VARIATION IN ROOT ARCHITECTURE OF COMMON BEAN AND EFFECTS ON PHOSPHORUS ACQUISITION

A Thesis in
Plant Physiology

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ABSTRACT

The theme of this thesis is the relation between root architecture and acquisition of diffusion limited nutrients. Three lines of research are presented that pertain to this theme. A laboratory experiment details potential roles of gravitropism in formation of root architecture, and two modeling projects detail how variation in root architecture affects nutrient acquisition efficiency.

Root architecture in *Phaseolus vulgaris* varies among genotypes, due in part to diversity in basal root growth angle. Basal roots are a specific type of secondary root that emerge from 2-4 whorls in a 1 cm section at the root-shoot interface. Basal roots grow at angles from 0 to over 90 degrees relative to gravity, and growth angles vary among genotypes and among whorls within genotypes. In some genotypes, basal root growth angle varies with phosphorus availability. These observations lead to the question of how much variation in graviresponsiveness accounts for differences in basal root growth angles. The objectives of this research were to examine the gravitropic response in basal roots of *P. vulgaris*, to determine whether variation in gravitropism exists among genotypes, phosphorus treatments, or whorls within a root system, and to test for effects of ethylene on graviresponsiveness of basal and tap roots. Five genotypes of *P. vulgaris* contrasting in basal root growth angles were tested for responsiveness to 45° gravistimulation in daily observations of tap and basal roots growing in clear pouches containing 0 or 1 mM phosphorus. Two contrasting genotypes were also tested for kinetics of root reorientation by time lapse imaging for 9 hours after gravistimulation. To examine the effect of ethylene on graviresponsiveness, plants were treated with five concentrations of ethylene and with the ethylene action inhibitor MCP. Both tap and
basal roots began to respond to gravistimulation within 20 minutes and reached maximum response within 3 hours. Basal root, but not tap root, responses varied significantly among genotypes and between phosphorus treatments. Basal roots of genotypes G19833 and G2333 responded less to gravistimulation than those of G19839. Responsiveness of G19833 to gravistimulation was less in 1 mM phosphorus than in 0 phosphorus. The decreased graviresponsiveness of G19833 basal roots in 1 mM phosphorus compared with no phosphorus was localized to lower whorls. In kinetics observations, there were significant differences between the two genotypes tested in both curvature and timing of reorientation of basal roots, but not tap roots. The genotype with deep basal roots, DOR364, responded more quickly and with less curvature than the genotype with shallow basal roots, G19833. Variance of curvature responses to gravistimulation was significantly affected by ethylene, and this affect was not attributable to growth rate differences among ethylene treatments. Genotype, phosphorus and basal root whorl effects on variance of curvature responses to gravistimulation were much less than those of ethylene and insignificant over most time points. Ethylene also significantly decreased the mean responsiveness of basal roots, but not of tap roots. Results of daily and kinetics observations suggest that variation in basal root growth angles among genotypes and whorls may be partially associated with variation in graviresponsiveness as measured by maximum curvature and time to reach maximum curvature. The effect of ethylene on response variance indicates that modulation of responsiveness by ethylene also plays a role in establishment of basal root growth angles. These experiments provide data supporting the hypothesis that graviresponsiveness and ethylene modulation play roles in the development of root architecture.
Adventitious rooting contributes to efficient phosphorus acquisition by enhancing topsoil foraging. However, metabolic investment in adventitious roots may retard the development of other root classes such as basal roots, which are also important for phosphorus acquisition. In this study we quantitatively assessed the potential effects of adventitious rooting on basal root growth and whole plant phosphorus acquisition in young bean plants. The geometric simulation model SimRoot was used to dynamically model root systems with varying architecture and C availability growing for 21 days at 3 planting depths in 3 soil types with contrasting nutrient mobility. Simulated root architectures, tradeoffs between adventitious and basal root growth, and phosphorus acquisition were validated with empirical measurements. Phosphorus acquisition and phosphorus acquisition efficiency (defined as mol phosphorus acquired per mol C allocated to roots) were estimated for plants growing in soil in which phosphorus availability was uniform with depth or was greatest in the topsoil, as occurs in most natural soils. Phosphorus acquisition and acquisition efficiency increased with increasing allocation to adventitious roots in stratified soil, due to increased phosphorus depletion of surface soil. In uniform soil, increased adventitious rooting decreased phosphorus acquisition by reducing the growth of lateral roots arising from the tap root and basal roots. The benefit of adventitious roots for phosphorus acquisition was dependent on the specific respiration rate of adventitious roots as well as on whether overall C allocation to root growth was increased, as occurs in plants under phosphorus stress, or was lower, as observed in unstressed plants. In stratified soil, adventitious rooting reduced the growth of tap and basal lateral roots, yet phosphorus acquisition increased by up to 10% when total C allocation to roots was high and adventitious root respiration was similar to that in
basal roots. With C allocation to roots decreased by 38%, adventitious roots still increased phosphorus acquisition by 5%. Allocation to adventitious roots enhanced phosphorus acquisition and efficiency as long as the specific respiration of adventitious roots was similar to that of basal roots and less than twice that of tap roots. When adventitious roots were assigned greater specific respiration rates, increased adventitious rooting reduced phosphorus acquisition and efficiency by diverting carbohydrate from other root types. Varying the phosphorus diffusion coefficient to reflect varying mobilities in different soil types had little effect on the value of adventitious rooting for phosphorus acquisition. Adventitious roots benefited plants regardless of basal root growth angle. Seed planting depth only affected phosphorus uptake and efficiency when seed was planted below the high phosphorus surface stratum. Our results confirm the importance of root respiration in nutrient foraging strategies, and demonstrate functional tradeoffs among distinct components of the root system. These results will be useful in developing ideotypes for more nutrient efficient crops.

Fractal analysis allows calculation of fractal dimension, fractal abundance and lacunarity. Fractal analysis of plant roots has revealed correlations of fractal dimension with age, topology or genotypic variation, while fractal abundance has been associated with root length. Lacunarity is associated with heterogeneity of distribution, and has yet to be utilized in analysis of roots. In this project, fractal analysis is applied to the study of root architecture and acquisition of diffusion limited nutrients. We tested the hypothesis that soil depletion and root competition are more closely correlated with a combination of fractal parameters than by any one alone. The geometric simulation model SimRoot was used to dynamically model roots of various architectures growing for up to 16 days in 3
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PREFACE

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BASAL ROOT GRAVITROPISM IN PHASEOLUS VULGARIS
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CHAPTER 3

ARCHITECTURAL TRADEOFFS BETWEEN ADVENTITIOUS AND BASAL ROOTS FOR PHOSPHORUS ACQUISITION
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CHAPTER 4

MODELING APPLICABILITY OF FRACTAL ANALYSIS TO EFFICIENCY OF SOIL EXPLORATION BY ROOTS
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CHAPTER 1
INTRODUCTION

The theme of this thesis is the relation between root architecture and acquisition of
diffusion limited nutrients. Significant progress has been made in understanding the
importance of root architecture for nutrient acquisition, but fertile areas for research
remain. Pursuing such research is potentially beneficial for agriculture worldwide. In
developing countries, a better understanding of how root architecture affects nutrient
acquisition may aid in the breeding of more productive crops in low input systems.
Meanwhile, in developed countries, such research may help reduce dependence on inputs
and decrease the environmental impacts of agriculture. In this thesis, three lines of
research are presented that pertain to the overall theme of how root architecture relates to
nutrient acquisition. A laboratory experiment details potential roles of gravitropism in
formation of root architecture, and two modeling projects detail how variation in root
architecture affects nutrient acquisition efficiency.

BACKGROUND

Root architecture

Root architecture is the most comprehensive description of root geometry (Lynch, 1995). If root architecture is known, then all component measures of macroscopic root
growth can be determined. The specifics of root architecture have been explored at least
twice (Fitter, 1991; Lynch, 1995), and there is general agreement that root architecture is
the three dimensional form of a root system. Topological parameters, which describe
how root branches are connected, have been used as descriptors of root architecture
(Fitter, 1991). However, since topology does not convey information about how roots change or how they are oriented or distributed in a soil volume, it is not sufficient for a complete description of root architecture. Dynamic root rotation and growth angles, along with the distribution of roots in the soil must be accounted for as complements to topological parameters in order to produce a complete representation of root architecture.

Topological parameters have been explored in detail (Fitter, 1991). A basic unit of root topology is a link or internode. An external link is a portion of root between a meristem and branch, and an internal link lies between two branches or a branch and stem. Components of root topology include link length, link diameters, the number of branches arising from a node, branch angles, altitude and magnitude. Link lengths and diameters are straightforward measures of distance typically expressed in cm or mm. Magnitude is the number of external links or root tips. Fitter (1991) defined the size of a root system as equal to magnitude, but two root systems of equal magnitude can vary widely in extent of soil explored due to variation in link lengths. Within a root system, one or more root tips is separated from the base of the root by the highest number of links. The number of links in the path from this root tip to the base is the altitude of the root system. Branch angles were defined as the angle at which a daughter root emerges from its parent (Fitter, 1991). Yet, growth angles change as roots grow (Bonser et al., 1996), so an accurate definition of branch angles needs to include measures of static initial parameters and dynamic temporally varying aspects. Finally, in addition to uncertainty about size and the failure to account for dynamics of root growth angles, topology does not consider rotation within branches (Lynch, 1995), which produces variation in spatial configurations among root systems, even when branching angles are equivalent.
Root distribution in soil refers to the presence of roots through a soil volume (Lynch, 1995). Gradients in the presence of roots are measured either through soil depth or distance from a plant. Distribution is typically estimated by sampling a portion of the root system and reported in terms of length or mass of roots per volume of soil, or number of intercepts of roots with a trench face. The reason for measuring root distribution is to determine how many roots are exploring given slices or volumes of soil. Information about topology and the orientation of root axes is usually not available in sampling for root distribution and is, therefore, not considered.

A complete description of macroscopic root growth is formulated only by considering root architecture, which denotes the spatial configuration of the complex assemblage of subunits that make up a root system (Lynch, 1995). If root architecture is known, then topology and distribution are as well. In theory, each link can be considered independently and root architecture can be described as the sum of individual link lengths, diameters, points of origin, initial orientation in space, and dynamics of rotation or orientation as the link develops. This is an approach that, along with branching parameters, is applied in simulations of root growth. For empirical work it is not feasible to measure and analyze all aspects of root architecture, so roots are sampled and components of architecture are measured as required for the objectives of the research.

Nutrient acquisition

Mineral nutrients are acquired from soil through root interception, diffusion, or mass flow (Marschner, 1995). Root interception requires roots growing into the source of nutrients. Since roots occupy a small portion of soil volume, interception is typically
an insignificant form of acquisition. Mass flow provides nutrients that are carried in the transpiration stream of water. Many nutrients, including nitrogen, calcium and magnesium are often supplied predominantly through mass flow. Water moves relatively freely through soil, but there are still gradients in water availability, particularly with depth, so root architecture has some relevance for acquisition of nutrients through mass flow. When mass flow does not provide sufficient quantities of a given nutrient, then the concentration of that nutrient near the root will decrease and diffusion from surrounding soil is required to meet plant demands. Diffusion limited nutrients, including phosphorus and potassium, are typically much more prevalent in the solid phase than in solution, and they have low mobility (< 1 mm/day) through soil. This leads to volumes of soil solution being depleted of diffusion limited nutrients surrounding roots and undepleted soil a cm or less away. The spatial configuration, or architecture of a root system is vital to the acquisition of these diffusion limited nutrients.

Root architecture is important for acquisition of diffusion limited nutrients in two ways. First, diffusion limited nutrients are heterogeneously distributed in most soils. Roots with architecture that allows proliferation in volumes of high nutrient availability will acquire more nutrients than those that proliferate in other volumes. Second, competition results when soil volumes are depleted by two or more root branches. With efficiency defined as the amount of nutrient acquired per unit of investment by the root, then root systems with architecture that minimizes competition acquire nutrients more efficiently than those with root proliferation in previously explored soil.

Efficiency of soil exploration is relevant because phosphorus is a primary constraint to productivity over much of the earth (Vance et al., 2003). Large areas of
tropical and subtropical soils in Africa, South America, and Asia have phosphorus availability limited by high phosphorus fixation (Sanchez and Uehara, 1980). A small increase in solution phosphorus requires a large increase in sorbed phosphorus, so fertilization is often not cost effective or economically possible. Root architecture contributes to phosphorus acquisition efficiency for both of the reasons mentioned in the previous paragraph. Soils are typically stratified in phosphorus availability with more near the surface (Barber, 1995; Huang, 2000), so plants with roots distributed in the phosphorus rich surface strata are expected to acquire more. Plus, phosphorus is typically diffusion limited with low mobility (Schenk and Barber, 1979). Therefore, root architecture that minimizes competition is expected to be beneficial for phosphorus acquisition efficiency. Efficiency of phosphorus acquisition allows plants to maintain allocation to shoot growth, which in turn feeds back to roots in the form of more resources for root growth. Over the course of a growing season, small advantages produced by marginal increases in phosphorus acquisition efficiency can result in large effects in vegetative growth and yield (Wissuwa, 2003).

Common bean

Low availability and immobility of phosphorus in many soils makes root architecture an agriculturally and ecologically relevant research topic for a wide range of plant species. The research presented in this thesis focuses on common bean (*Phaseolus vulgaris* L), which was selected for several reasons. *Phaseolus vulgaris* is a diverse species with a long history of domestication. A wide array of root architecture and low phosphorus tolerant phenotypes has been observed. Common bean is a primary source of
nutrition for many people in developing countries, so application of results could potentially benefit humankind. It is often grown in suboptimal conditions, with low phosphorus availability common. In these situations, increased understanding of phosphorus acquisition efficiency could aid in selection of higher yielding cultivars. Finally, this research adds to the growing amount of information concerning root architectural effects on nutrient acquisition that has been generated using common bean.

*Phaseolus vulgaris* is native to a wide range of habitats from Argentina to Mexico (Sauer, 1993). Morphological, isozyme, and seed protein variation indicates six different domestication events across this range, resulting in the cultivation of six major races (Sauer, 1993; Singh et al., 1991). The earliest evidence of domesticated bean seed is from 5500 BC in Peru, and domestication in Mexico appears to have occurred by 5000 BC (Sauer, 1993). Common bean was grown in Europe within a half century of Columbus’ voyage to the new world, and it spread through Africa faster than European exploration. Since then, *Phaseolus vulgaris* propagation has also spread to Asia and Australia.

Presently, common bean is cultivated in at least 111 countries (http://www.ciat.cgiar.org/beans/worldstat2.htm, http://apps.fao.org/default.jsp). Major producers include Brazil, Mexico, Argentina, India, China, Mayanmar, Uganda, United Republic of Tanzania, Rwanda, Democratic Republic of Congo, and Burundi. Average yield worldwide is 696 kg/ha, with a range of 250 kg/ha in Somalia to 4,594 kg/ha in Ireland. In many countries of significant production, including India, Mexico, and Uganda, yield is 150-250 kg/ha below the worldwide average.

Many conditions explain suboptimal common bean yield in various locations around the world, including cultural practices, disease, drought and mineral deficiency.
The purpose of this thesis is to present research concerning effects of root architecture on nutrient acquisition, not to comprehensively explore reasons for reduced yield, which is already being done (Voysest, 2001; Wortman et al., 1998). A widespread source of suboptimal yields that is relevant for this thesis is low phosphorus availability (Voysest, 2001; Wortman et al., 1998). As previously stated, large areas of tropical and subtropical soils in Africa, South America, and Asia have limited phosphorus availability (Sanchez and Uehara, 1980). Common bean is often cultivated in such soil. Economic constraints and high phosphorus fixation make application of phosphorus fertilizer untenable as a primary strategy for improving yields. The most promising strategy for increasing yields in these areas is selection of genotypes tolerant to low phosphorus stress.

Common bean is a diverse species composed, in part, of genotypes adapted to low phosphorus availability (Beebe et al., 1997; Lynch and Beebe, 1995). Variation in vegetative growth and yield responses to low phosphorus conditions has been identified (Yan et al., 1995a; b). Since phosphorus is typically stratified, root architecture has been an important trait to consider when identifying sources of phosphorus efficiency (Lynch and Brown, 2001).

Common bean has a tap root system, in which the tap root grows down and secondary roots branch off and typically grow at angles between straight down and horizontal. Basal roots are secondary roots that emerge from the root-shoot interface. Adventitious roots emerge from the subterranean portion of the hypocotyl. Tap, basal, and adventitious roots can each branch off 2 orders of lateral roots.

In this thesis, several aspects of common bean root architecture are researched for effects on phosphorus acquisition efficiency. Observations of basal root responses to
gravistimulation provide insight into how basal root growth angles are dynamically maintained. Effects of changes in allocation to adventitious rooting on distribution, magnitude and size of root systems, and resultant phosphorus acquisition, are examined in geometric simulations. Finally, the utility of fractal analysis for assessment of phosphorus efficiency is also addressed in simulations in which basal root angles, lateral root densities and adventitious numbers are varied.

RESEARCH PROJECTS

*Basal root gravitropism*

Basal roots are specialized secondary roots that emerge from the root-shoot interface. In *Phaseolus vulgaris*, basal roots emerge 2-3 days after seed germination. The diameter and growth rate of basal roots is intermediate between the tap root and tap laterals (personal observation). The growth angle of basal roots varies among common bean genotypes (Bonser et al., 1996; Liao et al., 2001), as well as within a single root system.

The growth angle of basal roots is initially determined by the angle at which each basal root emerges from the root-shoot interface. After emergence, basal roots grow at particular angles which might be maintained through gravitropism. If gravitropism is involved, the angle with respect to gravity at which the basal root tip is maintained is defined as the gravitropic setpoint angle (GSA) (Digby and Firn, 1995).

The genotypes included in this report represent a range of growth habits and exhibit a variety of basal root growth angles that have been extensively quantified in previous reports (Bonser et al., 1996; Liao et al., 2001). In comparisons of ability to grow in low phosphorus conditions, BAT477, G19833, and G19839 have been reported
as phosphorus efficient, G2333 as intermediate, and DOR364 as phosphorus inefficient (Liao et al., 2001; Yan et al., 1995a; b; 1996).

Each common bean plant has a number of whorls of basal roots that emerge from an approximately one cm section at the interface of roots and shoots. The number of whorls, which varies among genotypes, ranges from 2 to 4 in the genotypes included in this study. Genotypes DOR364 and BAT477 typically have 2-3, G19839, G19833 and G2333 have 3-4. Within a root system, basal roots tend to fan out from upper to lower whorls, with the topmost whorl growing at the largest angle with respect to gravity.

Mean shallowness of basal root growth angle has been correlated with efficiency of phosphorus acquisition (Liao et al., 2001; Rubio et al., 2003). The genotypes listed as phosphorus efficient above have more shallow basal roots than those found to be phosphorus inefficient. Several genotypes, including G19833 and G19839, have plasticity of basal root growth angle, defined as more shallow basal root growth angles under low phosphorus availability (Bonser et al., 1996; Liao et al., 2001).

In short, a number of reports describe a diversity of common bean root architectures generated in part by variation in basal root numbers and growth angles. While it seems apparent that gravitropism plays a role in producing variants, this has not been specifically tested. One report describes the lack of gravitropism in lateral roots of *Phaseolus vulgaris* (Ransom and Moore, 1983). However, it is unclear whether basal roots were included in their observations, and they used destructive sampling that did not allow for observation of real time responses to gravistimulation. On the other hand, gravitropism or geotropism of secondary roots has been observed in a number of species, including Arabidopsis, tea, and soybean (Kiss et al., 2002; Mitchell and Russell, 1971;
Ethylene has been shown to affect primary root gravitropism in maize (Lee et al., 1990). In addition, effects of ethylene on tomato shoot gravitropism have been observed (Madlung et al., 1999). Furthermore, the GSA of rye coleoptiles has been affected by ethylene (Edelmann et al., 2002). Yet, none of these systems require ethylene for gravitropism. The role it seems to play is as a modulator of responses. In common bean, ethylene has been shown to affect the growth angle of basal roots, specifically as a regulator of plastic responses among phosphorus treatments (Zhang, 2002). All of these observations taken together suggest that ethylene might act as a modulator of gravitropism in the establishment of growth angles of *Phaseolus vulgaris* basal roots and, ultimately, the resulting root architecture. This was studied in the present research by testing for effects of ethylene on responsiveness of basal roots to gravistimulation.

The objective of this research was to test for common bean basal root gravitropism by checking for the ability of basal roots to reorient towards previous growth angles upon gravistimulation. If gravitresponsiveness was observed, then basal root responses were checked for variation among genotypes and whorls in order to see if it could account for variation in growth angles observed among genotypes or whorls. Since growth angles of some genotypes vary with phosphorus treatment, 2 phosphorus treatments were included to test for effects of phosphorus nutrition on responsiveness to gravistimulation. In addition, time lapse photography was incorporated to test for difference in kinetics of graviresponsiveness. Finally, given the previously observed effect of ethylene on basal root growth angle, a study of ethylene effects on the kinetics
of graviresponsiveness was included in this project. These experiments were conducted to test the hypothesis that variation in basal root graviresponsiveness and modulation by phosphorus and ethylene are components of systems that establish root architecture.

Contribution of adventitious roots to phosphorus acquisition efficiency

Adventitious roots emerge from subterranean hypocotyls and grow near the soil surface. Basal roots vary in growth angle within a plant and among genotypes. The higher basal root growth angle is with respect to gravity, the more basal roots proliferate near the soil surface. With stratified phosphorus availability, both adventitious and basal roots may be beneficial for phosphorus acquisition. However, questions concerning what combinations of allocation to adventitious roots and basal root shallowness are most beneficial remain unsolved. Do basal roots and adventitious roots always complement each other in phosphorus acquisition, or do adventitious roots only benefit plants with deeper basal roots? Increasing allocation to adventitious roots means decreasing allocation to tap and basal roots, along with their laterals. What allocation to adventitious roots leads to peak efficiency of phosphorus acquisition, or is it always advantageous to allocate more resources to adventitious root production?

In addition to root architecture, variation in root respiration rates has been observed to affect phosphorus acquisition efficiency. As phosphorus availability decreases, the amount of carbon partitioned into root respiration increases (Nielsen et al., 2001). Phosphorus efficient genotypes can maintain higher growth rates by allocating less C to root respiration than inefficient genotypes under low phosphorus conditions (Nielsen et al., 2001). Furthermore, respiration varies among root types. In relative
terms, tap root respiration is low, basal root respiration is intermediate, and adventitious root respiration is high (Ho et al., 2003). Adventitious root respiration ranges from approximately 1-4 times greater than basal root respiration. In experiments with several phosphorus efficient and phosphorus inefficient genotypes, respiration in adventitious roots of phosphorus inefficient genotypes is in the high end of this range (Ho et al., 2003). Therefore, research into optimal allocation to adventitious rooting needs to address variation in respiration for conclusions to be valid.

For this project, we utilized the geometric simulation model \textit{SimRoot} (Lynch et al., 1997). Empirical data collected over many years has guided \textit{SimRoot} development, and has allowed for accurate representations of common bean roots. Past simulations have addressed a range of research topics, including carbon allocation, phosphorus acquisition, competition, and fractal analysis that have been verified with greenhouse and field data (Ge et al., 2000; Nielsen et al., 1994; Nielsen et al., 1997; Rubio et al., 2001; Walk et al., 2004). One modification was introduced into \textit{SimRoot} for this project. Carbon allocation to roots was estimated in greenhouse studies. Growth of roots in \textit{SimRoot} is then calculated by the amount carbon allocated, and after subtracting off respiration demands, dividing remaining carbon among growing root tips.

The soil in simulations that has had P concentration decreased by root uptake is referred to as the depletion volume, the size of which is affected by the diffusion coefficient of phosphorus through the soil. Phosphorus acquisition is estimated by multiplying depletion volume by factors empirically derived from greenhouse cultures in previously reported experiments (Ge et al., 2000; Rubio et al., 2001). Phosphorus acquisition efficiency is defined as moles of phosphorus acquired per mol of carbon allocated to the
root system. Some soil is depleted of P by more than one root from a single plant. The volume of soil explored by multiple roots is defined as competition volume.

The general hypothesis tested in this modeling project is that adventitious roots increase phosphorus acquisition and acquisition efficiency of plants growing in soils with stratified phosphorus availability. Related hypotheses and corollaries are: 1) Adventitious roots will functionally complement basal root architecture, i.e., plants with deep basal roots will benefit from adventitious roots more than plants with shallow basal roots. 2) Competition among adventitious roots for phosphorus acquisition will depend on the length of subterranean hypocotyl, as affected by e.g. planting depth 3) The effects of adventitious rooting on basal root development and whole plant phosphorus acquisition will be influenced by the relative respiratory costs of the respective root classes, e.g. if respiration rates in adventitious roots are high, then less carbon will be available for root proliferation and, therefore, phosphorus acquisition and efficiency could be reduced. 4) The benefit of adventitious roots for phosphorus acquisition will depend on the phosphorus mobility in the soil, i.e. with increased phosphorus mobility, phosphorus competition among roots increases, so the effect of adventitious roots on acquisition and efficiency decreases.

Utility of fractal analysis to estimate phosphorus acquisition efficiency

Annual crops produce several orders of roots as fine as a fraction of a mm in diameter, with a distance between branches ranging from millimeters to centimeters. With these branching patterns, roots can be characterized by fractal analysis over a range of scales from about a quarter of a millimeter to several centimeters (Tatsumi et al.,
The fractal dimension (FD) has been calculated for whole root systems (Tatsumi et al., 1989), as well as for separated branches (Lynch and van Beem, 1993), and planar sections (Eshel, 1998; Nielsen et al., 1997). In computer simulations, FD of planar sections was correlated with the FD of entire root systems (Nielsen et al., 1997), so analyzing subsamples may be appropriate for roots, which are often difficult to observe entirely intact.

A number of researchers have demonstrated that fractal analysis may be biologically relevant. Many aspects of morphological and physiological variation have been associated with variation in FD. As roots grow, the FD increases (Fitter and Stickland, 1992; Lynch and van Beem, 1993; Nielsen et al., 1998). Fractal dimension has been correlated with root topology (Fitter and Stickland, 1992), and root architecture (Nielsen et al., 1997). Differences in FD have been noted among 4 species of dicots and monocots (Fitter and Stickland, 1992), as well as among genotypes of sorghum, rice and common bean. (Izumi et al., 1995; Masi and Maranville, 1998; Nielsen et al., 1998). Genotypic variation of root mass and root to shoot ratio was correlated with variation in FD of roots (Masi and Maranville, 1998). Lastly, FD has been observed to vary with nitrogen availability for corn (Eghball et al., 1993), and phosphorus acquisition from low phosphorus soils in common bean (Nielsen et al., 1998).

Most research to date has focused on FD, even though other parameters can be easily calculated from the same data. Therefore, it remains unclear if FD is sufficient or the most appropriate aspect of fractal analysis that can be applied to the study of root system morphology and functioning. While FD appears to be a useful metric in some situations, there are more aspects of fractal analysis that might also be useful.
Fractal analysis in root biology typically utilizes box counting and the equation:

\[ N(L) = KL^{-D}, \]

where \( L \) is the box size, and \( N(L) \) is the number of boxes of size \( L \) needed to cover the object. A log-log plot of \( N(L) \) versus \( L \) returns \(-D\) as the slope and \( \log K \) as the intercept.

In terms of fractal analysis, \( D \) is the fractal dimension, and \( \log K \) has been associated with fractal abundance (FA) (Puche and Su 2001a; b). Fractal dimension is associated with the branching pattern, while FA is associated with the volume of space explored. To date, FD has been studied far more than FA in the plant sciences. Yet, it has been shown that FA may vary when fractal dimension does not. Corn roots that vary in size can have equivalent FD, but vary in FA (Eghball et al., 1993). Masi and Maranville (1998) speculated that FA might differentiate root systems with regard to their ability to exploit the soil profile, even when the FD are similar. However, no observations were made to test this speculation. Puche and Su (2001a; b) showed that the FD describes the intricacy of termite tunnels, while FA is associated with population density and foraging behavior. There could be an analogous situation with plants roots, whereby FD describes the branching pattern, while FA is associated with the size of the root system and extent of soil explored.

Another parameter that can be calculated in fractal analysis that might be useful in distinguishing objects of similar FD is lacunarity (L), which is a measure of the structural heterogeneity within an object (Smith et al., 1996). When the box counting method is used, \( L \) is an estimate of the variation of how many root segments are contained in the boxes. For example, some boxes might cover one root segment while other boxes cover 10 root segments. The FD does not account for this variation, but \( L \) does. One measure
of L is the coefficient of variation, which is the standard deviation divided by the mean of the number of root segments per box. This is the measure of L that is used in this paper. It is likely to be complementary to FD, and may or may not be a substitute or complement of FA.

The objective of this project was to determine the most appropriate fractal parameters to calculate for determining effectiveness of soil exploration by root systems. To be consistent with other research presented in this thesis and for simplicity, phosphorus is the nutrient by which exploration is measured. As in the adventitious root project described above, depletion volume is defined as the soil that has had P concentration decreased by root uptake, and competition volume is defined as the volume of soil explored by multiple roots. In contrast to the adventitious root model, phosphorus efficiency is defined in this project as competition volume divided by depletion volume. This definition has been used in geometric modeling to evaluate the P efficiency of contrasting root architectures (Ge et al., 2000; Rubio et al., 2001), which has been correlated with the ability of plants to maintain growth under low P conditions in greenhouse and field environments (Lynch and Brown, 2001; Rubio et al., 2003).

The FD of planar root sections can differentiate P efficient and P inefficient genotypes of common bean (Nielsen et al., 1998). Neither FA nor L was included in that study, so the question of whether these terms can improve our understanding of phosphorus acquisition efficiency remains.

Root systems can vary significantly in size and FA, yet have similar FD (Eghball et al., 1993). Therefore, a hypothesis tested in this study is that FD and FA are correlated with complementary aspects of uptake of diffusion limited nutrients. Specifically, FA is
correlated with absolute measures of soil exploration, such as depletion and competition volume, while FD is correlated with relative competition. Furthermore, with slow diffusion of nutrients, and, therefore low inter-root competition (Ge et al., 2000; Rubio et al., 2001), root length is likely to be a better estimate of depletion and competition than fractal abundance. As diffusion rate increases, and more inter-root competition results, FA may become a better estimate of depletion than root length.

The possible utility of lacunarity is not as clear. A low value of L implies homogeneity, which may be associated with roots that are uniformly distributed with either high or low competition. Therefore we hypothesize that L alone is not well correlated with estimates of soil exploration, but it may be useful as a complement to FD or FA in multiple regression to significantly increase correlations with estimates of soil exploration.

Since three-dimensional root architecture and nutrient depletion are not readily observable in field or greenhouse conditions, common bean root architectures were generated in SimRoot, and output was imported into associated programs for numerical determination of soil depletion and fractal analysis. Architectural parameters to be varied in this project are basal root angles, basal lateral density, and adventitious number. Fractal dimensions of entire root systems have been correlated with dimensions of root projections and planar sections (Nielsen et al., 1997), so simulations included 3 dimensional root systems, projections of root systems and 0.1 cm vertical slices taken from 3 positions. Empirical data were also examined to see if FD, FA and L could be used to differentiate common bean genotypes that varied in shoot mass and competitiveness in medium and high P field conditions.
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ABSTRACT
Root architecture in *Phaseolus vulgaris* varies among genotypes, due in part to diversity in basal root growth angle. Basal roots are a specific type of secondary root that emerge from 2-4 whorls in a 1 cm section at the root-shoot interface. Basal roots grow at angles from 0 to over 90 degrees relative to gravity, and growth angles vary among genotypes and among whorls within genotypes. In some genotypes, basal root growth angle varies with phosphorus availability. These observations lead to the question of how much variation in graviresponsiveness accounts for differences in basal root growth angles. The objectives of this research were to examine the gravitropic response in basal roots of *P. vulgaris*, to determine whether variation in gravitropism exists among genotypes, phosphorus treatments, or whorls within a root system, and to test for effects of ethylene on graviresponsiveness of basal and tap roots. Five genotypes of *P. vulgaris* contrasting in basal root growth angles were tested for responsiveness to 45° gravistimulation in daily observations of tap and basal roots growing in clear pouches containing 0 or 1 mM phosphorus. Two contrasting genotypes were also tested for kinetics of root reorientation by time lapse imaging for 9 hours after gravistimulation. To examine the effect of ethylene on graviresponsiveness, plants were treated with five concentrations of ethylene and with the ethylene action inhibitor MCP. Both tap and basal roots began to respond to gravistimulation within 20 minutes and reached maximum response within 3 hours. Basal root, but not tap root, responses varied significantly among genotypes and between
phosphorus treatments. Basal roots of genotypes G19833 and G2333 responded less to gravistimulation than those of G19839. Responsiveness of G19833 to gravistimulation was less in 1 mM phosphorus than in 0 phosphorus. The decreased graviresponsiveness of G19833 basal roots in 1 mM phosphorus compared with no phosphorus was localized to lower whorls. In kinetics observations, there were significant differences between the two genotypes tested in both curvature and timing of reorientation of basal roots, but not tap roots. The genotype with deep basal roots, DOR364, responded more quickly and with less curvature than the genotype with shallow basal roots, G19833. Variance of curvature responses to gravistimulation was significantly affected by ethylene, and this affect was not attributable to growth rate differences among ethylene treatments. Genotype, phosphorus and basal root whorl effects on variance of curvature responses to gravistimulation were much less than those of ethylene and insignificant over most time points. Ethylene also significantly decreased the mean responsiveness of basal roots, but not of tap roots. Results of daily and kinetics observations suggest that variation in basal root growth angles among genotypes and whorls may be partially associated with variation in graviresponsiveness as measured by maximum curvature and time to reach maximum curvature. The effect of ethylene on response variance indicates that modulation of responsiveness by ethylene also plays a role in establishment of basal root growth angles. These experiments provide data supporting the hypothesis that graviresponsiveness and ethylene modulation play roles in the development of root architecture.
INTRODUCTION

Root architecture is the three-dimensional distribution of a root system, which is determined by topological factors, branching angles and growth angles. Common bean (Phaseolus vulgaris L.) has a tap root system, in which the tap, or primary root grows at 0° with respect to gravity. Secondary roots branch off the tap root or hypocotyl and typically grow at angles between 0° to 90° with respect to gravity. The growth angle of secondary roots is initially determined by the angle at which each secondary root emerges from the tap root or hypocotyl. After emergence, secondary roots grow at particular angles that might be maintained through gravitropism. If gravitropism is involved, the angle with respect to gravity at which an organ is maintained is defined as the gravitropic setpoint angle (GSA) (Digby and Firn, 1995).

Basal roots are a genetically, anatomically, and physiologically unique set of secondary roots that emerge from the root-shoot interface (Zobel, 1975; 1986). In Phaseolus vulgaris, basal roots emerge 2-3 days after seed germination. The diameter and growth rate of basal roots is intermediate between the tap root and the lateral roots borne on the tap root. The growth angle of basal roots varies among genotypes (Bonser et al., 1996; Liao et al., 2001), as well as within a single root system (Fig 1a).

The genotypes of common bean included in this report exhibit a variety of basal root growth angles that have been extensively quantified in previous reports (Bonser et al., 1996; Liao et al., 2001). In comparisons of ability to grow in low phosphorus conditions, BAT477, G19833, and G19839 have been characterized as phosphorus efficient, G2333 as intermediate, and DOR364 as phosphorus inefficient (Liao et al., 2001; Nielsen et al., 1998; Nielsen et al., 2001; Yan et al., 1995; 1996). Phosphorus
efficiency has been correlated with basal root growth angle in laboratory, greenhouse and field experiments, with more efficient genotypes having more shallow basal roots (Liao et al., 2001; Rubio et al., 2003). Several genotypes, including G19833 and G19839, display plasticity of basal root growth angle, defined as shallower basal root growth angles under low phosphorus availability (Bonser et al., 1996; Liao et al., 2001). Basal root growth angle is also important for drought tolerance. Deep basal root growth is considered beneficial for enabling common bean plants to access water in periods of drought (Ho et al., 2004). Therefore, the observed diversity of basal root growth angles likely reflects genotypic variation selected by multiple constraints to growth in native common bean habitats.

Each common bean plant has a number of whorls of basal roots that emerge from an approximately one cm section at the interface of roots and shoots (Fig. 1a). The number of whorls, which varies among genotypes, ranges from 2 to 4 in the genotypes included in this study. Genotypes DOR364 and BAT477 have 2 or 3 whorls, while genotypes G19833, G19839 and G2333 have 3-4 whorls. Using root orientation consistent with that described by Weinhold (1967) and Zobel (1986), basal roots emerging towards the shoot apex are labeled as upper, and those emerging towards the root apex are lower. Within a root system, basal root growth angles tend to increase with respect to gravity from lower to upper whorls, with the uppermost whorl growing at the largest angle with respect to gravity (Fig 1a).

In short, a number of reports describe a diversity of common bean root architectures generated in part by variation in basal root numbers and growth angles. While it seems apparent that gravitropism plays a role in producing variants, this has not
been specifically tested. One report describes the lack of gravitropism in lateral roots of *Phaseolus vulgaris* (Ransom and Moore, 1983), while, on the other hand, gravitropism or geotropism of secondary roots has been observed in a number of species, including Arabidopsis, tea, and soybean (Kiss et al., 2002; Mitchell and Russell, 1971; Mullen and Hangarter, 2003; Yamashita et al., 1997). Additionally, questions remain regarding root gravitropism and how it might be involved in maintenance of GSA. Lateral root columella cells may contain amyloplasts (Kiss et al., 2002; Ransom and Moore, 1983, Yamashita et al., 1997), so, presumably, the gravity sensing apparatus is similar in primary and secondary roots. Given the uncertainty in whether basal roots of common bean are gravitropic, and, if they are, what role this plays in development of root architecture, we decided to test for gravitropic responsiveness in basal roots of various genotypes of *Phaseolus vulgaris*.

Another factor that has been implicated as playing a role in gravitropism is ethylene, which has been studied in roots and shoots of a wide range of species (Clifford et al., 1983; Harrison and Pickard, 1986; Lee et al., 1990; Madlung et al., 1999; Philosoph-Hadas et al., 1996; Wheeler and Salisbury, 1980; Woltering et al., 2005; Zobel, 1973). Yet there remains some controversy about what ethylene does. Ethylene increases primary root gravitropism in maize (Lee et al., 1990), but decreases tomato shoot gravitropism (Madlung et al., 1999). Furthermore, ethylene increases the GSA of rye coleoptiles (Edelmann et al., 2002). Yet, none of these systems requires ethylene for gravitropism. An explanation, therefore, for the diversity of responses is that ethylene acts as a response modulator (Clifford et al., 1983; Harrison and Pickard, 1986; Madlung et al., 1999), with the specific response dependent on the system and experimental conditions.
procedures. In common bean, ethylene has been shown to affect the growth angle of basal roots, specifically as a regulator of plastic responses among phosphorus treatments (Zhang, 2002). All of these observations taken together suggest that ethylene might act as a modulator of gravitropism in the establishment of growth angles of *Phaseolus vulgaris* basal roots and, ultimately, the resulting root architecture. This was studied in the present research by testing for effects of ethylene on responsiveness of basal roots to gravistimulation.

The objective of this research was to verify that common bean basal roots are gravitropic by measuring their reorientation towards previous growth angles upon gravistimulation. Since basal roots were found to be graviresponsive, but did not fully resume their previous growth angle, variation in response to gravistimulation was compared among genotypes and whorls to determine whether this could account for the observed variation in growth angles. Phosphorus affects basal root growth angles of some genotypes (Bonser et al., 1996; Liao et al., 2001), so two phosphorus treatments were included to test for effects of phosphorus nutrition on responsiveness to gravistimulation. Finally, given the previously observed effect of ethylene on basal root growth angle (Zhang, 2002), a study of ethylene effects on the kinetics of graviresponsiveness was conducted. These experiments were conducted to test the hypothesis that genetic variation in basal root graviresponsiveness and its modulation by ethylene are important components of root architecture.
MATERIALS AND METHODS

Plant materials

Seeds of bean genotypes BAT477, DOR364, G2333, G19833, and G19839 were obtained from the CIAT germplasm collection (Cali, Colombia). BAT477, DOR364, and G2333 are of Mesoamerican origin, while G19833 and G19839 are of Andean origin (Singh et al., 1991). These genotypes represent contrasting gene pools, shoot growth habits and basal root growth angles. Under phosphorus deficient conditions, shoot biomass and phosphorus content among these genotypes has been correlated with basal root shallowness (Bonser et al., 1996; Liao et al., 2001; Liao et al., 2004).

Genotype comparisons

Seed of the 5 genotypes listed above were surface sterilized for 3-5 minutes in 95% ethanol, followed after rinsing with purified water by 3-5 minutes in 10% bleach and another purified water rinse. Seed were germinated for 2 days in 0.5 mM CaSO$_4$ at 28°C. Germinated seedlings were transferred to a previously described pouch system (Liao et al., 2001), with the addition of a sheet of plexiglass clipped to the front of each pouch. Nutrient solutions consisted of (in µM) 3000 KNO$_3$, 2000 Ca(NO$_3$)$_2$, 500 MgSO$_4$, 25 KCl, 12.5 H$_3$BO$_3$, 1 MnSO$_4$, 1 ZnSO$_4$, 0.25 CuSO$_4$, 0.25(NH$_4$)$_6$Mo$_7$O$_24$, and 25 Fe-Na-EDTA. In high phosphorus pouches, 1 mM NH$_4$H$_2$PO$_4$ was included, which was substituted by 0.5 mM (NH$_4$)$_2$SO$_4$ in low phosphorus pouches. Containers were covered with aluminum foil to keep roots in the dark and grown at 25 °C. As hypocotyls elongated, a gap was opened in the middle of the cover to provide light and space for shoots. Root tip positions were recorded with marks made on the plexiglass and roots.
were photographed daily. Half of the pouches were tilted 45° just after marking root tip positions on day 2-3. Digital images were opened in Corel Draw (Corel Corporation, Ottawa, Ontario, Canada), Adobe Photoshop (Adobe Systems Incorporated, San Jose, CA, USA) or Image J (developed at the U.S. National Institutes of Health and available on the internet at http://rsb.info.nih.gov/ij/), and angles of basal and tap roots were measured with respect to gravity just before tilting and 12-24 hours later when it appeared that responses had maximized.

Kinetics

A genotype with deep basal roots, DOR364, and one with shallow basal roots, G19833, were observed for kinetics of responsiveness to gravistimulation. Surface sterilized seed were germinated for 2 days in either high or low phosphorus solution described above, and then transferred to pouches suspended in solution of the same composition. Containers were covered as described above. On days 2-5 after transferring to pouches, individual pouches had root tip positions marked and were then tilted 45° in matching nutrient solution with roots under constant fluorescent light of 100 μmol m$^{-2}$ s$^{-1}$ PAR. Photographs were taken automatically for 9 hours using a Nikon Coolpix 880 digital camera. Angles of basal and tap root tips were measured in Image J from images recorded at 0, 10, 20, 30, 40, 60, 90, 120, 150, 180, 210, 240, 300, 360, 420, 480, and 540 minutes after tilting. Lengths were measured from images taken each hour. Time required for roots to reach maximum curvature response to gravistimulation was assessed by visually inspecting kinetics data for each root and selecting the earliest time when curvature was within 10% of the maximum. An average growth rate was
calculated for each root by dividing change in length over the measurement period by time.

**Ethylene effects**

The two genotypes used to study kinetics, DOR364 and G19833, were subjected to an experiment testing the effects of ethylene on responsiveness to gravistimulation. Seed were surface sterilized, germinated, grown, tilted, photographed, and measured as described for kinetics. The only modification was that pouches were placed in air tight plexiglass chambers for gas treatments. Gas treatments included various concentrations of ethylene, the ethylene action inhibitor 1-methylcyclopropene (MCP), and control. In order for MCP to be active at the time of gravistimulation, plants were pretreated for 6 hours with 1 µL L\(^{-1}\) MCP in a dark chamber. Ethylene treated and control plants were pretreated for 6 hours in a dark chamber with no gas added. Plants were tilted at the time treatments were initiated with application of ethylene or 1 µL L\(^{-1}\) MCP into the chambers. Ethylene was injected through a septum, and MCP was added as EthylBloc powder (Floralife, Walterboro, South Carolina, USA), which was activated by injecting 2-3 ml EthylBloc buffer through a septum into the powder. Roots were photographed in these chambers with roots under constant fluorescent light of 100 µmol m\(^{-2}\) s\(^{-1}\) PAR. Control treatments had no MCP or ethylene added to either the pretreatment or the gravistimulation chamber. Data were collected in Image J from digital images taken at the times listed for the kinetics experiment up to 300 minutes.

Ethylene concentrations were determined by sampling 1 ml of air from the chambers and injecting it into a HP6890 gas chromatograph equipped with a flame
ionization detector and an activated alumina column (Hewlett-Packard Company, 2850 Centerville Road, Wilmington, DE 19801-1610, USA). Measured ethylene concentrations in ethylene treated chambers were (in nL/L ± SE) 186 ± 7, 250 ± 16, 441 ± 30, 747 ± 80, 1085 ± 82. Control chambers contained 78 ± 10 nL/L ethylene.

Data analysis

Whorls were numbered from upper to lower, with 1 being the uppermost whorl. In order to compare genotypes, data from the second basal root whorl and lower were pooled. Angle changes of basal and tap roots observed daily were analyzed by a general linear model analysis of variance in the SAS System for Windows, Release 8.02 (SAS Institute, Cary, NC, USA) with genotype, phosphorus, and whorl included as dependent variables. In kinetics and ethylene experiments, growth rates of basal and tap roots were analyzed by GLM ANOVA in SAS. The dependent variables were the same as with daily observations, but with the addition of ethylene in that experiment. Repeated measures ANOVA (SAS) was used to analyze root curvature over time in the experiments measuring kinetics of graviresponsiveness. Homogeneity of curvature variance was tested in the ethylene experiment using Levene’s test for homogeneity of variance in one way ANOVA (SAS) with genotype, phosphorus, whorl, and ethylene each tested separately. Conditions for GLM ANOVA were not met for analysis of ethylene treatment, so the nonparametric Kruskal-Wallis Chi-square test was used to analyze this data set for effects of ethylene on curvature responses to gravistimulation. Pairwise mean separation was conducted where applicable using the least squared means separation procedure. The critical value used for tests of significance was p = 0.05.
RESULTS

*Basal root graviresponse*

Both basal and tap roots of common bean responded to gravistimulation by reorienting growth with respect to gravity within 24 hours after tilting (Figs. 1b, 2a). Tap roots were growing at 0° with respect to gravity before tilting 45°, and returned about three quarters of the way to this angle after gravistimulation (Fig. 2a, b). Basal roots reoriented less than the tap roots (Fig. 2a, b). Untilted basal roots grew at nonzero angles with respect to gravity and curved as they grew. In a gravistimulated pouch, basal roots on one side were tilted up, while those on the other side were tilted down. The basal roots tilted up were stimulated to respond in the same direction as normal curvature, while those tilted down respond in the direction opposite normal curvature. Unsurprisingly, absolute curvature is greater in basal roots tilted up than in those tilted down. To correct for normal curvature, basal root angle changes were normalized by subtracting the mean curvature of untilted basal roots from the observed curvature of each tilted basal root. Each side was normalized independently. When data were normalized, basal roots tilted down still showed less response to gravistimulation than basal roots tilted up, and all basal roots responded less than the tap root (Fig. 2b). This appears to result from increased variation of response rather than a decreased potential response. About one quarter of basal roots tilted down showed little or no response. When those unresponsive roots were excluded, response of basal roots tilted down was similar to the response of basal roots tilted up (not shown). Therefore, in order to reduce variation and to focus on treatment effects, the results presented in the remainder of this report are generated from basal roots tilted up.
Variation among and within genotypes

Tap root response to gravistimulation was similar in all genotypes and phosphorus treatments (Table 1a). Growth angle of untilted basal roots (Fig. 3a) and graviresponsiveness of basal roots, defined as normalized angle change after gravistimulation, (Fig. 3b) varied among genotypes, but there was no correlation between normal growth angle and graviresponsiveness. For basal root graviresponsiveness genotype and phosphorus*whorl were significant factors (Table 1b, Figs. 3, 4). Among genotypic responses, that of DOR364 was significantly greater than those of BAT477 (p = 0.05) or G2333 (p = 0.005). Although the overall genotype*phosphorus effect was not significant, there were a number of differences resolved in pairwise testing, all of them involving DOR364 growing in 1 mM phosphorus or G19839 growing in 0 phosphorus (Fig. 3b).

Within genotypes, there were differences in responses among whorls. With phosphorus treatments pooled, the only significant whorl effect was that in G19839 the response of first whorl basal roots was less than that of lower whorls (p = 0.02). However, with phosphorus treatments separated, other significant within genotype effects of gravistimulation on basal root graviresponsiveness become evident. For example, G19833 basal roots in whorl 2 and below were less graviresponsive in 1 mM phosphorus than in 0 phosphorus (Fig. 4, p = 0.006). Graviresponsiveness of basal roots did not vary among whorls within the genotypes BAT477, DOR364, or G2333 in either phosphorus concentration tested.
Kinetics

Repeated measures analysis of curvature of tap roots over 9 hours subsequent to gravistimulation indicated no significant effect of genotype or phosphorus treatments on graviresponsiveness (Table 2a, Fig. 5a). All combinations of genotype and phosphorus resulted in tap root response that began within 20 minutes (Fig 5a).

Like tap roots, basal roots started to respond within 20 minutes (Fig. 5b, c). There was a significant difference between the genotypes, but not between phosphorus treatments or whorls in repeated measures analysis of graviresponsiveness (Table 2b). Basal roots of DOR364 were less responsive to gravistimulation than those of G19833 over the course of the kinetics observations. This genotypic variation was observed in both whorl 1 (p = 0.0279) and whorl 2 (p = 0.0358) basal roots (Fig. 5b, c). There was no significant variation among whorls within either genotype, but whorl 1 basal roots of DOR364 were less responsive than those of G19833 whorl 2 basal roots (p=0.0069).

There was no significant variation among tap roots in time required to reach maximum response to gravistimulation (Table 3a, Fig. 6a), but for basal roots there was a significant effect of genotype (Table 3b). With whorls and phosphorus treatments pooled, basal roots of DOR364 reached maximum response to gravistimulation in 116 ± 11 minutes, while basal roots of G19833 needed 180 ± 14 minutes to reach maximum response. When 1 mM phosphorus was provided, all whorls of G19833 basal roots required significantly more time to respond to gravistimulation than DOR364 basal roots (Fig. 6b, c). First whorl basal roots of G19833 in 1 mM phosphorus also took significantly longer to respond to gravistimulation than first whorl DOR364 basal roots growing in 0 mM phosphorus.
Growth rates of gravistimulated roots were evaluated during the 9 h period following gravistimulation. There was a significant interaction of genotype with phosphorus treatment, and a significant effect of root type (Table 4). For both DOR364 and G19833 in 1 mM phosphorus, growth rate of first whorl basal roots was significantly lower than those of the tap root, and for G19833, it was also lower than the growth rate of second whorl basal roots and below (Fig 7). There was no genotypic or phosphorus effect on tap root growth rates (Fig 7). Maximum basal root growth rates were similar to the growth of tap roots (Fig 7).

**Ethylene**

As with untreated roots (Fig. 5), ethylene treated roots started to respond to gravistimulation within 20 minutes, but ethylene appeared to suppress curvature responses to gravistimulation over time (Fig. 8). However, the most apparent effect of ethylene was to increase the deviation of curvature responses to gravistimulation (Fig. 9). Curvature responses of control and MCP treated roots had standard deviations of less than 20, with few exceptions, while roots exposed to higher concentrations of ethylene had up to 4-fold larger standard deviations. Therefore, testing homogeneity of variance was more likely to resolve treatment effects than ANOVA. Significant effects of ethylene on homogeneity of variance were observed in 20 minutes for basal roots and 30 minutes for tap roots, and this heterogeneity of variance became more pronounced over time (Table 5). In contrast, neither genotype nor phosphorus had any effect on homogeneity of variance of tap root response at any time point (Table 5). Homogeneity of basal root response variance was affected by genotype and phosphorus in less than half
of the time point observations and much less than by ethylene (Table 5). Whorl had no effect on homogeneity of basal root response variance (Table 5).

Heterogeneity of variance in responses to gravistimulation among ethylene treatments was not corrected by square root or log transformations, so the nonparametric Kruskal-Wallis test was applied in tests for effects of ethylene on mean graviresponsiveness. Ethylene did not significantly affect tap root response to gravistimulation at any time for either phosphorus treatment (Table 6). In contrast, pooled basal root response to gravistimulation was significantly affected within 40 minutes in both 0 and 1 mM phosphorus, and the difference became more significant over time (Table 6). Observations of root curvature indicate that, in general, reorientation in response to gravistimulation decreased with increasing ethylene (Figure 8). However, there were instances when ethylene treatment resulted in basal root response to gravistimulation that was greater than or equal to controls (Fig. 8). MCP did not significantly affect responses to gravistimulation (Fig. 8).

Ethylene did not affect time to reach maximum response to gravistimulation for any root class (Table 7), nor was there any significant interaction of ethylene with any other dependent variable on time to reach maximum response (Table 7). As in the kinetics experiment, there was a significant difference between the two genotypes in time to reach maximum response for basal roots, and this affect was also observed for tap roots in the ethylene experiment (Table 7). There was little correlation between growth rate and time to reach maximum curvature response to gravistimulation (0.0006 <= R2 <= 0.22) or maximum curvature response (0.0045 <= R2 <= 0.20) for any genotype by phosphorus by root type or basal whorl combination.
Growth rate was affected by significant interactions among genotype, phosphorus and ethylene treatments (Table 8). Growth rate was reduced by ethylene treatments, and in some cases increased by MCP treatments (Fig. 10). Tap roots of DOR364 were more significantly affected by ethylene than those of G19833, and first whorl basal roots were less affected by ethylene treatment than lower whorls (Fig. 10). The lowest ethylene treatment, 186 nL L$^{-1}$, reduced growth rate compared to controls of either tap or basal 2 roots in all genotype and phosphorus treatments (Fig. 10). Basal roots from the upper whorl showed significantly reduced growth rate at ethylene concentrations of 441 nL L$^{-1}$ or greater (Fig. 10). Phosphorus had opposite effects on growth rate responses to ethylene between the two genotypes. For DOR364, roots were more significantly affected by ethylene in 1 mM phosphorus than in 0 phosphorus, while for G19833, there was more of an effect in 0 phosphorus (Fig. 10). Ethylene did not affect variance of growth rate as evidenced by the similarity among error bars in Fig. 10.

DISCUSSION

Earlier reports have shown that basal root growth angles vary among genotypes and phosphorus treatments (Bonser et al., 1996; Liao et al., 2001). The present report is the first to use gravistimulation to confirm that basal roots of common bean are gravitropic and to quantify basal root graviresponsiveness. With this validation, there are at least two ways to explain how gravitropism is utilized to generate diversity in basal root growth angles among whorls and genotypes. First, potential graviresponsiveness might differ, which would be observed as variation in curvature in response to gravistimulation. Second, there could be equal ability to respond to gravistimulation, but
differences in downstream modulation of the gravity signal, possibly through ethylene. This could produce variation in growth angles within a root system composed of branches with equivalent potential responsiveness. Given the variation in responsiveness to gravistimulation, as well as the effects of ethylene observed in this study, our results indicate that both modes of gravitropism play a role in establishment of growth angle of common bean basal roots and resultant root architecture.

Tap roots typically grow down, so no differences among genotypes were expected for response of tap roots to gravistimulation. This was confirmed in both the 24 hour observations and in kinetics. Therefore, short term and long term responses do not appear to vary in tap roots among the genotypes tested. The lack of phosphorus effects on tap root responses indicates that tap root gravitropism is not regulated by phosphorus availability.

In contrast to tap roots, basal roots grow at a variety of angles. Observations of curvature responses to gravistimulation indicate that basal root graviresponsiveness varies among genotypes, with phosphorus availability and with whorl position. We did not record responses of other secondary root types (lateral and adventitious) to gravistimulation. Adventitious roots begin to emerge one week after germination, so were not observable in our experiments. The present results are limited to basal roots, which are a genetically distinct class of roots emerging from the root-shoot interface (Zobel, 1975; 1986).

Graviresponsiveness of basal roots has not been previously reported, but there are several descriptions of gravitropism in lateral roots of other species (Kiss et al., 2002; Mitchell and Russell, 1971; Mullen and Hangarter, 2003; Yamashita et al. 1997).
Cursory examination of lateral roots in this project suggests that lateral roots of common bean are also responsive to gravistimulation. This can be seen in the longest lateral root of the gravistimulated root system of genotype DOR364 shown in Fig. 1. Graviresponsiveness was observed in lateral roots of all genotypes in both phosphorus treatments. Initial estimates indicate that lateral roots responded to 45° reorientation by altering their growth angles by about 15-20°, less than the reorientation of basal or tap roots. However, the resolution of the images was too low to accurately quantify lateral responses, or to observe shorter lateral roots for graviresponsiveness. Nevertheless, these observations, along with the detailed measurements of basal root graviresponsiveness indicate that both of these classes of common bean secondary roots are gravitropic. This is in contradiction to previous reports of agravitropic lateral roots in *Phaseolus vulgaris* (Ransom and Moore, 1983). We conclude that basal roots of *Phaseolus vulgaris* are gravitropic, and lateral roots are likely gravitropic, as well.

Variation among genotypes and basal root whorls in responsiveness to gravistimulation suggests that graviresponsiveness plays a role in development of root architecture. If graviresponsiveness is wholly responsible for variations in root growth angle, we would expect to find higher graviresponsiveness in those roots with the deepest growth angles. This reasoning is supported by the observation that the tap root grows parallel to the gravity vector and is more graviresponsive than basal roots. However, several observations falsified this hypothesis. First, there was no clear relationship between basal root graviresponsiveness and growth angle of untilted control roots observed in this study (Fig. 3) or previously reported basal root depth (Bonser et al 1996.; Liao et al., 2001). Second, graviresponsiveness of two genotypes with deep basal roots,
DOR364 and G2333, differed more from each other than from shallow-rooted genotypes. Finally, response to gravistimulation varied among whorls only for G19833 and G19839, and the deeper basal roots of G1833 responded less than those of the uppermost whorl (Fig. 4), which is opposite to the trend predicted by comparing tap roots with basal roots.

The phosphorus effect on G19833 graviresponsiveness (Fig. 4) is in accordance with previous reports of plasticity of G19833 basal root growth angle with varying phosphorus availability (Bonser et al., 1996; Liao et al., 2001). Basal roots of G19839 have also been reported to increase growth angles with respect to gravity as phosphorus availability decreased, but less so than those of G19833 (Liao et al., 2001). The present report indicates that phosphorus effects on graviresponsiveness may account for at least some of the plasticity in G19833 basal root growth angle, but not in G19839. This result, along with the lack of a relationship between growth angle and graviresponsiveness highlighted in the previous paragraph, demonstrates that graviresponsiveness is not sufficient to explain all variation in basal root growth angle, an important component of root architecture.

Variation in time to reach maximum response to gravistimulation illustrates that timing differences between genotypes may be as relevant to understanding basal root gravitropism as measures of curvature alone. Basal roots of DOR364 grow more deeply and respond to gravistimulation more quickly than those of G19833. This correlation suggests a stronger response and possibly more sensitivity to gravistimulation in DOR364 basal roots compared to those of G19833. Strength of response may be derived from simple physical relations. The trajectory of a graviresponding root follows a curve that can be approximated as an arc of a circle. To follow a circular path there must be
radial acceleration. Curving more quickly means more radial acceleration. If growth zone and meristem masses are equal, then DOR364 basal roots respond to gravistimulation with more force than those of G19833. High resolution measurements of elongation zone lengths, diameters and masses would be required to test for differences in strength of response. As for sensitivity, it should be related to presentation time, with roots more sensitive to gravistimulation expected to have shorter presentation times than less sensitive roots. Clinostat experiments would discriminate differences in presentation time, but would require some development for use with common bean. Regardless of what factors determine the duration of graviresponsiveness, it can only be related to differences among genotypes in common bean. It does not explain variation in growth angles among basal root whorls. The diversity of basal root growth angles, therefore, appears to be derived from a complex set of interacting processes, including graviresponsiveness, strength or sensitivity of graviresponsiveness, and modulation of graviresponsiveness.

One potential modulator of gravitropism is ethylene. Effects of ethylene on root and shoot gravitropism have been well documented (Clifford et al., 1983; Harrison and Pickard, 1986; Lee et al., 1990; Madlung et al., 1999; Philosoph-Hadas et al., 1996; Wheeler and Salisbury, 1980; Zobel, 1973). An increase in ethylene production in gravistimulated tissues several hours after tilting has led to the consideration of ethylene as a modulator of gravitropism (Clifford et al., 1983; Kaufman et al., 1985). Further support of a role for ethylene in regulating gravitropism arises from molecular work demonstrating that genes for enzymes involved with ethylene synthesis or ethylene-responsive binding factors are differentially regulated in gravistimulated roots compared
to untilted roots (Kimbrough et al., 2004; Moseyko et al., 2002; Okushima et al., 2005).

In the present report, ethylene affected the mean and variance of basal root reorientation in response to gravistimulation, as well as variance of tap root responses, but the ethylene action inhibitor MCP had no significant effect (Table 6, 7; Fig. 9, 10). These results support the hypothesis that ethylene acts as modulator of the response to gravistimulation, but is not necessary for the response. The role of ethylene appears to be to decrease graviresponsiveness in common bean roots. Decreasing graviresponsiveness may explain how ethylene is involved in the production of more shallow basal roots (Zhang, 2002). In addition, the low correlation between growth rate and reorientation after gravistimulation or time to reach maximum curvature means that ethylene effects on gravitropism are independent of effects on growth rate, which also corroborates previous observations (Madlung et al., 1999). The biological significance of our observations, along with an existing report of ethylene affecting basal root growth angles (Zhang, 2002), make ethylene a candidate signaling molecule for regulation of basal root GSA through modulation of gravitropism.

While observing roots exposed to ethylene and in analyzing curvature data, it became apparent that ethylene effects on variance of response were as significant as effects on mean responses. This is clearly evident from the increased size of boxes and the number of data points in extreme positions when plotted as a box and whisker plot (Fig. 10). In time-lapse images this is observed as root tips oscillating back and forth with increased amplitude as ethylene concentration was increased. Some root tips would eventually oscillate to curvature values well beyond one standard deviation of mean curvature responses. Previous researchers have focused on the effects of ethylene on
mean responses to gravistimulation. Yet, if variance was affected in other studies as it was here, then this may explain why researchers have presented a diversity of sometimes conflicting reports on effects of ethylene on responses to gravistimulation (Clifford et al., 1983; Madlung et al., 1999, Wheeler and Salisbury, 1980; Woltering et al., 2005; Zobel, 1973), such as stimulatory effects (Lee et al., 1990) or inhibitory effects (Madlung et al., 1999). This speculation is reinforced by our observations that variance of responses increased with increasing ethylene, and by noting that ethylene concentrations tested by most other researchers were at the high end of what we tested or greater. In our data, we see instances where ethylene treatment results in mean curvature responses to gravistimulation that are less than, equal to, or greater than controls (Fig. 9). The overall trend is for ethylene to inhibit mean basal root responsiveness to gravistimulation, and to have no effect on tap root mean response. Yet, it is understandable how researchers might come to other conclusions by focusing only on mean responses at certain time points where responses appeared to be unaffected or increased by application of ethylene. Our results suggest that variance should be considered as carefully as mean root responses to gravistimulation. Ethylene effects on variance of response to gravistimulation support the conclusion that ethylene acts as a modulator of root gravitropism, and not a primary signal.

The oscillations in root tip growth angles observed in this research may be related to previous reports of circumnutation or waving in roots (Buer et al., 2000; Johnsson, 1997; Okada and Shimura, 1990; Simmons and Migliaccio, 1995). Circumnutations are small circular motions of roots that may be part of normal root growth through soil that do not necessarily result in any obvious growth trajectory when roots are observed at one
time point. Root waving, in contrast, produces visible waves in root morphology when roots are grown on an inclined surface (Okada and Shimura, 1990). The cause of root waving has not been fully elucidated, but evidence indicates that it involves the interaction of gravitropism with circumnutation (Mullen et al., 1998; Simmons and Migliaccio, 1995) or thigmotropism (Thompson and Holbrook, 2004). Both root waving and circumnutation are affected by ethylene (Buer et al., 2000; 2003; Johnsson, 1997), as were the oscillations observed in this study. However, recent work suggests that root waving requires immobile root tips growing against an inclined medium (Thompson and Holbrook, 2004). Root tips in our pouch system moved considerably, and the surface was perpendicular to gravity, indicating that the oscillations observed are related to circumnutation, but not root waving. Observations over a period of days would be required to test if the oscillations of common bean basal roots affected by ethylene would lead to variation in root morphology consistent with root waving. Ultimately, observation in a 3-dimensional solid medium would clarify any function of the oscillations observed in our pouch system.

Ethylene may potentially modulate common bean basal root gravitropism in perception, signal transduction or response. Given that timing of the initiation of responses was similar among ethylene treatments and that transcription of ethylene responsive elements is not differentially regulated until at least 15 minutes after gravistimulation (Kimbrough et al., 2004), it seems unlikely that ethylene affects gravity perception, or if it does, then there are likely affects downstream as well. Lee et al. (1990) explained ethylene effects in terms of timing and duration of asymmetric auxin transport, which supports a role for ethylene in signaling or response to gravistimulation.
The increased variance of responses of ethylene treated roots in our study indicates that either auxin responses are dynamically regulated or that ethylene affects responses downstream of the establishment of asymmetric auxin distribution. Auxin gradients may shift in proportion to the degree of oscillations observed in this study, or ethylene may play a role in crosstalk with auxin, or modulation of auxin responses. The later possibility seems more likely, but neither potential mechanism has been ruled out.

Based on a review of relevant literature, the following elements are proposed in the pathway from gravity perception to modulation of gravitropism by ethylene. Circumnutation or gravistimulation leads to deviation of root growth angle from the GSA. Deviation in the direction of growth from the GSA is converted to asymmetric production of reactive oxygen species (ROS) (Joo et al., 2001) through phosphatidylinositol 3-kinase (Joo et al., 2005), calcium (Gehring et al., 1990; Pauw et al., 2004; Philosoph-Hadas et al., 1996; Plieth and Trewavas, 2002), and auxin signaling (Joo et al., 2001; Woltering et al., 2005). An auxin responsive factor, such as NPH4 or a homologue, is involved in transduction of the auxin signal (Harper et al., 2000; Stowe-Evans et al., 1998). Asymmetric ROS distribution stimulates production of asymmetric ethylene (Ke and Sun, 2004; Philosoph-Hadas et al., 1996; Watanabe et al., 2001). Asymmetric auxin distribution stimulates a curvature response, and asymmetric ethylene distribution then modulates the root curvature response so that growth in the direction of the GSA is reestablished. Differences in the GSA among root branches may result from variation in ethylene production, or differential expression of ethylene receptors or ethylene responsive elements (Kimbrough et al., 2004; Moseyko et al., 2002; Okushima et al., 2005). These elements could then feed back in cross talk with auxin and ethylene.
production or signal transduction elements at any of a number of common points in their respective pathways. Ethylene may affect asymmetric distribution of auxin or the rate of auxin transport (Schwark and Bopp, 1993; Schwark and Schierle, 1992) through auxin influx or efflux by AUX1 or EIR1 (Rahman et al., 2001), or auxin transport and auxin/ethylene crosstalk at Alh1 (Vandenbussche et al., 2003). On the other hand, another possibility as explained in the preceding paragraph is that ethylene modulates auxin responses without affecting auxin gradients. This could occur through ethylene effects on auxin and ethylene sensitivity at AXR1 (Lincoln et al., 1990), regulation of auxin response factors by NPH4 (Harper et al., 2000), or effects on auxin mediated oscillations of cytosolic free Ca and pH (Felle, 1988). To date, the above listed elements of a signaling pathway regulating the GSA of secondary roots have not been tested. Nor has any other coherent model of regulation of the GSA of secondary roots been adequately presented and verified. Therefore, a whole field of research remains to be addressed. The proposed mechanism outlined above contains elements that can be tested in basal roots of common bean, as well as in secondary roots of other species. For example, ROS can be tested for effects on basal root responsiveness to gravistimulation and growth angles. Also, ethylene may be more precisely measured among root branches to test for a correlation between ethylene production and root growth angle or responsiveness to gravistimulation. Potential roles of other elements outlined above may require use of plants that are more readily manipulated, such as Arabidopsis.

Growth rate differences do not appear to be related to differences in graviresponsiveness observed between genotypes, whorls or phosphorus treatments, because where growth rates vary among whorls or phosphorus treatments,
graviresponsiveness does not. For genotype G19833, basal root growth rate varies with phosphorus treatment and among whorls, but graviresponsiveness does not (Fig. 5, 6). In addition, growth rate did not significantly vary between basal whorl 1 roots of DOR364 and those of G19833 grown in 0 phosphorus, but responsiveness to gravistimulation did (Fig. 5, 6). Most significantly, no correlations were found between growth rate and maximum curvature response to gravistimulation or time to maximum curvature for any genotype, phosphorus treatment or basal whorl (0.00007 < r² < 0.08).

This report is significant for several reasons. First, we demonstrate that basal roots of common bean are gravitropic, and demonstrate that variation in gravitropic setpoint angles may not be fully explained by variation in the ability to respond to gravistimulation. Modulation of gravity responses must occur downstream of perception. Ethylene is implicated in this report as a modulating factor in gravitropism. Methodologically, time to reach maximum curvature and heterogeneity of response variance are as important as mean response in determination of significant effects on graviresponsiveness. Our results are put in context of previous reports to construct a model of regulation of root GSA which can be tested in a range of plant species. In the long run, this work contributes to understanding of root architecture, which may be useful in cultivation of crops that can be manipulated to grow roots in soil strata where limited resources are located. For example, if mechanisms underlying plasticity of G19833 basal root growth angles can be elucidated, then it may be possible to breed genotypes that produce shallow basal roots where phosphorus is limiting, or deep basal roots where water is limiting.
Overall, the results presented in this report demonstrate that basal roots of *P. vulgaris* are graviresponsive. Variation of basal root graviresponsiveness among genotypes and within a root system does not account for all of the variation in basal root growth angles. Differences in duration of responses indicate that sensitivity to gravistimulation or strength of responses is involved in the development of basal root growth angles. Additionally, effects of ethylene on variance of responses suggest that ethylene modulates gravitropism in common bean. The combination of these three processes, graviresponsiveness, sensitivity or strength of graviresponsiveness, and modulation by ethylene, may be important factors in establishment of 3-dimensional root architecture.

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Table 2.1. ANOVA of growth angle measured 1 day after gravistimulation as affected by genotype, phosphorus treatment, or basal root whorl.

a. Tap root

<table>
<thead>
<tr>
<th>Source</th>
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b. Basal roots

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<th>DF</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>4</td>
<td>2.76</td>
<td>0.0297</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>1</td>
<td>1.05</td>
<td>0.3070</td>
</tr>
<tr>
<td>Whorl</td>
<td>1</td>
<td>0.03</td>
<td>0.8598</td>
</tr>
<tr>
<td>Genotype*P</td>
<td>4</td>
<td>0.89</td>
<td>0.4741</td>
</tr>
<tr>
<td>P*whorl</td>
<td>1</td>
<td>3.11</td>
<td>0.0800</td>
</tr>
<tr>
<td>Genotype*whorl</td>
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<td>1.98</td>
<td>0.1002</td>
</tr>
<tr>
<td>Genotype<em>P</em>whorl</td>
<td>4</td>
<td>0.95</td>
<td>0.4368</td>
</tr>
</tbody>
</table>

Table 2.2. Repeated measures ANOVA of growth angle measured in the kinetics experiment as affected by genotype, phosphorus treatment, or basal root whorl.

a. Tap root

<table>
<thead>
<tr>
<th>Effect</th>
<th>DF</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>1</td>
<td>0.89</td>
<td>0.3538</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>1</td>
<td>1.81</td>
<td>0.1892</td>
</tr>
<tr>
<td>Genotype*P</td>
<td>1</td>
<td>1.38</td>
<td>0.2501</td>
</tr>
</tbody>
</table>

b. Basal roots

<table>
<thead>
<tr>
<th>Effect</th>
<th>DF</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>1</td>
<td>4.89</td>
<td>0.0030</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>1</td>
<td>0.34</td>
<td>0.4877</td>
</tr>
<tr>
<td>Whorl</td>
<td>1</td>
<td>1.18</td>
<td>0.5205</td>
</tr>
<tr>
<td>Genotype*P</td>
<td>1</td>
<td>0.75</td>
<td>0.3528</td>
</tr>
<tr>
<td>P*whorl</td>
<td>1</td>
<td>1.72</td>
<td>0.4522</td>
</tr>
<tr>
<td>Genotype*whorl</td>
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<td>3.29</td>
<td>0.5369</td>
</tr>
<tr>
<td>Genotype<em>P</em>whorl</td>
<td>1</td>
<td>7.21</td>
<td>0.9205</td>
</tr>
</tbody>
</table>
Table 2.3. ANOVA of time to reach maximum curvature in response gravistimulation as affected by genotype, phosphorus treatment, or basal root whorl.

a. Tap root

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>1</td>
<td>1.05</td>
<td>0.3150</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>1</td>
<td>0.38</td>
<td>0.5440</td>
</tr>
<tr>
<td>Genotype*P</td>
<td>1</td>
<td>0.35</td>
<td>0.5615</td>
</tr>
</tbody>
</table>

b. Basal roots

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>1</td>
<td>13.96</td>
<td>0.0003</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>1</td>
<td>0.51</td>
<td>0.4767</td>
</tr>
<tr>
<td>Whorl</td>
<td>1</td>
<td>0.99</td>
<td>0.3230</td>
</tr>
<tr>
<td>Genotype*P</td>
<td>1</td>
<td>2.04</td>
<td>0.1564</td>
</tr>
<tr>
<td>P*whorl</td>
<td>1</td>
<td>0.02</td>
<td>0.8844</td>
</tr>
<tr>
<td>Genotype*whorl</td>
<td>1</td>
<td>2.09</td>
<td>0.1514</td>
</tr>
<tr>
<td>Genotype<em>P</em>whorl</td>
<td>1</td>
<td>0.84</td>
<td>0.3607</td>
</tr>
</tbody>
</table>

Table 2.4. ANOVA of growth rates of gravistimulated roots as affected by genotype, phosphorus treatment, or root

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>1</td>
<td>2.41</td>
<td>0.1229</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>1</td>
<td>3.04</td>
<td>0.0836</td>
</tr>
<tr>
<td>Root</td>
<td>2</td>
<td>7.01</td>
<td>0.0013</td>
</tr>
<tr>
<td>Genotype*P</td>
<td>1</td>
<td>8.99</td>
<td>0.0032</td>
</tr>
<tr>
<td>P*root</td>
<td>2</td>
<td>2.58</td>
<td>0.0799</td>
</tr>
<tr>
<td>Genotype*root</td>
<td>2</td>
<td>0.74</td>
<td>0.4783</td>
</tr>
<tr>
<td>Genotype<em>P</em>root</td>
<td>2</td>
<td>1.59</td>
<td>0.2071</td>
</tr>
</tbody>
</table>

1Roots are categorized as tap, basal 1 (uppermost whorl), or basal 2 (all whorls below basal 1)
Table 2.5. Probability levels for homogeneity of curvature variance with respect to 4 independent variables tested for effects on reorientation in response to gravistimulation. Values obtained using Levene’s test for homogeneity of variance.

<table>
<thead>
<tr>
<th>Time</th>
<th>Ethylene Tap</th>
<th>Ethylene Basal</th>
<th>Genotype Tap</th>
<th>Genotype Basal</th>
<th>Phosphorus Tap</th>
<th>Phosphorus Basal</th>
<th>Whorl Tap</th>
<th>Whorl Basal</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.4839</td>
<td>0.0559</td>
<td>0.3507</td>
<td>0.2641</td>
<td>0.2683</td>
<td>0.1735</td>
<td>0.5785</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>0.2043</td>
<td>0.0143</td>
<td>0.6334</td>
<td>0.4881</td>
<td>0.3837</td>
<td>0.0905</td>
<td>0.7928</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>0.0382</td>
<td>0.0035</td>
<td>0.6459</td>
<td>0.6787</td>
<td>0.9579</td>
<td>0.0234</td>
<td>0.6831</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>0.0921</td>
<td>0.0766</td>
<td>0.6415</td>
<td>0.2686</td>
<td>0.9507</td>
<td>0.0673</td>
<td>0.4692</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>0.0401&lt;0.0001</td>
<td>0.4114</td>
<td>0.0535</td>
<td>0.5925</td>
<td>0.9873</td>
<td>0.7106</td>
<td></td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>0.0048&lt;0.0001</td>
<td>0.9507</td>
<td>0.0090</td>
<td>0.8430</td>
<td>0.1055</td>
<td>0.1928</td>
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<td></td>
</tr>
<tr>
<td>120</td>
<td>0.0023&lt;0.0001</td>
<td>0.5046</td>
<td>0.0410</td>
<td>0.5829</td>
<td>0.1780</td>
<td>0.8469</td>
<td></td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>0.0051&lt;0.0001</td>
<td>0.9231</td>
<td>0.2707</td>
<td>0.9065</td>
<td>0.0236</td>
<td>0.8491</td>
<td></td>
<td></td>
</tr>
<tr>
<td>180</td>
<td>0.0102&lt;0.0001</td>
<td>0.7988</td>
<td>0.1494</td>
<td>0.3282</td>
<td>0.0138</td>
<td>0.7344</td>
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<td></td>
</tr>
<tr>
<td>210</td>
<td>0.0168&lt;0.0001</td>
<td>0.8612</td>
<td>0.0723</td>
<td>0.8618</td>
<td>0.0210</td>
<td>0.6598</td>
<td></td>
<td></td>
</tr>
<tr>
<td>240</td>
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<td>0.1182</td>
<td>0.0052</td>
<td>0.9999</td>
<td>0.0389</td>
<td>0.7319</td>
<td></td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>&lt;0.0001&lt;0.0001</td>
<td>0.3405</td>
<td>0.0232</td>
<td>0.7039</td>
<td>0.1470</td>
<td>0.6002</td>
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<td></td>
</tr>
</tbody>
</table>
Table 2.6. Kruskal-Wallis nonparametric test for effect of ethylene on maximum curvature of tap and basal roots in response to gravistimulation. This test is based upon the difference between observed and expected values, assuming that ethylene has no effect on graviresponsiveness. The higher the values of $\chi^2$, the greater the deviation from expected, and the lower the probability that results can be explained by random variation. LP and HP plants were grown with 0 and 1 mM phosphorus, respectively. Seven ethylene treatments were included in all calculations as described in the materials and methods. DF = 6 for all $\chi^2$.

<table>
<thead>
<tr>
<th>Time</th>
<th>LP $\chi^2$</th>
<th>P &gt; $\chi^2$</th>
<th>LP $\chi^2$</th>
<th>P &gt; $\chi^2$</th>
<th>HP $\chi^2$</th>
<th>P &gt; $\chi^2$</th>
<th>HP $\chi^2$</th>
<th>P &gt; $\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0.4230</td>
<td>6.6780</td>
<td>0.3517</td>
<td>2.3353</td>
<td>0.8864</td>
<td>3.1886</td>
<td>0.7848</td>
</tr>
<tr>
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<td>8.8548</td>
<td>0.1819</td>
<td>8.2533</td>
<td>0.2201</td>
<td>4.9544</td>
<td>0.5497</td>
<td>3.4560</td>
<td>0.7498</td>
</tr>
<tr>
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<td>6.6680</td>
<td>0.3526</td>
<td>10.1205</td>
<td>0.1197</td>
<td>7.7799</td>
<td>0.2457</td>
<td>8.1256</td>
<td>0.2290</td>
</tr>
<tr>
<td>40</td>
<td>10.3884</td>
<td>0.1092</td>
<td>9.0317</td>
<td>0.1718</td>
<td>17.1795</td>
<td>0.0086</td>
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</tr>
<tr>
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<td>12.5509</td>
<td>0.0507</td>
<td>7.2119</td>
<td>0.3017</td>
</tr>
<tr>
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<td>3.7594</td>
<td>0.7092</td>
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<td>5.4321</td>
<td>0.4897</td>
<td>3.1977</td>
<td>0.7837</td>
</tr>
<tr>
<td>120</td>
<td>5.6190</td>
<td>0.4672</td>
<td>2.9611</td>
<td>0.8137</td>
<td>18.2192</td>
<td>0.0057</td>
<td>9.1203</td>
<td>0.1669</td>
</tr>
<tr>
<td>150</td>
<td>9.9353</td>
<td>0.1274</td>
<td>5.7599</td>
<td>0.4506</td>
<td>14.3140</td>
<td>0.0263</td>
<td>16.2029</td>
<td>0.0127</td>
</tr>
<tr>
<td>180</td>
<td>3.5342</td>
<td>0.7394</td>
<td>8.5337</td>
<td>0.2015</td>
<td>29.2485</td>
<td>&lt;0.0001</td>
<td>12.3202</td>
<td>0.0552</td>
</tr>
<tr>
<td>210</td>
<td>3.2277</td>
<td>0.7798</td>
<td>7.9074</td>
<td>0.2450</td>
<td>27.3335</td>
<td>0.0001</td>
<td>13.2722</td>
<td>0.0389</td>
</tr>
<tr>
<td>240</td>
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</tr>
<tr>
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<td>0.0013</td>
<td>27.4602</td>
<td>0.0001</td>
</tr>
</tbody>
</table>
Table 2.7. ANOVA of time to reach maximum curvature in response gravistimulation as affected by genotype, phosphorus treatment, ethylene, or basal root whorl.

a. Tap root

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
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<td>9.32</td>
<td>0.0031</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>1</td>
<td>3.09</td>
<td>0.0829</td>
</tr>
<tr>
<td>Ethylene</td>
<td>6</td>
<td>0.34</td>
<td>0.9128</td>
</tr>
<tr>
<td>P*ethylene</td>
<td>6</td>
<td>0.44</td>
<td>0.8526</td>
</tr>
<tr>
<td>Genotype*P</td>
<td>1</td>
<td>0.07</td>
<td>0.7943</td>
</tr>
<tr>
<td>Genotype*ethylene</td>
<td>6</td>
<td>0.10</td>
<td>0.9964</td>
</tr>
<tr>
<td>Genotype<em>P</em>ethylene</td>
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<td>0.53</td>
<td>0.7808</td>
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</table>

b. Basal roots

<table>
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<tr>
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<th>DF</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
<tr>
<td>Phosphorus</td>
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<td>0.8893</td>
</tr>
<tr>
<td>Ethylene</td>
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<td>0.3173</td>
</tr>
<tr>
<td>Whorl</td>
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<td>3.68</td>
<td>0.0560</td>
</tr>
<tr>
<td>P*ethylene</td>
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<td>0.20</td>
<td>0.9752</td>
</tr>
<tr>
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<td>0.00</td>
<td>0.9638</td>
</tr>
<tr>
<td>P*whorl</td>
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<td>0.8271</td>
</tr>
<tr>
<td>Genotype*ethylene</td>
<td>6</td>
<td>0.72</td>
<td>0.6335</td>
</tr>
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<td>Ethylene*whorl</td>
<td>6</td>
<td>0.42</td>
<td>0.8674</td>
</tr>
<tr>
<td>Genotype*whorl</td>
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<td>0.23</td>
<td>0.6350</td>
</tr>
<tr>
<td>Genotype<em>P</em>ethylene</td>
<td>6</td>
<td>0.49</td>
<td>0.8156</td>
</tr>
<tr>
<td>P<em>ethylene</em>whorl</td>
<td>6</td>
<td>1.70</td>
<td>0.1215</td>
</tr>
<tr>
<td>Genotype<em>P</em>whorl</td>
<td>1</td>
<td>3.56</td>
<td>0.0602</td>
</tr>
<tr>
<td>Genotype<em>ethylene</em>whorl</td>
<td>6</td>
<td>0.80</td>
<td>0.5719</td>
</tr>
<tr>
<td>Genotype<em>P</em>ethylene*whorl</td>
<td>6</td>
<td>0.86</td>
<td>0.5235</td>
</tr>
</tbody>
</table>
Table 2.8. ANOVA of growth rates of gravistimulated roots as affected by genotype, phosphorus treatment, ethylene, or basal root whorl

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
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<td>28.45</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Phosphorus</td>
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<td>8.40</td>
<td>0.0040</td>
</tr>
<tr>
<td>Ethylene</td>
<td>6</td>
<td>17.30</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Root&lt;sup&gt;1&lt;/sup&gt;</td>
<td>2</td>
<td>48.35</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>P*ethylene</td>
<td>6</td>
<td>2.24</td>
<td>0.0391</td>
</tr>
<tr>
<td>Genotype*P</td>
<td>1</td>
<td>5.08</td>
<td>0.0248</td>
</tr>
<tr>
<td>P*root</td>
<td>2</td>
<td>0.00</td>
<td>0.9989</td>
</tr>
<tr>
<td>Genotype*ethylene</td>
<td>6</td>
<td>1.98</td>
<td>0.0678</td>
</tr>
<tr>
<td>Ethylene*root</td>
<td>12</td>
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<td>0.1175</td>
</tr>
<tr>
<td>Genotype*root</td>
<td>2</td>
<td>1.08</td>
<td>0.3414</td>
</tr>
<tr>
<td>Genotype<em>P</em>ethylene</td>
<td>6</td>
<td>2.20</td>
<td>0.0421</td>
</tr>
<tr>
<td>P<em>ethylene</em>root</td>
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<td>1.15</td>
<td>0.3172</td>
</tr>
<tr>
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<td>2</td>
<td>0.13</td>
<td>0.8804</td>
</tr>
<tr>
<td>Genotype<em>ethylene</em>root</td>
<td>12</td>
<td>0.64</td>
<td>0.8042</td>
</tr>
<tr>
<td>Genotype<em>P</em>ethylene*root</td>
<td>12</td>
<td>0.64</td>
<td>0.8113</td>
</tr>
</tbody>
</table>

<sup>1</sup>Rosots are categorized as tap, basal 1 (uppermost whorl), or basal 2 (all whorls below to basal 1)
Figure 2.1. *Phaseolus vulgaris* root systems, (a) untilted DOR364 root system illustrating the tap root and 2 whorls of basal roots (basal 1 and basal 2), (b) DOR364 root showing response of tap and basal roots to gravistimulation.
Figure 2.2. Maximum curvature of tap and basal roots after 45° gravistimulation in 0 (LP) or 1 (HP) mM phosphorus, (a) change in angle of untilted (control) roots and roots tilted up or down 45°, (b) tilted root responses normalized by subtracting mean untilted root curvature from mean tilted root curvature. Basal roots growing on each side of the pouch and the tap root were normalized independently. Values represent the mean of 31-37 tap roots and 70-108 basal roots pooled over 5 genotypes. Error bars represent the standard error of the mean.
Figure 2.3. Genotypic variation and phosphorus effects on (a) growth angle of untilted roots, and (b) curvature of basal roots gravistimulated 45° in 0 (LP) or 1 (HP) mM phosphorus. Bars with the same letter above do not significantly vary at the p = 0.05 level. Values represent the mean of 12-23 basal roots from all whorls. Error bars represent the standard error of the mean.
Figure 2.4. Curvature response to 45° gravistimulation for basal roots divided by whorl for the common bean genotypes (a) BAT477, (b) DOR364, (c) G19833, (d) G19839, and (e) G2333 grown in 0 (LP) or 1 (HP) mM phosphorus. Basal whorl designations represent the upper (1) or lower (2) position of root origin as described in materials and methods. Bars within a graph with the same letter above do not significantly vary at the p = 0.05 level. Values represent the mean of 5-10 roots for basal whorl 1 and 7-16 for basal whorl 2. Error bars represent the standard error of the mean.
Figure 2.5. Curvature response to 45° gravistimulation for (a) tap root, (b) top basal whorl, and (c) pooled basal roots from second basal whorl and lower from 2 common bean genotypes grown in 0 (LP) or 1 (HP) mM phosphorus. Curvature was measured at 0, 10, 20, 30, 40, 60, 90, 120, 150, 180, 210, 240, 300, 360, 420, 480, and 540 minutes after gravistimulation. Values represent the mean of 6-9 roots for tap and basal whorl 1, and 10-20 roots for basal whorl 2.
Figure 2.6. Time required for gravistimulated basal roots to attain maximum curvature response for (a) DOR364 and (b) G19833 tap and basal roots in 0 (LP) or 1 (HP) mM phosphorus. Bars within a graph with the same letter above do not significantly vary at the $p = 0.05$ level. Values represent the mean of 5-11 roots for tap and basal whorl 1, and 17-22 for basal whorl 2. Error bars represent the standard error of the mean.
Figure 2.7. Growth rate in 0 (LP) or 1 (HP) mM phosphorus for each root type and basal whorl for common bean genotypes (a) DOR364 and (b) G19833 during observations for kinetics of responsiveness to gravistimulation. Bars within a graph with the same letter above do not significantly vary at the p = 0.05 level. Values represent the mean of 6-9 roots for tap and basal whorl 1, and 10-20 for basal whorl 2. Error bars represent the standard error of the mean.
Figure 2.8. Curvature response to 45° gravistimulation over time as affected by ethylene for pooled basal roots of (a) DOR364 in 1 mM phosphorus (HP), (b) DOR364 in 0 mM phosphorus (LP), (c) G19833 in HP, (d) G19833 in LP. Curvature was measured at 0, 10, 20, 30, 40, 60, 90, 120, 150, 180, 210, 240, 300, 360, 420, 480, and 540 minutes after gravistimulation. Data for control roots are connected with a solid line, and data for MCP treated roots are connected by a dashed line. Each point represents the mean angle of 7-26 roots.
Figure 2.9. Maximum curvature of gravistimulated roots to ethylene treatment. The ethylene concentration in control treatments (no ethylene or MCP added) was 78 nL L$^{-1}$. Plots show median as a horizontal line, 25$^{\text{th}}$ and 75$^{\text{th}}$ percentile within boxes, 10$^{\text{th}}$ and 90$^{\text{th}}$ percentiles within bars, and observations $< 10^{\text{th}}$ percentile or $> 90^{\text{th}}$ percentile marked as points outside the range of the bars. Indentations in boxes represent 95% confidence intervals. Values represent data from 12-29 roots for tap and basal whorl 1, and 27-57 roots for basal whorl 2.
Figure 2.10. Growth rate of tap and basal roots exposed to various ethylene treatments for (a) DOR364 growing in 1 mM phosphorus (HP), (b) DOR364 in growing 0 mM phosphorus (LP), (c) G19833 in HP, (d) G19833 in LP. Ethylene treatments leading to roots growing at different rates than in 78 ppb controls of the same root type are marked in rows below the curves for tap roots (T), first whorl basal roots (B1), and pooled basal roots from second and lower whorls (B2). Values represent the mean of 3-10 roots for tap and basal whorl 1, and 3-20 roots for basal whorl 2. Error bars represent the standard error of the mean.

* p <= 0.05, ** p < 0.005, *** p < 0.0005
CHAPTER 3

ARCHITECTURAL TRADEOFFS BETWEEN ADVENTITIOUS AND BASAL ROOTS FOR PHOSPHORUS ACQUISITION

ABSTRACT

Adventitious rooting contributes to efficient phosphorus acquisition by enhancing topsoil foraging. However, metabolic investment in adventitious roots may retard the development of other root classes such as basal roots, which are also important for phosphorus acquisition. In this study we quantitatively assessed the potential effects of adventitious rooting on basal root growth and whole plant phosphorus acquisition in young bean plants. The geometric simulation model SimRoot was used to dynamically model root systems with varying architecture and C availability growing for 21 days at 3 planting depths in 3 soil types with contrasting nutrient mobility. Simulated root architectures, tradeoffs between adventitious and basal root growth, and phosphorus acquisition were validated with empirical measurements. Phosphorus acquisition and phosphorus acquisition efficiency (defined as mol phosphorus acquired per mol C allocated to roots) were estimated for plants growing in soil in which phosphorus availability was uniform with depth or was greatest in the topsoil, as occurs in most natural soils. Phosphorus acquisition and acquisition efficiency increased with increasing allocation to adventitious roots in stratified soil, due to increased phosphorus depletion of surface soil. In uniform soil, increased adventitious rooting decreased phosphorus acquisition by reducing the growth of lateral roots arising from the tap root and basal
roots. The benefit of adventitious roots for phosphorus acquisition was dependent on the specific respiration rate of adventitious roots as well as on whether overall C allocation to root growth was increased, as occurs in plants under phosphorus stress, or was lower, as observed in unstressed plants. In stratified soil, adventitious rooting reduced the growth of tap and basal lateral roots, yet phosphorus acquisition increased by up to 10% when total C allocation to roots was high and adventitious root respiration was similar to that in basal roots. With C allocation to roots decreased by 38%, adventitious roots still increased phosphorus acquisition by 5%. Allocation to adventitious roots enhanced phosphorus acquisition and efficiency as long as the specific respiration of adventitious roots was similar to that of basal roots and less than twice that of tap roots. When adventitious roots were assigned greater specific respiration rates, increased adventitious rooting reduced phosphorus acquisition and efficiency by diverting carbohydrate from other root types. Varying the phosphorus diffusion coefficient to reflect varying mobilities in different soil types had little effect on the value of adventitious rooting for phosphorus acquisition. Adventitious roots benefited plants regardless of basal root growth angle. Seed planting depth only affected phosphorus uptake and efficiency when seed was planted below the high phosphorus surface stratum. Our results confirm the importance of root respiration in nutrient foraging strategies, and demonstrate functional tradeoffs among distinct components of the root system. These results will be useful in developing ideotypes for more nutrient efficient crops.
INTRODUCTION

Phosphorus is a primary constraint to productivity over much of the earth (Lynch and Deikman 1998; Vance et al., 2003). Large areas of tropical and subtropical soils in Africa, Latin America, and Asia have phosphorus availability limited by high phosphorus fixation (Sanchez and Uehara, 1980). Fertilization may not be economically feasible in such soils, and is rarely employed in much of sub-Saharan Africa (CGIAR, 1996; World Bank, 2004). The development of crops with greater productivity at low phosphorus availability (“phosphorus efficiency”) would be of considerable value in improving food security in these regions (Lynch, 1998; Vance, 2001).

Common bean is the most important food legume on earth and is an important source of nutrients in Latin America and eastern and southern Africa (Voyste1, 2001; Wortmann et al., 1998). Low phosphorus availability is a primary constraint to bean production in developing countries (Voyste1, 2001; Wortmann et al., 1998). Substantial genetic variation for phosphorus efficiency exists in bean germplasm (Beebe et al., 1997; Gabelman and Gerloff, 1978; Gerloff and Gabelman, 1983; Yan et al., 1995a; Yan et al., 1995b), which appears to be caused primarily by genetic differences in root architecture and morphology (Lynch and Beebe, 1995; Lynch and Brown, 2001).

The root system of common bean is composed of three main types of axes (Fig. 1). The tap root is positively gravitropic. Basal roots emerge at the root-shoot interface and grow with dynamic plagiogravitropism at angles from nearly horizontal to vertically down. Variation of basal root growth angles has been observed within and among genotypes (Bonser et al., 1996). Finally, adventitious roots emerge from the hypocotyl
below the soil surface and grow primarily near the surface. Each of these root types can produce 2 orders of lateral roots during vegetative growth.

In most natural and agricultural soils, phosphorus availability is greatest near the surface and decreases with depth (Huang, 2000). For this reason, root architectural traits that enhance topsoil foraging can improve phosphorus acquisition (Lynch and Brown, 2001). One such trait is the basal root growth angle, which determines the extent of topsoil foraging by the basal roots, an important root class that forms the structural scaffold upon which much of the root system develops (Bonser et al., 1996; Ge et al., 2000; Liao et al., 2001).

Efficiency of phosphorus acquisition is correlated with shallowness of basal roots (Bonser et al., 1996; Liao et al., 2001), which enhances root proliferation in phosphorus-rich surface soil, and reduces competition for phosphorus among roots of the same plant (Ge et al., 2000), but increases competition for phosphorus among roots of neighboring plants (Rubio et al., 2001; Rubio et al., 2003a). Adventitious rooting has also been associated with phosphorus efficiency, with efficient genotypes allocating more resources to adventitious root production than inefficient genotypes, particularly in phosphorus-limited conditions (Miller et al., 2003). Adventitious roots enhance phosphorus acquisition because they have shallow growth angles and also because they explore soil at less metabolic cost per unit length than other root types (Miller et al., 2003).

Root architecture is demonstrably important for phosphorus efficiency, but it is only one member of a complex set of chemical and biological processes that contribute to phosphorus acquisition (Barber 1995; Vance et al, 2003; Tinker and Nye, 2000). A brief
statement of general principles includes the following points. The source of phosphorus is minerals from which phosphate must be solubilized by weathering or biological activity. As soil evolves, phosphorus is divided among inorganic, organic, dissolved and biological fractions. Plants acquire phosphorus directly from the dissolved pool or indirectly through mycorrhizal associations. However, the dissolved pool of phosphorus is a minor component of the total quantity in the soil. Therefore, phosphorus availability may be increased by plant secretion of organic acids or phosphate solubilization by bacteria. Once available, phosphorus acquisition is affected by soil moisture, root surface area, root hair density and length, contact of root or mycorrhizal surfaces with the soil solution, and kinetics of uptake across root surfaces. Solubilization, uptake by plants, and return of phosphorus to the soil through litter make phosphorus availability stratified over time, with concentrations increasing towards the soil surface. Among plant species, a variety of strategies have evolved to improve phosphorus efficiency by increasing dissolved phosphorus, increasing the volume of soil explored, or by proliferating roots in phosphorus rich soil. As mentioned above, exploration of topsoil through shallow basal roots or adventitious roots are examples of strategies that have been documented in common bean (Bonser et al., 1996; Liao et al., 2001; Miller et al., 2003) and maize (Zhu et al., 2005). In general, the metabolic and ecological costs and tradeoffs associated with plant traits associated with phosphorus acquisition are poorly understood (Lynch and Ho 2005).

As phosphorus availability decreases, the proportion of carbon partitioned to root growth and respiration increases (Nielsen et al., 1998a; Nielsen et al., 2001). Phosphorus efficient genotypes can maintain higher growth rates by allocating less C to root
respiration than inefficient genotypes under low phosphorus conditions (Nielsen et al., 2001). Furthermore, respiration varies among root types. In relative terms, tap root respiration is low, basal root respiration is intermediate, and adventitious root respiration is high (Ho et al., 2003). Adventitious root respiration ranges from approximately 1-4 times greater than basal root respiration. In experiments with several phosphorus-efficient and phosphorus-inefficient genotypes, respiration in adventitious roots of phosphorus-inefficient genotypes is at the high end of this range (Ho et al., 2003).

Since basal root shallowness and adventitious rooting vary substantially among genotypes and are under distinct genetic control (Liao et al, 2004; Ochoa, personal communication) the interaction of these two traits may be important for whole plant phosphorus acquisition. Interactions could be positive or negative. Increased resource allocation to adventitious roots could reduce the growth of basal roots. Since adventitious and basal roots have distinct patterns of deployment in time and space (adventitious roots appear later than basal roots in seedling development), they may be complementary or competitive in exploiting soil resources. In this regard, it is possible that shallow basal roots are competitive with adventitious roots, while deep basal roots are complementary with adventitious roots. A better understanding of the interaction of these traits is needed in order to deploy them in plant breeding programs for more nutrient-efficient crop cultivars.

A robust evaluation of the value of adventitious rooting for phosphorus acquisition must therefore include variation in the respiratory requirements of distinct root classes as well as the precise spatial and temporal patterns of root deployment. To this end we employed the geometric simulation model SimRoot (Lynch et al., 1997).
SimRoot was developed with empirical data on the growth, architecture, and physiology of bean roots as well as roots of other species, and has been used to address a range of topics, including carbon allocation, phosphorus acquisition, intraplant and interplant root competition, morphological synergism of root hair traits for phosphorus acquisition, and fractal analysis of root systems, that have been verified with greenhouse and field data (Ge et al., 2000; Ma et al., 2001; Nielsen et al., 1994; Nielsen et al., 1997; Rubio et al., 2001; Walk et al., 2004).

The general hypothesis we tested is that adventitious roots increase phosphorus acquisition and acquisition efficiency of plants growing in soils with stratified phosphorus availability. Related hypotheses and corollaries are: 1) Adventitious roots will functionally complement basal root architecture, i.e., plants with deep basal roots will benefit from adventitious roots more than plants with shallow basal roots. 2) Competition among adventitious roots for phosphorus acquisition will depend on the length of subterranean hypocotyl, as affected by e.g. planting depth. 3) The effects of adventitious rooting on basal root development and whole plant phosphorus acquisition will be influenced by the relative respiratory costs of the respective root classes, e.g. if respiration rates in adventitious roots are high, then less carbon will be available for root proliferation and, therefore, phosphorus acquisition and efficiency could be reduced. 4) The benefit of adventitious roots for phosphorus acquisition will depend on the phosphorus mobility in the soil, i.e. with increased phosphorus mobility, phosphorus competition among roots increases, so the effect of adventitious roots on acquisition and efficiency decreases.
MATERIALS AND METHODS

Empirical estimation of root growth parameters

Two phosphorus-efficient common bean (*Phaseolus vulgaris* L.) genotypes, G2333 and G19839, which are landraces in the CIAT *Phaseolus* germplasm collection (CIAT, Cali, Colombia), were selected for estimation of parameters to guide simulations. These genotypes contrast in basal root and adventitious root responses to changes in phosphorus availability. G2333 is a landrace from the mesoAmerican gene pool, G19839 is a landrace from the Andean gene pool. With decreasing phosphorus availability, G19839 basal root systems become more shallow (Bonser et al., 1996; Liao et al., 2001), while G2333 maintains allocation to adventitious root mass and length (Miller et al., 2003).

Seed was germinated for 2 days in 0.5 mM CaSO\(_4\) and planted into 20 l pots containing 50% sand, 50% vermiculite and 1% (w/w) phosphorus provided by buffered alumina (Lynch et al., 1990). Plants were grown for 3 weeks in January and February, 2003 in a climate-controlled greenhouse at Penn State University (40°49’ N, 77°49’ W), which allowed for 2 weeks of adventitious root proliferation. Natural and artificial light were combined to provide 14 hour photoperiods with a maximum of 1200 µmol photons m\(^{-2}\) s\(^{-1}\) photosynthetically active radiation. Temperature was maintained at 27°C/22°C (day/night). Two phosphorus treatments were included, low phosphorus had alumina buffering at 1 µM phosphorus, and high phosphorus had alumina buffering at 278 µM phosphorus. Nutrients were supplied in a fertigation system that provided an average of 118 ml of solution per pot twice a day. Nutrient solutions consisted of (in µM) 3000
KNO$_3$, 2000 Ca(NO$_3$)$_2$, 500 MgSO$_4$, 25 KCl, 12.5 H$_3$BO$_3$, 1 MnSO$_4$, 1 ZnSO$_4$, 0.25 CuSO$_4$, 0.25(NH$_4$)$_6$Mo$_7$O$_{24}$, and 25 Fe-Na-EDTA.

At 7, 14, and 21 days after planting, or 0 to 2 weeks after initial adventitious root emergence, 3 plants of each genotype in each phosphorus treatment were harvested. Shoots were cut off at the soil surface, dried and weighed. Roots were stored in 25% ethanol until analysis. Roots were divided into adventitious, basal and tap root portions. Adventitious and basal roots were counted. Lateral roots were cut off of each main root axis, and, if present, second order laterals were also cut off from primary laterals. Second order laterals were observed only on tap and basal lateral roots over the course of data collection. This led to a total of 8 root types being collected, namely: tap, tap lateral, 2$^{nd}$ order tap lateral, basal, basal lateral, 2$^{nd}$ order basal lateral, adventitious, and adventitious lateral. Each root type was stained with 0.2% neutral red (Phenazine, 3-amino-7-(dimethylamino)-2-methyl-, hydrochloride) in water, scanned and analyzed for length and diameter in WinRhizo (Regent Instruments Inc, Quebec, Canada). Each root type was then dried and weighed. Length, diameter and dry weight data were used for development and verification of simulations.

Specific respiration rates were estimated based on specific respiration values presented by Bouma et al. (1997) and Ho et al. (2003). Bouma reported whole root specific respiration ranging from 30-50 nmol CO$_2$/g/s. Ho et al. measured specific respiration of tap roots in the range of 33-83 nmol O$_2$/g/s. For the current simulations, a tap root respiration rate of 50 nmol CO$_2$/g/s was selected. Basal root respiration rates measured by Ho et al. were 1-2 times greater than respiration rates of tap roots. An intermediate rate of 1.5 times tap respiration, 75 nmol CO$_2$/g/s was selected for basal root
respiration in these models. In order to test for effects on phosphorus acquisition and efficiency, 3 values of adventitious root respiration were simulated. These were 75, 100, and 200 nmol CO$_2$/g/s, which correspond to the equivalent of basal root respiration rate, double tap root respiration rate, and 4 times tap root respiration rate. These rates for adventitious root respiration, relative to tap and basal root respiration, cover the range measured by Ho et al. within individual genotypes, with the high rate of adventitious root respiration being measured for a phosphorus inefficient genotype under phosphorus stress.

Root masses were converted to carbon, assuming dry mass is 40% C (Broadley et al., 2004; Chaves et al., 2004). Since respiration is a flux, C allocated to mass was considered in terms of change in mass per unit time, which was expressed as µmol C/hr. Respiration for each root type was estimated based on specific respiration rates derived as described above. Root exudation was neglected, so C allocation to roots was assumed to be the sum of C allocated to mass and C used in respiration. Carbon allocation was estimated for plants harvested weekly for 3 weeks. To account for differences in photosynthate availability between high- and low-phosphorus plants, 2 functions of C allocation to roots over time were generated from greenhouse data for biomass accumulation, one for high C allocation represented by plants growing in high phosphorus, and one for low C allocation represented by plants growing in low phosphorus. Plots were fit with sigmoidal curves generated in SigmaPlot 2000 (SPSS Inc, Chicago, IL, USA). The equations generated were:

\[
C = \frac{342.6991}{1 + \exp(0-(t-490.6776)/84.7049]}, \quad (1a)
\]

\[
C = \frac{112.0183}{1 + \exp(0-(t-388.5365)/79.0913)}, \quad (1b)
\]
Where $C$ is hourly carbon allocation to roots in $\mu$mol, and $t$ is time from the onset of seed germination in hours. Equation (1a) represents high $C$ allocation to roots as observed in high-phosphorus plants, and eqn (1b) represents low $C$ allocation to roots, as observed in low-phosphorus plants.

*Description of root model and input*

The dynamic geometric model *SimRoot* (Lynch et al., 1997) was used to simulate root growth and architecture. Parts of the program were modified to make root growth a function of $C$ allocation. Equations (1a) and (1b) were introduced into SimRoot to make hourly growth and respiration a function of $C$ available in either high or low $C$ allocation conditions. Hourly respiration was calculated for each root segment based on the mass of the segment and the specific respiration of that root type. Carbon used for respiration was subtracted from the pool of available $C$, and the remaining $C$ was used for growth. Each root axis was allocated $C$ for growth based on relative sink strength approximated as the cross sectional area 3 mm from the tip. Cross sectional area was calculated from radius measurements of roots harvested and analyzed in WinRhizo as described above. The use of cross sectional area as an approximation of sink strength is similar to the use of apical diameter which has been correlated with growth rate of roots in greenhouse and field settings (Cahn et al., 1989; Lecompte et al., 2001; Thaler and Pages, 1996), and has been used as a component of simulated sink strength (Thaler and Pages, 1998). In the present simulations, relating sink strength to the cross sectional area 3 mm from the apex led to fairly realistic simulations of bean roots. However, we have observed that in this species lateral roots do not elongate indefinitely, so cross sectional area was multiplied by
an elongation factor. Elongation factors were read from input for each 12 hour period subsequent to emergence for each branch of the root system. This elongation factor was constant for tap, basal, and adventitious primary axes at all times, but was reduced over time for lateral roots to simulate cessation of lateral root elongation. Tap and basal lateral growth rates were nonzero for 288 and 180 hours, respectively. Adventitious lateral and 2nd order tap and basal lateral growth rates were nonzero for 72 hours.

An illustration of simulated roots with 0, 10 or 30 adventitious roots on plants with deep or shallow basal roots is shown Figure 1. The accuracy of simulated root growth was verified by comparing lengths and masses of each root type from 2 and 3 week old plants with simulated roots. As shown in Figure 2, there was good agreement between root type length for 3 week old bean roots and simulated roots containing 10 or 20 adventitious roots at high C and low C allocation. The agreement between 2 week old bean roots and simulated roots containing 10 or 20 adventitious roots was similar to that observed at 3 weeks.

With SimRoot development completed, simulations were run with adventitious root number, adventitious root respiration, basal root gravitropism, planting depth, and carbon allocation to roots varied as described in Table 1. Basal roots emerged from the base of the hypocotyl at the seed planting depth. Each root system was modeled in three soil types that varied in diffusion coefficient ($D_e$) as listed in Table 1. Three pseudoreplicates were generated for each simulated root and soil type by differential seeding of the random number generator that influences root growth angles and branching angles.
To test if the effect of adventitious roots on phosphorus acquisition and efficiency changes over time, several models were run to 4 weeks after germination. Carbon allocation for 4 weeks was extrapolated from curves made for 3 weeks. Adventitious respiration was 75 or 100 nmol/g/s. All 3 basal root growth angles were included and pooled, planting depth was 5 cm, and diffusion coefficient run in the tests was 10^{-8} \text{ cm}^2 \text{ s}^{-1}.

A number of assumptions were made to simplify the model, i.e.: 1) All adventitious primary axes were equivalent in terms of diameter and growth rate, as were all adventitious laterals. 2) Changes in allocation to adventitious roots were reflected in varying numbers of adventitious roots, not in changes of growth rate of individual adventitious roots. 3) Adventitious roots emerge from day 7-14 with uniform timing between emergence of successive adventitious roots determined by dividing 168 by the number of adventitious roots. 4) All root surfaces have equivalent phosphorus acquisition capacity. 5) Phosphorus uptake was simulated by multiplying depletion volumes by empirically derived phosphorus supply capacities as described by Ge et al. (2000). 6) Specific respiration was constant within a root type. 7) Neither age, time of day, or activity affected respiration. 8) Lateral roots respired at a rate equivalent to their parent root. 9) Decreases of respiration resulting from aerenchyma formation were not considered. 10) Soil temperature regime is isohyperthermic, typical for many tropical soils where common bean is grown.

*Soil phosphorus depletion volumes, phosphorus acquisition and efficiency*

Total phosphorus depletion volume with overlap (V_t) was calculated by *SimRoot*. The phosphorus depletion volume calculation has been published (Ge et al. 2000). It is
the sum of phosphorus depletion over all root segments, which are individually calculated as cylindrical volume:

\[
V = \pi R_{dz}^2 l, \quad (2)
\]

where \( l \) is the segment length and \( R_{dz} \) is the depletion radius. The depletion radius is calculated by using the equation:

\[
R_{dz} = r + 2(D_e t)^{1/2}, \quad (3)
\]

where \( r \) is the root radius, \( t \) is time in seconds, and \( D_e \) is the diffusion coefficient in \( \text{cm}^2/\text{s} \).

Output from Simroot was analyzed in DepZone to determine actual depletion volume \( (V_a) \) by subtracting duplications of overlapped volume as previously described (Ge et al., 2000; Rubio et al., 2001). Competition volume is defined as:

\[
C_v = V_t - V_a, \quad (4)
\]

and relative competition is:

\[
C_r = 100(V_t - V_a)/V_a, \quad (5)
\]

Simulated phosphorus acquisition was calculated by multiplying depletion volume by empirically determined factors to account for phosphorus concentration and soil buffering of phosphorus as previously reported (Ge et al., 2000; Rubio et al., 2001). Simulated soil was either uniform or stratified. Uniform soil contained 10 \( \mu \text{M} \) available phosphorus throughout the soil volume. Stratified soil contained 30 \( \mu \text{M} \) phosphorus in the top 5 cm, 10 \( \mu \text{M} \) phosphorus in the next 5 cm, and 2 \( \mu \text{M} \) phosphorus below 10 cm depth. Phosphorus efficiency was defined as moles of phosphorus acquired per mol of C allocated to roots.
Data analysis

Simulation data was analyzed in StatView, Version 5.0.1 (SAS Institute Inc, Cary, NC, USA). Analysis of variance was conducted using phosphorus depletion volume, competition volume, phosphorus acquisition, and phosphorus efficiency as dependent variables, with adventitious number and the parameter being tested as independent variables. For example, when basal root angle was being tested, adventitious number and basal root angle were independent variables. Mean separation was performed using the Fisher’s Protected LSD post-hoc test.

RESULTS

The numerically iterative nature of SimRoot resulted in variation in C allocation to roots among simulations. Allocation over 504 hours was 21030.1 ± 21.7 µmol C in low C simulations and 33845.9 ± 37.8 µmol C in high C simulations. Total C allocation to roots over 504 hours averaged 38% less in low C simulations than in high C simulations.

Empirical measurements and simulations showed that increased allocation to adventitious roots was associated with decreased allocation to tap and basal roots (Fig. 3a, b). The decrease in tap and basal root lengths was greater than the increase in adventitious root length, so overall root length decreased with increasing allocation to adventitious roots (Fig. 3c). This decrease in overall root length is largely attributable to the effects of adventitious root production on lateral roots more than primary axes of either tap or basal roots (Fig. 4). Secondary lateral roots lost a higher percentage of length than primary laterals. So, adventitious primary axes were produced mainly at the expense of thinner roots in deeper soil.
Soil exploration was affected in several ways by increased allocation to adventitious roots. With greater adventitious rooting, more root production in shallow soil led to increased phosphorus depletion and competition volumes in the surface 5 cm of soil (Fig. 5a). Decreased total root length resulted in a smaller depletion volume for the whole root system (Fig. 5b), while overall competition volume increased as a result of more root proliferation in a small volume of soil around the hypocotyl (Fig. 5b). In uniform soil, the combined effects of decreased phosphorus depletion and increased competition for the whole root system led to a decrease in phosphorus acquisition and efficiency with increasing allocation to adventitious roots (Fig. 6a, c). In contrast, in stratified soil, increased exploration of the high phosphorus surface stratum was sufficient to increase phosphorus acquisition and efficiency with increasing allocation to adventitious roots (Fig. 6b, d). If high levels of carbon were partitioned to root systems in stratified soil, phosphorus acquisition increased by over 10% with high adventitious root production, whereas with low C partitioning, adventitious roots increased phosphorus acquisition and efficiency by about 5%. Adventitious root effects peaked with 30 adventitious roots in both high and low C partitioning.

Diffusion coefficients in the range of $10^{-9}$ to $10^{-7}$ cm$^2$ s$^{-1}$ did not change the effect of adventitious roots on phosphorus acquisition. Plants with adventitious roots were able to acquire about 10% more phosphorus than those without adventitious roots under all diffusion coefficients tested. Figure 6 illustrates the effect of adventitious number in soil with a phosphorus diffusion coefficient of $10^{-8}$ cm$^2$/s. The only exception to this pattern was that for a plant partitioning high amounts of C to the root system in soil with a phosphorus diffusion coefficient of $10^{-7}$ cm$^2$/s, ten adventitious roots sufficed for
maximum phosphorus acquisition. Thirty adventitious roots were necessary for maximum phosphorus acquisition for all other soil and C partitioning combinations.

Basal root architectures included in these simulations are illustrated in Fig. 7. Phosphorus acquisition and efficiency in stratified soil were each significantly affected by basal root angle ($52.5 < F < 54.1$, $p < 0.0001$) and adventitious number ($45.1 < F < 51.3$, $p < 0.0001$). In stratified soil, shallow basal roots or increased allocation to adventitious roots resulted in more phosphorus acquisition and higher efficiency of phosphorus acquisition (Fig. 8a, b). There was no interaction between basal root angle and adventitious number on phosphorus acquisition and efficiency ($p > 0.9$). In stratified soil, adventitious roots increased phosphorus acquisition and efficiency in all root systems regardless of basal root deployment. For a given number of adventitious roots, plants with shallow basal roots acquired more phosphorus than those with deep basal roots, as expected (Bonser et al., 1996; Ge et al., 2000; Liao et al., 2001). Plants with deep basal roots required at least 20 adventitious roots in order to acquire as much phosphorus as plants with shallow basal roots.

Like adventitious root number, the effect of basal root growth angle on phosphorus acquisition is largely attributable to phosphorus depletion in the top 5 cm of soil (Fig. 9). Plants with shallow basal roots deplete more surface soil than those with deep basal roots, and this pattern holds across all numbers of adventitious roots tested. Competition in shallow soil was not affected by basal root growth angle at any number of adventitious roots.

The benefit of adventitious roots for phosphorus acquisition was significantly affected by the respiration rate of adventitious roots (Fig. 10a, b). Plants with
adventitious root respiration equal to basal root respiration acquired the most phosphorus; increasing adventitious respiration to 33% greater than that of basal roots and double that of the tap root still allowed for a small benefit of adventitious roots for phosphorus acquisition and efficiency. Increasing adventitious respiration further to 4 times greater than tap root respiration resulted in plants in which adventitious roots significantly decreased acquisition of phosphorus and efficiency of phosphorus acquisition.

Planting depth significantly affected phosphorus acquisition and efficiency, but not the contribution of adventitious roots to phosphorus acquisition or efficiency. Seed planted in the 5 cm high phosphorus stratum, either in the middle or at the bottom, produced roots that acquired more phosphorus than seed planted below this layer (Fig. 11a). Increased allocation to adventitious roots resulted in increased phosphorus acquisition and efficiency of acquisition for all planting depths (Fig. 11a, b). A seed planted below the phosphorus rich surface soil would have to produce 20 adventitious roots to match the phosphorus acquisition and efficiency of a seed planted within the high phosphorus stratum that produced 0 adventitious roots. Basal root laterals grow into the surface stratum, but when seed was planted below the phosphorus rich layer, there was insufficient basal lateral proliferation near the surface to make deep planting beneficial.

Simulations to 4 weeks after germination produced similar phosphorus acquisition and efficiency results as those run to 3 weeks after germination (Fig. 11). Phosphorus acquisition was increased by about 10% with low adventitious root respiration and 5% with intermediate root respiration. Acquisition peaked with 30 adventitious roots.
DISCUSSION

Conclusions about the benefit of adventitious roots for phosphorus acquisition in previous research were based on observations that phosphorus efficient genotypes produced more adventitious roots under phosphorus stress (Miller et al., 2003). In the present report, geometric simulations allowed for a more precise quantification of the benefits of adventitious roots, and provided insight into potential mechanisms by which adventitious roots affect whole plant phosphorus acquisition.

Adventitious roots increase exploration of shallow soil, but, in the current model, also reduce overall soil phosphorus depletion volume. This is due to decreased total root system length resulting from C diversion from first and second order laterals arising from tap and basal roots (Fig. 4). Lateral roots on the tap and basal roots have smaller diameters and larger specific root lengths than adventitious main axes. Therefore, there is a tradeoff of less proliferation of thin, inexpensive roots in deeper strata for thicker adventitious roots closer to the soil surface. The validity of this result is supported by correspondence between simulations and empirical measurements (Fig. 2, 3), as well as similar sensitivity of higher order laterals to assimilate partitioning observed in independent experiments and modeling (Thaler and Pages, 1998), and the sensitivity of phosphorus acquisition efficiency to lateral rooting in Zea maize (Zhu and Lynch, 2004).

While allocation to adventitious roots appears to be beneficial for phosphorus acquisition, there are limits beyond which further allocation to adventitious rooting provides diminishing additional benefits in phosphorus acquisition. In addition to increasing phosphorus depletion, increasing allocation to adventitious roots leads to higher overall root competition. Before adventitious roots can grow into unexplored
surface soil, they must first compete with basal roots in a small volume of soil near their origin. These trends in overall phosphorus depletion and competition limit the benefit of adventitious roots for phosphorus acquisition in plants growing in stratified soil, which peaked at 30 adventitious roots in these simulations. If phosphorus availability is relatively uniform in the rooting zone, then any number of adventitious roots is expected to be disadvantageous for acquisition.

Soil phosphorus mobility within the range tested in these models did not affect the relative contribution of adventitious roots to phosphorus acquisition or acquisition efficiency. As long as phosphorus availability is stratified with depth, which is common for many reasons including bioaccumulation, higher microbial activity near the surface and fertilization of agricultural soils (Lynch and Brown, 2001), then plants are expected to benefit from adventitious root proliferation, regardless of phosphorus mobility. It was expected that in soils with higher diffusion coefficients, basal roots would be capable of depleting much of the surface soil of phosphorus. In this case, adventitious roots would be redundant for topsoil exploitation, which would lead to increased root competition that would negate potential benefits. However, competition volume, expressed as a percent of soil explored, was not affected by diffusion coefficient, and surface phosphorus depletion volumes increased with increased adventitious number, therefore allocation to adventitious roots is advantageous in a wide range of soils.

Plants with shallow basal roots depleted more surface soil than those with deep basal roots, regardless of adventitious root number. Competition among roots for acquisition of phosphorus in surface soil was similar among basal root architectures, and increasing allocation to adventitious roots affected competition for phosphorus in the
topsoil similarly for all basal root architectures. These results indicate that for acquisition of immobile nutrients, such as phosphorus, adventitious roots complement basal roots. Surface soil is explored independently by adventitious and basal roots. Therefore, plants with shallow basal roots benefit as much from adventitious root production as plants with deep basal roots. Furthermore, the most phosphorus efficient genotypes are expected to have shallow basal roots and large numbers of adventitious roots.

Further evidence for the independence of soil exploration by basal and adventitious roots is provided by the effect of planting depth on phosphorus acquisition and efficiency. Adventitious root production is beneficial for plants seeded at any of the depths tested. However, if seed is planted below the layer of high phosphorus availability, 20 adventitious roots are necessary to make up for the phosphorus that would otherwise be acquired by basal roots. This amount of adventitious root production is in the upper range of values reported for cultivated common bean (Miller et al., 2003). As long as seed is planted within the phosphorus-rich surface soil, even if it is at the bottom of this layer, adventitious and basal roots will have enough access to keep phosphorus acquisition and efficiency high. This is relevant for cultural practices used for common bean. In practice, the deeper that beans are planted, the more adventitious roots will emerge from subterranean hypocotyls. However, growers need to be careful not to plant seed so deep that basal roots become ineffective for phosphorus acquisition. If seed is planted below the high phosphorus surface stratum, it is unlikely that plants will allocate sufficient C to adventitious roots to compensate for the loss of access to phosphorus by basal roots. On the other hand, in these simulations, planting seed at the bottom of phosphorus rich surface soil allows for the maximum number of adventitious roots to be
produced without affecting basal root contributions to phosphorus acquisition and efficiency. The conclusion is that growers can increase yields by determining how deeply their soil is stratified and planting at an appropriate depth for that soil. The combination of planting genotypes with shallow basal roots at an appropriate depth will maximize yields in phosphorus-limited fields. The practice of planting seed in the top 3-5 cm and mounding soil around emerged seeds, which is commonly practiced in developing countries, is a safe way to keep basal roots shallow while stimulating increased adventitious root production.

In nature, bean seeds typically germinate on or near the surface. In these simulations, there was no difference between plants producing equal numbers of adventitious roots at either 3 or 5 cm depths. Given the immobility of phosphorus in soils common in native bean habitats (Beebe et al., 1997; Sanchez and Uehara, 1980), competition among roots for phosphorus is not likely to be a significant factor until 30 or more adventitious roots emerge from a single hypocotyl. Genotypes producing high densities of adventitious roots are expected to be more efficient than those producing low densities. Bean varieties adapted to stratified and limited phosphorus conditions are predicted to be a source of high adventitious density phenotypes. As previously noted, phosphorus efficient cultivated genotypes have more allocation to adventitious roots than inefficient genotypes, but there is much more diversity of adventitious rooting in wild genotypes. In terms of mass, adventitious roots comprise less than 10% of the root systems of cultivated genotypes, but they can comprise up to 20% of wild bean root systems (Miller et al., 2003). This implies that there is a significant amount of diversity
in common bean adventitious rooting that may yet be exploited in breeding efforts to produce more phosphorus efficient phenotypes.

The benefit of adventitious root production for phosphorus acquisition is sensitive to variation in adventitious root respiration. Phosphorus acquisition increases only as long as the specific respiration of adventitious roots is similar to that of basal roots. Increasing the respiration of adventitious roots reduces C available for basal and adventitious root growth, which reduces phosphorus acquisition and acquisition efficiency. This is consistent with previous research in which phosphorus-inefficient genotypes respired more C per unit root growth than phosphorus-efficient genotypes (Nielsen et al., 2001), as well as more recent findings that phosphorus-inefficient genotypes have higher adventitious root respiration than phosphorus-efficient genotypes (Ho et al., 2003). The respiration rates used in these simulations were within ranges observed by previous researchers (Bouma et al., 1997; Lynch and Ho, 2005, Ho et al., 2003). Given the diversity observed in other common bean traits, it is possible that respiration rates in common bean roots vary more than what was simulated here. Therefore, the range of positive or negative impacts of allocation to adventitious roots on phosphorus acquisition may be wider than what is presented here.

A number of internal and external factors influence root respiration rates. One easily identifiable, yet difficult to measure, area of potential refinement is respiration rate within a root type. Respiration varies along each root axis (Bidel et al., 2000b; Nielsen et al., 1994). So the assumption of homogenous respiration within each root type is an average for the whole root. Nielsen et al. (1994) quantified variation of respiration along a bean root axis and used this in simulations of C costs in SimRoot. However, all root
axes were assumed to respire equivalently with distance from the tip, so their method would not allow for distinguishing respiratory costs of adventitious roots versus other root types. Therefore, we used the data generated by Ho et al. (2003), which distinguished respiration rates among root types, but not along root axes. In the future, this model may be improved by considering changes in respiration along root axes.

Another area worthy of consideration is the effect of temperature on root respiration rates. This model assumed isohyperthermic conditions throughout the soil volume, which is reasonable for tropical soils, such as those in which common bean is produced as a primary food source. However, even in tropical and subtropical soils, there are temperature fluctuations diurnally and with depth (Tenge et al., 1998). Respiration rates in bean roots have been observed to increase in the temperature range of 22-38 C, with a Q10 of 1.69-1.79 (Bouma et al., 1997). With higher temperature near the surface, adventitious roots are expected to respire at a higher rate than basal roots. In this case, allocating more C to adventitious rooting will be of limited utility or detrimental. On the other hand, reduced respiratory rate fluctuations through acclimation are possible, even to diurnal temperature fluctuations (Atkin and Tjoelker, 2003; Loveys et al., 2003). In addition, respiration rates may be decreased as aerenchyma form, particularly in adventitious roots (Fan et al., 2003). Finally, surface soil is more prone to drying, which may result in decreased respiration of adventitious roots (Bryla et al., 1997; Huang and Fu, 2000), although at a cost of decreased phosphorus availability (Sanchez and Uehara, 1980). So, the actual impact of fluctuating temperatures on respiration rates may be less over a growing season than is predicted from short term measurements. Introducing the factors discussed here into SimRoot in order to adequately address variation in respiration
rates would help to address the relative impacts of each of these influences. The expected outcome would be to refine the quantification of estimates, but the importance of adventitious root respiration rate relative to that in basal roots in estimation of phosphorus acquisition and efficiency would likely be unchanged.

Model estimations of phosphorus acquisition are similar to phosphorus content of common bean reported in field studies (Miller et al., 2003; Yan et al., 1995b), as well as amounts that can be calculated from other field and greenhouse experiments (Nielsen et al., 1998b; Snapp and Lynch, 1996; Rubio et al., 2003a). Root respiration in these models accounted for an estimated 20-45% of C assimilation, which is in accord with published results (Nielsen et al., 1998a, 2001). None of the data in the empirical studies cited here were used to develop the present model, and therefore they represent independent verification of our simulation results. In addition, the simulated phosphorus efficiency reported here is similar to efficiency reported for nonmycorrhizal Eucalyptus (Jones et al., 1998). The growth of roots in this model is based on C allocation to the root system, root tip cross sectional area, and empirically measured respiration. The only nonspecific parameter introduced is the elongation factor, which simulates the slowing and eventual cessation of lateral root elongation, as has been previously reported (Cahn et al., 1989) and confirmed in our observations. In making sink strength a function of cross sectional area, this model is similar to previously developed models that used root tip diameter (Drouet and Pages, 2003; Thaler and Pages, 1998) or volume (Bidel et al., 2000a) to estimate root growth rate or sink strength. Finally, decreasing benefit of adventitious roots for phosphorus acquisition and efficiency with reduced C allocation to roots provides an explanation as to why reduced C availability in Arabidopsis and maize
leads to decreased or delayed adventitious rooting (Gibson, 2005; Pellerin, 1991; Takahashi et al., 2003).

Slight differences between empirical measures and simulations suggest room for improvement of the simulation model. Tap, basal and total root system lengths were more sensitive to changes in allocation to adventitious roots in empirical observations than in simulations, particularly in low P cultures used to estimate low C allocation (Fig. 3a, b, c). This indicates that the costs of adventitious roots were not fully explained by this model. Perhaps soil temperature profile effects on respiration in our greenhouse system made adventitious roots more costly than was assumed. Alternatively, nutrient acquisition by adventitious roots may have greater respiration costs than was observed previously. However, variation between the two genotypes used in this research in characters other than the differences in allocation to adventitious roots likely confounds explanations based simply on adventitious number, length, or physiology.

The maximum benefit of adventitious roots in these 3 week simulations was to increase phosphorus acquisition by 10%. Running simulations out to 4 weeks did not change this relative impact. At 4 weeks, a 10% increase in phosphorus acquisition translates into 2 mg of phosphorus. Assuming this trend continues, then adventitious roots may account for over 10 mg of phosphorus acquisition over the course of a growing season. Even with the low estimate of effects of allocation to adventitious roots on other root types described in the preceding paragraph, there are still several reasons to consider the estimate of adventitious root benefits presented here to be conservative. Adventitious roots emerge over a longer time frame than assumed for these simulations, so adventitious roots explore surface soil throughout the growing season, while basal roots
explore surface soil predominantly early in the season. More importantly, a 10% increase of phosphorus acquisition allows for greater leaf expansion in the short term (Pellerin et al., 2000), which results in more photosynthesis that cycles back as higher C allocation to the root system. Over the course of a growing season, these feedback cycles may turn an initial 10% increase in phosphorus acquisition into a several fold gain in phosphorus content and yield (Wissuwa, 2003). Currently, SimRoot does not contain any feedback between phosphorus acquisition, leaf expansion, photosynthesis, and C allocation to roots. Therefore, it is likely that slowly respiring adventitious roots on plants growing in stratified soil account for phosphorus acquisition of well over 10 mg, possibly 25-50 mg, over the course of a growing season.

In the field, multiple stresses are typically encountered through the course of a growing season. Plants must allocate resources between competing sinks that fulfill a variety of functions. Optimal allocation may be determined by marginal benefits of alternative pathways (Lynch and Ho, 2005). In this way, a number of resources and demands may be balanced so that no one resource is in excess. For example, C allocation is drawn on one side by demands of root respiration, growth, and water and mineral acquisition, and on the other by requirements of shoot respiration and leaf expansion. Similarly, common bean plants have evolved adaptations to drought tolerance and low phosphorus availability. Drought selects for plants with deeper roots, while phosphorus stress selects for plants with shallow roots. The optimum allocation between these strategies results when the benefits of a given allocation are offset by costs (Ho et al., 2004). Deeper roots will be selected until the benefit of water obtained is offset by the extra costs associated with phosphorus acquisition by deep roots. Overall, plant
phenotype is a complex expression of competing interests that cannot be easily analyzed by considering only single resource limitations or multiple limitations (Rubio et al., 2003b). Thus, considering availability of water and other nutrients will be important elements of future efforts to assess the contribution of adventitious rooting to plant performance.

Complexity of tradeoffs can be illustrated by considering the resources of phosphorus and water, along with carbon allocation into adventitious roots or mycorrhizal symbiosis. Drought selects for deeper roots (Ho et al., 2004), and drought stress may result in decreased allocation to adventitious roots (Pardales and Yamauchi, 2003). On the other hand, mycorrhizal association appears to result in better performance of host plants in drought conditions (Al-Karaki et al., 2004; El-Tohamy et al., 1999; Sanchez-Blanco et al., 2004), along with increased acquisition of phosphorus in soil of low phosphorus availability (Martin and Stutz, 2004; West et al., 1993). In addition, propagules of mycorrhizal fungi tend to be more numerous near the soil surface (Abbott and Robson, 1991). Therefore, allocation of carbon to mycorrhiza through roots proliferating in shallow soil may still be beneficial in drought conditions. However, this remains uncertain, because mycorrhiza increase respiratory demands (Martin and Stutz, 2004; Nielsen et al., 1998a), and concentration of mycorrhizal fungal propagules near the soil surface may or may not lead to greater colonization of shallow roots than of deep roots (Nehl et al., 1999). Allocation to adventitious roots may still be beneficial for phosphorus and water acquisition in soil with low phosphorus availability under drought conditions, depending on how adventitious root proliferation affects the extent of mycorrhizal symbiosis in shallow and deep soil strata.
Despite several assumptions and simplifications, the model simulations produced reasonable results validated by empirical data as well as published reports. These results lead to predictions that may be useful in crop management and breeding. For example, planting seed deeper to stimulate more adventitious production is expected to be beneficial only as long as seed is planted in phosphorus rich surface soil where basal roots have access to high phosphorus strata. Our models predict little competition between adventitious and basal roots. Therefore, adventitious and basal roots are complementary for phosphorus acquisition. Finally, maintaining high C utilization efficiency by roots is necessary to derive maximal benefit of adventitious rooting. The most phosphorus efficient genotypes are expected to have shallow basal roots, significant allocation to adventitious roots, which respire at rates similar to basal roots, and an ability to utilize C efficiently for root growth.

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Table 3.1. Root growth parameters used in simulations of bean root growth

<table>
<thead>
<tr>
<th>Parameter</th>
<th>504/21</th>
<th>10^{-7}</th>
<th>10^{-8}</th>
<th>10^{-9}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (hr/d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>De (cm^{2}s^{-1})^a</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbon allocation to roots^b</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed depth (cm)</td>
<td>3</td>
<td>5</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Adventitious number</td>
<td>0</td>
<td>10</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>Adventitious respiration^c (nmol CO_{2}/g/s)</td>
<td>75</td>
<td>100</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Basal root architecture^d</td>
<td>shallow</td>
<td>fanned</td>
<td>deep</td>
<td></td>
</tr>
</tbody>
</table>

^aDiffusion coefficient of mineral nutrient through soil, based on (Schenk and Barber 1979)
^bBased on empirical measurements, see materials and methods
^cBased on Ho et al. (2003) and Bouma et al (1997)
^dBased on (Rubio et al. 2001), fanned roots have 4 shallow, 4 intermediate, and 4 deep basal roots
Figure 3.1. Geometric models of common bean root systems generated by SimRoot. Pictured roots grew for 504 hours (21 d) with seed planted at 5 cm depth and high C allocation to roots as described in the materials and methods, and have 0, 10 or 30 adventitious roots along with shallow or deep basal roots. Scale bar is in cm.
Figure 3.2. Length of 8 root types measured from common bean genotypes G2333 and G19839 in 3-week-old greenhouse cultures, as well as simulated root lengths generated by 504 hour models of roots producing 10 or 20 adventitious roots respiring at a rate of 100 nmol CO$_2$/g/s; a) high C allocation to the root system and b) low C allocation to the root system. Values are the mean of 3 replicates for models and 2-3 replicates for greenhouse cultures. Error bars represent standard error of the mean.
Figure 3.3. Effect of allocation to adventitious roots on a) tap root, b) basal root, or c) total root system length of 3 week old common bean or simulated roots. Each root type includes primary axes and lateral branches. Trend lines are shown for empirical data. Rate of simulated adventitious root respiration is $100 \text{ nmol CO}_2/\text{g/s}$. High C (HC) and low C (LC) allocation to root systems were determined as described in materials and methods. Each point represents one plant for empirical data (HC and LC) and 3 replicates for model data.
Figure 3.4. Simulated effect of allocation to adventitious and adventitious lateral roots respiring at a rate of 75 nmol CO$_2$/g/s on root lengths of common bean tap and basal roots, along with their first and 2nd order laterals expressed as percent of maximum. For adventitious root, 100% length occurs with 40 adventitious roots, while for all other root types 100% root length occurs with 0 adventitious roots. Values represent the mean of 3 replicates of data generated from simulations in plants with high C allocation to roots as described in the materials and methods.
Figure 3.5. Depletion volumes of phosphorus and competition volumes for phosphorus in a) the surface 5 cm, or b) the entire soil volume as affected by number of adventitious roots. Competition volume is soil depleted by 2 or more roots. Rate of simulated adventitious root respiration is 75 nmol CO$_2$/g/s. High C (HC) and low C (LC) allocation to root systems were determined as described in materials and methods. Values are the mean of 3 replicates from simulations. Error bars represent standard error of the mean.
Figure 3.6. Phosphorus acquisition and acquisition efficiency in uniform soil (a and c) or in stratified soil (b and d) as affected by adventitious number. Uniform soil contains P available at 10 µM throughout the soil profile. Stratified soil contains P available at 30 µM in the top 5 cm, 10 µM in the next 5 cm, and 2 µM below 10 cm depth. Rate of simulated adventitious root respiration is 75 nmol CO$_2$/g/s. High C (HC) and low C (LC) allocation to root systems were determined as described in materials and methods. Values are the mean of 3 replicates from simulations. Error bars represent standard error of the mean.
Figure 3.7. Deep (a), fanned (b), and shallow (c) basal root architectures used in simulations. Pictured roots grew for 504 hours (21 d) with seed planted at 5 cm depth and high C allocation to roots as described in the materials and methods. Each root system has 10 adventitious roots. Scale bar is in cm.
Figure 3.8. Phosphorus acquisition (a), and phosphorus acquisition efficiency (b) as affected by adventitious root number for plants with deep, fanned or shallow basal root architectures as illustrated in Fig. 7. Rate of simulated adventitious root respiration is 75 nmol CO$_2$/g/s. Values are the mean of 3 replicates from simulations. Error bars represent standard error of the mean.
Figure 3.9. Depletion volumes of phosphorus and competition volumes for phosphorus in the surface 5 cm of soil as affected by number of adventitious roots for plants with deep, shallow or fanned basal root architectures. Rate of simulated adventitious root respiration is 75 nmol CO$_2$/g/s. Values are the mean of 3 replicates from simulations. Error bars represent standard error of the mean.
Figure 3.10. Phosphorus acquisition (a) and phosphorus acquisition efficiency (b) as affected by adventitious root number for plants with low (75), intermediate (100), or high (200) specific root respiration of adventitious roots. Units for respiration are nmol CO$_2$/g/s. Values are the mean of 3 replicates from simulations. Error bars represent standard error of the mean.
Figure 3.11. Phosphorus acquisition (a) and phosphorus acquisition efficiency (b) as affected by adventitious number for plants seeded at depths of 3, 5, or 7 cm. Rate of simulated adventitious root respiration is 75 nmol CO$_2$/g/s. Values are the mean of 3 replicates from simulations. Error bars represent standard error of the mean.
Figure 3.12. Phosphorus acquisition as affected by adventitious number for plants with low (75) or intermediate (100) adventitious specific root respiration grown for 672 hours (28 d). Units for respiration are nmol CO$_2$/g/s. Error bars represent standard error of the mean.
CHAPTER 4

MODELING APPLICABILITY OF FRACTAL ANALYSIS TO EFFICIENCY OF SOIL EXPLORATION BY ROOTS

ABSTRACT
Fractal analysis allows calculation of fractal dimension, fractal abundance and lacunarity. Fractal analysis of plant roots has revealed correlations of fractal dimension with age, topology or genotypic variation, while fractal abundance has been associated with root length. Lacunarity is associated with heterogeneity of distribution, and has yet to be utilized in analysis of roots. In this project, fractal analysis is applied to the study of root architecture and acquisition of diffusion limited nutrients. We tested the hypothesis that soil depletion and root competition are more closely correlated with a combination of fractal parameters than by any one alone. The geometric simulation model SimRoot was used to dynamically model roots of various architectures growing for up to 16 days in 3 soil types with contrasting nutrient mobility. Fractal parameters were calculated for whole roots, projections of roots and vertical slices of roots taken at 0, 2.5 and 5 cm from the root origin. Nutrient depletion volumes, competition volumes, and relative competition were regressed against fractal parameters and root length. Root length was correlated with depletion volume, competition volume and relative competition at all times. In analysis of 3D, projected roots, and 0 cm slices, log(fractal abundance) was highly correlated with log(depletion volume) when times were pooled. Other than this, multiple regression yielded better correlations than regression with single fractal
parameters. Correlations decreased with age of roots and distance of vertical slices from the root origin. Field data was also examined to see if fractal dimension, fractal abundance and lacunarity can be used to distinguish common bean genotypes in field situations. There were significant differences in fractal dimension and fractal abundance, but not in lacunarity. These results suggest that applying fractal analysis to research of soil exploration by root systems should include fractal abundance, and possibly lacunarity, along with fractal dimension.

INTRODUCTION

Annual crops produce several orders of roots as fine as a fraction of a mm in diameter, with a distance between branches ranging from millimeters to centimeters. With these branching patterns, roots can be characterized by fractal analysis over a range of scales from about a quarter of a millimeter to several centimeters (Tatsumi et al., 1989). The fractal dimension (FD) has been calculated for whole root systems (Tatsumi et al., 1989), as well as for separated branches (Lynch and van Beem, 1993), and planar sections (Eshel, 1998; Nielsen et al., 1997). In computer simulations, FD of planar sections was correlated with the FD of entire root systems (Nielsen et al., 1997), so analyzing subsamples may be appropriate for roots, which are often difficult to observe entirely intact.

A number of researchers have demonstrated that fractal analysis may be biologically relevant. Many aspects of morphological and physiological variation have been associated with variation in FD. As roots grow, the FD increases (Fitter and Stickland, 1992; Lynch and van Beem, 1993; Nielsen et al., 1998). Fractal dimension has
been correlated with root topology (Fitter and Stickland, 1992), and root architecture (Nielsen et al, 1997). Differences in FD have been noted among 4 species of dicots and monocots (Fitter and Stickland, 1992), as well as among genotypes of sorghum, rice and common bean. (Izumi et al, 1995; Masi and Maranville, 1998; Nielsen et al, 1998). Genotypic variation of root mass and root to shoot ratio was correlated with variation in FD of roots (Masi and Maranville, 1998). Lastly, FD has been observed to vary with nitrogen availability for corn (Eghball et al, 1993), and phosphorus acquisition from low phosphorus soils in common bean (Nielsen et al, 1998).

Most research to date has focused on FD, even though other parameters can be easily calculated from the same data. Therefore, it remains unclear if FD is sufficient or the most appropriate aspect of fractal analysis that can be applied to the study of root system morphology and functioning. While FD appears to be a useful metric in some situations, there are more aspects of fractal analysis that might also be useful.

Fractal analysis in root biology typically utilizes box counting and the equation:

\[ N(L) = KL^{-D}, \] (1)

where L is the box size, and \( N(L) \) is the number of boxes of size L needed to cover the object. A log-log plot of \( N(L) \) versus L returns \(-D\) as the slope and log \( K \) as the intercept. In terms of fractal analysis, \( D \) is the fractal dimension, and log \( K \) has been associated with fractal abundance (FA) (Puche and Su, 2001a, b). Fractal dimension is associated with the branching pattern, while FA is associated with the volume of space explored. To date, FD has been studied far more than FA in the plant sciences. Yet, it has been shown that FA may vary when fractal dimension does not. Corn roots that vary in size can have equivalent FD, but vary in FA (Eghball et al, 1993). Masi and Maranville (1998)
speculated that FA might differentiate root systems with regard to their ability to exploit the soil profile, even when the FD are similar. However, no observations were made to test this speculation. Puche and Su (2001a, b) showed that the FD describes the intricacy of termite tunnels, while FA is associated with population density and foraging behavior. There could be an analogous situation with plants roots, whereby FD describes the branching pattern, while FA is associated with the size of the root system and extent of soil explored.

Another parameter that can be calculated in fractal analysis that might be useful in distinguishing objects of similar FD is lacunarity (L), which is a measure of the structural heterogeneity within an object (Smith et al., 1996). When the box counting method is used, L is an estimate of the variation of how many root segments are contained in the boxes. For example, some boxes might cover one root segment while other boxes cover 10 root segments. The FD does not account for this variation, but L does. One measure of L is the coefficient of variation, which is the standard deviation divided by the mean of the number of root segments per box. This is the measure of L that is used in this paper. It is likely to be complementary to FD, and may or may not be a substitute or complement of FA.

The objective of this project was to determine the most appropriate fractal parameters to calculate for determining effectiveness of soil exploration by root systems. To simplify the discussion, phosphorus will be the focus of this explanation. Diffusion limited elements, such as P, move slowly through soil (Schenk and Barber, 1979), so soil volumes in the immediate vicinity of roots are often depleted of P, while bulk soil 1-2 cm away has P concentrations that remain largely unchanged. The soil that has had P
concentration decreased by root uptake is referred to as the depletion volume, the size of which is proportional to the rate of diffusion of P through the soil. Some soil is depleted of P by more than one root from a single plant. The volume of soil explored by multiple roots is defined as competition volume. Since competition volume increases as the root system grows, it is often useful to calculate relative competition, which in this report is competition volume divided by depletion volume. Using these concepts of depletion and competition, P efficiency can be defined as minimization of relative competition. This definition has been used in geometric modeling to evaluate the P efficiency of contrasting root architectures (Rubio et al., 2001; Ge et al., 2000), which has been correlated with the ability of plants to maintain growth under low P conditions in greenhouse and field environments (Lynch and Brown, 2001; Rubio et al., 2003). The FD of planar root sections can differentiate P efficient and P inefficient genotypes of common bean (Nielsen et al., 1998). Neither FA nor L was included in these studies, so the question of whether these terms can improve our understanding of phosphorus acquisition efficiency remains.

Root systems can vary significantly in size and FA, yet have similar FD (Eghball et al., 1993). Therefore, a hypothesis tested in this study is that FD and FA are correlated with complementary aspects of uptake of diffusion limited nutrients. Specifically, FA is correlated with absolute measures of soil exploration, such as depletion and competition volume, while FD is correlated with relative competition. Furthermore, with slow diffusion of nutrients, and, therefore low inter-root competition (Ge et al., 2000; Rubio et al., 2001), root length is likely to be a better estimate of depletion and competition than
fractal abundance. As diffusion rate increases, and more inter-root competition results, FA may become a better estimate of depletion than root length.

The possible utility of lacunarity is not as clear. A low value of L implies homogeneity, which may be associated with roots that are uniformly distributed with either high or low competition. Therefore we hypothesize that L alone is not well correlated with estimates of soil exploration, but it may be useful as a complement to FD or FA in multiple regression to significantly increase correlations with estimates of soil exploration.

Since 3 dimensional root architecture and nutrient depletion are not readily observable in field or greenhouse conditions, we employed the geometric simulation model SimRoot (Lynch et al., 1997), and output was imported into associated programs for numerical determination of soil depletion and fractal analysis. Fractal dimensions of entire root systems have been correlated with dimensions of root projections and planar sections (Nielsen et al., 1997), so simulations included 3 dimensional root systems, projections of root systems and 0.1 cm vertical slices taken from 3 positions. Empirical data were also examined to see if FD, FA and L could be used to differentiate common bean genotypes that varied in shoot mass and competitiveness in medium and high P field conditions.

MATERIALS AND METHODS

*Description of root model and input*

The dynamic geometric model SimRoot (Lynch et al., 1997) was used to simulate root growth and architecture. Time, adventitious root number, basal root
gravitropism, and basal lateral root density were varied as described in Table 4.1 to produce 360 root models. Each root type was modeled in three soil types that varied in diffusion coefficient \((D_e)\), producing a total of 1080 simulations. An illustration of simulated roots with 15 adventitious roots showing all time points and basal root angles is shown in Figure 4.1.

**Root length and soil depletion volumes**

Root length and total depletion volume with overlap \((V_t)\) were calculated by *SimRoot*. The depletion volume calculation has been published (Ge *et al.*, 2000). It is the sum of depletion over all root segments, which are individually calculated as cylindrical volume:

\[
V = \pi R_{dz}^2 l, \quad (2)
\]

where \(l\) is the segment length and \(R_{dz}\) is the depletion radius. The depletion radius is calculated by using the equation:

\[
R_{dz} = r + 2(D_e t)^{1/2}, \quad (3)
\]

where \(r\) is the root radius, \(t\) is time in seconds, and \(D_e\) is the diffusion coefficient in \(\text{cm}^2/\text{s}\).

Output from *Simroot* was analyzed in *DepZone* to determine actual depletion volume \((V_a)\) by subtracting duplications of overlapped volume as previously described (Ge *et al.*, 2000; Rubio *et al.*, 2001). Competition volume is defined as:

\[
C_v = V_t - V_a, \quad (4)
\]

and relative competition is:

\[
C_r = 100(V_t - V_a)/ V_a, \quad (5)
\]
Fractal analysis

Fractal analysis of simulated roots was performed in a C program written to analyze output from SimRoot. Box counting was used to calculate FD and FA. Preliminary simulations showed that box sizes from 0.25 to 4.55 cm were appropriate for this analysis. A scaling factor of 1.25 between box sizes was used to provide 14 box sizes for the analysis. Both FD and FA are defined as previously reported (Eghball et al., 1993; Masi and Maranville, 1998), which is shown in eq. 1.

When root systems are covered with boxes, not all boxes contain the same amount of roots. A measure of this variation is lacunarity (Smith et al., 1996). In this study, lacunarity was defined as the coefficient of variation of the amount of roots per box. That is, the standard deviation of amount of roots per box was divided by the mean. Lacunarity was calculated in this manner for each box size, and the average of these 14 estimates was used as the overall lacunarity that was used to analyze and compare root systems.

Fractal analysis for whole roots was performed with the intact 3 dimensional root systems, and for projections of root systems onto a 2 dimensional plane. Projections were simulated by taking 3 dimensional root output from SimRoot and setting all X equal to 0. Two dimensional slices were also analyzed by simulating 0.1 cm vertical slices of roots taken at 0, 2.5, and 5 cm from the seed position. Root projections and root segments within the slices were analyzed in a 2 dimensional box counting program that used the same range of box sizes as were used for 3 dimensional analysis.
Field study

Data from a field study were reexamined to test the utility of fractal analysis. Details of the experiment have been published (Rubio et al, 2003). Common bean recombinant inbred lines (RILs) from parents that varied in rooting depth and ability to maintain growth in low P conditions were grown in high and medium phosphorus conditions in the field in South China. After five weeks, trenches were dug ten cm from the row and root positions were marked on plastic sheets. The results, in short, were that two phosphorus efficient RILs had more shallow roots than two phosphorus inefficient RILs, and phosphorus efficient RILs out competed phosphorus inefficient RILs in medium P plots where they were grown together.

For this study, plastic sheets that had been marked with intercepts of roots with trench faces were scanned and saved in TIF format. Image J (developed at the U.S. National Institutes of Health and available on the internet at http://rsb.info.nih.gov/ij/) was used in Windows XP to remove extraneous markings and to output text files containing coordinates of roots. These text files were then analyzed for FD, FA and L in a fractal analysis program written in C that counts boxes in 2 dimensions. Because of limited resolution of field measurements, 7 box sizes were used ranging in size from 1.25 to 4.77 cm, with successive box sizes varied by a factor of 1.25.

Data analysis

Regression analyses were conducted in StatView, Version 5.0.1 (SAS Institute Inc, Cary, NC, USA). Simulation data were analyzed by simple and multiple regression of depletion volume, competition volume, and relative competition against root length,
Fractal parameters calculated for root projections and 2 dimensional intersections were regressed with the fractal parameters derived from 3 dimensional root systems. Regressions were carried out for all time points pooled together to test for general trends, as well as for data from each time point to simulate single harvest dates. Coefficients of simple determination ($r^2$) and multiple determination ($R^2$) (Neter et al, 1989) for combinations of fractal parameters were calculated to decide when root length, FD, FA and L are relevant for understanding nutrient acquisition. Field data were analyzed by a general linear model analysis of variance in the SAS System for Windows, Release 8.02 with the dependent variables FD, FA and L, and independent variables RIL and phosphorus treatment. Mean separation was evaluated by the least squares mean procedure including main effects and the interaction of RIL with phosphorus.

RESULTS

Correlation among fractal parameters

Scatter plots of FD, FA and L from analyses of 3D and 2.5 cm planar intersections are presented in Figure 4.2. Fractal dimension had low correlations with FA and L in 3D ($r^2 < 0.25$), while there was a somewhat greater correlation between FD and L in projected roots ($0.36 < r^2 < 0.60$), as well as between FD and FA in planar intersections, with the highest correlation in intersections 2.5 cm from the root origin ($0.44 < r^2 < 0.68$). Fractal abundance was highly correlated with L in 3D or projected roots when analyzed at individual time points ($0.78 < r^2 < 0.95$), but not when all times were pooled ($r^2 < 0.33$). In contrast, FA was more closely correlated with L in planar...
intersections when times are pooled ($r^2 \sim 0.5$) than in individual time points ($0.00 < r^2 < 0.49$).

Fractal dimensions from root projections and 2.5 cm planar intersections were correlated well with 3D FD across all sampling times (Figure 4.3). Fractal dimensions from 0 and 5 cm intersections had correlations with 3D FD that increased with time, but were always less than the correlations between 3D FD and root projections or 2.5 cm intersections. Fractal abundance from root projections and all planar intersections was highly correlated with 3D FA when all time points were pooled (Figure 4.3). Only FA from root projections was correlated with 3D FA when data was analyzed from individual time points. Only L from root projections was correlated with 3D L (Figure 4.3), and this correlation decreased over time, or by pooling time.

**Correlations with depletion volume**

Scatter plots for depletion volume versus root length and 3D fractal parameters are presented in Figure 4.4. The diffusion coefficient had some effect on the value of the correlations, but not on the patterns among fractal parameters or across time. For example, $r^2$ for correlation of root length with depletion volume ranged from 0.90 to 0.93 across diffusion coefficients tested, while $r^2$ for the correlation of fractal abundance with depletion volume ranged from 0.83 to 0.85. Therefore, only the results for $D_e = 10^{-8}$ are presented.

In simple regressions of 3D data, length had better correlation with depletion volume than any of the fractal parameters when time points are analyzed separately (Figures 4.4 and 4.5). The correlation of FA with depletion volume improved as $D_e$
increases, and the correlation of root length with depletion decreased with increasing $D_e$, but the correlation of depletion with root length remained better than that for depletion volume with FA over all $D_e$ simulated (not shown).

When time data were separated, all plots were linear, but when time data were pooled, the plot of depletion volume with FA became nonlinear (Figure 4.4). To make it linear, a log-log transformation was employed. The correlation of log(depletion volume) with log(FA) using pooled data was at least as high as than any other simple correlation tested for depletion volume (Figure 4.5).

Multiple regression improved correlations of fractal parameters with depletion volume for 3D and root projection data over all separately analyzed time points (Figure 4.5). Correlations in planar intersection data were also improved by using multiple regression, but the benefit decreased with time and distance from the root origin (Figure 4.5). For pooled time data, only in 5 cm intersections did multiple regression make correlations equivalent to the regression of log(depletion volume) with log(FA). Using all 3 fractal parameters in multiple regression did not improve correlations for 3D, projection, or any planar intersection data (not shown).

**Correlations with competition volume**

Scatter plots for competition volume versus root length and fractal parameters are presented in Figure 4.6. The diffusion coefficient had some effect on the correlations, but not on the patterns among fractal parameters or across time, so only the results for $D_e = 10^{-8}$ are presented.
Root length had better correlation with competition volume than any of the 3D fractal parameters, at each separately analyzed time point (Figures 4.6 and 4.7). Simple regression of FD, FA or L with competition volume at any one time point was much worse than the regression of length with competition volume. When time data were pooled, the plot of competition volume with FA was nonlinear (Figure 4.6). To make it linear, a log-log transformation was employed. The regression of log(competition volume) with log(FA) using pooled data resulted in correlation that was equivalent to the correlation between length and competition volume in 3d, and at least as good as any other correlations for projection and planar intersection data (Figure 4.7).

Multiple regression improved correlations for 3D fractal data with competition volume to the extent that fractal parameters became equivalent to root length at any single time point (Figure 4.7). There was also an improvement of correlations using root projection data over all time points. Correlations in planar intersection data were improved by using multiple regression, but the benefit decreased with time and distance from the root origin (Figure 4.7). For pooled time data, only in 5 cm intersections did multiple regression make correlations equivalent to the log(competition volume) with log(FA) regression. Using all 3 fractal parameters in multiple regression did not improve correlations for 3D, projection, or any planar intersection data (not shown).

Correlations with relative competition

Scatter plots for relative competition versus root length and fractal parameters are presented in Figure 4.8. The diffusion coefficient had some effect on the correlations,
but not on the patterns among fractal parameters or across time, so only the results for $D_e = 10^{-8}$ are presented.

Root length had better correlation with relative competition than any single 3D fractal parameter, regardless of time (Figures 4.8 and 4.9). Correlation of FD with relative competition improved with time, and became similar to the correlation of root length with relative competition when time was pooled. Multiple regression improved correlations for 3D fractal data with relative competition to the extent that fractal parameters became slightly better than root length (Figure 4.9). There was also an improvement of correlations using multiple regression with root projection data over all time points. Correlations in planar intersection data were improved by using multiple regression, but the benefit decreased with time and distance from the root origin (Figure 4.9). Using all 3 fractal parameters in multiple regression did not improve correlations for 3D, projection, or any planar intersection data (not shown).

Field measurements

There were significant differences (critical $p = 0.1$) in FD among genotypes, but not between phosphorus treatments (Figure 4.10). Genotypic and P effects were observed with FA (Figure 4.10). The phosphorus efficient RILs, 7 and 17, had the higher FD and FA in comparison to the matched phosphorus inefficient RILs, 38 and 24, respectively. The P inefficient RIL 38 had significantly lower FD and FA than RIL 7 in medium P. In the other test, the P inefficient RIL 24 had lower FD than the efficient RIL 17 in both medium and high P, along with a lower FA in high P. The FD and FA of mixed plots were always similar to the values obtained from plots of the P efficient RIL
grown in monoculture. Lacunarity did not vary except for a significantly lower L of RILs 7 and 38 grown together in high P compared with RIL 7 grown in monoculture in medium P.

DISCUSSION

This study provides evidence that fractal analysis can be improved by including FA or L along with FD when conducting research with plant roots. Fractal dimension has been correlated with topological and architectural traits (Fitter et al., 1992; Lynch and van Beem, 1993), but the physiological significance of these correlations has not been clearly established. Here, it is shown that fractal parameters may be correlated with uptake of diffusion limited nutrients. Fractal dimension is most closely correlated with relative competition, while fractal abundance is most closely correlated with depletion volume. This is in accordance with the hypothesis presented in the introduction that FD is associated with relative measures of exploration, while FA is associated with absolute measures.

The most relevant application of fractal analysis to the study of roots appears to be when more than one fractal parameter is used. Lacunarity alone appears to be of little use, but when combined with FD or FA, it may add significantly to correlations with measures of soil exploration. Similarly, FD alone has poor correlation with measures of soil exploration, but it adds significantly to correlations when combined with FA or L.

The only fractal parameter that appears to be useful by itself is FA, and then only when data from root harvests over time are combined. In these situations, log(FA) might be a relevant parameter to explore as a variable that can explain differences in
nutrient acquisition. When roots of one age are observed, FA may be of most use when combined with FD or L.

The hypothesis that depletion volume is more closely correlated with root length at low diffusion coefficients, and with fractal abundance at high diffusion coefficients was not observed with the diffusion coefficients tested in this study. Root length was highly correlated with depletion volume and competition volume regardless of diffusion coefficient. Therefore, root length may always be relevant for understanding soil depletion and root exploration. However, it is often difficult to measure. In this study, evidence is presented that fractal analysis of planar intersect data can be correlated with soil exploration. Planar intersection data appears to be most suitable when collected from less than 5 cm from the stem and early in the season, or with collection dates spread out over a week or more. Further testing in field situations would help clarify the validity and accuracy of these conclusions.

Field data provided some support for the validity of the modeling results. In spite of being collected at 5 weeks and from trenches 10 cm distant from the row, the analyzed field data revealed genotypic variation for FD and FA. In addition, genotypes that are efficient in phosphorus acquisition had the highest FD and FA, which is consistent with hypothesized correlations of FD and FA with spatial exploration, as well as previous results (Nielsen et al, 1998). Finally, L varied in only one case. This is consistent with the fact that heterogeneity of root distribution is not necessarily related to exploration efficiency. Modeling indicated that there could be situations where FA or L correspond with genotypic variation when FD does not. For example, increased basal root gravitropism could be offset by decreased lateral root density. In that case FD would
remain constant, while FA would decrease. No trends like this were observed in this limited field study. Inclusion of more genotypes would increase the chances of observing such cases.

The correlation of projection and planar intersection FD with 3D FD confirms and extends previous results (Nielsen et al, 1997). The previous results were for horizontal slices, while the results in this study are for vertical slices. The combination of these results indicates that planar FD can be correlated with 3D FD by slicing either way. On the other hand, 3D FA and L are correlated with projection FA and L, but not with planar intersection FA and L. This suggests that fractal analysis of 2D intersections may be good for estimating 3D FD, but not 3D FA or L. However, correlations with depletion and competition are still improved by calculating all three fractal parameters from planar intersections. In the field, genotypic and nutrient availability differences may be associated with differences in FD and FA calculated from the intersection of roots with trench faces.

In summary, this study supports previous reports of the validity of applying fractal analysis to the study of plant roots, and it extends our understanding of how the analysis applies to the biology of nutrient acquisition. Two parameters that are frequently neglected in fractal analysis, FA and L, significantly improve the relevance of fractal analysis for understanding biological functioning. In addition, FA and L are easily calculated using data and methods that are used for calculating FD. Plus, situations might arise where FA or L can differentiate among treatments that are not resolvable by FD. Therefore, future fractal analysis of plant root systems may be most appropriately
conducted when fractal abundance or lacunarity is calculated along with fractal dimension.

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Table 4.1. Root growth parameters used in simulations of bean root growth.

<table>
<thead>
<tr>
<th>Time (hr/d)</th>
<th>192/8</th>
<th>240/10</th>
<th>288/12</th>
<th>336/14</th>
<th>384/16</th>
</tr>
</thead>
<tbody>
<tr>
<td>De (cm$^2$s$^{-1}$)$^a$</td>
<td></td>
<td>$10^{-7}$</td>
<td>$10^{-8}$</td>
<td>$10^{-9}$</td>
<td></td>
</tr>
<tr>
<td>Seed depth (cm)</td>
<td></td>
<td></td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adventitious number</td>
<td>0</td>
<td>5</td>
<td>10</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>Basal root architecture$^b$</td>
<td>shallow</td>
<td>intermediate</td>
<td>Deep</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal lateral internode length (cm)</td>
<td>0.25</td>
<td>0.35</td>
<td>0.50</td>
<td>0.75</td>
<td></td>
</tr>
</tbody>
</table>

$^a$Diffusion coefficient of mineral nutrient through soil, based on Schenk and Barber, 1979

$^b$Based on Ge et al, 2000
Figure 4.1. Geometric models of common bean root systems generated by SimRoot. Pictured roots have 15 adventitious roots and a basal lateral branch spacing of 0.5 cm. Lines differ in basal root depth. Numbers for columns indicate time after germination in hours. Scale bar has tick marks every 5 cm.
Figure 4.2. Scatter plots of fractal dimension, fractal abundance and lacunarity of simulated root systems. Values shown are for 3D root systems (top) and a vertical slice of the root system taken 2.5 cm from the root origin (bottom). Different symbols indicate different times (h).
Figure 4.3. Coefficient of simple determination ($r^2$) between fractal parameters calculated for 3D root systems and for projection and vertical planar intersections at 0, 2.5, and 5 cm from root origin.
Figure 4.4. Depletion volume versus root length, fractal dimension, fractal abundance, and lacunarity. Diffusion coefficient equals $10^{-8}$ cm$^2$/s. Each figure contains data from 360 simulations with 5 times (192, 240, 288, 336, 384 hours from germination), 3 basal root angles (shallow, medium, deep), 4 basal lateral densities (0.25, 0.35, 0.5, 0.75 cm), and 6 adventitious numbers (0, 5, 10, 15, 20, 25). Regression lines are fitted for each time.
Figure 4.5. Coefficient of simple determination ($r^2$) or multiple determination ($R^2$) for correlation of depletion volume with root length, fractal dimension, fractal abundance, and lacunarity. Times 240 and 336 are omitted for clarity.

*Fractal abundance and depletion volume are untransformed when time points are analyzed separately, but they are each log transformed when time points are pooled.
Figure 4.6. Competition volume versus root length, fractal dimension, fractal abundance, and lacunarity. Diffusion coefficient equals $10^{-8}$ cm$^2$/s. Regression lines are fitted for each time.
Figure 4.7. Coefficient of simple determination ($r^2$) or multiple determination ($R^2$) for correlation of competition volume with root length, fractal dimension, fractal abundance, and lacunarity. Times 240 and 336 are omitted for clarity.

*Fractal abundance and competition volume are untransformed when time points are analyzed separately, but they are each log transformed when time points are pooled.
Figure 4.8. Relative competition versus root length, fractal dimension, fractal abundance, and lacunarity. Diffusion coefficient equals $10^{-8}$ cm$^2$/s. Regression lines are fitted for each time.
Figure 4.9. Coefficient of simple determination ($r^2$) or multiple determination ($R^2$) for correlation of relative competition with root length, fractal dimension, fractal abundance, and lacunarity. Times 240 and 336 are omitted for clarity.
Figure 4.10. Fractal analysis of root trench data from 4 RILs grown in the field in South China (Rubio et al., 2003). Error bars represent standard error of the mean. Bars within a single graph with the same letter above are not significantly different when compared by LSMean separation with critical p = 0.10.
CHAPTER 5

SUMMARY

OVERVIEW

This research contributes to understanding of root architecture and how it impacts phosphorus acquisition. While building on previous reports, new insights were generated into root development, physiology, and ecology. Potential applications exist in fields as diverse as theoretical biology and agricultural field research. Future testing, clarification, and expansion of conclusions will determine how this work is applied in practice.

The overall theme of this thesis is to address aspects of how common bean root architecture develops, how it can be measured, and how it impacts phosphorus acquisition efficiency. Specific projects were conducted to study basal root gravitropism, benefits and tradeoffs of allocation into adventitious roots, and utility of fractal analysis for assessing phosphorus acquisition efficiency. Each of these projects yielded significant results that warrant further consideration and suggest future research or applications. A brief overview of each research project will now be presented and followed by more detailed descriptions in separate sections for each project.

Gravitropism of common bean basal roots was demonstrated in gravistimulation experiments. Comparisons among genotypes and basal root whorls were conducted to test which if any components of graviresponsiveness might play a role in development of root architecture. Differences in curvature response, as well as time to reach maximum curvature were observed among genotypes and basal root whorls. However, the observed
variation is not sufficient to account for the diversity of basal root growth angles. An experiment with ethylene indicates that this signaling molecule is a modulator of graviresponsiveness in common bean basal roots. Based on all of the results, it is hypothesized that architecture of common bean basal roots is determined by interactions of several elements of gravitropism, including curvature and timing of responsiveness, along with modulation of graviresponsiveness by ethylene.

In one predominantly model based project, the contribution of adventitious roots for phosphorus acquisition was quantitatively assessed. The conclusion is that adventitious roots are beneficial for phosphorus acquisition in stratified soil with more phosphorus available near the surface, but not in soil with phosphorus uniformly available throughout the profile. The benefit of peaked with 30 adventitious roots. There was no interacting effect of planting depth or basal root growth angle for a plant with a given number of adventitious rooting. On the other hand, the benefit of adventitious rooting for phosphorus acquisition was sensitive to the rate of respiration within adventitious roots. Adventitious roots with a respiration rate that is greater than the rate in basal roots become less beneficial for phosphorus acquisition. At rates double to that in basal roots, adventitious roots are detrimental for phosphorus acquisition. The relevance of this project is that breeders may use the information when testing for traits associated with phosphorus efficiency. According to these results, genotypes with shallow basal roots that produce large numbers (~30) of adventitious roots that respire at a low rate will be the most efficient in phosphorus acquisition.

The final project presented in this thesis is also predominantly based in modeling, but with the subject of exploring the utility of fractal analysis for
understanding efficiency of phosphorus acquisition. To do so, 3 terms of fractal analysis were included, one, fractal dimension, which has been commonly used for roots, another, fractal abundance, which has rarely been used in analysis of roots, and a third, lacunarity, which has not been previously applied to roots. The log of fractal abundance is correlated with the log phosphorus depletion when roots are observed over multiple time points. Other than this, fractal parameters are most useful for studying phosphorus acquisition and efficiency when combined in multiple regression analysis. Root length has higher correlation with phosphorus depletion or competition for phosphorus. Still, fractal parameters are beneficial, because they may be quantified from 2 dimensional slices, which are much easier to observe in practice than entire root systems. A field study corroborated the utility of fractal abundance and fractal dimension collected from 2 dimensional trench observations for distinguishing among bean genotypes that vary in phosphorus efficiency. The overall conclusion of this project is that root length and fractal parameters are all useful for understanding efficiency of phosphorus acquisition. Fractal parameters are most applicable when combined in multiple regression analysis.

BASAL ROOT GRAVITROPISM

This project followed previous observations of variation in basal root growth angles among common bean genotypes (Bonser et al., 1996; Liao et al., 2001). These observations, along with reports of gravitropism in secondary roots of other species (Kiss et al., 2002; Mitchell and Russell, 1971; Mullen and Hangarter, 2003; Yamashita et al., 1997), led to the hypothesis that basal roots of common bean are gravitropic, and that variation in graviresponsiveness exists among genotypes. In addition, phosphorus and
ethylene were hypothesized to have effects on basal root gravitropism based on previous reports of effects on shallowness (Bonser et al., 1996; Zhang, 2002).

The results presented in this thesis demonstrate that basal roots of *P. vulgaris* are gravitropic. Basal roots of all 5 of the genotypes observed responded to gravistimulation by reorienting growth angle with respect to gravity. Variation in graviresponsiveness was observed among genotypes and within individual root systems. Phosphorus was observed to have an effect on responsiveness of one genotype, G19833, that has also been reported to be affected by phosphorus in basal root growth angle. Experiments to test kinetics of graviresponsiveness resulted in observations of differences in curvature and timing to maximum curvature observed between the two genotypes tested, DOR364 and G19833. In addition, effects of ethylene in increasing variance of responses suggest that ethylene modulates gravitropism in common bean. Modulation of responses to gravistimulation by ethylene, without being required for graviresponsiveness is in accordance a previous report (Lee et al., 1990). The new aspect of ethylene effects presented here is how it contributes to concepts of root architectural development. The combination of several processes, curvature and time to reach maximum graviresponsiveness of basal roots, as well as modulation by ethylene, may be important factors in establishment of 3 dimensional root architecture.

Presently, the results of this project are difficult to explain, because there are no simple trends in responses among basal roots within a root system or among genotypes. First, there was no clear relationship between basal root graviresponsiveness and growth angle of untilted control roots observed in this study or previously reported basal root depth (Bonser et al., 1996; Liao et al., 2001). Second, graviresponsiveness of two
genotypes with deep basal roots, DOR364 and G2333, differed more from each other than from shallow-rooted genotypes. Finally, response to gravistimulation varied among whorls only for G19833 and G19839, and the deeper basal roots of G1833 responded less than those of the uppermost whorl, which is opposite to the trend predicted by comparing tap roots with basal roots. 

To fully explain any roles of gravitropism in development of root architecture, many questions remain. These include the following. Asymmetric basipetal auxin transport is considered important for gravitropic responses (Blancaflor and Masson, 2003). What role does this signaling molecule play in graviresponsiveness of common bean basal roots? Is any of the variation in graviresponsiveness observed among genotypes or among basal roots within a root system explainable in terms of variation in auxin transport? Ethylene appears to modulate graviresponsiveness, but how is unknown. Does endogenous ethylene production vary among basal roots within a root system or among genotypes? Where does ethylene act in the pathway of graviresponsiveness? Ethylene has previously been shown to affect basal root shallowness (Zhang, 2002) and graviresponsiveness in this report, but how much variation in root architecture does it explain? Basal root angle is determined by initial angle upon emergence and dynamic angle changes with growth. Are differences in graviresponsiveness and modulation by ethylene sufficient for explaining dynamic aspects of basal root growth angles? If two genotypes with equivalent basal root angles of emergence and graviresponsiveness have ethylene action blocked, will identical growth angles result? Finally, how does phosphorus affect basal root growth angles and
graviresponsiveness of some, but not all, genotypes? At what physiological step might phosphorus affect graviresponsiveness?

While more research is necessary to fully explain these basal root gravitropism results, there are still benefits to this work. As with other species, gravitropism of secondary roots is confirmed in common bean. In contrast to work with other species, a number of genotypes were tested in this work. Variation in basal root graviresponsiveness, along with the effect of ethylene on variance provide evidence for the involvement of two components of gravitropism, potential responsiveness and modulation of response, in development of root architecture. Observations in this work provide the background information useful to formulating more specific hypotheses that can be tested for further understanding of root architectural development. Root architecture is the visible product of complex genetics, biochemistry, and physiology. In the long term, further work building upon these results may help elucidate key molecular and biochemical components that can be selected or manipulated for increased productivity through matching root architecture with environment.

CONTRIBUTION OF ADVENTITIOUS ROOTS TO PHOSPHORUS ACQUISITION

Simulation results presented in this thesis support field and greenhouse observations by Miller et al. (2003) that adventitious roots contribute to efficiency of phosphorus acquisition. The contribution of these simulations is to quantify potential benefits, predict costs and tradeoffs, and to establish potential limits on the benefits of allocation to adventitious roots.
Simulated root systems closely resembled those harvested from 2 genotypes grown in a greenhouse setting. The results imply that adventitious root proliferation comes at the expense of tap and basal lateral roots. Thicker main axes roots grow near the surface at the expense of thinner lateral roots growing in deeper soil. Therefore, total root length decreases with increasing adventitious proliferation.

As a result of increased shallow root length and decreased total root length, production of adventitious roots increases depletion of shallow soil, but, also leads to a reduction of overall soil depletion. In addition to increasing depletion of topsoil, increasing allocation to adventitious roots leads to higher overall competition. However, the effect on competition is less than the effect on shallow soil depletion. Therefore, allocation to adventitious roots is beneficial in stratified soil with more phosphorus available near the surface, but not when phosphorus is uniformly available throughout the root zone. It is expected that adventitious roots will be even more beneficial than reported here if they can be produced at the expense of basal and tap main axes, but which maintain lateral root proliferation and total soil exploration.

The benefit of allocation to adventitious roots appears to be applicable under a wide range of conditions. The diffusion coefficient of phosphorus in soil did not affect the magnitude of adventitious benefit. Nor did basal root angle. Basal roots and adventitious roots seem to be complementary. The most efficient architecture for phosphorus acquisition is one with shallow basal roots and large numbers of adventitious roots. In other words, adventitious roots may be beneficial for phosphorus acquisition in a wide range of soil types and in combination with a diversity of root architectures. In addition, for a given number of adventitious roots, planting depth only changed
phosphorus acquisition when seed was planted below phosphorus rich surface soil, and thereby removing basal roots from access to highly available phosphorus. In practice, planting seed deeper results in more adventitious proliferation. Seed may be planted deeper to stimulate adventitious root production, without harming phosphorus acquisition, as long as the seed is in phosphorus rich surface soil.

Allocation to adventitious roots is beneficial only as long as respiration rates in adventitious roots are similar to those in basal roots. If adventitious root respiration is significantly higher than basal root respiration, then less carbon is available for growth and any benefits of proliferation near the surface are negated. This is relevant, because the range of adventitious root respiration rates has been observed to be much wider than rates of other root types, with phosphorus inefficient genotypes having higher respiration rates in low phosphorus conditions than inefficient genotypes (Ho et al., 2003). Simulation of adventitious root respiration in the high end of the range observed by Ho et al. results in allocation to adventitious roots being detrimental for phosphorus acquisition. In addition, temperature variation in the soil might affect respiration rates among genotypes. Genotypes with adventitious root respiration acclimated to warmer surface temperatures might be able to maintain relatively low adventitious respiration.

The maximum benefit of adventitious roots in these 3 week simulations was to increase phosphorus acquisition by 10%. Running simulations out to 4 weeks did not change this relative impact. At 4 weeks, a 10% increase in phosphorus acquisition translates into 2 mg of phosphorus. Assuming this trend continues, then adventitious roots may account for over 10 mg of phosphorus acquisition over the course of a growing season. Given that adventitious roots explore surface soil throughout a growing season,
and phosphorus content of over 200 mg has been reported for common bean (Nielsen et al., 2001), then it is reasonable to conclude that this 10 mg estimate is conservative. In addition, a 10% increase of phosphorus acquisition allows for greater leaf expansion in the short term (Pellerin et al., 2000), which results in more photosynthesis that cycles back as higher C allocation to the root system. Over the course of a growing season, these feedback cycles may turn an initial 10% increase in phosphorus acquisition into a several fold gain in phosphorus content and yield (Wissuwa, 2003). Currently, SimRoot does not contain any feedback between phosphorus acquisition, leaf expansion, photosynthesis, and C allocation to roots. Therefore, it is likely that slowly respiring adventitious roots on plants growing in stratified soil account for phosphorus acquisition of well over 10 mg, possibly 25-50 mg, over the course of a growing season.

These simulations were run assuming that phosphorus is the sole constraint on root growth. In the field, multiple stresses are typically encountered through the course of a growing season. Plants must allocate resources between competing sinks that fulfill a variety of functions. Optimal allocation may be determined by marginal benefit of alternative pathways (Lynch and Ho, 2004). Overall, plant phenotype is a complex expression of competing interests that cannot be easily summed up by single resource limitation or multiple limitation models (Rubio et al., 2003). This will affect the application of this model to observations of field situations and selection of optimal varieties for yield in a variety of environments.

Areas for future refinement of this model exist that could produce more accurate results and predictions. Respiration may be varied within a root type either temporally or spatially. Root respiration is expected to change diurnally and as roots age. Aerenchyma
formation could be included to vary respiration along a root axis. Respiration of laterals may be considered separately from parent roots. Changes in allocation to adventitious roots can be modeled as changes in growth rate along with changes in adventitious number. Possibly the most important refinement might be inclusion of feedback between multiple shoot and root processes.

The results presented in this thesis lead to predictions that may be useful in management and breeding situations. For example, planting seed deeper to stimulate more adventitious production is expected to be beneficial only as long as seed is planted in phosphorus rich surface soil where basal roots have access to high phosphorus strata. In addition, this report might aid in the selection of potential phosphorus efficient varieties. Simulations predict that adventitious and basal roots are complementary for phosphorus acquisition, and high C allocation to roots is necessary to derive maximal benefit of adventitious rooting. The most phosphorus efficient genotypes are expected to have shallow basal roots, significant allocation to adventitious roots, which respire at rates similar to basal roots, and, finally, an ability to maintain high allocation of C to the root system.

FRACTAL ANALYSIS

Simulated root systems were analyzed as fractal objects over a finite range of scales. The contributions of this work were to include parameters infrequently used in root biology, and to test for correlations of fractal parameters with efficiency of soil exploration. The fractal parameters investigated were fractal dimension (FD), fractal abundance (FA), and lacunarity (L).
This study provides evidence that fractal analysis can be improved by including FA or L along with FD when conducting research with plant roots. It is shown that fractal parameters may be correlated with uptake of diffusion limited nutrients. Fractal dimension is most closely correlated with relative competition, while fractal abundance is most closely correlated with depletion volume.

The most useful application of fractal analysis to the study of roots appears to be when more than one fractal parameter is used. Lacunarity alone appears to be of little use, but when combined with FD or FA, it may add significantly to correlations with measures of soil exploration. Similarly, FD alone has poor correlation with measures of soil exploration, but it adds significantly to correlations when combined with FA or L. The only fractal parameter that appears to be useful by itself is FA, and then only when data from root harvests over time are combined. In these situations, log(FA) might be a relevant parameter to explore as a variable that can explain differences in nutrient acquisition. When roots of one age are observed, FA appears to be of most use when combined with FD or L.

Root length was highly correlated with depletion volume and competition volume regardless of diffusion coefficient. Therefore, root length may always be relevant for understanding soil depletion and root exploration. However, it is often difficult to measure. In this study, evidence is presented that fractal analysis of planar intersect data can be correlated with soil exploration. Planar intersection data appears to be most suitable when collected from less than 5 cm from the stem and early in the season, or with collection dates spread out over a week or more. Further testing in field situations would help clarify the validity and accuracy of these conclusions.
Field data supported modeling results. In spite of being collected at 5 weeks and from trenches 10 cm distant from the row, the analyzed field data revealed genotypic variation for FD and FA. In addition, genotypes that are efficient in phosphorus acquisition had the highest FD and FA. Lacunarity was not useful for assessing genotypic variation in this limited field study. Modeling indicates that there could be situations where FA or L correspond with genotypic variation when FD does not. For example, increased basal root gravitropism could be offset by decreased lateral root density. In that case FD would remain constant, while FA would decrease. No trends like this were observed in this study. Inclusion of more genotypes would increase the chances of observing such cases.

The correlation of projection and planar intersection FD with 3D FD confirms and extends previous results (Nielsen et al., 1997). On the other hand, 3D FA and L are correlated with projection FA and L, but not with planar intersection FA and L. This suggests that fractal analysis of 2D intersections may be good for estimating 3D FD, but not 3D FA or L. However, correlations with depletion and competition are still improved by calculating all three fractal parameters from planar intersections.

In summary, this study supports previous reports of the validity of applying fractal analysis to the study of plant roots, and it extends understanding of how the analysis applies to the biology of nutrient acquisition. Two parameters that are frequently neglected in fractal analysis, FA and L, significantly improve the relevance of fractal analysis for understanding biological functioning. In addition, FA and L are easily calculated using data and methods that are used for calculating FD. Plus, situations might arise where FA or L can differentiate among treatments that are not resolvable by FD.
Therefore, future fractal analysis of plant root systems may be most appropriately conducted when fractal abundance or lacunarity is calculated along with fractal dimension.

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EDUCATION

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PUBLICATIONS

Architectural tradeoffs between adventitious and basal roots for phosphorus acquisition
Walk TC, Jarmillo R, Lynch JP
accepted

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for phosphorus acquisition in Arabidopsis thaliana: A modeling approach
Ma Z, Walk TC, Marcus A, Lynch JP
Plant and Soil 236 (2): 221-235 October 2001

Flow cytometric analysis and sorting of Heterodera glycines eggs

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