Influence of Vesicular-Arbuscular Mycorrhizal Fungi on the Response of Potato to Phosphorus Deficiency

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Morphological and biochemical interactions between a vesicular-arbuscular mycorrhizal (VAM) fungus (Glomus fasciculatum [Thaxt. sensu Gerdemann] Gerdemann and Trappe) and potato (Solanum tuberosum L.) plants during the development of P deficiency were characterized. Nonmycorrhizal (NM) plants grown for 63 d with low abiotic P supply (0.5 mm) produced 34, 52, and 73% less root, shoot, and tuber dry matter, respectively, than plants grown with high P (2.5 mm). The total leaf area and the leaf area:plant dry weight ratio of low-P plants were substantially lower than those of high-P plants. Moreover, a lower shoot/root dry weight ratio and tuber:plant dry weight ratio in low-P plants than in high-P plants characterized a major effect of P deficiency stress on dry matter partitioning. In addition to a slower rate of growth, low-P plants accumulated nonreducing sugars and nitrogen. Furthermore, root respiration and leaf nitrate reductase activity were lower in low-P plants than in high-P plants. Low abiotic P supply also induced physiological changes that contributed to the greater efficiency of P acquisition by low-P plants than by high-P plants. For example, allocation of dry matter and P to root growth was less restricted by P deficiency stress than to shoot and tuber growth. Also, the specific activities of root acid phosphatases and vanadate-sensitive microsomal ATPases were enhanced in P-deficient plants. The establishment of a VAM symbiosis by low-P plants was essential for efficient P acquisition, and a greater root infection level for P-stressed plants indicated increased compatibility to the VAM fungus. By 63 d after planting, low-P VAM plants had recovered 42% more of the available soil P than low-P NM plants. However, the VAM fungus only partially alleviated P deficiency stress and did not completely compensate for inadequate abiotic P supply. Although the specific activities of acid phosphatases and microsomal ATPases were only marginally influenced by VAM infection, VAM roots characteristically had a higher protein concentration and, consequently, enhanced microsomal ATPase and acid phosphatase activities on a fresh weight basis compared with NM roots. Morphological and ultrastructural details of VAM plants are discussed in relation to the influence of the VAM symbiosis on P nutrition of potato.

Second only to N, P is the mineral nutrient for which availability is most likely limiting to plant growth. The concentration of P in the soil solution is in the micromolar range, and moreover, this element diffuses slowly in soils. Hence, depletion of P in the root zone commonly limits further uptake of P by existing roots and, potentially, by the plant as a whole (Bieleski and Ferguson, 1983; Clarkson, 1985). The effects of P starvation on plant growth and development are pleiotropic and include the restriction of cell division and expansion, inhibition of photosynthesis and respiration, and impairment of root uptake and transport of other nutrients, such as NO3 (Bieleski and Ferguson, 1983; Baas et al., 1989; Fredeen et al., 1989; Rufty et al., 1989). For the potato (Solanum tuberosum L.), a crop of world-wide agronomic importance, these effects result in substantial reductions in yield (Harris, 1978; Pursglove and Sanders, 1981; MacKay et al., 1988).

Potato plants have been characterized as relatively inefficient at acquiring soil P (Pursglove and Sanders, 1981), but are capable of morphological and physiological adaptations during the development of P deficiency that may substantially improve P acquisition (Cogliatti and Clarkson, 1983). Such adaptations include changes in P and dry matter partitioning that favor growth of roots over shoots and the induction of a high-affinity P uptake and transport system in roots. These responses to P deficiency are not exclusive to potato, and, although not demonstrated specifically for potato, other mechanisms by which plants cope with inadequate P supply have been described (Clarkson, 1985). For example, excretion of Apases by roots and enhanced acidification of the rhizosphere by plasma membrane-associated ATPases are thought to speed dissociation of P from P-containing compounds, thus improving uptake from various soil P pools (Clarkson, 1985; Leonard, 1985; Goldstein et al., 1988). Promotion of root extension and root hair formation also contribute significantly to more efficient P uptake (Barber and Silberbush, 1984; Clarkson, 1985).

An alternate plant strategy for coping with P deficiency is the establishment of a mycorrhizal symbiosis with an appropriate fungus. External hyphae of VAM roots more rapidly exploit a given volume of soil for available P than roots of NM plants and, thus, speed the acquisition of soil P for an infected root (Harley and Smith, 1983). For plants such as potato, which have a low root density (Pursglove and Sand-

Abbreviations: ANOVA, analysis of variance; Apase, acid phosphatase; DAP, days after planting; FeEDDHA, ferric ethylenediamine di-(o-hydroxyphenyl) acetic acid; LAR, leaf area/plant dry weight ratio; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide; NM, nonmycorrhizal; pNPP, para-nitrophenol phosphate; NRS/RS, nonreducing sugars:reducing sugars dry weight ratio; Sw/Rw, shoot:root dry weight ratio; Tn/W, tuber:plant dry weight ratio; VAM, vesicular-arbuscular mycorrhizal.
ers, 1981) and high growth potential, the VAM symbiosis may be of particular significance in coping with P deficiency stress in natural ecosystems. This also appears to be true for the commercial production of potato, since significant yield increases due to VAM fungi have been recorded (Black and Tinker, 1977; McArthur and Knowles, 1991).

The compatibility of plants to VAM fungi is enhanced under conditions of P deficiency (Schwab et al., 1991) in a manner similar to those physiological processes implicated in the “rescue” of plants from P starvation (Goldstein et al., 1988). Factors that may be involved in plant compatibility to VAM fungi have recently been reviewed (Koide and Schreiner, 1992) and may be nutritional, based on either fungal symbiont recognition by plants, or by avoidance of a defense response by the fungal symbiont (Anderson, 1988; Schwab et al., 1991; McArthur and Knowles, 1992). Although the molecular mechanisms responsible for plant compatibility to VAM fungi have not been characterized, the plant’s susceptibility to VAM infection appears to be somehow linked to P starvation-induced physiology of plants (McArthur and Knowles, 1992).

The intent of this study was to characterize morphological and biochemical aspects of P deficiency in potato as affected by VAM fungi, specifically *Glomus fasciculatum* (Thaxt. sensu Gerdemann) Gerdemann and Trappe. The extent to which VAM infection altered physiological processes that were also affected in potato by P deficiency stress was central to our study. Because increased susceptibility to VAM fungi was demonstrated to be one response to P deficiency, we suggest that once identified, factors influencing susceptibility to VAM infection also be considered as components of P starvation-induced metabolism.

**MATERIALS AND METHODS**

**Inocula**

Pure pot culture of *Glomus fasciculatum* (Thaxt. sensu Gerdemann) Gerdemann and Trappe originated from single-sporic culture of chlamydospores isolated from roots of strawberry grown in Edmonton (53° 30’ N Lat. 113° 30’ W Long.). Differences between isolates were not evident and, moreover, chlamydospores produced in pot culture were of one type, which consistently matched the description by Walker and Koske (1987). Inocula for NM and VAM treatments consisted of soil and roots of clover (*Trifolium repens* L. cv Altaswede) plants grown in an autoclaved sand:soil (3:1 v/v) medium for 90 d. Plants were grown in 2-L pots and were fertilized weekly with 50 mL of a modified (minus P) Hoagland solution. Roots of NM clover plants were uninfected, but roots of VAM plants were 85% infected at 90 DAP and had many vesicles. Few mature chlamydospores were present on either the surface of roots or in the soil of the VAM inoculum. To quantify infection for plants at 40, 54, and 63 DAP (see below), roots were cut to 1-cm lengths and stored in a solution (5% formalin, 5% acetic acid, 45% ethanol) until they were cleared and stained (Phillips and Hayman, 1970). Percent infection was assessed by the gridline intersect method (Giovannetti and Mosse, 1980) with 100 counts per slide, and five (clover) or three (potato *Solanum tuberosum* L. cv Russet Burbank) slides per plant.

**Plant Growth Conditions and Experimental Design**

Certified potato seed-tubers, taken from a 4°C (95% RH) storage, were surface sterilized with 1.0% (w/v) sodium hypochlorite (3 min) and rinsed thoroughly with distilled H2O. Single-eye seedpieces (5.0 g) were cut from the mid-region of the tubers and rinsed. After air drying for 1 h, seedpieces were planted in vermiculite and sprouted in the dark for 10 d at 27°C (95% RH).

Single-sprout seedpieces were blocked for shoot-length prior to transplanting onto NM or VAM soil inoculum (100 g fresh weight pot⁻¹) in 20-cm diameter pots (1 seedpiece pot⁻¹). Each pot contained 3.4 kg (air dry) of an autoclaved soil medium consisting of 6:1 v/v sand:soil (5 µg g⁻¹ soil NaHCO₃-extractable P). An equal mix of fine and coarse sand was used in the medium, and the soil was a silt clay loam with a cation exchange capacity of 0.5 (millimhos cm⁻¹). A 2:1 (v/v) water extract of this soil had a pH of 6.2, no detectable P, 33 µg of total N, 40 µg of Ca, 11 µg of Mg, and 5 µg of K (g⁻¹ soil). Pots were placed in a growth chamber set at 25/20°C (day/night) with a 16-h photoperiod. A PPFD of 480 µmol m⁻² s⁻¹ was provided by fluorescent and incandescent lights maintained at 25 cm from shoot tips. In the first week, each pot received 50 mL of nutrient solution three times (40 mM KNO₃; 20 mM Ca(NO₃)₂; 20 mM MgSO₄ 185 µM H₂BO₃; 36.5 µM MnCl₂; 0.3 µM ZnSO₄; 1.3 µM CuSO₄; 0.065 µM H₃MoO₄; 2 mg/L FeEDDHA [pH 6.0]), and then 100 mL with added P (0.5 or 2.5 mm NaH₂PO₄) was given three times per week thereafter (24 applications total). Thus, treatments consisted of two levels of P (0.5 and 2.5 mm) and two inocula (NM or VAM) arranged factorially in a randomized complete block design. Growth data were collected for plants harvested for physiological studies and were grouped into harvest dates of 40 and 54 DAP (four blocks each). Plants harvested at 63 DAP (four blocks) were used for growth data also, and from this, LAR (LA/W), Sw/Rw (Tw/W at 63 DAP) were calculated. Shoots, tubers, and roots were lyophilized for P, carbohydrate, and N analyses. P accumulation/P available = (cumulative total of shoot P plus tuber P at each harvest date minus initial plant P at transplanting)/(cumulative total of P supplied at each harvest date, including total NaHCO₃-extractable soil P P pot⁻¹).

**Nitrate Reductase Activity**

Sixty uniform leaf discs for a total of 17 cm² plant⁻¹ were taken from each treatment and each of three blocks at 42 and 56 DAP. Samples were incubated in darkness in 10 mL of a 1 mM phosphate buffer (pH 7.5) containing 0.3 m KNO₃ at 25°C for 100 min. Nitrite concentrations were determined at 20-min intervals by the method of Sanderson and Cocking (1964), and nitrate reductase activity was expressed as µmol NO₃⁻ g⁻¹ dry weight h⁻¹.
Root Respiration

Root respiration was measured at 33, 38, 43, 45, 57, and 59 DAP (average 46). Roots of intact plants were gently washed in running H2O, rinsed again in cold deionized-distilled H2O, and stored briefly (maximum 30 min) in cold deionized-distilled H2O (4°C). Roots were then blotted dry with filter paper, and fine roots from the mid-region of each root system were cut into 1-cm lengths. Excised roots were placed in triplicate 15-mL disposable test tubes (1 g fresh root system were cut into 1-cm lengths. Excised roots were packed with 80/100 mesh HayeSep T (Hewlett-Packard). The He flow rate was 30 mL/min and the column was isothermal at 100°C. The 1-mL gas samples were replaced with an equal volume of air at each sampling. Excised roots were then lyophilized, and respiration was expressed as μmol CO2 g⁻¹ dry weight h⁻¹.

Microsomal ATPase and Apase Assays

Root microsomal ATPase activity was measured at 38, 47, and 53 DAP. All operations were carried out at 4°C. Roots from the mid-section of the root system were cut into 2-mm pieces and ground in a mortar and pestle for 3 min with homogenization buffer (7 g fresh weight to 21 mL) (250 mM sucrose, 2 mM EDTA, 2 mM DTT, 0.05% [w/v] BSA, and 0.5 mM PMSF in 50 mM Tris [Mes], pH 7.4). Homogenate was filtered through Miracloth, and the filtrate was centrifuged at 15,000g for 25 min. The supernatant was then centrifuged at 105,000g for 35 min. The pellet was gently resuspended with a glass homogenizer in 21 mL of resuspension buffer (250 mM sucrose, 2 mM DTT, 10 mM Tris [Mes], pH 7.4) and recentrifuged at 105,000g (Churchill and Sze, 1983). The pellet was then resuspended with 4 mL of resuspension buffer and stored overnight at 4°C.

Root microsomal ATPase activity was assayed by determination of P from ATP hydrolysis (Serrano et al., 1976) after 30 min of incubation at 37°C. In a total volume of 0.5 mL, the reaction was started by addition of 25 μL of membrane suspension (10-25 μg protein) to 50 mM Mes (Tris) buffer (pH 6.5) containing 2.5 mM MgSO4, and 1 mM molybdate. ATPase activity was terminated by addition of 25 μL of 24% (w/v) TCA, followed by 2 mL of 0.7% (w/v) (NH4)6Mo7O24·4H2O in 0.72 N H2SO4. The specific activity of ATPase (μmol P mg⁻¹ protein h⁻¹) was characterized over a pH range of 5.5 to 7.5 and showed a pH optimum of 6.0 (specific activity = -107 + 45.5[PH] - 3.8[pH]², r = 0.99).

In preliminary studies on ATPase activity, the inhibitors nitrate (50 mM KNO3), molybdate (1 mM Na2MoO4·2H2O), or azide (1 mM NaN3) were separately tested to estimate the presence of vacuolar ATPases, non-specific Apases, or mitochondrial ATPases, respectively. ATP hydrolysis was inhibited (P < 0.01) 14% by molybdate, but not by azide or nitrate. This concentration of molybdate strongly inhibits Apase activity, but only slightly decreases ATPase activity (Gallagher and Leonard, 1982). Hence, 1 mM molybdate was included in all further ATPase assays to inhibit Apase activity. Vanadate sensitivity (0.1 mM Na2VO4 or V2O5) was used to demonstrate the presence of plasma membrane ATPases in microsomes. After accounting for possible interference in the P assay by vanadate (an absorbance shift of -0.04), vanadate inhibition of the specific activity of ATPase was determined to be 24% (P < 0.01).

At 47 and 53 DAP, root microsomal Apase activity was assayed in a 50 mM Mes (Tris) reaction buffer (pH 5.5) containing 2.5 mM pNPP and 2.5 mM MgSO4 (Gallagher and Leonard, 1982). Nitrophenol was used as a standard. To start the reaction, 25 μL of the microsomal membrane preparation was added to 475 μL of reaction buffer and then incubated for 30 min at 37°C. The reaction was stopped with 1 mL of 1 N NaOH, and the A450 was determined. Apase activity in the 105,000g supernatant was similarly assayed at 47 DAP.

Soluble-N, Soluble Carbohydrate, and Total P Assays

For determination of plant N-metabolites, P, and carbohydrate content, lyophilized plant material from 40, 54, and 63 DAP was ground through a Wiley mill (40 mesh). Fifty milligrams were extracted (4°C) for 2 min (mortar and pestle) in 5 mL of 50 mM Hepes buffer (pH 7.4). Homogenate was centrifuged (1640g, 4°C) for 30 min, and free amino-N, soluble protein-N, and NO3-N were determined on 100, 200, and 50 μL of cold supernatant, respectively. Soluble protein-N was measured by a modified Lowry method (Bensadoun and Weinstein, 1976) with BSA (15.6% N) as the standard. Ninhydrin was used to assay free amino-N (Rosen, 1956) with leucine (10.7% N) as the standard. Nitrate-N was determined by the methods of Cataldo et al. (1975) with KNO3 as the standard. Reducing sugars were assayed colorimetrically by the method of Somogyi (1952) with a 100-μL aliquot of extract and glucose as the standard. Total soluble carbohydrates were determined on a 50-μL aliquot of supernatant with the phenol-sulfuric acid reagent (Dubois et al., 1956) and glucose as the standard. Plant material was dry ashed and total P was determined by the method of Serrano et al. (1976).

Microscopy

For dark-field microscopy, 1-cm root lengths were stained with lactophenol cotton blue. Localization of cellular dehydrogenase activity in VAM roots was determined by a 20-h incubation in 50 mM Hepes (pH 7.4) containing 2 mg MTT/mL. (An and Hendrix, 1988).

For EM, washed roots were cut to 3-mm lengths in 100 mM phosphate buffer (pH 6.8), transferred to phosphate buffer containing 2.5% glutaraldehyde, and incubated for 2 h at 23°C. Roots were then rinsed three times with phosphate buffer. For cryostage scanning EM, roots were frozen in liquid N2 and fractured with an Emitech K1250 Scanning EM Cryosystem. Water was sublimated from the fractured surface of frozen roots for 30 min before examination. Alternatively, roots were incubated in phosphate buffer containing 2%...
OsO₄ for 2 h and rinsed three times with phosphate buffer. Roots were then plunged into liquid N₂, fractured with a precooled scalpel, and lyophilized overnight. Roots were mounted on double-sided sticky tape on stubs, gold-sputter-coated, and viewed with a Cambridge Stereoscan 150 scanning electron microscope. Glutaraldehyde-osmium-fixed roots were also embedded in Spurrs resin, thin sectioned, and stained with uranyl acetate followed by lead citrate for viewing with transmission EM. For freeze-fracture replica processing, glutaraldehyde-treated roots were incubated in phosphate buffer (pH 7.4) containing 30% glycerol for 30 min (Hudson et al., 1981). Root pieces were oriented longitudinally onto gold discs, plunged into liquid-phase Freon-22, stored in liquid N₂, and then transferred into a precooled Balzers BAE 080 apparatus (Balzers Instruments, Lichtenstein). Roots were fractured at −110°C and shadow-coated with platinum and carbon. The replicas were cleaned with chromic acid and washed with distilled H₂O. Replicas and thin sections were mounted on formvar-coated grids and examined with either a Siemens Ehniskop 102 or a Hitachi H-7000 electron microscope.

Statistical Analyses

Growth and physiological data were subjected to ANOVA and, where appropriate, sums of squares were partitioned into individual degree of freedom components of both main effects and interactions. Percent infection data were arc-sine transformed to achieve homogeneity of variance prior to ANOVA. Based on the results of ANOVA, regression analysis was used to derive polynomial models for describing various relationships. In this study, Time deviation indicates a significant residual variance that is not explained by a linear model and with no further trend analysis possible.

RESULTS

Analysis of Plant Growth

Plant growth was clearly limited by low abiotic P supply (0.5 mM), as dry matter accumulation in shoots and tubers of low-P NM plants at 40 DAP was 35 and 71% lower, respectively, than that from high-P (2.5 mM) NM plants (Fig. 1, A and B). By 63 DAP, roots, shoots, and tubers of low-P NM plants had 34 (Fig. 1C), 52, and 73% less dry matter, respectively, than those from plants grown with high-P. Inoculation of plants with G. fasciculatum significantly enhanced dry matter production in shoots and roots of low-P plants relative to low-P NM plants. In spite of this growth enhancement, the total dry weight of high-P NM plants at 63 DAP was still 2-fold greater than that of VAM plants grown with low abiotic P supply. Under high-P conditions, VAM infection marginally increased root dry weight but did not significantly influence the final whole-plant dry weight.

The most appreciable influence of P nutrition on plant morphology was observed in leaf development. At 63 DAP, the total leaf area of high-P NM plants was less than half of that for high-P NM plants, due to a combination of a 35% reduction in area per leaf and 39% fewer leaves (Table I). Total leaf area at 63 DAP was greater at both abiotic P levels for VAM plants than for NM plants (Table I). Under low-P conditions, VAM plants had a significant increase in leaf area compared to NM plants. Figure 1. Main effects of time and abiotic P supply on dry matter accumulation of shoots (A) and tubers (B) for potato plants inoculated with NM (○, □) or VAM (C. fasciculatum) (●, ■) clover inoculum. Plants were grown with either 0.5 mM P (○, ●) or 2.5 mM P (□, ■). Treatment effects on the dry weight of roots at 63 DAP (C) are also shown. For dry matter accumulation of shoots, F-values for the main effects of Time, VAM, [P], and interactions Time × VAM × [P] were significant at the 0.01, 0.01, 0.01, 0.01, and 0.05 levels, respectively. For dry matter accumulation of tubers, F-values for the main effects of Time, VAM × [P], and the interaction Time × [P] were significant at the 0.01, 0.05, 0.01, and 0.01 levels, respectively. For root dry weights, F-values for the main effects of VAM and [P] were significant at the 0.05 and 0.01 levels, respectively.
Potato, Phosphorus Deficiency, and Mycorrhizal Fungi

Table I. Effect of a VAM fungus and P supply on morphology of potato plants

Plants were inoculated with NM or VAM (G. fasciculatum) clover-pot-culture and fertilized with a complete nutrient solution with either 0.5 or 2.5 mM P. Plants were harvested at 63 DAP.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Leaf Number</th>
<th>Leaf Area</th>
<th>Total Leaf Area</th>
<th>Leaf Area Ratio</th>
<th>Shoot Root*</th>
<th>Tuber*</th>
</tr>
</thead>
<tbody>
<tr>
<td>[P]</td>
<td></td>
<td>dm² leaf⁻¹</td>
<td>dm² plant⁻¹</td>
<td>dm² g⁻¹ dry weight</td>
<td>Weight Ratio</td>
<td>Weight Ratio</td>
</tr>
<tr>
<td>0.5</td>
<td>-</td>
<td>100</td>
<td>0.17</td>
<td>15.9</td>
<td>0.60</td>
<td>8.2</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>108</td>
<td>0.24</td>
<td>24.5</td>
<td>0.71</td>
<td>6.9</td>
</tr>
<tr>
<td>2.5</td>
<td>-</td>
<td>165</td>
<td>0.26</td>
<td>39.8</td>
<td>0.56</td>
<td>10.8</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>153</td>
<td>0.29</td>
<td>44.3</td>
<td>0.68</td>
<td>9.4</td>
</tr>
</tbody>
</table>

* Tuber weight ratio, Tuberc:plant dry weight ratio. Sources of variation. Significance levels for indicated sources of variation (ns = not significant).

A major influence of P nutrition on the concentrations of plant soluble carbohydrates during the 63-d growth period was apparent by the relative proportion of nonreducing sugars to reducing sugars (Table II). The ratio of nonreducing to reducing sugars was higher for shoots or roots of low-P plants than for high-P plants. Because shoot and root growth were restricted by low abiotic P supply (Fig. 1), the higher proportion of nonreducing sugars in low-P plants likely reflects a reduced capacity to utilize carbohydrates for growth and, hence, greater vacuolar storage of nonreducing sugars such as sucrose (Mares et al., 1985). In shoots, differences between the levels of reducing sugars and nonreducing sugars for low-P and high-P plants offset each other. In contrast, the lower root concentration of reducing sugars for low-P plants was only partially offset by the higher level of nonreducing sugars. Thus, total soluble carbohydrates were significantly lower in roots of low-P plants than in roots of high-P plants. Such altered root carbohydrate levels due to P deficiency have been reported for other plant species and are often associated with enhanced exudation of reducing sugars into the rhizosphere (Schwab et al., 1991). Such exudates may enhance VAM infection, but were not measured in our study. No influence of VAM infection on root carbohydrate levels was evident, although the concentration of shoot carbohydrates for VAM plants was marginally lower than that for NM plants (Table II).

The effects of abiotic P supply and VAM infection on soluble-N pools were also characterized for potato plants (Table III). Over the harvest interval, shoot concentrations of nitrate-N, free amino acid-N, and soluble protein-N were

Table II. Effect of a VAM fungus and P supply on soluble carbohydrates of potato plants

Plants were inoculated with NM or VAM (G. fasciculatum) clover-pot-culture and fertilized with a complete nutrient solution with either 0.5 or 2.5 mM P. Results from plants harvested at 40, 54, and 63 DAP were pooled.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Shoot Soluble Carbohydrates</th>
<th>Root Soluble Carbohydrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>[P]</td>
<td>Reducing sugars</td>
<td>Nonreducing sugars</td>
</tr>
<tr>
<td>0.5</td>
<td>24.1</td>
<td>44.6</td>
</tr>
<tr>
<td></td>
<td>22.8</td>
<td>42.3</td>
</tr>
<tr>
<td>2.5</td>
<td>31.6</td>
<td>35.4</td>
</tr>
<tr>
<td></td>
<td>28.5</td>
<td>35.2</td>
</tr>
</tbody>
</table>

* NRS/RS, Nonreducing sugars:reducing sugars dry weight ratio. Sources of variation. Significance levels for indicated sources of variation (ns = not significant).
high-P VAM plants was 20% greater than for comparable plants (Table III). Because the ratio of soluble reduced-N to total soluble-N was not altered by P nutrition, the results indicate that N-assimilation was perhaps slower, but not impaired. VAM infection had no effect on the concentrations of soluble shoot N-metabolites, although nitrate reductase activity for high-P VAM plants was 20% greater than for comparable NM plants. A similar enhancement of nitrate reductase activity was reported in roots and leaves of VAM-infected clover plants (Oliver et al., 1983).

In contrast to shoot N-pools, P nutrition had no significant effect on either free amino acid-N or soluble protein-N concentration in roots (Table III). However, the nitrate-N concentration was 35% greater in low-P than in high-P roots and, hence, the ratio of soluble reduced-N to total soluble-N was significantly lower for roots of low-P plants. The high root nitrate-N level in NM plants indicates that root N-assimilation or nitrate transport to shoots was unable to match uptake, resulting in more storage of nitrate in roots of low-P plants. Changes to root soluble-N pools in response to VAM infection were more obvious and were characterized by significantly lower nitrate-N and higher levels of reduced-N (mg g⁻¹ dry weight). This shift in soluble-N pools resulted in a 37% higher ratio of reduced-N to total soluble-N in roots of VAM plants than in NM plants.

Continuous plant growth with limited P supply resulted in a gradual decline in tissue P concentrations (Fig. 2). In spite of the slower growth of low-P plants, by 40 DAP, the P concentrations of roots, shoots, and tubers were already 36, 28, and 19% lower, respectively, than those of high-P NM plants (Fig. 2). Over the remaining harvest interval, the relative decline in tissue P was less pronounced in low-P plants than in high-P plants, reflecting the almost negligible growth of low-P plants between 54 and 63 DAP. Hence, by 63 DAP, the P concentrations of roots and shoots of high-P NM plants approached those values maintained in low-P NM plants. The absence of such a trend for tuber P concentration (Fig. 2B), despite substantial tuber dry mass accumulation up to 63 DAP for all treatments (Fig. 1B), suggests that the sink strength of tubers for P was high during this period.

Although plant growth was only moderately stimulated by the VAM symbiosis, tissue P concentrations of VAM plants were significantly greater than those of NM plants throughout the harvest interval at either abiotic P level (Fig. 2). Over the 23-d harvest period, P was 13, 12, and 54% more concentrated in shoot, tuber, and root tissues, respectively, of VAM plants than in NM plants. For low-P plants, VAM infection also increased total P accumulation at 63 DAP in shoots, tubers, and roots by 31, 53, and 112%, respectively (Table IV). For high-P plants, however, no net benefit of VAM infection on total P content was evident, as a marginal increase in shoot P was offset by a decrease in tuber P. Although roots of high-P VAM plants contained 79% more P than those of high-P NM plants, this increment to whole-plant P content (2.3 mg of P) was almost negligible.

The increase in P nutrition of host plants provided by the VAM symbiont did not totally compensate for low abiotic P supply, and low-P VAM plants had 57% less P at 63 DAP than NM plants with high P (Table IV). However, the VAM symbiosis definitely improved plant P nutrition over that of low-P NM plants, which had 70% less P than high-P NM plants. In spite of their relatively low P content, a much higher proportion of available P was recovered by low-P plants than by high-P plants (Table IV). Hence, roots of low-P NM and VAM plants were 30 and 65% more efficient at acquiring P, respectively, than roots of high-P NM plants (averaged over 40–63 DAP). The beneficial influence of VAM infection on the efficiency of plant P acquisition was absent under high P nutrition, possibly because high abiotic P supply to plants can produce a growth inhibition of external hyphae of C. fasciculatum associated with these plants (Abbott et al., 1984). The efficiency of P acquisition by roots increased over the harvest interval, reflecting increased growth of roots or external hyphae and roots, depending upon treatment.

The effects of P nutrition and VAM infection on root

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Soluble Shoot-N</th>
<th>Reduced total</th>
<th>Leaf NRA</th>
<th>Soluble Root-N</th>
<th>Reduced total</th>
</tr>
</thead>
<tbody>
<tr>
<td>[P] VAM NO₃⁻</td>
<td>Amino</td>
<td>Protein</td>
<td>mg g⁻¹ dry weight</td>
<td>μmol g⁻¹ dry weight h⁻¹</td>
<td>mg g⁻¹ dry weight</td>
</tr>
<tr>
<td>0.5</td>
<td>–</td>
<td>9.0</td>
<td>2.2</td>
<td>12.1</td>
<td>0.61</td>
</tr>
<tr>
<td>+</td>
<td>9.5</td>
<td>2.4</td>
<td>12.9</td>
<td>0.62</td>
<td>6.4</td>
</tr>
<tr>
<td>2.5</td>
<td>–</td>
<td>8.1</td>
<td>1.9</td>
<td>10.4</td>
<td>0.60</td>
</tr>
<tr>
<td>+</td>
<td>7.4</td>
<td>1.9</td>
<td>11.0</td>
<td>0.63</td>
<td>8.9</td>
</tr>
<tr>
<td>[P] VAM</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>ns</td>
<td>0.01</td>
</tr>
<tr>
<td>VAM</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>0.01</td>
</tr>
<tr>
<td>[P] X VAM</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>0.01</td>
</tr>
</tbody>
</table>

a NRA, Nitrate reductase activity, μmol NO₃⁻ g⁻¹ dry weight h⁻¹ at 42 and 56 DAP. b Sources of variation. c Significance levels for indicated sources of variation (ns = not significant).

Table III. Effect of a VAM fungus and P supply on soluble N-Pools and in vivo leaf nitrate reductase activity of potato plants.

Plants were inoculated with NM or VAM (C. fasciculatūm) clover-pot-culture and fertilized with a complete nutrient solution with either 0.5 or 2.5 mM P. Results for plants harvested at 40, 54, and 63 DAP were pooled.
respiration at 46 DAP are shown in Figure 3. Although gas fluxes may be expected to decrease within hours of root excision (Bloom and Caldwell, 1988), evolution of CO₂ and ethylene (McArthur and Knowles, 1992) by all root samples in our study was linear over the 5-h incubation interval. Although it is likely that root washing and excision perturbed the physiology of these roots, relative differences in respiration in response to treatments, as depicted in Figure 3, are representative of those for actual root respiration. Thus, the respiration of roots from low-P plants was 26% lower than that of roots from high-P plants (Fig. 3 and inset). A similar difference between low-P and high-P plants for root respiration was reported by Baas et al. (1989), who measured respiration for Plantago polaroographically and without root excision. In contrast to the Plantago study, which indicated a substantial increase in respiration in response to root infection by G. fasciculatum, VAM infection of potato roots did not significantly alter root respiration. This discrepancy likely reflects damage to the external hyphae during washing in our study and, possibly, disruption to intraradical hyphae. In this regard, metabolic activity for intraradical hyphae and vesicles, as demonstrated by fungal dehydrogenase activity, was still apparent for some time after root excision. Moreover, VAM roots had a 24% higher level of reduced N than NM roots, which indicates that a higher potential for respiration had existed in VAM roots relative to NM roots (Table III).

In spite of the reduced root respiration of low-P plants, the specific activity of vanadate-sensitive ATPases in root microsomes was 13% higher for low-P plants than for high-P plants (Table V). VAM infection had no effect on the specific activity of ATPase; however, because VAM roots had significantly more protein, ATPase activity on a fresh weight basis was 48% higher for microsomes of VAM roots than for those of NM roots. Root protein content was not affected by P nutrition and, hence, the influence of low P on ATPase activity on a fresh weight basis was similar to that observed for specific activity. Although molybdate was included in ATPase assays to inhibit nonspecific Apases, microsomal Apase activity could account for as much as 17% of the ATP hydrolysis recorded in our study (Table V). In this regard, Apase activity of microsomes and supernatants was inhibited by 57 (Table V) and 86% (data not shown, P < 0.01), respectively, by 1 mM molybdate, in contrast to the 14% inhibition by molybdate observed in assays for microsomal ATPase activity (see "Materials and Methods"). Apparently, some supernatant Apases were trapped in microsomes during processing, in spite of repeated washing of the pellet. However, the specific activity of Apases in supernatants was 2.5-fold greater on average than that in microsomal suspensions and 18 times greater on a fresh weight basis (Table V). In general, the contribution of these Apases to ATP hydrolysis under our assay conditions appeared to be minor and probably did not contribute significantly to treatment effects on ATPase activity.

The effects of P nutrition and VAM infection on root Apase activity in the absence of molybdate are also shown in Table V. The specific activity of Apases in supernatants and microsomes of low-P plants was 5 and 12% greater, respectively, than that of high-P plants. Associated with improved P nutrition and greater protein content of VAM plants, the specific activity of Apases in supernatants and microsomes of VAM roots was 51 and 10% lower, respectively, than that of NM roots. Because of the greater protein content in VAM roots, however, microsomal Apase activity of VAM roots on a fresh weight basis was 37% higher than that of NM roots. On the other hand, supernatant Apase activity of VAM roots

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**Figure 2.** Time course of P concentrations of shoots (A), tubers (B), and roots (C) of potato plants inoculated with NM (○, □) or VAM (●, ■) (G. fasciculatum) clover inoculum and grown with 0.5 mM (○, □) or 2.5 mM P (●, ■). For shoot P, F-values for the main effects of Time×P, Time×VAM, P, and the interaction Time×P × [P] were significant at the 0.01, 0.01, 0.01, and 0.05 levels, respectively. For tuber P, F-values for the main effects of Time×P, Time×VAM, P, and the interaction Time×P × [P] were significant at the 0.01, 0.01, 0.05, and 0.01 levels, respectively. For root P, F-values for the main effects of Time×P, Time×VAM, P, and the interaction Time×P × [P] were significant at the 0.01, 0.10, 0.01, 0.01, and 0.10 levels, respectively.
Table IV. Effect of a VAM fungus and P supply on P accumulation of potato plants

Plants were inoculated with NM or VAM (C. fasciculatum) clover-pot-culture and fertilized with a complete nutrient solution with either 0.5 or 2.5 mM P.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>P Accumulated/P Available</th>
<th>Shoot P</th>
<th>Tuber P</th>
<th>Root P</th>
<th>Plant P</th>
</tr>
</thead>
<tbody>
<tr>
<td>[P]</td>
<td>VAM 40 DAP 54 DAP 63 DAP</td>
<td>%</td>
<td></td>
<td></td>
<td>mg plant$^{-1}$ at 63 DAP</td>
</tr>
<tr>
<td>0.5</td>
<td>- 55 64 57 16.3 11.8 1.7 29.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>+ 65 77 81 21.4 18.1 3.6 43.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time$^b$</td>
<td>[P] 0.05$^c$</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>[VAM]</td>
<td>0.01</td>
<td>0.10</td>
<td>ns</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>[P] X VAM</td>
<td>0.01</td>
<td>0.10</td>
<td>ns</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Based on cumulative total of shoot P plus tuber P at each harvest date minus initial plant P at transplanting versus cumulative total of P supplied at each harvest date, including total NaHCO$_3$-extractable soil P pot$^{-1}$. $^b$ Sources of variation. $^c$ Significance levels for indicated sources of variation (ns = not significant).

on a fresh weight basis was only marginally increased in low-P plants, similar to that reported by others (Capaccio and Callow, 1982; Dodd et al., 1987).

VAM Root Morphology

Infection of potato roots by G. fasciculatum, as a function of time and level of P supply, is shown in Figure 4. Although the level of infection was extensive throughout the harvest interval, percent infection declined from 54 to 63 DAP. This reduction occurred during rapid tuber growth from 40 to 63 DAP and could be attributed to diversion of plant nutrients away from the fungus (Schwab et al., 1991). The extent of root infection was greater for plants grown with 0.5 mM P than with 2.5 mM P, indicating that P-deficient plants were more susceptible to infection. Low-P roots have a lower capacity for ethylene generation and also have less extracellular peroxidase activity than high-P roots (McArthur and Knowles, 1992). Reduction in such activities may be indicative of lower capacity for the induction of a defense response by the plant, resulting in higher levels of infection.

Root hair development was observed to be greater on NM roots than on VAM roots (Fig. 5, A and B). Incubation of excised VAM roots in MTT solution demonstrated that both extraradical and intraradical VAM structures had considerable dehydrogenase activity (Fig. 5B). Staining was uneven in uninfected root sections, where MTT was reduced to the insoluble, blue formazan product. This irregular pattern of staining probably reflects the low permeability of intact plant cells to MTT (Altman, 1976). In the infected sections of roots, lipids in spores, hyphae, and vesicles stained red after incubation with MTT due to the lipid-soluble nature of the formazan product. For the VAM fungus, the majority of formazan product appeared in root vesicles and spores, thus characterizing them as active sites of metabolism. Little formazan product appeared in external hyphae, possibly reflecting damage incurred during root washing.

A high proportion of parenchyma cells in root cortices was colonized (Fig. 5C), with many arbuscules interfacing cells directly adjacent to the endodermis. The fine, dichotomous hyphae of arbuscules in colonized cells often filled the cell lumen (Fig. 5D), facilitating maximum contact with the protoplast and favoring nutrient exchange (Smith and Smith, 1990). The cryostage scanning electron micrograph in Figure 5E shows various intracellular VAM hyphae in close association with a freeze-etched cell protoplast. Such host cells...
exhibit ultrastructural evidence of heightened metabolic activity (Cox and Tinker, 1976; Holley and Peterson, 1979).

Vesicles predominated over arbuscules and other VAM structures in this study, and over 400 vesicles/cm of root were occasionally observed. Transversely fractured roots (Fig. 5F) illustrate the physical impact of these lipid-filled vesicles on host cells. Transmission electron micrographs of replicas of freeze-fractured vesicles displayed an ultrastructure similar to that previously described from thin sections (Holley and Peterson, 1979) and provided additional details of the ultrastructure of organelles and vesicular architecture (Fig. 6). Fenestrated cisternae of ER were arranged in stacks and were often in close association with one of the many nuclei present. Nuclear pores were prominent, and these displayed an uneven arrangement. Lipid globules were numerous, whereas few mitochondria were observed (Fig. 6A). Vesicle walls appeared to be trilaminate in replicas (Fig. 6B), and this was later confirmed with ultrathin sections. The ultrathin sections (not shown) also demonstrated a very large number of lipid globules of various sizes in each vesicle, some of which were associated with mitochondria (cristae evident).

**DISCUSSION**

Our results characterize a pronounced P deficiency in NM and VAM potato plants grown with 0.5 mM P. Restricted leaf canopy development (Table I) and dry matter accumulation (Fig. 1) due to low abiotic P supply were prominent indicators of plant stress. Moreover, the accumulation of relatively high levels of nonreducing sugars and nitrates in shoots and roots indicated that these plants were neither carbohydrate nor N starved (Tables II and III). On the other hand, the P status of low-P plants was clearly inferior to that of high-P plants (Fig. 2; Table IV). These responses of potato plants to low abiotic-P supply were similar to those generally attributed to P deficiency stress caused by inadequate P supply (Harris, 1979; Fredeen et al., 1989) or short-term P deprivation (Cogliatti and Clarkson, 1983; Goldstein et al., 1988; Rufty et al., 1989).

A general restriction of plant metabolism by P deficiency stress was apparent by a number of metabolic indicators. Leaf nitrate reductase activity and root respiration were substantially lower for low-P plants than for high-P plants (Fig. 3). Because respiration is often proportional to cell growth and nitrate reduction constitutes a significant investment of energy for plant growth (Johnson, 1990), the results characterize a decrease in the amount of respiratory energy devoted directly to growth processes as a result of P deficiency stress. Indeed, the lower dry matter accumulation for low-P NM plants between 40 and 63 DAP (Fig. 1) implies minimal investment of energy from respiration into processes that directly contribute to growth. Hence, most of the energy derived from root respiration of low-P plants was likely expended to maintain existing biomass. Energy-dependent maintenance processes such as the transport of nutrients across cell membranes and against electrochemical gradients, including the reassimilation of leaked ions to balance ion efflux (Leonard, 1985; Baas et al., 1989; Johnson, 1990), may

![Figure 4. Time course of root infection by G. fasciculatum for potato plants grown with 0.5 or 2.5 mM P.](image-url)

**Table V. Effect of a VAM fungus and P supply on the activity of ATPases and Apases of roots of potato plants**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Microsomal ATPase</th>
<th>Microsomal Apase</th>
<th>Supernatant Apase</th>
</tr>
</thead>
<tbody>
<tr>
<td>[P] VAM</td>
<td>+Molybdate</td>
<td>+Molybdate</td>
<td>-Molybdate</td>
</tr>
<tr>
<td></td>
<td>µmol P mg⁻¹ h⁻¹</td>
<td>µmol P mg⁻¹ h⁻¹</td>
<td>µmol P mg⁻¹ h⁻¹</td>
</tr>
<tr>
<td>0.5</td>
<td>65.0</td>
<td>9.5</td>
<td>10.6</td>
</tr>
<tr>
<td>+</td>
<td>59.9</td>
<td>14.6</td>
<td>9.1</td>
</tr>
<tr>
<td>2.5</td>
<td>58.7</td>
<td>8.6</td>
<td>9.9</td>
</tr>
<tr>
<td>+</td>
<td>52.3</td>
<td>12.2</td>
<td>8.2</td>
</tr>
<tr>
<td>[P] × VAM</td>
<td>ns</td>
<td>0.01</td>
<td>0.05</td>
</tr>
</tbody>
</table>

* Roots were harvested at 47 and 53 DAP for microsomal Apase. Molybdate decreased activity (P < 0.01).  
* Roots were harvested at 47 DAP for supernatant Apase. Molybdate decreased activity (data not shown, P < 0.01).  
* Sources of variation.  
* Significance levels indicated for sources of variation (ns = not significant).
be a relatively greater sink for metabolic energy under P stress. The greater efficiency of low-P plants in acquiring what little P was available (Table IV) suggests that meeting the energy requirement for P uptake was a more significant energy-dependent component of the root’s total respiratory activity in low-P plants. Our results on the efficiency of P acquisition are consistent with observations of an enhanced root P-uptake system in potato under conditions of P deficiency (Cogliatti and Clarkson, 1983).

In spite of the inhibitory effects of P deficiency stress on overall growth and metabolism, specific physiological processes were actually stimulated by low abiotic P supply. For example, the allocation of photosynthates (Table I) and P to roots (Table IV) appeared to be less restricted by low abiotic P supply than that for shoots or tubers. This indicates an altered pattern of dry matter partitioning favoring root growth and, thus, mineral acquisition (Cogliatti and Clarkson, 1983). Vanadate-sensitive ATPase activity in root microsomes was stimulated in response to P deficiency stress (Table V). This response may also be relevant to enhanced P acquisition, because root plasma membrane-ATPases (which are also vanadate sensitive) are considered responsible for acid secretion, which may increase P availability in the rhizosphere (Clarkson, 1985). These ATPases also provide the proton motive force required by carrier proteins to facilitate active uptake of nutrients such as H$_2$PO$_4^-$ across membranes (Leonard, 1985). Soluble A pase activity (intracellular plus extracellular) in roots was also enhanced in response to P deficiency stress (Table V). In terms of improving the plant’s P nutrition, a role for soluble excreted Apases in scavenging organic P from the rhizosphere has been suggested (Goldstein et al., 1988, 1989). Moreover, an increase in the intracellular Apases may allow for more efficient utilization of P in primary metabolism (Duff et al., 1989). Although further investigation is required to establish a stronger connection between the low-P-induced increase in the efficiency of P acquisition and the activities of microsomal ATPases and soluble Apases, our results indicate that such an approach is warranted.

Collectively, the changes in plant metabolism in response to low abiotic P supply that we have characterized may constitute part of a “phosphate starvation rescue system” in higher plants (Goldstein et al., 1988; Lefebvre et al., 1990). Such changes in root physiology during the development of P deficiency are generally considered to be adaptive and to improve plant acquisition or conservation of P (Cogliatti and Clarkson, 1983; Clarkson, 1985; Goldstein et al., 1989; Lefebvre et al., 1990). The actual contribution of these physiological changes to the greater efficiency of P acquisition characterized for low-P plants in our study, however, is difficult to estimate. On the other hand, the contribution of the VAM symbiosis to plant P nutrition was more easily quantified.

The growth response of low-P plants to VAM infection (Fig. 1) and the P status of these plants (Table IV) demonstrated that the mycorrhizal symbiosis enhanced plant P nutrition beyond that which could be derived solely through adaptation of the plant grown independently. However, the VAM symbiosis was capable of only partially alleviating P deficiency stress and could not compensate for low abiotic P supply. In a separate study, it was demonstrated that the VAM symbiosis could completely compensate for a low abiotic P (1.5 mm) supply that was one-half that of the high-P supply (3.0 mm) (unpublished results). In the current study, the extent to which VAM infection improved plant growth was clearly a function of its P contribution (Table IV; Fig. 2). Moreover, the VAM benefit was limited by the amount of P available in low-P pots. A conservative estimate of 81% for the P acquisition efficiency for low-P VAM plants (root P was not included) indicates that mycorrhizal roots were able to account for most of the available P in the soil. Although more efficient P scavenging would likely be attributable to external hyphae (Fig. 5, A and B), increased P absorption due to VAM-stimulated root growth may also be important (Fig. 1C).

Coincident with the effects on plant P nutrition, the fungal symbiont appeared to have some influence on plant allocation of dry matter and leaf development. For example, leaf expansion was stimulated relatively more so than biomass accumulation (Fig. 1) and, hence, a main effect of VAM on LAR was evident (Table I). Enhanced leaf expansion for VAM plants was likely related to the fact that both P allocation to shoots (Table IV) and nitrate reductase activity in leaves (Table III) were increased in VAM plants, even in the absence of a growth response to the VAM symbiosis for high-P plants. Possibly reflecting an enhanced sink strength in roots as a result of symbiotic activity (Snellgrove et al., 1982; Baas et al., 1989), the relative increase in root dry weight gain in response to the symbiosis was more than that for shoots. This was demonstrated by a lower S$_{w}$/R$_{w}$ for VAM plants than for NM plants at 63 DAP, and in spite of a higher shoot mass for low-P VAM plants than for low-P NM plants.

Generally, the negative influence of VAM infection on the specific activity of root ATPases and Apases was consistent with an improved P nutrition in plants via the VAM symbiosis (Table V). The promotion of these enzyme activities on a fresh weight basis was a direct consequence of the enhanced

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**Figure 5.** Dark-field and scanning electron micrographs of potato roots infected with the VAM fungus, *G. fasciculatum.* A, Dark-field micrograph of a NM root with an external VAM hypha for comparison of root hair and hyphal length. r, Root hair; E, extraradical hypha (magnification, ×27). B, Dark-field micrograph of a VAM root and its associated extraradical hyphae (E). Roots were incubated for 20 h in Hepes buffer containing MTT. The open arrow indicates blue formazan crystals in a NM root section. Arrows and the curved arrow indicate red formazan in vesicles and a spore, respectively (magnification, ×27). C, Scanning EM of a transverse fracture of a root illustrating arbuscules (A) in the cortex and exclusion of the VAM fungus from the stele by the endodermis (E). H, Intracellular hyphae (bar = 40 μm). D, Arbuscular (A) and intracellular (H) hyphae within a cortical cell (bar = 10 μm). E, Cryostage scanning EM of a root showing intracellular (H) and arbuscular hyphae (A) amidst cytoplasmic contents (c) of the root cell (bar = 10 μm). F, Scanning EM of a transverse fracture exposing numerous vesicles and showing exclusion of the fungus from the stele (bar = 40 μm).
plasma membrane surface area of cells due to arbuscular growth (Fig. 5, C and D) (Cox and Tinker, 1976). Enzymic studies using cytochemical techniques to characterize the presence of ATPases and Apases also showed enhanced activities of these enzymes, specifically at interfaces between intraradical VAM structures and the plasma membranes of colonized root cells (Gianinazzi-Pearson et al., 1991). Although the contribution of fungal ATPases and Apases to these activities in VAM roots could not be estimated in our study, we did find some Apase activity in soluble extracts of isolated chlamydospores and vesicles (data not shown). On the other hand, during a preliminary characterization of Apases from NM and VAM roots by native PAGE, a soluble Apase activity that could be attributed to the VAM fungus was not detected in extracts from VAM roots.

In this and a related study (McArthur and Knowles, 1992), potato plants grown with low abiotic P supply were more susceptible to infection by G. fasciculatum than those grown with a high abiotic P supply. Also, the capacity for ethylene production and extracellular peroxidase activity in roots increased proportionally with increasing P supply, whereas VAM infection of roots substantially inhibited ethylene production. We further demonstrated that the VAM inhibition of 1-amino cyclopropane-1-carboxylic acid oxidase activity could be attributed to a soluble, possibly phenolic, substance in VAM root leachates. Hence, these results indicated a possible link between plant mineral physiology and compatibility of plants to VAM fungi. In the present study, P deficiency stress influenced metabolism in a fashion that might have limited the resources available for induction of a defense response to VAM infection and, thus, may have been a contributing factor to enhanced root susceptibility. However, inhibition of 1-amino cyclopropane-1-carboxylic acid oxidase in VAM roots was also present for plants grown with high abiotic P supply and exhibiting a high rate of root respiration (Fig. 3). Further investigations into a link between plant compatibility to VAM fungi and P starvation-induced metabolism might consider the possible influence of P nutrition on root susceptibility to VAM infection through changes to root phenolic metabolism (Siqueira et al., 1991).

In summary, the growth and yield of potato were shown to be very sensitive to inadequate P nutrition in this study. The partitioning of dry matter to tubers was most affected by P nutrition in our study (Fig. 1), because P deficiency stress developed and increased during the period when the requirement for P was greatest, i.e. during tuberization and bulking (Nelson et al., 1947; MacKay et al., 1988). Our results underscore the importance of preventing P deficiency during growth of potato and suggest a potential benefit from the VAM symbiosis under appropriate field conditions. It should be noted that even under a P regime in which no growth benefit was obtained, no detrimental effect of the VAM infection was observed. Finally, we suggest that a P starvation-induced increase in susceptibility of plants to VAM fungi be interpreted as another component of P starvation-induced metabolism. Elucidation of the metabolic basis for enhanced susceptibility to VAM infection may provide an improved understanding of developmental and physiological patterns observed in plants in response to various sources of stress.

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