ~18 μmol HCN per gram fresh weight). The genotypes we used are small-seeded landraces from Central and South America and were selected based on their chemical and morphological features from a larger set of lima bean genotypes which are in permanent culture in our laboratory. Plants were cultivated in a greenhouse with additional light provided by a combination (1:1) of HQI-BT 400 W (Osram) and RNP-T/LR 400 W (Radium) lamps with a light regime of 13:11 h L:D under photon flux density of 450–500 μmol photons m^{-2} s^{-1} at table height. Temperature was set at 30 °C during the light period and at 23 °C during the dark period. Relative air humidity was adjusted to 70–80 %. Plants were cultivated in plant-containers of $10 \times 10 \times 11$ cm (width, length, height; one plant per pot) in a 1:1 ratio of potting soil (Fox Farms, Arcata, CA, USA) and sand (grain size 0.5–2.0 mm). Plants were not inoculated with rhizobia (Thamer et al. 2011; Ballhorn et al. 2013c) and none of the experimental plants showed spontaneous nodulation. After development of the primary leaves all plants were fertilized with 80 mL of a 0.2 %

aqueous solution of Flory-3® [NPK + Mg (%); 15, 10, 15 +2-Fertilizer; EUFLOR, Munich, Germany] once to provide for an initial nutrient supply sufficient for plant growth. Plants were watered daily with tap water and were cultivated until the end of their reproductive phase (10–12 weeks after germination).

Insects

Mexican bean beetles (Coccinellidae: *Epilachna varivestis*) used in experiments on the effect of herbivory on plant–plant competition were reared on non-cyanogenic snap bean (*Phaseolus vulgaris*) to avoid conditioning of insects to lima bean cyanotype. These insects are oligophagous herbivores with a narrow host range including various legumes but with distinct preference for lima bean (Lapidus et al. 1963; Flanders 1984; Dover et al. 1988). Both adults and larvae feed on foliage. Mexican bean beetles were maintained in cages (1.0 × 1.0 × 0.8 m, l:h:w) under greenhouse conditions chosen for plant cultivation (see above). Freshly hatched larvae were used for herbivore treatments.

Experimental setup

We applied seven experimental treatments to test for competitive ability of HC- and LC-plants under herbivore-free conditions. Competition experiments were designed to display defense costs under increasing competitive intensity. This increasing competitive intensity ranged from no competition (plants grown alone, controls), to interspecific competition, to intra-specific competition with plants of a different or the same genotype. Plants of the same genotype are considered to require more similar resources as compared to plants of another genotype or another species (Broz et al. 2010). Specifically, in two treatments, individual HC- and LC-plants each were cultivated in combination with corn (Poaceae: *Zea mays*) (HC vs. corn; LC vs. corn). In another two treatments, HC-plants were competing against LC-plants (inter-genotypic competition, HC vs. LC), and in a further competition treatment, HC- and LC-plants were competing against plants from the same cyanotype (intra-genotypic competition, HC vs. HC; LC vs. LC). In control (no competition) experiments, single HC- and LC-plants were analyzed for growth and reproduction.

In addition to experiments under herbivore-free conditions, in three treatments, we tested for the impact of insect herbivory on HC- and LC-plants with and without competition. Larvae of the Mexican bean beetle were added to HC- and LC-plants growing individually in pots (five larvae per plant). In addition to these non-competition experiments, we tested the impact of herbivory on HC- and LC-plants competing against each other (HC vs.

LC; ten larvae per pot, five larvae were placed on each plant). Herbivores were added when plants had developed their first secondary (trifoliate) leaf about 2 weeks after germination and were left on the plants until the end of the experiment. While herbivores showed limited movement during their first 2–3 larval developmental stages, and thus were mostly restricted to the plant they were placed on, larger larvae were able to easily move from one plant to the other. The movement of herbivores between plants was built into the setups to allow the ecologically-relevant response of plant rejection as a food source by this oligophagous insect based on level of plant defense. Plants with insects were covered with gauze in pairs to avoid escaping of herbivores. In previous experiments covering plants with gauze ($n = 12$ covered and non-covered plants respectively) proved not to significantly affect plant growth (t test; $P = 0.49$) due to only slightly reduced light availability. In fact, plant biomass (dry weight) was almost identical for covered and non-covered plants (covered plants: 3.92 ± 0.38; non-covered: 3.89 ± 0.43; mean $\pm$ SD).

Pots were arranged in a block design (6 blocks with 1 pot of each of the 10 specific combinations of plants and herbivores). Position of pots of the different treatments within the blocks was set at random and the position of blocks within the greenhouse was exchanged every five days to avoid chamber effects.

Data collection and analyses

Lima bean plants were analyzed for production of above- and belowground biomass, biomass of reproductive structures (pods) as well as for number and weight of produced seeds. Roots of plants were separated from shoots, washed thoroughly until all soil was removed, and both below- and aboveground plant parts were dried in an oven (65 °C) until constancy of weight (5 days). Dry or almost dry pods were collected per plant, dried at room temperature for 2 weeks, seeds were removed, counted and individually weighed to the nearest 0.001 g (Mettler Toledo, New Classic MF MS303SE). The weight of pods was determined separately. Mean seed weight was calculated as total seed weight per plant divided by the number of seeds.

Since HC- and LC-plants grew together in the same pots in the inter-cyanotype competition and combined competition and herbivory treatments they cannot be treated as independent replicates in a common analysis. We therefore used half of the pots with LC-plants as focal plants and the other half with HC-plants as focal plants. Further, in the intra-cyanotype competition treatments we a priori defined one plant as target plant and the other plant as competitor. Values of the corresponding competitors were not used for the analyses.

We used the dataset to perform two different analyses. The first analysis focused on the effects of different types of competition on vegetative and generative traits. For this, we used all herbivore-free treatments and a nested ANOVA model with block factor, cyanotype, two levels of competition (without/with), and three types of competition (interspecific, inter-genotypic, intra-genotypic) nested within the factor competition. Significant effects of type of competition were followed by a post hoc test (Tukey's). To further assess differences in the responses of LC- and HC-plants to the different competition treatments, we calculated log-response ratios for the different variables and compared them using paired t tests. Log-response ratios are a commonly used method to determine and compare symmetrical and independent strengths of experimental factors (Hedges et al. 1999). In this case, log-response ratios (LR) for the different competition treatments were calculated for each block as LR = log (response variable in the competition treatment/response variable without competition). Thus, values above zero would indicate a positive effect of competition whereas values below zero would indicate negative effects of competition on the measured variable. We used the 95 % confidence interval to test for deviations from zero.

The second analysis included the treatments LC- and HC- plants alone, in competition with the other cyanotype, and with and without herbivores. For this analysis, we used log-transformed data since deciding if there is an interaction between competition and herbivory may depend on the scale of data. Both herbivory and competition affect per gram loss or growth rates, and their joint effects should be therefore multiplicative on a linear scale even if they do not interact and work independently. On a logarithmic scale, however, a non-significant interaction would in fact indicate an additive response. Thus, a biologically meaningful interaction between herbivory and competition is therefore not necessarily indicated by statistically significant interactions on a linear scale (Rees and Brown 1992; Schädler et al. 2007). We therefore analyzed log-transformed data by means of a four-factorial ANOVA with block, cyanotype, herbivory, and (inter-cyanotypic) competition. Only ANOVAs with a significant interaction between cyanotype, herbivory and/or competition were followed by a post hoc comparison (Tukey's test) of means. Since most LC-plants did not reproduce with herbivory, mean pod weight and mean seed weight have been excluded from the analysis due to the very low number of valid observations.

Interacting effects of competition and herbivory were analyzed in more detail using response indices (Haag et al. 2004; Schädler et al. 2007). For this, we calculated herbivory response (HR) as a measure of the separate effect of herbivory in the absence of competition as HR = response variable (with herbivores)/response variable (without

Table 1 Results of the nested ANOVAs on the effects of competition and type of competition (interspecific, inter-cyanotypic, intra-cyanotypic) on vegetative and generative traits of HC- and LC lima bean plants

	Competition (Co)	Competition type (Ct)	Cyanotype (Cy)	Co × Cy	Ct × Cy
Shoot mass	$F_{1,2} = 149.6^{***}$	$F_{2,15} = 1.24$	$F_{1,2} = 25.52^{***}$	$F_{1,2} = 0.04$	$F_{2,29} = 0.77$
Root mass	$F_{1,2} = 55.96^{***}$	$F_{2,15} = 1.97$	$F_{1,2} = 0.60$	$F_{1,2} = 4.25^{*}$	$F_{2,29} = 0.80$
Number of pods	$F_{1,2} = 336.8^{***}$	$F_{2,15} = 2.24$	$F_{1,2} = 43.26^{***}$	$F_{1,2} = 5.13^{*}$	$F_{2,29} = 5.39^{*}$
Number of seeds	$F_{1,2} = 193.0^{***}$	$F_{2,15} = 7.53^{**}$	$F_{1,2} = 301.5^{***}$	$F_{1,2} = 68.06^{***}$	$F_{2,29} = 5.13^{*}$
Seeds/pod	$F_{1,2} = 2.37$	$F_{2,29} = 2.95$	$F_{1,2} = 115.08^{***}$	$F_{1,2} = 0.21$	$F_{2,29} = 0.80$
Mean pod mass	$F_{1,2} = 247.0^{***}$	$F_{2,15} = 3.87^{*}$	$F_{1,2} = 5.72^{*}$	$F_{1,2} = 2.04$	$F_{2,29} = 4.11^{*}$
Mean seed mass	$F_{1,2} = 3.26$	$F_{2,15} = 0.22$	$F_{1,2} = 36.20^{***}$	$F_{1,2} = 0.69$	$F_{2,29} = 2.45$

Significance levels * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

herbivores) and competition response (CR) as a measure of the separate effect of competition in the absence of herbivory as CR = response variable (with competition)/response variable (without competition) within each block. The predicted total response (TR_{pred}) of plants to the combined effects of herbivory and competition in the absence of any interacting effects would be $TR_{pred} = HR \times CR$. The observed total response (TR_{true}) was calculated as TR_{true} = response variable (with herbivores and competition)/response variable (without herbivores and competition). Again, the response indices were log-transformed and the resulting log-response ratios were analyzed by means of a two-way ANOVA with block and the "method of calculation" (predicted vs. true) as factors. Interactions between herbivory and competition are indicated by a significant value for the "method of calculation" factor. Some of the LC-plants did not produce any pods in the herbivory treatments, resulting in zero values of the response variable (number of pods, number of seeds) which causes log ratios to be undefined. However, eliminating zero values would mean to ignore potentially meaningful responses. Thus, we added the lowest value of the response variable to all values of these variables, which gives a conservative estimate of the corresponding log-response ratio (see Viola et al. 2010). However, again mean pod weight and mean seed weight have been excluded from the analysis in this case due to the very low number of valid observations.

Results

Type of competition

When grown without competition, LC-plants produced more shoot biomass, more pods, more seeds, and more seeds per pod than HC-plants, whereas mean seed mass was lower for these plants (paired t tests, all $P < 0.05$). Remarkably, the number of seeds was about four times higher for LC-plants than for HC-plants. The total seed mass per plant was higher for LC-plants (mean ± standard deviation: 5.65 ± 3.30) than for HC-plants (1.85 ± 1.13, $P < 0.001$) indicating that the lower seed mass does not outweigh the higher seed production. Root biomass was not affected by cyanotype (paired t test, $P > 0.05$). Both HC- and LC-plants showed consistently lower shoot- and root biomass under competition than without competition (Table 1; Fig. 1a, b). The extent of this effect did not depend on the type of competition. The negative effect of competition on root biomass was stronger for HC-plants (significant competition x cyanotype interaction, Table 1).

The number of pods per plant was generally lower under competition and this effect was further mediated by the cyanotype and the type of competition (significant interaction; Table 1; Fig. 1c). Interspecific competition had a less strong negative effect than inter-cyanotypic competition only for LC-plants. The number of seeds and mean pod weight followed this pattern, i.e., a stronger reduction due to inter-cyanotype competition than due to interspecific competition was only observed for LC-plants (Fig. 1d, e). The number of seeds per pod and mean seed weight were not affected by the competition treatments.

Log-response ratios also revealed that most vegetative and generative traits were negatively affected by the three types of competition (Fig. 2a–c). In accordance with the analysis above, only for LC-plants, inter-cyanotypic competition caused stronger reductions in seed and pod number than interspecific competition. Differences between the responses of cyanotype were more obvious under interspecific competition with corn, resulting in often weaker effects on LC-plants. More specifically, there was no significant response of root biomass of LC-plants grown in competition with corn (Fig. 2a) and the same cyanotype (Fig. 2c). Mean seed mass generally did not respond to intra-cyanotypic competition for either cyanotype and to inter-cyanotypic competition in HC-plants. The number of seeds per pod was only (positively) affected for HC-plants grown in intra-cyanotypic competition (Fig. 2c). Apart from one exception (number of seeds per pod for
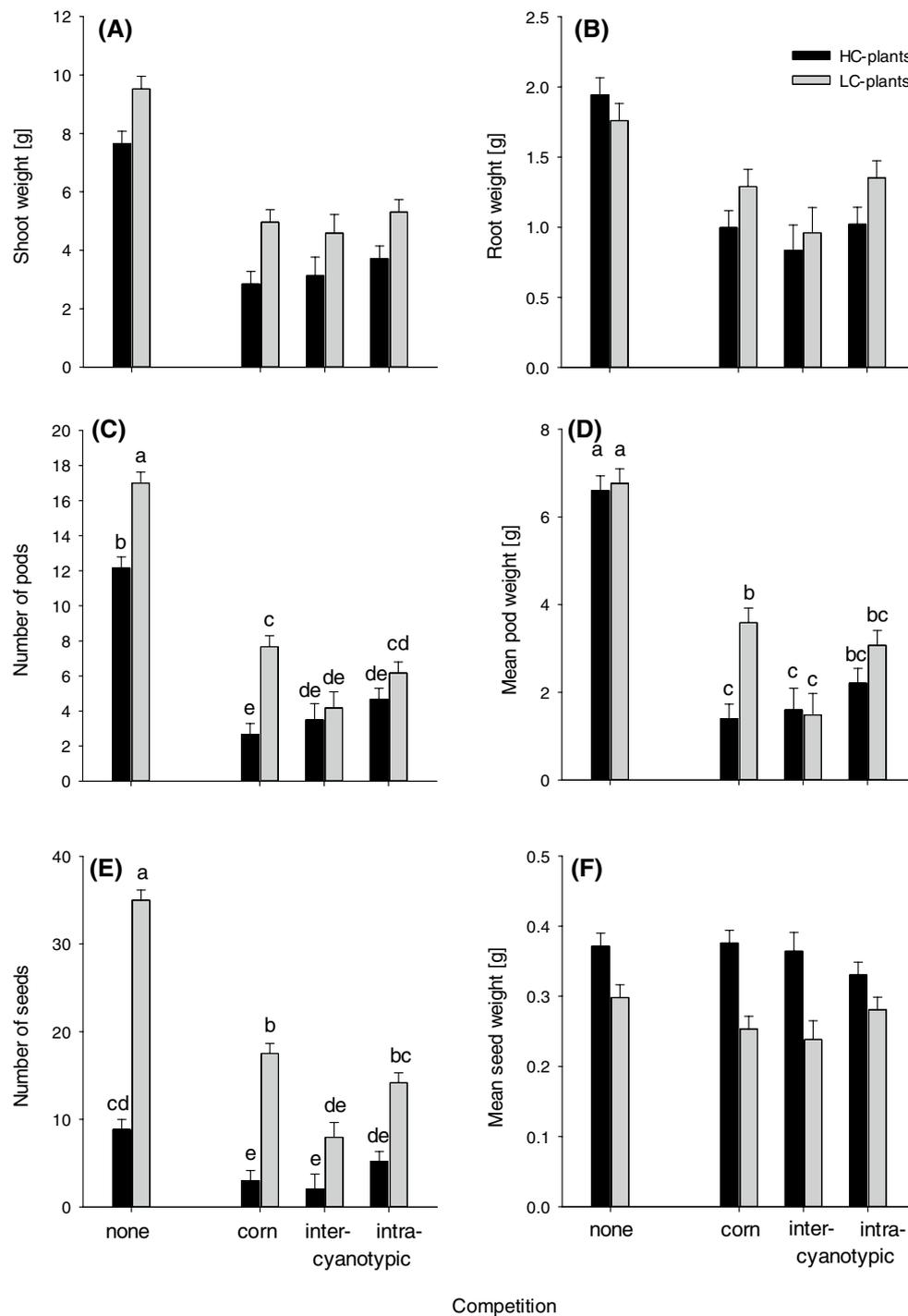
Fig. 1 Effects of different competition treatments and cyanotype of lima bean (mean ± SE). *None* no competition, *Corn* interspecific competition with corn, *Inter-cyanotypic* competition with a plant of the other cyanotype, *Intra-cyanotypic* competition with a plant of the same cyanotype. *Different letters* indicate significant differences between means following post hoc tests (Tukey's, $P < 0.05$). Note that only significant interactions are followed by a post hoc test

intra-cyanotypic competition), responses of both cyanotypes were only significantly different in the (interspecific) competition treatment with corn. Here, shoot biomass, the number of pods, and mean pod mass were less strongly negatively affected in LC-plants whereas mean seed mass was more strongly negatively affected in LC-plants and the number of seeds per pod more strongly positively affected for HC-plants.
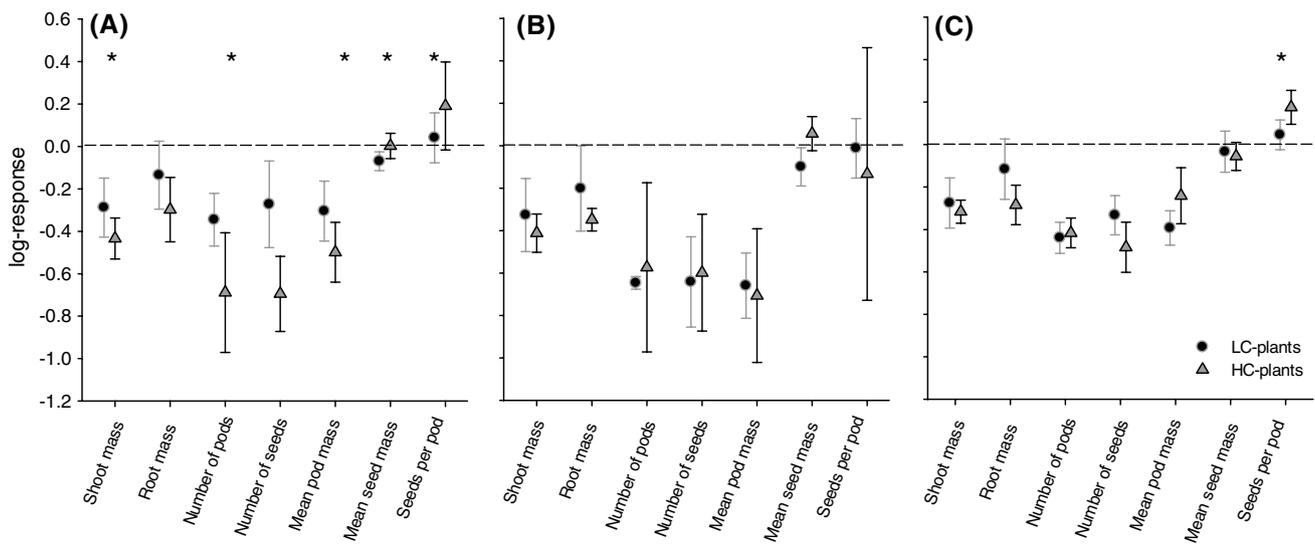
Fig. 2 Log-response ratios for vegetative and generative traits of HC- and LC-plants for the influence of **a** interspecific competition by corn, **b** inter-cyanotypic competition, and **c** intra-cyanotypic competition (means ± CI). Confidence intervals not overlapping the zero line indicate significant responses. *Asterisks* indicate significant differences between LC- and HC-plants based on a paired *t* test ($P < 0.05$)

Table 2 Results of ANOVAs on the effects of cyanotype, inter-cyanotypic competition and herbivory on vegetative and generative traits of HC- and LC lima bean plants

	Cyanotype (Cy)	Competition (Co)	Herbivory (H)	Cy × Co	Cy × H	Co × H	Cy × Co × H
Shoot mass	$F_{1,23} = 11.7**$	$F_{1,23} = 126.9***$	$F_{1,23} = 51.2***$	$F_{1,23} = 0.01$	$F_{1,23} = 102.8***$	$F_{1,23} = 47.8***$	$F_{1,23} = 5.30*$
Root mass	$F_{1,23} = 44.8***$	$F_{1,23} = 65.8***$	$F_{1,23} = 30.6***$	$F_{1,23} = 0.34$	$F_{1,23} = 53.8***$	$F_{1,23} = 20.8***$	$F_{1,23} = 5.40*$
Number of pods	$F_{1,23} = 40.7***$	$F_{1,23} = 66.2***$	$F_{1,23} = 187.2***$	$F_{1,23} = 4.88*$	$F_{1,23} = 56.9***$	$F_{1,23} = 21.7***$	$F_{1,23} = 0.16$
Number of seeds	$F_{1,23} = 6.60*$	$F_{1,23} = 53.9***$	$F_{1,23} = 91.5***$	$F_{1,23} = 2.68$	$F_{1,23} = 132.6***$	$F_{1,23} = 15.15***$	$F_{1,23} = 0.11$

Significance levels * $P < 0.05$, *** $P < 0.001$

Shoot biomass of corn was not affected by the cyanotype of lima bean (LC-plants: 6.60 ± 0.64; HC-plants: 6.44 ± 0.64, mean ± standard error; ANOVA: $F_{1,5} = 0.03$, $P = 0.87$). Root biomass of corn was considerably higher in competition with lima bean but this was only marginally significant for plants grown in competition with LC-plants (LC-plants: 1.49 ± 0.21; HC-plants: 0.85 ± 0.21, mean ± standard error; ANOVA: $F_{1,5} = 4.55$, $P = 0.086$).

Interacting effects of herbivory and competition

Vegetative traits were affected by interactive effects of cyanotype, competition and herbivory (significant three-way interaction; see Table 2; Fig. 3a, b). While LC-plants consistently showed a strong decrease of shoot and root biomass due to competition and herbivory, the responses of HC-plants to herbivory were weak without competition, or even positive under competition with LC-plants. As a result, HC-plants with herbivory did not suffer from competition in terms of above- and belowground biomass. The negative effect of herbivory was stronger for plants grown without competition and in LC-plants (significant two-way interactions); but the interactive effect of herbivory and competition was consistent across cyanotypes (no three-way interaction; Table 2; Fig. 4a, d).

The number of pods and the number of seeds were strongly reduced by herbivory in LC-plants, whereas in HC-plants, herbivory only had a negative effect on the number of pods (significant herbivory × cyanotype interaction; Table 2; Fig. 4d, e). Further, competition decreased the number of pods in both cyanotypes, but this effect was more pronounced for LC-plants (significant competition × cyanotype interaction; Table 2; Fig. 4c). In HC-plants, the number of seeds per pod was only affected by herbivory (Table 3; without herbivory: 0.71 ± 0.07, with herbivory 1.83 ± 0.07; mean ± standard error). Pod mass of HC-plants was higher for plants grown alone without herbivores than in all other treatments and seed mass of HC-plants was lower in plants grown with herbivores in competition than in all other
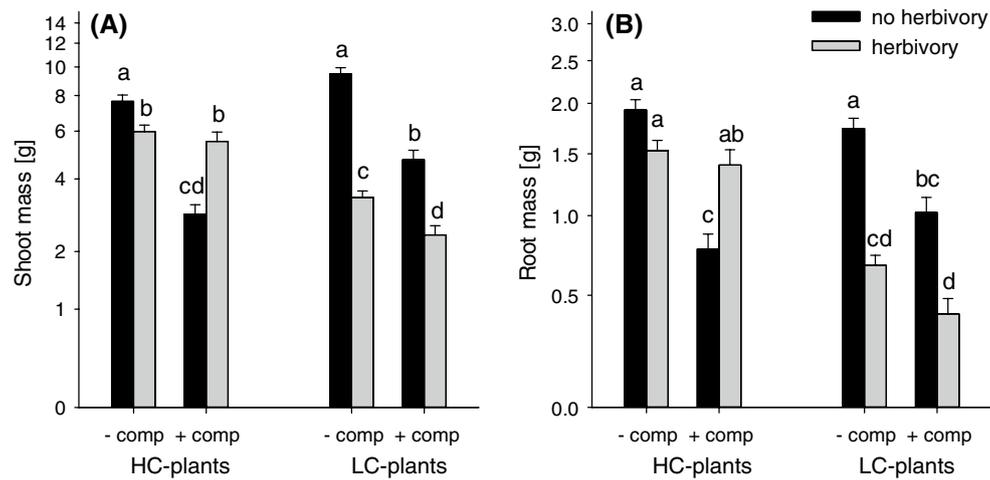
Fig. 3 Effects of inter-cyanotypic competition, cyanotype, and herbivory on vegetative traits of lima beans (mean ± SE). *Different letters* indicate significant differences between means following post hoc tests (Tukey's, $P < 0.05$). Note that only ANOVAs with a significant interaction between competition and herbivory are followed by post hoc tests. Note that the *y*-axes are on a log scale
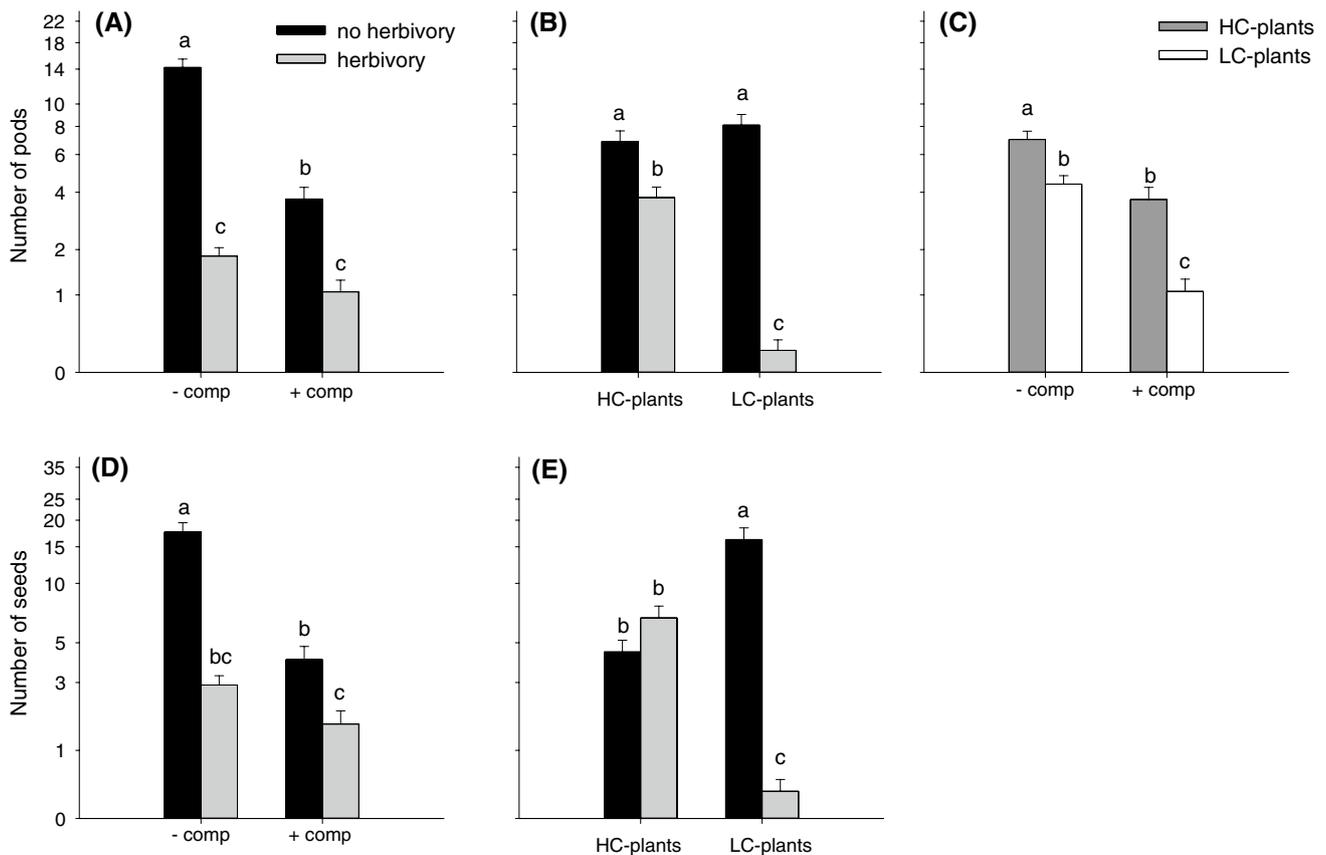
Fig. 4 Effects of inter-cyanotypic competition, cyanotype and herbivory on generative traits of lima beans (mean ± SE). *Different letters* indicate significant differences between means following post hoc tests (Tukey's, $P < 0.05$). *–comp* without competition, *+comp* with competition. Note that the *y*-axes are on a log scale

treatments (significant herbivory × competition interaction; Table 3; Fig. 5a, b).

For HC-plants, the predicted response to the combined effects of herbivory and competition (with the other cyanotype) was negative, and significantly stronger than the observed response for shoot weight (Fig. 6a; ANOVA, method of calculation: $F_{1,5} = 194.6$, $P < 0.001$), root biomass ($F_{1,5} = 18.8$, $P = 0.007$), number of pods

$(F_{1,5} = 17.4$, $P = 0.009)$, number of seeds ($F_{1,5} = 9.97$, $P = 0.03$) and mean pod weight ($F_{1,5} = 18.9$, $P = 0.007$). In contrast, for LC-plants we found no difference between observed and predicted values for shoot weight and root weight (Fig. 6b). Number of pods and number of seeds, however, showed the same pattern as for HC-plants.

Discussion

Few empirical studies highlight the competitive advantage of expensive defense traits in the presence of herbivore pressure and overall consensus among studies combining effects of defense and competitive ability is yet to be achieved (Viola et al. 2010; Moles et al. 2013). The data presented here are evidence of a negative correlation between a constitutive defense and competitive ability, supporting the predictions of the GDB hypothesis.

Table 3 Results of ANOVAs on the effects of inter-cyanotypic competition and herbivory on generative traits of HC lima bean plants

	Competition (Co)	Herbivory (H)	Co x H
Seeds/pod	$F_{1,9} = 0.79$	$F_{1,9} = 66.6$***	$F_{1,9} = 0.04$
Mean pod mass	$F_{1,9} = 40.1$***	$F_{1,9} = 17.9$**	$F_{1,9} = 28.9$***
Mean seed mass	$F_{1,9} = 14.1$**	$F_{1,9} = 4.37$	$F_{1,9} = 14.3$*

Significance levels * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Fig. 5 Effects of inter-cyanotypic competition and herbivory on generative traits of the HC-genotype of lima beans (mean ± SE). *Different letters* indicate significant differences between means following post hoc tests (Tukey's, $P < 0.05$). *–comp* without competition, *+comp* with competition. Note that the *y*-axes are on a log scale
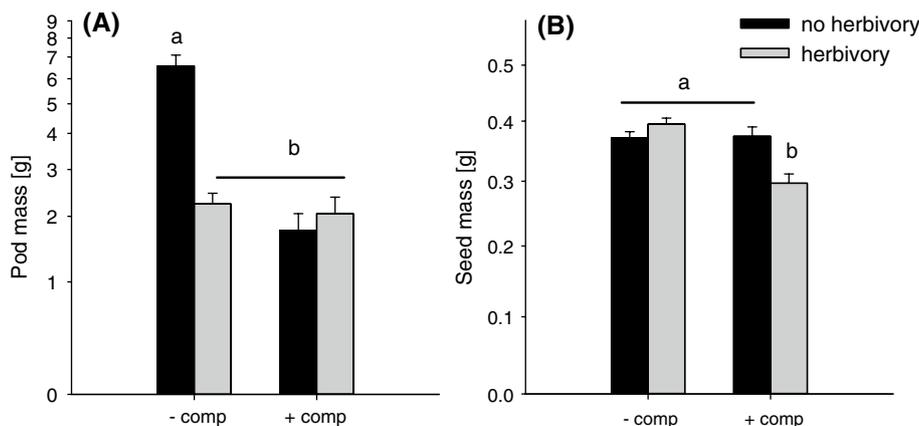
Fig. 6 Comparison of predicted (calculated from separate responses to competition and herbivory) and observed responses to the simultaneous effect of herbivory and competition for HC- and LC-plants of lima bean (mean ± CI). Confidence intervals not overlapping the zero line indicate significant responses. *Asterisks* indicate significant differences between predicted and true responses (ANOVA, method of calculation, $P < 0.05$)
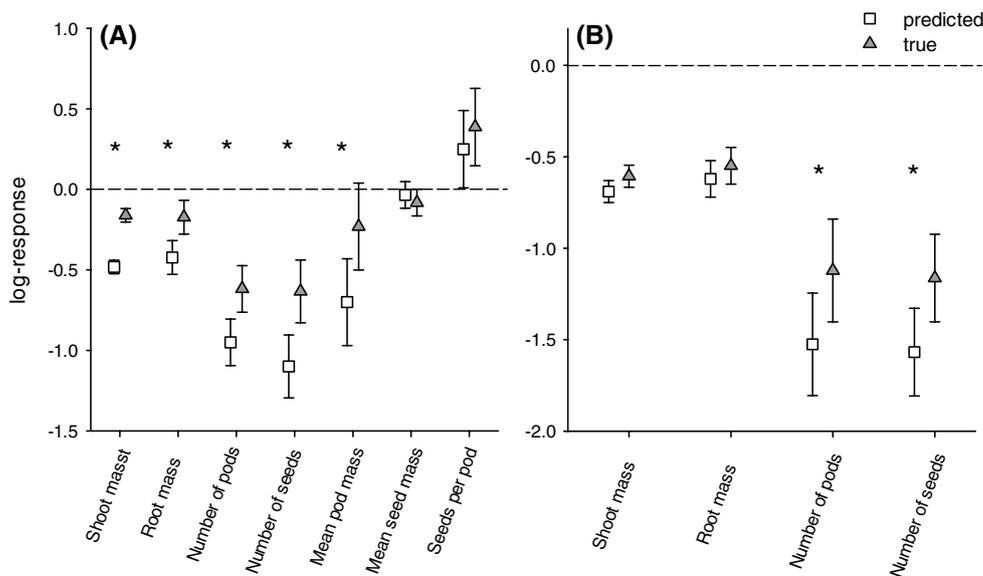
Individual plant growth strategies

In our study comparing the performance of differently defended plant genotypes under competition and herbivory, we considered a range of generative and vegetative plant traits either directly determining plant fitness (e.g., number of seeds produced per plant; weight of seeds) (Rees 1995; Fenner and Thompson 2005) or serving as indirect measures of plant fitness such as biomass (Caswell 1989; García and Ehrlén 2002). When grown without competition, LC-plants developed more shoot biomass, pods, seeds, number of seeds per pod as well as a greater mean pod weight when compared to HC-plants grown alone, indicating intrinsic costs of cyanogenesis. Cyanogenesis is a nitrogen-based plant defense, and as nitrogen often is limited, therefore considered costly for the plant (Kakes 1990). The nitrogen allocated to the synthesis of cyanogenic glucosides is not available for other nitrogen sinks associated with growth and reproduction. The cyanogenic glycosides linamarin and lotaustralin present in lima bean are derived from the proteinogenic amino acids L-valine and L-isoleucine, thus competing with protein biosynthesis (Ballhorn et al. 2005, 2011a). Beyond costs of the cyanogenic glycosides themselves, proteins involved in synthesis, cleavage and transportation of cyanogenic glucosides also require nitrogen, further increasing the cost of plant cyanogenesis. However, while most plant performance metrics noted in this study were lower in HC- compared to LC-plants, belowground biomass production was not significantly different between HC- and LC-lima bean plants. The additional necessity to acquire nitrogen to produce nitrogen-rich defenses may explain why HC-plants invest relatively more in root than in shoot production. Altogether, in treatments in which plants were grown alone, plants showed the highest values for biomass and reproductive structure production observed for both HC- and LC-plants, as opposed to treatments with competition or herbivore pressure. This indicates considerable impacts of both herbivory and plant–plant competition on growth and ultimately plant fitness, which is in accordance with the GDB hypothesis (Stamp 2003). Previous studies on the defense system in *Plantago* also support the GDB hypothesis by demonstrating: (1) a fitness cost to producing iridoid glycosides, (2) correlations in defense expression and leaf morphology, and (3) increased expression with plant ontogeny as opposed to decreasing with age, as supported by the optimal defense hypothesis (Marak et al. 2000, 2003; Barton 2007).

In their natural environment, lima bean plants and legumes in general are less dependent on soil nitrogen than other plants due to their association with nitrogen-fixing rhizobia (Kempel et al. 2011). However, as the maintenance of rhizobia requires extensive resource allocation towards the bacterial symbionts, obtaining nitrogen is associated with costs, even for legumes (Simms and Taylor 2002). Plants in this study were not inoculated with rhizobia and

remained rhizobia-free throughout the experiment. These costs of nitrogen-based defense through cyanogenesis were also found in other systems such as white clover (*Trifolium repens*) in which LC-plants showed increased biomass compared to HC-plants (Kempel et al. 2011).

Does cyanogenesis affect competitive ability?

In terms of shoot biomass, the effect of competition did not differ between lima bean cyanotypes. In contrast, HC-plants showed a stronger reduction of root biomass under competition than LC-plants. This finding may indicate the intensity of belowground competition, as HC-plants allocated similar amounts of biomass to roots as LC-plants, but produced far less shoot tissue. On the other hand, a high impact of competition on belowground biomass has also been reported for other plant systems. Grasses in a field study had reduced belowground performance under competitive pressure in both nitrogen-rich and nitrogen-poor soils (Wilson and Tilman 1991; Casper and Jackson 1997). In our system, the nitrogen demands for high cyanogenic plants likely increased the strength of belowground competition. Lowering the overall requirement for nitrogen may have given LC-lima bean plants a competitive advantage, especially when faced with intense belowground competition. Importantly, in our system, we show that competition in general, as well as different levels of competition, affected several (mainly generative) traits of high and low cyanogenic plants differently. Thus, not only plant species but also plant genotype may strongly impact the outcome of plant–plant competition (Noitsakis and Jacquard 1992; Johnson 2008). In the lima bean system, the tendency for inter-genotypic competition to act as a strong competitive force for the reproduction of LC-plants was observed, while interspecific competition with corn had weaker effects. In contrast, HC-plants showed no differentiation between the different levels of competition. In a direct comparison of the two cyanotypes, LC-plants can be seen as the better interspecific competitor in terms of reproduction, at least in a herbivore-free environment. Thus, the prediction that plants may tolerate interspecific competition better than intraspecific competition (Broz et al. 2010) we could only confirm for LC-plants.

The acyanogenic competitor, *Zea mays* (a plant not producing cyanogenic precursors or other nitrogen-based chemical plant defenses at significant levels) produced a similar amount of shoot tissue under competition with a LC-lima bean plant as when grown under competition with a HC-plant, indicating the cyanogenic potential of its competitor does not affect shoot production. However, in line with the aforementioned reduction of belowground biomass under competition in grasses (Wilson and Tilman 1991; Casper and Jackson 1997), the root tissue of *Z. mays* decreased greatly in the presence of a HC-lima bean plant as compared to a LC-lima bean plant. This

observation together with the strong effects on some reproductive traits of LC-plants suggest HC-plants are higher nutrient consumers and thus create a higher competitive pressure for neighboring plants. This is paralleled in other plants as cyanogenic morphs of white clover (*Trifolium repens*) were shown to utilize resources differently from acyanogenic morphs (Noitsakis and Jacquard 1992). Cyanogenic morphs experienced greater challenges under intra-morphic competition than did acyanogenic morphs (Noitsakis and Jacquard 1992). Since *Z. mays* root production was more intensely impacted by HC-lima bean plants as compared to LC-lima bean plants, the higher nitrogen requirement of the HC-genotypes seemingly limits the root growth of *Z. mays*. Belowground competition for resources may often be a stronger force than aboveground competition, and competition between plants in low nutrient environments was shown to take place mostly belowground, but, with additional nitrogen, the competitive pressure took place both above- and belowground (Connell 1983; Wilson and Tilman 1991; Casper and Jackson 1997).

Do benefits outweigh costs of defense in the presence of herbivores?

Expensive defense compound production is found in many different taxa and has evolved multiple times—the ability to defend can clearly lead to an increase in plant fitness (Campbell and Kessler 2013). However, in the context of these two ubiquitous phenomena, both competition and herbivory determine the benefits and costs of plant defense. The interacting nature of both phenomena is reflected in the growth of lima bean plants in our experiment. Both shoot and root biomass were strongly affected in LC-plants by herbivory rather independent of the competitive situation of the plant. In contrast, herbivores had only negligible effects on the biomass of HC-plants growing alone, while shoot and root biomass clearly increased in the competition treatment in the presence of herbivores. This can be attributed to the selective feeding of herbivores on the LC-plants thereby giving the HC-plants a competitive advantage.

The costs of herbivory and competition were also obvious for the generative growth of both cyanotypes. The number of pods and seeds decreased in response to both pressures, and the stronger response of LC-plants reflects the finding that LC-plants are especially sensitive to inter-cyanotypic competition (but not to competition in general, see above). Also, the negative effect of herbivory was pronounced for both traits in LC-plants, but (and according to the results of vegetative biomass) rather small for HC-plants in terms of number of pods, or even undetectable for the number of seeds. According to the lower number of pods with no change in number of seeds, the number of seeds per pod increased with herbivory in HC-plants. This effect is consistent across the competition treatments and may be interpreted as a kind of compensatory response. We consider the number of seeds as the most relevant indicator for generative fitness (notably in the absence of substantial effects on seed mass), which is therefore substantially affected by herbivory only in LC-plants, whilst HC-plants seemed to be able to compensate the lower number of pods with increased production of seeds within pods. This demonstrates the increase in relative fitness of plants that produce costly defensive metabolites under conditions that favor this investment. Herbivores in combined herbivory and competition treatments likely migrated between plants, probably resulting in selective feeding and consequently stronger herbivory on LC-plants, which would likely occur in co-occurring plants in nature. This also reflects situations in natural systems with a considerable variability of cyanogenesis within and between neighboring plant populations (Richards and Fletcher 2002). The resulting better plant performance of HC-plants demonstrates how investing resources into costly defense compounds can benefit highly defended plants. This benefit can be interpreted as a result of a competitive advantage of better defended plants due to the selective feeding of herbivores on less defended competitors. This suggests that strong herbivory acts as a selection pressure for increased defense production, despite the fitness costs the plant may endure due to lowered competitive ability (Bixenmann et al. 2013).

Our findings suggest that performance of a given plant genotype depends on the ecological context. In our experimental setting, high cyanogenic plants outperformed LC-plants when herbivore pressure was high. However, when herbivore pressure was low, LC-plants had a competitive advantage. Additional factors have to be considered. For example, cyanogenesis has been demonstrated to trade off with inducible indirect plant defenses against herbivores, the emission of herbivore-induced plant volatile organic compounds (HIVOCs) and the secretion of extrafloral nectar (Ballhorn et al. 2008a, 2013a; Kost and Heil 2008). Which of these anti-herbivore defense systems provides the highest fitness advantage under which conditions in natural settings remains to be studied (Ballhorn et al. 2010, 2011b). Furthermore, in natural systems, plants are frequently attacked by herbivores and pathogens simultaneously. Plant defenses efficient against one attacker might have fundamentally different effects on other plant antagonists. In cyanogenic rubber tree as well as in lima bean, high cyanogenic plant individuals are more susceptible to fungal pathogens than their low cyanogenic conspecifics (Lieberei 1988; Ballhorn et al. 2010, 2011b) adding another layer of complexity to the system. Moreover, a number of abiotic factors have been discussed to contribute to the ecological costs of cyanogenesis (see Kakes 1990; Hayden and Parker 2002 for review). Variable environmental conditions have led to the evolution of complex and plastic plant defensive systems even within a single plant species.

Conclusion

The low investment in plant defense resulted in the low cyanogenic genotypes being better able to allocate resources to shoot growth and seed production and, at the same time, better tolerate interspecific competition, whereas a high investment in defense limits the ability of HC-lima bean genotypes to do so, but allowed these HC-plants to resist herbivory more effectively. This could imply that intense competition may act as a selective force favoring low expression of expensive, constitutive defenses. Since these same plants also express a high level of inducible indirect defenses (Ballhorn et al. 2008a), selection may have acted positively on these alternative defense traits in the face of competition and limited resources. High cyanogenesis, in contrast, might represent a selective advantage when plants are exposed to constantly high herbivore pressure. This divergence of defense strategies—which occurs both in wild and cultivated forms of lima bean (Ballhorn et al. 2013)—and variable ability to compete for resources highlights the necessity to understand the ecological factors most intensely affecting plant growth and reproduction in natural populations. Several theories have been formulated to comprise current understanding of plant defense, yet a cohesive, community ecology-based approach is still lacking, which is frequently noted by the scientific community given the importance of understanding plant systems in a changing climate (Herms and Mattson 1992; Stamp 2003; Agrawal 2011). Therefore, generating empirical quantitative data on competitive ability of plants with varying degrees of expensive defense is critical to strengthening current understanding of plant community dynamics, as well as understanding the evolution of plant defenses.

References

Adler LS, Seifert MG, Wink M, Morse GE (2012) Reliance on pollinators predicts defensive chemistry across tobacco species. Ecol Lett 15:1140–1148. doi:10.1111/j.1461-0248.2012.01838.x

Agrawal AA (2007) Macroevolution of plant defense strategies. Trends Ecol Evol 22:103–109. doi:10.1016/j.tree.2006.10.012

Agrawal AA (2011) Current trends in the evolutionary ecology of plant defence. Funct Ecol 25:420–432. doi:10.1111/j.1365-2435.2010.01796.x

Agrawal AA, Fishbein M (2006) Plant defense syndromes. Ecology 87:S132–S149

Agrawal AA, Conner JK, Rasmann S (2010) Tradeoffs and negative correlations in evolutionary ecology. In: Bell MA, Eanes WF, Futuyma DJ, Levinton JS (eds) Evolution after Darwin: the first 150 years. Sinauer, Sunderland

Atsatt PR, O'Dowd DJ (1976) Plant defense guilds. Science (80-) 193:24–29

Baldwin IT, Hamilton W (2000) Jasmonate-induced responses of *Nicotiana sylvestris* results in fitness costs due to impaired competitve ability for nitrogen. J Chem Ecol 26:915–952

Baldwin IT, Sims CL, Kean SE (1990) The reproductive consequences associated with inducible alkaloidal responses in wild tobacco. Ecology 71:252–262

Ballhorn DJ (2011a) Cyanogenic glycosides in nuts and seeds. In: Preedy VR, Watson RR, Patel VB (eds) Nuts and seeds health and disease prevention (1st edn). Elsevier, Amsterdam, pp 129–136

Ballhorn DJ (2011b) Constraints of simultaneous resistance to a fungal pathogen and an insect herbivore in lima bean (*Phaseolus lunatus* L.). J Chem Ecol 37:141–144. doi:10.1007/s10886-010-9905-0

Ballhorn DJ, Lieberei R, Ganzhorn JU (2005) Plant cyanogenesis of *Phaseolus lunatus* and its relevance for herbivore–plant interaction: the importance of quantitative data. J Chem Ecol 31:1445–1473. doi:10.1007/s10886-005-5791-2

Ballhorn DJ, Heil M, Pietrowski A, Lieberei R (2007) Quantitative effects of cyanogenesis on an adapted herbivore. J Chem Ecol 33:2195–2208. doi:10.1007/s10886-007-9380-4

Ballhorn DJ, Kautz S, Lion U, Heil M (2008a) Quantitative variability of lima bean's VOC boquets and its putative ecological consequences. Plant Signal Behav 3:1005–1007. doi:10.1111/j.1365-2745.2008.01404.x. www.landesbioscience.com

Ballhorn DJ, Kautz S, Lion U, Heil M (2008b) Trade-offs between direct and indirect defences of lima bean (*Phaseolus lunatus*). J Ecol 96:971–980. doi:10.1111/j.1365-2745.2008.01404.x

Ballhorn DJ, Kautz S, Heil M, Hegeman AD (2009) Cyanogenesis of wild lima bean (Phaseolus lunatus L.) is an efficient and direct defense in nature. PLoS ONE e5450

Ballhorn DJ, Pietrowski A, Lieberei R (2010) Direct trade-off between cyanogenesis and resistance to a fungal pathogen in lima bean (*Phaseolus lunatus* L.). J Ecol 98:226–236. doi:10.1111/j.1365-2745.2009.01591.x

Ballhorn DJ, Kautz S, Jensen M, Schmitt I, Heil M, Hegeman AD (2011a) Genetic and environmental interactions determine plant defences against herbivores. J Ecol 99:313–326. doi:10.1111/j.1365-2745.2010.01747.x

Ballhorn DJ, Schmitt I, Fankhauser JD, Katagiri F, Pfanz H (2011b) CO_2-mediated changes of plant traits and their effects on herbivores are determined by leaf age. Ecol Entomol 36:1–13. doi:10.1111/j.1365-2311.2010.01240.x

Ballhorn DJ, Godschalx AL, Kautz S (2013a) Co-variation of chemical and mechanical defenses in lima bean (*Phaseolus lunatus* L.). J Chem Ecol 39:413–417. doi:10.1007/s10886-013-0255-6

Ballhorn DJ, Kautz S, Heil M (2013b) Distance and sex determine host plant choice by herbivorous beetles. PLoS ONE 8(2):e55602

Ballhorn DJ, Kautz S, Schädler M (2013c) Induced plant defense via volatile production is dependent on rhizobial symbiosis. Oecologia 172:833–846

Ballhorn DJ, Kay J, Kautz S (2014) Quantitative effects of leaf area removal on indirect defense of lima bean (*Phaseolus lunatus*) in nature. J Chem Ecol (in press)

Barton KE (2007) Early ontogenetic patterns in chemical defense in Plantago (Plantaginaceae): genetic variation and trade-offs. Am J Bot 94:56–66. doi:10.3732/ajb.94.1.56

Bennett AE, Bever JD, Deane Bowers M (2009) Arbuscular mycorrhizal fungal species suppress inducible plant responses and alter defensive strategies following herbivory. Oecologia 160:771–779. doi:10.1007/s00442-009-1338-5

Bernays E, Graham M (1988) On the evolution of host specificity in phytophagous arthropods. Ecology 69:886–892

Bixenmann RJ, Coley PD, Kursar TA (2013) Developmental changes in direct and indirect defenses in the young leaves of the neotropical tree genus Inga (Fabaceae). Biotropica 45:175–184

Broz AK, Broeckling CD, De-la-Peña C et al (2010) Plant neighbor identity influences plant biochemistry and physiology related to defense. BMC Plant Biol 10:1–14

Campbell SA, Kessler A (2013) Plant mating system transitions drive the macroevolution of defense strategies. Proc Natl Acad Sci USA 110:3973–3978. doi:10.1073/pnas.1213867110

Casper BB, Jackson RB (1997) Plant competition underground. Annu Rev Ecol Syst 28:545–570

Caswell H (1989) Matrix population models, 2nd edn. Sinauer, Sunderland

Chase JM, Abrams PA, Grover JP et al (2002) The interaction between predation and competition: a review and synthesis. Ecol Lett 5:302–315. doi:10.1046/j.1461-0248.2002.00315.x

Cipollini D, Heil M (2010) Costs and benefits of induced resistance to herbivores and pathogens in plants. CAB Rev Perspect Agric Vet Sci Nutr Nat Resour 5:1–25. doi:10.1079/PAVSNNR20105005

Connell JH (1983) On the prevalence and relative importance of interspecific competition: evidence from field experiments. Am Soc Nat 122:661–696

Cork SJ (1996) Optimal digestive strategies for arboreal herbivorous mammals in contrasting forest types: why koalas and colobines are different. Aust J Ecol 21:10–20

Dantas VDL, Batalha MA (2012) Can antiherbivory resistance explain the abundance of woody species in a Neotropical savanna? Botany 90:93–99. doi:10.1139/B11-087

Dover BA, Noblet R, Moore RF, Culbertson D (1988) An improved artificial diet for Mexican bean beetles based on host preference. J Agric Entomol 5:79–86

Ehrlich PR, Raven PH (1964) Butterflies and plants: a study in coevolution. Evolution (NY) 18:586–608

Fenner M, Thompson K (2005) The ecology of seeds. Cambridge University Press, Cambridge

Flanders RV (1984) Comparisons of bean varieties currently being used to culture the Mexican bean beetle (Coleoptera: Coccinellidae). Environ Entomol 13:995–999

Frehner M, Conn EE (1987) The linamarin beta-glucosidase in Costa Rican wild lima beans (Phaseolus lunatus L.) is apoplastic. Plant Physiol 84:1296–1300

García MB, Ehrlén J (2002) Reproductive effort and herbivory timing in a perennial herb: fitness components at the individual and population levels. Am J Bot 89:1295–1302. doi:10.3732/ajb.89.8.1295

Haag JJ, Coupe MD, Cahill JFJ (2004) Antagonistic interactions between competition and insect herbivory on plant growth. J Ecol 92:156–167

Hayden KJ, Parker IM (2002) Plasticity in cyanogenesis of Trifolium repens L.: inducibility, fitness costs and variable expression. Evol Ecol Res 4:155–168

Hedges LV, Gurevitch J, Curtis PS (1999) The meta-analysis of response ratios in experimental ecology. Ecology 80:1150–1156

Heil M (2008) Indirect defence via tritrophic interactions. New Phytol 178:41–61

Herms DA, Mattson WJ (1992) The dilemma of plants: to grow or defend. Q Rev Biol 67:283–335

Johnson MTJ (2008) Bottom-up effects of plant genotype on aphids, ants, and predators. Ecology 89:145–154

Jones PR, Møller BL, Høj PB (1999) The UDP-glucose: p-hydroxy-mandelonitrile-O-glucosyltransferase that catalyzes the last step in synthesis of the cyanogenic glucoside dhurrin in Sorghum bicolor. J Biol Chem 274:35483–35491

Kakes P (1990) Properties and functions of the cyanogenic system in higher plants. Euphytica 48:25–43

Kaplan I, Dively GP, Denno RF (2009) The costs of anti-herbivore defense traits in agricultural crop plants: a case study involving leafhoppers and trichomes. Ecol Appl 19:864–872

Kempel A, Schädler M, Chrobock T et al (2011) Tradeoffs associated with constitutive and induced plant resistance against herbivory. Proc Natl Acad Sci USA 108:5685–5689. doi:10.1073/pnas.1016508108

Kost C, Heil M (2008) The defensive role of volatile emission and extrafloral nectar secretion for lima bean in nature. J Chem Ecol 34:1–13. doi:10.1007/s10886-007-9404-0

LaPidus JB, Cleary RW, Davidson RH et al (1963) Chemical factors influencing host selection by Mexican bean beetle Epilachna varivestis Muls. Agric Food Chem 11:462–463

Lieberei R (1988) Relationship of cyanogenic capacity (HCN-c) of the rubber tree Heveabrasiliensis to susceptibility to Microcyclus ulei, the agent causing South American leaf blight. J Phytopathol 122:54–67

Marak HB, Biere a, Van Damme JMM (2000) Direct and correlated responses to selection on iridoid glycosides in Plantago lanceolata L. J Evol Biol 13:985–996. doi:10.1046/j.1420-9101.2000.00233.x

Marak HB, Biere A, Van Damme JMM (2003) Fitness costs of chemical defense in Plantago lanceolata L.: effects of nutrient and competition stress. Evolution 57:2519–2530

Massad TJ, Dyer LA, Vega CG (2012) Costs of defense and a test of the carbon-nutrient balance and growth-differentiation balance hypotheses for two co-occurring classes of plant defense. PLoS ONE 7:e47554. doi:10.1371/journal.pone.0047554

Moles AT, Peco B, Wallis IR et al (2013) Correlations between physical and chemical defences in plants: tradeoffs, syndromes, or just many different ways to skin a herbivorous cat? New Phytol 198:252–263. doi:10.1111/nph.12116

Noitsakis B, Jacquard P (1992) Competition between cyanogenic and acyanogenic morphs of Trifolium repens. Theor Appl Genet 83:443–450

Nomura M, Hatada A, Itioka T (2011) Correlation between the leaf turnover rate and anti-herbivore defence strategy (balance between ant and non-ant defences) amongst ten species of Macaranga (Euphorbiaceae). Plant Ecol 212:143–155. doi:10.1007/s11258-010-9810-1

Rask L, Andréasson E, Ekbom B et al (2000) Myrosinase: gene family evolution and herbivore defense in Brassicaceae. Plant Mol Biol 42:93–113

Read J, Sanson GD, Caldwell E et al (2009) Correlations between leaf toughness and phenolics among species in contrasting environments of Australia and New Caledonia. Ann Bot 103:757–767. doi:10.1093/aob/mcn246

Rees M (1995) Community structure in sand dune annuals: is seed weight a key quantity? Oecologia 83:857–863

Rees M, Brown VK (1992) Interactions between invertebrate herbivores and plant competition. J Ecol 80:353–360

Richards AJ, Fletcher A (2002) The effects of altitude, aspect, grazing and time on the proportion of cyanogenics in neighbouring populations of Trifolium repens L. (white clover). Heredity 88:432–436

Selmar D, Lieberei R, Conn EE, Biehl B (1989) a -Hydroxynitrile lyase in Hevea brasiliensis and its significance for rapid cyanogenesis. Physiol Plant 75: 97–101

Schädler M, Brandl R, Haase J (2007) Antagonistic interactions between plant competition and insect herbivory. J Ecol 88:1490–1498

Siemens DH, Garner SH, Mitchell-Olds T, Callaway RM (2002) Cost of defense in the context of plant competition: Brassica rapa may grow and defend. Ecology 83:505–517

Simms EL, Taylor DL (2002) Partner choice in nitrogen-fixation mutualisms of legumes and rhizobia. Integr Comp Biol 42:369–380

Stamp N (2003) Out of the quagmire of plant defense hypotheses. Q Rev Biol 78:23–55

Thamer S, Schädler M, Bonte D, Ballhorn DJ (2011) Dual benefit from a belowground symbiosis: nitrogen fixing rhizobia promote growth and defense against a specialist herbivore in a cyanogenic plant. Plant Soil 341:209–219. doi:10.1007/s11104-010-0635-4

Viola DV, Mordecai EA, Jaramillo AG et al (2010) Competition-defense tradeoffs and the maintenance of plant diversity. Proc Natl Acad Sci USA 107:17217–17222. doi:10.1073/pnas.1007745107

Wilson SD, Tilman D (1991) Component of plant competition along an experimental gradient of nitrogen availability. Ecology 72:1050–1065