Angiosperm root evolution: the relationship between root anatomy and mycorrhizal colonization among Angiosperm trees.

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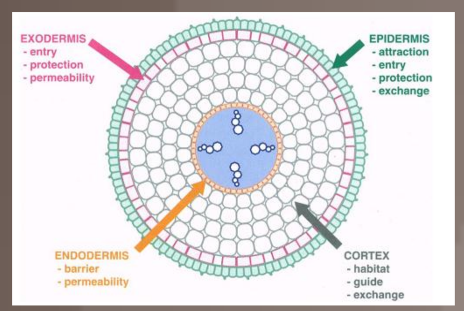
Introduction

The association between plants and arbuscular mycorrhizal fungi (AMF) is possibly the most prevalent symbiotic relationship in nature. In this relationship, roots rely on AMF for the acquisition of nutrients; in return the plant provides carbon and habitat to the fungi.

• **Anatomically, plant dependence should be reflected in a higher cortex area with respect to the vascular tissue**, thus providing a proportionally larger area for fungal habitat.

• **Chemically, high lignin and low nitrogen content are usually associated with secondary growth in roots**, which is correlated with decreased mycorrhizal colonization.



It has been hypothesized that flowering plants (angiosperms) were initially highly dependent on their AMF and became more independent as they evolved over time. However, there are relatively few studies describing how changes in root anatomy and chemistry occur across angiosperm lineages and how this is related to AMF colonization rates. In this study we evaluated trends in fine root evolution in angiosperms and the possible consequences on mycorrhizal colonization using 34 woody species grown in two common garden experiments.

Hypothesis/ Purpose

Assuming that root systems in angiosperms have evolved to be more independent from AMF associations, we expected :

1) a decrease in cortex area as a proportion of the root cross section in more derived angiosperm lineages.
2) an increase in distal root lignin content, and decrease in nitrogen content, in more derived angiosperm species.
3) a negative relationship between stele:root and lignin:N ratios with AMF colonization resulting in lower colonization in more derived angiosperm lineages.

Expected Results

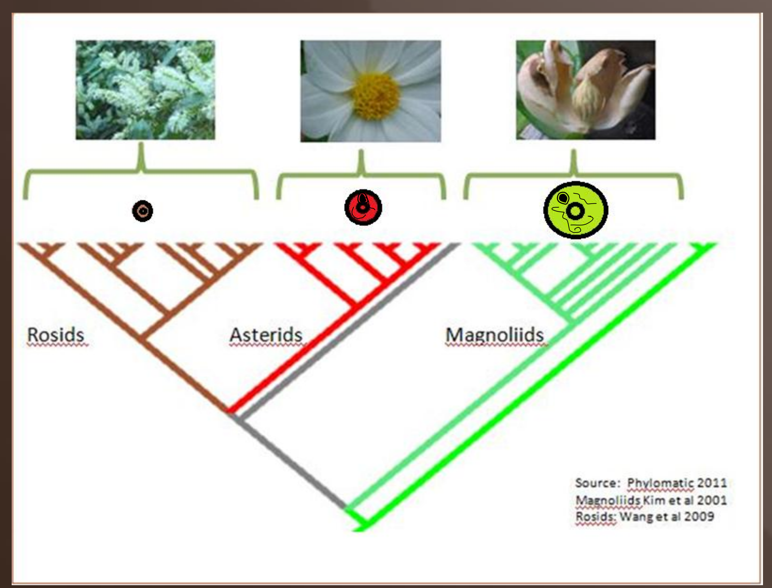


Figure 1. Expected distribution of root diameter among three main clades of angiosperms based on dependency of mycorrhizal association.

Sample Collection and Analysis

• Samples of roots were collected at The Holden Arboretum and Boone County Arboretum. Roots from two individuals of 34 species of woody Angiosperms were extracted using a soil corer (10 cm diameter X 15 cm deep).

• Soil samples were soaked in water over night and roots were carefully removed from soil. Root systems representing the most distal portion of the roots were dissected and ~0.2 g first order roots used for chemical analysis.

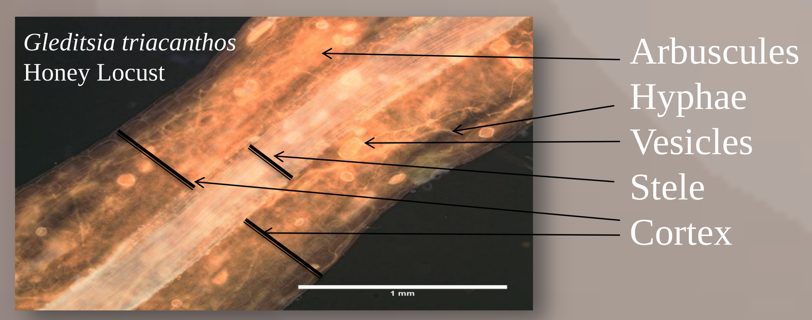
• We measured N content using a Costech elemental analyzer. Lignin content was estimated as the residual of a series of methanol, dichloromethane and sulfuric acid digestions following Melillo et al. (1990).

Estimation of AMF colonization and Root Anatomy

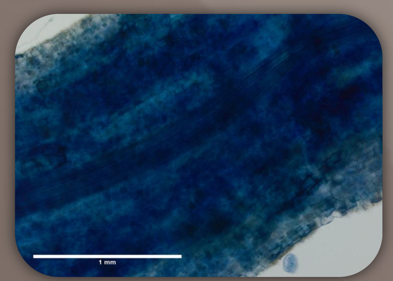
•Dry root systems were rehydrated and bleached sequentially with a solution of 1% KOH and 5% Acetic acid.

•Samples were then dyed with Trypan blue, dissected and mounted on to slides.

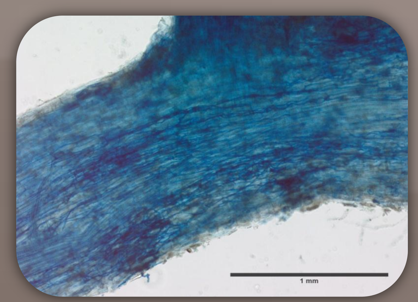
•Root and stele diameter measurements were taken as well as percentage of colonization using a light microscope.



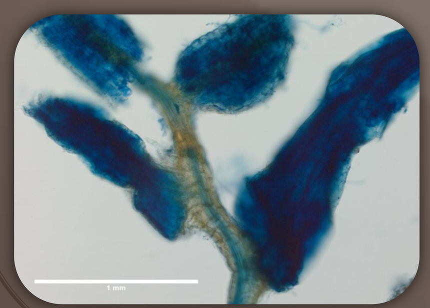
Examples of Morphological Variation in First Order Roots



Magnolia acuminata
Magnoliid
(Thick, heavily colonized roots)



Catalpa bignonoides
Asterid



Ulmus americana
Rosid
(Thin and less extensively colonized)

Statistical Analysis

•To test whether the distribution of morphological and chemical traits in angiosperms is structured phylogenetically we used an autocorrelative model (Moran Index) that tested if the distribution of traits across species is more similar among closely related species than expected by chance.

•Since most root traits show some degree of phylogenetic structure, we employed a generalized least square (GLS) approach, using phylogenetic correlation structures as the error structure of the model to test the influence of stele:root ratio and lignin:N ratio on AMF colonization.

Results: Phylogenetic Structure

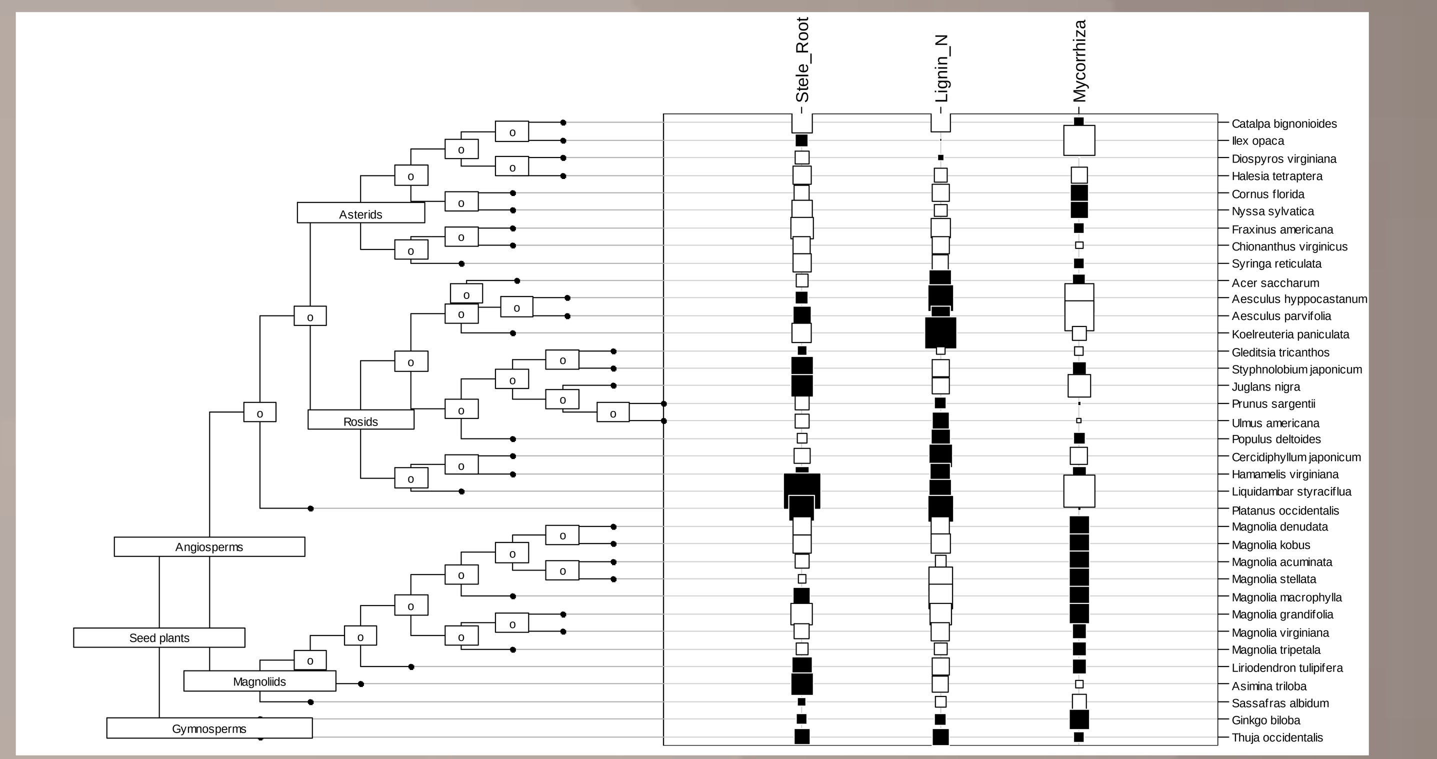


Fig. 2. Phylogenetic relationship and trait value distribution for 36 woody species grown in common gardens in Midwest USA. All values refer to first order roots. Associated p-values were 0.45;<0.001; and <0.001 for stele:root ratio; lignin:N ratio; and mycorrhizal colonization respectively. Symbols indicated relative trait values for each species, black squares represent values above the mean and white values below the mean. Brach lengths were standarized to have the same length. The gymnosperms *Gingko biloba* and *Thuja occidentalis* were used as root group for the tree.

Results: Controls on AMF Colonization

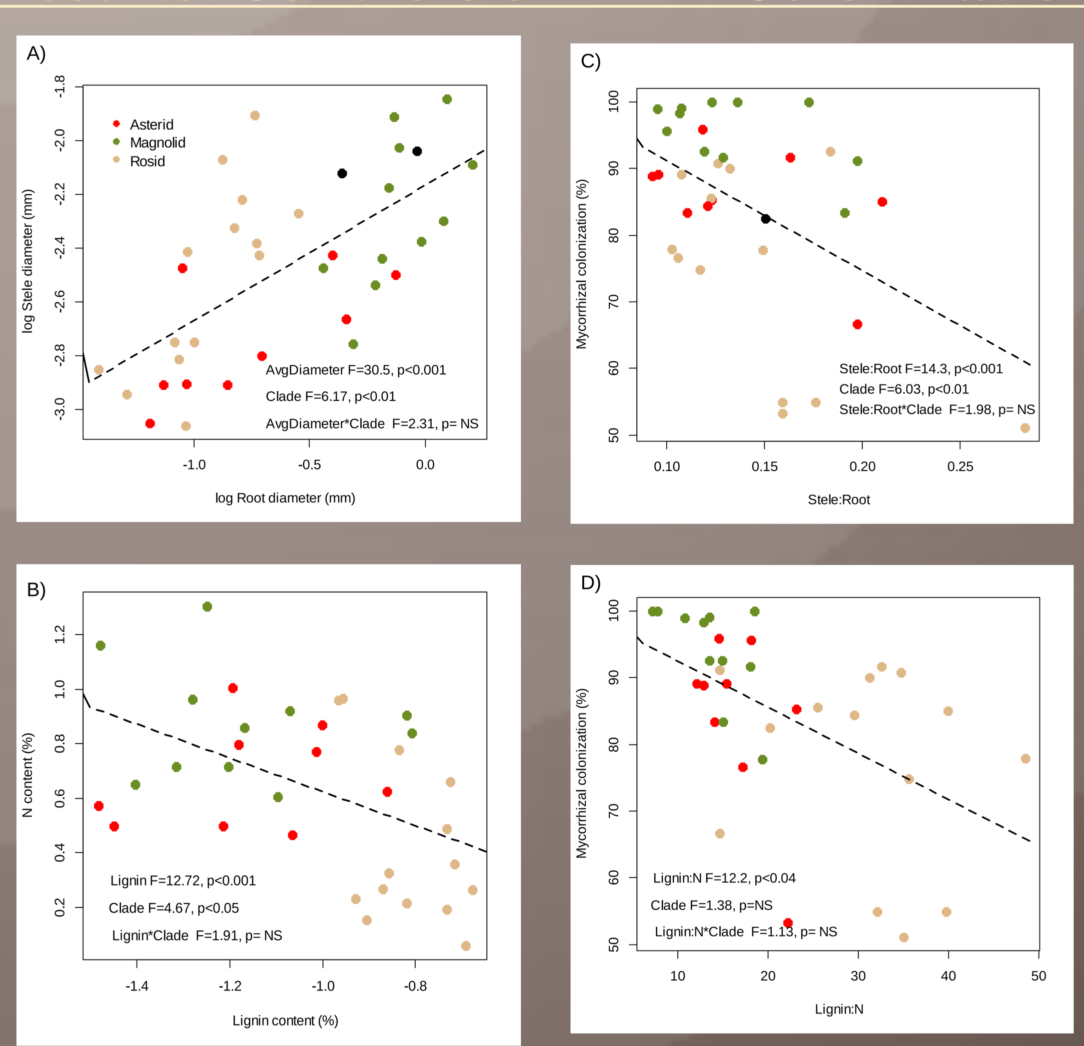


Fig. 3. Relationship between root traits and with mycorrhizal colonization for first order roots of 36 woody species grown in common gardens in Midwest, USA. A) Relationship between first order stele and root diameter; B) relationship between lignin (measured as non-acid soluble carbon compounds) and N content in first order roots; C) effect of stele:root ratio and D) lignin:N ratio on mycorrhizal colonization for first order roots.

Discussion

•Our results suggest divergences in root anatomy and chemistry along evolutionary pathways between angiosperms. All variables except stele:root ratio showed a significant phylogenetic signal (Fig. 2). Rosids showed thinner roots, less cortex area, higher lignin content and lower N content than magnoliids, whereas asterids showed intermediate values between the other two groups.

•Root diameter was thinner in rosid than in magnoliid , but not stele diameter (Fig 3A). This result indicates that rosids maintain less cortex area on average per unit of root area than magnoliids, suggesting less habitat for AMF fungi among rosid trees.

•Chemically, rosids showed a significantly negative relationship between lignin and N levels, whereas the relationship was not significant among other trees (Fig 3B). This result suggests a strong divergence in root chemistry between rosid trees and other angiosperms.

•In accordance with our hypotheses, both stele:root and lignin:N ratios were negatively associated with mycorrhizal colonization (Fig 3 C and D). In fact, both factors were significant when included in a single model (p= 0.04 and 0.01 for stele:root and lignin:N ratio respectively), suggesting that they function as additive mechanisms of AMF colonization control.

•In conclusion, our results support the idea of a decrease in mycorrhizal dependency during angiosperm evolution. Decreases in root diameter seems to be a common feature in more derived groups, although some asterids still maintain thick roots. Additionally, rosids seem to have undergone further chemical changes, possibly associated with early secondary growth that enhance limitations for AMF colonization.

Future Research

• We will measure AMF colonization using qPCR techniques and try to define more accurately the total fungal biomass supported per unit of root biomass.
• We will sequence fungal DNA extracted from root samples to test to what extent root morphology influences AMF community composition.

Acknowledgements

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