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RAPID COMMUNICATION

Constraints of Simultaneous Resistance to a Fungal Pathogen and an Insect Herbivore in Lima Bean (*Phaseolus lunatus* L.)

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Abstract The existence of tradeoffs among plant defenses is commonly accepted, however, actual evidence for these tradeoffs is scarce. In this study, I analyzed effects of different direct defenses of wild lima bean plants (*Phaseolus lunatus*) that were simultaneously exposed to a fungal pathogen (*Colletotrichum lindemuthianum*) and an insect herbivore, the Mexican bean beetle (*Epilachna varivestis*). Although plants were derived from spatially widely separated populations, I observed a common tradeoff between resistance to pathogens and herbivores. Plants with high levels of anti-herbivore defense (cyanogenesis) showed low levels of resistance to pathogens (polyphenol oxidase activity and phenolic compounds), and *vice versa*. Competition for resources generally is considered to be the basis for tradeoffs. However, I report direct inhibition of polyphenol oxidase by cyanide, making simultaneous expression of both defenses at high levels impossible. I argue that populations composed of individuals investing in one type of defense have an advantage in environments that periodically favor either pathogen or herbivore plant antagonists.

Key Words Tradeoff · Plant defense · *Colletotrichum lindemuthianum* · *Epilachna varivestis* · *Phaseolus lunatus* · Defense syndrome · Cyanogenesis · Polyphenol oxidase · Phenolics

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Introduction

In their natural environment, plants are exposed to multiple antagonists simultaneously, including pathogens as well as herbivores. Consistent with the broad range of attackers, plants generally do not rely on a single defense mechanism, but express multiple defenses (Walling 2000). Theory predicts that constraints on resource allocation produce negative genetic correlations (tradeoffs) between individual traits (Agrawal et al., 2010). In theory, tradeoffs occur where multiple traits are favored by natural selection and compete for shared resources (Thaler et al., 1999), but in practice tradeoffs are rarely found (Agrawal et al., 2010). Such discrepancies between the theory and evidence are difficult to explain without a mechanistic understanding of how tradeoffs affect plant interactions in ecological networks.

Tradeoffs may reflect physiological interference among defensive traits instead of direct resource competition. In lima bean for example, extensive cyanogenesis (anti-herbivore defense) inhibits the activity of polyphenol oxidases (PPO), which are mainly targeted against pathogens. Cyanide binds to the metal-containing active site of PPO (Lieberei et al., 1989). Cyanide-resistant defensive enzymes such as lipoxygenases (LOX) have been demonstrated in some plant species (Tscharntke et al., 2001), but PPOs in lima bean are cyanide-sensitive (Ballhorn et al., 2010a).

I asked how two traits that act against different types of antagonists (Ballhorn et al., 2010a,b) functionally interact when lima bean plants are under simultaneous attack by fungal pathogens and insect herbivores. Under experimental greenhouse conditions, I used wild lima bean plants derived from three different populations in North America, Central America, and the Caribbean to test the concerted effects of cyanogenesis and PPO activity on resistance to a fungal bean pathogen (*Colletotrichum lindemuthianum*) and a specialist insect herbivore, the Mexican bean beetle (*Epilachna varivestis*).

Methods and Materials

Plants Lima bean plants were grown from seeds collected at natural sites from a Mexican (near Puerto Escondido, Pacific coast of Oaxaca 15°55′31.80″N, 97°9′4.68″W, 8 m a.s.l.), a Costa Rican (near Asserri, San Jose 9°51′48.02″N, 84°5′42.07″W, 1900 m a.s.l.), and a Cuban (Montecristo, Guantanamo 20°7′12.15″N, 74°14′58.01″W, 168 m a.s.l.) population. Plants were cultivated under greenhouse conditions as described in Ballhorn et al., (2007) for 5 wk prior the experiments.

Pathogens and Insects The anthracnose-causing fungal bean pathogen *Colletotrichum lindemuthianum* (strain 12250 purchased from the DSMZ, Braunschweig, Germany), was maintained on oat flake medium (30 g oat flakes, 15 g agar, 1000 ml H_2O) according to DSMZ instructions. To induce sporulation, sterile young snap bean pods (*Phaseolus vulgaris*) were inoculated with a suspension of *C. lindemuthianum* mycelia and incubated at 22±1°C for 14 d in the dark. From these cultures, conidial suspensions were prepared by filtering the homogenate through 3 cm cotton in a 60 ml syringe to remove mycelial fragments. Spore concentration in pooled solutions was adjusted to 10^5 conidia ml^{-1} with sterile distilled water using an Improved-Double-Neubauer counting-chamber. Mexican bean beetles (MBB) were maintained on snap bean as described in Ballhorn et al., (2010b). Freshly molted third-instar larvae were used for the experiments.

Experimental Setup Twenty-four plants (in blocks of 6×4 plants) from each population were used for the experiment in growth chambers under conditions similar to those used for greenhouse cultivation (Ballhorn et al., 2007). Plants of the same block (= population) had contact with each other, whereas the different blocks did not. At the end of the light period, every plant was sprayed from the lower side with 5 ml spore suspension. On the following day, one MBB larva was placed on each plant. Third-instars are relatively mobile and move between contacting plants searching for suitable hosts but do not leave their food plants completely.

Data Collection and Chemical Analysis After an experimental period of 8 d, the number of fungal lesions was counted, and feeding damage was quantified for every plant. Missing leaf area was quantified with a LICOR LI-3100 Area Meter (Walz GmbH, Effeltrich, Germany). Every leaf was scanned three times, and the mean was used for further analysis. To characterize defensive features of plants, leaf discs from undamaged areas of young leaves (Ballhorn et al., 2010c) were analyzed chemically. Leaf cyanogenic potential was analyzed by complete enzymatic degradation of cyanogenic glycosides in leaf extracts followed by 20 min of incubation at 30°C in gas-tight glass vessels (Thunberg-vessels) and spectrophotometric measurement at 585 nm using the Spectroquant® cyanide test (Merck, Darmstadt, Germany) (Ballhorn et al., 2011). Enzymatic activity of PPO was determined polarographically, using the Clark electrode system (Yellow Springs Instruments, Yellow Springs, OH, USA) to measure O_2−consumption during the oxidation of polyphenols and their derivates to quinones (Ballhorn et al., 2010a). Total phenolics were determined in aqueous acetone extracts of leaves with the Folin method using epicatechin (Sigma, Deisenhofen, Germany) as the standard.

Statistical Analyses One-way ANOVAs were applied to test for significant differences of chemical traits, number of lesions, and consumed leaf area among populations. *Pearson's correlations* were used to test for co-variation of variables. Analyses were carried out with PASW Statistics 18.

Results and Discussion

Lima bean plants varied by a factor of 5.15 between the lowest and highest values measured for cyanogenic potential (HCNp, total amount of cyanogenic precursors in leaf tissue). There also was variation in PPO activity (factor of 16.01), and in concentration of total phenolics (factor of 2.76). As I observed no significant differences of traits among lima bean plants from the different origins (HCNp: $F_{2,69}$=0.670, P=0.515; PPO activity: $F_{2,69}$=0.048, P=0.953; total phenolics: $F_{2,69}$=2.469, P=0.092; according to *one-way ANOVA*), data were pooled for further analyses. Cyanogenic potential of plants was correlated negatively with PPO activity and concentration of total phenolics, whereas PPO activity and phenolics showed a positive correlation (according to *Pearson's correlation*; Fig. 1a–c). Consumed leaf area was correlated negatively with HCNp but showed a significantly positive correlation to PPO activity and phenolics (Fig. 1d–f). The number of lesions showed a positive correlation to HCNp (Fig. 1g) but was correlated negatively to PPO activity and concentration of phenolics (Fig. 1h,i). The observed defensive effects of cyanide on the insect herbivore as well as of PPO activity on the fungal pathogen are in line with previous studies (Gleadow and Woodrow 2002; Ballhorn et al., 2010a,b). However, the positive correlation between consumed leaf area and PPO activity (Fig. 1e) or total phenolics (Fig. 1f) is more likely due to negative effects of HCN on insects than to

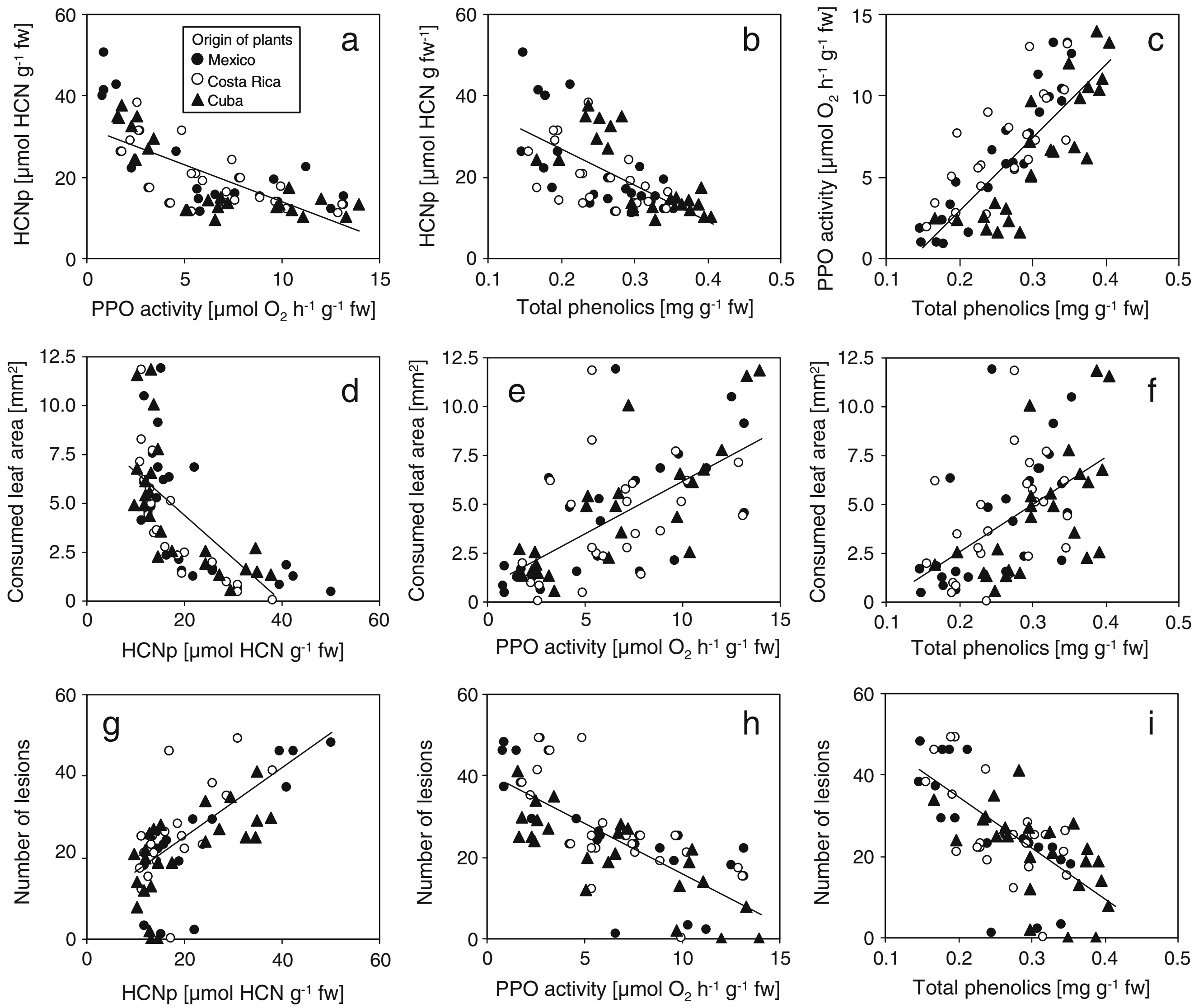

Fig. 1 Correlations between plant traits and plant resistance to fungal and herbivore antagonists. Lima bean plants from three different populations (Mexico, Costa Rica, and Cuba) were analyzed for cyanogenic potential (HCNp; total amount of cyanogenic precursors in leaf tissue), polyphenol oxidase (PPO) activity and concentration of total phenolics. In experiments in which plants were simultaneously exposed to a fungal pathogen (*Colletotrichum lindemuthianum*) and a specialist insect herbivore (Mexican bean beetle; *Epilachna varivestis*), damage caused by each plant antagonist was quantified. To search for potential tradeoffs among plant traits and resistance to fungal pathogens and insect herbivores, the following *Pearson's correlation* were conducted (**a**: $r=-0.716$, $P<0.001$; **b**: $r=-0.604$, $P<0.001$; **c**: $r=0.710$, $P<0.001$; **d**: $r=-0.682$, $P<0.001$; **e**: $r=0.646$, $P<0.001$; **f**: $r=0.534$, $P<0.001$; **g**: $r=0.659$, $P<0.001$; **h**: $r=-0.732$, $P<0.001$; **i**: $r=-0.558$, $P<0.001$)

positive effects of PPOs and phenolics (Ballhorn et al., 2010a). It also is likely that the negative correlations between HCNp and PPO activity as well as between HCNp and phenolic content are the underlying cause of the positive correlation obtained here between the number of pathogen lesions and HCNp (Fig. 1g).The complex interdependence of various chemical plant traits and the resulting effects on different plant antagonists shows that correlations have to be interpreted with care—in particular when only one trait and one corresponding effect is analyzed—as some correlations might not be of functional importance.

The present study on wild lima beans demonstrates that tradeoffs between cyanogenesis and PPO activity as well as cyanogenesis and phenolics occur in natural bean populations distributed over large spatial scales. More importantly, the work shows that these tradeoffs actually result in an ecologically important tradeoff between resistance to pathogens and herbivores when plants were exposed to both antagonists simultaneously. Among all plants, feeding damage and lesion number showed a highly significant negative correlation ($r=-0.600$, $P<0.001$ according to *Pearson's correlation*). From the ecological and population-genetics perspective, the expression of either anti-fungal or anti-herbivore defenses may be interpreted as an adaptation to variable environmental conditions, which either favor the occurrence of pathogens (humid conditions) or enhance the pressure by herbivores (dry periods) in the natural habitats of lima bean (pers. observation). Thus, variability of defensive traits allows populations to overcome periodically changing environmental conditions not only on the seasonal level but also over longer periods for example due to El Niño climate episodes. In evolutionary terms, periodically changing environmental conditions may represent a stabilizing factor for maintaining this tradeoff between anti-pathogen and anti-herbivore defenses in natural lima bean populations.

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