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The effect of phosphorus availability on the carbon economy of contrasting common bean (*Phaseolus vulgaris* L.) genotypes

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Abstract

A common response to low phosphorus availability is increased relative biomass allocation to roots. The resulting increase in root : shoot ratio presumably enhances phosphorus acquisition, but may also reduce growth rates by diverting carbon to the production of heterotrophic rather than photosynthetic tissues. To assess the importance of increased carbon allocation to roots for the adaptation of plants to low P availability, carbon budgets were constructed for four common bean genotypes with contrasting adaptation to low phosphorus availability in the field ('phosphorus efficiency'). Solid-phase-buffered silica sand provided low (1 μM), medium (10 μM), and high (30 μM) phosphorus availability. Compared to the high phosphorus treatment, plant growth was reduced by 20% by medium phosphorus availability and by more than 90% by low phosphorus availability. Low phosphorus plants utilized a significantly larger fraction of their daytime net carbon assimilation on root respiration (*c.* 40%) compared to medium and high phosphorus plants (*c.* 20%). No significant difference was found among genotypes in this respect. Genotypes also had similar rates of P absorption per unit root weight and plant growth per unit of P absorbed. However, P-efficient genotypes allocated a larger fraction of their biomass to root growth, especially under low P conditions. Efficient genotypes had lower rates of root respiration than inefficient genotypes, which enabled them to maintain greater root biomass allocation than inefficient genotypes without increasing overall root carbon costs.

Key words: Biomass allocation, *Phaseolus vulgaris* L. (common bean), phosphorus efficiency, root respiration, root:shoot ratio.

Introduction

Allocation of carbohydrates to various plant parts and functions is a governing parameter of plant survival and success. The carbon resources generated through photosynthesis are either utilized in respiration, exported to the rhizosphere or used for construction of plant tissues and make up most of the dry weight (DW) of its organs. The fraction of carbohydrates that is lost through respiration is determined by the overall metabolic efficiency of the plant. Plant ontogenetic developmental processes and environmental conditions affect the size of this fraction. The success of plants under stress conditions may be determined by their ability to control carbohydrate utilization for metabolic energy. The remaining carbohydrates are allocated to the construction of the various plant organs and accumulated as reserves.

Terrestrial plants acquire resources from two parts of their environment. Carbon dioxide fixation and light absorption takes place in the plant shoot parts, mostly leaf blades. All the other materials are taken up by the roots from the soil. The partitioning of structural material among the various plant organs is determined by their genetic traits, ontogenetic development and environmental conditions. Performance of most food crops is evaluated by their ability to allocate enough materials to the seeds at the end of the season. Two contrasting theories have been proposed for description of the rules that govern carbohydrate partitioning between plant

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shoots and roots. The 'Functional Equilibrium' theory (Brouwer, 1983) suggests that allocation of materials between shoots and roots is determined dynamically by the relative availability of resources in their respective parts of the environment. Whenever edaphic resources are in ample concentration more carbohydrates will be allocated to the shoot resulting in shoot growth and increased capacity for carbon assimilation. However, when edaphic resources are scarce an excess amount of carbohydrates will be accumulated in the shoot and will not be utilized for further growth because of lack of minerals. In such a case, these carbohydrates will be transported to the roots increasing their size and improving mineral supply. The consequence of this theory is that environmental conditions are the major determinant of root:shoot ratio of the plant.

The alternative view (Hunt, 1975, 1990) emphasized the inherent allometric relationships among the different organs of the plant. According to this theory the size ratios among the different parts of the plant are governed by a power function characteristic to each genotype. The exponent, called the 'Allometric Coefficient' (K), determines how the root:shoot ratio changes with plant size. In most plants K is less than unity, meaning that root:shoot ratio decreases as overall size of the plant grows larger. Seedlings of most plants grow their root before they grow the stem and leaves. This relative dominance of the root over the shoot diminishes gradually as the plant grows. Root:shoot ratio is thus in tight relationship with overall plant size. If the plant growth is inhibited by stressful conditions in the root environment it tends to maintain the higher root:shoot ratio characteristic of small plants. Genotypes differ in the magnitude of K, and thus in their allocation of materials to the various organs throughout their life. Internal control mechanisms that act through hormone action, sink:source relationships and other growth control functions, will determine the root:shoot ratio of the plant at all times.

Phosphorus-deficient plants exhibit reduced shoot growth and increased root:shoot DW ratio (Whiteaker *et al*., 1976). The decrease in shoot growth of P-deficient plants is a direct consequence of a reduction of leaf expansion and reduced leaf initiation (Lynch *et al*., 1991), possibly caused by decreased root hydraulic conductance (Radin and Eidenbrock, 1984) and reduced transport of cytokinins from root to shoot (Horgan and Wareing, 1980; Salama and Wareing, 1979).

The reduction of bean growth that is caused by low P availability has been associated with relatively increased below-ground biomass and reduction of leaf appearance rate, rather than with decreased specific C assimilation by the leaves (Lynch, *et al*., 1991; Lynch and Beebe, 1995). In a recent study it was shown that root respiration of bean plants grown under low P conditions, represented as a fraction of the whole plant C budget, was approximately twice that of plants grown under moderate P stress (Nielsen *et al*., 1998).

The performance of plants when grown on soils with low P availability is determined by their P efficiency. Here efficiency is defined as plant growth and seed yield with suboptimal P availability. Several possible mechanisms have been proposed for improving the P efficiency of crop plants, such as reduced tissue P requirements and increased seed reserves (Lynch and Beebe, 1995), mycorrhizal symbiosis (Koide, 1991) and root architecture and plasticity (Bonser *et al*., 1996; Fitter and Stickland, 1991; Kirk and Du, 1997; Lynch and Beebe, 1995; Nielsen *et al*., 1994; Vanvuuren *et al*., 1997). Furthermore, since root systems are composed of various root types that have distinct properties with respect to nutrient uptake (Eshel and Waisel, 1996) and construction cost (Eissenstat, 1992) a change of root system composition may also improve its P uptake efficiency. Variation in growth and architecture of roots among genotypes within a species has been observed in a wide range of species, for example, barley (Grando and Ceccarelli, 1995), bean (Lynch and van Beem, 1993), rye (Baon*et al*., 1994), soybean (Pantalone *et al*., 1996), white clover (Caradus *et al*., 1995), and the desert shrub *Gutierrezia sarothrae* (Wan *et al*., 1996). Plasticity in root properties such as root growth, root architecture, root system composition, and mycorrhizal infection could be beneficial for plants adapting to unfavourable soil conditions, such as low P, as often observed in weathered tropical soils (CIAT, 1987).

Carbon (C) expended in root respiration can amount to 10–30% of net photosynthesis under favourable conditions (Lambers *et al*., 1996) and has been found to increase under unfavourable conditions such as temperature extremes (Sisson, 1983; Bouma *et al*., 1997*a*), drought (Sisson, 1989), low light intensity (Hansen and Jensen, 1977), low nitrate supply (van der Werf *et al*., 1992), and low phosphorus (P) availability (Lynch and Beebe, 1995; Nielsen *et al*., 1998). Root respiration can be partitioned into costs for growth, maintenance (McCree, 1970; Thornley, 1970; Lambers and Steingröver, 1978) and ion uptake (Veen, 1980; van der Werf *et al*., 1988; Bouma *et al*., 1996). Poorter *et al*. speculated about fast growing species having lower specific respiratory costs for ion uptake than slow growing species (Poorter *et al*., 1991). However, Scheurwater *et al*. determined specific costs for growth, maintenance of biomass and ion transport in fast- and slow-growing grass species and concluded that respiratory costs associated with ion uptake are clearly higher in the slow-growing species (Scheurwater *et al*., 1998). This is mostly due to the relatively high nitrate efflux (inefficient net nitrate uptake) in slow-growing grasses (Scheurwater *et al*., 1999). When plants are deprived of phosphate for extended periods, vacuolar P stores become exhausted, and this is followed by reductions in cytoplasmic P concentration (Lauer *et al*., 1989).

Other work suggests that alternative pathways of glycolytic carbon flow and mitochondrial electron transport allows respiration to proceed in plants during severe P deficiency (Theodorou and Plaxton, 1993). Respiration of pea roots was not found to be affected by P starvation (Rychter *et al*., 1992). When plants are P stressed to an extent that growth is reduced, respiratory costs of growth and nutrient uptake will be affected.

The objective of this study was to test the hypothesis that differences in the P efficiency of plants can be due to differences in below-ground C allocation between root respiration and root growth and, more specifically, that the respiratory costs of roots are higher in inefficient plants than in efficient ones. Plants, contrasting genetically in P efficiency, were used in order to determine if these processes are important in accounting for P efficiency differences.

Materials and methods

Plant material

Seeds of common bean (*Phaseolus vulgaris* L.) genotypes DOR364, G19839, G1937, and 'Porillo Sintetico' (CIAT germplasm accession G4495) were obtained from CIAT (Cali, Colombia). The genotypes DOR364 and 'Porillo Sintetico' have been characterized as P-inefficient and the genotypes G19839 and G1937 have been characterized as P-efficient based on plant growth and seed yield in relation to availability of P. The Mesoamerican genotype DOR364 has an indeterminate erect bush habit (Type II) and in field studies has been characterized as P-inefficient yet responsive to P fertilization (CIAT, 1987). The land race 'Porillo Sintetico' (P.S.) from El Salvador is also an indeterminate erect bush habit (Type II), and yields well in favourable soil environments, but poorly under low P conditions. Its physiology has been studied extensively at CIAT (Laing *et al*., 1984; Lynch and van Beem, 1993). The genotype G19839 is of Andean origin and has an indeterminate prostrate growth habit (Type III), shallow root system, large seeds, and efficient P acquisition leading to excellent adaptation to low P soil environment. The genotype G1937 has also been found to yield well under low soil P availability. It has an indeterminate prostrate growth habit (Type III), shallow root system, and medium sized seeds. The experiment was started on 5 February, 1995. Seeds were surface-sterilized in 7 mM NaOCl and 0.1% Triton X-100 (Sigma Chemical Co., St Louis, MO) for 10 min, and germinated in rolls of germination paper (Anchor Paper Co., St Paul, Minnesota) soaked with 0.5 mM $CaSO_4$ for 48 h at 25 °C. The seedlings were then planted at a depth of 3 cm. Planting as well as gas exchange measurements and destructive harvests were staggered for 2 d among the four main blocks, giving a total of four replicates per treatment.

Growth conditions

Plants were grown in 20 l plastic pots filled with acid-rinsed solid-phase-buffered silica sand (Lynch *et al*., 1990) providing a constant availability of low (1 μM), medium (10 μM), and high (30 μM) P concentration in the soil solution. Twice daily (07.00 h and 14.00 h), the pots were irrigated with nutrient solution containing (in mM) 3.1 NO_3, 1.8 K, 1.2 Ca, 1.4 SO_4, 1.0 NH_4, 0.825 Mg, 0.05 Cl, and (in μM) 5 Fe-EDTA, 2 B, 1.5 Mn, 1.5 Zn 0.143, Mo, and 0.5 Cu in addition to the concentration of KH_2PO_4 as described above for the different P treatments. The plants were grown in a temperature-controlled greenhouse in University Park, Pennsylvania (40°49′ N, 77°49′ W), in February, March, and the beginning of April 1995. Temperature ranged from a minimum of 22 °C (night) to a maximum of 30 °C (day). Maximum midday photosynthetic flux densities reached 1440 μmol photons m^{-2} s^{-1} on clear days and 450 μmol photons m^{-2} s^{-1} on days with heavy cloud cover. The plants were 8-weeks-old at the last harvest. Tissue DW and P content was measured at planting, and at 14 d (early vegetative), 28 d (late vegetative), 42 d (flowering), and 56 d (podfill) after planting (DAP).

Experimental design

Each genotype was grown at each P treatment level (high, medium, and low). The four replicates were planted and measured 2 d apart to minimize the effect of daily differences in environmental conditions on gas exchange parameters. Plant and treatment positions were arranged in a randomized complete block design with time of planting and measurement as the block.

Gas exchange measurements

Extensive studies of bean and citrus root respiration in soil (Bouma *et al*., 1997*a*, *b*), showed that in such conditions root respiration was not affected by soil moisture content or soil CO_2 concentration, and there was no significant difference observed in root respiration when comparing 'head space' measurements to 'perfusive' measurements. Rate of root respiration was in these studies strongly affected by diurnal fluctuations in temperature.

At 28 DAP the root system was sealed off from the shoot by a PVC plate positioned on top of the pot. An air pump provided a constant flow of air through the 'head space' compartment of the pot, ensuring that the 'head space' CO_2 concentration remained relatively low and that the 'head space' temperature only increased slightly during daytime measurements. The shoots were enclosed for a short (*c*. 5 min) measurement period in a clear plexiglass chamber of known volume sealed on top of the PVC plate. Shoot and root CO_2 exchange rates were measured separately with a portable infra-red gas analyser (Li-Cor 6200, Li-Cor, Lincoln, Nebraska) connected via tubes in a closed-system mode either to the plexiglass chamber enclosing the shoot or the pot 'head space'. In order to estimate the daily course of C flux, measurements were taken four times each day: mid-morning (09.30–10.30 h), noon (11.30–12.30 h), mid-afternoon (15.30–16.30 h), and 2 h after sunset (20.30–21.30 h). Root respiration was measured at the same times. A preliminary experiment had shown that shoot and root respiration rates did not change throughout the night. Immediately before the root respiration measurement, the airflow going through the 'head space' of the pot was interrupted, and it was connected to a closed-system IRGA. The rate of CO_2 accumulation to *c*. 1000 ppm in the 'head space' was measured within a few minutes. Numerical analysis was used to integrate shoot and root CO_2 flux measurements and determine daytime net shoot C assimilation, night-time shoot respiration, and root respiration (Lynch and Rodriguez, 1994).

Biomass determination

Plants were harvested at planting and 14, 28, 42, and 56 DAP. Shoots were separated into leaves, stem, and flowers and pods.

Petioles were included in the stem fraction. Roots were separated from the sand and rinsed in deionized water. Leaves, stems, and roots were dried at 60 °C for 48 h prior to DW determination. Root–shoot allometric coefficient (K) was derived from series of paired measurements of root DW and shoot DW by linear regression of the form:

$$K=(\log R - \log b)/\log S$$

where R is root DW (g), S is shoot DW (g), b is a constant, and K is the allometric coefficient (from Hunt, 1990). Relative growth rate (RGR) at 14, 28 and 42 DAP was calculated using numerical differentiation formulas based on least squares fitting of 2nd degree polynomials to three biomass measurements evenly spaced over time (Erickson, 1976).

Tissue analysis and rate parameter calculations

Dry samples of root, stem, and leaf blade tissue were ground and analysed for P content colorimetrically (Murphy and Riley, 1962). Specific P absorption rate (SPAR) is a measure of the net P absorption rate per unit root DW (mg P g^{-1} root DW d^{-1}), over the interval t_1 to t_2

$$\text{SPAR}=(M_2 - M_1)/(t_2 - t_1)\times(\log_e R_2 - \log_e R_1)/(R_2 - R_1)$$

where M is the P content per plant, and R is root DW (g) (from Hunt, 1990).

Specific P utilization rate (SPUR) is a measure of DW return for P uptake (the productive efficiency). SPUR expressed DW increase rate per unit of P content (g DW mg^{-1} P d^{-1}), over the interval t_1 to t_2

$$\text{SPUR}=(W_2 - W_1)/(t_2 - t_1)\times(\log_e M_2 - \log_e M_1)/(M_2 - M_1)$$

where M is the P content per plant, and W is plant DW (g) (from Hunt, 1990).

Statistical analysis

Data were analysed with SAS JMP statistical package version 3.2.1 (SAS Institute, 1996). Gas exchange data (C used in root respiration, shoot respiration, and net C gain presented as fraction of total C fixation), SPAR, SPUR, plant DW, plant P content, and allometric coefficient were analysed by multiple ANOVA (randomized block design) for main effects and first order interactions. Analysis of covariance was applied for the interaction between RGR and rate of root respiration (SAS Institute, 1996). Bean genotypes (G1937, G19839, DOR364, and P.S.), P levels (high, medium, and low), and time (14, 28, 42, and 56 DAP for specific absorption rate and harvest data and 28 DAP for gas exchange data) were the independent variables.

Results

Plant growth

Plant DW increased over time for all genotypes and all P treatments. The growth of medium P plants was reduced by approximately 20% and low P plants by more than 90% compared to high P plants at the last harvest date (Fig. 1). The decrease in plant DW under low P conditions was more severe in the two inefficient genotypes, DOR364 and Porrillo Sintetico, than in the efficient genotypes, G1937 and G19839 (Fig. 1, Low P), and the RGR of the efficient genotypes was higher as indicated by the higher slope, especially in G1937. Under low P conditions this was significantly different from 42 DAP, whereas it was less pronounced under medium and high P conditions.

The root–shoot allometric coefficient (Hunt, 1990) was higher in efficient compared to inefficient genotypes (Fig. 2; Table 1). As the plants grew larger the efficient genotypes allocated a larger fraction of their assimilated C towards root growth, as indicated by their higher root-shoot allometric coefficient (Table 1). Inefficient genotypes had significantly lower root–shoot allometric coefficients when grown under low P conditions. This is indicated by the significant interaction between genotype and P-level in the ANOVA (Table 1).

Phosphorus uptake and utilization

P uptake continued throughout the experiment in all treatments and plant P content increased over time together with plant DW for all genotypes (Fig. 3). The low P plants were seriously P stressed, especially inefficient genotypes. At 56 DAP the efficient genotypes had managed to take up twice the amount of P at low P compared to inefficient genotypes.

Specific P absorption rate (SPAR) increased by increasing P availability (Fig. 4). There was no significant

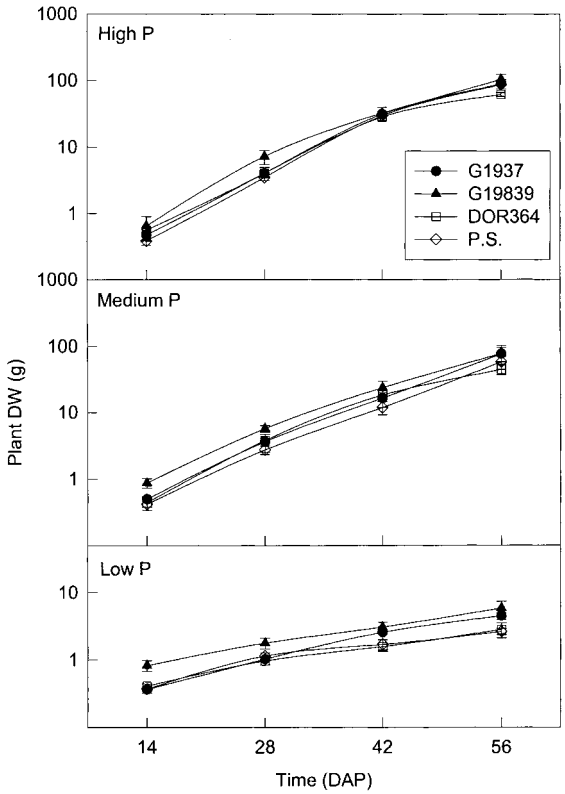

Fig. 1. Plant dry weight (log scale) at 14, 28, 42, and 56 d after planting of four common bean genotypes, grown under low, medium, and high P conditions. Data shown are mean ± standard error of the mean (n=4).

difference among genotypes in their P absorption rates, although G19839 had higher absorption rates early after planting than the other genotypes. For plants grown under low P conditions SPAR was very low and not significantly different among genotypes.

Specific P utilization rate (SPUR) was lower in the period from planting to 14 DAP than in the time after that (Fig. 5), indicating that seed P reserves were not depleted in that period. There was no significant difference among genotypes in SPAR and SPUR and there was no distinct effect of soil P availability on SPUR. The lack of genotypic differences could indicate that the differences in P efficiency among the four contrasting genotypes are not related to uptake and utilization characteristics. As SPAR describes net absorption of P it does not give

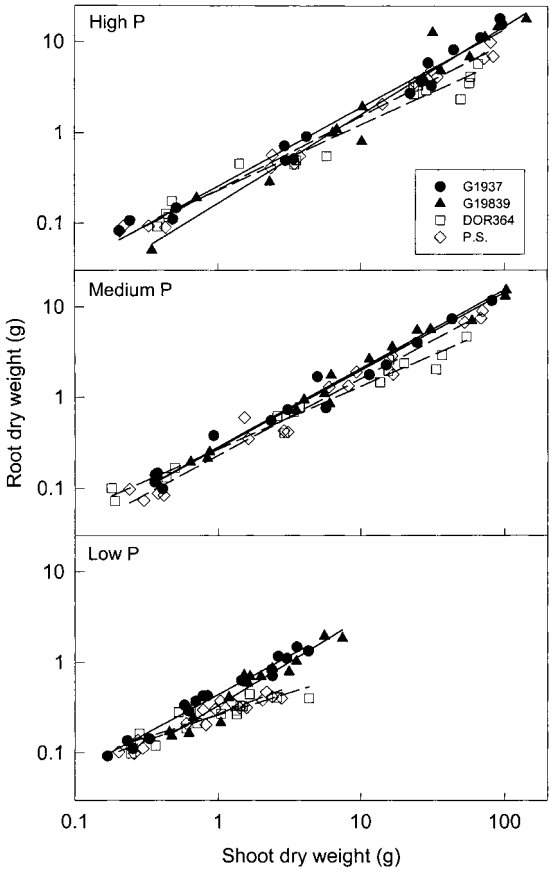

Fig. 2. Allometric relationships between the root dry weight (log scale) and shoot dry weight (log scale) of four common bean genotypes, grown under low, medium and high P conditions measured at 14, 28, 42, and 56 d after planting. Correlation coefficients for linear regressions for the efficient genotypes (solid lines) and inefficient genotypes (dashed lines) were all higher than 0.89 (Table 1) (n=16), thus significant.

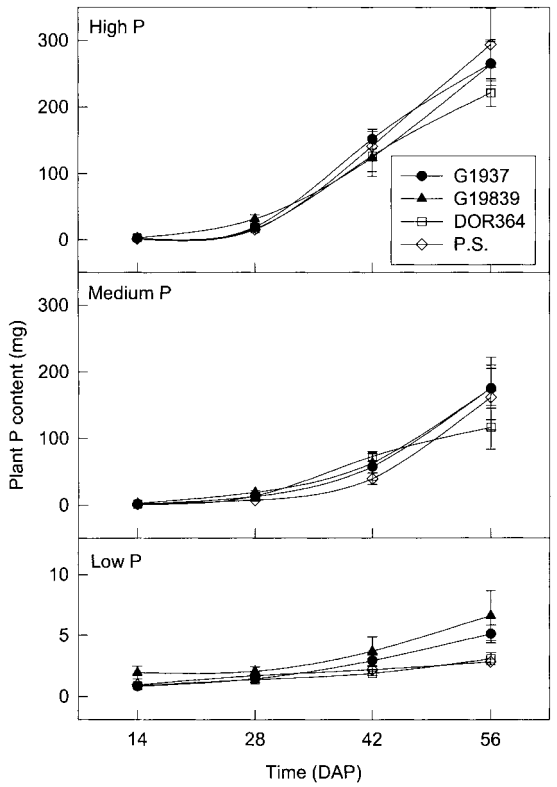

Fig. 3. Plant P content of four common bean genotypes, grown under low, medium, and high P conditions, measured at 14, 28, 42, and 56 d after planting. Data shown are mean ± standard error of the mean (n=4).

Table 1. *Root:shoot allometric coefficient (*K*) of contrasting common bean genotypes as influenced by P availability*

Each value is derived from paired measurements of root DW and shoot DW (Hunt, 1990), measured at 14, 28, 42, and 56 d after planting (n=4). Within a group of three values in columns, coefficients followed by the same letter are not significantly different (P=0.05).

P-level	G1937		G19839		DOR364		P.S.	
	K	r^2	*K*	r^2	*K*	r^2	*K*	r^2
H	0.86 a	0.99	0.93 a	0.98	0.72 a	0.98	0.80 a	0.99
M	0.89 a	0.98	0.86 a	0.99	0.69 a	0.99	0.84 a	0.99
L	0.85 a	0.99	0.95 a	0.97	0.49 b	0.89	0.60 b	0.95
F from ANOVA								
Block	0.686ns							
Genotype	34.6***							
P-level	4.88*							
Genotype × *P*-level	5.97***							

*, **, *** Significant at $P \leq 0.05$, 0.01, and 0.001, respectively; ns, not significant.

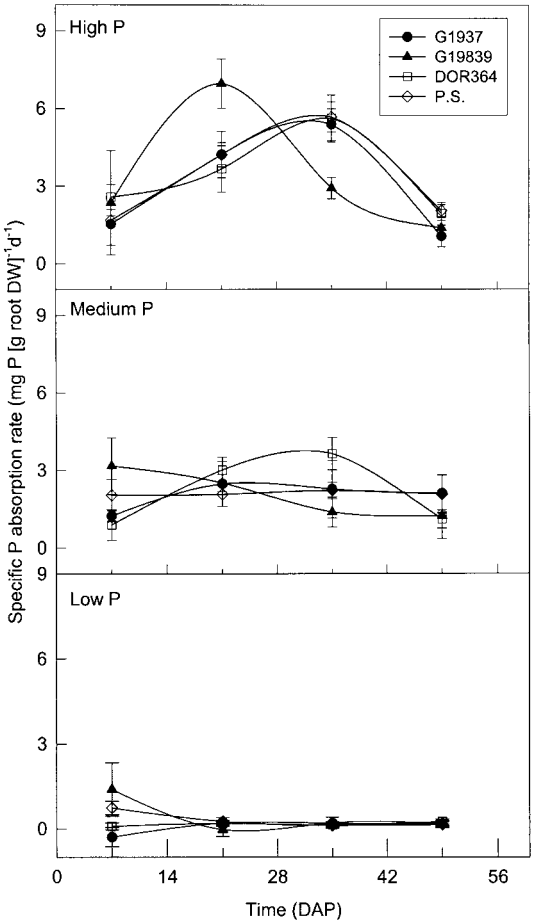

Fig. 4. Specific P absorption rate (SPAR) at 7, 21, 35, and 49 d after planting of four common bean genotypes, grown under low, medium and high P conditions. The values represent the midpoint of the interval SPAR was measured over. Data shown are mean ± standard error of the mean (n=4).

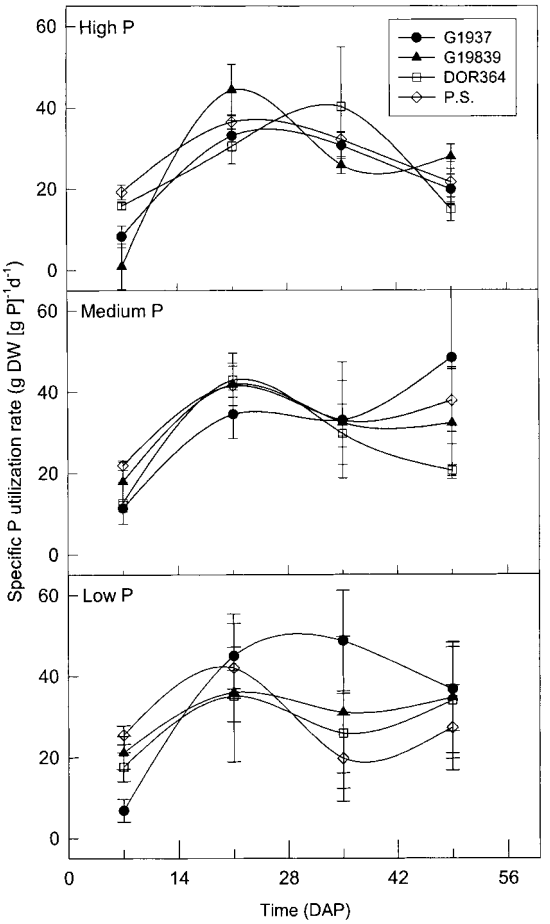

Fig. 5. Specific P utilization rate (SPUR) at 7, 21, 35, and 49 d after planting of four common bean genotypes, grown under low, medium and high P conditions. The values represent the midpoint of the interval SPUR was measured over. Data shown are mean ± standard error of the mean (n=4).

any information regarding efflux of P and the efficiency of resource utilization related to P uptake.

Gas exchange measurements

Root and shoot C flux at 28 DAP are presented in Fig. 6. The fraction of daytime shoot C fixation used for root respiration in low P plants was significantly higher than in medium and high P plants. Shoot respiration did not vary significantly among cultivars or P-levels, but C fixation and net C gain expressed as a fraction of C fixation was significantly higher in high and medium P plants compared to low P plants (Table 2). No significant difference was found in C fixation and in the C allocation among C used in root respiration, night-time shoot respiration and net C gain, among the four contrasting genotypes used in this study. The significant block effect observed for shoot respiration and net C gain expressed as a fraction of C fixation and for daily C fixation was due to differences in light conditions on days of gas exchange measurements that were staggered among blocks. Light conditions and differences in C fixation and shoot respiration and net C gain expressed as a fraction of C fixation did not cause significant differences in C used in root respiration among blocks (Table 2).

A correlation was found between root RGR and rate of root respiration in efficient and inefficient genotypes (Fig. 7). The two P-inefficient genotypes (DOR364 and P.S.) had a significantly higher rate of root respiration ($P<0.05$) than the two P-efficient genotypes (G1937 and G19839). Low P plants had a significantly lower rate of root respiration than medium and high P plants ($P<0.001$), and medium P plants had a significantly lower rate of root respiration than high P plants ($P<0.001$). No significant interaction was found between root relative growth rate and P efficiency (inefficient compared with efficient genotypes), suggesting that the rate of root respiration per unit root RGR was higher in P-inefficient genotypes than P-efficient genotypes independent of P availability.

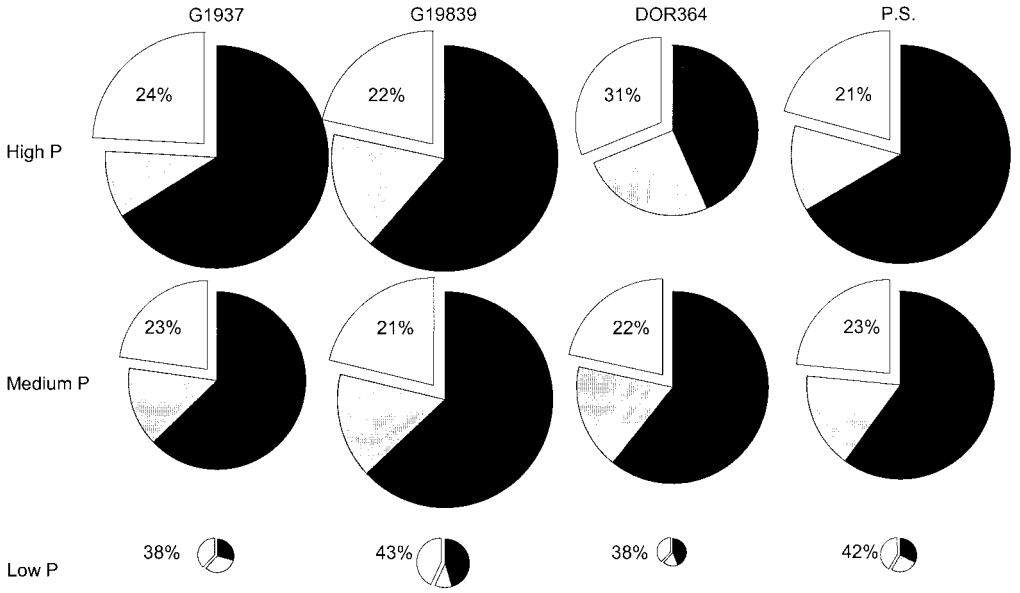

Fig. 6. Allocation of daytime net C assimilation 28 d after planting, as percentage of C used in root respiration (open sections), night-time shoot respiration (gray sections), and net C gain (closed sections) of four common bean genotypes, grown under low, medium and high P conditions. The numbers indicate the percentage of the diurnal C fixation used in root respiration. Pie diameter is a relative measure of overall daytime net C assimilation. Each value is the mean of four replicates.

Table 2. F *from ANOVA of root respiration, shoot respiration, and C gain as percentage of daily C fixation and daily C fixation for two P-efficient (G1937 and G19839) and two P-inefficient (DOR364 and P. S.) bean genotypes, as influenced by P availability*

F from ANOVA	Root respiration	Shoot respiration	C gain	C fixation
Genotype	0.108^{ns}	0.295^{ns}	0.193^{ns}	0.882^{ns}
P-level	14.47***	0.776^{ns}	6.289**	47.02***
Block	1.880^{ns}	3.837*	3.227*	12.04***
Genotype × P-level	0.474^{ns}	1.443^{ns}	0.892^{ns}	0.308^{ns}

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively; ns, not significant.

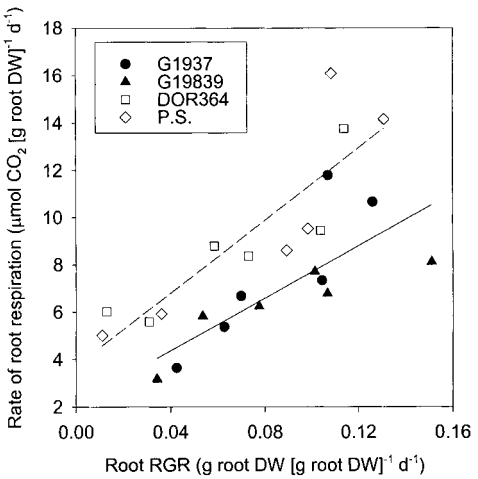

Fig. 7. Relationship of the rate of root respiration and root RGR of four common bean genotypes, grown under low, medium and high P conditions at 28 and 42 d after planting. Linear regressions for efficient genotypes (G1937 and G19839) for all P levels (solid line; rate of root respiration=55.39 × Root RGR + 2.16; r^2=0.62; n=12; $P \leq 0.05$) and for inefficient genotypes (DOR364 and P.S.) for all P levels (dashed line; rate of root respiration=76.72 × Root RGR + 3.73; r^2=0.76; n=12; $P \leq 0.01$). Each data point represent the mean of four replicates.

Discussion

A number of physiological traits could contribute to P efficiency, by improving P acquisition from the soil (P acquisition efficiency) or by improving the utilization of acquired P in growth and reproduction (P utilization efficiency). Although common bean is a diverse species that includes genotypes with contrasting P efficiency (Beebe *et al.*, 1997), it does not appear to have large differences in P utilization efficiency (Lynch and Beebe, 1995). This is not surprising: at the cellular level, efficient utilization of a commonly limiting nutrient would have been subject to natural selection for æons, and as a result utilization efficiency at the whole plant level is more related to fundamental differences in form, size and phenology. These differences are not large within an annual herbaceous crop species. Comparisons of bean genotypes in diverse soil environments showed that genetic differences in P acquisition were not due to interactions with specific soil symbionts such as mycorrhizas, or differential ability to mobilize P from distinct soil pools, such as might be possible through differential production of root exudates (Yan *et al.*,

1995*a*, *b*). Large genetic variation for root growth and architecture are present in bean germplasm (Lynch and van Beem, 1993). Geometric modelling showed that architectural variation in bean root systems could substantially affect P acquisition efficiency, by determining the extent of inter-root competition, and in heterogeneous soils, by determining the exploitation of P-rich soil domains (Nielsen *et al*., 1994; Lynch *et al*., 1997; Ge *et al*., 2000). Modelling also showed that root C costs could be a significant factor in the reduction of plant growth by low P availability (Lynch and Beebe, 1995), which was confirmed by C budgets of actual bean plants (Nielsen *et al*., 1998).

Phosphorus status of the soil affected all aspects of C partitioning in the bean plants. The fraction of C used in root respiration was higher in low P plants (Fig. 6; Table 2). The remaining amount of carbon resources left for organ growth was smaller. The cumulative result of this process over time could have been responsible for the smaller plant size under the low P. No significant differences were found in C fixation, root and shoot respiration among genotypes at 28 DAP (Fig. 6; Table 2). This indicates that differences in root biomass allocation (Fig. 2; Table 1) as the plant biomass increases during the vegetative growth phase must be due to factors other than C used in root respiration. Plants that have a higher efficiency of P acquisition per unit C spent for root construction and maintenance, would perform better under low P conditions (Nielsen *et al*., 1994). A plant would have this capacity if it had a large root system due to lower root construction costs or if the root system required a smaller amount of C to be respired due to a lower rate of root respiration. This appears to be the case among the genotypes compared; SPAR did not vary consistently among genotypes, but rate of root respiration per unit root RGR was higher in the inefficient genotypes.

Biomass allocation to roots could be regulated by hormonal relations between the root and the shoot, that regulate the physiological mechanisms controlling the sink strength of the shoot (Aiken and Smucker, 1996). It is well documented that reduced availability of nitrogen (Ingestad and Lund, 1979; McDonald *et al*., 1986) and P (Lynch *et al*., 1991) results in a rapidly reduced sink strength of the shoot. Specific assimilation rate is reduced less than leaf area growth rate, thus there is an oversupply of carbohydrates in the shoot and translocation of carbon to the root system is increased, promoting root growth. Ingestad and Ågren concluded that the main processes controlling biomass allocation occur in the shoot, where the dominant factors seem to be, firstly, the ability of the plant to retain photosynthetic C for growth and, secondly, the consequences of plant nutrient status on the photosynthetic rate (Ingestad and Ågren, 1991). In the four bean genotypes studied here it appears that the ability of the plant to retain photosynthetic C for growth varies with ontogenetic development and among genotypes. The allometric coefficient (K) was not altered by P level in the efficient genotypes, but the inefficient ones had a lower K under low P conditions (Fig. 2; Table 1). This indicates that they could not maintain a large enough root:shoot ratio needed for supply of P under these conditions. The efficient genotypes conformed to the 'allometric relationship' theory maintaining the same K for plants of all ages and all treatments. In the inefficient genotypes K changed with P level, but in a direction counter to what would have been expected from the 'Functional Equilibrium' theory. Under low P conditions K decreased, meaning that for the same overall plant size, those under low P had a lower root:shoot ratio.

The study reported here did not include detailed observations of root anatomy, morphology or architecture, and therefore does not permit a mechanistic analysis of how some genotypes maintain greater root biomass allocation with reduced rate of root respiration. Several possibilities merit further investigation. It has been observed that P-efficient bean genotypes (including one of the P-efficient genotypes used in the present study, G19839) produce more adventitious roots under P stress than P-inefficient genotypes (including genotype DOR364) (Miller, 1998; Miller *et al*., 1998). This is significant because adventitious roots differ from basal and tap roots in bean. For example, they have higher specific root length (metres of root length g^{-1} root DW) than basal and tap roots (Miller, 1998). Adventitious roots had a lower metabolic construction cost than basal roots, based on elemental composition (Miller, 1998). It has also been observed that P stress induces aerenchyma formation in bean roots, especially in P-efficient genotypes (Eshel *et al*., 1995; Lynch and Brown, 1998). Aerenchyma may decrease the rate of root respiration per unit absorbing surface area by eliminating cortical tissue, which may shift the balance of root weight to tissues with less maintenance respiration per gram, such as sclerenchyma (Lynch and Brown, 1998). Similarly, P stress affects secondary root development differently in different genotypes (Eshel *et al*., 1995), which may change the rate of bulk root respiration by changing the proportion of root weight devoted to various tissues. The P-efficient genotype G19839 had greater secondary root growth than did the P-inefficient DOR364. This is consistent with these present data, since tissue resulting from secondary growth is expected to increase the proportion of biomass in non-respiring xylem and sclerenchyma tissue as compared to primary growth.

Another possibility is that genotypic differences in root architecture could lead to differences in utilization of carbohydrates between respiration and biomass deposition (growth), by determining the proportion of growing and non-growing tissue (Nielsen *et al*., 1994). The genotypes employed in this study differ in root architecture, although late in vegetative growth, some of

these differences may have been obscured by physical restriction from the growth containers. One of the architectural features that differs among bean genotypes is the gravitropism of basal roots, with P-efficient genotypes having more shallow root systems (Bonser *et al*., 1996). In previous studies it was observed that G19839 has a shallow root system, whereas DOR364 has a deep root system (Bonser *et al*., 1996). This could be significant in the context of the present study since root depth may be correlated with root temperature, and therefore with C used in root respiration, although temperature profiles in pots in the greenhouse would be smaller than such gradients in field soils. Although soil temperature profiles in the pots were not measured, temperature was expected to be greatest at the surface, which would have increased the amount of C used in root respiration of genotypes with shallow roots, such as G19839, which is not consistent with the trends observed here. Any differences in root architecture manifested in the pots were not reflected in P acquisition efficiency, perhaps because the pots had a homogeneous P distribution.

The hypothesis was tested that differences in the P efficiency of plants can be due to differences in respiratory costs of their roots. Bean plants contrasting genetically in P efficiency were used in order to determine if these processes are important in accounting for differences in P efficiency. Low phosphorus plants utilized a significantly larger fraction of their daytime net carbon assimilation on root respiration (*c*. 40%) compared to medium and high phosphorus plants (*c*. 20%). No significant difference was found among genotypes in this respect. Genotypes also had similar rates of P absorption per unit root weight and plant growth per unit of P absorbed. Efficient genotypes had lower rates of root respiration than inefficient genotypes, which enabled them to maintain greater root biomass allocation than inefficient genotypes without increasing overall root carbon costs.

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References

Aiken RM, Smucker AJM. 1996. Root system regulation of whole plant growth. *Annual Review of Phytopathology* **34,** 325–346.

Baon JB, Smith SE, Alston AM. 1994. Growth response and phosphorus uptake of rye with long and short root hairs: interactions with mycorrhizal infection. *Plant and Soil* **167,** 247–254.

Beebe S, Lynch JP, Galway NW, Tohme J, Ochoa I. 1997. A geographical approach to identify phosphorus-efficient genotypes among landraces and wild ancestors of common bean. *Euphytica* **95,** 325–336.

Bonser AM, Lynch J, Snapp S. 1996. Effect of phosphorus deficiency on growth angle of basal roots in *Phaseolus vulgaris*. *New Phytologist* **132,** 281–288.

Bouma TJ, Nielsen KL, Eissenstat DM, Lynch JP. 1997*a*. Estimating respiration of roots in soil: Interactions with soil CO_2, soil temperature and soil water content. *Plant and Soil* **195,** 221–232.

Bouma TJ, Nielsen KL, Eissenstat DM, Lynch JP. 1997*b*. Soil CO_2 concentration does not affect growth or root respiration in bean and citrus. *Plant, Cell and Environment* **20,** 1495–1505.

Bouma TJ, Broekhuysen AGM, Veen BW. 1996. Analysis of root respiration in *Solanum tuberosum* as related to growth, ion uptake and maintenance of biomass. *Plant Physiology and Biochemistry* **34,** 795–806.

Brouwer R. 1983. Functional equilibrium: sense or nonsense? *Netherlands Journal of Agricultural Sciences* **31,** 335–348.

Caradus JR, Mackay AD, Dunlop J, Vandenbosch J. 1995. Relationships between shoot and root characteristics of white clover cultivars differing in response to phosphorus. *Journal of Plant Nutrition* **18,** 2707–2727.

CIAT (International Center for Tropical Agriculture). 1987. *CIAT annual report 1987*. Cali, Colombia: CIAT.

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

Erickson RO. 1976. Modeling of plant growth. *Annual Review of Plant Physiology* **27,** 407–434.

Eshel A, Waisel Y. 1996. Multiform and multifunction of various constituents of one root system. In: Waisel Y, Eshel A, Kafkafi U, eds. *Plant roots: the hidden half,* 2nd edn. New York: Marcel Dekker Inc., 175–192.

Eshel A, Nielsen KL, Lynch JP. 1995. Response of bean root systems to low level of phosphorus. In: *Plant roots—from cells to systems*. 14th Long Ashton International Symposium. Bristol, England: IACR-Long Ashton Research Station, 63.

Fitter AH, Stickland TR. 1991. Architectural analysis of plant root systems. 2. Influence of nutrient supply on architecture in contrasting plant species. *New Phytologist* **118,** 383–389.

Ge Z, Rubio G, Lynch JP. 2000. The importance of root gravitropism for inter-root competition and phosphorus acquisition efficiency: results from a geometric simulation model. *Plant and Soil* **218,** 159–171.

Grando S, Ceccarelli S. 1995. Seminal root morphology and coleoptile length in wild (*Hordeum vulgare* ssp. *spontaneum*) and cultivated (*Hordeum vulgare* ssp. *vulgare*) barley. *Euphytica* **86,** 73–80.

Hansen GK, Jensen CR. 1977. Growth and maintenance respiration in whole plants, tops and roots of *Lolium multiforum*. *Physiologia Plantarum* **39,** 155–164.

Horgan JN, Wareing PF. 1980. Cytokinins and the growth of seedlings of *Betula pendula* Roth. and *Acer pseudoplatanus* L. to nitrogen and phosphorus deficiency. *Journal of Experimental Botany* **31,** 525–532.

Hunt R. 1975. Further observations on root-shoot equilibria in perennial ryegrass (*Lolium perenne* L.). *Annals of Botany* **39,** 745–755.

Hunt R. 1990.*Basic growth analysis*. London: Unwin Hyman Ltd.

Ingestad T, Ågren GI. 1991. The influence of plant nutrition on biomass allocation. *Ecological Applications* **1,** 168–174.

Ingestad T, Lund AB. 1979. Nitrogen stress in birch seedlings. I. Growth technique and growth. *Physiologia Plantarum* **45,** 137–148.

Kirk GJD, Du LV. 1997. Changes in rice root architecture, porosity, and oxygen and proton release under phosphorus deficiency. *New Phytologist* **135,** 191–200.

Koide RT. 1991. Nutrient supply, nutrient demand and plant response to mycorrhizal infection. *New Phytologist* **117,** 365–386.

Laing DRL, Jones PG, Davis JHC. 1984. Common bean (*Phaseolus vulgaris* L.). In: Goldsworthy PR, Fisher NM, eds. *The physiology of tropical field crops*. New York: John Wiley and Sons.

Lambers H, Scheurwater I, Atkin OK. 1996. Respiratory patterns in roots in relation to their functioning. In: Waisel Y, Eshel A, Kafkafi U, eds. *Plant roots: the hidden half,* 2nd edn. New York: Marcel Dekker Inc., 323–362.

Lambers H, Steingröver E. 1978. Efficiency of root respiration of a flood-tolerant and flood-intolerant *Senecio* species as affected by low oxygen tension. *Physiologia Plantarum* **42,** 179–184.

Lauer MJ, Blevins DG, Sierputowska-Gracz H. 1989. ^{31}P-nuclear magnetic resonance determination of phosphate compartmentation in leaves of reproductive soybeans as affected by phosphate nutrition.*Plant Physiology* **89,** 1331–1336.

Lynch JP, Beebe SE. 1995. Adaptation of bean (*Phaseolus vulgaris* L.) to low phosphorus availability. *HortScience* **30,** 1165–1171.

Lynch J, Brown K. 1998. Regulation of root architecture by phosphorus availability in common bean. In: Lynch JP, Deikman J, eds. *Phosphorus in plant biology: regulatory roles in ecosystem, organismic, cellular, and molecular processes*. Rockville, MD: American Society of Plant Physiologists, 148–156.

Lynch J, Epstein E, Läuchli A, Weigt GE. 1990. An automated greenhouse sand culture system suitable for studies of phosphorus nutrition. *Plant, Cell and Environment* **13,** 547–554.

Lynch J, Läuchli A, Epstein E. 1991. Vegetative growth of the common bean in response to phosphorus nutrition. *Crop Science* **31,** 380–387.

Lynch JP, Nielsen KL, Davis RD, Jablokow AG. 1997. *SimRoot*: Modelling and visualization of botanical root systems. *Plant and Soil* **188,** 139–151.

Lynch J, Rodriguez HN. 1994. Photosynthetic nitrogen-use efficiency in relation to leaf longevity in common bean. *Crop Science* **34,** 1284–1290.

Lynch J, van Beem JJ. 1993. Growth and architecture of seedling roots of common bean genotypes. *Crop Science* **33,** 1253–1257.

McDonald AJS, Lohammar T, Ericson A. 1986. Growth response to step-decrease in nutrient availability in small birch (*Betula pendula* Roth). *Plant, Cell and Environment* **9,** 427–432.

Miller CR. 1998. Root architecture of common bean (*Phaseolus vulgaris* L.): dynamic nature and adaptive responses to low phosphorus. MS thesis, Penn State University.

Miller CR, Nielsen KL, Lynch JP, Beck D. 1998. Adventitious root response in field-grown common bean: a possible adaptive strategy to low-phosphorus conditions. In: Flores HE, Lynch JP, Eissenstat D, eds. *Radical biology: advances and perspectives on the function of plant roots*. Current Topics in Plant Physiology **18,** 394–396.

McCree K. 1970. An equation for the rate of respiration of white clover plants grown under controlled conditions. In: Setlik I, ed. *Prediction and measurement of photosynthetic productivity*. Preceedings IBP/PP Technical Meeting, 221–230.

Murphy J, Riley JP. 1962. A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta* **27,** 31–36.

Nielsen KL, Bouma TJ, Lynch J, Eissenstat DM. 1998. Effects of phosphorus availability and vesicular-arbuscular mycorrhizas on the carbon budget of common bean (*Phaseolus vulgaris*). *New Phytologist* **139,** 647–656.

Nielsen KL, Lynch JP, Jablokow AG, Curtis PS. 1994. Carbon cost of root systems: an architectural approach. *Plant and Soil* **165,** 161–169.

Pantalone VR, Rebetzke GJ, Burton JW, Carter TE. 1996. Phenotypic evaluation of root traits in soybean and applicability to plant breeding. *Crop Science* **36,** 456–459.

Poorter H, van der Werf A, Atkin OK, Lambers H. 1991. Respiratory energy requirements of roots vary with the potential growth rate of a plant species. *Physiologia Plantarum* **83,** 469–475.

Radin JW, Eidenbrock MP. 1984. Hydraulic conductance as a factor limiting leaf expansion of phosphorus deficient soybean plants. *Plant Physiology* **75,** 372–377.

Rychter AM, Chauveau M, Bomsel J-L, Lance C. 1992. The effect of phosphate deficiency on mitochondrial activity and adenylate levels in bean roots. *Physiologia Plantarum* **84,** 80–86.

Salama AMS, Wareing PF. 1979. Effects of mineral nutrition on endogenous cytokinins in plants of sunflower (I L.). *Journal of Experimental Botany* **30,** 971–981.

SAS Institute. 1992. *SAS JMP Version 3.1.2*. Cary NC: SAS Institute Inc.

Scheurwater I, Clarkson DT, Purves JV, Van Rijt G, Saker LR, Welschen R, Lambers H. 1999. Relatively large nitrate efflux can account for the high specific respiratory cost for nitrate transport in slow-growing grass species. *Plant and Soil* **215,** 123–134.

Scheurwater I, Cornelissen C, Dictus F, Welschen R, Lambers H. 1998. Why do fast- and slow-growing grass species differ so little in their rate of root respiration, considering the large differences in rate of growth and ion uptake? *Plant, Cell and Environment* **21,** 995–1005.

Sisson WB. 1989. Carbon balance of *Panicum coloratum* during drought and non-drought in the northern Chihuahuan desert. *Journal of Ecology* **77,** 799–810.

Sisson WB. 1983. Carbon balance of *Yucca elata* Engelm. during a hot and cool period in situ. *Oecologia* **57,** 352–360.

Theodorou ME, Plaxton WC. 1993. Metabolic adaptations of plant respiration to nutritional phosphate deprivation. *Plant Physiology* **101,** 339–344.

Thornley JHM. 1970. Respiration, growth and maintenance in plants. *Nature* **227,** 304–305.

van der Werf A, Kooijman A, Welschen R, Lambers H. 1988. Respiratory costs for the maintenance of biomass, for growth and for ion uptake in roots of *Carex diandra* and *Carex acutiformis*. *Physiologia Plantarum* **72,** 483–491.

van der Werf A, Welschen R, Lambers H. 1992. Respiratory losses increase with decreasing inherent growth rate of a species and with decreasing nitrate supply: A search for explanations for these observations. In: Lambers H, Van der Plas LHW, eds. *Molecular, biochemical and physiological aspects of plant respiration*. The Hague: SPB Academic Publishing bv, 421–432.

Vanvuuren MMI, Robinson D, Fitter AH, Chasalow SD, Williamson L, Raven JA. 1997. Effects of elevated atmospheric CO_2 and soil water availability on root biomass, root length, and N, P and K uptake by wheat. *New Phytologist* **135,** 455–465.

Veen BW. 1980. Energy costs of ion transport. In: Rains DW, Valentine RC and Hollaender A, eds. *Genetic engineering of osmoregulation: impact on plant productivity for food, chemicals and energy*. New York: Plenum Press, 187–195.

Wan CG, Sosebee RE, McMichael BL. 1996. Lateral root development and hydraulic conductance in four populations of *Gutierrezia sarothrae*. *Environmental and Experimental Botany* **36,** 157–165.

Whiteaker G, Gerloff GC, Gabelman WH, Lindgren D. 1976. Intraspecific differences in growth of beans at stress levels of phosphorus. *Journal of the American Society of Horticultural Science* **101,** 472–475.

Yan X, Lynch JP, Beebe SE. 1995*a*. Genetic variation for phosphorus efficiency of common bean in contrasting soil types. I. Vegetative response. *Crop Science* **35,** 1086–1093.

Yan X, Beebe SE, Lynch JP. 1995*b*. Genetic variation for phosphorus efficiency of common bean in contrasting soil types. II. Yield response. *Crop Science* **35,** 1094–1099.