Lack of evidence for programmed root senescence in common bean (*Phaseolus vulgaris*) grown at different levels of phosphorus supply

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Summary

• The influence of phosphorus (P) availability on root lifespan in common bean (*Phaseolus vulgaris* cv. Carioca) is reported here, as well as patterns of root survivorship in relation to vegetative and reproductive development.

• Plants were grown in a glasshouse in a sand-culture system with varying P availability, and in a high-P field soil with greater biotic activity. Root dynamics were assessed using a minirhizotron system.

• Phosphorus limitation, which reduced plant growth by 90%, did not diminish root survivorship. At nonlimiting and limiting P, root survivorship was not synchronized closely with shoot development and senescence; a substantial portion of the roots were present and seemingly alive even beyond reproductive maturity. In field-grown beans and in beans under severe P limitation in sand culture, survivorship fell approx. 50%, but only after pod fill was nearly complete.

• Common bean does not exhibit programmed root senescence during and immediately following pod fill. Roots died after the shoot had undergone other senescence processes, such as leaf drop and pod dry-down. Under field conditions, soil organisms are likely to modulate the effects of phosphorus deficiency and pod fill on root death.

Key words: root death, root turnover, minirhizotrons, common bean (*Phaseolus vulgaris*), phosphorus, plant nutrition, senescence.

Introduction

Common bean (*Phaseolus vulgaris*) is the most important food legume on earth, providing scarce nutrients to many people in developing countries (Pachico, 1999). Low soil fertility, particularly low soil phosphorus (phosphorus) availability, is a primary constraint to bean production in developing countries (Lynch & Beebe, 1995). One obstacle to the selection or breeding of common bean cultivars adapted to low-phosphorus soils is that relatively little is known about the traits comprising an optimal root system (Lynch & Beebe, 1995). Root lifespan is a trait that could be important for phosphorus acquisition. In particular, root lifespan may be important for sustained phosphorus uptake during reproduction, for nutrient translocation into developing seeds, and for whole-plant carbon budgets during reproduction.

Root death has been reported in annual species (Cheng *et al.*, 1990; Krauss & Deacon, 1994). Root death in annuals is often considered a preprogrammed response that occurs when plant resources, such as carbon, nitrogen and phosphorus, are diverted from root growth and maintenance to flowering and fruiting (Cheng *et al.*, 1990; Box & Ramseur, 1993). However, programmed senescence in roots has never been examined critically by tracking the survivorship of different cohorts of roots at different stages of growth under carefully controlled soil conditions.

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Snapp & Lynch (1996) studied how phosphorus availability in common bean affected phosphorus uptake and phosphorus remobilization from roots to seeds using radiophosphorus and destructive harvests. Regardless of phosphorus availability, 50% of the phosphorus in the grain came from remobilization of leaf phosphorus (Snapp & Lynch, 1996). In addition, < 20% of the phosphorus absorbed into the roots appeared in the shoot if any part of the root system was experiencing phosphorus stress. If no part of the root was experiencing phosphorus stress, then 80% of the absorbed phosphorus moved to the shoots. During the course of that study, root length did not seem to change over time in high-phosphorus plants, but low-phosphorus plants exhibited a slight decrease in root length after pod fill.

In the study of Snapp & Lynch (1996), the fate of individual roots was not followed. Growth and mortality of roots usually occur concurrently, making it difficult to estimate root longevity from multiple destructive harvests over time (Dubach & Russelle, 1995). Minirhizotron technology permits the tracking of specific roots from birth until death, allowing a more direct assessment of root demography (Cheng *et al.*, 1990; Hendrick & Pregitzer, 1992). By taking multiple pictures of the roots over the course of the plant's life cycle, we hoped to observe correlations between root death, phosphorus stress and reproduction.

The primary objectives of this study were to investigate whether the incidence of bean root death differed under adequate, deficient, and severely deficient phosphorus levels and how mortality patterns were related to periods of high reproductive effort (pod fill). We hypothesized that bean root mortality would increase with a decrease in available phosphorus and that mortality would be highest during pod fill. We observed very little root mortality for plants deficient or sufficient in phosphorus, even well after pod fill when the leaves had nearly completely senesced.

These results led to a second hypothesis: if roots are not under biotic pressure from herbivores and weak parasites, then there may be little root mortality (based on root disappearance or removal). Root mortality, while often considered a single event, may represent gradual senescence of the epidermal, cortical and vascular tissues. Death of a weakened, poorly defended root may be accelerated by the presence of soil organisms (Eissenstat & Yanai, 1997; Eissenstat *et al.*, 2000). Techniques used to study root mortality in soil often rely on visual cues for assessment of root mortality, which may include some root decomposition (Comas *et al.*, 2000). Thus, the amount of biological activity in the soil may strongly influence interpretations of root mortality in response to phosphorus stress or shoot phenology. Weakened roots may give off chemical-signals that attract herbivores. Liljeroth (1995) claims that increased root cortical death (RCD) leads to increased saprophytic-fungal attack on roots. This may be due in part to the leakage of cellular nutrients. Deacon (1987) reviewed work that indicates a propensity of saprophytic fungi colonize dead cortical cells. Thus, the second objective of this study was to examine mortality patterns observed in field soil as a comparison with soil of low biological activity (sand culture).

Materials and Methods

Glasshouse study

Common bean (*Phaseolus vulgaris* L., cv. 'Carioca') (CIAT accession G4017) is a bush, indeterminate (Type III) Brazilian landrace of the Mesoamerican gene pool. Carioca is the single most widely grown bean genotype in the tropics, perhaps because of its tolerance of infertile soils. Plants were grown in a glasshouse sand culture system at University Park, PA, USA (Long. 77°5′ W, Lat. 40°43′ N) from June through October, 1995. Active heating and cooling systems maintained daytime temperatures < 30°C and nighttime temperatures > 18°C. No supplemental lighting was provided. Seeds were surface sterilized for 1 min in 10% NaOCl, rinsed with deionized water for 5 min, and planted at a depth of 3 cm in 18.9-l plastic containers filled with silica sand (flint shot 3.0, US Silica Co., Ottawa, IL, USA). Three seeds per pot were planted and thinned to one plant per pot 10 d after planting (DAP). Plants were grown for 125 DAP, through physiological maturity, until the shoots were largely senescent.

Nutrient solution was provided twice daily at 07.00 h and 14.50 h using drip irrigation. Nutrient solutions contained in mM: 1.5 KNO_3, 1.2 $Ca(NO_3)_2$, 0.5 $MgSO_4$, 0.4 NH_4NO_3, 0.3 K_2SO_4, 0.3 $(NH_4)_2SO_4$, 0.025 $MgCl_2$, 0.0015 $MnSO_4$, 0.005 FeEDTA, 0.0015 $ZnSO_4$, 0.0005 $CuSO_4$, 0.0005 NaB_4O_7, and 0.00014 $NH_4Mo_7O_{24}$. A solid-phase-buffer system employing activated alumina was used to maintain phosphorus availability at stable low levels (Lynch *et al.*, 1990). The system was used to generate three different levels of phosphorus availability: 27, 1.0 and 0.4 μM phosphate, corresponding to adequate, limiting and severely deficient phosphorus availability for common bean (Lynch *et al.*, 1991).

Root longevity was monitored starting 25 DAP and every 10–14 d thereafter (i.e. 25, 37, 48, 69, 82, 98, 109, 112, 122 DAP) using a miniature video camera (BTC 1.125 – Bartz Technology Corporation, Santa Barbara, CA, USA) connected to a camcorder (Sony video Hi – 8 mm ccd-fgx710) in minirhizotrons (clear polybuterate tubes). The minirhizotron tubes were 3.7 cm outside diameter and 30 cm in length. Minirhizotrons were scribed with three rows of frames, 1.5 wide × 1 cm high, along the length of the tube with each row space 120° apart. Minirhizotrons were orientated horizontally at depths of 12, 20, and 28 cm from the top of the bucket. Video images were captured on the computer and then root images assessed for colour change and disappearance. Absence of roots indicated root death. Dates of image capture corresponded with growth stages in the following sequence: third trifoliate leaf (V4), 25 DAP; preflowering (R5), 37 DAP; mid pod formation (R7), 48 DAP; mid pod filling (R8), 69 DAP;

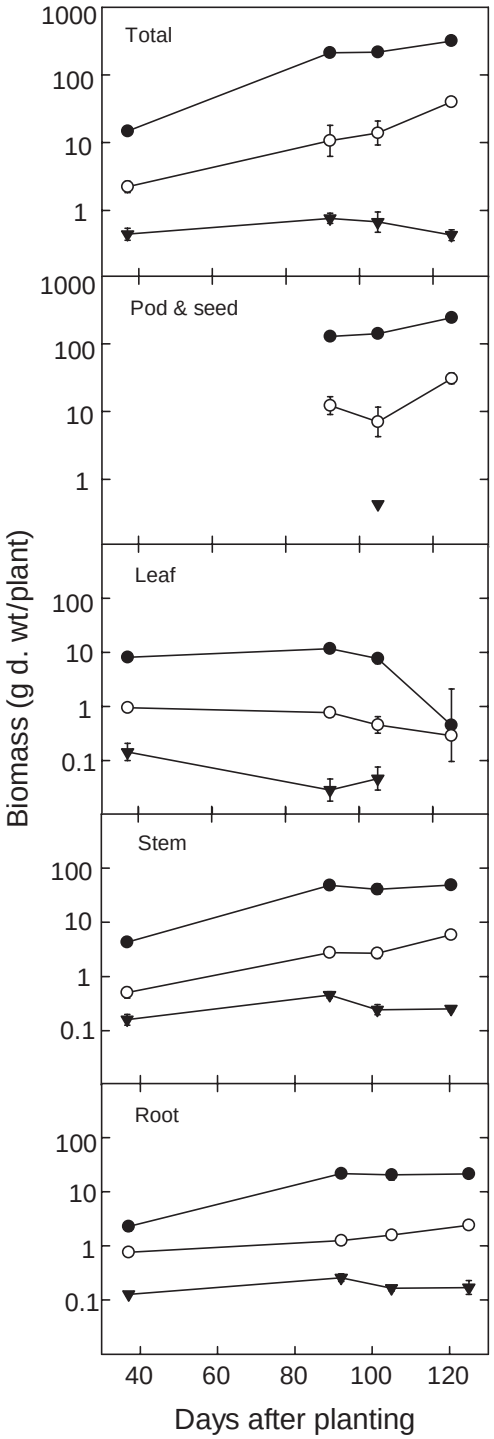

Fig. 1 Dry weight of leaves, stems, roots, pods and seed, and total plant biomass for common bean grown at severely deficient (0.4 μM, triangles), limiting (1.0 μM, open circles) and sufficient (27.0 μM, closed circles) P concentrations in the sand culture medium. Plants were destructively sampled four times during the growth period (37, 92, 105 and 125 days after planting (DAP)). Data were plotted on a $\log_{10}$ scale (± SE, on many dates SE is covered by the symbol). Sample size of treatment means were $n = 3$ for 37, 92 and 125 DAP and $n = 6$ for 105 DAP. At 92 DAP, most leaves had turned yellow and by 105 DAP leaves had mostly turned brown.

plant maturation, maximum production reached, most leaves yellowing (R9), 82 DAP; and beyond 82 DAP, dry down of leaves, stem and pods (growth stage nomenclature follows that of Centro Inernacional de Agricultura Tropical (1982)). Cohort analysis was based on the date the roots were first observed. Cohort 1 represented roots first observed 25 DAP; cohort 2, 37 DAP; cohort 3, 48 DAP; and cohort 4, 69 DAP. Rate of mortality was determined from the slope of the relationship of percent survivorship (on a Log_{10} scale) with time.

Plants were harvested 30 (V4), 37 (R5), 41 (R6), 92 (R9), 105 (just beyond pod dry down) and 125 DAP (well beyond pod dry down; plants were still green with 3–4 green trifoliates at the top of the medium- and high-phosphorus plants). Tissue phosphorus content was determined spectrophotometrically after ashing at 495°C for a minimum of 10 h (Murphy & Riley, 1962).

Field study

The field study was conducted at the Russell E. Larsen Agricultural Research Center near State College, PA, USA. The soil was a Hagerstown silt loam, 0–3% slope (fine, mixed mesic, typic Hapludalf) subject to standard tillage before planting (9 cm depth). At the end of the study, soil samples were analysed by the Pennsylvania State University Agricultural Analytical Service Laboratory and found to have a pH of 6.7, CEC of 7.8–11.1 cmol kg^{-1}, and available phosphorus (Mehlich III extraction) of 332–435 kg ha^{-1}, available K of 462–703 kg ha^{-1}, and available Ca of 4080–4710 kg ha^{-1}. On June 22, 1995 seeds (uninoculated with rhizobium) of common bean (*P. vulgaris*) were planted in three rows with 75 cm between rows at each of four locations. Seeds were planted 2 cm deep and 20 cm apart within rows and thinned at V1 (6 DAP) to 40 cm apart. At each location, five experimental plants were chosen from the middle row for minirhizotron observations.

The field received 37.4 cm of precipitation from June to October. Temperatures ranged from 15.6 to 29.4°C, from planting to physiological maturity (R9). Beyond R9, temperatures were between 5.6 and 21.1°C. Average temperatures were 19.7°C in June, 22.2°C in July, 22.4°C in August, 15.8°C in September, and 12.4°C in October. Plants were watered using drip irrigation as needed. Malathion insecticide (mercaptan) was applied as needed for insect control. Plots were hand weeded every 8–14 d.

Minirhizotron tubes were 45 cm in length and made of polybuterate plastic. A permanent marker was used to scribe four columns of frames along the length of the tube, 90° apart. Each frame was 1 cm high × 1.5 cm wide. For root tracking purposes, 30 frames per angle were numbered sequentially. Tubes were installed 10 cm from the plant at a 45° angle from the soil surface at 25 DAP (vegetative stage V3). Tubes were inserted to a depth of 27 cm, leaving 7 cm protruding above the ground. To protect the protruding

tube-end from rain and sunlight, the tops were wrapped with electrical tape, capped with a rubber stopper, and covered with a white aluminium can.

Roots images were recorded as previously described at 32 (R5), 40 (R6), 54 (R8, early pod fill), 65 (R8, mid pod fill), 79 (R9, mature pods), 93 (R9, dried pods), 102 (beyond dry down), and 105 DAP. Root images were processed as described previously for the glasshouse experiment. In the field study we captured images of a total of 1458 individual roots for population and cohort analysis. No root nodulation was observed in the minirhizotron images.

The above-ground portion of the plants was harvested on 19 October 1995 (121 DAP, beyond physiological maturity). Seed weights were measured and harvest index (the ratio of seed d. wt to shoot d. wt) was determined after drying at 70°C. Seeds were shelled to determine harvest index. After harvest, roots were collected and sent to The Pennsylvania State University Plant Disease Clinic for identification of possible pathogenic fungi.

Statistics

Root survivorship was analysed using the Wilcoxon log-rank test (SAS Institute, Cary, IN, USA). Probabilities of significant treatment effects were reported for nonlimiting, limiting and severely limiting phosphorus conditions. Comparisons of the effects of pod fill on root survival rate ($\log(\%)\ d^{-1}$) were made within each phosphorus treatment using log-linear regression using SAS. A *t*-test was used to determine whether slopes differed significantly from zero.

Results

Glasshouse study

Plant growth in response to phosphorus supply Plants had more leaves with increasing phosphorus availability, exhibiting a fivefold to 10-fold increase in leaf d. wt with each increase in phosphorus availability (Fig. 1). Only the nonlimiting-phosphorus treatment had an increase in leaf biomass from flowering until pod-dry-down (92 DAP). All phosphorus treatments lost leaf d. wt after pod-dry down. Stem d. wt in nonlimiting and limiting phosphorus treatments (27.0 μM & 1.0 μM phosphate) increased over time, in contrast to plants at severely low phosphorus, in which stem d. wt decreased after maturity.

Pod and seed d. wt in the severely deficient phosphorus treatment were low and quite variable. Pod d. wt in nonlimiting and limiting phosphorus treatments (27.0 μM & 1.0 μM phosphate) increased steadily from pod dry down until 125 DAP (well beyond pod dry down with approx. 3–9 green leaves with the beginnings of new pods on each plant). Pod d. wt in the limiting phosphorus treatment was only 10% the pod d. wt of the nonlimiting phosphorus treatment (Fig. 1). Seed d. wt in the nonlimiting, limiting and severely deficient phosphorus treatments followed the same pattern as that of the pods, in that the d. wt decreased by (roughly) a factor of 10 with each phosphorus level. As expected, seed d. wt increased over time at nonlimiting and limiting phosphorus. At severely deficient phosphorus pods were observed only once – most plants had no pods.

Root d. wt reached a maximum at 92 DAP in the nonlimiting phosphorus treatment and then remained constant to the end of the experiment (Fig. 1). Root d. wt in the severely deficient phosphorus treatment also reached a maximum at 92 DAP, but then declined. Variability in root d. wt, however, was much higher in the severely deficient phosphorus than the nonlimiting phosphorus treatment. In the limiting-phosphorus treatment, root biomass increased over the entire duration of the experiment and variability was comparable to that in nonlimiting phosphorus roots.

Plant phosphorus concentration in relation to phosphorus supply By contrast to the fivefold to 10-fold variation in biomass among phosphorus treatments, phosphorus concentration only varied one- threefold between the severely deficient and nonlimiting phosphorus treatments (Fig. 2). At 92 DAP leaf phosphorus concentration was typically threefold higher in the leaves than in the roots, regardless of phosphorus treatment. For the severely deficient and limiting phosphorus treatments, there was a marked decline in leaf phosphorus concentration after pod-dry down. For the nonlimiting phosphorus treatment, average leaf phosphorus concentration increased, mainly because the old leaves had fallen off the plants and new leaves appeared at this time.

By contrast to leaves, stem and root phosphorus concentration varied little over the course of plant development. In particular there was little evidence of a sharp decrease at pod fill in the limiting phosphorus treatment (1.0 μM phosphate). Root phosphorus concentration declined in the severely deficient phosphorus treatment, which probably reflected the contribution of dead intact roots, which could not be separated from living roots at harvest.

Concentration of phosphorus in the seeds peaked at 105 DAP. The decline in seed-phosphorus concentration between 105 and 125 DAP probably represents biomass dilution as the seeds filled at this time primarily with carbohydrates (Fig. 3). Although only one plant produced seeds in the severely deficient phosphorus treatment, the concentration of phosphorus in these seeds was similar to those in the limiting phosphorus treatment and only 25% lower than those in the nonlimiting phosphorus treatment (Fig. 2). Thus, seed phosphorus concentration seems to be highly conserved among plants that vary widely in phosphorus concentration.

Plant seed mass and harvest index Total seed mass per plant was approx. eightfold greater in nonlimiting phosphorus plants than limiting phosphorus plants at either 92 or 125

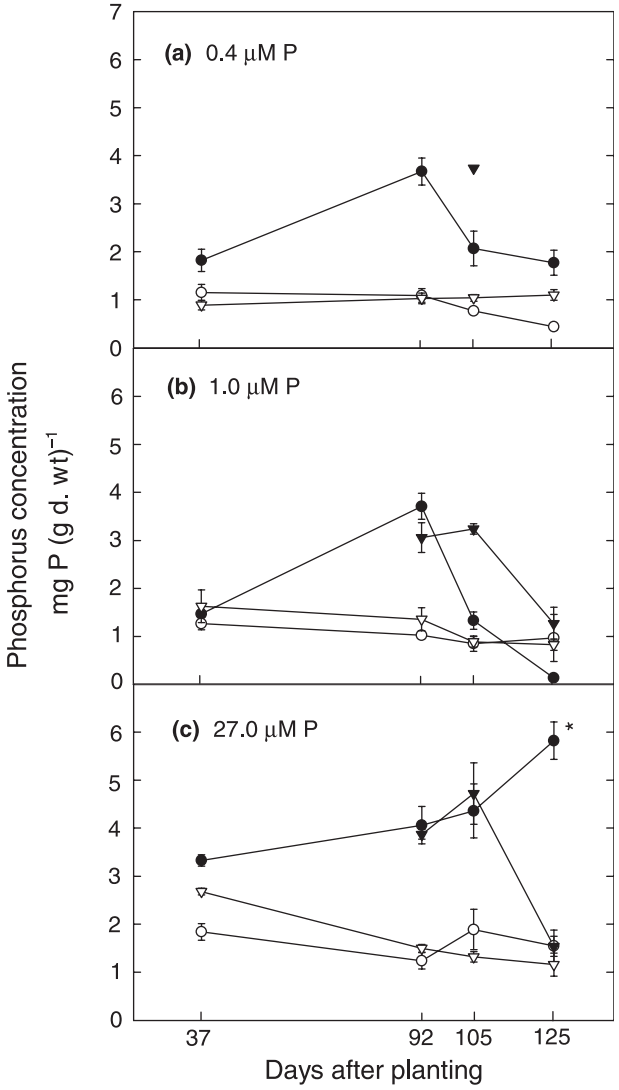

Fig. 2 Phosphorus concentration (± SE) of leaves (closed circles), stems (open triangles), roots (open circles), and seed (closed triangles) for common bean grown at severely deficient ((a) 0.4 μM), limiting ((b) 1.0 μM) and sufficient ((c) 27.0 μM) P concentrations in the sand culture medium. Plants were destructively sampled four times during the growth period (37, 92, 105 and 125 days after planting (DAP)). Asterisk indicates P concentrations of newly formed leaves in (c). At 92 DAP, most leaves had turned yellow and by 105 DAP leaves had mostly turned brown.

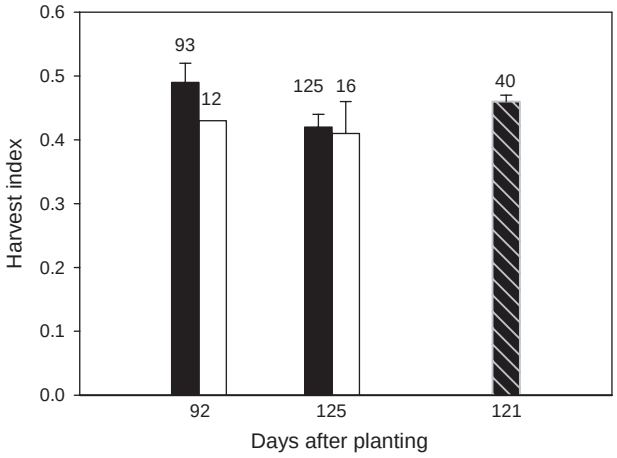

Fig. 3 Harvest index (seed d. wt/shoot d. wt) (+ SE) of common bean grown under limiting (1.0 μM, open columns) and sufficient (27.0 μM, closed columns) P concentrations in glasshouse sand culture conditions and of plants grown under field conditions (hatched columns). For sand culture plants, n = 3–6 except limiting P plants at 92 days after planting (DAP) (n = 1). Sample number for field-grown plants was 20. Numbers above bar indicates average seed d. wt per plant (g).

DAP (Fig. 3). Harvest index, however, was very similar in nonlimiting and phosphorus-limited plants, but was almost nonexistent in severely deficient plants. Harvest index did not increase beyond initial pod dry-down (92 DAP), while total seed weight increased approx. 34% from 92 to 125 DAP (Fig. 3).

Root survivorship Root survivorship was significantly affected by phosphorus supply ($P < 0.0001$), however, the differences in survivorship between limiting (1.0 μM phosphate) and nonlimiting phosphorus (27 μM phosphate) were slight. Root survivorship in the nonlimiting phosphorus treatment was 100% in cohort 1 when followed from the third trifoliate leaf to beyond maturity (25–125 DAP) (Fig. 4). At pod dry down, all four cohorts exhibited greater than 90% survival rate. The high survivorship suggests that unlike leaves, root mortality is not closely synchronized with pod fill (69 DAP) under nonlimiting-phosphorus conditions (27 μM phosphate). Beyond pod dry down, however, some root mortality occurred in cohorts 3 and 4, in the range of 15–25%. Under limiting phosphorus conditions, root survivorship in root cohorts 1–4 remained above 90% from time of first observation until well beyond pod dry down (122 DAP) (Fig. 4). By contrast, under severely deficient phosphorus conditions only 50% of roots in cohort 1 (roots first observed at the third trifoliate leaf: 25 DAP) survived to 122 DAP (well beyond pod dry down), with a 20% decrease in root survivorship observed at the time of pod maturation (82 DAP) (Fig. 4). Cohorts 2, 3 and 4 exhibited a 30% to 40% decrease in root survivorship by the end of the study (125 DAP). The first cohort (roots observed at the third trifoliate leaf) showed the highest mortality rate followed by cohorts 3 (preflowering), 4 (flowering through pod-filling) and 2 (third trifoliate).

The effects of pod fill on root survival were examined by testing the significance of the slopes of the survivorship curves (log% d^{-1}) before and after pod fill (Table 1). In the nonlimiting phosphorus treatment in sand culture, cohort 1 exhibited no decline in survival rate; cohort 2 exhibited a modest decline after pod fill. Roots in the limiting phosphorus treatment exhibited no significant mortality for either cohort

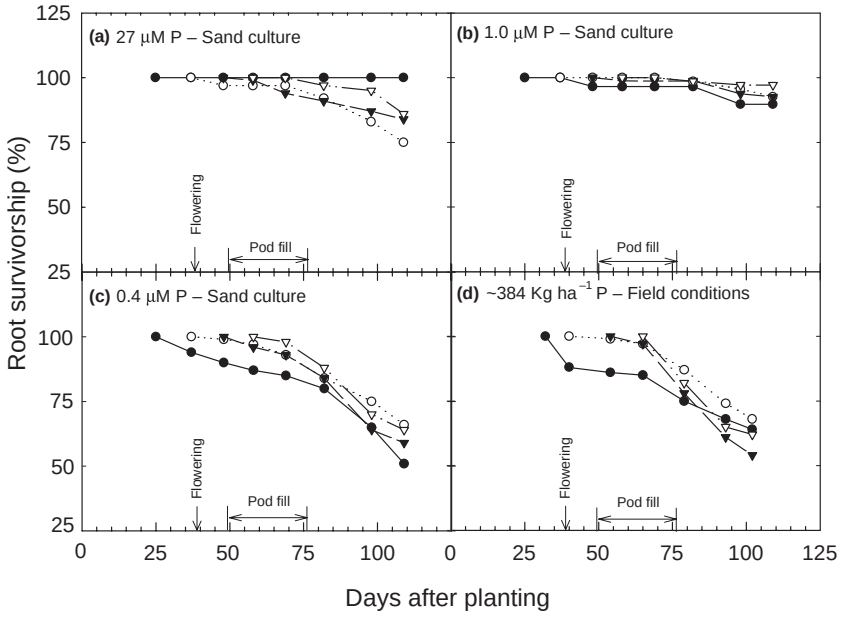

Fig. 4 Survivorship of four cohorts of roots for common bean grown at three levels of P availability under glasshouse sand culture conditions and in the field with high P availability. Cohort 1 (closed circles) represents roots first observed at 25–32 days after planting (DAP) (preflowering, R5; $n = 27, 29, 141$ and 103 for a, b, c and d, respectively), cohort 2 (open circles) represents roots born between the first observation and 37–40 DAP (flowering, R6; $n = 115, 67, 138, 889$ for a, b, c and d, respectively), cohort 3 (triangles) represents roots born between 37 and 40 DAP and 48–54 DAP (pod formation, R7; $n = 176, 80, 95, 432$ for a, b, c and d, respectively), and cohort 4 (open triangles) represents roots born between 48 and 54 DAP and 65–69 DAP (pod fill, R8; $n = 200, 71, 81, 34$ for a, b, c and d, respectively). Periods of flowering and pod fill are indicated. Leaves were beginning to turn yellow by late pod fill; by 92 DAP most leaves were yellow and by 105 DAP leaves had mostly turned brown and were falling off the plant.

Table 1 Survival rate (log percentage/day) of common bean roots up to and following mid-podfill (65 days after planting (DAP)). Cohort 1 represents roots first observed before 25 DAP (glasshouse study), or 32 DAP (field study) and cohort 2 represents roots first observed between 25 and 37 DAP (glasshouse study) or between 32 and 40 DAP (field study). Statistical analyses done for each cohort individually

Treatment	Cohort	Survival rate up to mid-podfill (x10^{-3})	Survival rate following mid-podfill (x10^{-3})
Field	1	−1.03	−9.00***
	2	No mortality	−5.78***
27.0 μM P	1	No mortality	No mortality
	2	−0.12	−3.48**
1.0 μM P	1	−0.16	−2.08
	2	No mortality	−0.81
0.4 μM P	1	−0.79	−8.32***
	2	−0.07	−5.92***

Significance values indicate if rate of survival was significantly different from zero (**$P < 0.01$ and ***$P < 0.001$).

before or after pod fill. Roots in the severely deficient phosphorus treatment exhibited strongly significant decreases in survivorship for both cohorts after pod fill.

Field study

Harvest index was very similar between field plants and nonlimiting sand culture plants (Fig. 3). Seed production in the field was intermediate of that in the limiting-phosphorus and nonlimiting-phosphorus sand culture plants.

In the field, root survivorship declined from 100% to 80% during podfill (Fig. 4). The order of decrease in survivorship among cohorts followed closely that observed in sand culture in the severely limiting phosphorus treatment, in that the first cohort exhibited the greatest mortality compared to subsequent cohorts. After reproductive maturity, root cohorts first observed between stages associated with the third trifoliate leaf and preflowering declined approx. 20%. Roots first observed during preflowering and pod filling exhibited high survivorship until after maturation of the pods, after which survivorship decreased to 50–60%. In the root cohort first observed at pod filling, there was a rapid decline in survivorship, so that root survivorship was as low as 40% by pod maturity. Root survival rate showed a substantial decline after pod fill in both cohort 1 and cohort 2 (Table 1).

At seed harvest, roots of field grown plants had propagules of *Fusarium* sp., *Mucor* sp., *Penicillium* sp., *Rhizoctonia* sp., *Trichoderma* sp., *Pythium* sp., and *Verticillium* sp. In spite of these fungi being identified, the roots were in stable condition and appeared intact and healthy. *Mucor* sp., *Penicillium* sp., and *Trichoderma* sp. are saprophytic fungi, whereas *Pythium* sp., *Verticillium* sp. and *Rhizoctonia* sp. may or may not be pathogenic on bean roots.

Discussion

In this study we employed two environments: a fertile field typical of high-input agroecosystems; and a solid-

phase-buffered sand culture supplying varying phosphorus availability, from adequate to severely limiting. The solid phase buffer provides controlled yet realistic phosphorus regimes in the range encountered in naturals soils. Since 3 μM phosphate is the critical threshold for bean growth in this system (Lynch *et al.*, 1990), the high phosphorus treatment (27 μM) was abundantly adequate without being unrealistically high (by comparison, conventional nutrient solution contains several mM phosphate). The medium phosphorus treatment (1 μM) approximates phosphorus availability in typical low-input tropical bean production environments. The lowest phosphorus treatment induced severe deficiency, at the lower limit of what might be encountered in agricultural production. As has been reported previously, phosphorus availability had substantial effects on plant growth and development in common bean (Lynch *et al.*, 1991). Phosphorus availability had only minor effects on harvest index (Fig. 3; Beebe *et al.*, 1997) and on seed P concentration (Fig. 2; Lynch *et al.*, 1990), suggesting that relative reproductive sink strength remained high in all phosphorus regimes. Shoot senescence was synchronized with pod filling, as observed in previous reports (Lynch & White, 1992; Snapp & Lynch, 1996). Our growth data extend previous reports (Laing *et al.*, 1984) documenting continued seed filling well into physiological maturity in this indeterminate genotype. These results provide a whole-plant context for root demographics during late reproductive development.

In the sand-culture system, phosphorus supply had a statistically significant effect on root survivorship. Biologically, the effects of limited phosphorus supply on root survivorship were generally modest – when comparing nonlimiting-phosphorus to limiting-phosphorus plants, there was a < 11% change in survivorship in spite of a 10-fold difference in plant d. wt. This pattern of high survivorship in the limiting and nonlimiting phosphorus treatments (1.0 and 27 μM phosphate) held true regardless of shoot phenological stage. As plants transitioned from vegetative to reproductive growth, root survivorship was maintained despite a large increase in leaf mortality.

The lack of root senescence during pod fill may be attributed to several factors. Unlike leaves, phosphorus concentrations tend to be fairly low in roots (Fig. 2) and the amount of phosphorus mobilized from root tissue to reproduction may be less than the amount that can be obtained by retaining roots and allowing for continued phosphorus uptake. In addition, roots serve an important function in water acquisition and transport, which is required for cell expansion, a necessary part of podfill. Thus breeding for root systems that senesce at podfill may diminish pod development due to both phosphorus and water stress.

Besides phosphorus, the carbon cost of maintaining and defending roots may outweigh the benefits of root survivorship, especially in monocarps when carbon is demanded in large quantities for reproduction. Root carbon costs are a significant limitation to the growth of common bean plants grown under phosphorus stress (Nielsen *et al.*, 1998, 2001). During pod fill, reducing the carbon costs for root maintenance and defense would allow more carbon to be allocated to reproduction. Even if root senescence is not preprogrammed as in leaves, simply expending less carbon on root maintenance and defense would potentially enhance reproduction.

Under the biological pressure of root herbivores and pathogens in a normal field environment, there was up to 49% disappearance of roots. The increase in root mortality primarily occurred during late pod filling (Fig. 4; Table 1). When comparing field results where plants were sufficient in phosphorus (332–435 kg ha^{-1}) to those in the nonlimiting-phosphorus (27 μM phosphate), sand culture conditions, two explanations seem possible. One is that roots in both systems, field and sand culture, were dead or nearly dead at pod-fill but roots in the field environment were removed by decomposers like insects, fungi and bacteria. Unlike leaves, when a root senesces, it is supported by its soil surroundings so that its death may be less obvious. A lack of disappearance may be attributed to a lack of microorganisms to remove the root from its location. However, most roots at the end of the sand culture experiment in the limiting and nonlimiting phosphorus treatments appeared white and healthy while in the field many had turned brown and appeared unviable (root surface exhibited significant deterioration), which argues against the dead-but-still-intact explanation. An alternate explanation is that herbivores and parasites such as *Pythium* or *Rhizoctonia* accelerated root death in the field environment, in contrast to the glasshouse environment. Perhaps bean has evolved to hold on to all roots for as long as possible until herbivores or parasites remove them. We favour an explanation intermediate between these two extremes: roots are somewhat less defended against herbivores and pathogens during podfill, causing more mortality at this time if herbivores or pathogens are present. There is abundant evidence that weak root pathogens and herbivores are more aggressive when a plant is carbon stressed (Graham, 1995; Eissenstat & Yanai, 1997). That would explain why the roots in the sand culture environment remained on the plant until well past pod fill compared to roots grown in a soil with an abundance of soil decomposers, herbivores and weak parasites.

There has been much debate on the effects of soil fertility on root longevity (Nadelhoffer, 2000; Burton *et al.*, 2000). Most studies have focused on the effects of nitrogen supply on root longevity, with results indicating both an increase (Burton *et al.*, 2000) and a decrease (Majdi & Kangas, 1997; Johnson *et al.*, 2000) in root longevity with an increase in available nitrogen. The effects of phosphorus supply have received considerably less attention, despite the abundance of soils low in available phosphate in most tropical and arctic regions, as well as in many temperate regions (Soil Taxonomy, 1999). Using sequential coring (a method that does not allow tracking the fate of individual roots), Ostertag (2001)

found root longevity to decrease with increased fertility (nitrogen and phosphorus) along a natural fertility gradient in Hawaii. In addition, phosphorus fertilization decreased root longevity compared to unfertilized plots at sites where the treatment was significant. Our results were similar to those of Ostertag (2001). In the sand-culture experiment, high fertility slightly diminished root longevity (Table 1; Fig. 4). We suspect that compared to the phosphorus-deficient plants, the high fertility plants may have been more limited by carbon in their growth and reproduction, since mineral nutrients were supplied in sufficient amounts. Thus, the slightly greater root mortality during and after pod-fill in the unstressed phosphorus treatment may have reflected the benefit these plants may have gained by reducing the carbon demand of excess roots not required for mineral nutrient acquisition.

Under severe phosphorus deficiency, where plants were unable to produce more than two seeds and the plant could not support the growth of more than two leaves at a time, root longevity was considerably reduced. Moreover, survivorship patterns mimicked almost exactly those of roots grown in the field. Roots of the first cohort declined in survivorship slightly almost immediately after they were first observed. Roots in other cohorts exhibited high survivorship until pod filling stage after which they decline rapidly. Our data indicate that the first root cohorts produced may be more at risk for mortality than roots that are produced later, which would indicate that time of emergence is a stronger factor in risk of root mortality than physiological stage.

While root mortality was generally higher in the field than in sand culture, there was still more than 50% root survival in any cohort at the end of the experiment even though all the leaves had senesced weeks earlier (Fig. 4). This genotype of bean, Carioca, is indeterminate. In indeterminate genotypes, roots may remain viable as a strategy to allow the plant to have a second flush of seed production if rainfall permits. In this study, after pod fill was complete and leaf senescence had occurred, new leaves began to emerge from the apical meristematic regions of shoots in both nonlimiting and limiting phosphorus treatments.

In conclusion, unlike leaf senescence, the timing of root senescence in common bean did not coincide with pod filling under controlled conditions, unless phosphorus was severely limited. Under field conditions in fertile soil, the timing of root mortality did coincide with pod filling, suggesting that a rich soil biota is required for mortality patterns to be expressed when phosphorus is not severely limited. In the limiting-phosphorus treatment, restricting phosphorus supply so that plant growth was reduced 90% slightly increased root survivorship compared to nonlimiting phosphorus conditions. By contrast, severe phosphorus deficiency decreased root survivorship approx. 40% by the end of the study. Our data suggest that unlike leaves, root senescence is not a preprogrammed response in annual plants.

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