

SPECIAL FEATURE

PLANT-MEDIATED INTERACTIONS BETWEEN ABOVE- AND BELOW-GROUND COMMUNITIES

Plant defence theory re-examined: nonlinear expectations based on the costs and benefits of resource mutualisms

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Summary

1. General theories of plant defence often fail to account for complex interactions between the resources required for defence expression. For example, the carbon that is used for carbon-based defence is acquired using nutrient-rich photosynthetic pigments, while nutrient gain itself requires substantial carbon allocation belowground. We should therefore expect the expression of plant defence to reflect the tight linkage between carbon and nutrient gain, yet mechanistic studies linking resource gain with plant defence theory have been slow to emerge.

2. The overwhelming majority of plants participate in nutrition mutualisms with fungal or bacterial symbionts. We propose the resource exchange model of plant defence (REMPD) in which the costs and benefits associated with nutrition mutualisms affect plant resource status and allocation to growth and defence. The model predicts quadratic relationships between mutualist abundance and expression of defence. Within plant genotypes, both plant biomass and defence expression are maximized at optimal nutrient exchange among mutualistic partners, and as a consequence, the two are positively associated.

3. We tested the model by growing *Asclepias syriaca*, the common milkweed, with two mycorrhizal fungal species in nine fungal abundance treatments. Plant growth and defence traits and mycorrhizal colonization were quantified after 14 weeks of plant growth. Linear, quadratic, saturating and exponential decay models were fit to curves relating the proportion of root colonized by mycorrhizal fungi to plant traits, and compared using AICc.

4. As predicted by our model, increasing colonization by *Scutellospora pellucida* produced quadratic responses in plant growth, latex exudation and cardenolide production. In contrast, *Glomus etunicatum* appeared to act as a parasite of *A. syriaca*, causing exponential decline in both plant growth and latex exudation. As predicted by our model, plant growth was positively correlated with all defences quantified.

5. *Synthesis.* The REMPD combines cost–benefit analysis of mutualisms with plant resource acquisition strategies to predict the expression of plant defence. The effects of *S. pellucida* and *G. etunicatum* on defence expression differ; however, both provide support for the model and suggest that resource mutualisms affect the expression of defence in a predictable nonlinear fashion.

Key-words: arbuscular mycorrhizal fungi, *Asclepias syriaca*, carbon–nutrient balance hypothesis, *Glomus etunicatum*, growth–differentiation balance hypothesis, plant–herbivore interactions, resource exchange model of plant defence, *Scutellospora pellucida*

Introduction

Predicting the expression of plant defence against herbivores in natural and managed ecosystems is essential to modelling and

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managing these systems. However, current general theories of plant defence are incomplete (Hamilton *et al.* 2001; Stamp 2003). Recent advances have improved our understanding of the evolution of defensive strategies among plant species and genotypes (Fine *et al.* 2006; Agrawal & Fishbein 2008), but the prediction of individual phenotypic expression of plant defence remains challenging. While early hypotheses relied on plant nutrient availability in ecological time to predict plant allocation to defence or growth (Bryant, Chapin & Klein 1983), many of the underlying assumptions have since been challenged (Gershenzon 1994; Hamilton *et al.* 2001). Plant nutrient status alters not only the availability of precursor compounds for the synthesis of defence, but also changes plant physiology and allocation patterns (Bloom, Chapin & Mooney 1985; Herms & Mattson 1992; Shipley & Meziane 2002) and influences the ability of plants to acquire other resource types (Hamilton *et al.* 2001). For example, *Populus tremuloides* plants grown under elevated CO₂ are limited by nitrogen (N) availability, but plants that are able to acquire more N through increased carbon (C) allocation belowground improve subsequent C acquisition through increased photosynthesis (Zak *et al.* 2000). Carbon and nutrient acquisition are coupled through alternate allocation to roots and shoots (Ingstad & Agren 1991). When resource acquisition is uncoupled and resources become limiting, trade-offs become evident (Herms & Mattson 1992; Mole 1994; Donaldson, Kruger & Lindroth 2006). Here, we develop a general model that integrates the coupled acquisition and expenditure of resources in an ecological context and generates novel predictions regarding the expression of defence. By incorporating into defence theory the complex interactions among nutrients during resource acquisition and allocation, we may gain a better understanding of phenotypic variation in defence expression (Glynn *et al.* 2007).

Current models of plant defence (Stamp 2003), as well as models of optimal resource allocation within plants (Shipley & Meziane 2002), fail to incorporate the biotic interactions that mediate resource acquisition and alter plant allocation patterns between growth and defence. Soil microbes are intimately coupled with root function, but can induce changes in plant physiology not predicted by models of nutrient uptake by roots alone (Wright, Read & Scholes 1998a). For example, mycorrhizal fungi and rhizobia can act to stimulate plant photosynthesis (Kaschuk *et al.* 2009) and net assimilation rate (Wright, Read & Scholes 1998a) independently of plant nutrition. Conversely, symbionts also require resources for their own growth, which can result in parasitism and growth depressions in host plants (Peng *et al.* 1993; Johnson, Graham & Smith 1997). As a result, incorporating microbial associations into models of plant defence expression may result in novel predictions.

PLANT RESOURCE ACQUISITION THROUGH NUTRITION MUTUALISMS

Over 80% of land plants acquire mineral nutrients from soil microbes at the expense of C (Wang & Qiu 2006; Smith & Read 2008). As a consequence, general theories of plant

defence should include the feedback among resources mediated by plant–microbe interactions. Additionally, resource mutualisms represent a convenient framework in which to examine plant allocation patterns, the interactions among nutrients, and resulting effects on plant defence expression. Incorporating resource exchanges among organisms into plant defence theory will increase our understanding and prediction of plant defence expression in an ecological context.

Plant interactions with mycorrhizal fungi are among the most common nutrition mutualisms and provide an excellent opportunity to explore the interactions among primary currencies and the expression of plant defences. More than 80% of all plant species examined host symbiotic fungi within their roots (Wang & Qiu 2006) and transfer hexose sugars to fungal partners in exchange for mineral nutrients and water (Smith & Read 2008). We focus on the interaction between arbuscular mycorrhizal fungi (AMF) and their plant partners because this symbiosis is the most common nutrition mutualism among plant species (Wang & Qiu 2006). Although the exact currencies of transfer may vary, other types of nutrition mutualisms, including symbiotic N-fixing bacteria and additional types of mycorrhizal fungi, are likely to function comparably in their effects on defence (Kempel, Brandl & Schädler 2009), and our model seeks to generalize to plants participating in these mutualisms as well. Because the vast majority of land plants exchange vital resources with soil symbionts, these interactions may be a key (and underappreciated) variable in the expression of plant defence.

Although other models of defence incorporate resource uptake from roots (Herms & Mattson 1992), resource dynamics resulting from exchange with mycorrhizal fungi differ from those of nutrient uptake via roots (Wright, Scholes & Read 1998b), in part because there can be fitness conflicts between partners (Kiers & Denison 2008). We propose that the fundamental exchange of resources within the mycorrhiza mediates the expression of plant defence. How might variation in mycorrhizal associations alter the expression of plant defence?

Mycorrhizal associations are typically classified as mutualistic interactions, but intrinsically involve both costs and benefits (Koide & Elliott 1989; Fitter 1991). Plant responses to colonization are largely a function of these exchanges. The costs and benefits of the currencies transferred, and therefore the outcome of mycorrhizal associations, vary within natural and agricultural systems (Johnson, Graham & Smith 1997). Plant and fungal identity, ontogeny and abiotic resource availability alter the costs and benefits of association among partners, and therefore mediate the outcome of mycorrhizal interactions (Johnson, Graham & Smith 1997; Hoeksema *et al.* 2010). However, one aspect of the mutualism notably missing from this discussion is the importance of partner abundance (but see Gange & Ayres 1999). While the abundance of mutualist partners can affect mutualist performance and population dynamics (Holland, DeAngelis & Bronstein 2002), and is tied to partner performance in non-mycorrhizal systems (Morris, Vazquez & Chacoff 2010), recent work on mycorrhizae has not emphasized the importance of fungal abundance. We argue that the factors that alter resource exchange (costs and

benefits of association) between soil mutualists and plants in large part determine the effect of soil mutualists on the expression of plant defence (Jones & Last 1991).

Experimental evidence demonstrates that mycorrhizal fungi can substantially alter insect performance (Goverde *et al.* 2000; Gange 2001), often increasing aphid performance and that of specialist insects, while decreasing the performance of generalist chewing insects (Hartley & Gange 2009; Koricheva, Gange & Jones 2009). However, the effect of mycorrhizal fungi on insect herbivores and secondary metabolites varies substantially among studies (Hartley & Gange 2009). Our model offers a framework for interpreting and reconciling these results in terms of resource stoichiometry and effects on plant defence.

Model description – the resource exchange model of plant defence

Plants are predicted to allocate optimally to obtain limiting resources (Bloom, Chapin & Mooney 1985), and have associated for 465 million years with mycorrhizal fungi (Brundrett 2002), which aid in acquisition and uptake of macro and micronutrients (Smith & Read 2008). Although mycorrhizal fungi confer multiple benefits to plants including pathogen protection and improved water relations (Auge 2001; Borowicz 2001), we focus on nutrient benefits, a key factor in predicting the outcome of AMF symbioses (Johnson 2010) and the expression of plant defence (Herms & Mattson 1992; Gershenzon 1994).

When mineral nutrients limit plant growth, plants increase C allocation belowground, increasing the root : shoot ratio (Bloom, Chapin & Mooney 1985; Shipley & Meziane 2002) or allocation to mycorrhizal fungi (Treseder & Allen 2002). We refer to allocation to fungi as the carbon 'cost' associated with nutrient acquisition through mycorrhizal symbionts. In return, mycorrhizal fungi transfer phosphorus (P), nitrogen (N) and micronutrient 'benefits' to plants (Smith & Read 2008). The cost associated with hosting mycorrhizal fungi can be substantial, from 4 to 20% of net photosynthetic intake (Jakobsen & Rosendahl 1990). Nutrient returns are also considerable: some plants receive in excess of 50% of total P inflow from AMF (Li *et al.* 2006), and substantial N influx via AMF has been documented as well (Govindarajulu *et al.* 2005). A stronger C sink in roots and an increase in nutrients with which to construct photosynthetic apparatus allow plants to increase the rate of photosynthesis so that under some circumstances, fungi essentially 'pay' for themselves (Kaschuk *et al.* 2009).

Previous models have described the costs and benefits of mycorrhizal fungi (Koide & Elliott 1989; Fitter 1991; Gange & Ayres 1999), and postulated that they may alter the expression of plant defence against herbivores (Jones & Last 1991; Bennett, Alers-Garcia & Bever 2006), and we build upon these previous efforts. Specifically, we propose that the costs and benefits of symbiosis are dynamic, depend intrinsically upon the abundance of soil mutualists, and affect the expression of plant defences. After describing the basic model, we illustrate

how environmental or biotic variation may shift the shape of the cost or benefit curves and alter plant defence expression. We conclude by incorporating our predictions with those of the growth differentiation balance hypothesis (GDBH) (Herms & Mattson 1992) to generate the novel predictions of the REMPD.

Mycorrhizal fungal abundance varies substantially within and among ecosystems (Treseder & Cross 2006). As a result, plant associations with fungi also vary among habitats and ecosystems. Limited fungal abundance in the environment can constrain the formation of the mycorrhiza and associated resource exchange in greenhouse, agricultural and natural systems (Lekberg & Koide 2005). Greater fungal abundance can increase colonization of plant roots and resource exchange (Sanders *et al.* 1977; Fitter 1991) (Fig 1a), due to a greater extraradical biomass and nutrient flux to the root. Indeed, the proportion of root colonized is significantly correlated with AMF biomass, quantified using phospholipid fatty acids (van Diepen *et al.* 2007) and hyphal length outside the root (Miller, Reinhardt & Jastrow 1995). We use the proportion of root length colonized as a proxy for the abundance of a single fungal species with which a plant associates (Hart & Reader 2002a) because it is easily quantified and reported in most studies. Although we acknowledge that the proportion of root colonized does not perfectly represent nutrient flux between partners (Li *et al.* 2008), we use it to represent the maximum nutrient transfer rate within the symbiosis. Using this assumption, we hypothesize benefit and cost curves based on fungal colonization of root tissue.

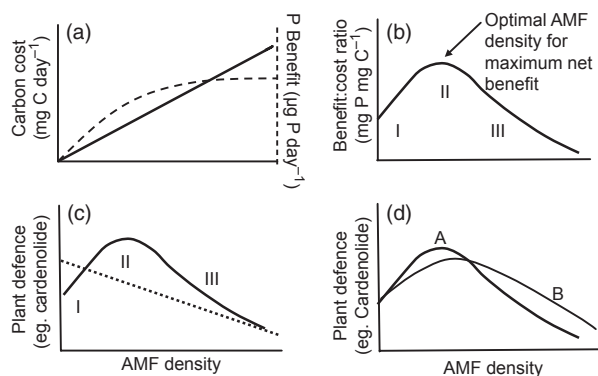


Fig. 1. Hypothesized relationships between increasing arbuscular mycorrhizal fungi (AMF) mutualist density and (a) carbon costs and nutrient benefits. Costs increase with increasing mutualist density, while benefits saturate. As a result (b) the benefit : cost ratio is nonlinearly related to mutualist density. Zone I represents limited fungal abundance and nutrient transfer, zone II represents optimal exchange with mutualistic fungi and maximal nutrient benefits, and zone III represents fungal parasitism, where carbon costs exceed nutrient benefits. The benefit : cost ratio translates directly to the (c) expression of plant defences predicted by our model (solid), in comparison to CNB (dotted). (d) The shapes of the phenotypic response curves to fungal abundance vary among plant genotypes (A and B).

As mycorrhizal fungal abundance in the environment increases and plants increasingly associate with these fungi, mycorrhizal interactions and nutrient exchange between plants and fungi increases; however, the carbon cost associated with hosting fungi also increases (Fig 1a), owing mainly to the construction and maintenance costs of fungal tissue (Douds, Johnson & Koch 1988; Peng *et al.* 1993). Some plants have developed adaptations to limit the extent of root colonization to prevent parasitism (Koide & Schreiner 1992), while others are unable to limit fungal colonization and exhibit growth depressions (Klironomos 2003).

We use the ratio of the nutrient benefit to gross carbon cost afforded by the mycorrhiza to represent the net effect of the mycorrhizal symbiosis (Fig. 1b). At low fungal densities, nutrient return for C investment is high, and increased photosynthetic capability can allow plants to keep up with or overcompensate for the C cost of the fungi (Kaschuk *et al.* 2009). However, at high colonization density and fungal abundance, carbon costs of fungal tissue construction and respiration can exceed P benefits (Douds, Johnson & Koch 1988) and result in net parasitism (Johnson, Graham & Smith 1997). As a result, the benefit : cost ratio curve (Fig. 1b) suggests that benefits obtained from mycorrhizal fungi are maximized at intermediate colonization densities, where carbon costs are balanced by nutrient gains associated with the mycorrhiza. Optimal colonization density will depend on plant and fungal identity, as well as abiotic context. What then are the consequences for defence?

Specifically, the resource exchange model predicts three zones of fungal abundance, nutrient transfer and associated zones of plant defence expression (Fig 1c). First, when plants are colonized by no or few fungal propagules, both growth and defence are limited by nutrient and carbon availability. Carbon costs associated with the symbiosis are low and balanced by any increase in nutrients transferred within the mycorrhiza. Within this zone, increasing nutrient acquisition should increase the expression of both growth and defence (Glynn *et al.* 2007).

The second zone of fungal abundance represents maximal C : nutrient exchange efficiency and an optimal association with soil mutualists (Zone II, Fig 1b,c). Within this range, photosynthetic rates are maximal, plants are co-limited by C and nutrients, and we predict that defence expression is also maximized (Fig 1c). High nutrient availability facilitates enzymatic synthesis of both carbon and nutrient-based defences (Gershenzon 1994), and precursor molecules are also predicted to be available. Within this zone, plant genotypes may vary in their relative allocation to growth and defence (Fig. 1d); however, both should be expressed maximally within any individual plant. In other words, we expect genetic trade-offs between growth and defence, but that individual-based trade-offs will not be manifest in this zone. Co-evolved plant–fungal symbioses at equilibrium are predicted to function primarily in zone II (Johnson *et al.* 2010).

The third zone represents fungal parasitism. Arbuscular mycorrhizal fungi, as obligate symbionts, must acquire carbon from plants in order to grow and reproduce, and

although some plants can decrease allocation to AMF, others are unable to limit the extent of infection (Koide & Schreiner 1992; Johnson, Graham & Smith 1997). As a result, plants can exhibit growth depressions associated with supporting the construction and maintenance costs of a large amount of mycorrhizal fungi (Peng *et al.* 1993). We predict that at high levels of fungal colonization, the expression of defences, and potentially plant growth, will decline (Fig 1c) due to a reduction in C available for the construction of primary and secondary metabolites.

PREDICTIONS

From the conceptual model presented above, the following predictions can be made regarding the expression of defence. First, the relationship between defence expression and fungal colonization will be nonlinear, increasing to a local maximum, and decreasing at high fungal abundance. The shape of this relationship should hold both for plant growth and defence, as plants that are exchanging nutrients at an optimal rate will grow and defend maximally. However, we expect the expression of defence to decline earlier than any decline in growth at high levels of AMF colonization (Herms & Mattson 1992; Glynn *et al.* 2007).

Secondly, since nutrient benefits conveyed by mycorrhizal symbioses are contingent upon abiotic nutrient availability (Johnson, Graham & Smith 1997), the shape of the cost and benefit curves will depend on soil fertility. Specifically, plants that can access sufficient N and P without AMF will experience only a C cost to hosting mycorrhizal fungi, and therefore experience parasitism at most levels of colonization by mycorrhizal symbionts. We predict that increasing environmental P availability will diminish the benefits gained through mycorrhizal fungi, and as a result, decrease the ideal AMF abundance. In addition, the trade balance model of AMF functioning (Johnson 2010) predicts that the costs and P benefits of association with AMF are dependent on N availability. With sufficient N, rates of photosynthesis compensate for the C cost of AMF, and plants are more likely to exhibit positive growth responses to elevated P. The benefit : cost curves in Fig. 1 may be extended to a plane with two or more nutrients to represent the interactions among these resources (see ‘Discussion’ section for integration with the GDBH). What are the consequences for defence? In high nutrient environments, plants are not likely limited by nutrient availability, but fungal parasitism may limit the C available for defence expression in those plants unable to control C flow to fungi. In contrast, plants growing in nutrient-poor environments may rely heavily on mycorrhizal fungi, and may not experience fungal parasitism. Plant defence expression in these plants would be positively correlated with fungal colonization and nutrient benefits.

The REMPDP was developed for plants hosting a single species of mycorrhizal fungus, but fungal species vary in nutrient gathering ability and carbon demand (Hart & Reader 2002b). The balance of nutrients conveyed and the carbon required to support the construction of a hyphal network determine the net benefit of the interaction. In reality,

plants are associated with multiple species of fungi (Opik *et al.* 2006), which may access a greater range of nutrients than a single fungal species (Koide 2000; Jansa, Smith & Smith 2008). As a result, the slope and maximum of the nutrient benefit curve may increase; however, the costs to hosting multiple fungi may also be greater. Plant defence expression will still be determined by the net benefit : cost ratio curve.

An initial test of the resource exchange model of plant defence

STUDY SYSTEM

As an initial test of the REMPDP, we inoculated *Asclepias syriaca* L. (common milkweed) plants with a series of mycorrhizal fungal soil treatments. *A. syriaca* is a perennial herb that grows throughout eastern North America and is associated with mycorrhizal fungi throughout its range (Landis, Gargas & Givnish 2004). *A. syriaca* is attacked by a variety of insect herbivores and expresses traits that deter damage by herbivores or reduce herbivore growth and reproduction (Dussourd & Hoyle 2000; Zalucki *et al.* 2001; Agrawal 2005). Cardenolides, toxic, bitter-tasting steroids, can decrease the survival and performance of the specialist herbivore *Danaus plexippus* (Zalucki *et al.* 2001). Latex, a sticky polyisoprene polymer that contains cardenolides and other compounds, is stored within pressurized laticifers and can engulf small herbivores and inhibit the feeding of larger ones (Zalucki & Malcolm 1999; Zalucki *et al.* 2001). Trichomes, produced on the upper and lower lamina and leaf veins of *A. syriaca*, may inhibit feeding by herbivores (Levin 1973). These defensive traits are primarily composed of carbon, but synthesis of such compounds and structures requires nutrient-rich enzymes (Gershenson 1994). While *A. syriaca* does not require mycorrhizal fungi for growth, plants at our field site are associated with AMF in colonization levels ranging from 10% to 80% root length colonized (authors' unpublished data). Mycorrhizal fungal species *Glomus etunicatum* and *Scutellospora pellucida* associate with *A. syriaca* at our field site.

MATERIALS AND METHODS

To investigate the effect of mutualist abundance on the expression of plant defences, we manipulated the density of mycorrhizal fungi available to milkweed clones. We delineated five genets of *A. syriaca* growing in a natural population in northern Michigan (Pellston, MI, USA) based on morphological, phenological and chemical similarity. Clonal structure at this site has since been verified using microsatellite markers (Kabat, Dick & Hunter 2010). Rhizomes of *A. syriaca* were unearthed, bleached in 5% bleach solution, and freed from all fine roots. This process removes mycorrhizal fungi from *A. syriaca* roots. Rhizomes were then overwintered at 3 °C in a refrigerator. Cultures of *Glomus etunicatum* (MI210B) and *Scutellospora pellucida* (NC118), were obtained from INVAM and cultured on *Sorghum* roots to obtain sufficient inoculum. In spring, rhizomes were cut into 5 cm pieces containing meristem buds and were planted into fungal density treatments. Rhizome biomass was recorded and did not differ among

fungal treatments (ANOVA for *S. pellucida*: $F_{1,87} = 0.08$, $P = 0.77$, and *G. etunicatum*: $F_{1,145} = 0.78$, $P = 0.37$). Conical Deepots™ (Steuwe and Sons Inc., Tangent, OR, USA), with a diameter of 6.4 cm and depth of 25 cm, were filled with 600 mL 1 : 1 autoclaved Sunshine Metromix : sand including mycorrhizal fungal inoculum which contained spores, hyphae, and colonized sorghum root pieces, in 9 dilutions ranging from 150 to 4 mL mixed inoculum/pot. These inoculation densities were determined from an initial trial with *A. syriaca* in order to generate a wide range of colonization densities. Arbuscular mycorrhizal fungi (AMF) density treatments were established separately with *Glomus etunicatum* and *Scutellospora pellucida* species. Due to some plant mortality, sample sizes varied among treatments (*G. etunicatum* $n = 9-22$, *S. pellucida* $n = 4-17$ per fungal density) and clones were pooled to provide replicates of AMF treatments. Rhizome pieces were planted in inoculated soil, maintained in a greenhouse and watered daily.

HARVEST AND ANALYSIS OF PLANT TRAITS

At the end of 4 months, plants were destructively harvested, foliar defence levels were assessed and above- and below-ground biomass measured. Five hole punches (424 mm²) of fresh leaf tissue were taken from one half of the two largest leaf pairs on each plant, placed immediately into 1 mL of methanol and stored at -10 °C for cardenolide analysis (below). Five identical leaf discs were taken from the opposite half of the leaf pairs and stored in glassine envelopes to provide estimates of sample dry mass and measures of other leaf traits (below). Latex that flowed from the first five holes punched was collected on a pre-weighed cellulose disc (1 cm diameter), dried and weighed. Trichomes on the lower surface of the leaf were counted under a dissecting microscope. Plant chemical defences were assessed following established protocols (Zehnder & Hunter 2007). Briefly, cardenolides were separated and quantified by extracting plant material in methanol. Samples were run on a HPLC (Waters Inc., Milford, MA, USA) with digitoxin as an internal standard, and peaks with symmetrical absorbance between 218 and 222 nm were quantified as cardenolides. Total cardenolides were calculated as the sum of individual peaks.

A subset (*c.* 0.5 g) of fresh fine root tissue was sampled from each plant, cleared with 10% KOH for 10 min, acidified using 2% HCl and stained in 0.05% trypan blue in 1 : 1 : 1 water : glycerine : lactic acid. Roots were mounted on slides and scored using the magnified gridline intersect method (McGonigle *et al.* 1990). A site was considered colonized if AM hyphae, arbuscules, or vesicles were present. Non-AMF hyphae were also detected at low levels (<0.05%), and occurrence did not differ among treatments.

Above- and below-ground plant tissues were collected, dried and weighed; total biomass was calculated from dry mass plus estimates of tissue removed for cardenolide and root analysis.

STATISTICAL ANALYSIS

The resource exchange model of plant defence (REMPDP) predicts that plant defences will respond nonlinearly to changes in AMF colonization (Fig. 1c). We therefore examined a series of linear and nonlinear model fits to the plant traits measured during our experiments (Motulsky & Ransnas 1987). We fit linear, quadratic, Michaelis–Menten, and negative exponential functions to relationships between defence traits and AMF density using the stats package in R v. 2.11.0 (Team 2010). The first three models were fit to increasing or null relationships, but only linear and exponential decay

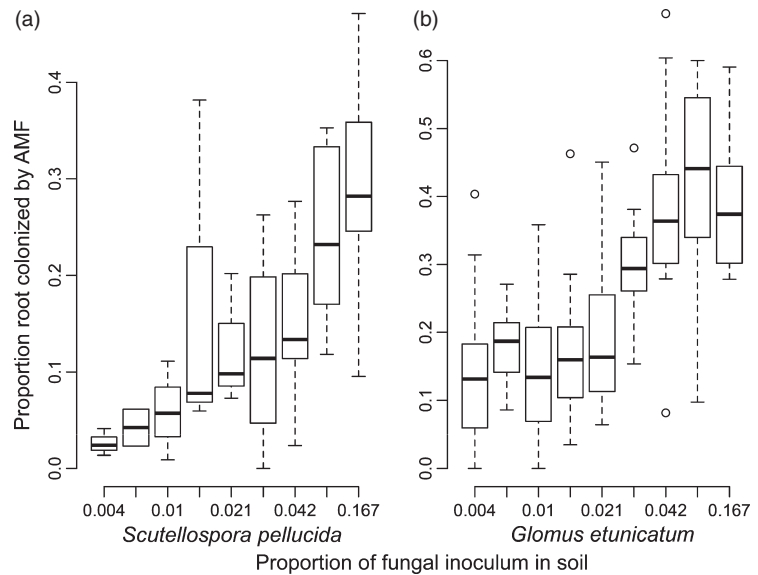


Fig. 2. Box and whiskers plot of *Asclepias syriaca* root tissue colonized by (a) *Scutellospora pellucida* and (b) *Glomus etunicatum* in response to experimental inoculum manipulation.

functions were fit to decreasing relationships in order to limit regressions to hypothesized and biologically realistic relationships. Mean trait values at each colonization density were weighted by variance⁻¹ in the trait value and fit to either a linear ($y = a + bx$), quadratic ($y = a + bx + cx^2$), Michaelis–Menten ($y = ax/(k + x)$), or negative exponential ($y = ae^{-bx}$) model. Data were plotted and log-transformed if necessary to reduce heteroscedasticity. We used weighted regression (Sokal & Rohlf 1995), because fungal colonization followed neatly the treatments imposed (Fig. 2). Measures of model fit including AICc (McQuarrie & Tsai 1998), and adjusted R^2 were extracted from each model using package qpcR (Spiess & Ritz 2010). Adjusted R^2 was calculated as $1 - (1 - R^2) n - 1/(n - p - 1)$, where n = sample size and p is the total number of regressors. R^2 , defined broadly, was calculated for all models as 1-residual sums of squares/total sums of squares. Model selection was performed using AICc; models with the lowest AICc are presented in the results and the results of all model fits are presented in Appendices S1 and S2 in Supporting Information.

Additionally, we assessed correlations among plant biomass and defence traits among all plants from all treatments using Pearson product–moment correlations using the stats package in R v. 2.11.0.

Results

As is required to test REMPDP, we succeeded in generating a wide range of AMF colonization densities on *A. syriaca* plants for both fungal species (Fig 2a,b). *Glomus etunicatum* colonized *A. syriaca* root length to a greater extent than did *S. pellucida*, with maxima of 45% and 28% root length colonized, respectively. Proportion root length colonized by arbuscules was correlated with total mycorrhizal colonization ($F_{1,127} = 360.8, P < 0.0001, R^2 = 0.73$). Plant growth and defence traits varied in the shapes of their responses to AMF abundance, from linear through saturating to quadratic. The statistics underlying model fits are provided in detail in Tables 1 and 2 and in Appendices S1 and S2. We report general trends and refer back to the tables for statistical support.

As predicted by REMPDP, *A. syriaca* biomass responded nonlinearly to colonization by *S. pellucida* (Fig. 3a, Table 1), increasing at low to mid fungal abundance, and decreasing at high fungal abundance. In contrast, the relationship between *A. syriaca* biomass and colonization by *G. etunicatum* was best represented by an exponential decay model, as if *G. etunicatum*

Table 1. Best-fit regression models and adjusted R^2 values for effects of *Scutellospora pellucida* on plant traits

Trait	Best-fit model	Adj R^2
Plant biomass	Quadratic*	0.58
Foliar cardenolides	Quadratic*	0.54
Latex	Quadratic*	0.64
Trichomes	Linear	0.21

* $P < 0.05$, ** $P < 0.01$.

Best-fit models were selected using AICc from weighted linear, quadratic, negative exponential and Michaelis–Menten regression analyses of the effects of *Scutellospora pellucida* colonization on *Asclepias syriaca* growth and defence traits. All analyses were performed in R (v. 2.11).

Table 2. Best-fit regression models and adjusted R^2 values for effects of *Glomus etunicatum* on plant traits

Trait	Best-fit model	Adj R^2
Plant biomass	Negative exponential**	0.76
Foliar cardenolides	Linear	0
Latex	Negative exponential	0.15
Trichomes	Linear	0

* $P < 0.05$, ** $P < 0.01$.

Best-fit models were selected using AICc from weighted linear, quadratic, negative exponential and Michaelis–Menten regression analyses of the effects of *Glomus etunicatum* colonization on *Asclepias syriaca* growth and defence traits. All analyses were performed in R (v. 2.11).

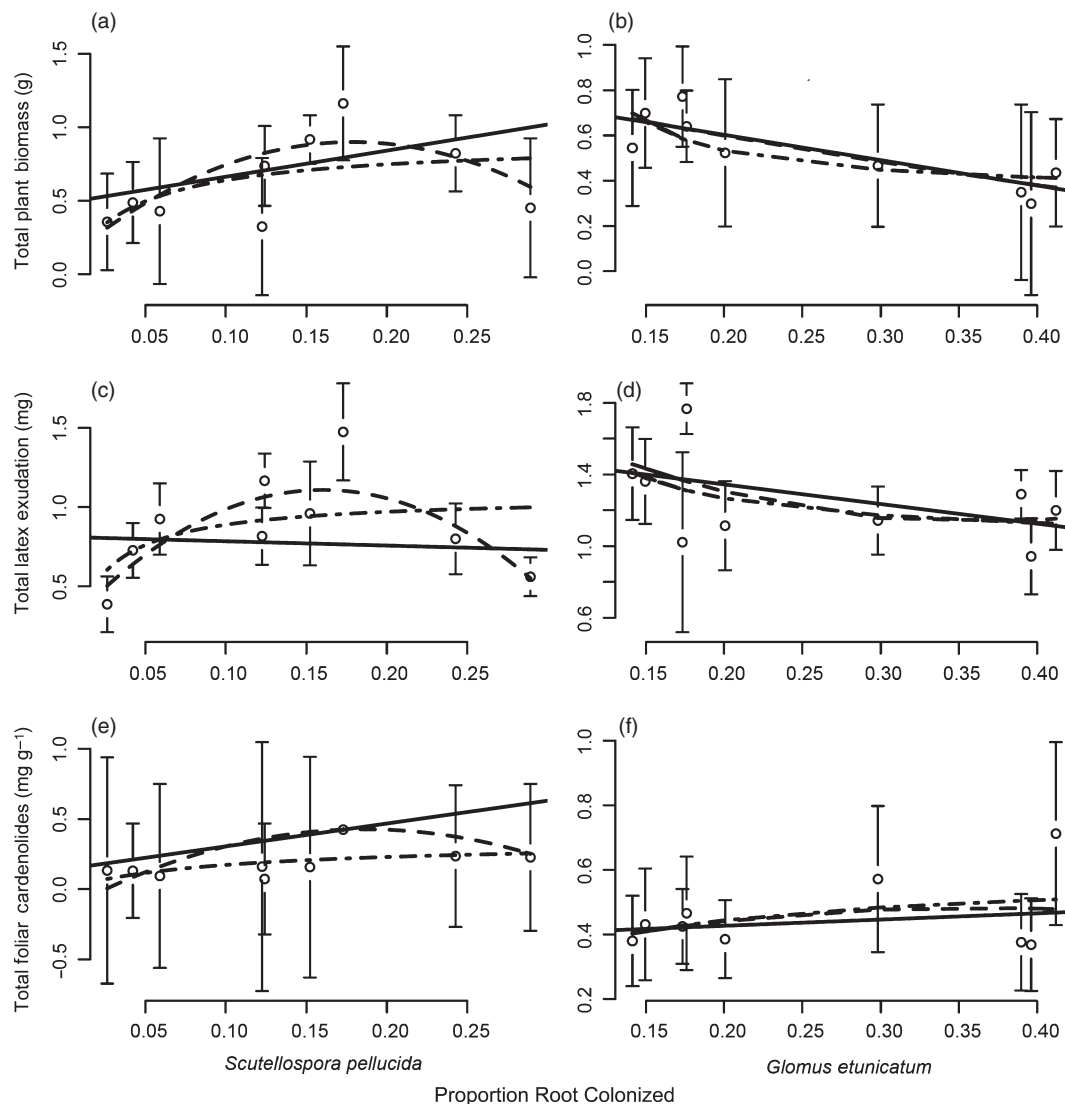


Fig. 3. Expression of *Asclepias syriaca* defensive traits when grown under experimental manipulation of fungal inoculum density. The left column illustrates responses to colonization by *Scutellospora pellucida*, while the right column illustrates responses to colonization by *Glomus etunicatum* inoculum. Solid lines represent the best-fit linear regression model, dashed lines represent the best-fit quadratic regression model, while dotted and dashed lines represent the nonlinear best fit Michaelis–Menten or negative exponential regression model. Trait means ± 1 SE represented are (a, b) plant biomass, (c, d) latex exudation, and (e, f) total foliar cardenolide concentration.

was acting only as a parasite (Fig. 3b, Table 2). As predicted by REMP, the relationship between latex exudation by *A. syriaca* and colonization by *S. pellucida* was best represented by a quadratic function (Fig. 3c, Table 1), maximized at intermediate levels of fungal colonization. The expression of foliar cardenolides was also best represented by a quadratic function (Fig. 3e), maximized at intermediate *S. pellucida* density (Table 1). In contrast, colonization by *G. etunicatum* tended to decrease latex exudation, best represented by an exponential decay model (Fig. 3d, Table 2). *Glomus etunicatum* did not affect cardenolide expression in *A. syriaca* (Fig. 3f, Table 2). Trichome density was not statistically related to the abundance of either fungal species (Tables 1 and 2), but tended to increase in response to colonization by *S. pellucida* (Table 1).

Analysis of Pearson correlations revealed that all defence traits measured, including latex exudation, foliar cardenolide

expression and trichome density, were positively correlated with plant biomass (Table 3), as predicted by REMP. Cardenolide concentration was negatively correlated with trichome

Table 3. Pearson product–moment correlations between plant biomass and the expression of various defence traits in *Asclepias syriaca*, $n = 234$

Plant trait	Latex	Cardenolides	Trichomes
Plant biomass	0.328***	0.134†	0.1423*
Latex	1	0.0017	0.0503
Cardenolides		1	-0.174*
Trichomes			1

† $P < 0.10$, * $P < 0.05$, *** $P < 0.001$.

density, while other relationships among defence traits were not statistically significant (Table 3).

Discussion

Our model incorporates ecologically realistic nutrient exchange dynamics between plants and soil mutualists to generate novel predictions regarding the expression of defence. An initial test of the model provides good support for REMPDP with the fungal species *S. pellucida*, but results contrary to expectations with the fungal species *G. etunicatum*. We detected quadratic responses in *A. syriaca* biomass, latex exudation and cardenolide expression in response to colonization by *S. pellucida* (Fig. 3a,c,e), as predicted by REMPDP (Fig. 1c). In contrast, increasing colonization by *G. etunicatum* led to exponential declines in both plant biomass and latex exudation. The quadratic relationships predicted by our model are based on the assumption that soil symbionts act as mutualists over some range of colonization densities; in this case, *G. etunicatum* appears to be acting only as a parasite. As a consequence, we should expect to see only the 'right-hand side' of Fig. 1c expressed. Both growth (Fig. 3b) and latex defence (Fig. 3d) declined with *G. etunicatum* colonization, suggesting that the increasing carbon cost associated with hosting *G. etunicatum* seems to have outweighed any nutrient benefits received from the interaction. The different plant responses to the two fungal species were likely due to intrinsic differences in the biology of the fungi. *Glomus* species tend to invest heavily in intraradical structures and relatively little outside the root and as a result tend to confer fewer nutrient benefits (Powell *et al.* 2009). In contrast, *Scutellospora* species often display lower rates of root colonization but more extensive extraradical hyphal growth (Hart & Reader 2002c), and tend to increase plant growth. These differences in fungal biology were reflected in the plant phenotypic response. Fungal life-history and allocation patterns may aid predictions of the effects of other fungal species on plant growth and defence expression.

The positive correlations between plant biomass and defence traits also support REMPDP. Although cardenolides, latex and trichomes are all composed primarily of carbon, the benefits associated with *S. pellucida* colonization at intermediate densities allowed for increased allocation to both growth and defence. In consistent fashion, parasitism by *G. etunicatum* decreased resource availability for allocation to both growth and latex defence. Overall, these results suggest that allocation to growth and defence are coupled, as the model predicts. However, defence traits were not uniformly correlated with one another and some defence traits may receive preferential allocation over others. We recognize that 'defence' is not a univariate trait and suites of traits may co-occur or trade-off (Rasmann & Agrawal 2009). Specifically, resistance and tolerance (Vandermeijden, Wijn & Verkaar 1988), as well as constitutive and induced resistance (Karban & Baldwin 1997) should be included in the broad definition of defence. They should be quantified in future work to construct a complete description of *A. syriaca* defence across a range of AMF densities. Overall, we predict that defence viewed and quantified

broadly will respond nonlinearly to fungal colonization and resource exchange.

Additional variation in our results may be due to multiple plant genotypes used in our experiment. Previous work has demonstrated that *A. syriaca* genotypes vary in the expression of growth and defence traits (Agrawal 2005; Vannette & Hunter 2010). Genotypic differences in allocation patterns and nutrient requirements may interact with fungal nutrient exchange dynamics to shift the shape of plant response to fungal colonization (Fig. 1d) (Garrido *et al.* 2010). Future experiments will allow us to partition variation in defence among effects of plant genotype, fungal colonization and their interaction.

Additional support for our model can be found in previous research that documents the effects of fungal density on plant phenotype. Gange & Ayres (1999) proposed that the increasing costs and diminishing benefits conveyed by mycorrhizal fungi would result in a nonlinear response of plant biomass to fungal abundance. They describe numerous examples where plant 'benefit' was nonlinearly related to arbuscular colonization intensity. More recently, Garrido *et al.* (2010) manipulated the density of mycorrhizal fungi within the roots of *Datura stramonium* (jimson weed), and found a curvilinear response – increasing, then decreasing – of root mass, seed production and leaf area to increasing fungal colonization. However, the tolerance of jimson weed to herbivory decreased with increasing mycorrhizal colonization. Although plant tolerance of simulated herbivory did not seem to follow our predicted pattern, we suggest that unmeasured plant resistance traits may respond in kind with root biomass and reproduction. It is a combination of tolerance and resistance traits that define the defensive strategy of plants; this combination should follow the predictions of REMPDP.

SYNTHESIS

While previous plant defence theory has ignored the role of soil mutualists, these symbionts play a crucial role in mediating nutrient acquisition for the majority of plants (Smith & Read 2008). The identity and abundance of soil symbionts vary, and accordingly, alter nutrient exchange with plants. Although AMF are an integral part of roots, the benefit : cost ratio of the association can change dramatically depending on plant and fungal genotypes and environmental resource availability (Johnson, Graham & Smith 1997; Hoeksema *et al.* 2010). As a consequence, AMF can act parasitically (Johnson, Graham & Smith 1997), a condition not accounted for in models of plant defence based only on optimal allocation models.

The model we develop here (REMPDP) offers both complementary and novel predictions when compared with previous theories of plant defence. In order to facilitate a comparison of REMPDP to GDBH (Herms & Mattson 1992), we present both models independently in Fig. 4 (note the difference in the *x*-axis) and their integration in Fig. 5. Both models predict a nonlinear response in plant defence to environmental variation that is ultimately linked to internal nutrient availability (Fig. 4). However, the models differ

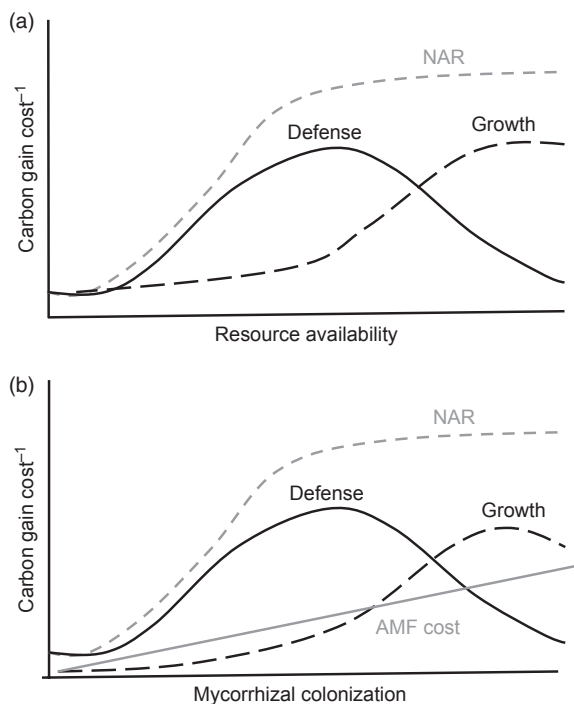


Fig. 4. Comparison of (a) the growth differentiation balance hypothesis (GDBH, after Herms & Mattson 1992) and (b) resource exchange model of plant defence (REMPD). Note the different x -axes in the figures. In (b) mycorrhizal colonization is assumed to increase plant internal nutrient availability and increase net assimilation rate (NAR). REMPD predicts that increasing arbuscular mycorrhizal (AM) costs will decrease defence expression, and decrease plant growth at high colonization levels.

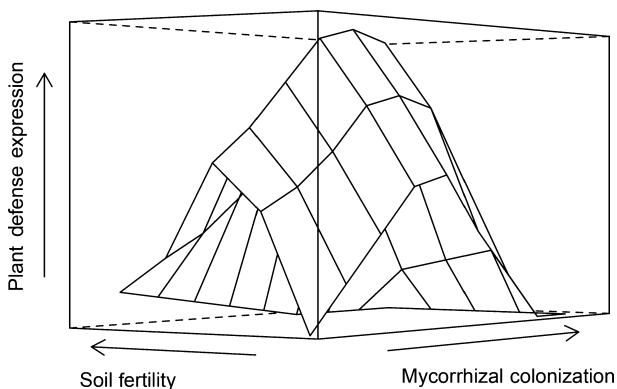


Fig. 5. An integration of the Growth Differentiation Balance Hypothesis with the Resource Exchange Model of Plant Defence. Soil fertility alters the benefits associated with mycorrhizal fungal colonization and the subsequent effects on defence expression. When soil fertility is very high, mycorrhizal fungi act only as parasites, and increasing mycorrhizal costs result in declines in defence expression. When mycorrhizal colonization is very high, defence expression is insensitive to variation in soil fertility.

in two specific ways. First, two different mechanisms may account for decreasing defence expression: a resource-based trade-off between growth and defence, as posited by GDBH (Fig. 4a), or the increasing resource demands of soil micro-

bial symbionts (REMPD). Secondly, REMPD predicts that plant growth rate may also decline at fungal abundance, as a result of the increasing cost of root symbionts. Our results (Fig 3a, b), as well as those from other studies (Gange & Ayres 1999), confirm that plant biomass can decline with increasing fungal colonization.

Additionally, the costs and benefits of fungal colonization may be altered by soil fertility (Hoeksema *et al.* 2010), especially in plants unable to limit fungal colonization at high nutrient availability. To facilitate predictions of the integrative effects of symbiotic exchange and varying environmental nutrient availability on defence expression (Kleczewski, Herms & Bonello 2010), we constructed a response surface to illustrate plant defence as a function of environmental nutrient availability and fungal abundance (Fig. 5), using the following assumptions: (i) When resource availability is very low, the effect of AMF on defence should be quadratic (this is our basic model). (ii) Likewise, when AMF density is very low, the effect of resources on defence should be quadratic (this is the prediction from GDBH). (iii) When resource availability is high, and nutrient gain is therefore already saturated, the only effect of AMF on plants is carbon parasitism and defences should decline with increasing AMF (Fig. 5). (iv) When AMF density is high, nutrient gain has already saturated, and increasing resources will have no effect on defence (Fig. 5). Therefore, one important difference between the predictions of our combined model and that of GDBH is that we predict that defence expression will be insensitive to soil fertility at the highest levels of fungal colonization. In Fig. 5, we extend our model predictions to a single dimension of soil fertility but acknowledge that extending the model to consider multiple soil nutrients would also be valuable (Johnson 2010).

In summary, REMPD proposes that positive feedbacks mediated by ecological interactions between nutrient and C availability can increase the availability of precursor compounds and enzymes available for growth and the synthesis of defence (Gershenzon 1994), and increase allocation to both demands (Bennett, Alers-Garcia & Bever 2006). Both our initial experiment and data from previous work in mycorrhizal systems support the potential for the resource exchange mechanism as a useful framework for understanding plant defence expression and tritrophic interactions. In addition, the model makes novel predictions about the ecological costs that may limit defence expression and it offers insight into the interactions among resources that control defence expression. Further experimental tests of REMPD will determine the generality of the cost : benefit approach and its effects on plant defence expression.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Weighted regression of root colonization by mycorrhizal fungus *Scutellospora pellucida* on the expression of *Asclepias syriaca* growth and defense traits. All analyses were performed in R (v. 2.11).

Appendix S2. Weighted regression of root colonization by mycorrhizal fungus *Glomus etunicatum* on the expression of *Asclepias syriaca* growth and defense traits. All analyses were performed in R (v. 2.11).

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