REGULAR ARTICLE

Fine root diameters can change in response to changes in nutrient concentrations

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Abstract Plant roots function in the critical role of water and nutrient uptake. Although extensive data exist on functioning of seedling roots, little is known of the actual functionality of the fine roots of mature plants. Although this class of root represents 90% or more of the total root length of a given mature plant, their small size has inhibited detailed studies. Commonly, the critical metrics for studies of root function are root length and total weight, expressed as Specific Root Length. The metric that classifies "fine roots," root diameter, is rarely a focus except as average diameter, even though this is the primary characteristic from which accurate estimates of surface area and volume can be calculated. Using data from several preliminary experiments, this study shows consistent changes in measured fine root diameter with changes in concentration of some nutrients. Twelve different species demonstrated concentration dependent diameter increases, or decreases, in response to increasing concentrations of nitrate, phosphorus, aluminum or tannic acid. On the other hand, Cacao (*Theobroma cacao* L) fine roots changed diameter in response to changes in nitrate concentration, but not ammonium. Clearly pattern of diameter change in response to nutrient concentration is dependent on nutrient, species and their interaction. It is suggested that the routine assessment of fine root diameter will be essential to understanding nutrient uptake dynamics.

Keywords Fine roots · Root diameter · Nutrients · AMMI-VC · Relative diameter class length · Diameter class

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Introduction

Much of our knowledge about plant root function is based on seedling responses. Technically, seedling research is faster and easier to deal with, than intact pre- and full-flowering plants. As a consequence, we have much information about young seedling root function. This includes classical physiological studies and more modern molecular research. Taking a tongue-in-cheek approach, the operating paradigm for much existent root research appears to be: in a relative absence of data to the contrary, it must be assumed that all roots have similar if not identical functional characteristics.

Our laboratory does not subscribe to this paradigm, but rather to the opposite paradigm: In an absence of evidence to the contrary, it must be assumed that differences in size, shape and ontogeny herald differences in function. Previous publications from this laboratory suggest that, in mature seedlings, there are up to four distinct types of root with unique functional patterns (Zobel 1975, 2005a, b; Zobel et al. 1992; Bushamuka and Zobel 1998). We believe this can be extended to roots <1 mm in diameter. Recently, Zobel et al. (2006) demonstrated that in chicory, fine roots can display three different cultivar-specific responses to phosphorus deprivation: first, a reduction in length of one diameter class of fine roots in favor of a second, thinner diameter class; second, a reduction in fine root mass density without a concomitant change in length; third, the reverse of the first type of response. In the first response type, the larger roots averaged 0.86-mm diameter, and the smaller averaged 0.28-mm diameter (approx. 75% of total root length). The images, from which these data were taken, were photographed at 317 dpi (12.5 p mm^{-1}), and, with the diameter class length plotted on a log scale, the polygonal histogram displayed dips/humps in the curve, suggestive of distinct diameter classes. Two of these humps appeared to correspond with the 0.28 and 0.86 diameter classes. Similar dips and humps in histograms have been demonstrated with three other species (Zobel 2005a).

Ryser and Lambers (1996) and Ryser (1998; cf p 452 – fig7) present several root histograms that appear to show shifts in Dactylis glomerata L. (Orchardgrass) root diameter with shifts in nutrient level. These apparent shifts are on the level of 10% of the nominal diameter of the roots, a change that can not be accurately assessed with current image analysis software (this issue will be covered in a separate paper). In the latter of these two wide ranging articles, Ryser (1998) demonstrates differential changes in mass density of the roots, such that, in one species Specific Root Length (SRL – Length of root per unit mass) changed with changes in length and with another, SRL did not change though root length and mass did.

Discussion of changes in root diameter and mass per unit length, necessarily raises the question of changes in anatomy, and function. McCully's lab has a long history of coupling anatomical and physiological studies. In a summary of much of their research (McCully 1987), it was pointed out that the finest

roots of maize (approx 0.07-mm diameter) were anatomically rudimentary, and routinely shed their apex after several days of growth. This pattern of development has not been described for larger roots, and suggests that fine roots may indeed have some very interesting structural/functional relationships. A first step along the way to discovering these relationships is to demonstrate that changes in fine root diameter, in response to changes in nutritional environment, are a common occurrence.

To determine whether fine root diameter shifts are real, were anomalies, or were possibly a consequence of some form of experimental error, we re-examined the data from three preliminary experiments from our laboratories. These experiments studied the response of various species to differing concentrations of nutrients or toxicants. This then is a preliminary report on diameter changes. On-going experiments designed to assess anatomical and physiological concomitants of diameter change will be the grist of future papers.

Materials and methods

Plant materials consisted of *Aeschynomene americana* L. (Joint-vetch), *Cajanus cajan* (L.) Millsp. (Pigeon-pea), *Calapogonium muconoides* Desv. (Calopo), *Canavalia ensiformis* (L.) DC (Jackbean), *Crotalaria spectabilis* Roth (Showy Crotalaria), *Desmodium heterocarpon* (L.) DC. (Ea-Ea), *Mucuna pruriens* L. DC. (Mucuna), *Pueraria phaseoloides* (Roxb.) Benth (Puero), *Sesbania sesban (L.) Merr. Subsp. punctata* (DC.) (Sesbania), *Theobroma cacao L.* (Cacao), *Triticum aestivum* L. cv Scout. (Wheat), and *Vigna unguiculata* (L.) Walp. (Cowpea). ILDIS World Database of Legumes, version 10.01, was used to verify the names of legume species; http://www.ildis.org/LegumeWeb/.

Roots were scanned in a tray made from a glass sheet fitted with Plexiglas walls that was laid on the scanner-imaging surface and filled with 1- to 2-mm-deep reverse-osmosis water. Roots were spread out in the water on the glass and scanned in positive transparency mode and 8-bit grey scale, using the attached light lid. All other scanner settings, such as sharpening, etc. were turned off. A Dell Optiplex GX270 (Trade names are mentioned for the benefit of the reader, and are not recommendations by the USDA) was used to drive the scanner (an Epson Perfection

Fig. 1 Histogram of Cacao relative diameter class length by diameter class for roots grown at four different nitrate concentrations (data for the lowest treatment concentration in this experiment are missing). Images were scanned at 24 p mm^{-1}, and were analyzed with WR
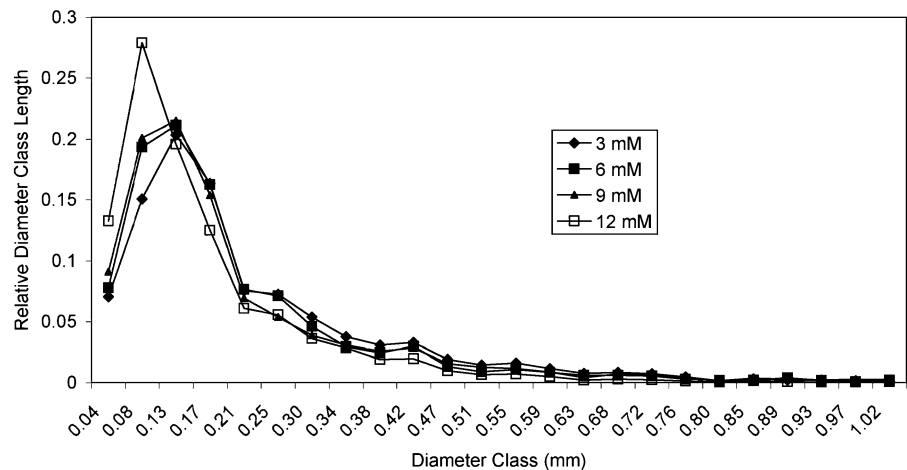
4870 PHOTO; non-interpolated image capability is 4,800×9,600 dpi [roughly 200×400 p mm^{-1}]; the 9600 axis is accomplished with a stepping motor, such that images at 4,800 dpi are 4,800×4,800), store the images, and analyze the images with WinRhizo, v 2005b (regent.qc.ca). Routine settings for WinRhizo were diameter interpolation, maximum diameter sensitivity, and automatic thresholding (company confidential processes). Diameter class size was set to the scanner resolution+0.00001 p mm^{-1} (required to adjust for an artifact which occurs when diameter class size is set to the exact scanner resolution). Specific resolutions are indicated in the experimental designs.

Since many treatments can result in differential plant sizes, the data were routinely transformed: The value of a given Diameter Class Length (DCL, the sum of root lengths within a diameter class) was divided by total root length, yielding a proportion of total root length (or relative Diameter Class Length – rDCL). This normalizes much of the disparity between plants of different sizes, and can highlight areas where DCL responses occur.

Statistical analyses were routinely made with the AMMI (Additive Main effects and Multiplicative Interaction) analysis (Zobel and Wallace 1995) in its Tukey (1962) 'vacuum cleaner' mode (AMMI-VC). This variant consists of extracting the grand mean, treatment means, and associated statistics, then running joint regression, diameter class regression, and treatment regression on the residual and, where significant, extracting their variance from the residual, before applying singular value decomposition to the new residual. The resulting ANOVA table is con-

densed by placing the degrees of freedom and variances of the nonsignificant terms into the error term *df* and SS respectively, providing a very parsimonious ANOVA model.

In addition to the statistical analyses cited above, nonlinear regression analyses of the datum sets were conducted. Nonlinear regression analysis has been used extensively for the description and statistical analysis of data (Draper and Smith 1998). This method has been especially helpful in the quantitative resolution of multiple toxic and ameliorative effects of ions upon root elongation (Blair and Taylor 1997; Kinraide 1999; Kinraide 2003). Nonlinear regression analysis is useful too because the functional relationships thus obtained may then be used for graphic presentations. Here we present our use of the method to describe root-diameter responses to the addition of solutes to the growth medium.

To find an appropriate equation to describe the data, several of the datum sets in the study were subjected to a survey for equations by TableCurve 2D 5.01 (Systat Software Inc., Richmond, CA 94804-2028). Examples of the data are presented in the Figs. 1, 2a, 3 and 5 in which root rDCL (or DCL) is plotted against diameter class. These data include replications and treatments (different concentrations of solute in the rooting media) that were not considered in this initial survey for equations. The chosen equation met these criteria: (1) r^2 was among the largest for the many equations surveyed. (2) The number of coefficients was relatively small. (3) Each coefficient evaluated significantly; that is, the 95% confidence interval did not encompass zero. (4) Each coefficient has a simple meaning (see below).

Fig. 2 Histograms of Cacao relative diameter class length for roots grown at five different nitrate concentrations(plots **a**, **c**) and five different ammonium concentrations (plot **b**). Images (252 p mm^{-1}) were analyzed with WR. Note the co-incident dips in the plots (between treatment concentrations) in **a**. **a** Un-modified relative length data (diameter class length divided by total root length). Histograms of the data generated by the non-linear model for ammonium treatments (**b**) and nitrate treatments (**c**). Differences in peak height and diameter class are clearly shown in plots **b** and **c** as compared to plot **a**
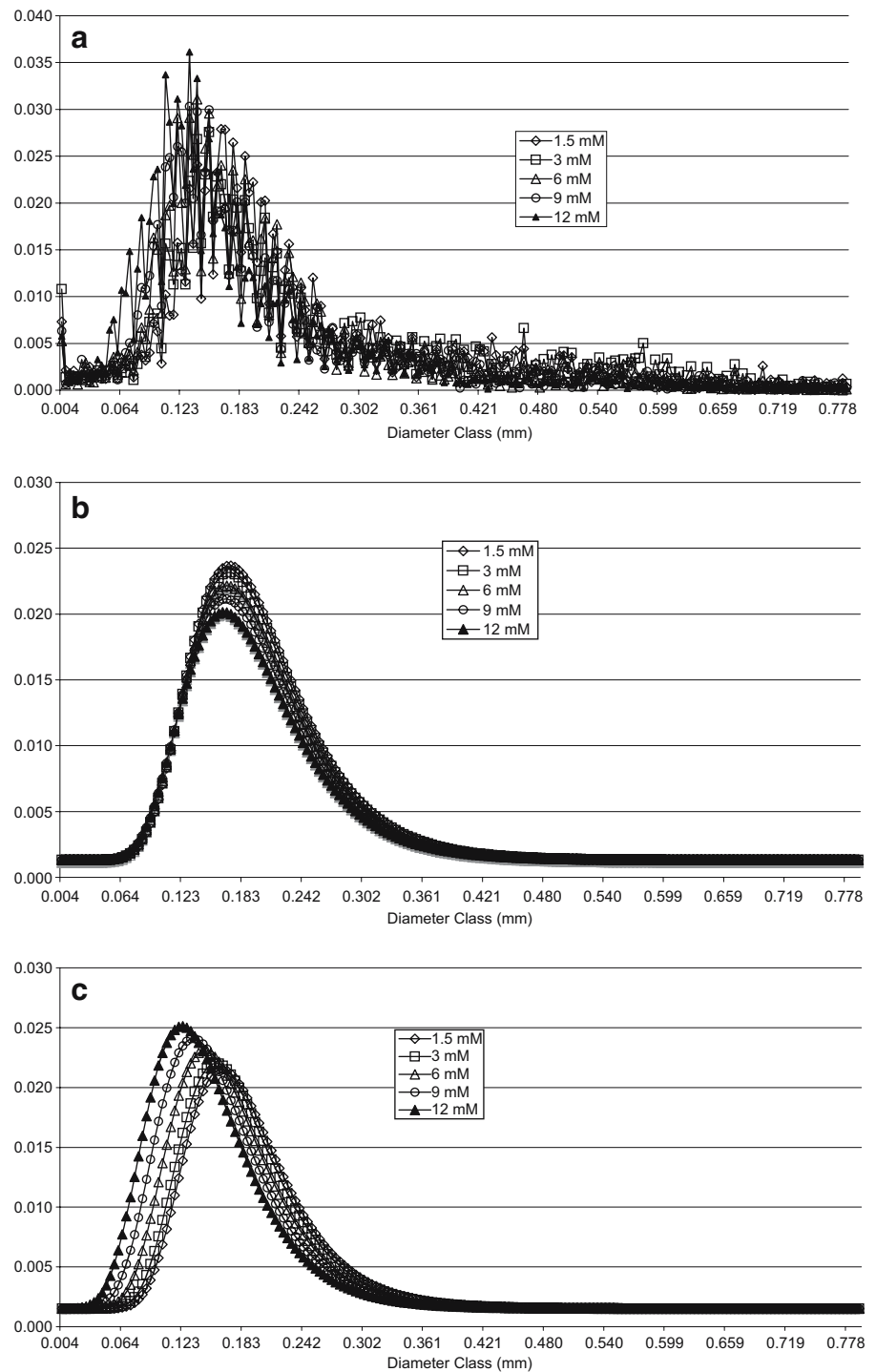

The chosen equation, written below, is referred to as 'Extreme Value' by TableCurve.

$$y = a + b \exp\left[-\exp\left[-\frac{x-c}{d}\right] - \frac{x-c}{d} + 1\right] \quad (1)$$

In our study, y refers to DCL or rDCL, and x refers to diameter class. In general, the coefficient a is the value approached by y as x approaches positive or negative infinity, b is the peak value for y minus a, c is the x-axis location of b, and d is related to the

Fig. 3 Histograms of the WR analysis of four randomly selected images (legend indicates image number) from the collection of 300 legume species images. Data points were connected with smoothed lines and then the point markers (except for legume065) removed from the figure. Note the coincidence of dips and peaks in this randomly selected data. The markers on the line for legume065 are one pixel apart. Note that there are generally three or four markers for each peak/dip pair. This implies a systematic artifact in the software
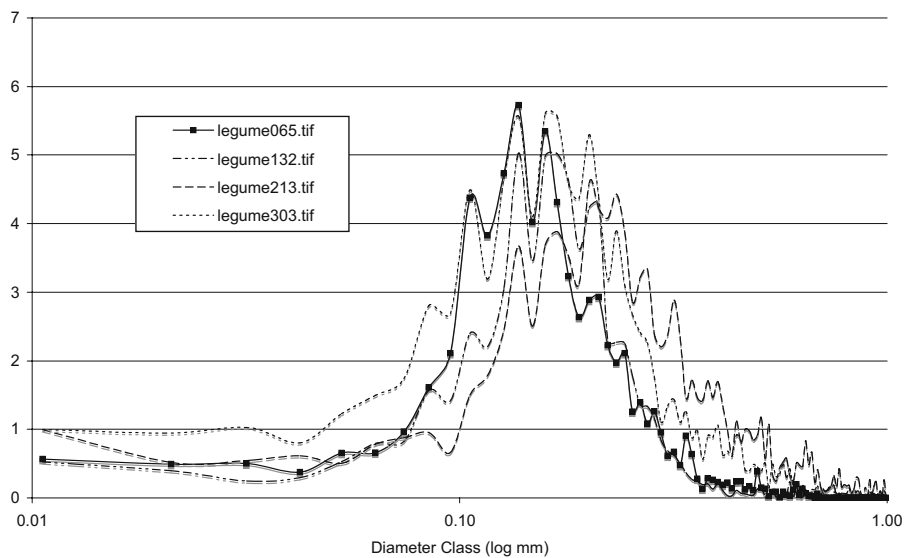
width across the curve at half maximum $(b/2+a)$ so that width at half maximum equals $2.446\ d$ (User's Manual for TableCurve 2D 5.01).

The principal objective in this portion of the study was to determine whether solutes in the rooting media influence root characteristics, especially whether roots become thinner or thicker in response to treatments. That is, do changes in solute concentrations shift the curve leftward (roots become thinner) or rightward (roots become thicker)? To determine the influence of treatment, the coefficients in Eq. 1 were expanded to include treatment level (TR) so that as TR increases the values of the expanded coefficients increase.

$$y = a + b(1 + b_1 \mathrm{TR})\ \exp$$

$$\left[-\exp\left[-\frac{\mathrm{DC} - c(1 + c_1 \mathrm{TR})}{d} \right] - \frac{\mathrm{DC} - c(1 + c_1 \mathrm{TR})}{d} + 1 \right] \tag{2}$$

If coefficient b_1 evaluates as a significantly positive number, then the treatment enhances the peak height and thus the total root length, unless d changes significantly. If coefficient c_1 evaluates as a significantly positive number, then the treatment enhances root thickness. Coefficient a is problematical since roots cannot have a diameter <0, nor can there be any roots, in seedlings at least, that are very thick. Thus one would expect that a would approximate 0, however it is

retained here for mathematical balance. In most cases d did not change with treatment; therefore, both a and d are treated as constants that are characteristic of the species being studied. To evaluate the coefficients in Eq. 2 we used SYSTAT for DOS, Version 6, but later editions of the software are available.

Experiment 1

Fifteen-day-old Cacao seedlings, selected for uniformity, were grown in 2-l plastic pots containing vermiculite. Plants were placed in a growth chamber at 30°C, 75% relative humidity, and 14 h PPFD of 350 µmol m^{-2} sec^{-1}. Plants were watered with five concentrations of NO_3 (1.5, 3.0, 6.0, 9.0, and 12.0 mM with N as $Ca(NO_3)_2\ 2H_2O$) or ammonium (1.5, 3.0, 6.0, 9.0, and 12.0 mM as $(NH_4)_2SO_4$) in modified Snyder nutrient solution (Hewitt 1966 [The original for Hoagland and Arnon 1938]) at pH 5.5, a nutrient solution containing all the other essential nutrients to support good Cacao growth. Pots were surface irrigated with nutrient solution for 5 days with 100 ml of a given nutrient treatment, and on the sixth day each container was rinsed with deionized water until dripping from the bottom to reduce nutrient accumulation. The treatments were replicated three times. Plants were harvested on the 57th day of growth, and washed in deionized water.

Roots were separated from the shoot, cut into approximately 2-cm long pieces, rinsed, thoroughly mixed, and a 1-g (fresh weight) representative sample removed for assessment of root diameters. The roots were scanned first at 24 p mm^{-1} (600 dpi), and, subsequently, at 252 p mm^{-1} (6,400 dpi, with interpolation from 4,800 dpi – in hindsight, this was a mistake). At the higher resolution, four square images (10 cm on a side) of different portions of the glass plate were scanned into memory. The images were subsequently analyzed with WinRhizo. For the 252 p mm^{-1} images, the DCL data were summed across the four sub-images to produce a sample value.

Experiment 2

Calopo, Cowpea, EaEa, Jackbean, Jointvetch, Mucuna, Pigeonpea, Puero, Sesbania, and Showy Crotalaria were grown in 2-l plastic pots containing washed Ottawa sand in a growth room at 30°C and 75% relative humidity with 14 h of PPFD of 400 μmol m^{-2} sec^{-1}. Plants were grown in modified Snyder solution (Hewitt 1966) at three phosphate concentrations (0.1, 1.6, and 3.2 mM). Pots were flushed with 100-ml nutrient solution daily and flushed with distilled water every third day to remove accumulations. Roots were prepared as in Experiment 1. Images were produced in a fashion similar to Experiment 1, scanning at 94 p mm^{-1} (2,400 dpi). Images were analyzed with WinRhizo, and lengths of sub-samples were added together to produce a sample value.

Experiment 3

Seeds of cv. Scout Wheat were germinated on filter paper and 2-day-old seedlings, selected for uniformity, were placed in foam pieces and floated (six seedlings per beaker) on 500-ml solutions of continuously aerated 1 mM $CaCl_2$ supplemented with tannic acid and $AlCl_3$ (Al), in a factorial design and adjusted to pH 4.5. The concentrations used were 0, 1.2, 3.6, 10.8, 32.4, or 97.2 mg l^{-1} tannic acid or 0, 3, 6, or 9 mM Al. The beakers were then placed in a dark incubator. The seedlings were harvested 6 days later; whole root systems were scanned at 47 p mm^{-1} (1,200 dpi); and the images were analyzed with WinRhizo.

Results

Experiment 1

Visual inspection of a plot of the low resolution WinRhizo images (24 p mm^{-1}) of Cacao roots grown at four concentrations of nitrate (Fig. 1) might not convince readers of a significant effect of nitrate concentration upon root diameter. Statistical analysis of the relative diameter class length (rDCL) data, however, indicated a significant pattern of interaction between root diameter and nitrate concentration (data not presented). This suggests that the apparent single pixel diameter class difference between the peak rDCL of the 12-mM treatment and the other concentrations of nitrate represents a significant change in root diameter. To more closely explore this apparent one pixel shift in diameter, the roots were scanned again at 252 p mm^{-1}, relying on scanner interpolation to increase the effective resolution from 189 p mm^{-1} to 252 p mm^{-1} (Fig. 2a).

The high-resolution data were analyzed with the AMMI-VC statistical model (Table 1), and the analysis indicated significant nitrate regression variance

Table 1 Cacao Ammonium and Nitrate high resolution (252 p mm^{-1}) reduced AMMI-VC tables

Ammonium

Source	df	SS	MS	Prob.	Sig.
Total	799	234.25	0.293		
Treatment	399	204.02	0.511	0.0000	***
Ammonium	4	10.39	2.599	0.0000	***
Diameter Class	79	158.51	2.006	0.0000	***
Joint Regr (K=3.40)	1	23.82	23.826	0.0000	***
Ammonium Regrs	3	2.61	0.871	0.0000	***
Error	712	38.90	0.054		
Grand mean	0.352				

Nitrate

Total	799	221.55	0.277		
Treatment	399	206.30	0.517	0.0000	***
Nitrate	4	1.59	0.397	0.0000	***
Diameter Class (DC)	79	178.97	2.265	0.0000	***
Joint Regr (K=4.43)	1	6.97	6.978	0.0000	***
Nitrate Regrs	3	0.64	0.216	0.0002	***
DC Regrs	78	12.56	0.161	0.0000	***
Error	634	20.79	0.032		
Grand mean	0.381				

(49% of the non-additive treatment variance) and a significant though relatively weak ammonium regression variance, suggesting the appropriateness of a non-linear regression analysis (Table 2). Modelled curves for ammonium (Fig. 2b) demonstrate an additive variance effect (decreasing rDCL with increasing ammonium) but no interaction with diameter class. The modeled curves for the five nitrate treatments (Fig. 2c) demonstrate a 0.04-mm (approx. 10 pixels) separation of the 1.5-mM (diameter class= 0.17 mm), and the 12-mM (diameter class=0.13 mm) nitrate peaks. Peak rDCL also increased from 0.021 to 0.025 mm as nitrate concentration increased from 1.5 to 12 mM.

Experiment 2

The legume images from the 10 different species and three concentrations of phosphorus, resulted in WR histograms that show co-incident dips in the curves across all 300+ images (Fig. 3). The pattern of pixel representation in these plots suggests a consistent artifact induced by the software. The legumes responded to differing concentrations of phosphorus in one of four ways (Table 2). As phosphorus concentration increased, Jackbean, Calopo, and JointVetch roots became thinner and attained a larger peak rDCL (Fig. 4a). Phosphorus increase caused the fine roots of Sesbania and Pigeonpea to become thicker and

Table 2 Root responses to solutes in the rooting medium modeled according to Eq. 2 in which positive or negative values for b_1 indicate increases or decreases, respectively, of root elongation and positive or negative values for c_1 indicate a thickening or a thinning, respectively

Species	a	b	b_1	c	c_1	d	r^2
Experiment 1							
Treatment: $(NH_4)_2SO_4$ at 1.5, 3.0, 6.0, 9.0, or 12.0 mM							
rCacao	0.00132	0.0229	−0.0147	0.170	−0.0031 ns	0.0511	0.852
Treatment: $Ca(NO_3)_2$ at 1.5, 3.0, 6.0, 9.0, or 12.0 mM							
rCacao	0.00152	0.0195	0.0176	0.167	−0.0224	0.0456	0.873
Experiment 2							
Treatment: Phosphate at 0.1, 1.6, or 3.2 mM							
rSesbania	0.0063	0.0539	0.0794	0.0808	0.0584	0.0430	0.892
rPigeonpea	0.0073	0.0422	0.1060	0.121	0.0286	0.0488	0.845
rCowpea	0.0122	0.0315	0.0188 ns	0.148	0.0125 ns	0.0569	0.833
rMucuna	0.0059	0.0229	0.2060	0.205	0.0032 ns	0.0689	0.766
rPuero	0.0077	0.0312	0.2450	0.134	0.0016 ns	0.0497	0.788
rEa Ea	0.0103	0.0447	0.0953	0.106	−0.0182 ns	0.0395	0.849
rShowy Crotalaria	0.0044	0.0316	0.1290	0.119	−0.0183 ns	0.0731	0.729
rJackbean	0.0044	0.0446	0.1230	0.155	−0.0414	0.0497	0.819
rCalopo	0.0054	0.0307	0.2150	0.195	−0.0437	0.0616	0.801
rJointvetch	0.0049	0.0472	0.1550	0.103	−0.0645	0.0468	0.858
Experiment 3a							
Treatment: Tannic acid at 0, 1.2, 3.6, 10.8, 32.4, or 97.2 mg L^{-1}							
Wheat	0.114	5.18	−0.0328	0.267	0.0359	0.0503	0.806
Experiment 3b							
Treatment: $AlCl_3$ at 0, 3.0, 6.0, or 9.0 mM							
Wheat	0.101 ns	6.32	−0.0835	0.265	0.0367	0.0494	0.836

Lower case 'r' preceding the species common name indicates analysis on the basis of relative diameter class length (rDCL)

Fig. 4 Non-linear regression model based histograms of 3 of the 10 legume species treated with three concentrations of phosphate. **a** Jackbean, showing an increase in relative diameter class length and decreased diameter in response to increasing phosphorus; **b** sesbania, showing an increase in relative diameter class length and increased diameter with increasing Phosphorus; **c** cowpea, the one line with no response to the different phosphorus concentrations
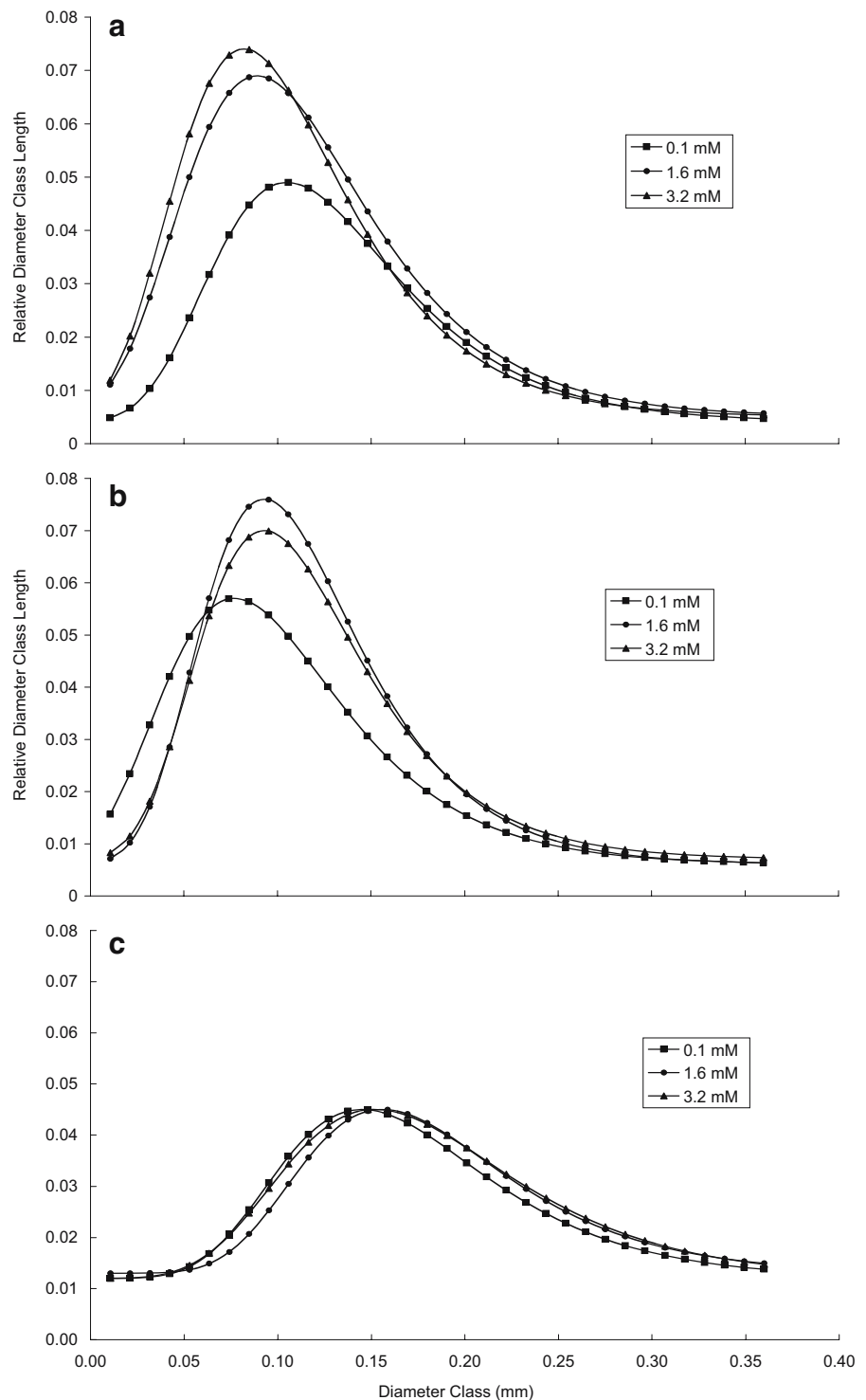
to attain a larger peak rDCL (Fig. 4b). On the other hand, phosphate increase had no significant effect on the root fineness of Mucuna, Puero, EaEa, or Showy Crotalaria, but the increase did cause the fine roots of these species to assume a larger peak rDCL. Finally, the fine roots of Cowpea did not show any significant response to changes in phosphate concentrations (Fig. 4c). The nominal peak diameter class (*c* at

Table 3 Modeled curve maximum height and corresponding diameter class for Al and tannic acid treatments

Aluminum (mM)	Peak DCL (cm)	Peak Diameter Class (mm)	Tannic Acid (mg l⁻¹)	Peak DCL (cm)	Peak Diameter Class (mm)
0	6.42	0.265	0	5.29	0.267
3	4.84	0.294	1.2	5.09	0.279
6	3.25	0.323	3.6	4.68	0.302
9	1.67	0.353	10.8	3.46	0.371
			32.4	0.95	0.440
			97.2	0.86	0.440

The values for the two highest concentrations of Tannic acid were estimated from images because the linear nature of the treatment adjustments to the model gave very un realistic values at these levels

TR=0) differed amongst the 10 species from a low of 0.08 mm for Sesbania to a high of 0.21 mm for Mucuna (Table 2).

Experiment 3

Wheat seedling roots respond to Al concentrations by becoming thicker and shorter at higher Al concentrations (Tables 2 and 3). Roots thickened from 0.265 mm at 0 Al to 0.353 mm at 9 mM Al. Al suppressed DCL at all concentrations (DCL was used rather than rDCL because the analyzed roots were seminal roots only). There was a similar response to tannic acid concentrations (Table 3) where roots thickened from 0.267 mm at 0 tannic acid to 0.440 mm at 97.2 mg l⁻¹

tannic acid. Tannic acid, at the two lowest concentrations, had no significant effect on root length compared to the 0 tannic acid treatment. The higher concentrations (10.8, 32.4, and 97.2 mg l⁻¹) all significantly suppressed root length. Manual (pixel counting from enlarged images) measurement of the diameters in the root images from which the histogram in Fig. 5 was produced, indicate that the visible severe dips in the plots, at 0.32 and 0.4 mm, are software analysis artifacts and not root or image characteristics.

Discussion

As scanner and camera resolutions are increased with the intent to accurately measure very fine roots, the presence of extensive 'noise' in image analysis data becomes apparent (Fig. 2a). Some of this noise is undoubtedly due to natural variation in root diameters (increasing or decreasing one cell layer will result in roughly a 20 μ [0.02 mm] change in root diameter while cellular swelling or shrinking might cause smaller changes) and surface characteristics, such as root hairs, branching and sloughed tissues, which can affect the analysis. However, as the plots in Figs. 2a and 3 indicate, there is a systematic pattern of artifacts (dips and peaks) that must be due to either the imaging or analysis processes. Since pixel analysis of the images from which the data in Fig. 5 was produced do not confirm the very strong dips at 0.32 and 0.4 mm, it must be concluded that the software generates these artifacts, rather than the imaging scanner. Data modeling through non-linear

Fig. 5 Histograms of the diameter class lengths of wheat roots grown in four concentrations of Aluminum. Note the severe dips in the curves at 0.32 and 0.4 mm. These images were of whole root systems and the roots were more or less parallel
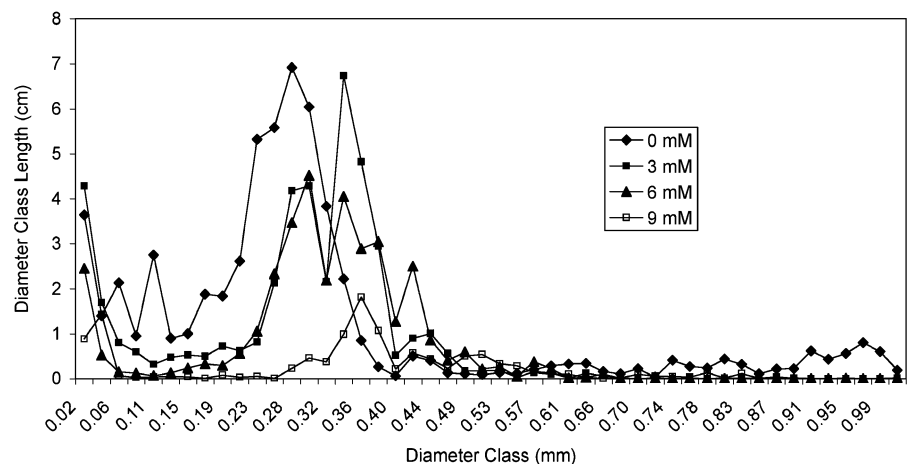
regression techniques was able to demonstrate that there are significant patterns of proportional root length increase and diameter change which are visually obscured by these software induced artifacts (Fig. 2a vs. 2c, and 3 vs. 4).

Increased concentrations of Al or tannic acid resulted in reduced rates of root elongation and increased root diameter. The diameter increases at the lower concentrations of Al were general increases in diameter with no obvious localized swelling (data not presented) as seen at higher concentrations and described in the literature on Al toxicity (Blancaflor et al. 1998). This suggests that there may be two separate physiological responses involved. Since the two lowest concentrations of tannic acid produced no significant reduction in DCL, the increase in diameter at the lower concentrations, per se, may not be a toxic response. On the other hand, Cacao fine roots become longer (either more rapid elongation or initiation, or both, as demonstrated by Smilauerova and Smilauer (2002)) and thinner with increasing nitrate, a typical response to increasing nitrate levels (Marschner 1995). However, ammonium differences had no significant effect on root diameter.

Experiment 2 demonstrated the potential complexity of measuring root elongation and diameter and relating them to functional aspects of the plant root system. There were four distinctly different patterns of response amongst the 10 species studied. One species, Cowpea, demonstrated no response (i.e. no significant change in diameter class, rDCL, or DCL) to the three concentrations of phosphate. Four species (Mucuna, Puero, EaEa, and Showy Crotalaria) demonstrated increased relative elongation with increased P concentration, but no change in fine root diameter. The third pattern (Sesbania and Pigeonpea) was an increase in relative elongation and root diameter with increasing phosphate. The fourth pattern was increasing relative elongation and decreasing diameter with increasing phosphate (Jackbean, Jointvetch, and Calopo), a pattern that is often seen at low phosphorus levels (Marschner 1995).

Assessments of morphological changes in fine root populations and their relationship to changes in functionality will have to be assessed on a case-by-case basis with clear documentation of root length, diameter, number of root tips, and mass density changes. The data from Smilauerova and Smilauer (2002) demonstrated that one species (*Poa angustifolia*) reduces the number of root tips (number of fine roots)

when nutrients are increased, while *Luzula campestris* and *Plantago lanceolata* do not change numbers of roots but rather change the length of roots. In that study, *L. campestris* increased in root length and *P. lanceolata* decreased in root length, in response to increased nutrient concentrations. Number of root tips is an additional factor to be documented, because *P. angustifolia* did not demonstrate any change in total root length with increasing nutrients, just numbers of roots. It should be noted that the Smilauerova and Smilauer (2002) research did not describe any assessment of diameters.

Specific Root Length is an excellent tool to easily demonstrate plant root functional differences/changes. The above discussion and the work of Ryser (1998) suggest that there are a number of phenomena responsible for the observed changes in SRL. To thoroughly understand the structural functional relationship of fine roots to their environment, each of these phenomena must be taken into account. It is possible that anatomical/physiological studies will indicate additional characteristics that should be documented if we are to thoroughly understand fine roots. In addition, some significant improvements in image analysis software, and the routine use of imaging at the 200 p mm^{-1} resolution range, will be required to accurately separate root tips from broken ends of root segments as well as 10% changes in diameter.

The diversity of nominal diameters amongst the legumes in Experiment 2 (from 0.08 to 0.21 mm) suggests that initial imaging with a new cultivar or species should be done at the highest possible resolution to determine the minimum resolution necessary to document possible changes in diameter and identification of root tips. Lyford (1975) states that the smallest roots of oak, maple, and birch are in the 60-μ diameter range. This, taken with the Cacao, Jointvetch and Sesbania data here and the data of Wright et al. (1999), suggests that it would be judicious to do an initial scan of roots of all species at 200 p mm^{-1}, at least, in order to correctly identify the resolution best able to image and analyze the fine roots under study. (N.B. Epson [http://www.Epson.com] has just announced a 6400 dpi [252 p mm^{-1}] scanner). The histograms of Fig. 3, clearly suggest that current image analysis technology lacks the necessary precision to carry out these analyses without reliance on data smoothing techniques like non-linear regression.

Much additional research on fine roots is also necessary. McCully (1987) described the anatomy of 0.07-mm diameter corn lateral roots. She pointed out that these roots had only an epidermis, hypodermis, and an endodermis – one cell layer for each – and no parenchymatous cortical cells or pericycle. Miller (1981) first demonstrated corn roots in the <0.1-mm diameter range having a rudimentary vascular system. McCully (1987) followed this up and found that the stele had either one small xylem vessel or none at all. McCully's (1987) research and later that of Cahn et al. (1989), Varney and McCully (1991) and Varney et al. (1991) also described these roots as determinate and without an organized apex when at full length. Zobel (2005b citing the work of Cahn et al. 1989 and others) suggested that the smallest diameter roots in most species have fixed lengths and a short lifespan. Cell size at these root diameters can be on the order of 10 mm or less, indicating that a decrease in diameter of 0.01 mm (10 μ) may represent the loss of a cortical cell layer or a dramatic shrinking of a large number of cells. Decreasing the number of cortical cell layers (relatively low mass density) should result in a mass density increase for the root. Clearly, anatomical studies, along with physiological studies of fine roots that change diameter with changes in the environment, are desperately needed.

The data from these three experiments with 11 species, when taken as a whole, suggest that a change in diameter of very fine roots may be a normal response to changes in some solute concentrations. Many more species, cultivars, and solutes will need to be surveyed before any generalizations can be made or causal theories developed. Most discussion of diameter change in response to solutes has been in terms of toxicity responses such as those of Experiment 3. This demonstration of differential species/cultivar changes in root fineness, in response to changes in single nutrients, underscores the conclusions of Zobel (2005b) that the finest roots have a response pattern that may be distinct from that of larger roots. True, large roots also change diameter, but those changes should have minimal impact on their ability to function. Future research with fine roots will need to document anatomical characteristics as well as diameter, length, and mass density. If root imaging and other technologies can be further developed to support separation of roots into diameter classes followed by determinations of mass density, anatomy, and function,

a much clearer picture of the structure/function relationships of fine roots relative to changes in their environment will be obtained.

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