

# Regulation of arbuscular mycorrhizal development by plant host and fungus species in alfalfa

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## SUMMARY

Two cvs of alfalfa (*Medicago sativa* L.), Gilboa and Moapa 69, were inoculated in glasshouse pots with three arbuscular mycorrhizal (AM) fungi to investigate the efficacy of mycorrhizas with respect to the extent of colonization and sporulation. *Paspalum notatum* Flugge also was inoculated to describe fungal parameters on a routine pot culture host. Percentage root length of *P. notatum* colonized by *Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe, *Glomus intraradices* Schenck & Smith, and *Gigaspora margarita* Becker & Hall increased from 10 to 21 wk, and all fungi sporulated during that period. In alfalfa, only colonization by *G. intraradices* increased over that time period, and it was the only fungus to sporulate in association with alfalfa at 10 wk. *Glomus mosseae* did not sporulate after 16–21 wk despite having colonized 30–35 % of the root length of both alfalfa cvs. *In vitro* experiments in which Ri T-DNA-transformed roots of alfalfa were inoculated with AM fungi showed normal mycorrhizal formation by *G. intraradices* and a hypersensitivity-like response to *Gi. margarita*. Colonized cells became necrotic, and HPLC analysis indicated increased concentrations of phenolics and isoflavonoids in these root segments. These data strongly support the existence of a degree of specificity between AM fungi and host that might rely on specific biochemical regulatory processes initiated in the host as a result of the attempts at colonization by the fungus.

Key words: AM fungi, specificity, hypersensitivity-like response.

## INTRODUCTION

Arbuscular mycorrhizal (AM) fungi have wide host ranges, yet certain host and fungus combinations are more effective from either the perspective of the fungus, i.e. greater spore/hyphae production (Struble & Skipper, 1985) or from that of the host, i.e. enhanced growth (Pope *et al.*, 1983), nutrient acquisition (Jensen, 1982), or pathogen resistance (Schenck, 1987). The performance of the symbiosis can be affected by various environmental and physiological factors, of which soil P availability is most studied (Bethlenfalvai *et al.*, 1982). Growth of both plant and fungus is limited at extremely low P whereas growth of the fungus can be inhibited at high levels of P. Between the extremes of P

availability, mycotrophy can occur towards the low range, and non-mycotrophy, in which the fungus is a carbon drain on the host without providing a benefit, can occur in the high range. In addition, host mutants exist which limit the growth of the AM fungus at different stages of colonization (Gianinazzi-Pearson *et al.*, 1991), but this restriction of colonization does not relate to nutrient level and might depend on biochemical processes which occur during the attempted colonization.

Sporulation of AM fungi has been shown to be related to seasonal periodicity in host phenology (Gemma & Koske, 1988) and in host-plant nutrition (Douds & Schenck, 1990). The onset of sporulation of some AM fungi might coincide with the achievement of a threshold level of colonized root length (Gazey, Abbott & Robson, 1992; Heldreth & Morton, 1996). Frequently, however, levels of sporulation and colonization are not directly related (Giovannetti *et al.*, 1988). Recent evidence suggests the carbon flow through a mycorrhiza will differ at

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different levels of P availability (Douds, 1994). This would also affect the ability of an AM fungus to sporulate.

The ability of the host root to regulate the development and sporulation of its AM fungus partner was studied in glasshouse and root organ culture experiments. Three AM fungi were inoculated onto alfalfa (*Medicago sativa*) in the presence and absence of *Rhizobium meliloti*. Colonization and sporulation of the fungi on alfalfa was compared to that on the routine pot culture host bahiagrass (*Paspalum notatum*). This, and experimentation with Ri T-DNA-transformed alfalfa roots, indicated an unusual behaviour for a mycorrhizal host plant in that roots can resist colonization by a specific AM fungus.

## MATERIALS AND METHODS

### Experiment 1

This experiment was conducted as a complete factorial design with two factors: plant host (three levels: bahiagrass (*Paspalum notatum* Flugge) and two cvs of alfalfa (*Medicago sativa* L.)), and AM fungus inoculation (four levels), with 14 replicates of each treatment combination. *Paspalum notatum* was included to demonstrate colonization and sporulation of the AM fungi on a 'control' host and contrast this with the alfalfa cvs.

Four seeds of alfalfa cv. Gilboa (supplied by Hazera Co., Heifa, Israel) or cv. Moapa 69 (supplied by D. A. Phillips, University of California, Davis, CA, USA), or one germinated *P. notatum* seedling, were sown into conical plastic pots (165 cm<sup>3</sup>) (Super cell C-10, Stuewe & Sons, Corvallis, OR 97333, USA) containing a 0.75:1:1:0.75 (v/v) autoclaved potting mixture of soil (Comly silt loam (fine-loamy, mixed, mesic Typic Fragiudalf) with 165 mg kg<sup>-1</sup> available P):sand:vermiculite:calcined clay (Turface®, Applied Industrial Materials Corp., Deerfield, IL 60015 USA), respectively, with one of three mycorrhizal fungus inocula or with inoculum filtrate (control). Inocula used were pot culture media (prior host *P. notatum*) of the following AM fungi: *Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe (INVAM 336) (final concentration of spores 1.7 spores cm<sup>-3</sup>), *Gigaspora margarita* Becker & Hall (DAOM 194757) (final concentration of spores 1.0 cm<sup>-3</sup>), and *Glomus intraradices* Schenck & Smith (from Les Tourbieres Premier, Rivière du Loup, Quebec, Canada) (infected root pieces and hyphae as inoculum with very few soil-borne spores). Inoculum of *Gi. margarita* was washed through a 39 µm sieve and the filtrate was added to the non-inoculated controls to equilibrate non-mycorrhizal microflora.

Plants were grown in a glasshouse under natural photoperiods with a day/night temperature range of

30 °C/10 °C beginning 23 June 1993. No nutrients were added throughout the experiment. Seven plants of each treatment were harvested after weeks 10 and 21. Heights of the two longest shoots were recorded. Spores of AM fungi were isolated by wet sieving (Gerdemann & Nicolson, 1963) and centrifugation (Jenkins, 1964) from a 43 cm<sup>3</sup> section of the soil column in the middle of the pot, and quantified under a dissection microscope. Colonization of roots by AM fungi, nodule numbers, and spore production by *G. intraradices* were quantified using roots in the soil section used to isolate spores. *Glomus intraradices* did not produce soil-borne spores under the conditions of this experiment. Roots were cleared in KOH, stained with trypan blue (Phillips & Hayman, 1970), and total colonization measured by the gridline-intersection method (Newman, 1966). Arbuscular colonization was measured according to the method of McGonigle *et al.* (1990) on a subsample of the roots sampled at 21 wk. Shoot and root d. wt were measured after 2–3 d at 80 °C. Shoot N and P contents were measured after H<sub>2</sub>O<sub>2</sub>–H<sub>2</sub>SO<sub>4</sub> digestion, by the methods of Wall & Gehrke (1975) and Murphy & Riley (1962), respectively.

### Experiment 2

A second experiment was conducted to explore the performance of the three AM fungi in the presence of *Rhizobium* inoculation. Six seeds of alfalfa cv. Gilboa were sown on 25 October 1995 into the potting mix described above, which was preinoculated with AM fungi to yield 1.7 spores cm<sup>-3</sup> of *Gi. margarita*, 13.2 spores cm<sup>-3</sup> of *G. mosseae*, or *P. notatum* roots and pot culture soil colonized by *G. intraradices*. The control potting mix was inoculated with a 1-yr-old non-mycorrhizal *P. notatum* 'pot culture' to supply the micro-organisms which normally colonize a pot culture over time. All pots were inoculated with 2 ml of a suspension of *Rhizobium meliloti* strain 102F28 (absorbance 0.2 at 520 nm, c. 2–3 × 10<sup>7</sup> cells ml<sup>-1</sup>). Pots were watered with distilled water for 2 wk, then seedlings were thinned to two per pot and watered with tap water.

Plants were grown in a glasshouse with supplemental light (metal halide lamps) to provide 14-h daylengths. Seven pots per AM fungus treatment were harvested at weeks 8 and 16 after sowing of seeds. Data were collected at harvest as outlined for expt 1. In addition, spores of *Gi. margarita* collected at the 16-wk harvest were inoculated onto *P. notatum* seedlings. Six weeks later, these plants were sampled for colonization to test the infectivity of these spores.

### In vitro experiments

Ri T-DNA-transformed roots of alfalfa cv. Moapa 69 were inoculated *in vitro* with *Gi. margarita* and *G. intraradices* (DAOM 181602, supplied by J. A.

Fortin, Institut Recherche Biologie Végétale, Montréal, Québec, Canada) to test their susceptibility to mycorrhizal colonization. Roots were grown in M medium (Bécard & Fortin, 1988) made with 0.4% gellan (Gelgro®, ICN Biochemicals, Cleveland, OH, USA) and amended with 40 g l<sup>-1</sup> sucrose. Roots were inoculated with *Gi. margarita* according to Bécard & Piché (1992), and with *G. intraradices* by the *in vitro* technique of St. Arnaud *et al.* (1996). Colonized Ri T-DNA-transformed roots of *Daucus carota* L. were grown on one side of a divided Petri dish in M medium (10 g l<sup>-1</sup> sucrose). Six to 8 wk later the hyphae of *G. intraradices* grew over the barrier into the second compartment containing M medium amended with 40 g l<sup>-1</sup> sucrose. Alfalfa cv. Moapa 69 roots then were transplanted into this compartment and grown for 8 wk. Roots were recovered from gels using 10 mM Na citrate, pH 6.0 (Doner & Bécard, 1991) and cleared, stained, and assayed for colonization as described for expt 1.

Root segments for HPLC analysis were selected carefully from cultures of transformed roots of alfalfa with *Gi. margarita*. They were either uncolonized (complete absence of hyphae around them) or colonized by *Gi. margarita*. Root phenolics were extracted as described by Graham (1991). Less than 20 mg of fresh root tissue was ground in 80% ethanol, immediately centrifuged at 18000 g for 3 min and filtered (0.2 µm) before injection for HPLC analysis. Separations were carried out on a Spectra-Physics® (San Jose, CA) system equipped with a scanning u.v. Spectra-Focus® detector, a Rainin® (Woburn, MA) C18 reverse-phase packing analytical column (4.6 × 250 mm), and a 20 µl loop injector. The u.v.-absorbing compounds were separated at low pH (4% acetic acid) for 30 min by a linear gradient elution of 30–70% methanol followed by 8 min of isocratic elution with 70% methanol at 1.0 ml min<sup>-1</sup>. Standard isoflavonoids were purchased from ICN, Costa Mesa, CA (daidzein); Indofine Chemical Co., Sommerville, NJ (genistein, formononetin); and Sigma, St. Louis, MO (biochanin A).

#### Data analysis

Data were analysed using ANOVA after arcsin (percentage root length colonization) or SQRT(X+1) (spore population) transformations. Measurements for which significant treatment effects were found were characterized further using Tukey's Method of Multiple Comparisons ( $\alpha = 0.05$ ).

## RESULTS

### Experiment 1

*Development and sporulation of mycorrhizas.* Though each fungus colonized all hosts in this experiment, there were significant differences in both colonization

and sporulation among different host–fungus combinations (Table 1). All fungi were well established on *P. notatum* at 10 wk and colonization levels significantly increased by 21 wk (Table 2). All fungi colonized both alfalfa cvs, but percentage root length colonized increased from 10 to 21 wk only for *G. intraradices* on 'Moapa 69' (Table 2). Colonization levels remained constant or declined over this period for all other combinations.

*Glomus intraradices* and *Gi. margarita* sporulated in association with *P. notatum* at 10 wk (Table 2). Spore populations of all AM fungus species were greater at 21 wk than at 10 wk when grown with this host. *Glomus intraradices* produced abundant spores within the roots of both alfalfa cvs at the first sampling (Table 2). Populations of *G. mosseae* spores remained at initial levels throughout the experiment, indicating that even though there was colonization there was no net spore production. Although *Gi. margarita* sporulated on *P. notatum* by 10 wk, it exhibited no net increase in spores with either cv. of alfalfa at that time and, by the end of the experiment, sporulated only in association with 'Gilboa'.

*Paspalum notatum* colonized by *G. mosseae* and *Gi. margarita* had greater percentage of root length with arbuscules than either alfalfa cv. colonized by these fungi (Fig. 1). Arbuscule development by *G. intraradices* on alfalfa equalled or exceeded that on *P. notatum*.

### Effects of AM fungi upon the growth and mineral nutrition of alfalfa

The effect of inoculation treatments upon the growth of alfalfa was the same for both cvs studied, so data are presented only for 'Moapa 69'. *Glomus intraradices* had significant effects upon the growth of alfalfa at 10 wk (Table 3). These effects were more demonstrable at 21 wk. Plants colonized by *G. intraradices* had significantly less shoot and root growth at the end of the experiment than did plants of other treatments.

Phosphorus concentrations and contents of shoots were not significantly different between controls and plants colonized by *G. mosseae* and *Gi. margarita* (Fig. 2a, b). *Glomus intraradices* significantly increased P concentrations and contents of alfalfa shoots, even though these plants had lower shoot weights than did the other treatments (Table 3).

Although plants were not inoculated with *Rhizobium* sp. in this experiment, they were exposed to these bacteria via the air or mycorrhizal inoculum and filtrate. There were significantly fewer nodules in plants colonized by *G. intraradices* (data not shown) which was reflected in shoot N levels. Alfalfa colonized by *G. intraradices* had lower shoot N contents and concentrations than did non-mycorrhizal controls or plants colonized by *G. mosseae* or *Gi. margarita* (Figs. 3a, b).

**Table 1.** ANOVA of mycorrhizal parameters for three different AM fungi colonizing two cvs of *Medicago sativa* and *Paspalum notatum* after 10 and 21 wk of growth

Source	d.f.	% root length colonized		Sporulation		d.f.	Arbuscular colonization (% root length) 21 wk
		10 wk	21 wk	10 wk	21 wk		
Host	2	0.0876**	0.0697**	51.8	352.2**	2	0.0538**
AMF	2	0.7495**	1.8940**	44838.0**	44655.1**	2	0.0713**
Host × AM	4	0.0547**	0.1650**	625.6**	2820.9**	4	0.0531**
Error	54	0.0075	0.0047	108.2	67.3	9	0.0023

Values are mean squares for main effects, interaction, and error terms. Those followed by \*\* are significant at  $P > F = 0.01$ .

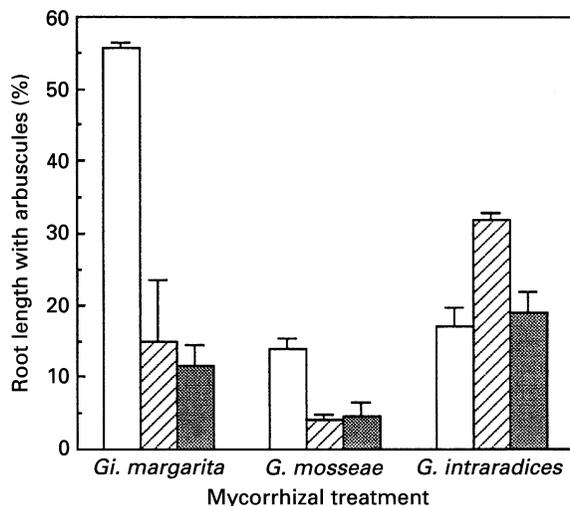
Colonization and sporulation data were analysed after arcsin and SQRT(X + 1) transformations, respectively.

**Table 2.** Colonization of roots and sporulation by three AM fungi grown in association with two cvs of *Medicago sativa* and *Paspalum notatum*

Host	AM fungus	Colonization (% root length)		Sporulation*		
		10 wk	21 wk	10 wk	21 wk	
<i>M. sativa</i>	cv. Gilboa	<i>Gigaspora margarita</i>	57.7 a	35.9 b	72 b	175 a
		<i>Glomus mosseae</i>	34.2 a	36.6 a	75 b	63 a
		<i>Glomus intraradices</i>	73.6 a	78.4 a	10100 a	82000 a
	cv. Moapa 69	<i>Gigaspora margarita</i>	40.8 a	24.1 b	54 a	52 a
		<i>Glomus mosseae</i>	32.6 a	34.9 a	57 a	57 a
		<i>Glomus intraradices</i>	64.3 b	83.9 a	8900 b	14400 a
<i>P. notatum</i>	<i>Gigaspora margarita</i>	55.2 b	63.2 a	521 b	1659 a	
	<i>Glomus mosseae</i>	32.3 b	37.0 a	78 b	157 a	
	<i>Glomus intraradices</i>	57.2 b	75.2 a	6600 b	10400 a	

Values are the means of seven observations. Numbers for a pairwise comparison across time within a host–fungus combination followed by the same letter are not significantly different ( $\alpha = 0.05$ ).

\* Spores per 43 cm<sup>3</sup> sampled section in centre of pot. Initial estimated populations of spores in the same volume of potting mix: *G. margarita* = 43 and *G. mosseae* = 73.



**Figure 1.** Percentage root length of *Paspalum notatum* (□) and *Medicago sativa* cv. Moapa 69 (▨) and Gilboa (▩) with arbuscules ± SEM. Plants were 21-wk-old and colonized by one of three AM fungi.

### Experiment 2

Experiment 2 was conducted to test whether the observations of expt 1 would be reproduced under

more controlled nitrogen fixation conditions. Alfalfa cv. Gilboa was studied in an experiment in which plants were inoculated with *R. meliloti* and the three AM fungi.

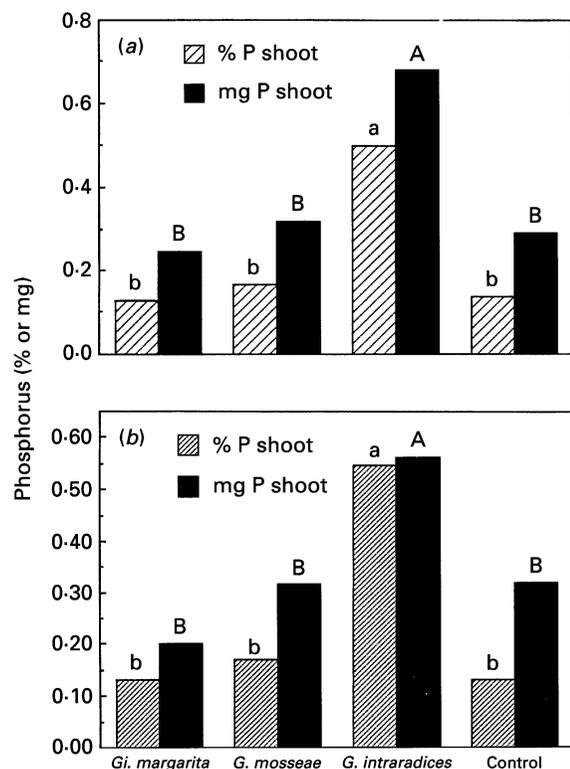
### Colonization and sporulation of mycorrhizas

Each AM fungus colonized roots of cv. Gilboa in this experiment, but colonization by *Gi. margarita* was very low (Table 4) and decreased over the period 8–16 wk. Percentage root length colonized by *G. mosseae* and *G. intraradices* increased significantly over the course of the experiment. *Glomus intraradices* sporulated profusely whereas *Gi. margarita* and *G. moseae* did not sporulate. Indeed, the number of spores of *Gi. margarita* and *G. mosseae* was nearly identical from 8 to 16 wk and only 25–43% of that estimated to be present at the start of the experiment. Although *M. sativa* roots were < 1% colonized by *Gi. margarita*, and it was unlikely that any new spores were produced, spores recovered after 16 wk produced 6.8% colonized root length in *P. notatum* 6 wk after inoculation, indicating that the spores were infective.

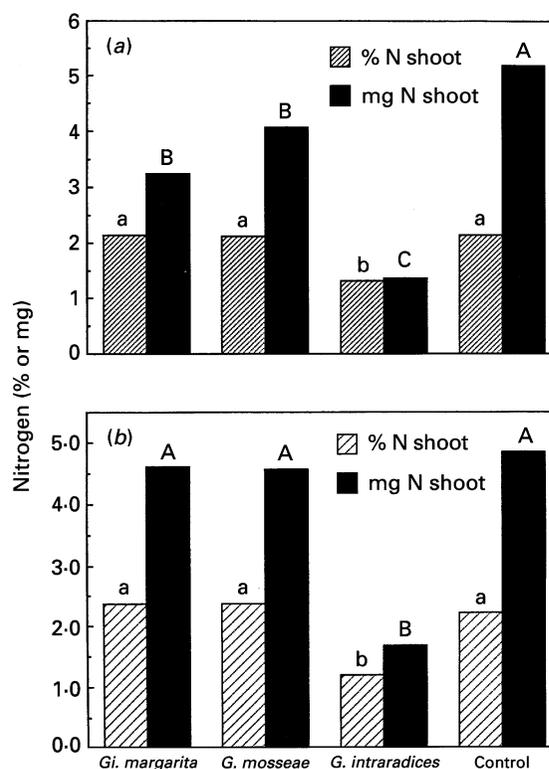
**Table 3.** Effects of mycorrhiza upon the growth of *Medicago sativa* cv. *Moapa 69* after 10 and 21 wk of growth, expt 1

AM fungus	Height (cm)		Root d. wt (g)		Shoot d. wt (g)	
	10 wk	21 wk	10 wk	21 wk	10 wk	21 wk
Non-inoculated	14.3 a	17.3 a	0.3683 a	1.0905 a	0.2526 a	0.8388 a
<i>Gigaspora margarita</i>	8.8 b	14.6 a	0.2722 b	1.0282 a	0.1539 cb	0.7680 a
<i>Glomus mosseae</i>	13.2 a	15.6 a	0.2791 ab	1.1585 a	0.1893 b	0.7561 a
<i>Glomus intraradices</i>	13.2 a	9.9 b	0.2321 b	0.3049 b	0.1074 c	0.1291 b

Values are the means of seven observations. Numbers for a comparison within a column followed by the same letter are not significantly different ( $\alpha = 0.05$ ).



**Figure 2.** Phosphorus concentrations and contents of shoots of 10-wk-old *Medicago sativa* cv. Gilboa (a) and Moapa 69 (b) non-inoculated or inoculated with one of three AM fungi. Means of seven, comparisons among mycorrhizal treatments followed by the same letter (% P, lower case; total P content, upper case) are not significantly different ( $\alpha = 0.05$ ).



**Figure 3.** Nitrogen (% of d. wt and total N content of shoots) in (a) 10-wk-old *Medicago sativa* cv. Moapa 69 and (b) 10-wk-old *M. sativa* cv. Gilboa non-inoculated or inoculated with one of three AM fungi. Means of seven, comparisons among mycorrhizal treatments followed by the same letter (%N, lower case; total N, upper case) are not significantly different ( $\alpha = 0.05$ ).

#### Plant growth, N and P contents, and nodulation

Though there were no differences in plant height or shoot d. wt between inoculation treatments, plants colonized by *G. mosseae* and *G. intraradices* had significantly greater root d. wts at 8 and 16 wk (data not shown) than controls, and significantly greater P concentrations in roots and shoots than those of plants inoculated with *Gi. margarita* (Table 5). Nitrogen concentrations of roots and shoots were similar among mycorrhizal treatments. Nodulation in this experiment was increased by *G. intraradices* and was least in controls and plants inoculated with *Gi. margarita* (Fig. 4).

#### In vitro inoculations

Ri T-DNA-transformed roots of alfalfa cv. Gilboa became colonized by *G. intraradices* ( $23 \pm 3\%$  colonized root length with  $5 \pm 1\%$  root length with arbuscules,  $n = 4$ ), and exhibited normal formation of mycorrhiza (Fig. 5a). In contrast to previous reports using tomato roots and transformed carrot roots (Bécard & Piché, 1992), these alfalfa roots did not support symbiotic growth of *Gi. margarita* *in vitro*. Hyphae from germinating spores grew normally and showed the characteristic stimulation of growth which occurs in the presence of host roots before colonization (Bécard & Piché, 1990). Clearing

**Table 4.** Colonization of roots of *Medicago sativa* cv. *Gilboa* and sporulation by three AM fungi after 8 and 16 wk of growth in expt 2

Treatment	Sporulation*		Colonization†		Arbuscular colonization‡	
	8 wk	16 wk	8 wk	16 wk	8 wk	16 wk
<i>Gigaspora margarita</i>	21 a	18 a	1.8 a	0.6 b	—	—
<i>Glomus mosseae</i>	246 a	245 a	16.5 b	35.1 a	43.5 a	10.5 b
<i>Glomus intraradices</i>	962 b	6231 a	31.1 b	43.4 a	63.2 a	68.6 a

Means of seven observations. Statistics as in Table 1.

\* Spores  $43 \text{ cm}^{-3}$  section in centre of pot. Initial estimated populations of spores in the same volume of potting mix: *G. margarita* = 73, *G. mosseae* = 568.

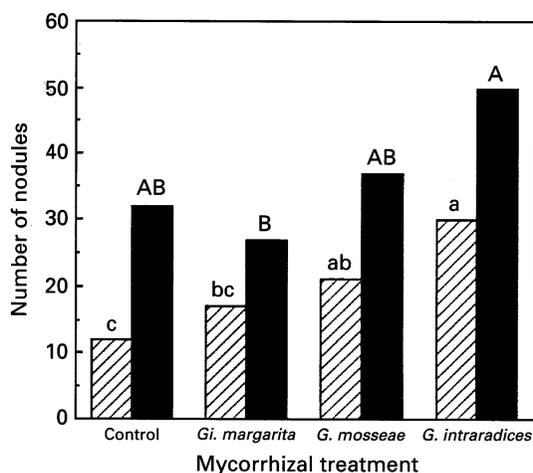
† Percentage of root length colonized by mycorrhizal fungi.

‡ Percentage of the colonized root length occupied by arbuscules.

**Table 5.** Nitrogen and phosphorus concentrations and contents in *Medicago sativa* cv. *Gilboa* after 8 wk of growth, expt 2

Treatment	Root		Shoot		total plant	
	% N	% P	% N	% P	N (mg)	P (mg)
Control	2.64 a	0.28 bc	2.97 a	0.23 bc	7.62 b	0.64 c
<i>Gigaspora margarita</i>	2.78 a	0.22 c	2.58 a	0.21 c	8.76 ab	0.70 c
<i>Glomus mosseae</i>	2.80 a	0.32 ab	2.76 a	0.27 ab	8.86 ab	0.93 b
<i>Glomus intraradices</i>	2.91 a	0.38 a	2.91 a	0.30 a	10.28 a	1.17 a

Means of seven observations. Numbers in the same column followed by the same letter are not significantly different ( $\alpha = 0.05$ ).



**Figure 4.** Number of nodules in the middle third of the root system of *Medicago sativa* cv. *Gilboa* after 8 (▨) and 16 wk (■) of growth. Plants were inoculated with *Rhizobium meliloti* and either non-mycorrhizal pot culture soil or *Gigaspora margarita*, *Glomus mosseae*, or *Glomus intraradices*. Columns within a sample date with the same letter (lower case for 8 wk, upper case for 16 wk) are not significantly different ( $\alpha = 0.05$ ).

and staining of the root–fungus contacts showed formation of appressoria, root penetration, and arbuscule formation. Further colonization was arrested by the host’s hypersensitive-like response (Fig. 5b). Colonized cells became dark coloured, indicating necrosis and accumulation of phenolics.

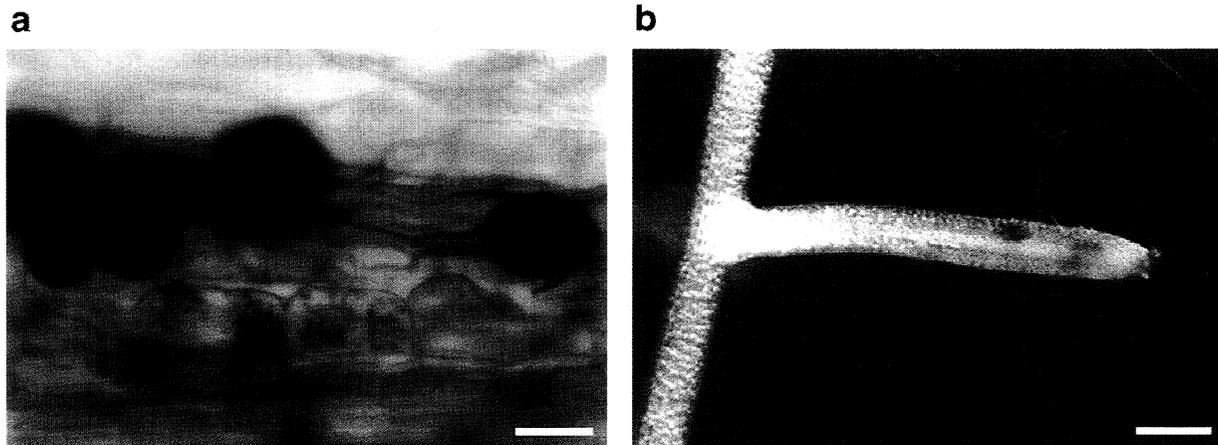
No further intraradical growth of the fungus was observed. Overall hyphal growth in the medium was limited to that typically produced *in vitro* by germinated spores.

The accumulation of phenolics in root tissue colonized by *Gi. margarita* was confirmed by HPLC analysis. Chromatograms of colonized tissue showed more peaks at the early elution times corresponding to phenolic acids (Fig. 6). Later peaks at elution times (and with u.v. spectra, not shown) corresponding to those of isoflavonoids were also in much larger amounts (4.5-fold to 6.8-fold greater in colonized tissue for major peaks) than found in uncolonized root segments.

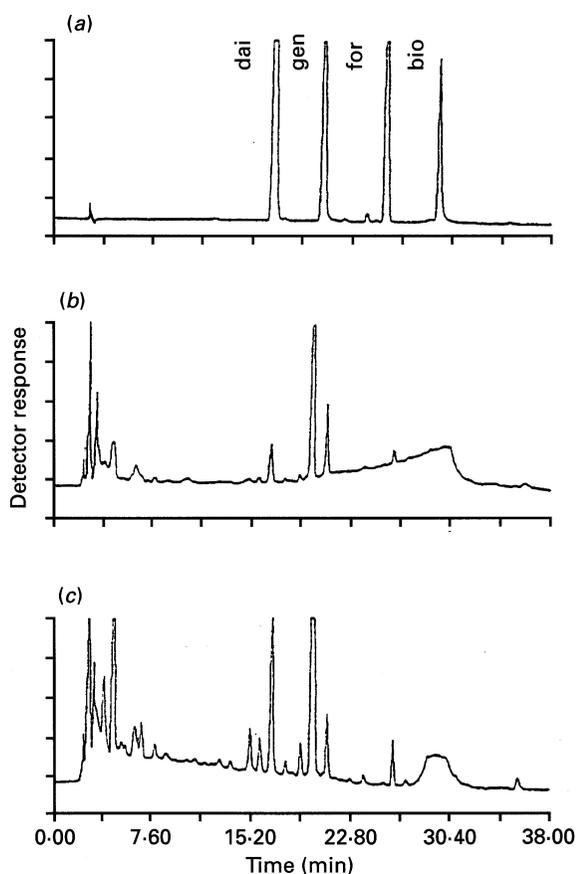
#### DISCUSSION

The observation that the approx. 150 spp. of AM fungi (Morton & Bentivenga, 1994) colonize an estimated 225 000 species of plants (Law & Lewis, 1983) has led to the conclusion that AM fungi have wide host ranges. We presented experiments with alfalfa cvs and three common species of AM fungi which showed host–fungus interactions which ranged from a normal symbiotic relationship to incompatibility.

*Paspalum notatum* readily supported colonization and abundant sporulation by the three AM fungi studied here. The alfalfa cvs interacted differently with these fungi. Colonization by *G. mosseae* and *Gi.*



**Figure 5.** Light micrographs of mycorrhizal colonization of Ri T-DNA-transformed roots of *Medicago sativa* cv. Moapa 69. (a) Normal mycorrhiza formation with *Glomus intraradices*. Bar = 48  $\mu\text{m}$ . (b) Hypersensitive-like reaction in response to colonization by *Gigaspora margarita*. Bar = 540  $\mu\text{m}$ .



**Figure 6.** High-performance liquid chromatograms. (a) Isoflavonoid standard mixture of daidzein (dai), genistein (gen), formononetin (for) and biochanin A (bio), all at  $83 \mu\text{g ml}^{-1}$  methanol. (b) Extract of Ri T-DNA-transformed roots of *Medicago sativa* cv. Moapa 69. (c) Extract of the same roots colonized by *Gigaspora margarita*.

*margarita* developed but sporulation was either completely inhibited or at least delayed relative to that on *P. notatum*. Neither of these fungi appeared to benefit the P or N nutrition of the host. *Glomus intraradices* had a very disruptive, negative effect upon both alfalfa cvs in the absence of *R. meliloti*

inoculation, yet increased the P content of the plants. It had the greatest colonization and produced abundant intraradical spores.

We used the simple *in vitro* system of root organ culture to investigate the regulatory mechanisms that make the host treat a mycorrhizal fungus like an opportunistic partner. Despite the artificial nature of the *in vitro* system, the relative susceptibility/receptivity of 'Moapa 69'-transformed roots toward *Gi. margarita* and *G. intraradices* was similar to that expressed *in vivo*. *Glomus intraradices* formed typical mycorrhizas whereas *Gi. margarita*, although capable of forming arbuscular colonizations, could not increase the size of an infection unit, receive the carbon necessary to support extraradical hyphal growth, or sporulate. The root expressed a hypersensitivity-like response at each colonization site that was absent for *G. intraradices* colonizations *in vitro*. This response was characterized by a localized necrosis of the root tissue and an accumulation of phenolic compounds including isoflavonoids. Accumulation of isoflavonoids in roots of legumes infected with AM fungi has been reported (Morandi, Bailey & Gianinazzi-Pearson, 1984; Harrison & Dixon, 1993, 1994; Volpin *et al.*, 1994). However, this isoflavonoid accumulation, or the enzymatic activities involved in their accumulation, was generally transient or very localized, and subsequently suppressed. Suppression of the defence reactions in host plants in response to AM fungus colonization has been hypothesized (Harrison & Dixon, 1993; Lambais & Mehdy, 1993; Volpin *et al.*, 1994; Kapulnik *et al.*, 1996). The roots of alfalfa cv. Moapa 69 colonized by *Gi. margarita* behaved like a resistant host with apparently no suppression of isoflavonoid biosynthesis after colonization.

This unusual root response toward a specific AM fungus was not an early response since arbuscules were formed. Gollote *et al.* (1993) also described aborted colonization by *G. mosseae* of a 'locus a'

myc<sup>-</sup> pea mutant. The plant response was 'early' because colonization was stopped at the appressorium stage. Localized plant defence reactions were exhibited. It was hypothesized that 'locus a' could be an important symbiosis gene. The expression of such plant genes could serve to prevent the plant from recognizing the symbiotic partner as a pathogen. Another myc<sup>-</sup> mutant of pea exists which has been termed a 'late' mutant (Gianinazzi-Pearson *et al.*, 1991). The AM fungus colonizes the root, sometimes to a significant extent, but no arbuscules are formed and there is insufficient transfer of nutrients between the symbionts to support extraradical hyphal growth (Kling *et al.*, 1996).

Our observations suggest that regulation of mycorrhizal development is not only dependent upon the host plant, as indicated by myc<sup>-</sup> mutant studies, but also upon the colonizing fungus. The fact that specific stages in the plant-fungus interaction are affected suggests that definite genetic characteristics are involved in the restriction of colonization. The induction of compatibility or incompatibility reactions is determined by the particular host-fungus combination.

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