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ROOT ANATOMICAL MECHANISMS INVOLVED IN HOST PLANT CONTROL OF ARBUSCULAR MYCORRHIZAL COLONIZATION

A Dissertation in

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by

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ABSTRACT

Mycorrhizal host plants are widely known to be capable of controlling the degree to which their root systems are colonized by arbuscular mycorrhizal fungi. Previous studies to elucidate mechanisms involved in host plant control of colonization have not considered root anatomical changes as potential control mechanisms. Here, I examined three root anatomical variables hypothesized to influence mycorrhizal colonization: the percentage of root length with a suberized hypodermis, the distribution of hypodermal passage cells, and the percentage of root volume as intercellular air space.

To test whether changes in these root anatomical variables influence mycorrhizal colonization, interspecific variation in each trait was correlated with mycorrhizal colonization in a diverse assortment of plant species. Hypodermal passage cell distribution was the only variable capable of influencing mycorrhizal colonization via its effect on fungal penetration point formation. Mycorrhizal colonization was not found to be significantly influenced by either the percentage of root length suberized or the percentage of root volume as intercellular air space.

Next, I evaluated the effect of P treatment on each of these variables in order to explore whether they might be potential mechanisms involved in phosphorus-mediated control of mycorrhizal colonization. Fifteen species from 13 families were grown at two P availabilities, and the effect of plant P concentration on each variable was assessed. When each plant species was analyzed as a replicate, only the distribution of passage cells was significantly affected by P status; high P plants possessed a reduced percentage of root length with passage cells. Other examined traits did not respond significantly to P

status, and individual species responses to P treatment varied widely. I conclude that, while no single anatomical mechanism appears to be responsible in all species, in species with passage cells, altering the percentage of root length with passage cells could serve as one mechanism for P-mediated control of mycorrhizal colonization.

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Finally, I praise my Creator for the intricate works of His hands, of all that is seen and unseen. "Be exalted, O God, above the heavens; let your glory be all over the earth." (Psalm 57:5).

Chapter 1

Summary

Arbuscular mycorrhizal (AM) fungi are obligate biotrophs, utilizing living plants as their sole source of reduced carbon. Colonization of terrestrial root systems by AM fungi is very common, but the degree of colonization can be quite variable among species. The mycorrhizal colonization process comprises several stages of biochemical communication and/or interaction between the AM fungus and plant host. Control of mycorrhizal colonization by the plant host could occur at any or all of these stages. Previous studies have revealed that root exudate quality and quantity can influence spore germination, hyphal branching, and appressorium formation on the surface of the root. However, little is known of the mechanisms used to control mycorrhizal colonization during later stages of colonization. During these stages, AM fungi attach and grow between the cells of the epidermis, penetrate through the cells of the hypodermis, and grow through the root cortex. Although a large degree of physical contact between symbionts is necessary during these stages, the role of root anatomy has not been explored as a mechanism capable of limiting colonization.

The first major objective of the current study was to address this paucity of knowledge through an investigation of the efficacy of three root anatomical variables in controlling mycorrhizal colonization: hypodermal passage cell distribution in dimorphic hypodermal species, percentage of suberized root length in uniform hypodermal species, and percentage of root volume as intercellular air space in plant species which form Arum-type arbuscules. Each of these traits was examined in multiple plant species from a diverse range of plant families, whereby interspecific variability in each root anatomical variable was correlated to interspecific

variability in mycorrhizal colonization. To identify any general trends that exist across plant family lines, mean values obtained for each anatomical variable were correlated to mean values for mycorrhizal colonization.

Specifically, the following hypotheses were tested:

1. Interspecific variability in mycorrhizal colonization is accounted for by interspecific variation in passage cell distribution (Chapter 2, overall Hypotheses 1a, b).

2. Interspecific variability in mycorrhizal colonization is accounted for by interspecific variation in percentage of root length suberized (Chapter 3, overall Hypothesis 2).

3. Interspecific variability in mycorrhizal colonization is accounted for by interspecific variation in root volume as intercellular air space (Chapter 3, overall Hypothesis 3).

The second major objective of this study was to identify whether any of these root traits may be involved in P-mediated control of mycorrhizal colonization. This classic example of plant control of mycorrhizal colonization is characterized by the reduction in mycorrhizal colonization associated with greater soil or plant P concentrations. This particular phenomenon has been observed in numerous plant species, but the responsible mechanisms have not been completely elucidated. As was the case for the first objective, root anatomical variables have been largely ignored as potential control mechanisms contributing to this phenomenon. In order to determine whether any or all of the root anatomical variables listed above may be involved in this form of plant control of mycorrhizal colonization, the responsiveness of each trait to soil P availability was evaluated. Based on specific mechanisms by which each root anatomical variable might limit colonization, each was hypothesized to respond to soil P availability in a

manner which would explain the observed change in mycorrhizal colonization. The following three hypotheses were tested in multiple host plant species, and mean values from each species were considered as individual replicates.

4. For plant species with a uniform hypodermis, a larger percentage of the hypodermis is suberized at higher plant P concentrations (Chapter 4, overall Hypothesis 4).

5. For plant species with a dimorphic hypodermis, a higher plant P concentration results in a lower percentage of root length with passage cells and/or a lower density of passage cells (Chapter 4, overall Hypothesis 5a, b).

6. The percentage of root volume as intercellular air space is lower in plants of higher P concentrations (Chapter 4, overall Hypothesis 6).

Through this work, I contribute to our knowledge of how root anatomy influences mycorrhizal colonization. In addition, as very few studies have characterized the plasticity of various root anatomical traits in their ability to respond to abiotic factors such as P availability, the second objective of this study will prove to be valuable in that regard. As multiple host plant species from different plant families were used to test each hypothesis, my findings should be applicable to a wide range of plant species.

Chapter 2

Can hypodermal passage cell distribution limit root penetration by mycorrhizal fungi?

Introduction

Colonization of terrestrial root systems by arbuscular mycorrhizal (AM) fungi is very common (Smith & Read, 1997), but the degree of colonization can be quite variable among species. For example, the percentage of root length that becomes colonized can range from 8 to 64% among various crop species (Hayman *et al*., 1976), 20 to 70% among various citrus rootstocks (Graham *et al*., 1991), and 11 to 75% among tallgrass prairie species (Wilson & Hartnett, 1998). Substantial interspecific variation in mycorrhizal colonization has also been observed among plant species in shrublands and grasslands (Wilson & Hartnett, 1998; Roumet *et al.*, 2006) and in tropical forest ecosystems (St John, 1980; Zangaro *et al*., 2005). A number of studies have identified various anatomical or morphological traits as well as ecological factors that strongly correlate with mycorrhizal colonization. These include root diameter or coarseness (Baylis, 1975; St John, 1980; Cook *et al*., 1988; Eissenstat, 1992), the presence and length of root hairs (Lackie *et al*., 1988), and even successional status of the plant (Zangaro *et al*., 2007). Although these traits may help predict the extent to which the roots of various species are colonized and the degree of mycorrhizal dependence, they do not actually explain *how* plants control the extent of colonization. This is the focus of present study.

Initial contact of roots by an AM fungal hypha occurs when the appressorium forms on epidermal cells and anchors the hypha to the root (Hayman *et al*., 1976; Brundrett *et al*., 1985;

Garriock *et al*., 1989). After penetrating the epidermis from the appressorium, the hypha encounters the hypodermis, the outermost layer of cortical cells (Bonfante-Fasolo, 1984). A hypodermis with a Casparian band is often called an exodermis. As I make no note of the presence or absence of Casparian bands in roots examined in this study, I hereafter refer to this layer as a hypodermis. The hypodermis is often anatomically differentiated from more interior layers of the cortex. For example, the outer tangential wall of some hypodermal cells may become suberized, rendering the cells impenetrable to fungi (Perumalla *et al*., 1990; Enstone *et al*., 2002). In order for AM fungal colonization of the cortex to proceed, a penetrating hypha must pass through a hypodermal cell with no suberin lamella (Mosse, 1973; Smith *et al.*, 1989; Brundrett & Kendrick, 1990; Matsubara *et al.*, 1999). Once past the hypodermis, the hypha is then free to grow longitudinally through the cortex either through intercellular air spaces or by intracellular hyphal coiling from one cell to another (Brundrett *et al*., 1985; Dickson, 2004), and to form arbuscules in cells of the inner cortex (Smith & Read, 1997).

Many plant species possess a dimorphic hypodermis, which comprises a matrix of suberized cells with a limited number of unsuberized "passage cells" interspersed therein (Shishkoff, 1987; Perumalla *et al*., 1990). The passage cells are so-called because, unlike suberized cells, they are able to conduct water and ions into the interior of the root (Peterson & Enstone, 1996). Passage cells are also apparently the only cells through which AM fungi gain access to the inner cortex (Mosse, 1973; Smith *et al.*, 1989; Brundrett & Kendrick, 1990; Matsubara *et al.*, 1999). Because unsuberized passage cells are easily differentiated from suberized cells on the basis of their appearance following the staining of partially cleared roots (Shishkoff, 1987), I was able to examine the root systems of eight plant species, each of which possess a dimorphic hypodermis, in order to quantify the distribution of passage cells in relation

to AM fungal penetration into the root cortex. In so doing, I tested the hypothesis that *interspecific variability in mycorrhizal colonization is explained, in part, by interspecific variability in the distribution of hypodermal passage cells* (overall Hypothesis 1). I am unaware of any previous test of this hypothesis.

Materials and Methods

Plant material

To test the hypothesis, I required a collection of dimorphic hypodermal species from a diverse range of plant families (Table 2.1).

Table 2.1. Species, family (tribe), for all species examined in this study, according to the classification system of the Angiosperm Phylogeny Group II (APG II, 2003). Planting date and duration of growth for each species are also listed.

Plant Species	***Family (Tribe)***	***Planting date***	***Duration (DAP)***
Allium cepa L.	Liliaceae	19 Oct 2006	49
Asclepias tuberosa L.	Asclepiaceae	24 Oct 2006	48
Asparagus officinalis L.	Asparagaceae	30 Oct 2006	38
Coreopsis grandiflora Hogg ex Sweet. Per.	Asteraceae (Heliantheae)	22 Sep 2006	47
Ipomoea purpurea L.	Convulvulaceae	26 Jan 2007	19
Ocimum basilicum L.	Lamiaceae	27 Mar 2007	22
Rudbeckia fulgida Ait.	Asteraceae (Astereae)	18 Jul 2006	43
Vinca minor L.	Apocynaceae	15 Feb 2007	40

I referred to Shishkoff (1987) as a guide to identify dimorphic hypodermal genera before confirming myself that those species' roots exhibited this property. I finally selected the following species with a dimorphic hypodermis: *Allium cepa* L., *Asclepias tuberosa* L., *Asparagus officinalis* L., *Coreopsis grandiflora* Hogg ex Sweet. Per., *Ipomoea purpurea* L., *Ocimum basilicum* L., *Rudbeckia fulgida* Ait., *and Vinca minor* L. Sixteen plants of each species were grown separately in 600 ml Deepots (Stuewe and Sons, Inc.) in a greenhouse, with air

temperatures maintained at 24°- 27°C. Growth medium was autoclaved field soil, medium grade sand, and *Glomus intraradices* Schenck and Smith inoculum from pot cultures, mixed to a final volume ratio of 1:4:1. Pot culture inoculum contained field soil and sand (1:4), as well as *G. intraradices* spores and hyphae and colonized roots of *Sorghum sudanense*. Field soil was a Buchanan silt loam (fine-loamy, mixed, semiactive mexic, Aquic Fragiudult), collected from two unfertilized fields at the Russell E. Larson Agricultural Research Center in Rock Springs, PA. It contained 9.2 μg g^{-1} $NaHCO_3$-extractable P.

I harvested each of the sixteen plants from each of the eight species before their root systems became potbound. Some roots of potbound plants will grow at the interface between the soil and the pot wall. Because I could not be assured that the anatomy of such roots would be representative of those growing in soil, I did not use potbound plants. Because each species grew at a different rate, the length of time allowed for growth also varied by species (Table 2.1). At harvest, plants were removed from pots, and shoots and roots were separated. Whole root systems were rinsed carefully in tap water to remove soil and stored in 50 % ethanol.

Clearing and staining roots for mycorrhizal colonization and passage cells

Each root system was divided into taproots and lateral roots. Because the vast majority of colonized root length comprised lateral roots, I excluded taproots from the analysis. Lateral roots were suspended in a beaker of water. Approximately 1.0-2.0 g (fresh weight) of root pieces was randomly selected for analysis. Roots were cleared overnight in 10 % KOH at room temperature, and then rinsed several times in deionized water before soaking for 1 min in 5% HCl to acidify them. To reveal the presence of AM hyphae in the roots as well as hyphae coiling

through hypodermal cells (penetration points), roots were stained overnight in a Trypan blue solution containing 0.06 % Trypan blue in a solution of 2.5 % glacial acetic acid, 47.5 % deionized water, and 50 % glycerol. The percentage of root length colonized by AM fungi, was determined using the line intersect method (Koide and Mooney, 1987).

Measurement of passage cell and penetration point distributions and colonization (%)

The percentage of root length colonized by AMF, hereafter referred to as colonization (%), was determined using the line intersect method (Koide & Mooney, 1987). Both passage cell distribution and penetration point distribution were characterized using two different methods. The first was analogous to the one used to determine colonization (%). In other words, I determined the percentage of root length with passage cells (passage cells (%)) and the percentage of root length with penetration points (penetration points (%)) using the line intersect method (Koide & Mooney, 1987), calculated as the percentage of intersections where either passage cells or penetration points were present along the line intersecting the root segment. As this method takes into account only the presence/absence of features at a point of intersection, a second method was used to determine passage cell density and penetration point density. In this second method, ten intersections were randomly selected for each root sample, and the number of passage cells and penetration points present in each of the ten 1-mm segments of root to the left of the intersect line was recorded. The root intersections possessing no passage cells and thus no penetration points were excluded from the overall species means calculations. The reported values therefore represent the densities of passage cells or penetration points in sections where passage cells actually exist.

To confirm that AM fungal penetration occurred only through passage cells, I noted while assessing penetration point densities in all species whether penetration occurred through passage cells or non-passage cells.

Statistical analyses

To determine whether significant correlations existed between colonization, penetration, and passage cell distribution, means of each species were plotted and correlation coefficients were determined among traits. Linear, quadratic, and cubic fitted regression analyses were performed in Minitab (1998), and the model that explained the most variation (R^2) was used. The significance of each model was determined from the p-value for the regression line. A one-way analysis of variance (Minitab, 1998) was used to determine the significance of species to the mean number of penetration points per ten passage cells.

Results

For each species, the sixteen replicates yielded anywhere from several hundred to over a thousand penetration points. For each species, all penetration points occurred through passage cells. There were no exceptions (Table 2.2).

Table 2.2. Total number of penetration points occurring through passage cells and non-passage cells, and mean number (standard error) of penetration points formed per 10 passage cells for each species. n=16 per species.

Plant Species	***No. penetration points through passage cells***	***No. penetration points through non-passage cells***	***Mean (s.e.) no. penetration points per 10 passage cells***
Ocimum basilicum L.	215	0	0.963 (0.134) ab
Rudbeckia fulgida Ait.	264	0	0.300 (0.037) c
Coreopsis grandiflora Hogg ex Sweet Per.	355	0	0.501 (0.057) c
Vinca minor L.	392	0	0.483 (0.060) c
Allium cepa L.	404	0	0.851 (0.079) b
Ipomoea purpurea L.	757	0	0.467 (0.069) c
Asclepias tuberosa L.	863	0	0.506 (0.063) c
Asparagus officinalis L.	1280	0	1.214 (0.095) a

However, there was a low density of penetration points relative to the density of passage cells. For every ten passage cells, the mean number of fungal penetration points ranged from 0.3 to 1.2, depending on the species (Table 2.2).

A regression analysis revealed that passage cells (%) and passage cell density (the two measurements used to characterize hypodermal passage cell distribution) were not significantly correlated ($R^2 = 0.324$, $p = 0.141$; data not shown).

The correlation among species between passage cell density and penetration point density (Figure 2.1) was not statistically significant ($R^2 = 0.128$; $p = 0.383$).

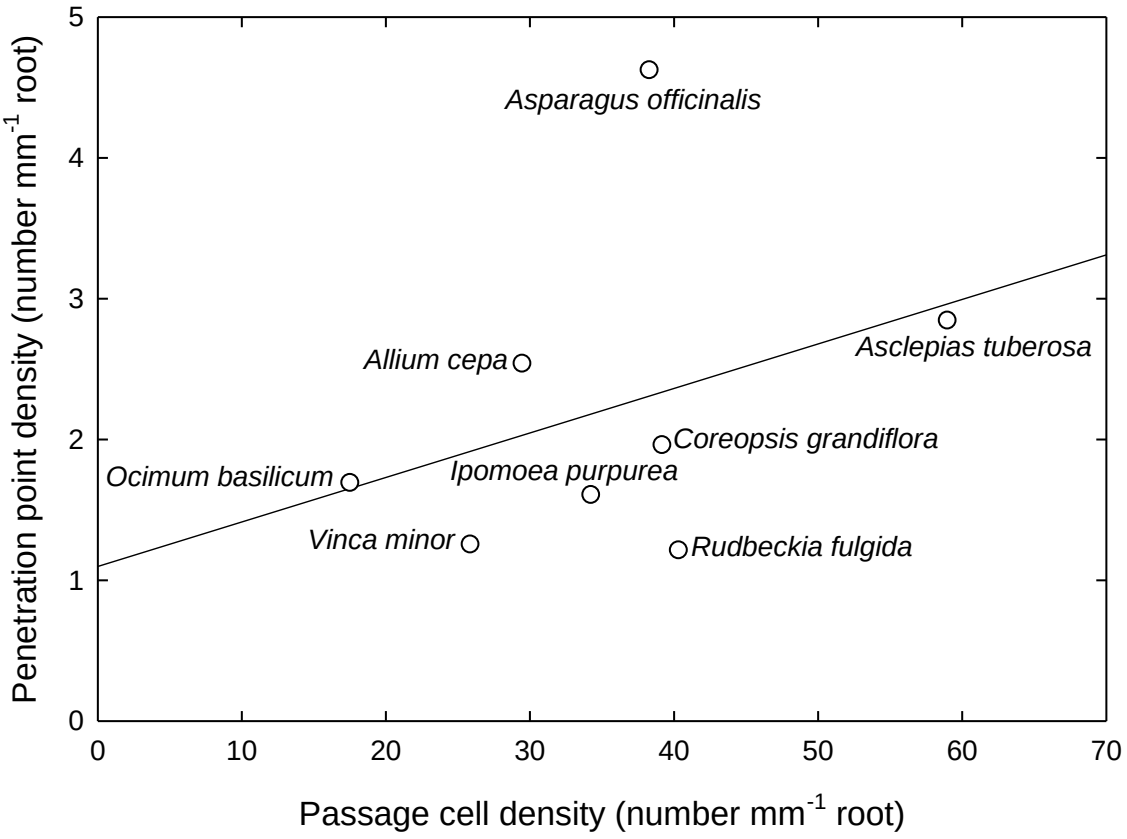

Figure 2.1: Correlation between penetration point density (number mm^{-1} root) and passage cell density (number mm^{-1} root). Each datapoint represents the mean from the sixteen replicates of that species. $Y = 26.9 + 3.88x$; $R^2 = 0.128$; $p = 0.383$.

In contrast, there was a strong positive relationship between passage cells (%) and penetration points (%) ($R^2 = 0.987$; $p < 0.0001$, Figure 2.2).

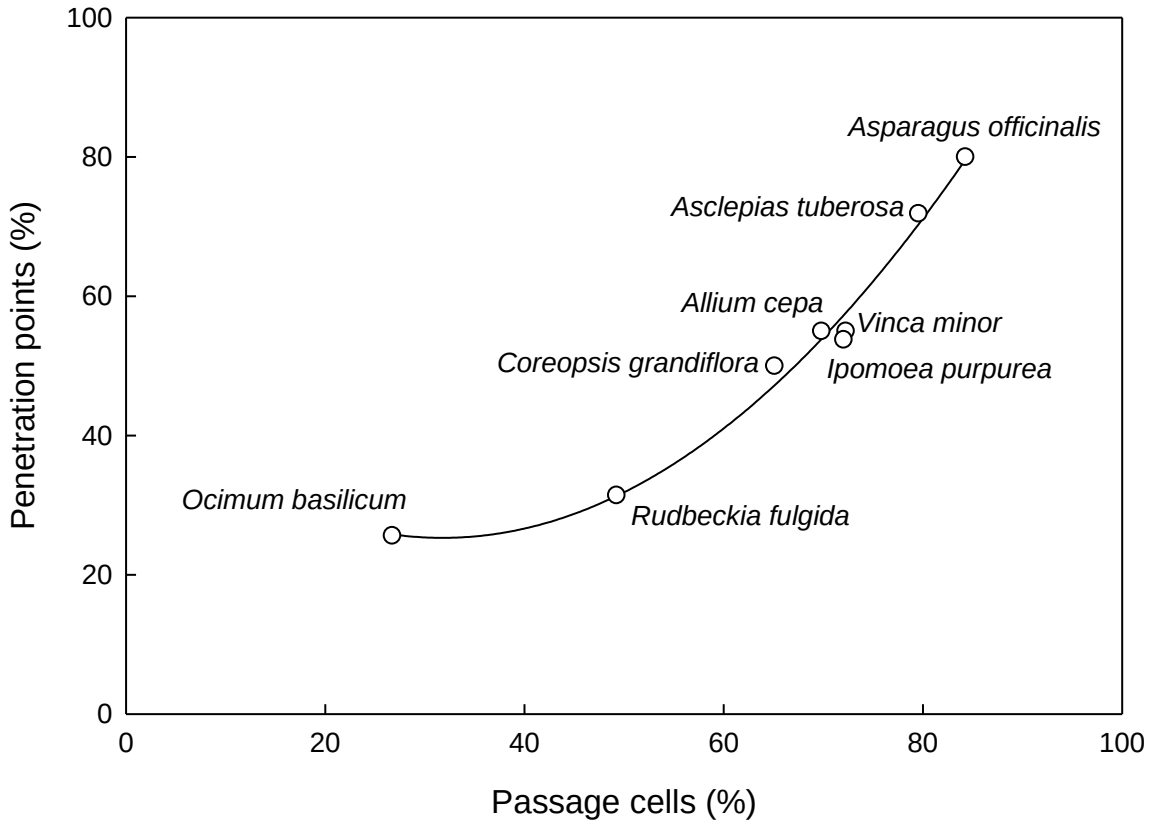

Figure 2.2: Correlation between penetration points (%) and passage cells (%). Each datapoint represents the mean from the sixteen replicates of that species. $Y = 45.2\text{-}1.25x + 0.02x^2$; $R^2 = 0.987$; $p < 0.0001$.

The correlation between penetration points (%) and colonization (%) (Figure 2.3) was nearly significant ($R^2 = 0.441$; $p = 0.072$).

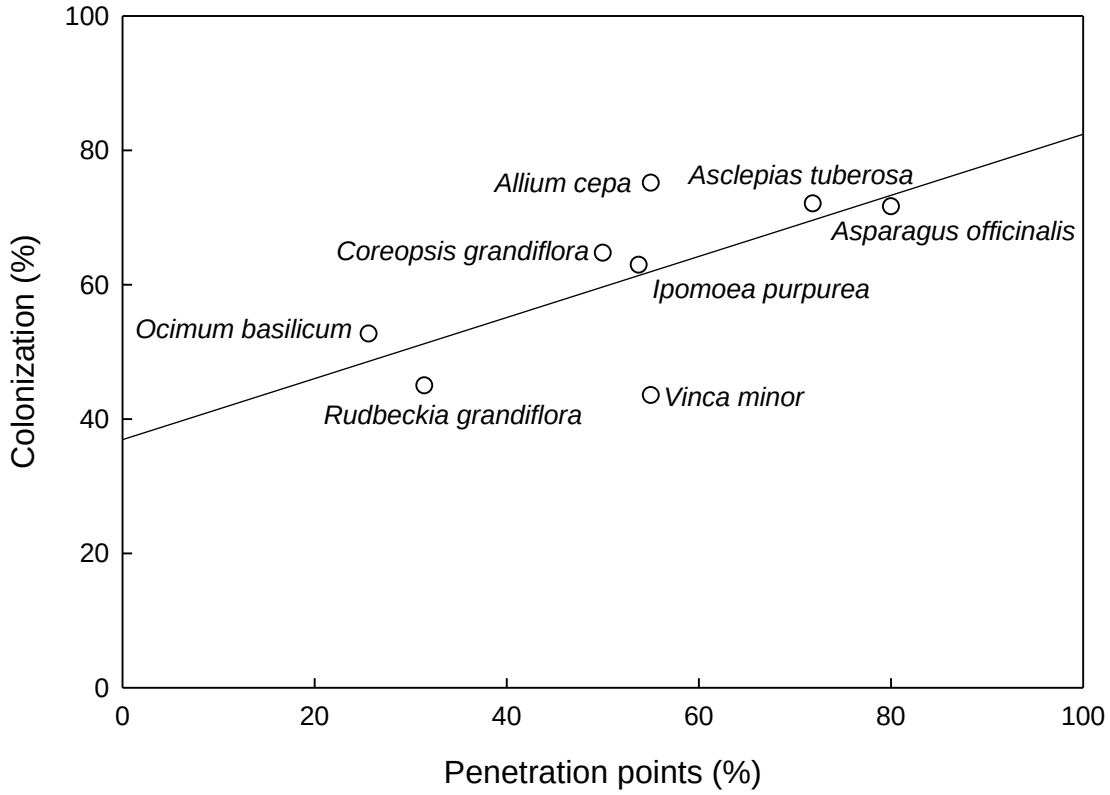

Figure 2.3: Correlation between colonization (%) and penetration points (%). Each datapoint represents the mean from the sixteen replicates of that species. Y = 36.94 + 0.45x; R^2 = 0.441; p = 0.072.

Discussion

For dimorphic hypodermal species, AMF penetration into the cortex apparently occurs only through passage cells (Mosse, 1973; Smith *et al.*, 1989; Brundrett & Kendrick, 1990; Matsubara *et al.*, 1999). My results confirmed this for the eight species included in this investigation. Passage cells are apparently also important for root colonization by other microorganisms including the pathogens *Pyrenochaeta* (Becker, 1976), *Fusarium* (Kamula *et al*., 1994) and *Pythium* (Shishkoff, 1989). It seems reasonable to hypothesize, therefore, that for dimorphic hypodermal species, interspecific variation in the degree of colonization is related to variation in passage cell distribution.

I examined two aspects of hypodermal passage cell distribution, including the percentage of root length containing passage cells (passage cells (%)) and the number of passage cells per mm root length (passage cell density). These variables were uncorrelated, indicating that they were independent characterizations of passage cell distribution (data not shown). For the eight species included in this study, I examined the relationships between 1) passage cell density and penetration point density and 2) the percentage of root length containing passage cells (passage cells (%)) and the percentage of root length containing penetration points (penetration points (%)).

Passage cell density was not significantly correlated with penetration point density (Figure 2.1). From many observations, it became clear why this may have been the case. In most instances, passage cells in a given length of root were far more dense than penetration

points (Figure 2.4), such that for every ten passage cells the mean number of penetration points formed was, on average, less than 1 (Table 2.2).

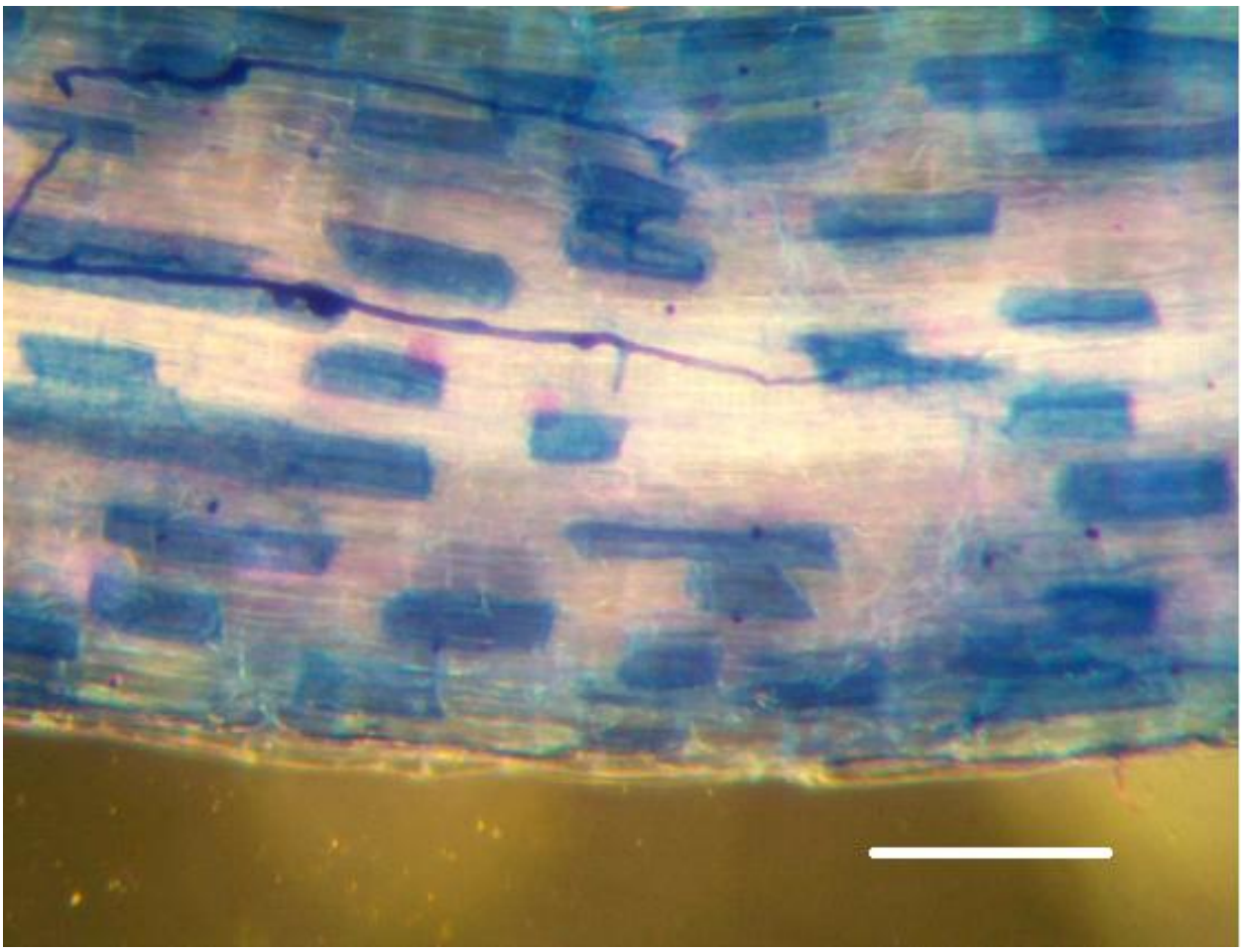

Figure 2.4: Longitudinal view of *Allium cepa* root stained with Trypan blue. Hypodermal passage cells are stained blue. Mycorrhizal hyphae penetrating into the cortex through the hypodermal cells are stained darker blue. Scale bar: 0.1 mm.

Thus, at the inoculum potential of this experiment, it is likely that passage cell density did not limit the density of penetration points. It is possible that at higher inoculum potentials, with a greater number of hyphae attempting to colonize the roots, passage cell density could prove to be more limiting.

In contrast, the correlation between the percentage of root length containing passage cells (passage cells (%)) and the percentage of root length containing penetration points (penetration

points (%)) was very strong (Figure 2.2). Nearly 99% of the interspecific variation in penetration points (%) was accounted for by interspecific variation in passage cells (%). The most parsimonious explanation of this significant correlation is that the percentage of the root system that is colonizable (contains passage cells) limits penetration of the hypodermis by AM fungi.

Because fungal penetration of the hypodermis precedes colonization of the cortex, one might expect a good correlation between penetration points (%) and colonization (%). This correlation (Figure 2.3) was nearly significant ($p = 0.072$, $R^2 = 0.441$). That only 44% of the variability in colonization is accounted for by hypodermal penetration may reflect the fact that in addition to penetration, the rate of longitudinal spread through the cortex from the site of penetration will also influence total cortical colonization. The longitudinal rate of spread may be influenced, for example, by the amount of air space within the cortex (Brundrett *et al*., 1985), or by the rate of carbon transfer from host to fungus (Koide & Schreiner, 1992).

Based on these results, I conclude that the percentage of root length containing passage cells can serve as one point of control of mycorrhizal colonization via its effects on fungal penetration into the cortex. The eight species I examined exhibited a wide range of both passage cells (%) and penetration points (%), varying from less than 30% to nearly 90% of root length. The broad amplitude in % passage cells makes this particular trait an effective one with which to control colonization.

Because I assayed for passage cells and not for suberin *per se*, I cannot unequivocally conclude that penetration occurred only through passage cells because they were unsuberized. Other factors may have been involved in attracting fungi to these particular cells, such as chemotactic exudates or galvanotactic electrical fields (Shishkoff, 1989; Smith *et al*., 1989;

Nagahashi *et al.*, 1996a; Tawaraya *et al.*, 1998; Matsubara *et al.*, 1999). Nevertheless, it is doubtful whether exudates or electrical fields could be operative if passage cells were suberized because both require the movement of materials across the cell wall. Moreover, suberization apparently prevents colonization of the cortex by fungi and related mycelial organisms (Becker, 1976; Shishkoff, 1989; Kamula *et al*., 1994). Thus, the lack of suberization of passage cells appears to be a key feature of a dimorphic hypodermis insofar as colonization by AMF is concerned.

There are a number of factors unrelated to root anatomy that may control mycorrhizal colonization, including root growth rate (Buwalda *et al*., 1984), root exudation (Nagahashi *et al*., 1996a, b), and root age (Hepper, 1985), but it is not surprising to find evidence consistent with the hypothesis that a simple anatomical trait contributes significantly to the control of mycorrhizal colonization. Roots are conduits of photosynthate into the soil, which contains numerous fungi and bacteria capable of using roots and root compounds as sources of nutrition. One might naturally expect the protection of the root cortex from such microorganisms to include physical barriers such as suberin lamellae covering the surface of hypodermal cells (Peterson, 1989; Peterson, 1992). The admission into the root cortex of nutrient ions or beneficial fungi would therefore require barrier-free "passage cells". The notion that passage cells could influence mycorrhizal colonization was first mentioned by Demeter (1923), who observed special passage cells with thickened cell walls through which fungi rapidly penetrated into the cortex (Demeter, 1923). Since then, multiple studies have confirmed that fungal entry is limited to passage cells in dimorphic hypodermal species (Mosse, 1973; Becker, 1976; Shishkoff, 1989; Smith *et al*., 1989; Kamula *et al*., 1994; Matsubara *et al*., 1999). However, I believe this is the first report to present evidence that variation in the distribution of passage cells

among plant species may contribute to variation in colonization. Considering the tight coevolution that has occurred between roots and mycorrhizal fungi (Brundrett, 2002), it is reasonable to hypothesize that variability in passage cell distribution among plant species has evolved to control mycorrhizal colonization.

While I have performed this study with only a single species of AMF, it seems likely that the pattern I observed would also exist for other species. Other researchers have found that, for dimorphic hypodermal plant species, entry into the cortex is limited to passage cells for other species of AMF including *Glomus mosseae* (Mosse, 1973), *Glomus etunicatum* and *Glomus sp. (WUM 16) "City Beach"* (Smith *et al.*, 1989), as well as *Glomus intraradices* (Matsubara *et al.*, 1999), the species I utilized.

There are undoubtedly numerous other anatomical factors that contribute to the level of colonization of a particular plant species, and further studies are needed to identify these. Some of these include the anatomical properties of the root surface, which may influence the formation of appressoria (Nagahashi & Douds, 1997), suberization of the hypodermis, and the extent of cortical air space, which may influence the rate of longitudinal growth of the hyphae (Brundrett *et al*., 1985). The next chapter examines the role of the latter two in controlling mycorrhizal colonization.

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References

APG II. 2003. An update of the Angiosperm Phylogeny Group classification for orders and families of flowering plants: APG II. *Botanical Journal of the Linnaean Society* **141**: 399-436.

Baylis GTS. 1975. The magnolioid mycorrhiza and mycotrophy in root systems derived from it. In: Sanders FE, Mosse B, Tinker PB, eds. *Endomycorrhizas*. New York, USA: Academic Press, 373-389.

Becker WN. 1976. Quantification of onion vesicular arbuscular mycorrhizae and their resistance to *Pyrenochaeta terrestris.* Ph.D. dissertation, University of Illinois, USA.

Bonfante-Fasolo P. 1984. Anatomy and morphology of VA mycorrhizae. In: Powell CL, Bagyaraj DJ, eds. *VA Mycorrhizas*. Boca Raton: CRC Press, 5-33.

Brundrett M, Kendrick B. 1990. The roots and mycorrhizas of herbaceous woodland plants.II. Structural aspects of morphology. *New Phytologist* **114**: 469-479.

Brundrett MC. 2002. Coevolution of roots and mycorrhizas of land plants. *New Phytologist* **154**: 275-304.

Brundrett MC, Piche Y, Peterson RL. 1985. A developmental-study of the early stages in vesicular-arbuscular mycorrhiza formation. *Canadian Journal of Botany-Revue Canadienne de Botanique* **63**: 184-194.

Buwalda JG, Stribley DP, Tinker PB. 1984. The development of endomycorrhizal root systems.V. The detailed pattern of development of infection and the control of infection level by host in young leek plants. *New Phytologist* **96**: 411-427.

Cook BD, Jastrow JD, Miller RM. 1988. Root and mycorrhizal endophyte development in a chronosequence of restored tallgrass prairie. *New Phytologist* **110**: 355-362.

Demeter VK. 1923. Über "Plasmoptysen"-Mykorrhiza. *Flora* **116**: 405-456.

Dickson S. 2004. The Arum-Paris continuum of mycorrhizal symbioses. *New Phytologist* **163**: 187-200.

Eissenstat DM. 1992. Costs and benefits of constructing roots of small diameter. *Journal of Plant Nutrition* **15**: 763-782.

Enstone DE, Peterson CA, Ma FS. 2002. Root endodermis and exodermis: Structure, function, and responses to the environment. *Journal of Plant Growth Regulation* **21**: 335-351.

Garriock ML, Peterson RL, Ackerley CA. 1989. Early stages in colonization of *Allium porrum* (leek) roots by the vesicular arbuscular mycorrhizal fungus, *Glomus versiforme*. *New Phytologist* **112**: 85-92.

Graham JH, Eissenstat DM, Drouillard DL. 1991. On the relationship between a plant's mycorrhizal dependency and rate of vesicular-arbuscular mycorrhizal colonization. *Functional Ecology* **5**: 773-779.

Hayman DS, Barea JM, Azcon R. 1976. Vesicular-arbuscular mycorrhiza in southern Spain - its distribution in crops growing in soil of different fertility. *Phytopathologia Mediterranea* **15**: 1-6.

Hepper CM. 1985. Influence of age of roots on the pattern of vesicular arbuscular mycorrhizal infection in leek and clover. *New Phytologist* **101**: 685-693.

Kamula SA, Peterson CA, Mayfield CI. 1994. Impact of the exodermis on infection of roots by *Fusarium culmorum*. *Plant and Soil* **167**: 121-126.

Koide RT, Mooney HA. 1987. Spatial variation in inoculum potential of vesicular arbuscular mycorrhizal fungi caused by formation of gopher mounds. *New Phytologist* **107**: 173-182.

Koide RT, Schreiner RP. 1992. Regulation of the vesicular-arbuscular mycorrhizal symbiosis. *Annual Review of Plant Physiology and Plant Molecular Biology* **43**: 557-581.

Lackie SM, Bowley SR, Peterson RL. 1988. Comparison of colonization among half-sib families of *Medicago sativa* by *Glomus versiforme* (Daniels and Trappe) Berch. *New Phytologist* **108**: 477-482.

Matsubara Y, Uetake Y, Peterson RL. 1999. Entry and colonization of *Asparagus officinalis* roots by arbuscular mycorrhizal fungi with emphasis on changes in host microtubules. *Canadian Journal of Botany-Revue Canadienne de Botanique* **77**: 1159-1167.

MINITAB 1998. Release 12 for Windows. Minitab. State College, PA, USA.

Mosse B. 1973. Plant-growth responses to vesicular-arbuscular mycorrhiza.IV. In soil given additional phosphate. *New Phytologist* **72**: 127-136.

Nagahashi G, Douds DD, Abney GD. 1996a. Phosphorus amendment inhibits hyphal branching of the VAM fungus *Gigaspora margarita* directly and indirectly through its effect on root exudation. *Mycorrhiza* **6**: 403-408.

Nagahashi G, Douds DDJ, Abney GD 1996b. A rapid microinjection technique allows for the sensitive detection of root exudate signals which stimulates the branching and growth and germinated VAM fungus spores. In: Szaro TM, Bruns TD, eds. *First International Conference on Mycorrhizae*. University of California, Berkeley, USA.

Nagahashi G, Douds DD. 1997. Appressorium formation by AM fungi on isolated cell walls of carrot roots. *New Phytologist* **136**: 299-304.

Perumalla CJ, Peterson CA, Enstone DE. 1990. A survey of angiosperm species to detect hypodermal Casparian bands.I. Roots with a uniseriate hypodermis and epidermis. *Botanical Journal of The Linnean Society* **103**: 93-112.

Peterson CA. 1989. Significance of the exodermis in root function. In: Loughman BC, Gasparikova O, Kolek J, eds. *Structural and functional aspects of transport in roots*. Dordrecht, The Netherlands: Kluwer Academic Publishers, 35-40.

Peterson CA, Enstone DE. 1996. Functions of passage cells in the endodermis and exodermis of roots. *Physiologia Plantarum* **97**: 592-598.

Peterson RL. 1992. Adaptations of root structure in relation to biotic and abiotic factors. *Canadian Journal of Botany-Revue Canadienne de Botanique* **70**: 661-675.

Roumet C, Urcelay C, Diaz S. 2006. Suites of root traits differ between annual and perennial species growing in the field. *New Phytologist* **170**: 357-368.

Shishkoff N. 1987. Distribution of the dimorphic hypodermis of roots in angiosperm families. *Annals of Botany* **60**: 1-15.

Shishkoff N. 1989. Zoospore encystment pattern and germination on onion roots, and the colonization of hypodermal cells by *Pythium coloratum*. *Canadian Journal of Botany-Revue Canadienne de Botanique* **67**: 258-262.

Smith SE, Long CM, Smith FA. 1989. Infection of roots with a dimorphic hypodermis: possible effects on solute uptake. *Agriculture Ecosystems & Environment* **29**: 403-407.

Smith SE, Read DJ. 1997. *Mycorrhizal symbiosis*. 2nd Ed. London: Academic Press.

St John TV. 1980. Root size root hair and mycorrhiza infection a reexamination of Baylis's hypothesis with tropical trees. *Acta Amazonica* **10**: 141-146.

Tawaraya K, Hashimoto K, Wagatsuma T. 1998. Effect of root exudate fractions from P-deficient and P-sufficient onion plants on root colonisation by the arbuscular mycorrhizal fungus *Gigaspora margarita*. *Mycorrhiza* **8**: 67-70.

Wilson GWT, Hartnett DC. 1998. Interspecific variation in plant responses to mycorrhizal colonization in tallgrass prairie. *American Journal of Botany* **85**: 1732-1738.

Zangaro W, Nishidate FR, Camargo FRS, Romagnoli GG, Vandressen J. 2005. Relationships among arbuscular mycorrhizas, root morphology and seedling growth of tropical native woody species in southern Brazil. *Journal of Tropical Ecology* **21**: 529-540.

Zangaro W, Nishidate FR, Vandressen J, Andrade G, Nogueira MA. 2007. Root mycorrhizal colonization and plant responsiveness are related to root plasticity, soil fertility and successional status of native woody species in southern Brazil. *Journal of Tropical Ecology* **23**: 53-62.

Chapter 3

Hypodermal suberization in a uniform hypodermis and intercellular air space do not limit mycorrhizal colonization

Introduction

Significant interspecific variability in mycorrhizal colonization rates has been documented in a wide range of species and ecosystems (Hayman *et al*., 1976; Graham *et al*., 1991; Wilson & Hartnett, 1998; Roumet *et al*., 2006; St John, 1980; Zangaro *et al*., 2005). Several studies have identified anatomical, morphological and ecological traits which correlate with mycorrhizal colonization levels, including root diameter or coarseness (Baylis, 1975; St John, 1980; Cook *et al*., 1988; Eissenstat, 1992), presence and length of root hairs (Lackie *et al*., 1988), and successional status of the plant (Zangaro *et al*., 2007). While these traits allow us to better predict the mycorrhizal status of a given plant, they do not explain the mechanisms responsible for the observed differences in mycorrhizal colonization levels across species. In the previous chapter, I demonstrated how hypodermal passage cell distribution is able to influence mycorrhizal colonization via its effects on penetration point formation. In the current chapter, I examine two other root anatomical traits: suberization of a uniform hypodermis and percentage of root volume of intercellular air space.

Shortly after initial contact of roots by an AM fungal hypha, the appressorium forms on epidermal cells and anchors the hypha to the root (Hayman *et al*., 1976; Brundrett *et al*., 1985; Garriock *et al*., 1989). After penetrating the epidermis from the appressorium, the hypha encounters the hypodermis, the outermost layer of cortical cells (Bonfante-Fasolo, 1984). In

contrast to ‘dimorphic’ hypodermal species, such as those examined in the previous chapter, the current chapter focuses on ‘uniform’ hypodermal species, in which the hypodermis does not contain hypodermal passage cells. In this type of hypodermis, hypodermal cells become suberized in a developmentally regulated fashion (Peterson & Perumalla, 1984; Ma & Peterson, 2003). The rate of suberization varies with plant species, and is responsive to various environmental stimuli (Hose *et al*., 2001; Enstone *et al*., 2003). Because hyphae can only penetrate an unsuberized portion of the hypodermis, one hypothesis was that in species with a uniform hypodermis, *those with a lower percentage of suberized root length would have higher mycorrhizal colonization (%)* (overall Hypothesis 2).

Once past the hypodermis, hyphae spread longitudinally through the cortex either by growing between cells or by traversing through a series of cells (Brundrett *et al*., 1985; Dickson, 2004), forming arbuscules in cells of the inner cortex (Smith & Read, 1997). Arum-type arbuscules are formed within inner cortical cells as offshoots from intercellular hyphae, whereas Paris-type arbuscules form on hyphal coils. The amount of air space is hypothesized to allow for more rapid longitudinal growth of hyphae in roots of plants that form Arum-type arbuscules (Brundrett *et al*., 1985; Dickson, 2004). Thus, the next hypothesis was that *for plant species which form Arum-type arbuscules, those with a higher percentage of their root volume as intercellular air space will have an enhanced mycorrhizal colonization (%)* (overall Hypothesis 3).

Materials and Methods

Plant material

Multiple species from different plant families were used to test both hypotheses (see Table 3.1).

Table 3.1: Listing of each plant species, their family (tribe) (APG, 2003), planting dates, duration of growth prior to harvest, and the hypotheses* for which they were utilized. *: SUH: Suberization of a uniform hypodermis (overall Hypothesis 2); IAS: Percentage of root volume as intercellular air space (overall Hypothesis 3).

Species	**Family**	**Planting date**	**Duration (d)**	**Hypotheses***
Allium cepa L.	Liliaceae	9 Oct 2006	49	IAS
Antirrhinum majus L.	Scrophulariaceae	8 May 2007	38	SUH, IAS
Aquilegia vulgaris L.	Ranunculaceae	22 Sep 2006	53	SUH, IAS
Asclepias tuberosa L.	Asclepiaceae	24 Oct 2006	48	IAS
Asparagus officinalis L.	Asparagaceae	30 Oct 2006	38	IAS
Coreopsis grandiflora Hogg. ex Sweet Per.	Asteraceae (Heliantheae)	22 Sep 2006	47	IAS
Cucurbita sativa L.	Cucurbitaceae	8 May 2007	18	IAS
Daucus carota L.	Apiaceae	8 May 2007	39	SUH, IAS
Helianthus annuus L.	Asteraceae (Heliantheae)	14 Aug 2007	21	SUH
Ipomoea purpurea L.	Convulvulaceae	16 Aug 2007	15	IAS
Rudbeckia fulgida Ait.	Asteraceae (Astereae)	18 Jul 2006	43	IAS
Solanum tuberosa L.	Solanaceae	8 May 2007	37	SUH, IAS
Zea mays L.	Poaceae	25 Aug 2006	18	SUH, IAS

Sixteen plants from each species were grown according to the methods described in Chapter 2. Briefly, each individual plant was grown separately, in a sand and soil mixture amended inoculated with *Glomus intraradices* inoculum. The soil contained 9.2 $\mu g\ g^{-1}$ $NaHCO_3$-extractable P. Nutrients were supplied to all plants in the form of one-third strength Hoagland's

nutrient solution (for which 1 mmol L^{-1} KH_2PO_4 was replaced by 1 mmol L^{-1} KCl) (Machlis & Torrey, 1956). Plants were harvested before their root systems became potbound. Each species grew at a different rate, so the length of time I allowed for growth also varied by species (Table 3.1).

At harvest, plants were removed from the pots, and shoots and roots were separated. Whole root systems were rinsed carefully in tap water, and then each root system was divided into taproots and lateral roots. Because the vast majority of colonized root length comprised lateral roots, and because I knew from previous examination that taproots had significantly higher suberized root length (%) as well as intercellular air space (%), I excluded taproots from the analysis. Lateral roots were suspended in a beaker of water, and approximately 1.0 – 2.0 g (FW) of root pieces were randomly selected and assessed for suberized root length (%), intercellular air space (%), and colonization (%). Separate subsets of roots were used for each of these measurements. Roots selected for intercellular air space (%) measurements were processed immediately, whereas those used to obtain suberized root length (%) and colonization (%) were stored in 50 % ethanol and processed at a later date, as described below.

Clearing and staining roots for colonization (%)

Roots were cleared and stained according to the methods described in Chapter 2. Briefly, roots were cleared overnight in 10 % KOH at room temperature, then stained in a Trypan blue solution overnight to reveal AM hyphae. The percentage of root length colonized by AM fungi (colonization (%)), was determined using the line intersect method (Koide & Mooney, 1987).

Measurement of suberized root length (%)

Prior to staining for suberin, roots were lightly cleared overnight at room temperature in a 10 % KOH solution to allow better stain permeation. Roots were removed from the KOH, rinsed in 5 % HCl to acidify them, and stained overnight at room temperature in a Sudan red 7B stain solution, made up according to the method of Brundrett *et al*. (1991). The suberized portions of the hypodermis appeared red or light pink against a white background. To make the red or pink staining more obvious, following the staining in Sudan red 7B, roots were counterstained overnight in Trypan blue solution (as described above). The resulting light blue background allowed us to better visualize the pink or red suberized hypodermis. Using the line intersect method (Koide & Mooney, 1987), I calculated the suberized root length (%) as the percentage of root intersections with red or pink stain relative to total root intersections.

Measurement of intercellular air space (%)

The percentage of root volume as intercellular air space (intercellular air space (%)) was calculated using a pycnometer, a small vial capable of holding a pre-determined volume of de-gassed water. According to Archimedes' Principle, one can calculate the volume of gas in porous tissue by determining the weight of the water displacing the gas (Raskin, 1983). The following calculation was made to determine intercellular air space (%):

Intercellular air space (%) = $(W3 - W2) / (Wr + W1 - W2) \times 100$

Where:

Wr = weight of roots (g)

W1 = weight of water-filled pycnometer (g)

W2 = weight of pycnometer with roots and water (g)

W3 = weight of pycnometer with ground roots and water (g)

To determine the weight of the water in the pycnometer (W1), the pycnometer was filled with de-gassed water and weighed. Water was de-gassed by boiling and was cooled to room temperature in a closed glass bottle. Fresh lateral roots from individual plants were placed in a beaker of water to uniformly hydrate them. A subsample of roots was bundled into a polyester mesh envelope, and this was then placed on top of a paper towel stuffed into a test tube. The test tube was centrifuged in a clinical desktop centrifuge at moderate speed for 40 s to remove excess water, and roots were weighed immediately to determine the fresh weight (Wr). To determine the volume of these roots when they contain air space, the fresh roots were placed into the pycnometer filled with water, and weighed immediately (W2). Finally, the roots were removed and ground with mortar and pestle to eliminate air space. The ground roots were added back into the pycnometer, which was then filled with water once more, and reweighed (W3).

Statistical analyses

To determine whether significant correlations existed between colonization, percentage of suberized root length, and percentage of root volume as intercellular air space, means of each species were plotted and correlation coefficients were determined among traits. Linear regression analyses were performed in Minitab (1998). The significance of each model was determined from the p-value for the regression line. Tukey's Honest Significant Difference test

was used to analyze significant differences in means across species within each hypothesis. Intercellular air space data were square root transformed after 1 was added to each datapoint.

Results

All of the arbuscules observed in this study were the Arum-type. Mean values of suberized root length (%) ranged from approximately 5 to 35 % (Figure 3.1).

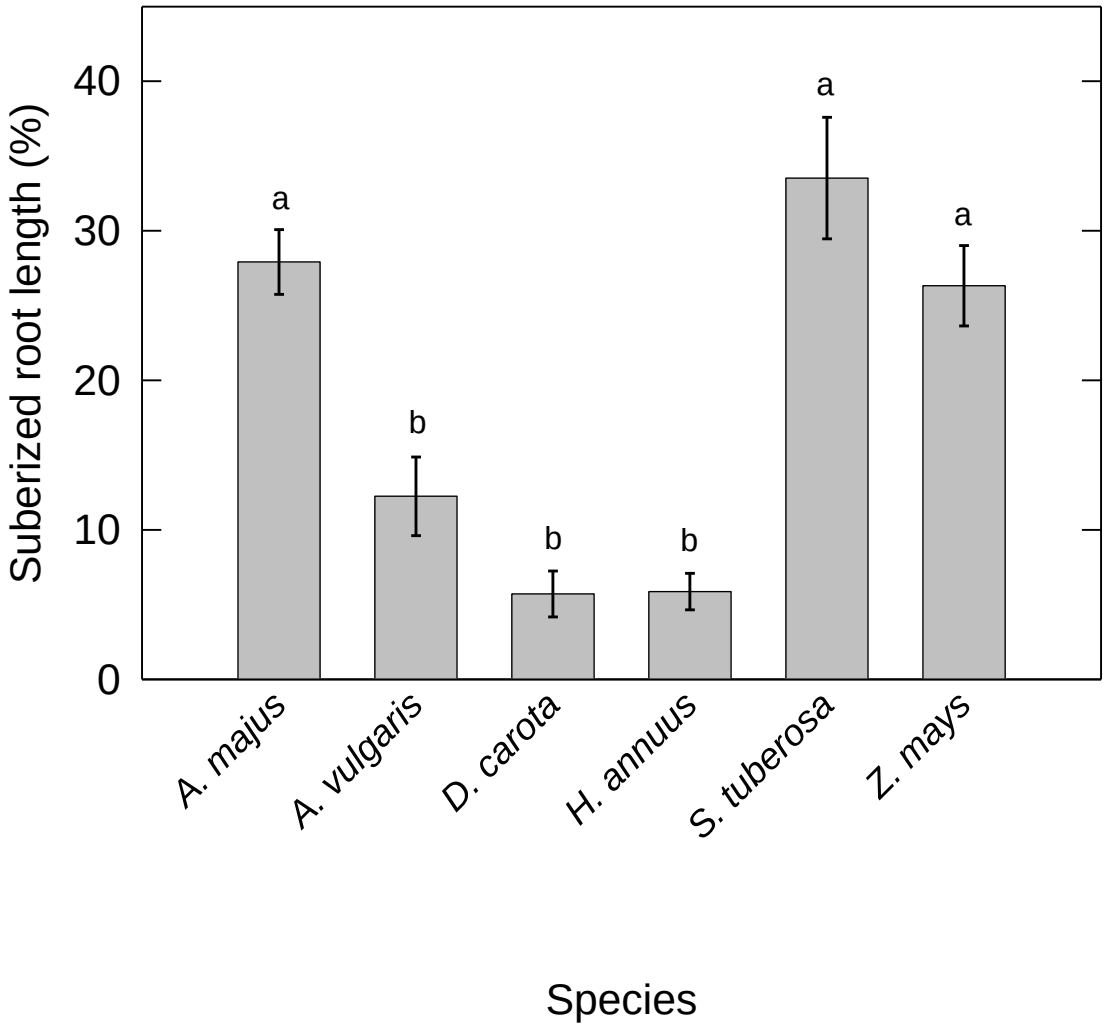

Figure 3.1: Mean suberized root length (%) for each species examined for overall Hypothesis 2. Error bars represent ± 1 s.e.m. Letters above bars represent significant differences between species means, as determined by the Tukey's Honest Significant Difference (HSD) test.

Suberized root length (%) was not significantly correlated with colonization (%) (Figure 3.2).

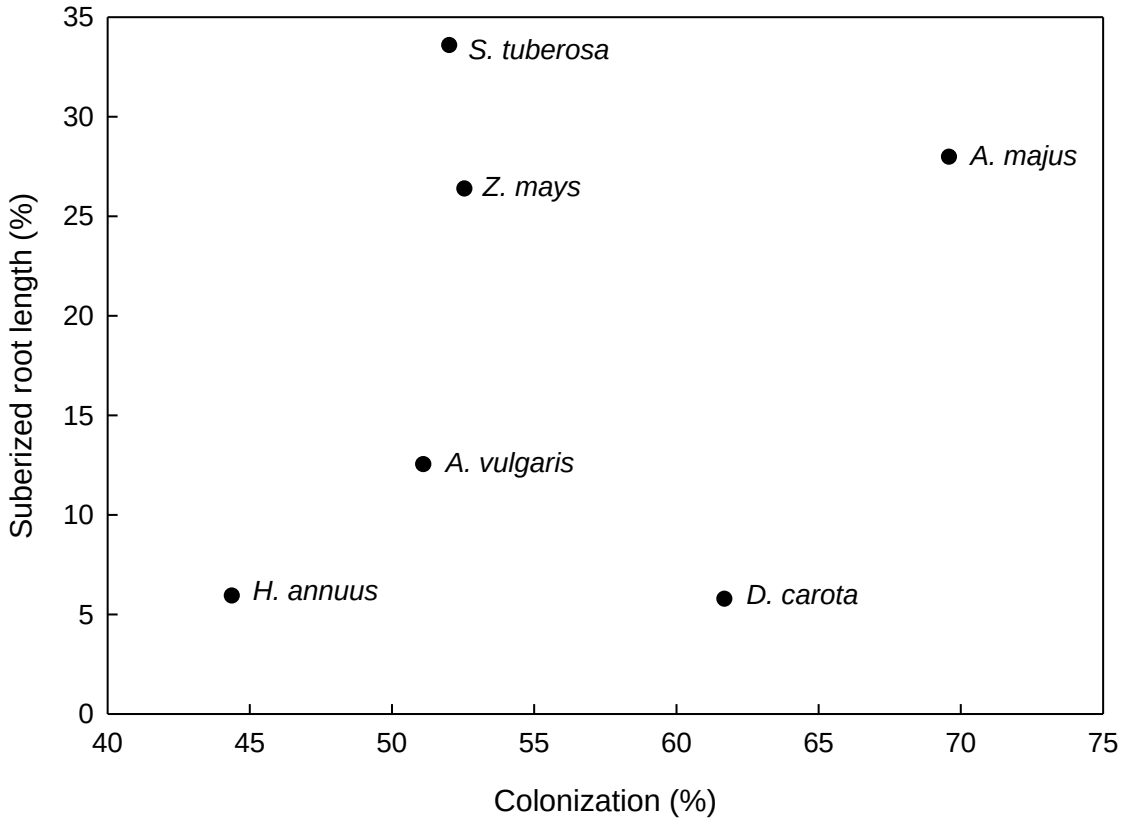

Figure 3.2: Correlation between mycorrhizal colonization and the percentage of root length possessing a suberized hypodermis (suberized root length (%)). Each datapoint represents the mean from 16 replicate plants of each species. $R^2 = 0.072$, $p = 0.608$.

Mean values of intercellular air space (%) ranged from approximately 2 to 5 % (Figure 3.3).

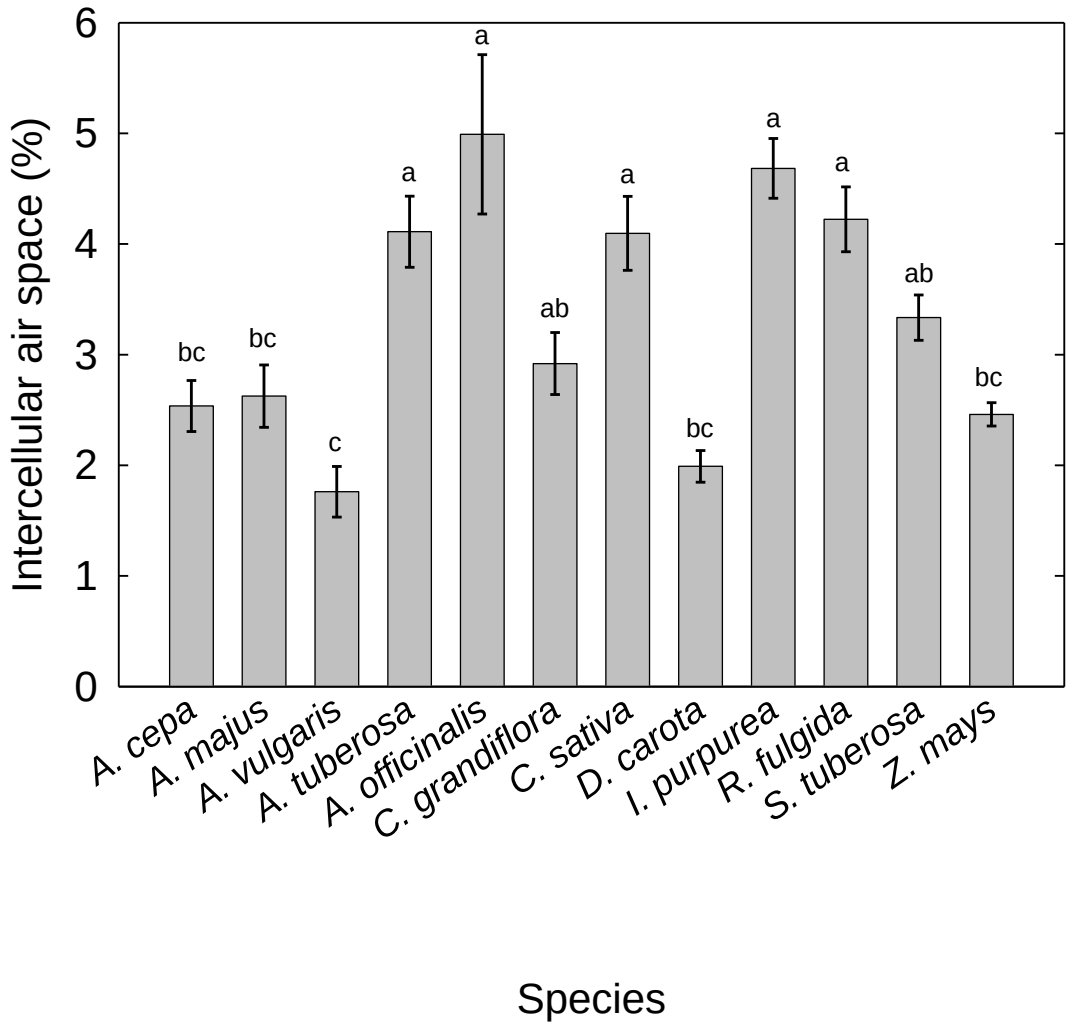

Figure 3.3: The percentage of root volume as intercellular air space for all species examined for overall Hypothesis 3. Error bars represent ± 1 s.e.m. Letters above the bars represent significant differences in species means, as determined by the Tukey's Honest Significant Difference (HSD) test.

Intercellular air space (%) was not significantly correlated with colonization (%) (Figure 3.4).

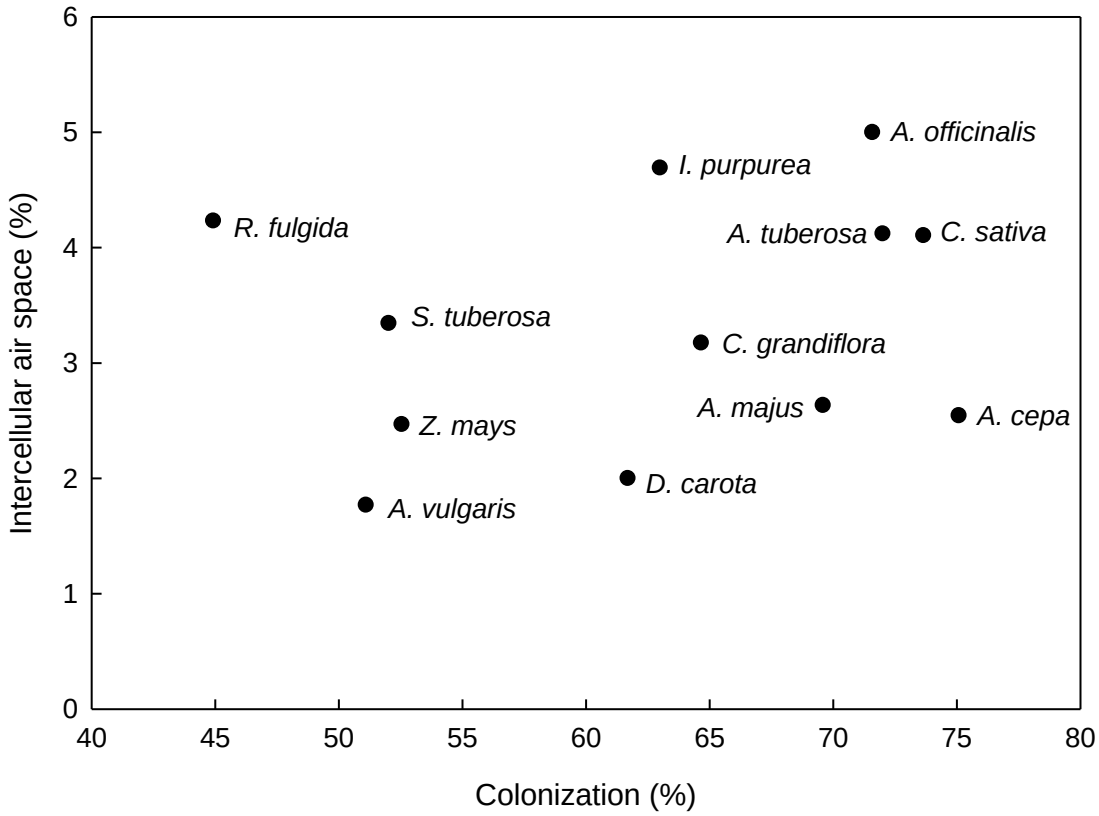

Figure 3.4: Correlation between colonization (%) and the percentage of root volume as intercellular air space. Each datapoint represents the mean from 16 replicate plants of each species. $R^2 = 0.054$, $p = 0.467$.

Mycorrhizal colonization (%) ranged from approximately 45 to 75 %.

Discussion

The objective of this study was to test whether interspecific variation in two root anatomical variables, suberized root length (%) and intercellular air space (%), could explain interspecific variation in mycorrhizal colonization. Although significant interspecific variability was observed in colonization (%) as well as in the two root anatomical variables, I found that neither of the anatomical variables was correlated with colonization (%).

The degree of hypodermal suberization may limit mycorrhizal colonization via its effect on fungal penetration into the cortex. For example, in the previous chapter (Sharda & Koide, 2008), I found the percentage of root length with penetration points to be highly correlated with colonization (%). However, in the current study, mean values of suberized root length (%) ranged from approximately 5 to 35 % (Figure 3.1), which indicates that 95 to 65 % of the root length was unsuberized and thus potentially susceptible to mycorrhizal colonization. Notably, at intersections where a suberized hypodermis was noted, suberization was extremely patchy, and thus the entire circumference of the root hypodermis was not always suberized. It is possible that at these low levels, suberization was ineffective in limiting the formation of penetration points (Figure 3.2).

While there was certainly significant interspecific variability in intercellular air space (%), the absolute range was not great (Figure 3.3). Mean values for intercellular air space (%) ranged from approximately 2 to 5 % in the lateral roots of species examined in this study. I have no evidence supporting the hypothesis that intercellular air space (%) within this range could control colonization (Figure 3.4). It is possible that other species possess greater values of

intercellular air space (%), but my study included an examination of twelve species in eleven families in order to increase the probability of finding a wide range of intercellular air space. Moreover, as has been suggested by others (Brundrett *et al*., 1985; Dickson, 2004), the *tortuosity* of the intercellular hyphal pathway or the amount of *continuous* air space may be more important than the proportional volume of intercellular air space in determining how quickly hyphae grow through the root cortex.

Finally, it is possible that both suberized root length (%) and intercellular air space (%) did influence the formation of penetration points into the cortex and hyphal growth within the cortex, respectively, but that these influences were not manifest as significant effects on colonization because other, unaccounted, factors also influence mycorrhizal colonization. For example, interspecific variation in suberization of the hypodermis could have had significant effects on the number of penetration points per root, but subsequent differences in the development of infection from the points of penetration could result in no net effect on colonization. Similarly, interspecific variation in intercellular air space may have had significant effects on the longitudinal growth of hyphae within roots, but significant differences in the number of infection units growing longitudinally through roots could also result in no net effect on colonization. Both the rate of development of infection from the points of penetration and the number of infection units on roots could be influenced by root exudation (Nagahashi *et al*., 1996; Tawaraya *et al*., 1998). I do conclude, however, that neither interspecific variation in suberization of the hypodermis nor interspecific variation in intercellular air space was solely responsible for significant variation in colonization.

I have hereby demonstrated how variability in three different root anatomical traits may or may not contribute to the observed interspecific variability in mycorrhizal colonization.

In the next chapter, I address whether each of these root anatomical traits may be involved in another form of control of mycorrhizal colonization—that which is mediated by phosphorus concentrations within individual plant species.

References

APG II. 2003. An update of the Angiosperm Phylogeny Group classification for orders and families of flowering plants: APG II. *Botanical Journal of the Linnaean Society* **141**: 399-436.

Baylis GTS. 1975. The magnolioid mycorrhiza and mycotrophy in root systems derived from it. In: Sanders FE, Mosse B, Tinker PB, eds. *Endomycorrhizas*. New York, USA: Academic Press, 373-389.

Bonfante-Fasolo P. 1984. Anatomy and morphology of VA mycorrhizae. In: Powell CL, Bagyaraj DJ, eds. *VA Mycorrhizas*. Boca Raton: CRC Press, 5-33.

Brundrett MC, Piche Y, Peterson RL. 1985. A developmental-study of the early stages in vesicular-arbuscular mycorrhiza formation. *Canadian Journal of Botany-Revue Canadienne de Botanique* **63**: 184-194.

Brundrett MC, Kendrick B, Peterson CA. 1991. Efficient lipid staining in plant material with Sudan red 7B or Fluorol yellow 088 in polyethylene glycol-glycerol. *Biotechnic and Histochemistry* **66**: 111-116.

Cook BD, Jastrow JD, Miller RM. 1988. Root and mycorrhizal endophyte development in a chronosequence of restored tallgrass prairie. *New Phytologist* **110**: 355-362.

Dickson S. 2004. The Arum-Paris continuum of mycorrhizal symbioses. *New Phytologist* **163**: 187-200.

Eissenstat DM. 1992. Costs and benefits of constructing roots of small diameter. *Journal of Plant Nutrition* **15**: 763-782.

Enstone DE, Peterson CA, Ma FS. 2003. Root endodermis and exodermis: Structure, function, and responses to the environment. *Journal of Plant Growth Regulation* **21**: 335-351.

Garriock ML, Peterson RL, Ackerley CA. 1989. Early stages in colonization of *Allium porrum* (leek) roots by the vesicular arbuscular mycorrhizal fungus, *Glomus versiforme*. *New Phytologist* **112**: 85-92.

Graham JH, Eissenstat DM, Drouillard DL. 1991. On the relationship between a plant's mycorrhizal dependency and rate of vesicular-arbuscular mycorrhizal colonization. *Functional Ecology* **5**: 773-779.

Hayman DS, Barea JM, Azcon R. 1976. Vesicular-arbuscular mycorrhiza in southern Spain - its distribution in crops growing in soil of different fertility. *Phytopathologia Mediterranea* **15**: 1-6.

Hose E, Clarkson DT, Steudle E, Schreiber L, Hartung W. 2001. The exodermis: a variable apoplastic barrier. *Journal of Experimental Botany* **52**: 2245-2264.

Koide RT, Mooney HA. 1987. Spatial variation in inoculum potential of vesicular arbuscular mycorrhizal fungi caused by formation of gopher mounds. *New Phytologist* **107**: 173-182.

Lackie SM, Bowley SR, Peterson RL. 1988. Comparison of colonization among half-sib families of *Medicago sativa* by *Glomus versiforme* (Daniels and Trappe) Berch. *New Phytologist* **108**: 477-482.

Ma R, Peterson CA, 2003. Current insights into the development, structure, and chemistry of the endodermis and exodermis of roots. *Canadian Journal of Botany-Revue Canadienne de Botanique* **81**: 405-421.

Machlis L, Torrey JG. 1956. *Plants in action*. San Francisco, USA: Freeman.

MINITAB 1998. Release 12 for Windows. Minitab. State College, PA, USA.

Nagahashi G, Douds DD, Abney GD. 1996. Phosphorus amendment inhibits hyphal branching of the VAM fungus *Gigaspora margarita* directly and indirectly through its effect on root exudation. *Mycorrhiza* **6**: 403-408.

Peterson CA, Perumalla CJ. 1984. Development of the hypodermal Casparian band in corn and onion roots. *Journal of Experimental Botany* **35**: 51-57.

Raskin I. 1983. Volume, density, thickness, and internal gas volume. *HortScience* **18**: 698-699.

Roumet C, Urcelay C, Diaz S. 2006. Suites of root traits differ between annual and perennial species growing in the field. *New Phytologist* **170**: 357-368.

Sharda JN, Koide RT. 2008. Can hypodermal passage cells limit penetration by mycorrhizal fungi? *New Phytologist* **180**: 696-701.

Smith SE, Read DJ. 1997. *Mycorrhizal symbiosis*. 2nd Ed. London: Academic Press.

St John TV. 1980. Root size root hair and mycorrhiza infection a reexamination of Baylis's hypothesis with tropical trees. *Acta Amazonica* **10**: 141-146.

Tawaraya K, Hashimoto K, Wagatsuma T. 1998. Effect of root exudate fractions from P-deficient and P-sufficient onion plants on root colonisation by the arbuscular mycorrhizal fungus *Gigaspora margarita*. *Mycorrhiza* **8**: 67-70.

Wilson GWT, Hartnett DC. 1998. Interspecific variation in plant responses to mycorrhizal colonization in tallgrass prairie. *American Journal of Botany* **85**: 1732-1738.

Zangaro W, Nishidate FR, Camargo FRS, Romagnoli GG, Vandressen J. 2005. Relationships among arbuscular mycorrhizas, root morphology and seedling growth of tropical native woody species in southern Brazil. *Journal of Tropical Ecology* **21**: 529-540.

Zangaro W, Nishidate FR, Vandressen J, Andrade G, Nogueira MA. 2007. Root mycorrhizal colonization and plant responsiveness are related to root plasticity, soil fertility and successional status of native woody species in southern Brazil. *Journal of Tropical Ecology* **23**: 53-62.

Chapter 4

Exploring the role of root anatomy in P-mediated control of arbuscular mycorrhizal colonization

Introduction

Arbuscular mycorrhizal fungi (AMF) are obligate biotrophs, utilizing living plants as their sole source of reduced carbon (Smith & Read, 1997). Most terrestrial plant species are colonized by AMF, which may contribute significantly to uptake of phosphorus (P) by the host plant (Mosse, 1973; Smith & Read, 1997; Smith *et al*., 2003). Although colonized plants lose a certain amount of reduced carbon to mycorrhizal fungi, with relatively few exceptions (Koide, 1985; Modjo & Hendrix, 1986; Peng *et al*., 1993; Johnson *et al*., 1997), host plants derive a benefit from the symbiosis. One reason why plants may be able to maintain a net benefit from the symbiosis is that they are able to control the extent of mycorrhizal colonization in their roots (Hayman, 1983; Koide & Schreiner, 1992; Pinior *et al*., 1999; Vierheilig, 2004), which could help to minimize carbon loss.

A well-known example of such control is that due to P concentration; greater soil or plant P concentrations are frequently associated with reductions in fractional colonization of root length (Baylis, 1967; Daft & Nicolson, 1969; Clarke & Mosse, 1981; Buwalda *et al*., 1982; Thomson *et al*., 1986; Koide & Li, 1990; Braunberger *et al*., 1991). Some of this reduction in fractional colonization is undoubtedly due to increased growth of root systems at higher P concentrations, leading to a "dilution" of colonization. However, absolute length of colonized

root may also be reduced at higher P concentrations (Abbott *et al*., 1984; Amijee *et al*., 1989; Koide & Li, 1990).

A few mechanisms have been suggested for P-mediated control of mycorrhizal colonization. For example, root membrane permeability may decrease when plant P concentration is increased, resulting in a reduction in overall root exudation (Ratnayake *et al*., 1978). As plant P concentration increases, carbohydrate availability may decline, resulting in lower concentrations of soluble carbohydrates within roots (Jasper *et al*., 1979; Same *et al*., 1983) as well as the concentration of carbohydrates exuded from roots (Graham *et al*., 1981; Schwab *et al*., 1983). Additionally, significantly less host carbon is available to the fungus when host plants are not limited by P concentrations (Olsson et al., 2002). The quality of exudates also may change with host plant P status, resulting in less hyphal elongation (Elias & Safir, 1987; Tawaraya *et al*., 1996), branching (Nagahashi *et al*., 1996) and appressorium formation (Tawaraya *et al*., 1998) when plant P concentrations are high. Specific compounds present in root exudates are capable of stimulating or inhibiting colonization, often through their effects on hyphal growth and appressorium formation (Gianinazzi-Pearson *et al*., 1989; Nair *et al*., 1991; Siqueira *et al*., 1991; Becard *et al*., 1992; Xie *et al*., 1995; Akiyama *et al*., 2002). Variation in the concentration of such active compounds may be at least partly responsible for control of colonization by plant P concentration.

In previous attempts to elucidate the mechanisms of control of mycorrhizal colonization by plant P concentration, root anatomy has been largely ignored. In the current study, I examine the response to P treatment by several root anatomical variables which could potentially control colonization through their effects on the colonization process. In order to determine whether any or all of these may serve as mechanisms responsible for host P-mediated

control of mycorrhizal colonization, I hypothesized that each of these variables responded to plant P concentrations in a manner that would alter colonization levels.

The first event of contact between the AM hypha and the root occurs when an appressorium forms on the epidermis, which anchors the hypha to the epidermis (Brundrett *et al*., 1985; Garriock *et al*., 1989). AM hypha then grow between epidermal cells, and encounter the hypodermis, the outermost layer of cortical cells (Bonfante-Fasolo, 1984). The outer tangential walls of hypodermal cortical cells become suberized at various points in development, rendering them impenetrable to fungi (Perumalla *et al*., 1990), so fungal penetration into the cortex is therefore limited to unsuberized hypodermal cells (Mosse, 1973; Smith *et al*., 1989; Shishkoff, 1989; Brundrett & Kendrick, 1990; Matsubara *et al*., 1999). Some plant species possess a so-called "uniform" hypodermis that becomes suberized in a developmentally regulated fashion. Suberization of the uniform hypodermis is somewhat responsive to drought and flooding (Peterson, 1989; Enstone *et al*., 2002), but it is unknown whether it responds to soil P availability. Thus, I tested the hypothesis that for plant species with a uniform hypodermis, *a larger percentage of the hypodermis is suberized at higher plant P concentrations* (overall Hypothesis 4).

Many plant species possess a "dimorphic hypodermis" comprising unsuberized "passage cells" amidst a matrix of suberized cells. "Dimorphic" refers to the fact that in many species, the passage cells are significantly smaller than the non-passage cells (Shishkoff, 1987; Perumalla *et al*., 1990; Peterson & Enstone, 1996). In dimorphic hypodermal plant species, fungal penetration occurs only through the unsuberized passage cells. From previous work (Sharda & Koide, 2008), I know that interspecific variability in hypodermal passage cell distribution is highly correlated to that of mycorrhizal colonization. To determine whether plant P concentrations

might also control hypodermal passage cell distribution, the next hypothesis I tested was that for plant species with a dimorphic hypodermis, *a higher plant P concentration results in a lower percentage of root length with passage cells and/or a lower density of passage cells* (overall Hypothesis 5a, b).

Once past the hypodermis, hyphae may grow longitudinally through the root cortex either by growing through intercellular air spaces or passing through one cell to the next (Brundrett *et al*., 1985; Smith & Smith, 1997; Dickson, 2004). Arbuscules are formed in inner cortical cells as offshoots from these hyphae (Smith and Read, 1997). For plant species which spread primarily by hyphal growth through intercellular air spaces, the amount of intercellular air space has been hypothesized to influence hyphal spread (Brundrett & Kendrick, 1990; Smith & Smith, 1997). The final hypothesis, therefore, is that *the percentage of root volume as intercellular air space is lower in plants of higher P concentrations* (overall Hypothesis 6).

Materials and Methods

Plant culture

Each hypothesis was tested using several plant species from a diverse range of plant families. All measurements were made in colonized plants in accordance to the reality that the majority of penetration and hyphal spread occurs in colonized plants, as the only non-mycorrhizal stage of plant development is the very short time following seed germination. In each case, the experimental unit was the species, and there were 16 individuals of each species for each of two P treatments. Because there were 15 plant species in this study, there were a total of 480 individual plants grown. For the first hypothesis (overall Hypothesis 4), I used six of the 15 plant species that possess a uniform hypodermis (Table 4.1).

Table 4.1: Listing of each plant species, their family (tribe) (APG, 2003), amount of P added in the High P treatment, planting dates, days after planting (DAP) prior to harvest, and the hypotheses for which they were utilized. SUH: suberization of a uniform hypodermis (overall Hypothesis 4); PCD: passage cell distribution in a dimorphic hypodermis (overall Hypothesis 5); IAS: percentage of root volume as intercellular air space (overall Hypothesis 6). *: mg elemental P added per kg soil in the High P treatment. The soil in the Low P treatment received no additional P.

Species	Family	High P treatment*	Planting date	Duration (d)	Hypotheses
Allium cepa L.	Liliaceae	50	9 Oct 2006	49	PCD, IAS
Antirrhinum majus L.	Scrophulariaceae	100	8 May 2007	38	SUH, IAS
Aquilegia vulgaris L.	Ranunculaceae	50	22 Sep 2006	53	SUH, IAS
Asclepias tuberosa L.	Asclepiaceae	50	24 Oct 2006	48	PCD, IAS
Asparagus officinalis L.	Asparagaceae	50	30 Oct 2006	38	PCD, IAS
Coreopsis grandiflora Hogg. ex Sweet Per.	Asteraceae (Heliantheae)	50	22 Sep 2006	47	PCD, IAS
Cucurbita sativa L.	Cucurbitaceae	100	8 May 2007	18	IAS
Daucus carota L.	Apiaceae	100	8 May 2007	39	SUH, IAS

Helianthus annuus L.	Asteraceae (Heliantheae)	100	14 Aug 2006	21	SUH
Ipomoea purpurea L.	Convulvulaceae	80	16 Aug 2007	15	PCD, IAS
Ocimum basilicum L.	Lamiaceae	100	27 Mar 2007	23	PCD
Rudbeckia fulgida Ait.	Asteraceae (Astereae)	200	18 Jul 2006	43	PCD, IAS
Solanum tuberosa L.	Solanaceae	100	8 May 2007	37	SUH, IAS
Vinca minor L.	Apocynaceae	50	15 Feb 2007	40	PCD
Zea mays L.	Poaceae	100	25 Aug 2006	18	SUH, IAS

For the second hypothesis (overall Hypothesis 5), I used 8 of the 15 species that possess a dimorphic hypodermis (Table 4.1). I referred to Shishkoff (1987) as a guide to identify genera with either a uniform or dimorphic hypodermis before confirming the hypodermis type by microscopic observation. For the final hypothesis (overall Hypothesis 6), twelve species were examined (Table 4.1).

Each of the sixteen individuals for each P treatment and for each species was grown separately, according to the methods described in Chapter 2. Briefly, each individual plant was grown separately, in a sand and soil mixture amended with *G. intraradices* inoculum. The soil contained 9.2 μg g^{-1} $NaHCO_3$-extractable P. The High P treatment plants received additional P in the form of finely ground triple superphosphate $Ca(H_2PO_4)_2$. The Low P treatment plants received no additional phosphorus. For the High P treatment variable amounts of P were added to the soil medium depending on the species in order to avoid P toxicity in some species. To determine the appropriate amount of phosphorus needed for the High P treatments in each species, I grew each species at four different P availabilities (50, 80, 100, and 200 mg elemental P kg^{-1} soil) in a preliminary experiment, and the P availability that yielded the highest shoot biomass was selected (Table 4.1, Appendix A). Other nutrients were supplied to all plants as

needed by watering with 100 ml of one-third strength Hoagland's nutrient solution (in which 1 mmol L^{-1} KH_2PO_4 was replaced by 1 mmol L^{-1} KCl) (Machlis & Torrey, 1956).

I harvested all plants from each species before their root systems became potbound, to assure that the anatomy of such roots would be representative of those growing in soil. Because each species grew at a different rate, the length of time allowed for growth also varied by species (Table 4.1). At harvest, plants were removed from the pots, and shoots and roots were separated. Whole root systems were rinsed carefully in tap water to remove soil. Roots used to test overall Hypotheses 4 and 5 were then stored in 50 % ethanol. Roots used for the analysis of intercellular air space (overall Hypothesis 6) were processed immediately. Shoots were dried and, following digestion at 400˚C in a mixture of concentrated H_2SO_4 and 30 % H_2O_2, shoot P concentrations were determined by the molybdo-phosphate method (Watanabe & Olsen, 1965).

Staining for AM hyphae and measurement of colonization (%)

Each root system was divided into taproots and lateral roots. Because these two types of roots often differ significantly in their thickness and certain aspects of their anatomy, and because the majority (in most cases, more than 90 %) of colonized root length comprised lateral roots, I chose to exclude the taproots from our analyses. After being detached from the taproots, the lateral roots were suspended in a beaker of water. Approximately 1.0-2.0 g (FW) of root pieces was randomly selected for analysis. These were cleared and stained according to the methods described in Chapter 2. Briefly, roots were cleared overnight in 10 % KOH at room temperature. Following a rinse in deionized water and 5 % HCl, they were stained overnight at room temperature in a Trypan blue solution to reveal AM hyphae in the roots as well as hyphae

coiling through hypodermal cells (penetration points). The percentage of root length colonized by AM fungi, hereafter referred to as colonization (%), was determined using the line intersect method (Koide & Mooney, 1987).

Measurement of the percentage of root length possessing a suberized hypodermis (overall Hypothesis 4)

The percentage of root length possessing a suberized hypodermis was determined according to the methods described in Chapter 3. Briefly, prior to staining for suberin, roots were lightly cleared overnight at room temperature in 10 % KOH, and then stained overnight at room temperature in a Sudan red 7B stain solution. To make the red or pink staining more obvious, roots were counterstained overnight in the Trypan blue solution used to reveal AM hyphae and passage cells. The resulting light blue background allowed us to better visualize the pink or red suberized hypodermis. I calculated the suberized root length (%) as the percentage of intersections with red or pink stain relative to total root intersections using the line intersect method (see above).

Measurement of passage cell and penetration point distributions (overall Hypothesis 5a, b)

Passage cell and penetration point distribution was determined according to the methods described in Chapter 2. The same clearing and staining method used to reveal AM hyphae also reveals passage cells. A cell was considered to be a passage cell if it stained with Trypan blue (Shishkoff, 1987), as suberized hypodermal cells do not stain. To characterize passage cell and

penetration point distribution, I used the line intersect method (Koide & Mooney, 1987) to determine the percentage of root length with passage cells and the percentage of root length with penetration points, hereafter referred to as passage cells (%) and penetration points (%), respectively. Passage cell density and penetration point density were also determined (see Chapter 2 for details). Root intersections possessing no passage cells and thus no penetration points were excluded from the overall species means calculations. The reported values therefore represent the densities of passage cells or penetration points in sections where passage cells actually exist. Notably, fungal penetration occurred only through passage cells, and did not occur at the very tip of the root where the hypodermis was not yet developed.

Measurement of percentage of root volume as intercellular air space (overall Hypothesis 6)

Percentage of root volume as intercellular air space, hereafter referred to as intercellular air space (%), was calculated as described in Chapter 3. Briefly, a pycnometer was used to determine the volume of gas in porous tissue by determining the weight of the water which displaced the gas volume in the root tissue (Raskin, 1983). While some methods employing a pycnometer use vacuum infiltration of the porous tissue, I found this to be inadequate for my purposes, and eliminated gas space in the tissue by grinding roots with a mortar and pestle. For the specific equation used to calculate intercellular air space (%), see Chapter 3.

Statistical analyses

The response to P treatment in terms of plant P concentration, colonization (%), and shoot dry weight was analyzed using a General Linear Model ANOVA (Minitab, 1998) using Species, P treatment, and the Species*P treatment interaction as factors. A post-hoc Tukey's test was performed for each species to analyze individual responses to P treatment.

To test each of the three hypotheses, I calculated the means of 16 plants (per P treatment) of each individual species, as the species were considered units of replication. These means were analyzed using a General Linear Model ANOVA (Minitab, 1998) with Species and P treatment as factors. In order to document variation within individual species in the responses of their various root traits to P treatment, I also conducted single-factor analyses of variance (Minitab, 1998) for each species utilizing all 16 replicates for each P treatment.

Results

The High P treatment influenced shoot P concentration in the hypothesized manner by significantly increasing shoot P concentrations compared to the Low P treatment. This was true for each species according to separate single-factor analyses of variance. The magnitude of the response to P treatment was species-dependent in the sense that the difference in shoot P concentration between Low and High P treatments depended on the species as indicated by the significant Species*P treatment interaction (Table 4.2).

Table 4.2: Table of means for all species plant P concentration, colonization (%), and shoot dry weight. Different letters indicate a significant effect of P treatment within each species, as determined by the post-hoc Tukey's test. n=8 for shoot P concentrations. n=16 for colonization (%) and shoot dry weight.

Species	***P treatment***	***Shoot P concentration (mg P g^{-1} dry wt.)***	***Colonization (%)***	***Shoot dry wt. (g)***
Allium cepa L.	High	5.38a	32.7b	0.125a
	Low	2.42b	75.1a	0.075b
Antirrhinum majus L.	High	7.40a	19.3b	0.134a
	Low	3.11b	69.6a	0.173a
Aquilegia vulgaris L.	High	9.03a	30.4b	0.055a
	Low	3.00b	51.1a	0.052b
Asclepias tuberosa L.	High	4.60a	45.6b	0.107a
	Low	3.11b	72.1a	0.116a
Asparagus officinalis L.	High	4.19a	51.5b	0.132a
	Low	2.57b	71.6a	0.099b
Coreopsis grandiflora Hogg ex. Sweet Per.	High	4.48a	42.8b	0.266a
	Low	2.08b	64.7a	0.275a
Cucurbita sativa L.	High	12.89a	36.5b	0.358a
	Low	2.44b	73.7a	0.263b

Daucus carota L.	High	10.16a	41.8b	0.280a
	Low	2.13b	61.7a	0.217b
Helianthus annuus L.	High	9.93a	12.4b	0.762a
	Low	2.20b	44.4a	0.481b
Ipomoea purpurea L.	High	7.24a	48.0b	0.289a
	Low	1.51b	63.0a	0.188b
Ocimum basilicum L.	High	8.58a	26.9b	0.037a
	Low	2.55b	52.7a	0.010b
Rudbeckia fulgida Ait.	High	11.83a	29.1b	0.335a
	Low	2.27b	45.0a	0.257b
Solanum tuberosa L.	High	12.94a	28.9b	0.268a
	Low	2.42b	52.1a	0.234b
Vinca minor L.	High	4.56a	23.8b	0.039a
	Low	2.15b	43.5a	0.030b
Zea mays L.	High	9.15a	36.2b	0.484a
	Low	3.27b	52.6a	0.377b

Analysis of variance

Factor				
Species		**<0.001**	**<0.001**	**<0.001**
P treatment		**<0.001**	**<0.001**	**<0.001**
Interaction		**<0.001**	**<0.001**	**0.111**

The High P treatment also decreased colonization (%), as predicted. This was true for each species according to separate single-factor analyses of variance. The response to P treatment was species-dependent as the difference in colonization (%) between Low and High P

treatments depended on the species as indicated by a significant Species*P treatment interaction. Because P treatment influenced shoot P concentration and colonization (%) in the predicted manner, each of the species could be used as a replicate to test the various hypotheses. Shoot dry weight was significantly greater for plants in the High P treatment, but the Species*P treatment interaction was not significant.

Phosphorus treatment did not significantly influence suberized root length (%) ($p = 0.367$, Figure 4.1a), passage cell density ($p = 0.899$, Figure 4.1c), or intercellular air space (%) ($p = 0.116$, Figure 4.1d). However, a significantly lower passage cells (%) was observed in the High P treatment ($p = 0.015$, Figure 4.1b).

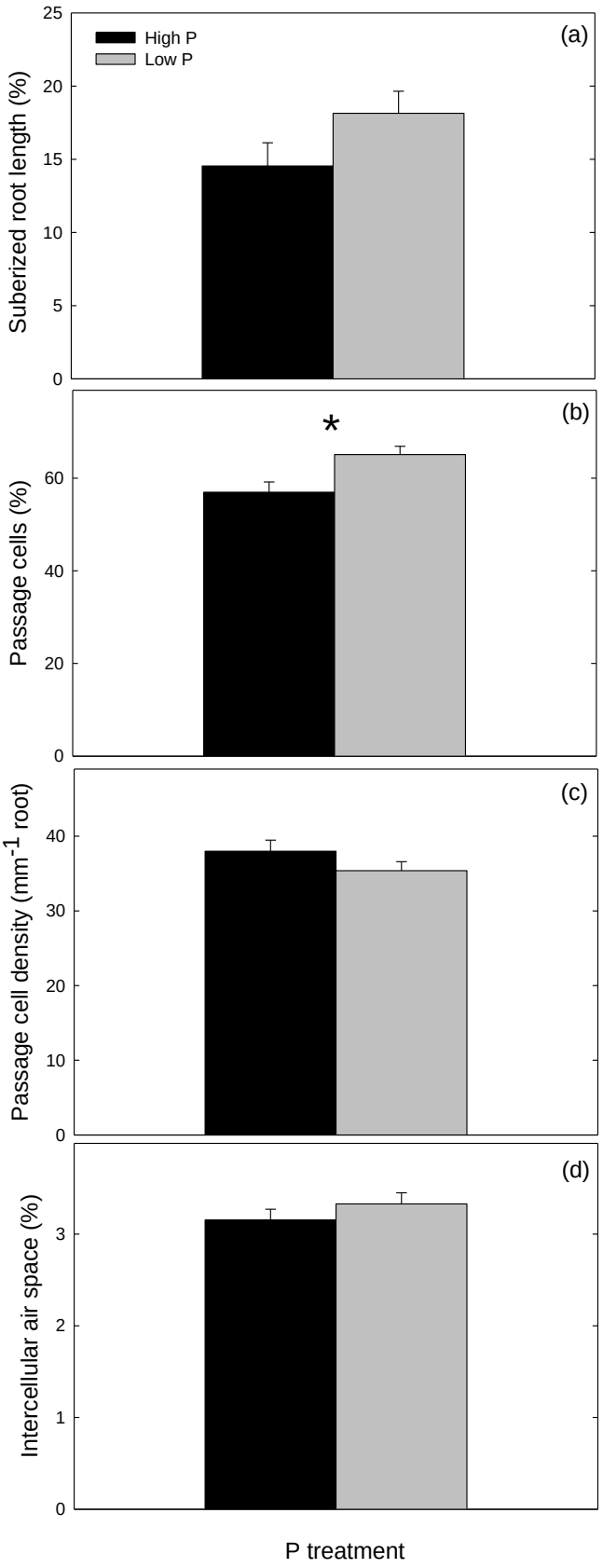
High P
Low P
(a)
Suberized root length (%)
25
20
15
10
5
0
(b)
*
Passage cells (%)
60
40
20
0
(c)
Passage cell density (mm-1 root)
40
30
20
10
0
(d)
Intercellular air space (%)
3
2
1
0
P treatment

Figure 4.1a-d: Effect of P treatment on the various root traits. Each mean represented for each trait and P treatment is the mean of all species means (each species mean is the mean of the 16 individuals examined). 4.1a. Percentage of root length possessing a suberized hypodermis (suberized root length (%), overall Hypothesis 4). 4.1b. Percentage of root length with passage cells (passage cells (%), overall Hypothesis 5a). 4.1c. Passage cell density (overall Hypothesis 5b). 4.1d. Percentage of root volume as intercellular air space (intercellular air space (%), overall Hypothesis 6). Means from each P treatment are displayed. Asterisks indicate a significant effect of P treatment. Vertical bars represent ± 1 s.e.m.

The effect of P treatment on suberized root length (%), passage cells (%), passage cell density, and intercellular air space (%) varied by species (Figures 4.2-4).

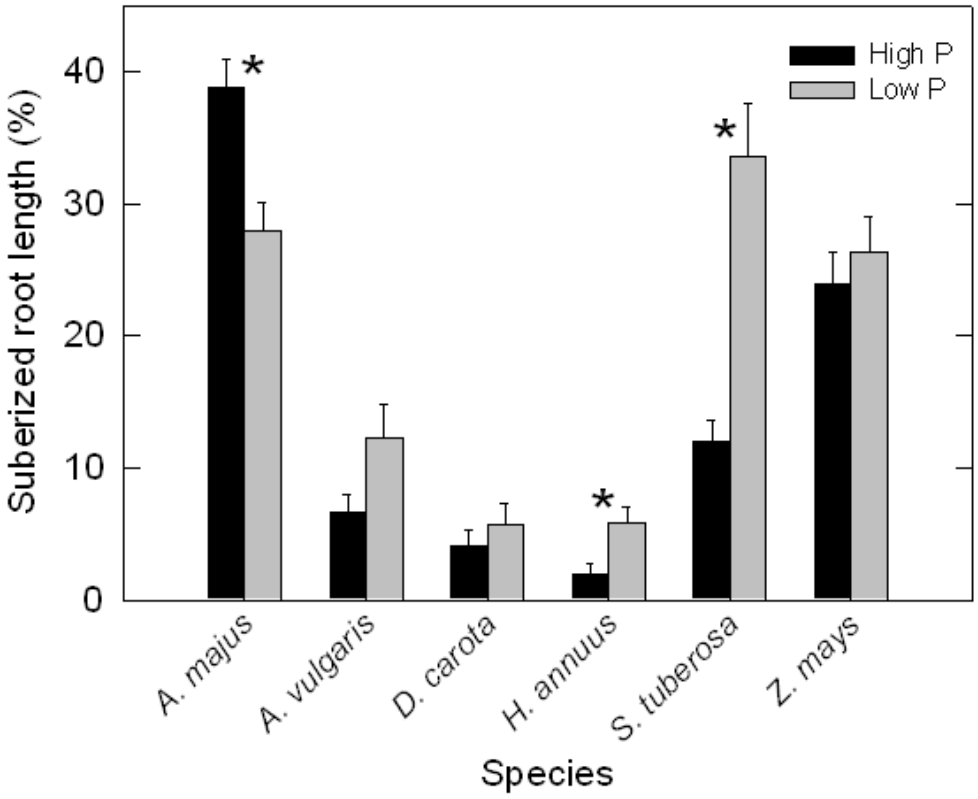

Figure 4.2: The effect of P treatment on the percentage of root length possessing a suberized hypodermis (suberized root length (%)) in individual species (overall Hypothesis 4). Asterisks indicate a significant effect of P treatment, as determined by the single factor analyses of variance. Vertical bars represent ± 1 s.e.m.

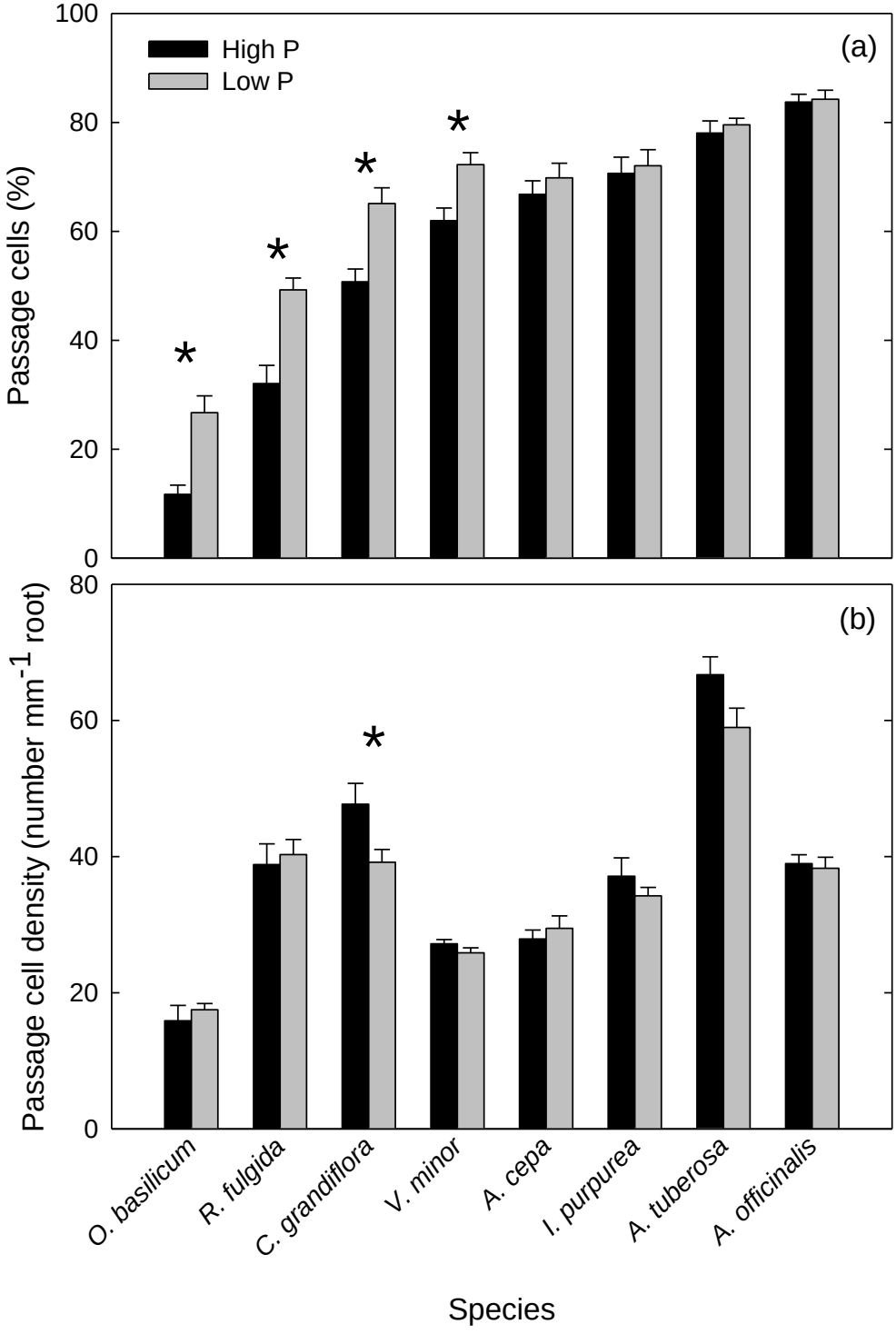

Figure 4.3a,b: The effect of P treatment on the percentage of a) root length with passage cells (passage cells (%)), and b) passage cell density in individual species (overall Hypothesis 5a,b). Asterisks indicate a significant effect of P treatment, as determined by the single factor analyses of variance. Vertical bars represent ± 1 s.e.m.

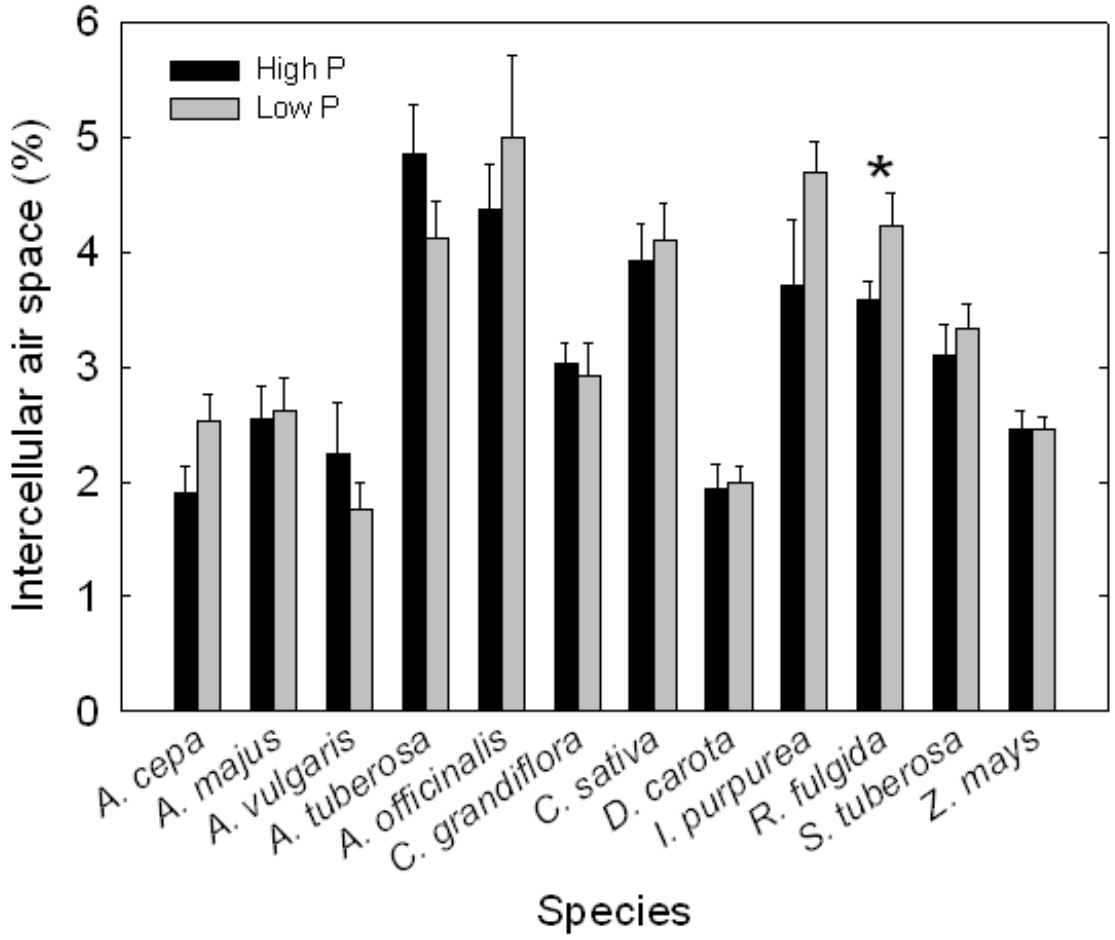

Figure 4.4: The effect of P treatment on percentage of root volume as intercellular air space (intercellular air space (%)) in individual species (overall Hypothesis 6). Asterisk indicates a significant effect of P treatment, as determined by single factor analyses of variance. Vertical bars represent ± 1 s.e.m.

In *A. majus*, suberized root length (%) was significantly higher in the High P treatment, but in *H. annuus* and *S. tuberosa*, suberized root length (%) was significantly lower in the High P treatment. The remaining three species (*A. vulgaris*, *D. carota*, and *Z. mays*) showed no significant response to P treatment (Figure 4.2). Passage cells (%) was significantly lower in the High P treatment for *O. basilicum*, *V. minor*, *R. fulgida*, and *C. grandiflora*, but *A. cepa*, *I. purpurea*, *A. tuberosa*, and *A. officinalis* did not respond significantly to P treatment (Figure 4.3a). Passage cell density was significantly higher in plants from the High P treatment in C.

grandiflora, which was the opposite response to what was hypothesized. The remaining seven species did not respond significantly to P treatment (Figure 4.3b). Both penetration points (%) and penetration point density were significantly lower in the High P treatment for all plant species (data not shown). Of the twelve species examined for changes in intercellular air space (%), only one species (*R. fulgida*) responded significantly to P treatment. It possessed significantly lower intercellular air space (%) in the High P treatment (Figure 4.4).

Discussion

To my knowledge, this is the only examination of the potential for root anatomy to contribute to P-mediated control of mycorrhizal colonization. I measured the responsiveness to P by three root traits either known to or hypothesized to affect colonization. These included suberization of a uniform hypodermis, hypodermal passage cell distribution in a dimorphic hypodermis, and the percentage of root volume as intercellular air space. The ability of each root trait to respond to plant P treatment was assessed in several angiosperm host species from different families.

In all species examined, plants in the High P treatment possessed significantly higher shoot P concentrations and significantly lower colonization (%). These results are consistent with those from many other studies (Baylis, 1967; Daft & Nicolson, 1969; Clarke & Mosse, 1981; Buwalda *et al*., 1982; Thomson *et al*., 1986; Koide & Li, 1990; Braunberger *et al*., 1991), and they were necessary before I could use these species to test the hypotheses. Examination of P-mediated changes in shoot dry weight allowed me to confirm that plants were not experiencing P toxicity. In most species, shoot dry weight was significantly higher for plants in the High P treatment and in no case did the High P treatment result in a significant reduction of growth.

The first hypothesis (overall Hypothesis 4) I tested was that for species possessing a uniform hypodermis, suberized root length (%) would be significantly higher in the High P treatment. In fact, no significant response to P treatment was found for this root trait (Figure 4.1a). The suberized root length (%) of most replicates (species) was less than 30 %, and suberization was patchy along the root, as has been reported by others (Wang *et al*., 1995;

Enstone and Peterson 1997). From previous studies (Mosse, 1973; Smith *et al*., 1989; Brundrett & Kendrick, 1990; Matsubara *et al*., 1999), it is well known that fungi selectively penetrate unsuberized hypodermal cells. However, given the lack of a significant response to P treatment, it is unlikely that suberized root length (%) in species with a uniform hypodermis is a general mechanism in angiosperms involved in P-mediated control of mycorrhizal colonization. Notably, a suberized hypodermis is known to protect roots from various water stresses, so root water potentials may influence suberization rates more than root P concentrations. While suberization of a uniform hypodermis may not be a general response to P treatment among angiosperms, it may be an effective means to reduce mycorrhizal colonization in some species. Suberized root length (%) increased in *A. majus*, decreased in *H. annuus* and *S. tuberosa*, and did not change significantly in *A. vulgaris*, *D. carota*, and *Z. mays* in response to the High P treatment (Figure 4.2).

The second hypothesis (overall Hypothesis 5) I tested was that for species possessing a dimorphic hypodermis, increased P concentration would result in a lower percentage of root length with passage cells (passage cells (%)) or a lower passage cell density. I found that passage cells (%) was significantly lower in the High P treatment ($p = 0.015$), as hypothesized (Figure 4.1b). In contrast, no significant response to P treatment was observed for passage cell density ($p = 0.899$) (Figure 4.1c). In a previous study (Sharda & Koide, 2008), interspecific variation in passage cells (%) was found to explain a large amount of the variability observed in the percentage of root length with penetration points (penetration points (%)). Variation in passage cell density was also examined in that study but, as in the current study, it was not found to be a significant determinant of penetration point density. These studies together indicate that passage cells (%), which is correlated with penetration point (%), is responsive to plant P

concentration, while passage cell density, which is not correlated with penetration point density, did not respond to plant P concentration. I therefore conclude that variation in passage cells (%) may serve as a mechanism contributing to P-mediated control of mycorrhizal colonization in dimorphic hypodermal species. Single-factor analyses of variance for each species revealed that the response to P treatment was species-dependent. For example, passage cells (%) was significantly lower in the High P treatment for *O. basilicum, V. minor, C. grandiflora,* and *R. fulgida*, while no significant response to P treatment was observed for *I. purpurea, A. cepa, A. tuberosa,* and *A. officinalis* (Figure 4.3a). It is interesting to note that the four significantly responsive species possessed the lowest mean passage cells (%). It is possible, therefore, that for species with inherently smaller percentages of root length with potential entryways for fungal penetration, variation in passage cells (%) can wield at least some control on colonization. This hypothesis could be tested further with more plant species possessing a wide range of passage cells (%). *Coreopsis grandiflora* responded to the High P treatment with significantly higher passage cell density, contrary to what was hypothesized. It is unclear why this species responded in this manner, while the remaining seven species did not respond significantly to P treatment (Figure 4.3b). I conclude, therefore, that variation in passage cell density is not a likely mechanism employed in host control of mycorrhizal colonization.

The third hypothesis was that the percentage of root volume as intercellular air space (intercellular air space (%)) would be lower in the High P treatment. However, no significant response to plant P concentration was observed ($p = 0.116$) (Figure 4.1d). I do not know whether roots with higher intercellular air space (%) would allow for more rapid intercellular hyphal spread but it is thought that intercellular air space does facilitate rapid growth of arbuscular mycorrhizal fungi through roots (Brundrett & Kendrick, 1990; Smith &

Smith, 1997). Given the lack of response to P treatment, however, I conclude that change in intercellular air space (%) is not likely to serve as a mechanism of P-mediated control of colonization for any of the species I examined in this study. Only one species (*R. fulgida*) responded significantly to P treatment with a significant decrease in intercellular air space (%) in the High P treatment (Figure 4.4). The remaining eleven species did not respond significantly to P treatment. The reason for the significant response by *R. fulgida* is unknown.

While there was an overall significant effect of P treatment on passage cells (%), the effect of P treatment on each root trait was species-dependent. I conclude that variation in none of these traits appears to serve as a general mechanism by which all species achieve P-mediated control of mycorrhizal colonization. Instead, each species may be idiosyncratic with respect to the mechanisms used to effect a P-mediated control of mycorrhizal colonization. Because I found that penetration points (%) and penetration point density were significantly lower in the High P treatment of all species, including those species for which no significant response to P treatment was observed in terms of any of the measured root traits, it is clear that penetration point formation must be affected by other factors. Identifying other features of roots that are controlled by plant P status may help us identify mechanisms for P-mediated control of colonization. Such features may include cytoskeleton structure (Takemoto & Hardham, 2004), recognition factors in the host cell wall (Nagahashi & Douds, 1997) or the quality and quantity of root exudates (Nagahashi *et al*., 1996, Tawaraya *et al*., 1998). Plant defense mechanisms may also be involved, and some have argued that the reduction of colonization under high P occurs because the plant has sufficient P nutrition to launch various defense mechanisms, and that high levels of colonization under P-deficient conditions are a result of poor plant health (McArthur & Knowles, 1992).

References

Abbott LK, Robson AD, De Boer G. 1984. The effect of phosphorus on the formation of hyphae in soil by the vesicular-arbuscular mycorrhizal fungus, *Glomus fasciculatum. New Phytologist* **97:** 437-446**.**

Akiyama K, Matsuoka H, Hayashi H. 2002. Isolation and identification of a phosphate deficiency-induced C-glycosylflavonoid that stimulates arbuscular mycorrhiza formation in melon roots. *Molecular Plant-Microbe Interactions* **15**: 334-340.

Amijee F, Tinker PB, Stribley DP. 1989. The development of endomycorrhizal root systems. VII. A detailed study of effects of soil phosphorus on colonization. *New Phytologist* **111**: 435-446**.**

APG II. 2003. An update of the Angiosperm Phylogeny Group classification for orders and families of flowering plants: APG II. *Botanical Journal of the Linnaean Society* **141**: 399-436.

Baylis, GTS. 1967. Experiments on the ecological significance of phycomycetous mycorrhizas. *New Phytologist* **66**: 231-243.

Becard G, Douds DD, Pfeffer PE. 1992. Extensive in vitro hyphal growth of vesicular-arbuscular mycorrhizal fungi in the presence of CO_2 and flavonols. *Applied and Environmental Microbiology* **58**: 821-825.

Bonfante-Fasolo P. 1984. Anatomy and morphology of VA mycorrhizae. In: Powell CL, Bagyaraj DJ, eds. VA Mycorrhizas. Boca Raton: CRC Press, 5-33.

Braunberger PG, Miller MH, Peterson RL. 1991. Effect of phosphorus nutrition on morphological characteristics of vesicular-arbuscular mycorrhizal colonization of maize. *New Phytologist* **119**: 107-113.

Brundrett MC, Piche Y, Peterson RL. 1985. A developmental-study of the early stages in vesicular-arbuscular mycorrhiza formation. *Canadian Journal of Botany-Revue Canadienne de Botanique* **63**: 184-194.

Brundrett M, Kendrick B. 1990. The roots and mycorrhizas of herbaceous woodland plants.II. Structural aspects of morphology. *New Phytologist* **114**: 469-479.

Brundrett MC, Kendrick B, Peterson CA. 1991. Efficient lipid staining in plant material with Sudan red 7B or Fluorol yellow 088 in polyethylene glycol-glycerol. *Biotechnic and Histochemistry* **66**: 111-116.

Buwalda JG, Ross GJS, Stribley DP, Tinker PB. 1982. The development of endomycorrhizal root systems. IV. The mathematical analysis of effects of phosphorus on the spread of vesicular-arbuscular mycorrhizal infection in root systems. *New Phytologist* **92**: 391-399.

Clarke C, Mosse B. 1981. Plant growth responses to vesicular-arbuscular mycorrhiza. XII. Field inoculation responses of barley at two soil P levels. *New Phytologist* **87**: 695-703.

Daft MJ, Nicolson TH. 1969. Effect of Endogone mycorrhiza on plant growth. II. Influence of soluble phosphate on endophyte and host in maize. *New Phytologist* **68**: 945-952.

Dickson S. 2004. The Arum-Paris continuum of mycorrhizal symbioses. *New Phytologist* **163**: 187-200.

Elias KS, Safir GR. 1987. Hyphal elongation of *Glomus fasciculatus* in response to root exudates. *Applied and Environmental Microbiology* **53**: 1928-1933.

Enstone DE, Peterson CA. 1997. Suberin deposition and band plasmolysis in the corn (*Zea mays*) root exodermis. *Canadian Journal of Botany-Revue Canadienne de Botanique* **75**: 1188-1190.

Enstone DE, Peterson CA, Ma FS. 2002. Root endodermis and exodermis: Structure, function, and responses to the environment. *Journal of Plant Growth Regulation* **21**: 335-351.

Garriock ML, Peterson RL, Ackerley CA. 1989. Early stages in colonization of *Allium porrum* (leek) roots by the vesicular arbuscular mycorrhizal fungus, *Glomus versiforme*. *New Phytologist* **112**: 85-92.

Gianinazzi-Pearson V, Branzanti B, Gianinazzi S. 1989. In vitro enhancement of spore germination and early hyphal growth of a vesicular-arbuscular mycorrhizal fungus by host root exudates and plant flavonoids. *Symbiosis* **7**: 243-255.

Graham JH, Leonard RT, Menge JA. 1981. Membrane-mediated decrease in root exudation responsible for phosphorus inhibition of vesicular-arbuscular mycorrhiza formation. *Plant Physiology* **68**: 548-552.

Hayman DS. 1983. The physiology of vesicular-arbuscular endomycorrhizal symbiosis. *Canadian Journal of Botany-Revue Canadienne de Botanique* **61**: 944-963.

Jasper DA, Robson AD, Abbott LK. 1979. Phosphorus and the formation of vesicular-arbuscular mycorrhizas. *Soil Biology and Biochemistry* **11**: 501-505.

Johnson NC, Graham JH, Smith FA. 1997. Functioning of mycorrhizal associations along the mutualism-parasitism continuum. *New Phytologist* **135**: 575-585.

Koide RT. 1985. The nature of growth depressions in sunflower caused by vesicular-arbuscular mycorrhizal infection. *New Phytologist* **99**:449-462.

Koide RT, Mooney HA. 1987. Spatial variation in inoculum potential of vesicular arbuscular mycorrhizal fungi caused by formation of gopher mounds. *New Phytologist* **107**: 173-182.

Koide RT, Li M. 1990. On host regulation of the vesicular-arbuscular mycorrhizal symbiosis. *New Phytologist* **114**: 59-74.

Koide RT, Schreiner RP. 1992. Regulation of the vesicular-arbuscular mycorrhizal symbiosis. *Annual Review of Plant Physiology and Plant Molecular Biology* **43**: 557-581.

Matsubara Y, Uetake Y, Peterson RL. 1999. Entry and colonization of *Asparagus officinalis* roots by arbuscular mycorrhizal fungi with emphasis on changes in host microtubules. *Canadian Journal of Botany-Revue Canadienne de Botanique* **77**: 1159-1167.

McArthur DAJ, Knowles NR. 1992. Resistance responses of potato to vesicular-arbuscular mycorrhizal fungi under varying abiotic phosphorus levels. *Plant Physiology* **100**: 341-351.

Machlis L, Torrey JG. 1956. Plants in action. San Francisco, USA: Freeman.

MINITAB 1998. Release 12 for Windows. Minitab. State College, PA, USA.

Modjo HS, Hendrix JW. 1986. The mycorrhizal fungus *Glomus macrocarpum* as a cause of tobacco stunt disease. *Phytopathology* **76**: 688-691.

Mosse B. 1973. Plant growth responses to vesicular-arbuscular mycorrhiza.IV. In soil given additional phosphate. *New Phytologist* **72**: 127-136.

Nagahashi G, Douds DD, Abney GD. 1996. Phosphorus amendment inhibits hyphal branching of the VAM fungus *Gigaspora margarita* directly and indirectly through its effect on root exudation. *Mycorrhiza* **6**: 403-408.

Nagahashi G, Douds DD. 1997. Appressorium formation by AM fungi on isolated cell walls of carrot roots. *New Phytologist* **136**: 299-304.

Nair MG, Safir GR, Siqueira JO. 1991. Isolation and identification of vesicular-arbuscular mycorrhiza-stimulatory compounds from clover (*Trifolium repens*) roots. *Applied and Environmental Microbiology* **57**: 434-439.

Olsson PA, van Aarle IM, Allaway WG, Ashford AE, Rouhier H. 2002. Phosphorus effects on metabolic processes in monoxenic arbuscular mycorrhiza cultures. *Plant Physiology* **130**: 1162-1171.

Peng S, Eissenstat DM, Graham JH, Williams K, Hodge NC. 1993. Growth depression in mycorrhizal citrus at high-phosphorus supply: analysis of carbon costs. *Plant Physiology* **101**: 1063-1071.

Perumalla CJ, Peterson CA, Enstone DE. 1990. A survey of angiosperm species to detect hypodermal Casparian bands.I. Roots with a uniseriate hypodermis and epidermis. *Botanical Journal of The Linnean Society* **103**: 93-112.

Peterson CA. 1989. Significance of the exodermis in root function. In: Loughman BC, Gasparikova O, Kolek J, eds. Structural and functional aspects of transport in roots. Dordrecht, The Netherlands: Kluwer Academic Publishers, 35-40.

Peterson CA, Enstone DE. 1996. Functions of passage cells in the endodermis and exodermis of roots. *Physiologia Plantarum* **97**: 592-598.

Pinior A, Wyss U, Piche Y, Vierheilig H. 1999. Plants colonized by AM fungi regulate further root colonization by AM fungi through altered root exudation. *Canadian Journal of Botany-Revue Canadienne de Botanique* **77**: 891-897.

Raskin I. 1983. Volume, density, thickness, and internal gas volume. *HortScience* **18**: 698-699.

Ratnayake M, Leonard RT, Menge JA. 1978. Root exudation in relation to supply of phosphorus and its possible relevance to mycorrhizal formation. *New Phytologist* **81**: 543-552.

Same BI, Robson AD, Abbott LK. 1983. Phosphorus, soluble carbohydrates and endomycorrhizal infection. *Soil Biology and Biochemistry* **15**: 593-597.

Schwab SM, Menge JA, Leonard RT. 1983. Quantitative and qualitative effects of phosphorus on extracts and exudates of sudangrass roots in relation to vesicular-arbuscular mycorrhiza formation. *Plant Physiology* **73**: 761-765.

Sharda JN, Koide RT. 2008. Can hypodermal passage cells limit penetration by mycorrhizal fungi? *New Phytologist* **180**:696-701.

Shishkoff N. 1987. Distribution of the dimorphic hypodermis of roots in angiosperm families. *Annals of Botany* **60**: 1-15.

Shishkoff N. 1989. Zoospore encystment pattern and germination on onion roots, and the colonization of hypodermal cells by *Pythium coloratum*. *Canadian Journal of Botany-Revue Canadienne de Botanique* **67**: 258-262.

Siqueira JO, Safir GR, Nair MG. 1991. Stimulation of vesicular-arbuscular mycorrhiza formation and growth of white clover by flavonoid compounds. *New Phytologist* **118**: 87-93.

Smith SE, Long CM, Smith FA. 1989. Infection of roots with a dimorphic hypodermis: possible effects on solute uptake. *Agriculture Ecosystems & Environment* **29**: 403-407.

Smith FA and Smith SE. 1997. Structural diversity in (Vesicular)-arbuscular mycorrhizal symbioses. *New Phytologist* **137**: 373-388.

Smith SE, Smith FA, Jakobsen I. 2003. Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth response. *Plant Physiology* **133**: 16-20.

Smith SE, Read DJ. 1997. Mycorrhizal symbiosis. 2nd Ed. London: Academic Press.

Takemoto D, Hardham AR. 2004. The cytoskeleton as a regulator and target of biotic interactions in plants. *Plant Physiology* **136**: 3864-3876.

Tawaraya K, Watanabe S, Yoshida E, Wagatsuma T. 1996. Effect of onion (*Allium cepa*) root exudates on the hyphal growth of *Gigaspora margarita*. *Mycorrhiza* **6**: 57-59.

Tawaraya K, Hashimoto K, Wagatsuma T. 1998. Effect of root exudate fractions from P-deficient and P-sufficient onion plants on root colonisation by the arbuscular mycorrhizal fungus *Gigaspora margarita*. *Mycorrhiza* **8**: 67-70.

Thomson BD, Robson AD, Abbott LK. 1986. Effects of phosphorus on the formation of mycorrhizas by *Gigaspora calospora* and *Glomus fasciculatum* in relation to root carbohydrates. *New Phytologist* **103**: 751-765.

Vierheilig H. 2004. Regulatory mechanisms during the plant-arbuscular mycorrhizal fungus interaction. *Canadian Journal of Botany-Revue Canadienne de Botanique* **82**: 1166-1176.

Wang XL, McCully ME, Canny MJ. 1995. Branch roots of *Zea*. V. Structural features that may influence water and nutrient transport. *Botanica Acta* **108**: 209-219.

Watanabe FS, Olsen SR. **1965.** Test of an ascorbic acid method for determining phosphorus in water and $NaHCO_3$ extracts from soil. *Soil Science Society of America Proceedings* **29**: 677-678.

Xie ZP, Staehelin C, Vierheilig H, Wiemken A, Jabbouri S, Broughton WJ, Vögeli- Lange R, Boller T. 1995. Rhizobial nodulation factors stimulate mycorrhizal colonization of nodulating and nonnodulating soybeans. *Plant Physiology* **108**: 1519-1525.

Chapter 5

Concluding Remarks

In this study, three root anatomical variables found in a wide range of angiosperm species were examined as mechanisms that may contribute to 1) interspecific variability in mycorrhizal colonization, and 2) P-mediated control of mycorrhizal colonization. The traits comprised the distribution of hypodermal passage cells in dimorphic hypodermal species, percentage of suberized root length in uniform hypodermal species, and percentage of root volume as intercellular air space.

The first objective was to determine whether interspecific variability in each trait might explain some of the observed interspecific variability in mycorrhizal colonization. By correlating these two factors in species spanning a wide range of plant families, I determined that of the three root anatomical traits, the distribution of hypodermal passage cells was the only significant contributor to mycorrhizal colonization, through its effect on penetration point formation. As interspecific variability in percentage of suberized root length and root volume as intercellular air space did not significantly affect overall colonization values, and thus it does not seem that these two variables are capable of limiting mycorrhizal colonization. The conclusions from the specific hypotheses are as follows:

1. In dimorphic hypodermal species, passage cells appear to be key determinants of mycorrhizal colonization because they are the cells through which fungal penetration of the hypodermis occurs. Variation among such species in mycorrhizal colonization may be at least partly determined by variation in the percentage of root length with passage cells.

2. In uniform hypodermal species, percentage of suberized root length, at least at the levels observed in the current study, does not seem to be capable of limiting mycorrhizal colonization. While successful penetration of AM hyphae may be limited to portions of roots lacking a suberized hypodermis, overall colonization levels are not affected by variation in percentage of suberized root length.

3. I found no evidence to support the hypothesis that percentage of root volume as intercellular air space can limit mycorrhizal colonization.

The second objective was to determine whether any of these traits responded to soil P availability in a manner by which they might contribute to P-mediated control of mycorrhizal colonization. All species examined were grown at high and low soil P availabilities, and the various traits were analyzed at the different P availabilities. Only the distribution of hypodermal passage cells (specifically, the percentage of root length with passage cells) responded significantly to P availability in the manner hypothesized, in contrast to the other traits, which did not respond significantly. The conclusions from this part of the study are as follows:

4. In uniform hypodermal species, percentage of suberized root length did not respond significantly to soil P availability, and therefore is not likely to serve as a mechanism responsible for P-mediated control of mycorrhizal colonization.
5. In species with hypodermal passage cells, altering the percentage of root length with passage cells could serve as one mechanism for P-mediated control of mycorrhizal colonization.

6. The percentage of root volume as intercellular air space did not respond significantly to soil P availability, is not likely to serve as a mechanism responsible for P-mediated control of mycorrhizal colonization.

Aside from the distribution of hypodermal passage cells, it appears that there is little support for the hypothesis that the other root anatomical variables are involved in host plant control of mycorrhizal colonization. It is noteworthy that the distribution of hypodermal passage cells was found to be both effective in limiting mycorrhizal colonization according to the analysis of interspecific variability and responded to soil P availability. In contrast, interspecific variability in the other two variables was not correlated with mycorrhizal colonization, and did not respond to soil P availability. As soil P availability and plant P concentrations are crucial variables that seem to mediate mycorrhizal colonization, only the variables capable of limiting mycorrhizal colonization (e.g., hypodermal passage cell distribution) may be the ones to benefit from the development of responsiveness to P availability. On the other hand, for those variables incapable of limiting mycorrhizal colonization, there may be no need to respond to P availability.

Finally, it is important to point out that each species has different mechanisms by which they may control mycorrhizal colonization, which most likely developed over time, depending on each species' environmental and genetic circumstances. While the mechanisms used to achieve control of mycorrhizal colonization vary significantly across species, it is remarkable that control of mycorrhizal colonization is a nearly universal phenomenon observed in the majority of mycorrhizal plant species examined to date. It is apparently a crucial survival mechanism in mycorrhizal plants, necessary for survival as a partner in this symbiosis, and future studies should strive to identify other mechanisms involved in this control.

Appendix

Phosphorus response curve. Shoot dry weight (g) as a measure of response to varying soil P treatments (mg elemental P kg^{-1} soil) to determine optimal "High P" treatments for each species. *: plants showed severe phosphorus toxicity symptoms, and shoot dry weight measurements were not taken.

Species	**50 mg P**	**80 mg P**	**100 mg P**	**200 mg P**
Allium cepa L.	0.13	0.10	*	*
Antirrhinum majus L.	0.13	0.13	*	*
Aquilegia vulgaris L.	0.06	0.05	*	*
Asclepias tuberosa L.	0.11	*	*	*
Asparagus officinalis L.	0.13	0.11	*	*
Coreopsis grandiflora Hogg ex Sweet Per.	0.27	*	*	*
Cucurbita sativa L.	0.29	0.34	0.36	*
Daucus carota L.	0.23	0.26	0.28	*
Helianthus annuus L.	0.56	0.73	0.76	*
Ipomoea purpurea L.	0.25	0.29	0.28	*
Ocimum basilicum L.	0.01	0.03	0.04	*
Rudbeckia fulgida Ait.	0.26	0.28	0.30	0.34
Solanum tuberosa L.	0.25	0.25	0.27	*
Vinca minor L.	0.04	0.04	*	*
Zea mays L.	0.42	0.46	0.48	*

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Publications

1. Wu T, **Sharda JN**, Koide RT. 2003. Exploring interactions between saprotrophic microbes and ectomycorrhizal fungi using a protein-tannin complex as an N-source by red pine (*Pinus resinosa). New Phytologist* **159** (1): 131-139.

2. Koide RT, Xu B, **Sharda JN.** 2005. Contrasting belowground views of an ectomycorrhizal fungal community. *New Phytologist* **166**: 251-262.

3. Koide RT, Xu B, **Sharda JN**, Lekberg Y, Ostiguy N. 2005. Evidence of species interactions within an ectomycorrhizal fungal community. *New Phytologist* **165** (1):305-316.

4. Koide RT, Shumway DL, Xu B, **Sharda JN**. 2007. On temporal partitioning of a community of ectomycorrhizal fungi. *New Phytologist* **174** (2): 420-429.

5. Koide RT, **Sharda JN**, Herr JR, Malcolm GM. 2008. Ectomycorrhizal fungi and the biotrophy-saprotrophy continuum. *New Phytologist* **178** (2): 230-233.

6. **Sharda JN**, Koide RT. 2008. Can hypodermal passage cells limit penetration by mycorrhizal fungi? *New Phytologist* **180**: 696-701.

Posters and Presentations

June 2006: "Mycorrhizal research at Penn State University". Presented at Beijing University (Beijing), Northeast Forestry University (Harbin), and South China Agricultural University (Guangzhou), as part of a trip to develop collaborations between Penn State and these three universities in China.

March 2007: "Investigating the role of root anatomy in phosphorus-mediated regulation of mycorrhizal colonization." Poster presentation at the MEEC (Mideast Ecology and Evolution Conference), Kent State University.

July 2007: "The role of hypodermal passage cells in P-mediated regulation of mycorrhizal colonization." Poster presentation at the ASPB (American Society of Plant Biologists) conference, Chicago, Illinois.