Oecologia (1997) 112:534–542 

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# A fungal root symbiont modifies plant resistance to an insect herbivore

Received: 28 February 1997 / Accepted: 23 June 1997

**Abstract** Vesicular-arbuscular mycorrhizal (VAM) fungi are common root-colonizing symbionts that affect nutrient uptake by plants and can alter plant susceptibility to herbivores. I conducted a factorial experiment to test the hypotheses that colonization by VAM fungi (1) improves soybean (*Glycine max*) tolerance to grazing by folivorous Mexican bean beetle (*Epilachna varivestis*), and (2) indirectly affects herbivores by increasing host resistance. Soybean seedlings were inoculated with the VAM fungus *Glomus etunicatum* or VAM-free filtrate and fertilized with high-[P] or low-[P] fertilizer. After plants had grown for 7 weeks first-instar beetle larvae were placed on bagged leaves. Growth of soybean was little affected by grazing larvae, and no effects of treatments on tolerance of soybeans to herbivores were evident. Colonization by VAM fungus doubled the size of phosphorus-stressed plants but these plants were still half the size of plants given adequate phosphorus. High-[P] fertilizer increased levels of phosphorus and soluble carbohydrates, and decreased levels of soluble proteins in leaves of grazed plants. Colonization of grazed plants by VAM fungus had no significant effect on plant soluble carbohydrates, but increased concentration of phosphorus and decreased levels of proteins in phosphorus-stressed plants to concentrations similar to those of plants given adequate phosphorus. Mexican bean beetle mass at pupation, pupation rate, and survival to eclosion were greatest for beetles reared on phosphorus-stressed, VAM-colonized plants, refuting the hypothesis that VAM colonization improves host plant resistance. VAM colonization indirectly affected performance of Mexician bean beetle larvae by improving growth and nutrition of the host plant.

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**Key words** Plant nutrition · Vesicular-arbuscular mycorrhizal fungus · *Glycine max* · *Epilachna varivestis* · Indirect effects

## Introduction

Environmental factors such as nutrient availability lead to considerable intraspecific variation among plants in susceptibility to herbivory. Nutrient limitation may alter the pattern of allocation to defense (Bryant et al. 1983; Wilkens et al. 1996), affect the nutritional value of foliage as food for herbivores (Bentz et al. 1995; Holopainen et al. 1995), or affect growth of a grazed plant (Fay et al. 1996). Vesicular-arbuscular mycorrhizal (VAM) fungi are common rhizosphere microorganisms that influence the availability of limiting nutrients for terrestrial plants and thus may affect susceptibility to herbivory (Gange and West 1994; Gehring and Whitham 1994). The intimate associations formed by VAM fungi and roots increase uptake of nutrients, particularly phosphorus, by hosts growing in nutrient-limited soil (Bolan 1991). Depending upon environmental conditions, colonization can alter the mineral and lipid composition of host tissue (Pacovsky et al. 1986; Pacovsky and Fuller 1988), increase the rate of photosynthesis (Paul and Kucey 1981; Harris and Paul 1987), and affect investment in growth and reproduction (Bethlenfalvay et al. 1982; Lu and Koide 1994).

By altering growth and nutrition of hosts, VAM colonization can affect susceptibility of host plants to enemies such as herbivores. Because well-nourished plants tend to grow better under stress than do poorly nourished plants (Maschinski and Whitham 1989), improved nutrition resulting from VAM colonization may enhance the plant's ability to sustain damage without a decrease in productivity (= tolerance). In greenhouse studies VAM colonization commonly improves tolerance of crops to other forms of biotic stress, such as nematodes (Hussey and Roncadori 1982; Calvet et al. 1995). For example, soybeans colonized by *Glomus*

*fasciculatum* can support greater numbers of nematodes and still produce more biomass than nonmycorrhizal plants (Francl and Dropkin 1985). Greater tolerance of mycorrhizal plants to fungal pathogens has also been demonstrated (Davis and Menge 1980).

As symbionts of plants, VAM fungi can indirectly affect herbivores by reducing acceptability of the plant tissue, altering the nutritional quality, or increasing concentrations or types of defense compounds in the plant tissue (Gange and West 1994), i.e., VAM fungi may alter host resistance. These effects of mycorrhizal colonization may result from improved uptake of limiting nutrients or from induced resistance that follows colonization of roots by fungal hyphae. There is mounting evidence that both mechanisms operate in resistance to fungal pathogens (e.g., Newsham et al. 1995; Matsubara et al. 1995) and nematodes (e.g., Smith et al. 1986; Cooper and Grandison 1986, 1987; Carling et al. 1989). Experiments testing effects of VAM colonization on herbivore performance are few and have not distinguished between nutritional and induced effects. Nonetheless, studies of leaf-chewing caterpillars (Rabin and Pacovsky 1985; Gange and West 1994) and root-feeding weevil larvae (Gange et al. 1994) point towards some form of increased resistance with VAM colonization.

Although VAM colonization frequently improves growth of plants growing in phosphorus-deficient soil, the host plant invests significant amounts of photosynthates in mycorrhizae. In soybeans, 8–17% of total photosynthates produced by the plant are devoted to the fungal component (Harris et al. 1985). Because of this greater drain on photosynthates, herbivory that reduces plant photosynthetic area may impose a greater burden on the carbon budget of mycorrhizal than nonmycorrhizal plants and make mycorrhizal plants less tolerant of herbivory, particulary if phosphorus is not limiting.

By improving the nutrient status of the host plant, VAM colonization may also improve the nutritional quality of plant tissue for herbivores. For example, if colonization increases nitrogen uptake or increases the level of soluble carbohydrates, insects may grow better on mycorrhizal plants (Gehring and Whitham 1994). Consequently, colonization by VAM fungi may indirectly exert a positive effect on herbivore performance.

I examined effects of VAM colonization and phosphorus level on tolerance and resistance of soybean, *Glycine max* (L.) Merr., to Mexican bean beetle, *Epilachna varivestis* Mulsant (Coleoptera: Coccinellidae). The Mexican bean beetle's host range is narrow and limited to some wild and cultivated species of legumes, including soybean (Turnipseed and Kogan 1976). Adults invade fields early in the season and generations may overlap much of the summer (Turnipseed and Kogan 1976). Larvae and adults feed on leaves by rasping the surface and sucking up juices. The scraped area dries, leaving numerous small windows in the leaves, bordered by the tissue that was scraped aside. Mexican bean beetles are sensitive to changes in host quality induced by previous damage (Lin and Kogan 1990) and cultivars are bred for constitutive resistance to this economically important pest.

Using a factorial design I tested the following hypotheses:

1. VAM colonization directly improves tolerance of soybeans to herbivory by improving the nutrient status of the host plant. *Prediction*: The effects of VAM will be limited to phosphorus-stressed plants. Under low-phosphorus conditions, mycorrhizal plants will be less affected by grazing than will nonmycorrhizal plants. Under high phosphorus conditions mycorrhizal and nonmycorrhizal plants will exhibit similar responses to grazing.

2. VAM colonization exerts an indirect, negative effect on insect herbivores by increasing host resistance. *Prediction*: If the effect of colonization on herbivores is due to improved nutrient uptake by the plant, VAM colonization will decrease insect performance only under low phosphorus conditions. *Prediction*: If penetration of host tissue by hyphae induces plant resistance, VAM colonization will decrease insect performance on both phosphorus-stressed and well-nourished plants.

## Methods

### Plants

The soybean cultivar "Williams 82", which is known to exhibit induced resistance (Lin and Kogan 1990), was used for this experiment. Germinated seeds were planted in pots containing approximately 1.25 l of autoclaved 1:1:1 sand:prairie topsoil:perlite. The soil in each VAM-inoculated pot was mixed with 50 ml of fine roots and soil from *Glomus etunicatum* (INVAM UT-316) stock pots containing sudangrass. Pots without VAM fungus received filtrate from a slurry of stock pot soil that had passed through a 43 μm sieve. Pots were then randomly positioned in a growth chamber and maintained on a 16:8 L:D schedule at 25°C. Seedlings were later thinned to one per pot, for a total of 44 plants.

For the first 3 weeks plants were fertilized three times a week and watered as needed with solutions buffered to a pH of 6.9. Plants assigned low-[P] treatment were watered to excess with (P-free) basal fertilizer and plants assigned the high-[P] treatment were watered with basal fertilizer + 0.4 mM $KH_2PO_4$ (Pacovsky and Fuller 1988). At 3 weeks many plants showed some brown speckling of leaves suggesting manganese toxicity. Because speckling was more severe and overall growth was poorer in low-[P] plants the fertilizer for this treatment was changed to include 0.04 mM $KH_2PO_4$, or 10% of high-[P] treatment. All plants were fertilized four times per week for the remainder of the experiment. On 20 May 1996, 7 weeks after germination, the plants were positioned randomly on a greenhouse bench and positions were rotated weekly. The number of trifoliate leaves on each plant was recorded to provide an index of the effects of VAM colonization and [P] fertilizer at the start of the grazing treatment. Insects were placed on 28 of the 44 plants (7 plants for each [P] fertilizer/VAM colonization combination), with the remaining plants serving as controls for the insect treatment. Natural light was supplemented with artificial light part of the day. After transfer to the greenhouse the plants began flowering and all had developing pods by harvest. The experiment was terminated approximately 12 weeks after germination.

Plant roots were sampled for percent colonization (percentage of roots sites inspected that had VAM fungus arbuscules), and roots, vegetative shoots, and pods were separated, oven-dried, and weighed. The masses were analyzed as variables in a multivariate analysis of variance (MANOVA, SAS 1987). Phosphorus fertilizer (low/high), VAM treatment (no/yes), and insect treatment (no/yes) were main effects. Bonferroni comparisons of least squares means from individual ANOVA's were made at experimentwise $\alpha = 0.05$ when an interaction was significant.

Phosphorus, soluble carbohydrate, and soluble protein concentrations of ground, dried leaves were analyzed for a randomly chosen subset of plants on which insects were reared. Leaves that abscised prior to harvest were not included. Phosphorus concentration was determined following procedures outlined by Murphy and Riley (1962). Soluble proteins were analyzed with the Bio-Rad reagent (BioRad Inc., Richmond, Calif., USA) using the method of Bradford (1976), with bovine serum albumin for the standard. Extracts were obtained by soaking leaf samples in water at room temperature for 4 h and centrifuging. Extracts were examined spectrophotometrically at 595 nm. Soluble carbohydrates were analyzed by the anthrone procedure (Roe 1955), which measures sugars and some polysaccharides. Extracts were obtained through hot water extraction (2 h) and filtered extracts were examined spectrophotometrically at 600 nm. Data were analyzed with a two-way fixed effects ANOVA.

### Insects

Of the 11 plants in each [P]-fertilizer/VAM colonization combination 4 were randomly assigned to be controls. These were bagged or caged without insects. The remaining 7 plants in each treatment were assigned to receive insects. Eggs of Mexican bean beetle were obtained from a colony at the Illinois Natural History Survey. First instar larvae were selected from four simultaneously-hatching egg clusters such that each egg cluster was represented on every plant. The number of larvae placed on a plant varied with leaf number. Plants with eight or fewer trifoliate leaves received four larvae. Larger plants received one additional larva per four leaves, up to nine larvae. Within a plant all larvae were placed on the oldest available trifoliate leaf and enclosed in a nylon bag. Several leaves that were checked on high [P] plants over the course of the first 3 days wilted as a result of petioles being crushed during re-bagging. If the larvae were active they were moved to a new leaf and one day was subtracted from the final count of number of days to pupation. If larvae were inactive they were all replaced by fresh larvae on a fresh leaf, and this was considered day 0 in the count of days to pupation. After larvae were large enough they were allowed to roam freely on their plants, which were bagged in toule or placed in a cage covered with plastic screen. These two techniques for enclosing plants were equally represented for each treatment. As each larva pupated it was weighed and isolated in a tripour beaker until adult eclosion, when sex could be determined. Two larvae in the high-[P] treatments (one on a VAM-free plant and one on a VAM-colonized plant) had not yet pupated when the experiment was terminated, 35 days after they had been placed on plants. These were considered dead.

The group of males or females on each plant constituted the experimental unit for statistical analysis of mass at and time to pupation. For males the mean mass at pupation and mean number of days to pupation were variables in a multivariate analysis of variance with VAM treatment and [P] fertilizer as main effects (GLM procedure, SAS 1987). For females median mass at pupation and mean days to pupation better fit the assumptions of normality and homogeneity of variance. Bonferroni comparisons of least squares means from individual ANOVA's were made at experimentwise $\alpha = 0.05$ when an interaction was significant.

During the course of the experiment some larvae died from causes unrelated to plant quality (e.g., drowned in a water droplet on a leaf, crushed during handling), and most of this mortality occurred in the first 3 days. I analyzed survival using a censored data set consisting of all larvae alive 4 days after the start of the experiment, minus any larvae that subsequently died due to accidents. Some larvae disappeared and could not be found. These larvae were assumed to have succumbed to natural causes and were included in the analysis. For the censored data set the proportion that survived to eclosion was analyzed with a two-way fixed effects ANOVA. A significant interaction was examined with Bonferroni comparisons of least squares means within treatments at experimentwise $\alpha = 0.05$.

## Results

### Plants

Several plants treated with VAM-free filtrate were colonized (2 low-[P]/+insects, 2 high-[P]/+insects, 1 low-[P]/−insects, 1 high-[P]/−insects). Data from these plants and insects on them were not included in analyses. At the time larvae were added, plants given high-[P] fertilizer had significantly more trifoliate leaves than low-[P] plants ($F = 63.64$, $P = 0.0001$; least squares means ($\pm$SE): high = 10.8 (0.4) leaves, low = 6.6 (0.4) leaves), but mycorrhizal plants did not differ from VAM-free plants ($F = 1.12$, $P = 0.2978$). At harvest all plants inoculated with *Glomus etunicatum* were colonized and plants treated with high-[P] fertilizer had generally lower levels of colonization (Fig. 1). However, pairwise comparisons of least squares means indicate that high- and low-[P] plants inoculated with VAM fungi differed in percent colonization only when plants were grazed by insects (Fig. 1).

Multivariate analysis indicated that insects did not significantly affect the pattern of plant growth (Table 1), indicating that proportional allocation to the various components of biomass was not affected by grazing. Although insect grazing was associated with

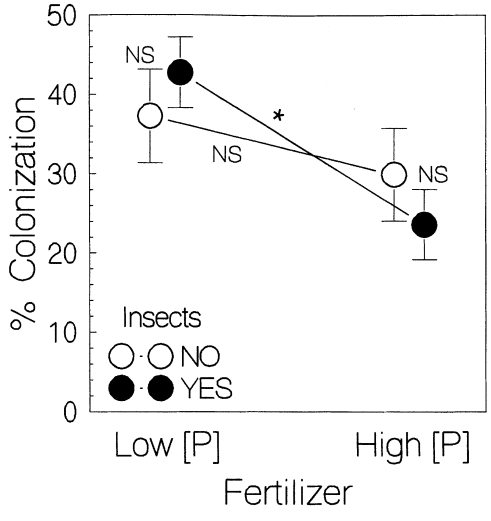


**Fig. 1** Mycorrhizal colonization of VAM-inoculated plants fertilized with low-[P] or high-[P] fertilizer and grazed or not grazed by Mexican bean beetle larvae; * indicates significant differences at experimentwise $\alpha = 0.05$ using Bonferroni comparisons within treatments. Shown are least squares means $\pm 2$ SE

slightly smaller root and shoot mass, no interactions of VAM and insects were significant (Table 1). VAM colonization neither increased, nor decreased plant tolerance to herbivory. Thus, hypothesis 1 was not supported.

The MANOVA indicated that VAM and phosphorus treatments affected biomass allocation and the multivariate effect was primarily due to effects on shoot mass (Table 1). All plants invested proportionally more in shoot mass than in root mass, and so factors that improved plant growth especially improved shoot growth. The multivariate VAM effect was due to differences in vegetative mass rather than pod mass (significant $F$ tests, Table 1). Significant interaction of VAM and phosphorus treatments indicated that the effect of VAM depended upon fertilizer treatment (Table 1). For plants given low-[P] fertilizer,VAM doubled the size of roots ($P = 0.0001$), shoots ($P = 0.0001$), and pods ($P = 0.0112$). VAM had no effect on plants given high-[$P$] fertilizer ($P > 0.05$ for all components) (Fig. 2). Plants given high-[P] fertilizer were twice the size of VAM-colonized, low-[P] plants ($P = 0.0001$ for all comparisons) (Fig. 2). Thus VAM improved growth for phosphorus-stressed plants but did not equal the positive effects of high-[P] fertilizer.

Phosphorus fertilizer treatment, VAM colonization, and the interaction of these factors influenced concentrations of nutrients in leaves (Table 2). Standardized canonical coefficients show that concentrations of phosphorus and soluble proteins were negatively correlated (Table 2). Treatments that increased phosphorus concentration decreased soluble proteins (Fig. 3A, B). Although growth of phosphorus-stressed, VAM colonized plants lagged behind that of plants given adequate fertilizer, phosphorus concentration in leaves did not differ among these treatments (Fig. 3A). However, VAM-free, phosphorus-stressed plants had significantly lower levels of foliar phosphorus (Fig. 3A). Mycorrhizal colonization reduced the concentration of soluble proteins in phosphorus-stressed plants to levels similar to concentrations in plants given adequate phosphorus (Fig. 3B). The concentration of soluble carbohydrates was significantly greater in leaves from plants given high-[P] fertilizer but was unaffected by VAM treatment (Table 2; Fig. 3C).

**Table 1** MANOVA and ANOVA results for three biomass components of VAM-free or VAM-colonized soybeans fertilized with low- or high-[P] fertilizer and grazed or not grazed by Mexican bean beetle larvae

| Source | *df* | *F* root (*P*) | *F* shoot (*P*) | *F* pods (*P*) | Pillai's trace (*P*) |
|---|---|---|---|---|---|
| [P] | 1 | 170.73 (0.0001) | 455.90 (0.0001) | 87.82 (0.0001) | 0.943 (0.0001) |
| VAM | 1 | 9.23 (0.0049) | 29.80 (0.0001) | 0.60 (0.4438) | 0.510 (0.0001) |
| [P] × VAM | 1 | 11.63 (0.0019) | 39.35 (0.0001) | 9.30 (0.0048) | 0.600 (0.0001) |
| Insect | 1 | 4.64 (0.0393) | 5.24 (0.0292) | 1.54 (0.2240) | 0.187 (0.1172) |
| [P] × Insect | 1 | 0.14 (0.7085) | 0.79 (0.3799) | 0.45 (0.5062) | 0.372 (0.7822) |
| VAM × Insect | 1 | 0.11 (0.7462) | 1.73 (0.1979) | 0.11 (0.7480) | 0.089 (0.4482) |
| [P] × VAM × Insect | 1 | 0.67 (0.4197) | 0.00 (0.9561) | 3.45 (0.0729) | 0.123 (0.2903) |
| Error | 30 | | | | |

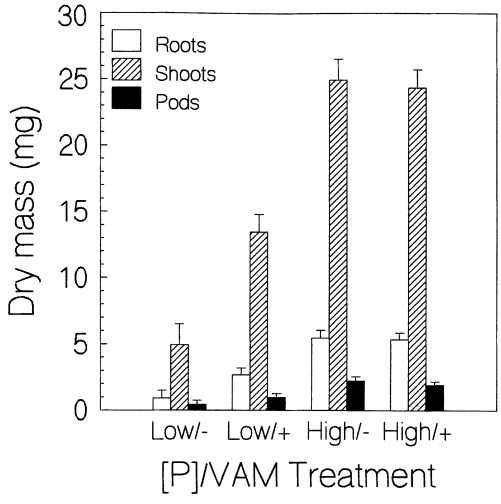


**Fig. 2** Dry mass of roots, shoots, and pods of VAM-free (–) or VAM-inoculated (+) plants fertilized with low-[P] or high-[P] fertilizer. Shown are least squares means ±2 SE

**Table 2** ANOVA results for concentration of phosphorus, soluble proteins, and soluble carbohydrates in leaves of VAM-free or VAM-colonized plants given low- or high-[P] fertilizer and fed to insects

| Source | *df* | *F* phosphorus | *F* proteins | *F* carbohydrates | Pillai's trace | Standardized canonical coefficients | | |
|---|---|---|---|---|---|---|---|---|
| | | (*P*) | (*P*) | (*P*) | (*P*) | Phosphorus | Proteins | Carbohydrates |
| [P] | 1 | 14.42 (0.0025) | 18.91 (0.0009) | 17.44 (0.0013) | 17.5070 (0.0003) | 1.2711 | −1.3451 | 0.5715 |
| VAM | 1 | 14.04 (0.0028) | 5.40 (0.0385) | 0.00 (0.9544) | 8.5757 (0.0041) | 1.7250 | −1.3325 | −0.2278 |
| [P] × VAM | 1 | 5.27 (0.0406) | 6.06 (0.0299) | 0.35 (0.5644) | 4.9957 (0.0227) | 1.5334 | −1.5227 | −0.0048 |
| Error | 12 | | | | | | | |

Insects

VAM treatment, phosphorus treatment, and their interaction were significant in multivariate analysis of both female and male performance (Tables 3 and 4). The magnitude of standardized canonical coefficients indicate that the multivariate effect was primarily due to effects on mass at pupation (Tables 3 and 4). VAM colonization significantly increased both these measures of larval performance but only when plants were grown with low-[P] fertilizer (Fig. 4). This result indicates that VAM colonization decreased resistance to herbivory in phosphorus-stressed plants. Mass and developmental rate of larvae on plants given high-[P] fertilizer was not affected by VAM colonization and did not differ from performance on VAM-free plants given low-[P] fertilizer (Fig. 4).

In the analysis of survival neither treatment yielded a significant main effect but the interaction of phosphorus and VAM treatments was significant ($F = 14.44$, $P = 0.0011$). VAM colonization improved survival of larvae on phosphorus-stressed plants but did not significantly affect larvae on plants given high-[P] fertilizer (Fig. 5). Thus, all three measures of insect performance refute hypothesis 2.

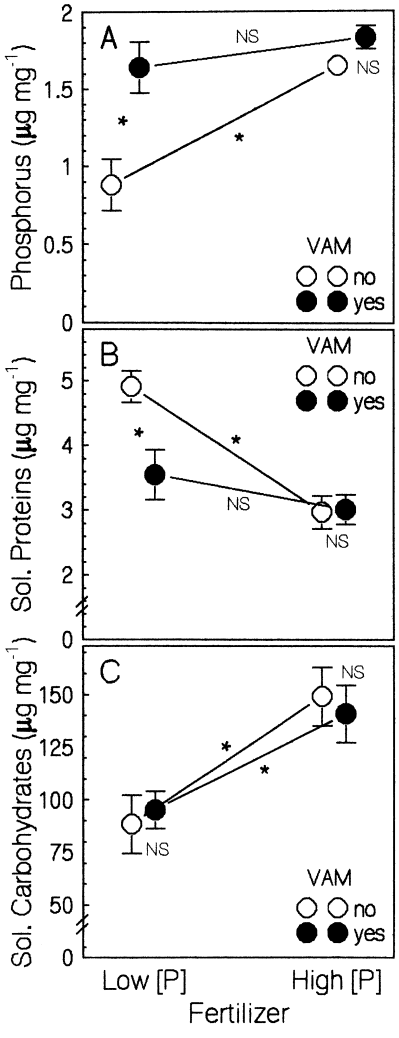


**Fig. 3** Concentrations of **A** phosphorus, **B** soluble proteins, and **C** soluble carbohydrates in leaves of plants fed to Mexican bean beetle larvae; * indicates significant differences at experimentwise $\alpha = 0.05$ using Bonferroni comparisons within treatments. Shown are means $\pm 1$ SE

## Discussion

The results of this experiment did not support the hypothesis that VAM colonization increases tolerance of plants to insect grazing. Colonization by VAM fungi improved growth of phosphorus-stressed plants and increased foliar phosphorus to concentrations similar to levels in leaves of high-[P] plants. However, there was no evidence that VAM colonization reduced the negative effects of grazing. Although many leaves were damaged by larvae, effects of grazing were marginal. Soybeans are generally tolerant of defoliation (Turnipseed and Kogan 1976) but the magnitude of effects depends upon the stage of development of the plant and the degree of defoliation. In my experiment defoliation occurred when pods were first forming and expanding, the stage of development when yield was most reduced by Mexican bean beetle grazing in a field test of "Williams 82" soybean (Nolting and Edwards 1989). However, effects on yield depend upon the duration and intensity of defoliation (Nolting and Edwards 1989). It is possible that VAM colonization alters tolerance of soybeans under very intense grazing or over a lifetime of growth and

**Table 3** MANOVA and ANOVA results for performance of female Mexican bean beetles reared on VAM-free or VAM-colonized plants given low- or high-[P] fertilizer

| Source | *df* | *F* mean pupation rate (*P*) | *F* median mass (*P*) | Pillai's trace (*P*) | Standardized canonical coefficients | |
|---|---|---|---|---|---|---|
| | | | | | Mean pupation rate | Median mass |
| [P] | 1 | 1.08 (0.3148) | 11.40 (0.0042) | 0.4437 (0.0165) | −0.3784 | 2.1611 |
| VAM | 1 | 9.06 (0.0088) | 5.60 (0.0318) | 0.4011 (0.0276) | 1.1621 | 0.7115 |
| [P] × VAM | 1 | 10.70 (0.0052) | 16.36 (0.0011) | 0.5514 (0.0037) | 0.5866 | 1.4679 |
| Error | 15 | | | | | |

**Table 4** MANOVA and ANOVA results for performance of male Mexican bean beetles reared on VAM-free or VAM-colonized plants given low- or high-[P] fertilizer

| Source | *df* | *F* mean pupation rate | *F* mean mass | Pillai's trace | Standardized canonical coefficients | |
|---|---|---|---|---|---|---|
| | | (*P*) | (*P*) | (*P*) | Mean pupation rate | Mean mass |
| [P] | 1 | 1.28 (0.2751) | 26.94 (0.0001) | 0.7048 (0.0002) | −0.9654 | 3.3012 |
| VAM | 1 | 5.25 (0.0369) | 15.09 (0.0015) | 0.5033 (0.0075) | −0.1612 | 2.7203 |
| [P] × VAM | 1 | 17.44 (0.0008) | 55.72 (0.0001) | 0.7904 (0.0001) | −0.2375 | 2.7963 |
| Error | 15 | | | | | |

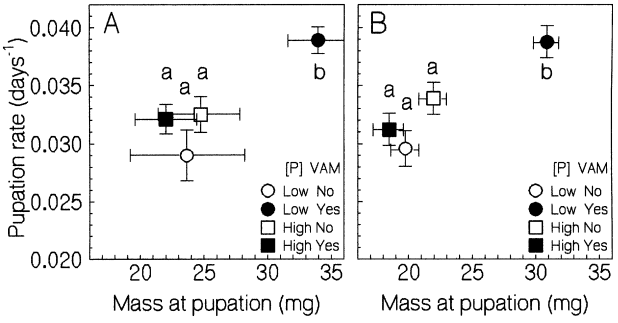


**Fig. 4** Mass at pupation vs. pupation rate for **A** female and **B** male Mexican bean beetle larvae reared on VAM-free or VAM-inoculated plants fertilized with low-[P] or high-[P] fertilizer. Values are least squares means (±1 SE) of mean pupation rate and median mass for females; least squares means (±1 SE) of mean pupation rate and mean mass for males. Within a panel values with the same *letter* do not differ significantly in Bonferroni comparisons at experimentwise $\alpha = 0.05$

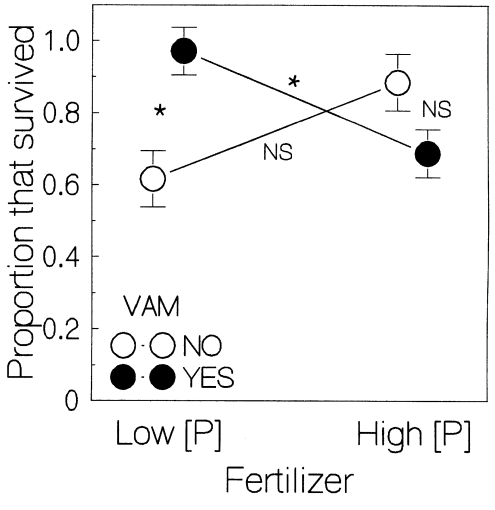


**Fig. 5** Survival to eclosion for larvae reared on VAM-free or VAM-inoculated plants fertilized with low-[P] or high-[P] fertilizer; * indicates significant differences at experimentwise $\alpha = 0.05$ using Bonferroni comparisons within treatments. Shown are least squares means ±1 SE for combined males and females

reproduction, conditions that could not be examined in this study.

I hypothesized that VAM colonization would alleviate phosphorus stress and make phosphorus-stressed plants similar to well-nourished plants in terms of chemistry and resistance to herbivory. By the end of the experiment mycorrhizal low-[P] plants were indeed similar to high-[P] plants in both phosphorus concentration of leaves and soluble proteins. However insects grew bigger, faster, and survived better on these mycorrhizal low-[P] plants than on all other fertilizer/VAM-colonization treatments. VAM colonization increased, rather than decreased, resistance to Mexican bean beetle.

The primary effect of VAM colonization is alleviation of phosphorus stress, and nutrient stress is often associated with increased damage by foliage-feeding arthropods (White 1984). However, the response of herbivores is frequently not linear (Louda and Collinge 1992). Insects often damage moderately-stressed plants more than either severely or minimally-stressed plants (Mattson and Haack 1987; English-Loeb 1989). Mechanisms proposed to explain this pattern of damage by insects link the quality of the foliage as food for insects to insect performance. At one extreme, severely stressed plants may provide a nutrient-poor diet for herbivores or may be heavily defended by carbon-based compounds that result from an imbalance of resources (Bryant et al. 1983). At the other extreme, unstressed plants may be well-defended by nitrogen-based compounds or have more complex proteins that herbivores cannot easily metabolize (Mattson and Haack 1987). Intermediate levels of nutrient stress can improve quality of foliage for herbivores by increasing concentrations or the balance of nonstructural carbohydrates and soluble nitrogenous compounds (White 1984; Mattson and Haack 1987; Louda and Collinge 1992). Moderate stress can also leave plants poorly defended because allocation to defense generally receives lower priority than allocation to growth (Bazzaz et al. 1987).

In my experiment, VAM-colonized, phosphorus-stressed plants exhibited intermediate growth suggesting intermediate stress, and yielded the best performance of insects, consistent with the hypothesis that moderate stress favors herbivores (Fig. 6). By reducing phosphorus stress and improving plant growth, VAM colonization may have improved the value of foliage for insects (Gehring and Whitham 1994). However, chemical assays did not identify likely mechanisms for the intermediate stress effect on insect performance. VAM-colonized,

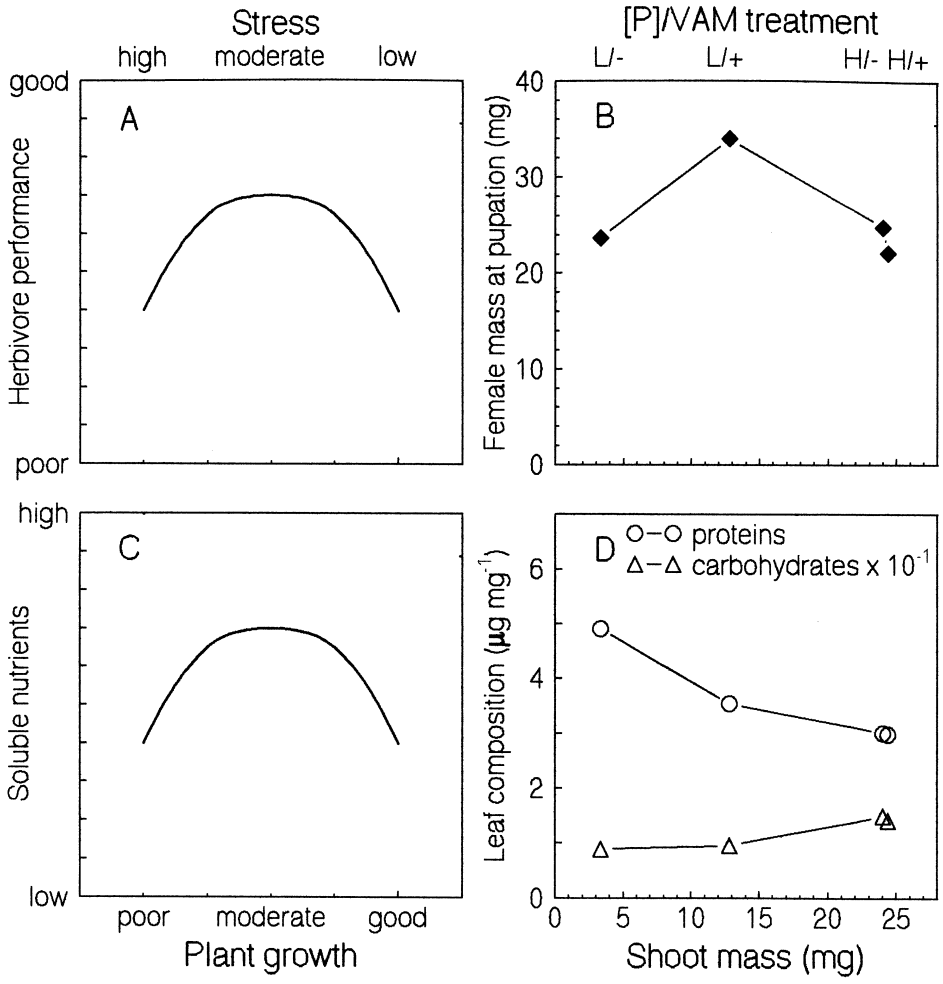


**Fig. 6A–D** Predictions of the hypothesis that intermediate stress reduces herbivory, and observed results from soybeans. **A** Predicted relationship of herbivore performance and plant growth. **B** Observed relationship of herbivore mass and shoot mass. **C** Predicted relationship of plant tissue nutrient content and plant growth. **D** Observed relationship of leaf protein and carbohydrate contents and shoot mass. Data in **B** and **D** are means from Figs. 2–4. *Error bars* have been omitted for clarity

phosphorus-stressed plants, which yielded the best performance by Mexican bean beetles, did not exhibit the highest levels of either soluble carbohydrates or soluble proteins (Fig. 6), as predicted for plants under moderate stress (White 1984; Mattson and Haack 1987; Louda and Collinge 1992). However, both qualitative and quantitative differences in nutrient composition of VAM-colonized and P-fertilized soybeans have been shown (Rabin and Pacovsky 1985; Pacovsky and Fuller 1988) but no significant correlations between leaf nutrient levels (e.g., N content, P content, reducing sugars, or amino acids) and insect performance have emerged (Rabin and Pacovsky 1985). Insects in my experiment may have responded to unmeasured qualitative differences between treatments.

Alternatively, differences in insect performance may have resulted from differences in plant allocation to chemical defense. In order to gain the benefit of increased phosphorus uptake, mycorrhizal plants must devote photosynthates to the root symbiont and this investment is costly for source-limited plants (Tinker et al. 1994). Soybeans are adapted to environments with higher light levels than those of the greenhouse used in this experiment and low-[P] plants released from phosphorus stress by mycorrhizal colonization may have become light-limited. Because they were dependent upon VAM fungi for P uptake they maintained a high level of colonization when grazed, unlike high-[P] plants (Fig. 1). The high investment in VAM fungi by grazed low-[P] plants may have come at the expense of allocation to defense.

The results of my experiment contradict results of three studies with leaf and root chewers and leaf miners. *Spodoptera frugiperda* (fall armyworm) and *Helicoverpa zea* (corn earworm) larvae fed excised leaves from VAM-colonized soybeans grew more slowly and weighed less at pupation than larvae fed leaves from P-fertilized plants (Rabin and Pacovsky 1985). This trend was similar whether the cultivars were susceptible to these insects or bred for resistance to multiple insect species. VAM-colonized soybeans of several cultivars are known to differ from P-fertilized soybeans in uptake of some micronutrients and fatty acid composition (Pacovsky et al. 1986; Pacovsky and Fuller 1988). Superior performance of larvae fed P-fertilized leaves could have been due to decreased nutritional quality, increased feeding deterrents, or increased toxins in VAM-colonized plants.

In a field study of *Plantago lanceolata*, leaf chewers and leaf miners inflicted heavier damage when plants

were treated with a fungicide that reduced VAM colonization (Gange and West 1994). *Arctia caja* caterpillars grew faster and ate more leaf tissue from fungicide-treated plants in complementary laboratory trials. These plants had higher levels of total nitrogen and lower levels of iridoid glycosides, which are known carbon-based feeding deterrents. Gange and West (1994) hypothesized that VAM colonization of plants in a high-light environment increased the carbon/nutrient ratio which in turn led to greater investment in carbon-based defenses.

Colonization of *Taraxacum officinale* (dandelion) by the VAM fungus *Glomus mosseae* increased mortality and reduced mass at pupation for surviving root-feeding *Otiorhynchus sulcatus* (black vine weevil) (Gange et al. 1994). Mycorrhizal plants were less affected by grazing than were VAM-free plants. This effect was attributed to reduced damage by herbivores (= resistance) rather than increased growth by the plant (= tolerance).

Although these studies with leaf- and root-chewing insects support the hypothesis that VAM colonization decreases susceptibility to herbivory, mode of feeding may be an important determinant of herbivore response to mycorrhizal plants. Two studies of sucking insects support this hypothesis. VAM colonization of sorghum had no effect on reproductive behavior and feeding by the aphid *Schizaphis graminum* (Pacovsky et al. 1985), and aphids [*Myzus persicae* (Sulzer)] grew better on well-colonized *Plantago lanceolata* than on fungicide-treated plants – opposite of the response exhibited by *Arctia caja* caterpillars (Gange and West 1994). Furthermore, Lin and Kogan (1990) found that performance of Mexican bean beetle larvae was more sensitive to induced resistance in soybean than was performance of soybean looper caterpillars (*Pseudoplusia includens*). Compared to leaf-chewing insects, Mexican bean beetles ingest relatively little structural tissue as they scrape the leaf surface to release juices. Insects that feed primarily on plant juices may be more influenced by factors that alter concentrations of soluble nutrients and toxins than are insects that process a great deal of structural tissue.

The difference between my results with Mexican bean beetles, and the results of Rabin and Pacovsky (1985) with caterpillars is particularly striking because both studies examined soybeans. A number of differences between the experiments may have contributed to the lack of agreement, yet the results of each study are equally valid. Our experiments differed in the cultivars and VAM fungus examined, and inevitably, in environmental conditions. Low-[P], VAM-colonized plants (Rabin and Pacovsky 1985) also exhibited higher levels of colonization (overall mean% infection from four cultivars: 63% compared to 43% in my study). It is possible that a threshold level of colonization must be surpassed before VAM fungus improves tolerance or resistance. However, high levels of VAM colonization did not increase resistance of sorghum to aphids (Pacovsky et al. 1985). Even low levels of colonization by the VAM fungus *Glomus mosseae* reduced black vine weevil growth on dandelion (Gange et al. 1994). A seasonal mean of 26.7% colonization of *Plantago lanceolata* reduced performance of caterpillars and improved performance of aphids (Gange and West 1994). Levels of colonization that were comparable to levels in my experiment inhibited egg production by root-knot nematodes in the soybean cultivar "Ransom" (Carling et al. 1989). Consequently, if there is a threshold effect, the level required to affect resistance appears to vary widely among herbivores and pathogens, and perhaps even among plants within species.

Research on plant-VAM fungus interactions has typically focussed on direct effects of the fungus on the growth and nutrition of the host plant. However, by altering the nutrition of a plant the fungus can indirectly, but significantly, alter the quality of food for the next higher trophic level. This type of indirect effect, where one organism modifies the relationship between two other organisms, is referred to as "interaction modification" (Wootton 1994). My experiment and those of others studying the effect of VAM fungus on plant-herbivore relations (Rabin and Pacovsky 1985; Gange and West 1994; Gange et al. 1994) demonstrate that the nature of the interaction modification depends on the environment(e.g., P availability) and the particular organisms engaged in the interaction. VAM colonization can improve foliage as food for herbivores, or reduce its value. How this effect influences herbivore populations remains to be explored.

VAM fungi may also exert other types of indirect effects. For example, the fungus and the herbivore both consume photosynthates, and so may engage in exploitative competition. If photosynthates are limiting, increased grazing would reduce amount of photosynthates available to the VAM fungus. Reduced VAM colonization with grazing is not uncommon (Gehring and Whitham 1994), consistent with the hypothesis that VAM fungi and herbivores compete. At the same time, colonization by VAM fungus may increase primary productivity and thus benefit herbivores by increasing the amount of food available. Indirect effects of VAM colonization on herbivore performance may be common and complex. If there is a general trend in the effects of VAM colonization on plant-herbivore relations, it will not be apparent until more fungus-plant-herbivore systems are examined over a range of environmental conditions.

**Acknowledgements** I thank S. A. Juliano and R. C. Anderson for useful comments and discussion, A. Khanna and J. Hillyard for assistance in a pilot study, R.L. Preston and D. J. Schmidt for technical advice and assistance, J. Morton for advice regarding VAM fungi, and C. Helm for providing beetles. This research was supported by USDA NRI Competitive Grants Program award # 91-37100-6595.

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