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Resistance Responses of Potato to Vesicular-Arbuscular Mycorrhizal Fungi under Varying Abiotic Phosphorus Levels[1]

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ABSTRACT

In mycorrhizal symbioses, susceptibility of a host plant to infection by fungi is influenced by environmental factors, especially the availability of soil phosphorus. This study describes morphological and biochemical details of interactions between a vesicular-arbuscular mycorrhizal (VAM) fungus and potato (*Solanum tuberosum* L. cv Russet Burbank) plants, with a particular focus on the physiological basis for P-induced resistance of roots to infection. Root infection by the VAM fungus *Glomus fasciculatum* ([Thaxt. sensu Gerdemann] Gerdemann and Trappe) was extensive for plants grown with low abiotic P supply, and plant biomass accumulation was enhanced by the symbiosis. The capacity of excised roots from P-deficient plants to produce ethylene in the presence or absence of exogenous 1-amino cyclopropane-1-carboxylic acid (ACC) was markedly reduced by VAM infection. This apparent inhibition of ACC oxidase (ACC_{ox}) activity was localized to areas containing infected roots, as demonstrated in split-root studies. Furthermore, leachate from VAM roots contained a potent water-soluble inhibitor of ethylene generation from exogenous ACC by nonmycorrhizal (NM) roots. The leachate from VAM-infected roots had a higher concentration of phenolics, relative to that from NM roots. Moreover, the rates of ethylene formation and phenolic concentration in leachates from VAM roots were inversely correlated, suggesting that this inhibitor may be of a phenolic nature. The specific activity of extracellular peroxidase recovered in root leachates was not stimulated by VAM infection, although activity on a fresh weight basis was significantly enhanced, reflecting the fact that VAM roots had higher protein content than NM roots. Polyphenol oxidase activity of roots did not differ between NM and VAM roots. These results characterize the low resistance response of P-deficient plants to VAM infection. When plants were grown with higher abiotic P supply, the relative benefit of the VAM symbiosis to plant growth decreased and root infection was lower. The in vivo ACC_{ox} activity was also greater in roots of plants grown on high levels of P compared with those grown on low levels, although the influence of VAM infection was partially to counteract the nutritional effect of P on ACC_{ox} activity. Similar to ACC_{ox} activity, extracellular peroxidase activity of roots increased linearly with increasing abiotic P supply, thus indicating a greater potential for resistance to VAM infection. These findings suggest that VAM fungi may alter phenolic metabolism of roots so as to hinder ethylene production and the root's ability to invoke a defense response. Raising the abiotic P supply to plants at least partially restores the capacity of roots to produce ethylene and may, in this way, increase the root's resistance to VAM infection.

The VAM[2] fungi are a small and distinct group of obligate, symbiotic fungi that are capable of association with roots of most terrestrial vascular plants (20). In exchange for photoassimilates, VAM fungi contribute to the plant acquisition of P and other mineral nutrients and, by this means, stimulate the growth of host plants. Although genotypic and environmental factors influence the symbiosis, a prolonged, stable, and compatible association between plants and VAM fungi is based primarily upon the transfer of P from the symbiont to a host that has a net P deficit. When available P in the soil solution is nonlimiting to plant growth, roots exclude or limit VAM infection. Hence, plants appear capable of exerting substantial control in their interactions with these symbionts, although the physiological, biochemical, and genetic bases for host control are not well understood (29, 32).

Mechanisms by which plants restrict VAM growth appear to involve nutritional or non-nutritional factors as their basis, and may include both (32). The ability of plants to regulate partitioning of carbohydrates appears to play a central role in their control of the VAM infection process. Evidence for this comes from experiments on how plant developmental stages, nutritional components (including P), and the VAM symbiosis alter source to sink relations, root exudation, and photosynthetic activity (22, 29). On the other hand, recent investigations into the influence of phenolic compounds on hyphal growth and root colonization implicate several secondary metabolites (31) as promoters of VAM growth and development. Insight into this area of research can be gleaned from investigations of legume-rhizobium interactions, where specific metabolites of the phenylpropanoid pathway play a key role in the establishment of the symbiosis. Thus, the observed suppression of fungal colonization of roots when the external, i.e. abiotic, P supply increases may be due to the plant somehow limiting the availability of photoassimilates needed for fungal nutrition, or specific regulatory signals from the plant that directly control symbiont growth.

Also meriting consideration is the observation that the invasive growth of VAM fungi during infection is morphologically similar to that in many host-pathogen interactions (3, 16). VAM infection hyphae can induce papilla-like thick-

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[2] Abbreviations: VAM, vesicular-arbuscular mycorrhizal; POX, peroxidase; ACC, 1-amino cyclopropane-1-carboxylic acid; ACC_{ox}, ACC oxidase; PPO, polyphenol oxidase; NM, nonmycorrhizal; Fe-EDDHA, ferric ethylenediamine di(*o*-hydroxyphenyl) acetic acid; DAP, days after planting; ANOVA, analysis of variance; PSI, P starvation inducible; SEM, scanning electron microscope.

enings in epidermal cells, and the activities of cell wall-bound POXs (involved in lignin formation) and chitinases are enhanced during the early stages of infection by VAM fungi (33, 34). Such observations are consistent with a pathogen-induced defense response by plants (37). In this regard, roles for phenolic metabolism and lignin production in the resistance responses by plants to various types of mycorrhizae have been suggested (3).

Many resistance responses of plants to parasites involve the ethylene and phenylpropanoid biosynthetic pathways (13, 19, 37). Activation of these pathways can stimulate the generation of fungitoxic compounds (e.g. oxidized polyphenols from increased PPO activity) and create lignin barriers (e.g. the actions of extracellular POX on ferulic acid) that may physically impede infection (37). Involvement of the plant defense response in host-VAM fungi interactions appears feasible, because, in addition to the above observations, VAM infection increases the level of phenolic compounds in roots (24), and, moreover, ethylene (ethrel) treatment of roots or shoots inhibits VAM infection (4, 27). A coincident increase in phytoalexins has been associated with a lower level of infection after ethylene treatment (27).

This article describes morphological and biochemical details of interactions between VAM fungi and potato (*Solanum tuberosum* L. cv Russet Burbank) roots after infection. The extent to which the plant's defense responses are involved in P-induced resistance to VAM fungi (*Glomus fasciculatum* [Thaxt. sensu Gerdemann] Gerdemann and Trappe) is central to our investigation. Our objectives were to characterize the effects of abiotic P supply and VAM fungal infection on root ethylene production and phenolic metabolism. The results demonstrate that the root's capacity for ethylene production via ACC_{ox} activity is restricted by VAM infection. In contrast, the activities of other enzymes that have been implicated in plant defense responses, such as PPO and extracellular POX, were unaltered or only mildly stimulated in response to VAM infection. However, as abiotic P supply increased, the root capacity for ethylene production and extracellular POX activity also increased, whereas VAM infection of roots declined. The physiological basis for this P-induced resistance to VAM infection and the paradox that plants cope with in shifting their priorities between P nutrition and defense are discussed.

MATERIALS AND METHODS

Inocula

Inocula for NM and VAM (*Glomus fasciculatum* [Thaxt. sensu Gerdemann] Gerdemann and Trappe) treatments consisted of soil and roots of clover (*Trifolium repens* L. cv Altaswede) plants grown in an autoclaved sand:soil (v/v, 3:1) 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, whereas those of VAM plants were 85% infected 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, roots were cut into 1-cm lengths and were stored in FAA solution (5% formalin, 5% acetic acid, 45% ethanol) until further processing by clearing and staining (28). Percent infection was assessed using the gridline intersect method (17) with 100 counts per slide and 5 (clover) or 3 (potato [*Solanum tuberosum* L. cv Russet Burbank]) slides per plant.

Plant Growth Conditions

Certified potato seed-tubers, taken from a 4°C (95% RH) storage, were surface-sterilized with 1.0% (v/v) sodium hypochlorite (3 min) and rinsed thoroughly with distilled H_2O. Single-eye seedpieces (5.0 g) were cut from the midregion of the tubers and rinsed. After air-drying for 1 h, the seedpieces were planted in vermiculite and sprouted in the dark for 10 d at 27°C (95% RH).

Single-sprout seedpieces were blocked for sprout 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 $NaHCO_3$-extractable P). 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 the shoot tips. Each pot received nutrients as indicated below. For split-root studies, sprouted seedpieces were produced as above and transplanted with half of their roots positioned in a separate uninoculated pot and the other half in a VAM-inoculated pot. Each root-half received half of the total nutrient solution containing 0.5 mM P as described below. In the first week, each pot received 50 mL of nutrient solution three times (40 mM KNO_3; 20 mM $Ca(NO_3)_2$; 20 mM $MgSO_4$; 185 μM, H_3BO_3; 36.5 μM, $MnCl_3$; 0.3 μM, $ZnSO_4$; 1.3 μM $CuSO_4$; 0.065 μM H_2MoO_4; 2 mg/L Fe-EDDHA [pH 6.0]), and then 100 mL with added P (0.5 or 2.5. mM NaH_2PO_4) was given three times per week thereafter.

A second study was completed with sprouted seedpieces produced and treated as in the previous experiment, except that 50 g of soil inoculum from NM or VAM clover plants was used (VAM roots were 80% infected, with spores absent, but vesicles abundant). Pots were placed under growth conditions as above, but with a PPFD of 540 μmol m^{-2} s^{-1}, and the lights were maintained 14 cm from shoot tips. Essential nutrients were provided as described above.

Experimental Design and Analytical Methods

In the first study, treatments consisted of all four combinations of two levels of P (0.5 and 2.5 mM) and two inocula (NM or VAM) in a randomized complete block design (five blocks). Blocks were harvested at 33, 38, 43, 45, and 57 (average 43) DAP for measurement of ethylene production and the apparent ACC_{ox} activity in root systems. Two supplementary experiments were also initiated. In the first, plants were inoculated as above but grown on nutrient solution containing only the 0.5 mM level of P. These plants were harvested at 83 DAP (two blocks) to examine the effect of VAM-root leachate (pooled samples from the 43 DAP study) on root ACC_{ox} activity. In the second, plants grown with their roots divided (with or without inoculum) were harvested at 85 DAP (four blocks) for determination of ACC_{ox} activity.

Roots of intact plants were gently washed in running water, rinsed in cold deionized, distilled H_2O, and stored briefly (max 30 min) in cold deionized, distilled H_2O (4°C). The roots were then blotted dry with filter paper, and groups of fine roots from the midregion of each root system were cut into 1-cm lengths. Excised roots were placed in triplicate 15-mL disposable test tubes (1 g fresh weight tube^{-1}) containing 2 mL of 50 mM Mes (Tris) buffer (pH 6.5), with or without 10 mM ACC. Tubes were capped with serum stoppers and incubated at 23°C for up to 7.5 h. Ethylene production by excised roots was measured at 2.5-h intervals by injecting a 1-mL gas sample from each tube into a gas chromatograph (Hewlett-Packard, model 5890A) equipped with a flame ionization detector. The 1-mL gas samples were replaced with an equal volume of air at each sampling. The remaining shoots, tubers, and roots were lyophilized for further analyses.

In another experiment, treatments consisted of four harvest dates (21, 28, 35, and 42 DAP), three levels of nutrient solution P (0.5, 2.5, and 5.0 mM), and two inocula (NM or VAM) arranged factorially in a randomized complete block design (three blocks). Measurements (in triplicate) of root ACC_{ox} activity were as described above; however, each tube contained 0.5 g fresh weight of roots and 1 mL of buffer containing 10 mM ACC. Roots were incubated for 2.5 h at 25°C. In addition, 15-mL test tubes (in duplicate), each containing 0.5 g fresh weight of roots in 5 mL of 50 mM Mes (Tris) (pH 6.5) buffer with 25 mM NaCl, were simultaneously incubated for measurement of leached phenolics and POX. Filtrates were collected for the assays, and the roots were lyophilized. The remaining roots for each plant were frozen (−20°C) for later extraction. Phenolics and POX were extracted (mortar and pestle) at 4°C in Mes (Tris) buffer (50 mM; pH 6.5) containing 2.5 mM $MgSO_4$ (1 g fresh weight roots to 10 mL of buffer). Extracts were filtered through a double layer of Miracloth and centrifuged at 15,000*g* for 20 min (4°C). Pellets were discarded and supernatants were used for determinations of POX and total soluble phenolics. Protein was assayed by the method of Bensadoun and Weinstein (6) with BSA as a standard.

Phenolics in 1 mL of leachate or 0.5 mL of root extract were assayed by the method of Amerine and Ough (2). Samples were incubated at 25°C in a shaking water bath for 15 min and read at A_{765}. Total phenolics were quantified with 0.5 mM ferulic acid (in 0.5% ethanol) as a standard. For estimation of polyphenol oxidase activity (5), 100 μL of root extract was added to 3 mL of 5 mM 3,4-dihydroxyphenylalanine, and the change in A_{475} was followed over a 2-min interval. The linear phase was used to calculate PPO activity, which was expressed as the change in absorbance min^{-1} on a grams fresh weight or a milligrams protein basis. Peroxidase activity was determined at A_{470} (5). A 50-μL aliquot of filtrate (25 μL of root extract) was added to 3 mL of 50 mM sodium acetate buffer (pH 5.5) containing 2.5 mM $MgSO_4$, 1.0 mM $CaCl_2$, 0.03% H_2O_2, and 15 mM guaiacol (made fresh before use). Enzyme activity was expressed as the change in absorbance s^{-1} on a grams fresh weight or a milligrams protein basis.

Microscopy

Washed roots were cut into 3-mm pieces, transferred to phosphate buffer (100 mM; pH 6.8) containing 5% glutaraldehyde, and incubated for 2 h at 23°C. The root pieces were rinsed three times with phosphate buffer and then incubated in phosphate buffer containing 2% osmium for 2 h. After three additional rinses with phosphate buffer, roots were plunged into liquid N_2, fractured with a precooled scalpel, and lyophilized overnight. Roots were mounted with double-sided sticky tape on stubs, gold-sputter-coated, and viewed with a Cambridge Stereoscan 150 SEM. Some glutaraldehyde-fixed roots were frozen in liquid N_2 and fractured in an Emitech K1250 SEM Cryosystem. Water was sublimated from the fractured surface of frozen roots for 30 min before examination with the SEM.

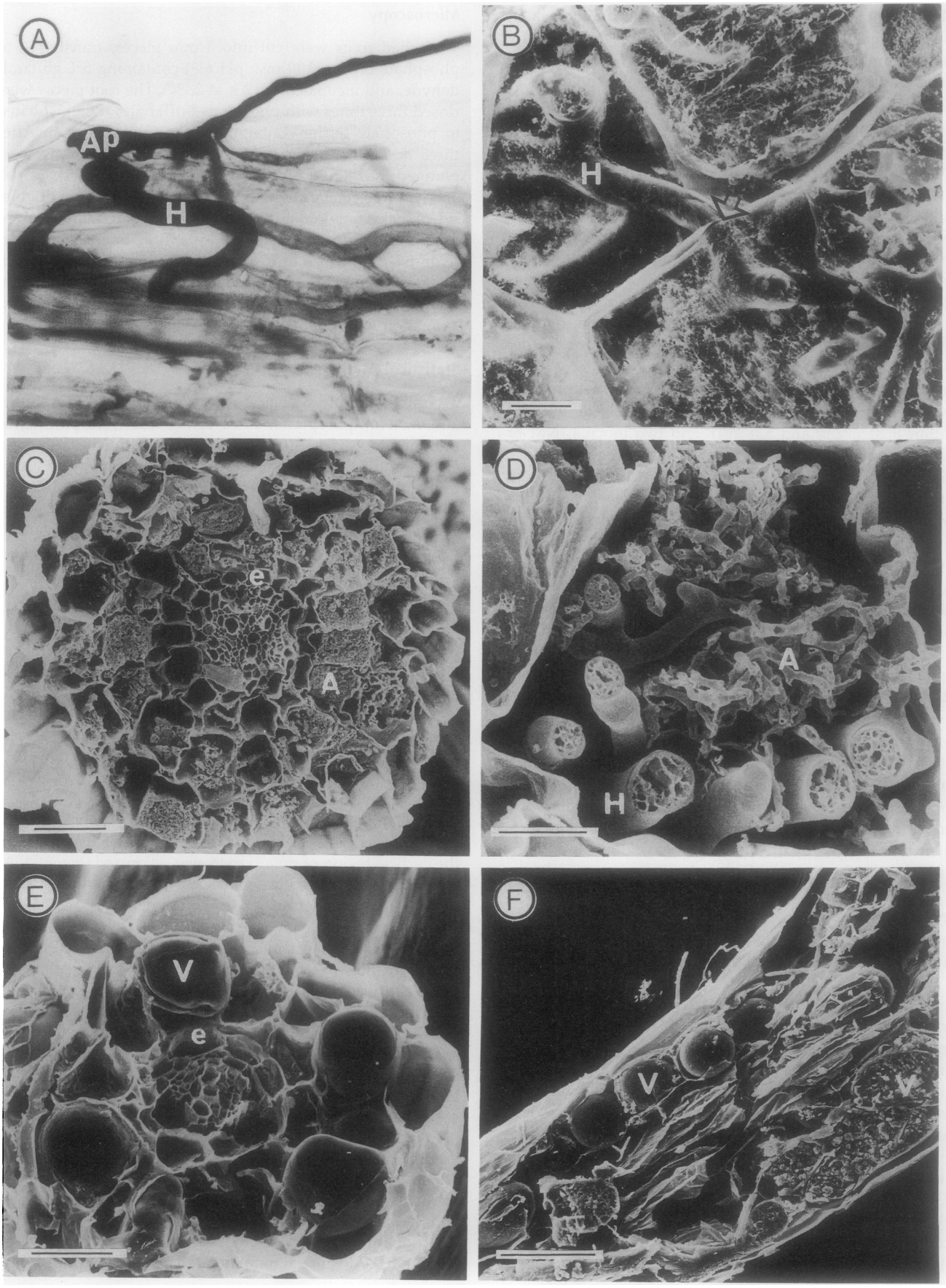
Statistical Analyses

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 arcsin transformed to achieve homogeneity of variance prior to ANOVA. Based on the ANOVA results, regression analysis was used to derive polynomial models describing the various relationships.

RESULTS

The invasive nature of VAM fungi in roots and a perspective of the plant-fungus interface are illustrated by the micrographs in Figure 1. Extraradical hyphae adhering to the epidermal surface produced swollen appressoria, whereas the hypha penetrating through the cell wall constricted to facilitate entry (Fig. 1A). Once through the cell wall, the hypha again attained a swollen appearance before returning to a size more typical of an intracellular hypha (Fig. 1, A and B). Hyphal movement through cortical cell walls appeared less difficult and often did not involve visible alterations in hyphal diameter (Fig. 1B). A high proportion of parenchyma cells in the cortical region of potato roots was colonized (Fig. 1C). As a result, many arbuscules interfaced with cells directly adjacent to the endodermis, thus increasing the potential for nutrient interception by the fungus (15). The fine, dichotamous hyphae of the arbuscule filled most of the cell lumen; however, these terminal structures eventually collapsed, whereas thicker intraradical hyphae continued growth into other cells (Fig. 1D). Although arbuscules and vesicles (Fig. 1E) differ in size and form, both appeared to have a major spatial and physical impact on the infected root cells. Vesicles frequently appeared to fill the cell lumen (Fig. 1, E and F), and counts of vesicles in highly colonized roots often exceeded 250 per cm.

In the initial study, vesicles appeared to predominate over arbuscules and other VAM structures, although no formal attempt was made to quantify either. Moreover, high levels of root colonization were evident in VAM roots at 33 DAP, and these levels were maintained thereafter (e.g. 89 and 76% infection for plants grown on 0.5 and 2.5 mM P, respectively). Although VAM infection stimulated plant growth at either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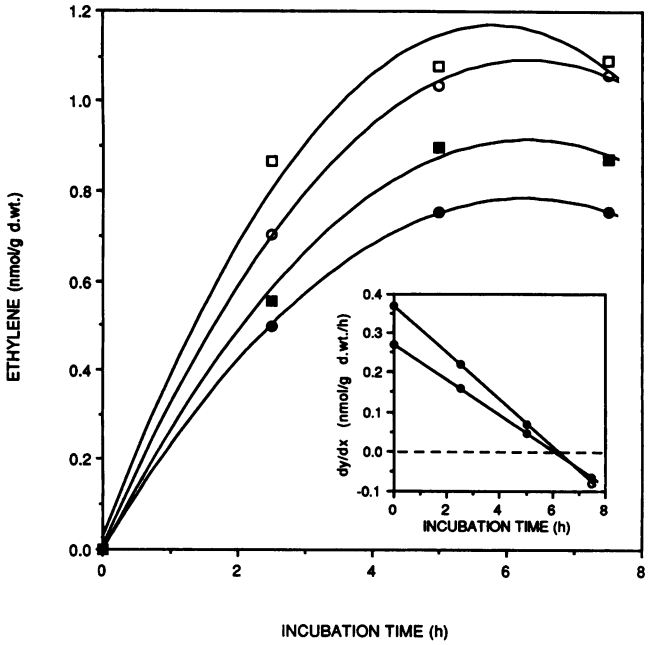

Figure 2. Ethylene production (ACC_{ox} activity) by excised roots from 43-d-old potato plants grown with 0.5 mM (○,●) or 2.5 mM P (□,■) and inoculated with NM (○,□) or VAM (*G. fasciculatum*) (●,■) clover inoculum. Roots were incubated in Mes (Tris) buffer (pH 6.5) at 23°C for 7.5 h. *F*-values for the main effects of VAM, $Time_{linear}$, and $Time_{quadratic}$ were significant at the 0.05, 0.01, and 0.01 levels, respectively. Inset: Change in ethylene production rate for NM (○) and VAM (●) roots during incubation.

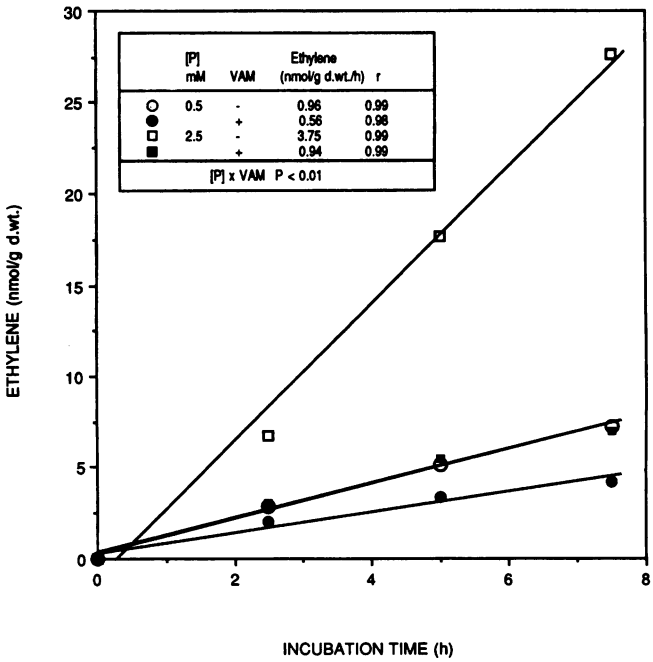

Figure 3. Ethylene production (ACC_{ox} activity) by excised roots from 43-d-old potato plants grown with 0.5 mM or 2.5 mM P and inoculated with NM or VAM (*G. fasciculatum*) clover inoculum. Roots were incubated in Mes (Tris) buffer (pH 6.5) containing 10 mM ACC at 23°C for 7.5 h. *F*-values for the main effects of [P], VAM, and $Time_{linear}$ and the interactions [P] × VAM, [P] × $Time_{linear}$, VAM × $Time_{linear}$, and [P] × VAM × $Time_{linear}$ were all significant at the 0.01 level. Inset: Linear regression and correlation coefficients for ACC_{ox} activity of the various treatments.

level of abiotic P supply (e.g. at 63 DAP, root growth was 2.5 and 3.0 g for VAM plants versus 1.8 and 2.7 g for NM plants for low and high P, respectively; $P < 0.05$), and increased root P concentrations (for low and high P, respectively, 1.4 and 1.7 mg g dry weight^{-1} for VAM plants versus 0.9 and 1.1 mg g dry weight^{-1} for NM plants; $P < 0.01$), the high abiotic P supply had a negative influence on VAM infection ($P < 0.01$). High P nutrition thus somehow altered the host's physiology, rendering the plant more resistant to VAM infection.

Although changes in host compatibility in response to P supply may reflect alterations in many physiological processes (e.g. the flux of nutrients from plant to fungus), the plant's defense response is likely to be a good indicator of the degree of compatibility between host and symbiont (3, 37). Because ethylene biosynthesis by plants is known to be a component of their defense response, the interactive effects of P and VAM infection on ethylene biosynthesis were examined.

For roots without ACC in the incubation buffer, ethylene production from either NM or VAM plants at 43 DAP was relatively low (Fig. 2), and the rate of ethylene production (maximum 0.37 nmol g dry weight^{-1} h^{-1}) decreased over the 7.5-h incubation period (Fig. 2, inset). However, even at these low levels, VAM-infected roots produced significantly less (26%) ethylene over the incubation interval than did NM roots. Root ethylene biosynthesis was much higher when ACC was included in the buffer, indicating that the probable source of ethylene-generating activity was ACC_{ox} (Fig. 3). Consistent with previous findings (12), roots of plants grown with higher levels of P exhibited higher ACC_{ox} activities than those grown with lower P. For plants grown with high P, the inhibitory effect of VAM infection on the ability of roots to convert ACC to ethylene, presumably via ACC_{ox}, was even more pronounced. ACC_{ox} activity of VAM roots was 42 and 75% lower than that from NM roots of plants grown under

Figure 1. Light and scanning electron micrographs of potato roots infected with the VAM fungus *G. fasciculatum*. A, An extraradical hypha penetrating the root epidermis via an appressorium (Ap) and producing intracellular hyphae (H) (magnification ×2000). B, Cryostage SEM of a root showing an intracellular hypha (H) penetrating through a cell wall (arrow) (bar = 10 μm). C, Transverse fracture of a root illustrating the predominance of arbuscules (A) in the cortex, and exclusion of the VAM fungus from the stele by the endodermis (e) (bar = 40 μm). D, An arbuscule and intracellular hyphae within a cortical cell (bar = 10 μm). E, Transverse fracture exposing numerous vesicles and showing exclusion of the fungus from the stele by the endodermis (bar = 40 μm). F, Longitudinal fracture showing adjacent intact vesicles and fractured vesicles (right) (bar = 100 μm).

low and high levels of P, respectively (Fig. 3, inset), reflecting a highly significant [P] × VAM interaction ($P < 0.01$). In this regard, the inhibitory effect of VAM infection on the ability of roots to produce ethylene is somewhat at odds with the potential enhancement of P nutrition for plants provided by the fungus. The P concentration in roots of VAM-infected plants (1.8 mg g dry weight^{-1}) was 50% greater (averaged over P levels and time) than roots of NM plants (1.2 mg g dry weight^{-1}) in this study. Hence, VAM infection somehow interferes with the root's capacity to produce ethylene over and above the main effects of P.

Roots of plants harvested as late as 85 DAP displayed the inhibitory effect of VAM infection on ACC_{ox} activity and, thus, ethylene production (Fig. 4 and inset). By utilizing plants grown with their roots split into two compartments (with or without inoculum), we demonstrated that the inhibition (82%) of ACC_{ox} activity was localized to the VAM-infected side of the root system. Thus, the uninoculated half of roots from a VAM plant had an ACC_{ox} activity comparable to that of the uninoculated roots of a NM plant. Moreover, ACC_{ox} activity in roots exposed to NM inoculum was not significantly different than that from the uninoculated sides of NM or VAM plants.

When leachate from VAM roots was added to the incubation buffer of NM roots, ACC_{ox} activity and ethylene accumulation were inhibited by 75% relative to NM controls (Fig. 5 and inset). Furthermore, VAM-root leachate reduced the level of ACC_{ox} activity of NM roots to a level equivalent to that of VAM roots. No such inhibitory effect of VAM-root leachate was apparent when added to the VAM roots. Hence, it appeared that a potent water-soluble factor, unique to VAM roots, was responsible for the inhibition of ACC_{ox} activity. The results of these studies suggest that VAM fungi and increasing abiotic P supply may influence the ethylene component of a plant's defense response in opposite ways, despite the fact that the net effect of either treatment was to enhance the plant's P nutrition.

In addition to the effects of abiotic P supply and VAM infection on ACC_{ox} activity, other components of the defense response were examined in a separate study. Specifically, changes in phenolic production and the activities of POX and PPO in roots, key components of root defense responses to pathogens (5, 37), were quantified as a function of infection and level of P nutrition. These components may reflect the degree of resistance expressed by plants to VAM infection. From the data in Figure 6, it is evident that VAM colonization of roots in this study was less than that observed previously. The lower infection level was likely a consequence of less inoculum (50 versus 100 g in the previous studies), earlier harvest dates, and a higher average concentration of P in roots (e.g. low P NM plants averaged 1.47 mg P g dry

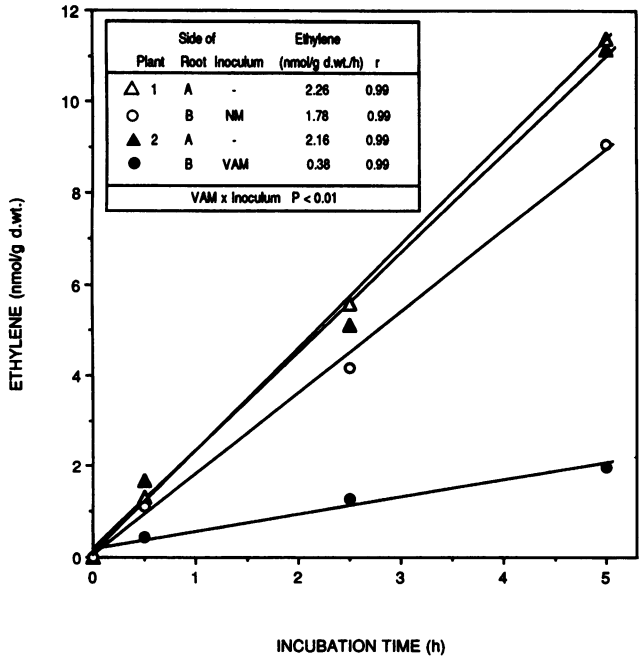

Figure 4. Ethylene production (ACC_{ox} activity) by excised roots from 85-d-old potato plants that had one-half of their roots uninoculated and the other half inoculated with NM or VAM (*G. fasciculatum*) clover inoculum. Roots were incubated in 50 mM Mes (Tris) buffer (pH 6.5) containing 10 mM ACC at 23°C for 5.0 h. *F*-values for the main effects of VAM, inoculum, $Time_{linear}$ and the interactions VAM × inoculum, VAM × $Time_{linear}$, and inoculum × $Time_{linear}$ were significant at the 0.05, 0.01, 0.01, 0.05, 0.10, and 0.01 levels, respectively. Inset: Linear regression and correlation coefficients for ACC_{ox} activity of the various treatments.

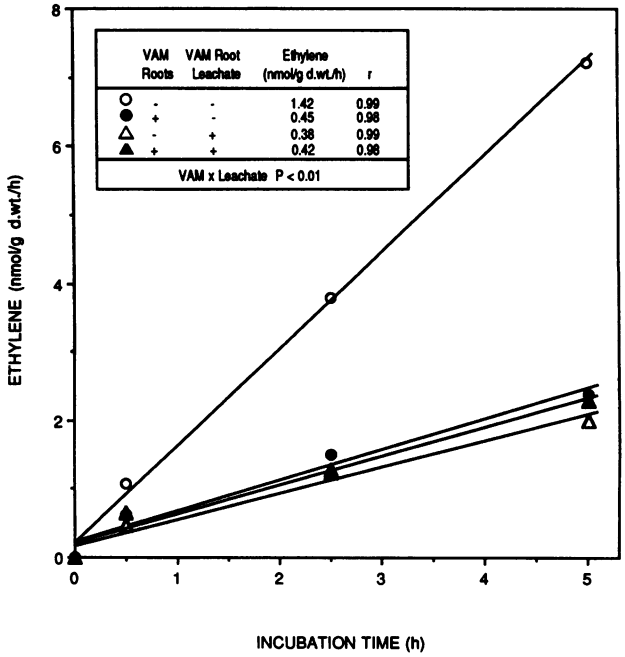

Figure 5. Ethylene production (ACC_{ox} activity) by excised roots from 83-d-old potato plants inoculated with NM or VAM (*G. fasciculatum*) clover inoculum and treated with 100 μL of incubation buffer or VAM root leachate. Roots were incubated in Mes (Tris) buffer (pH 6.5) containing 10 mM ACC at 23°C for 5.0 h. *F*-values for the main effects of VAM, leachate, and $Time_{linear}$, and the interaction of VAM × leachate were all significant at the 0.01 level. Inset: Linear regression and correlation coefficients for ACC_{ox} activity of the various treatments.

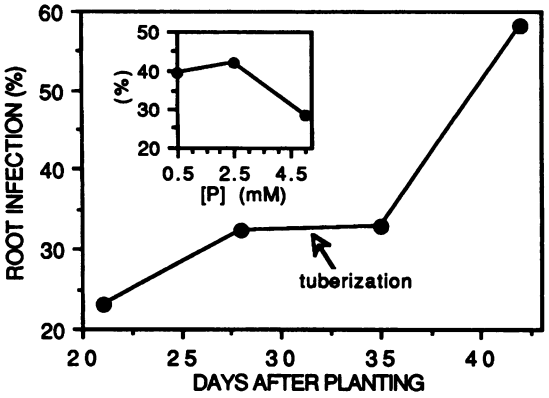

Figure 6. Main effect of time on percent roots infected by *G. fasciculatum* for potato plants grown with 0.5, 2.5, or 5.0 mM P, and inoculated with VAM clover pot culture. *F*-values for the linear, quadratic, and deviation trends were significant at the 0.01, 0.05, and 0.05 levels, respectively. Inset: Main effect of increasing abiotic P supply on percent of roots infected. *F*-values for the linear and deviation trends were significant at the 0.01 and 0.05 levels, respectively.

weight^{-1} versus 0.98 mg P g dry weight^{-1} in the previous study). Increasing the abiotic P supply from 0.5 to 5.0 mM significantly reduced VAM colonization of roots (Fig. 6, inset); however, plants receiving 2.5 mM P showed the same level of infection as those receiving 0.5 mM P in this study. Moreover, fewer vesicles were present in the roots, and arbuscules and coiled hyphae were more prevalent as compared with that observed in the previous study. Although the level of infection increased from 21 to 42 DAP, infection levels remained constant between 28 and 35 DAP. It is interesting that this period coincided with tuberization of the VAM plants; tubers first appeared at 35 DAP. The reduced ability of VAM fungi to increase colonization during tuberization may be a consequence of a change in source to sink relationships favoring an increased flux of photoassimilates to tubers at the expense of roots and/or fungi, as has been suggested (22). On the other hand, our observations may reflect a more direct increased resistance response by the plant that is linked to developmentally induced changes in host compatibility.

With the exception of 35-d-old VAM roots, root ACC_{ox} activity decreased over the 42-d growth period (Fig. 7A), and the levels of activity in this study were higher than those observed previously (Fig. 3). The higher activities probably relate to higher root P concentrations in this study. Again, roots of plants supplied with higher levels of P had higher ACC_{ox} activities than those grown with low P. For every 1 mM increase in P supplied to plants during growth, root ACC_{ox} activity increased by 0.292 nmol g dry weight^{-1} h^{-1} (ACC_{ox} = 0.292[P] + 4.889, r = 0.99, $P < 0.05$). As in the previous study, VAM infection significantly decreased the ability of roots to produce ethylene from ACC, although the response was somewhat less. In this regard, the extent of inhibition of ACC_{ox} activity by VAM reflected the lower VAM infection levels in general, and especially the lapse in root colonization between 28 and 35 DAP. Unlike the earlier study, the [P] × VAM interaction on ACC_{ox} activity was not significant.

The effect of VAM infection on production of leachable phenolics from roots is illustrated in Figure 7B. Levels of leachable phenolics were not significantly affected by P nutrition or an interaction between VAM and [P]. Hence, Figure 7B shows leachable phenolics averaged over the three levels of P. The amount of leachable phenolics increased signifi-

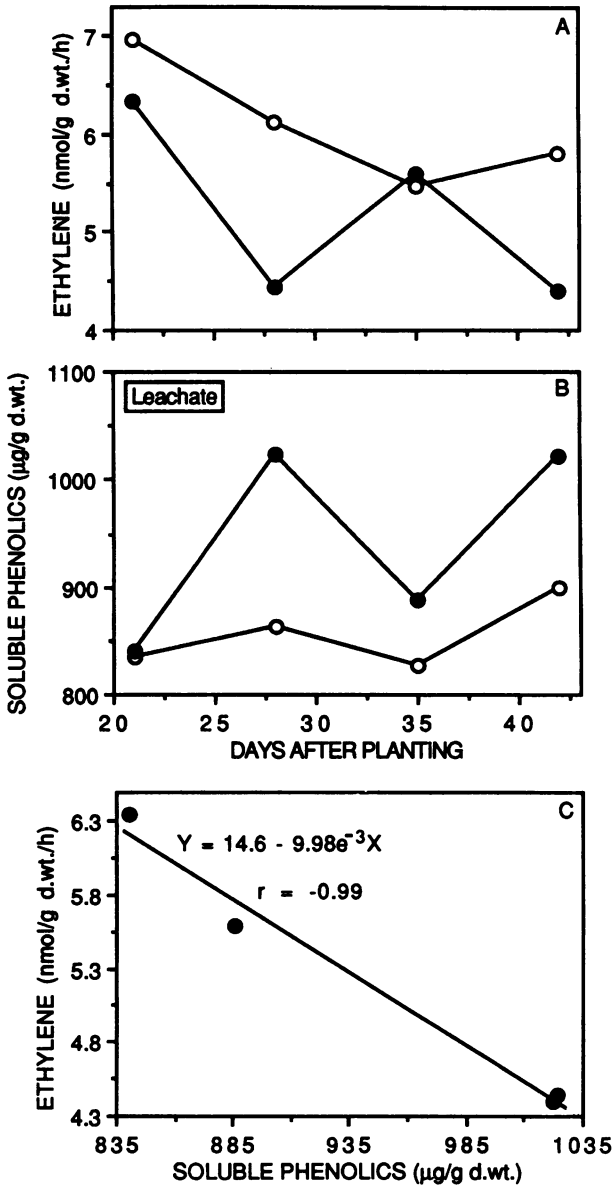

Figure 7. Time course of root ACC_{ox} activity (A) and soluble phenolic levels (B) in leachate of roots excised from NM (O) and VAM (*G. fasciculatum*) (●) infected potato plants. Roots were incubated in Mes (Tris) buffer at 25°C for 2.5 h. For ACC_{ox} activity, the buffer contained 10 mM ACC. *F*-values for the main effects of VAM and $Time_{linear}$ were significant at the 0.10 and 0.05 levels, respectively, for ACC_{ox} activity; for soluble phenolics, VAM, $Time_{linear}$, and $Time_{deviations}$ were significant at the 0.05, 0.10, and 0.05 levels, respectively. C, Correlation between ACC_{ox} activity and leachate phenolic level for VAM roots. For NM roots, the correlation between ACC_{ox} activity and soluble phenolics was not significant (r = −0.19).

cantly with increasing DAP. Furthermore, VAM-infected roots leached significantly more phenolics than NM roots at each harvest date, although the differences between VAM and NM plants were lower at 35 DAP, reflecting the lack of increase in root infection during tuberization. For total extracted phenolics, the main effects of P and VAM infection were not significant; however, a highly significant VAM × $Time_{linear}$ interaction ($P < 0.01$) indicated that the increase in leached phenolics may be partly due to a 56% greater synthesis of phenolics by the VAM roots (4.3 versus 6.7 μg phenolics g fresh weight^{-1} d^{-1} for NM and VAM roots, respectively). On a grams fresh weight basis, the proportion of leached to extractable soluble phenolics was also significantly ($P < 0.01$) greater for VAM roots relative to NM roots (average of 29 and 34% for NM and VAM roots, respectively), which would also contribute to the higher levels of phenolics in leachates from VAM roots. A significant inverse correlation ($r = -0.99$) between the amount of leached phenolics and ACC_{ox} activity was apparent for VAM roots (Fig. 7C), whereas no such relationship existed for NM roots (data not shown; $r = -0.19$). These data suggest that the soluble component from VAM roots that inhibits ACC_{ox} activity (Fig. 5) may be of a phenolic nature.

Root polyphenol oxidase activity, which has been negatively correlated with susceptibility to pathogen invasion (5), increased to a maximum of 8.3 (ΔA_{475} g fresh weight^{-1} min^{-1}) at 42 DAP in NM roots (Fig. 8). PPO activity on a fresh weight basis was not affected by VAM infection through most of the study, although VAM roots at 35 DAP had significantly higher activity ($LSD_{0.05}$ = 1.07 ΔA_{475} g fresh weight^{-1} min^{-1}) than NM roots. The difference at 35 DAP may relate to the aforementioned lapse in VAM colonization (Fig. 6) due to tuberization between 28 and 35 DAP. The specific activity of PPO (data not shown) was significantly ($P < 0.01$) less (15%) in VAM roots due to an 18% higher rate of soluble protein accumulation in VAM roots, relative to

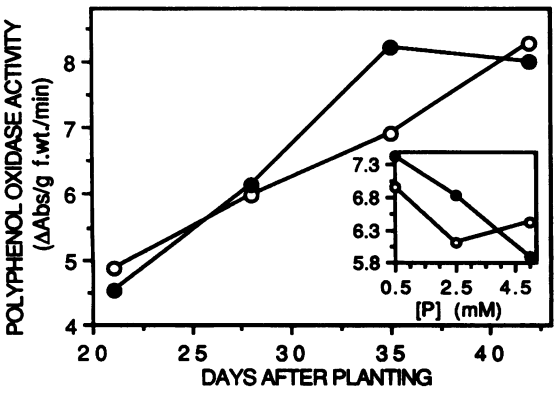

Figure 8. Main effect of time on polyphenol oxidase activity of roots from NM (O) or VAM (*G. fasciculatum*) infected (●) potato plants that were grown with 0.5, 2.5, or 5.0 mM P. *F*-values for the main effect of $Time_{linear}$ and the interaction of VAM × $Time_{quadratic}$ were significant at the 0.01 and 0.05 levels, respectively ($LSD_{0.05}$ = 1.07). Inset: Main effect of increasing abiotic P supply on polyphenol oxidase activity of roots for NM or VAM plants. *F*-value for the linear trend was significant at the 0.01 level.

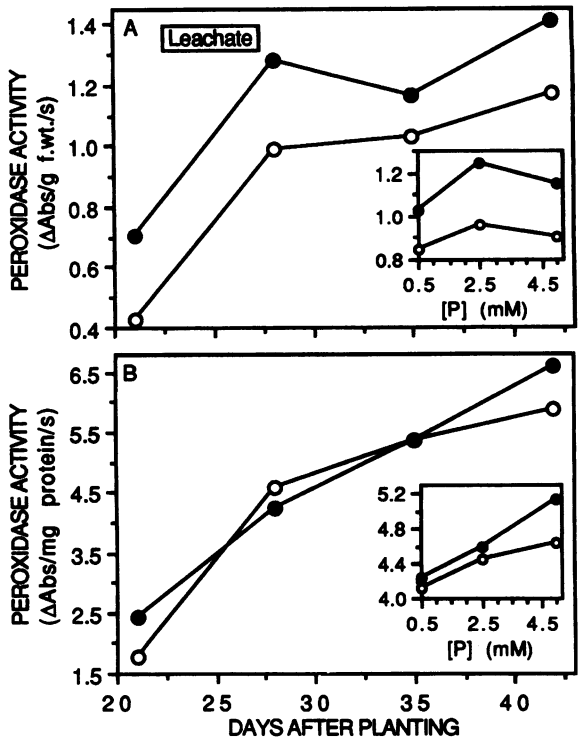

Figure 9. Time course of peroxidase activity in leachate from roots excised from potato plants inoculated with NM (O) or VAM (*G. fasciculatum*) (●) clover inoculum, and grown with 0.5, 2.5, or 5.0 mM P. Roots were incubated in 50 mM Mes (Tris) buffer (pH 6.5) containing 25 mM NaCl at 25°C for 2.5 h. A, POX on a grams fresh weight basis, *F*-values for the main effects of VAM, $Time_{linear}$, $Time_{quadratic}$, $Time_{deviation}$, and $[P]_{deviation}$ (inset) were all significant at the 0.01 level. B, POX specific activity, *F*-values for the main effects of $Time_{linear}$, $Time_{quadratic}$, and $[P]_{linear}$ (inset) and the interaction of VAM × $Time_{quadratic}$ were significant at the 0.01, 0.01, 0.01, and 0.05 levels, respectively.

that for NM roots (VAM × $Time_{linear}$ was significant at $P < 0.01$). Oddly, whereas increasing abiotic P supply stimulated root ACC_{ox} activity and decreased VAM infection, suggesting a greater potential for a defense response, increased abiotic P supply had a negative influence on PPO activity on a protein (data not shown) and fresh weight basis (Fig. 8, inset). Hence, our results provide no evidence supporting a role for PPO in the P-induced resistance response to VAM infection.

Peroxidase activity of roots, either in leachates (Fig. 9) or extracts (data not shown), increased over the harvest period. The specific activity of POX was 2.3-fold (NM) or 2.9-fold (VAM) greater in leachates relative to extracts. This enrichment of POX activity in leachate likely reflects the higher extracellular specific activity of POX seen in many plant tissues (21). On a fresh weight basis, VAM infection increased the POX activity of leachates, but had no effect on POX activity in extracts from roots. Hence, infection significantly ($P < 0.01$) increased the proportion of leachable to extractable POX activity (27 and 34%, respectively, for NM and VAM roots). This result was similar to that established for phenolics and may reflect a role for some of these phenolics as substrates for extracellular POX (which is involved in lignin

biosynthesis). In contrast, VAM infection did not appear to have a stimulatory influence on the specific activity of extracellular POX (Fig. 9B), because the specific activity of POX in VAM root extracts was less than that of NM root extracts (data not shown). These results are also similar to those established for PPO activity and probably reflect the greater protein content of roots in response to VAM infection. Because the proportion of leached POX to extracted POX activity on a milligrams protein basis was significantly increased by VAM infection (similar to that of POX on a fresh weight basis), it is likely that VAM fungi are somehow able to enhance POX activity in potato roots.

An influence of abiotic P supply on POX activity was also observed, and this effect was similar to that for ACC_{ox} activity. The activity of POX in root leachates was 10% greater on a fresh weight basis (Fig. 9A, inset) and 17% greater on a protein basis (Fig. 9B, inset), at the highest relative to the lowest level of P. Activity of extracted POX was not significantly influenced by VAM infection as mentioned, but POX activity in extracts (fresh weight basis) was increased slightly by higher levels of abiotic P supply ($P < 0.10$), similar to that for leachable POX activity. Electrophoretic studies of POX in our lab indicated that neither abiotic P supply nor VAM infection altered the root isozymic patterns (unpublished data). This is not unusual because others have observed that roots, unlike other tissues, often constitutively express the plant's complete complement of POX isozymes (25).

DISCUSSION

The negative effects of P deficiency on plant growth have long been documented, and there is an increasing body of knowledge on the physiological changes that occur in response to P deprivation (8, 18). These changes have been characterized as constituting a PSI metabolism (18). A detailed characterization of the influence of VAM infection on mineral physiology of potato during development of P deficiency showed that although low P plants had significantly lower rates of root respiration, the activities of root microsomal ATPases and extracellular acid phosphatases were higher (McArthur and Knowles, unpublished data). Concomitant with these heightened enzyme activities were lower activities of ACC_{ox} (Fig. 3, inset) and extracellular POX (Fig. 9B, inset) and higher levels of root infection by the VAM fungi (Fig. 6, inset). As abiotic P supply increased, these trends reversed, suggesting that the plant's susceptibility to VAM infection is somehow linked to PSI metabolism of the roots. In this regard, choices between defense against infection by VAM fungi and adequate P nutrition appear to have been determined. It is interesting that the effects of increased nitrate supply on root ethylene biosynthesis and the extent of nodulation in legumes (26) appear analogous to the effects of P on ethylene biosynthesis and VAM infection.

In many host-parasite interactions, ethylene is usually associated with or induces various biochemical pathways considered integral to a plant defense response. For example, increased levels of ethylene produced by the plant upon infection can stimulate lignification of cell walls through increasing phenylalanine ammonia-lyase and extracellular POX activities. Enzymes involved in the biosynthesis of phytoalexins (e.g. chalcone synthase) and other antimicrobial compounds (e.g. PPO), as well as chitinases, are also enhanced in response to increases in ethylene during infection (7, 13, 37). Because increasing abiotic P supply enhanced both ethylene production and extracellular POX activities in our studies, a similar defense response by potato roots may have been the basis for the observed P-induced resistance to VAM infection. Indeed, considering the aggressive growth and invasive association that VAM fungi display within potato roots (Fig. 1), it seems likely that the plant would have some mechanism to limit invasion if abiotic P supply was not limiting to plant growth.

It is interesting that studies by others have shown that VAM infection initially stimulates a high level of cell wall-bound POX (34) and chitinase (33) activities, which, after about 20 DAP, decline to levels below those of controls. In those studies, plants were grown with only one level of abiotic P supply (0.06 mM), and although tissue P concentrations were not reported, they would likely decline over time under such conditions. Hence, the initial stimulation observed in chitinase and POX activities in those studies may have reflected the plant's defense response to infection. With further plant growth and declining tissue P concentration, a shift away from defense activities by plant roots to PSI metabolism and, therefore, to an increased compatibility to the VAM infection would be expected. Because these resistance-response enzymes are inducible by ethylene, their lower activities may have been in response to a VAM-induced suppression of ACC_{ox} activity, similar to that characterized in our studies.

Impairment of ACC_{ox} activity has been observed in other host-parasite interactions, such as during infection of grapefruit by *Penicillium digitatum* (1). Related studies have shown that inhibition of either ACC synthase or ACC_{ox} activity can result in redirection of *S*-adenosylmethionine into the polyamine biosynthetic pathway and, as a consequence, result in further reductions of ACC_{ox} activity (14). Increased barley leaf polyamine levels implicate a parasite-induced enhancement of polyamine biosynthesis in the "green island" (10) effect and a coincident decrease in ethylene biosynthesis by leaves. Similarly, VAM infection has been shown to increase the level of intermediates (e.g. arginine) and activity of enzymes involved in polyamine biosynthesis (11). A potential role for polyamines in the VAM-induced decrease in ethylene production of potato roots merits investigation.

The localized inhibition of ACC_{ox} activity by VAM infection (Fig. 4, inset), inhibition of ACC_{ox} activity by VAM root leachates (Fig. 5, inset), and an inverse correlation (Fig. 7C) between ACC_{ox} activity and total soluble phenolics from VAM root leachates suggest a role for phenolic metabolism in the mechanism by which VAM fungi are able to suppress the plant's defense response. An influence of VAM fungi on phenolic metabolism was suggested by the higher level of phenolics in root leachate (Fig. 7B) and in root extracts (24). An important function for specific phenolics produced by roots, such as ferulic acid or cinnamyl alcohol, is as a substrate for extracellular POX in the lignin biosynthetic pathway. Lignin acts to strengthen cell walls, thereby providing a physical barrier to microbial invasion. Although not examined in our studies, observations by others suggest that roots

show cell wall apposition to VAM hyphae; however, lignification is not enhanced at infection sites or even later after the infection has been well established (9, 16). It is interesting that ferulic acid and its conjugate 1-O-feruloyl-β-D-glucose have been reported to be potent inhibitors of ACC_{ox} activity (30). If such phenolic compounds are involved in the observed inhibition of ACC_{ox} activity by VAM infection, a role for a conjugate of ferulic acid seems more likely, because ferulic acid can inhibit plant P uptake and VAM fungal growth (23, 36). It is worth noting that some fungi are capable of metabolizing plant phenolic compounds or producing phenolics themselves (31); however, the capabilities of VAM fungi in this regard have not been investigated.

Apart from the P-induced decrease in VAM infection of roots, it appeared that the host plant was exerting a certain degree of developmentally linked control over fungal growth (Figs. 6, 7, and 8). The lapse in the rate of infection from 28 to 35 DAP coincided with tuberization and was very similar to that observed during flowering of chrysanthemum (22). In the latter case, the initiation of flower buds coincided with a transient reduction in VAM infection. It was found that bud initiation correlated with decreased levels of root metabolites and exudates, and, thus, the inhibitory effect of flowering on VAM growth was attributed to a plant influence on fungal nutrition. Although such an effect was not investigated in our study, VAM roots at 35 DAP had a level of ACC_{ox} activity (Fig. 7A) that was comparable to that of NM roots. Furthermore, the low ACC_{ox} activity of VAM roots during tuberization coincided with lower levels of soluble phenolics (Fig. 7B) and significantly higher root PPO activity (Fig. 8). It is interesting that some authors have suggested a role for endogenous ethylene production in tuber initiation (35), and such ethylene production may have inadvertently triggered a defense response in the present study.

The results of our studies demonstrate that several components of a potato plant's defense response to microbial invasion are positively influenced by abiotic P supply and suggest a role for the defense response in the observed P-induced decrease of VAM infection in roots. Under conditions of inadequate available P (plant P deficit), the potato plant shifts its priorities to P acquisition (PSI metabolism) and roots become more susceptible to VAM invasion. In this regard, ethylene production and phenolic metabolism in VAM roots appear to be of particular significance, although details of their interaction require further investigation. Elucidation of the metabolic basis for these changes in plant response will provide further insight to the mechanisms of compatibility of this symbiosis and will possibly be relevant to mechanisms of resistance in other plant-parasite interactions.

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