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A Vesicular Arbuscular Mycorrhizal Fungus (*Glomus intraradix*) Induces a Defense Response in Alfalfa Roots[1]

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Flavonoid accumulation and activities of phenylalanine ammonia-lyase (PAL), chalcone isomerase (CHI), and chitinase were followed during early colonization of alfalfa roots (*Medicago sativa* L. cv Gilboa) by vesicular arbuscular (VA) fungi (*Glomus intraradix*). Formononetin was the only flavonoid detected that showed a consistent increase in the inoculated roots. This increase depended only on the presence of the fungus in the plant rhizosphere; no colonization of the root tissue was required. CHI and chitinase activities increased in inoculated roots prior to colonization, whereas the increase in PAL activity coincided with colonization. After reaching a maximum, activities of all enzymes declined to below those of uninoculated roots. PAL inactivation was not caused by a soluble inhibitor. Our results indicate that VA fungi initiate a host defense response in alfalfa roots, which is subsequently suppressed.

VA fungi form mycorrhiza with plant roots. The VA fungi are obligate symbionts and are not host specific. VA mycorrhiza occur in about 80% of plants (Bonfante-Fasolo, 1987).

Plant tissue infected by pathogenic fungi responds with significant metabolic changes (Dixon and Harrison, 1990). Secondary metabolites, some with antibiotic activities (phytoalexins) (Dixon et al., 1983), accumulate, and several new proteins are produced (Van Loon, 1985). Some of these proteins, e.g. chitinases and β-1,3-D-glucanases, have the potential to hydrolyze polymers of the fungal cell wall (Mauch et al., 1988). Although VA fungi are not considered to induce typical defense responses in host plants (Codignola et al., 1989), chitinase (Spanu et al., 1989) and peroxidase (Spanu and Bonfante-Fasolo, 1988) activities were induced in leek roots during early stages of root colonization by VA fungi. Also, soybean roots colonized by *Glomus mosseae* or *Glomus fasciculatus* accumulated more glyceollin I, coumestrol, and daidzein than nonmycorrhizal roots (Morandi et al., 1984). These results indicate that VA fungi can initiate a defense-like response when colonizing some host roots.

Alfalfa cell suspensions respond to fungal elicitors by weak induction of chitinase and glucanase activities. Likewise, enzymes from the phenylpropanoid pathway are activated, and their products accumulate (Dalkin et al., 1990). Some of these products have antimicrobial activities (Dixon et al., 1983), and some apparently function as signal molecules in symbiotic interactions (Phillips, 1992). The fact that all Myc$^-$ pea mutants induced by chemical mutagenesis also were Nod$^-$ (Gianinazzi-Pearson et al., 1991) suggests that a common early infection event is required in both types of symbiosis. The role of flavonoids as signal molecules in the establishment of the mycorrhizal plant is not clear, but some flavonoids enhance germination and hyphal growth of VA fungi (Gianinazzi-Pearson et al., 1989; Tsai and Phillips, 1991) and promote VA fungal colonization of white clover roots (Siqueira et al., 1991).

In the present study, the influence of *Glomus intraradix* on flavonoid production by alfalfa roots was studied during the early stages of colonization. At the same time, the ability of the fungi to induce PAL, CHI, and chitinase activities during the infection process was measured.

MATERIALS AND METHODS

Plant Culture

Pregerminated, surface-sterilized seedlings of *Medicago sativa* L. cv Gilboa were grown *Rhizobium* free under microbiologically controlled conditions (Patterson et al., 1990) in 3.5-kg pots (100 seedlings per pot) containing autoclaved sand. Plants were transferred to a phytotron (16-/8-h day/night cycle and 27/22°C day/night temperature). Natural day length (April–November) was extended with incandescent illumination (6 μE m^{-2} s^{-1}) at plant level. All plant ages in this study were recorded as days after germination. The pots were watered to field capacity twice a week with a modified Johnson solution (Johnson et al., 1957) containing 8 mM NH_4NO_3 and 0.2 mM KH_2PO_4. In treatments referred to as high-P treatments, the KH_2PO_4 concentration was 2 mM. A commercial inoculum containing spores of *Glomus intraradix* (obtained from Native Plant, Inc., Salt Lake City, UT) (10–40 spores per seedling) was placed in a layer 4 cm below the

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Abbreviations: CHI, chalcone isomerase; *d*, doublet; *dd*, double doublet; Eu, enzyme unit; FAB, fast atom bombardment; δ_H, chemical shift of proton; 1H, one proton, etc.; H-1, proton on carbon one, etc.; *m*, multiplet; MeOH, methanol; *m/z*, mass/charge ratio; PAL, phenylalanine ammonia-lyase; *s*, singlet; VA, vesicular arbuscular.

soil surface in the pots prior to planting. In experiments in which the physiological effects of the inoculum components were studied, the carrier containing the spores was dissolved in sterile distilled water and sieved through a 26-μm nylon mesh. The wet-sieved inoculum contained the solid particles and accompanying microorganisms and spore extracts but no viable VA fungal spores. The treatments designated "with carrier" refer to plants grown in pots treated with the sieved carrier applied in the same manner as described above for mycorrhizal inoculation.

At each harvest, the roots were immediately frozen in liquid nitrogen and kept at −70°C until assayed. In some experiments, the roots were separated into upper (0–5 cm below the soil surface) and lower (5–12 cm below soil surface) portions. Initial experiments showed that mycorrhizal colonization was restricted to the upper root portions throughout the 26 d of growth.

Dry weight of plants was measured after plant samples (shoots or roots) were dried at 80°C for 48 h. The experiment was conducted with four replicates, and each replicate contained 10 plants. All other assays were conducted in two to three replicates, each replicate containing roots from four pots (400 seedlings). Following analysis of variance, treatment means were compared by LSD, and when appropriate, single degree of freedom σ^2 values for populations were calculated. All statistical tests were conducted using $P = 0.05$. Mycorrhizal colonization was estimated colorimetrically by measuring glucosamine released from fungal chitin (Hepper, 1976).

Isolation and Purification of Flavonoids

Frozen, ground root tissue (2 g) was thawed in 30 mL of 80% MeOH, extracted for 2 h at 4°C, and centrifuged at 10,000*g* at 4°C for 25 min. Supernatant was partitioned twice against hexane, freeze dried, and dissolved in 200 μL of 50% MeOH. Aliquots (100 μL) were loaded onto a Waters HPLC system (Millipore Corp., Milford, MA) fitted with a 125- × 4-mm LiChrospher 100 RP-18 column and eluted at 0.5 mL min^{-1} from 0 to 65 min with a linear gradient from 69:30:1 (v/v/v) water:MeOH:acetic acid to 99:1 (v/v) MeOH:acetic acid. The analysis continued isocratically at that concentration for another 10 min. Flavonoids from 15 g of frozen tissue were purified to homogeneity for identification. Extraction was performed as described above. Eluant fractions were collected every 60 s with a fraction collector (FRAC-100; Pharmacia), combined when associated with absorbance peaks, and dried under vacuum. Closely eluting contaminants were removed during a second HPLC separation on a 250- × 10-mm Econosyl C_{18} semipreparative column with an elution rate of 4 mL min^{-1} with appropriate concentrations of MeOH for each peak.

Identification of Flavonoids

UV/visible spectral shift analyses (Mabry et al., 1970) were done with a Lambda 6 dual-beam spectrophotometer (Perkin-Elmer, Norwalk, CT). One-dimensional proton NMR measurements were done in [U-^{2}H]MeOH (Maxwell and Phillips, 1990), and FAB-MS measurements were made on samples dissolved in MeOH (Maxwell and Phillips, 1990). Data obtained were compared with authentic standards and analyzed by each of the above procedures.

Enzyme Extraction and Assay

All enzyme assays were performed with crude extracts. PAL was assayed by measuring the conversion of L-Phe (Sigma) to cinnamic acid at 270 nm (Lamb et al., 1979). One Eu is defined as the formation of 1 μM cinnamic acid s^{-1} kg^{-1} of enzyme. CHI was assayed by measuring the rate of disappearance of the chalcone isoliquiritigenin (synthesized according to the method of Nadkarni and Wheeler [1938]) at 400 nm (Dixon and Bendall, 1978). One enzyme unit is defined as the disappearance of 1 mM isoliquiritigenin s^{-1} kg^{-1} of enzyme. The chitinase assay was based on a colorimetric determination of monomeric GlcNAc released from colloidal chitin (Boller and Mauch, 1988). One enzyme unit is defined as the release of 1 mg of GlcNAc s^{-1} kg^{-1} of enzyme. Percentage of activity of control was calculated by considering the activity of the untreated control from each sampling as 100% activity.

RESULTS

Effect of VA Fungi on Plant Growth

Growth of plants in pots with the commercial inoculum or wet-sieved carrier (no viable VA fungal spores) or untreated control plants was compared 14 and 21 d after germination (Table I). Except for a minor transient increase in shoot dry weight in the control treatment with carrier, no differences could be found in the growth characteristics of the plants (shoots or roots) within the growth period used in our experiments.

Flavonoid Identification

HPLC chromatograms of MeOH extracts from alfalfa roots revealed four major and several minor peaks that were related to different stages of VA mycorrhizal establishment in the inoculated roots. Peak 4 (Fig. 1) on chromatograms of extracts of inoculated roots showed a consistent increase during infection of the roots by the VA fungi (Fig. 2) compared with

Table I. *Dry weight of alfalfa plants treated with commercial mycorrhizal inoculum or wet-sieved carrier of the inoculum (no viable VA fungal spores) and untreated controls*

Values are means of four replicates, each replicate containing 10 plants. Different letters within columns show significant differences according to Duncan's multiple range test ($P = 0.05$).

Treatment (carrier/spores)	14 d after Germination		21 d after Germination	
	Shoot	Root	Shoot	Root
	g^{-1} dry wt			
−/−	0.063 b	0.118 a	0.074 a	0.153 a
+/−	0.082 a	0.112 a	0.089 a	0.151 a
+/+	0.068 b	0.120 a	0.088 a	0.161 a

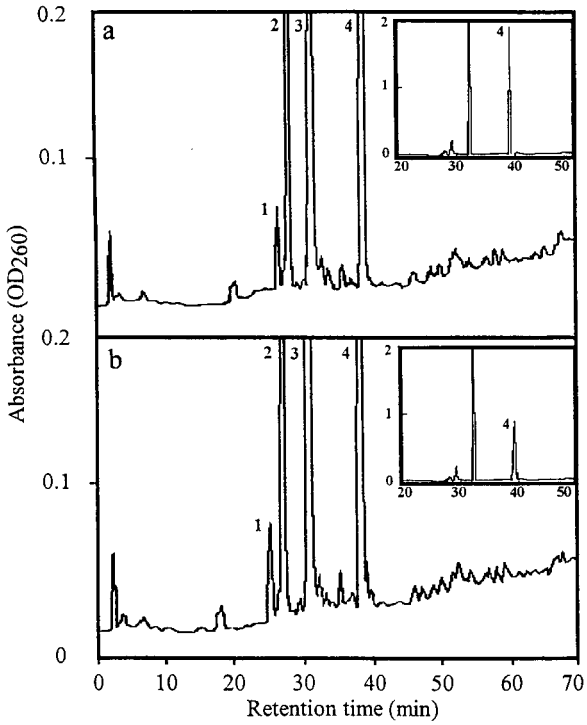

Figure 1. HPLC characteristics at A_{260} of MeOH extracts from 18-d-old alfalfa roots inoculated with *G. intraradix* (a) or uninoculated (b). Peaks 3 and 4 are identified here as a formononetin-7-*O*-glucoside and formononetin, respectively. Inset, Full-scale A_{260} of the four major peaks (retention time, 20–50 min).

peaks from extracts of uninoculated controls. Peak 3 (Fig. 1) showed a transient increase at d 21 (Fig. 2).

Absorbance measurements of peak 3 (Fig. 1) produced a UV-visible spectrum similar to that of formononetin-7-*O*-glucoside, and peak 4 (Fig. 1) produced a spectrum similar to that of formononetin (Mabry et al., 1970). UV-visible spectroscopic shift analysis of peak 4 and authentic formononetin gave equivalent results, comparable to published data for formononetin (Mabry et al., 1970).

Peak 3 had the following proton resonances: δ_H ppm ([U-^{2}H]MeOH); 8.23 (1H, *s*, H-2), 8.17 (1H, *d*, J = 8.5 Hz, H-5), 7.51 (2H, *d*, J = 5.0 Hz, H-2′, 6′), 7.29 (1H, *s*, H-8), 7.21 (1H, *dd*, J = 1.9, 2.4 Hz, H-6), 6.98 (2H, *d*, J = 8.5 Hz, H-3′, 5′), 5.11 (1H, *d*, J = 3.7 Hz, H-1″), 3.91 (1H *d*, J = 12 Hz, H-6″a), 3.83 (3H, *s*, OCH_3), 3.68 (1H, *dd*, J = 12, 13 Hz, H-6″b), 3.6 to 3.4 (4H, *m*, H-2″-5″). FAB-MS analyses produced major ions at m/z = 431 and 269. The data obtained for peak 3 were comparable with previously published results for formononetin-7-*O*-glucoside (Dakora et al., 1993) and consistent with those obtained with authentic formononetin-7-*O*-glucoside. We concluded that peak 3 was formononetin-7-*O*-glycoside. The sugar moiety was not identified because of limited amounts of the compound.

Peak 4 and authentic formononetin had identical proton resonances: δ_H ppm ([U-^{2}H]MeOH); 8.11 (1H, *s*, H-2), 7.99 (1H, *d*, J = 9.1 Hz, H-5), 7.45 (2H, *d*, J = 9.1 Hz, H-2′, 6′), 6.96 (2H, *d*, J = 9.2 Hz, H-3′, 5′), 6.91 (1H, *dd*, J = 3.1, 4.9 Hz, H-6), 6.89 (1H, *s*, H-8), 3.83 (3H, *s*, OCH_3). FAB-MS analysis gave a molecular ion signal at m/z = 269, which corresponded to MH^+ of formononetin. Based on those results, we concluded that peak 4 was formononetin.

Effect of VA Fungi and P on Flavonoid Content of Roots

VA fungal infection was detected 19 to 23 d after germination (Fig. 2a). At that time, internal hyphae were visible in the infected tissue (results not shown).

When flavonoids were extracted from the roots, only the peak identified as formononetin was consistently higher in the inoculated roots when compared with the uninoculated controls. Several other peaks, not yet identified, showed transient increases at different stages of development of the symbiosis (results not shown). The amount of formononetin in the inoculated roots remained above that of the control roots from 15 d after germination until the end of the experiment (Fig. 2b). The presence of the carrier alone did not affect formononetin levels (Table II). The formononetin-7-*O*-glycoside level was significantly greater in the inoculated roots only on d 21 (Fig. 2c).

When effects of phosphate nutrition were examined, it was evident that glucosamine content was elevated only in the upper part of the low-P, inoculated roots (Table III). After the low-P treatment, formononetin content was elevated in

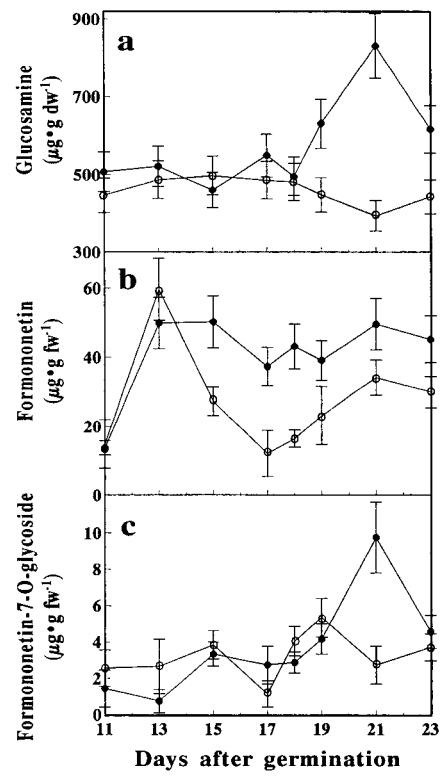

Figure 2. Effect of VA fungal inoculation on glucosamine (a), formononetin (b), and formononetin-7-*O*-glycoside (c) content of alfalfa (*M. sativa* L. cv Gilboa) roots inoculated with *G. intraradix* (●) or uninoculated (○) 11 to 23 d after germination. Values are means ± σ^2 of 800 plants grown in eight individual pots (100 plants each). fw, Fresh weight; dw, dry weight.

Table II. *Glucosamine content, formononetin content, and enzyme activities in upper portion of alfalfa roots from plants grown with the commercial inoculum or wet-sieved carrier (no viable VA fungal spores) and untreated control plants*

Values are means from duplicate determinations, each replicate containing 400 plants. Different letters within the same days and columns show significant differences according to Duncan's multiple range test (P = 0.05).

d after Germination	Carrier/ Spores	Glucosamine	Formononetin	PAL	CHI	Chitinase
		$\mu g\ g^{-1}$ dry wt	$\mu mol\ g^{-1}$ dry wt		Eu	
14	−/−	376.4 b	21.35 a	14.27 a	0.22 a	10.49 a
	+/−	294.2 b	23.47 a	14.79 a	0.23 a	12.85 a
	+/+	677.1 a	26.89 a	14.56 a	0.23 a	11.77 a
17	−/−	402.3 b	18.26 b	12.32 b	0.20 b	7.66 b
	+/−	399.1 b	20.41 b	12.87 b	0.22 b	7.98 b
	+/+	689.7 a	40.87 a	16.55 a	0.33 a	13.23 a
21	−/−	388.1 b	22.87 b	10.97 b	0.19 b	5.90 b
	+/−	391.3 b	25.79 b	10.42 b	0.20 b	7.02 b
	+/+	981.2 a	42.88 a	14.97 b	0.28 a	10.03 a

both the upper and lower part of the inoculated roots (100 and 80%, respectively) as compared with the uninoculated controls, whereas at the high-P-level, a 50% increase was detected in the upper part of the inoculated roots (Fig. 3a). Formononetin-7-*O*-glycoside content in both the upper and lower segments after the low-P treatment was about twice that of the high-P-treated roots but was not affected by inoculation (Fig. 3b).

Effects of VA Fungi on Chitinase, PAL, and CHI Activities

Chitinase activity increased in the inoculated roots between d 13 and 16 relative to uninoculated control roots. On d 19, the activity in the colonized roots decreased below the control roots and remained at that level until the end of the experiment (Fig. 4).

Based on glucosamine measurements, VA hyphae in the upper portion of the root system increased significantly between d 17 and 19 (Fig. 5a). The elevated glucosamine content in upper portions of inoculated roots as compared to the nearly identical values for lower portions of inoculated roots and the uninoculated controls may reflect the presence of hyphae on the root surface, because no internal hyphae were visible (results not shown). In lower portions of the root system, glucosamine content of inoculated roots did not differ from that of uninoculated controls at any time, indicating that only the upper portion was colonized by the fungi.

PAL activities in the upper portions of the inoculated root system were more than twice those of the uninoculated control plants on d 17 (Fig. 5b). However, within 24 h the activity in the inoculated roots declined to a value 50% below

Table III. *Glucosamine content in upper and lower portions of alfalfa roots grown with (+VA fungi) or without (−VA fungi) VA mycorrhizal inoculum at high (+P) or low (−P) levels of phosphate 26 d after germination*

Values are means of three replicates, each replicate containing 400 plants. Different letters within rows show significant differences according to Duncan's multiple range test (P = 0.05).

Segment	Glucosamine Content			
	+VA fungi		−VA fungi	
	+P	−P	+P	−P
	$\mu g\ g^{-1}$ dry wt			
Upper	366 b	1456 a	431 b	349 b
Lower	517 a	627 a	387 a	402 a

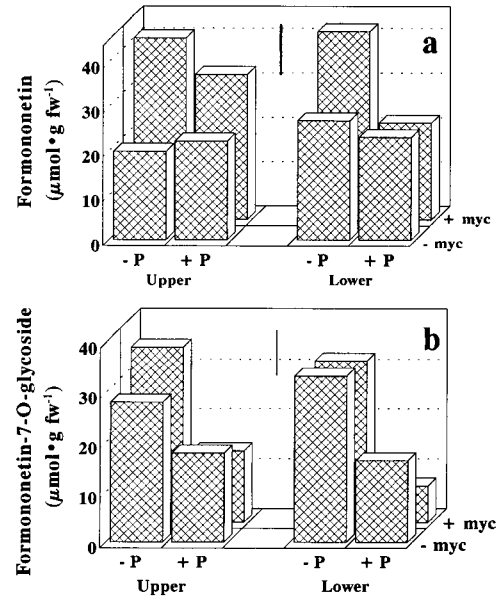

Figure 3. Effect of VA fungal inoculation on formononetin (a) and formononetin-7-*O*-glycoside (b) in roots grown with high or low levels of P, inoculated (+myc) or not (−myc) with VA mycorrhizal inoculum. Values are means of 800 plants grown in eight individual pots (100 plants each). The bars represent LSD (P = 0.05) for these samples taken 26 d after germination. fw, Fresh weight.

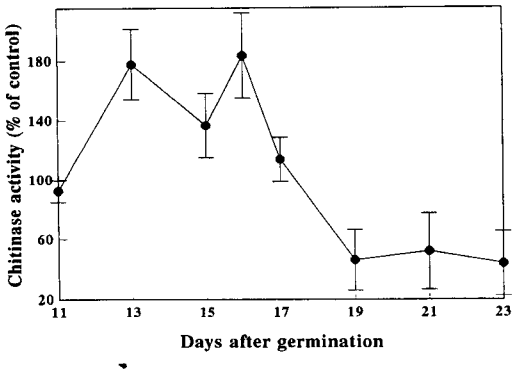

Figure 4. Relative activity of chitinase in alfalfa roots inoculated with VA fungi, expressed as a percentage of control. The uninoculated control values (100%) on d 11 to 23 ranged from the release of 17 to 2 mg of GlcNAc s^{-1} kg^{-1} of protein, respectively. Values are means ± σ^2 of 800 plants grown in eight individual pots (100 plants each).

that measured in the uninoculated control and stayed below the uninoculated control for the remainder of the experiment. In the lower part of the inoculated root system, PAL activity followed the pattern of the uninoculated controls until d 16 and thereafter declined to 60 to 80% of that in the control (Fig. 5b).

CHI activity in the upper part of the inoculated roots increased on d 14 and reached a maximum on d 16. Between 19 and 21 d after germination, CHI activity in the upper part of the inoculated roots declined to levels below that of the control roots (Fig. 5c). The CHI activity in the lower part of the inoculated roots did not differ from that of the control roots at any time.

PAL, CHI, and chitinase activities in the upper part of plant roots grown with carrier and untreated controls did not differ among the treatments. Only inoculation with the complete commercial inoculum containing viable VA mycorrhizal spores had an influence on the enzyme activities (Table II).

There was no evidence that a soluble PAL inhibitor was present in the inoculated roots, because mixing crude extracts from inoculated roots with extracts from uninoculated controls had no effect on PAL activity (Table IV).

DISCUSSION

Physiological and biochemical changes that take place at early stages of development of the symbiosis in alfalfa roots inoculated with a VA mycorrhizal fungi were found not only in the upper, colonized parts but also in the lower, noncolonized segments of the inoculated roots. The reported changes were shown to be due solely to the presence of the mycorrhizal fungi because no differences could be observed in the corresponding control treatments. Furthermore, the growth characteristics of the plants treated with mycorrhiza and the plants receiving control treatments, as expressed by dry weight, were similar, showing that the observed effects were a response to the presence of the mycorrhiza and not caused indirectly by a changed growth pattern.

HPLC profiles of flavonoids extracted from VA fungus-

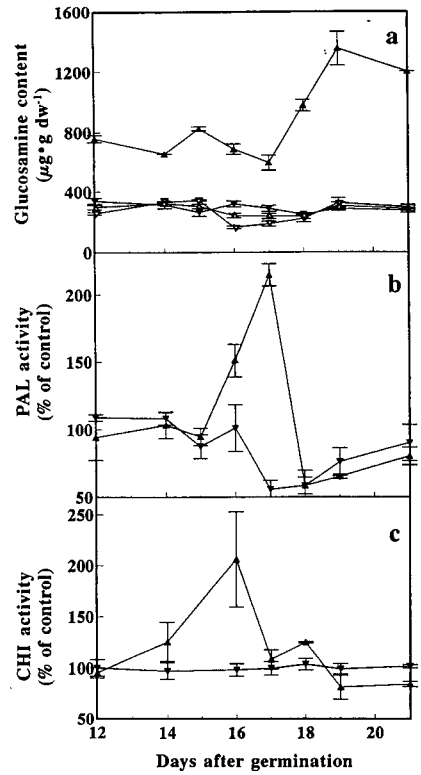

Figure 5. Effect of VA fungal inoculation on glucosamine content (a) and PAL (b) and CHI (c) activities in alfalfa roots. Mean ± σ^2 values for upper (▲) or lower (▼) inoculated root portions of 800 plants grown in eight individual pots (100 plants each) are given. For glucosamine content (a), values for upper uninoculated (Δ) and lower uninoculated (∇) are also included. The 100% PAL on d 12 to 21 ranges from formation of 13 to 9 μmol of cinnamic acid s^{-1} kg^{-1} of enzyme in the upper portion of roots and 0.16 to 0.14 mmol of cinnamic acid in the lower portion. The 100% CHI activity reflects the disappearance of 0.2 to 0.3 mol of isoliquiritigenin s^{-1} kg^{-1} of protein. dw, Dry weight.

Table IV. *PAL activity in various combinations of crude root extracts from inoculated (VA) and uninoculated (control) roots*

PAL activity in the mixture is expressed as a percentage of the expected activity calculated from unmixed controls. Values are means of two replicates, each replicate containing 400 plants.

d after Germination	Ratio VA/Control	Activity
		% of calculated
15	2:3	103
	4:1	102
16	2:3	98
	4:1	101
17	2:3	102
	4:1	105
19	2:3	104
	4:1	100

inoculated alfalfa roots differed from those of uninoculated control roots during the early stages of mycorrhizal establishment. Two of the major peaks were identified as formononetin and formononetin-7-*O*-glycoside. The concentration of formononetin increased when VA fungus was present in the rhizosphere, even in high-P-treated plants that were not colonized by the fungus (Fig. 3a), suggesting that formononetin production in the roots is induced merely by the presence of the fungus in the host rhizosphere and that apparent colonization of the tissue is not required. In the lower part of the low-P-treated roots, the increase in formononetin concentration seemed to be dependent on the colonization of the upper part. This could be either a systemic response to the colonization of the upper part or a response to the presence of external mycorrhizal hyphae in the soil that reach the lower part of the roots and thus induce formononetin production. Under the high-P treatment, when no apparent colonization occurred, hyphal growth probably was limited, and therefore, no formononetin production was induced in the lower part. Medicarpin-malonyl-glucoside, formononetin-malonyl-glucoside, daidzein, coumestrol, and 4',7-dihydroxyflavone have also been found to accumulate in VA mycorrhizal roots of *Medicago truncatula* (Harrison and Dixon, 1993), showing that phytoalexins and their precursors are induced by VA-fungal inoculation.

Increases in formononetin levels have been attributed to P stress in white clover roots (Nair et al., 1991). However, in our work uninoculated alfalfa plants showed similar concentrations of formononetin at the two P levels, indicating that the elevated formononetin concentrations observed were not a result of P stress but a direct response to the fungus present.

Formononetin has not been shown to have antimicrobial activities but is a precursor of the isoflavonoid phytoalexins produced in alfalfa in response to stresses and microbial infections (Dalkin et al., 1990). Formononetin is not usually found in the exudates of young (8 d old) alfalfa roots but is secreted from stressed roots (Maxwell and Phillips, 1990) and is produced in leaves of alfalfa inoculated with *Ascochyta imperfecti* (Olah and Sherwood, 1971). Formononetin added to the soil increased colonization of white clover by *G. intraradix* (Siqueira et al., 1991). Under in vitro conditions, formononetin promoted germination and hyphal growth of an undescribed *Glomus* species (Nair et al., 1991) but inhibited germination of *Glomus etunicatum* and *Glomus macrocarpum* (Tsai and Phillips, 1991). In accordance with these results, formononetin secreted in the presence of mycorrhiza could play a significant role in competition between indigenous VA species, favoring colonization of the roots by those species for which formononetin promotes germination and enhances hyphal growth. Nevertheless, in alfalfa, formononetin does not seem to be a signal for the actual colonization process because the level of formononetin was elevated in the high-P treatment in response to VA fungal inoculation, although colonization was inhibited. Whether formononetin is produced in alfalfa roots in response to other VA strains and whether it is secreted from the roots in the presence of VA mycorrhizas remains to be investigated.

The formononetin-7-*O*-glycoside level, which was not influenced by the fungus, was significantly higher in roots from the low-P than the high-P treatments (Fig. 3b). Formononetin-7-*O*-glycoside is secreted from alfalfa roots inoculated with *Rhizobium meliloti* (Dakora et al., 1993). Whether this compound is secreted in the presence of VA fungi or has any influence on VA fungal development has not yet been tested.

The increase in formononetin coincided with the increase in CHI but not with PAL activity (Fig. 5, b and c). In elicited alfalfa cell suspensions, the increases in CHI and PAL activities occurred concomitantly (Dalkin et al., 1990). However, in our work, CHI activity in VA-colonized roots increased prior to that of PAL (Fig. 5, b and c), even prior to penetration signs, whereas increased PAL activity seemed to be more associated with penetration of the VA fungus into the root tissue. The timing of induction of PAL and CHI activities in the mycorrhizal roots was different from that usually seen in elicitor-treated alfalfa cell suspension (Dalkin et al., 1990). One possible explanation is that a pool of chalcones is present in the roots and that only after depletion of this pool does PAL activity increase.

Chitinase activity was induced in the VA-inoculated roots with a timing similar to that of CHI. Accordingly, chitinase activity (Spanu and Bonfante-Fasolo, 1988) and peroxidase activity (Spanu et al., 1989) increased in VA-inoculated leek roots during early stages of colonization. Those results, together with our data from alfalfa (Figs. 2, 4, and 5), support the assumption that mycorrhizal fungi initiated a host defense response. The observed response to VA mycorrhizal inoculation is probably a part of a general, nonspecific induction of phytoalexin biosynthesis, as has been found in bean in response to inoculation with pathogenic and saprophytic bacteria (Jacobek and Lindgren, 1993). At this stage it would be too speculative to compare the magnitude of the increase in enzyme activities associated with this response in VA-colonized roots with that of roots colonized by pathogenic fungi. Supposedly, differences in magnitude can be attributed to localized responses in the immediate vicinity of VA fungal penetration (Cypers et al., 1988), thus diluting the activities measured in extracts prepared from whole roots or segments thereof. In our experimental system "invasion rate" along the roots was not estimated.

In elicitor-treated alfalfa cell suspensions, the induced increase in PAL, CHI, and chitinase activities is followed by an inactivation of PAL but not of CHI and chitinase. PAL activity does not decline to below that of the unelicited cells (Dalkin et al., 1990). After reaching maximum levels of PAL, CHI, and chitinase, the activities in the infected roots all declined rapidly to below those of the uninoculated roots. PAL activity in elicited cell suspensions of *Phaseolus vulgaris* was inhibited by the addition of cinnamic acid (Bolwell et al., 1986). When extracts from VA-inoculated roots were mixed with extracts from control roots, resulting PAL activities did not differ from those expected by calculation (Table IV), indicating that the lower activity was not caused by the presence of a soluble PAL inhibitor. Corresponding to this, chitinase (Spanu and Bonfante-Fasolo, 1988) and peroxidase (Spanu et al., 1989) activities in leek roots with established VA colonization have been found to be lower than those of the control roots. Similarly, the transcription of endochitinase, β-1,3-endoglucanase, and CHI was suppressed in bean roots following VA mycorrhizal colonization (Lambais and Mehdy, 1993). Together with our results, this implies that the induced host

defense response was actively suppressed. Such a suppression of defense responses has recently been demonstrated in bean leaves inoculated with a compatible *Pseudomonas syringae* (Jacobek et al., 1993). Decreased enzyme activities due to a lack of recognition between host and fungi would not be expected to cause a decrease in activities to below the levels of those in the control roots. Taken together, it is reasonable to suggest that following VA fungal colonization, a nonspecific defense response is induced, which is subsequently specifically depressed.

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