Seedling growth and survival, in relation to seed size and phosphorus content, of six Fynbos Proteaceae species deprived of single mineral nutrients

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Abstract

Seedlings of six Fynbos Proteaceae species (four acid sand and two limestone species) with a range of seed sizes were grown under nutrient conditions where all essential elements except one were supplied.

Initial seed size had a positive effect on plant mass, while there was no relationship between seed size and relative growth rate. Phosphorus content of cotyledons was positively related to seed size and the initial phosphorus content had a positive effect on plant mass. There was no difference in phosphorus content of cotyledons between acid sand and limestone species.

High concentrations of nutrients had an adverse effect on Proteaceae seedlings. Pottasium was the most limiting nutrient where plants had high root to shoot ratios. Phosphorus was the least limiting nutrient and plants were able to survive on cotyledonary reserves.

The lack of an external phosphorus requirement is more enhanced in the acid sand species and may be an adaptation of particular species to survive in nutrient poor edaphic environments.

Introduction

The reproductive strategy of a particular plant species may involve the partitioning of its seed output into many small seeds or a few large ones, depending on the environmental constraints placed on the allocation of its resources (Fenner 1985). Seed size seems to be a fixed characteristic of each species, but the size of a seed must be a compromise between effective dispersal (favouring small seeds) and successful establishment (favouring large seeds) (Fenner 1985).

Large seeds are characteristic of plants that are constrained by some environmental factor such that it is more advantageous to produce fewer seeds, but larger and more nutrient rich so that the seedlings have enough energy and nutrients for successful establishment and survival (Fenner 1985). Small seeds, on the other hand, are produced in large numbers and are characteristic of plants with short life cycles without environmental constraints and often form large seed banks favouring the dispersal of the species rather than necessary establishment (Fenner 1985).

Large seeds generally produce large seedlings (Chapin et al. 1989, Zhang & Maun 1993, Jurado & Westoby 1992, Fenner 1985) which enhances the competitive ability of a particular plant species in hostile environments (Fenner 1983, Stanton 1984, Fenner 1985). Large seedlings also have better survivorship under adverse conditions such as in shade, high light intensity, low water and low nutrient availability (Fenner 1985, Fenner 1983, Stock, Pate & Delfs 1990, Jurado & Westoby 1992). However,

large seedling size is often negatively correlated with relative growth rate (Fenner 1983, Fenner & Lee 1989, Jurado & Westoby 1992, Fenner 1986b, Fenner 1986a) and therefore, large seedlings may be disadvantaged by low potential growth rates in environments that are prone to disturbance.

It has been shown that seedling size is proportional to nutrient quality of the seed (Fenner 1983), but that the absolute quantity of nutrients in the seed may not always influence seedling size (Fenner & Lee 1989). Often, seed stored nutrients are in forms that are unuseable by the seedlings (Kuo, Hocking & Pate 1982) and therefore only the spectrum of useable nutrients will positively influence seedling size. Furthermore, the absolute amounts of nutrients are often imbalanced in the seeds in relation to the requirements of the seedlings (Fenner & Lee 1989). However, there is an indication that the amount of phosphorus stored in seeds is balanced in terms of seedling requirement (Fenner & Lee 1989).

In the Fynbos floral region of South Africa and the Kwongan floral region of South Western Australia the soils are nutrient poor, particularly in phosphorus and nitrogen (Thwaites & Cowling 1988, Stock & Lewis 1986). In addition, the vegetation is fire prone and the reproductive strategies of plants in these regions are often dependant on fire (Groves, Hocking & McMahon 1986, Jongens-Roberts & Mitchell 1986). Many members of the Proteaceae require fire for seed dispersal or as germination cues. Members of the South African and Australian Proteaceae characteristic of these regions often produce few, large seeds (Esler et al. 1989, Stock et al. 1989, Mustart & Cowling 1993). Species of the

genera Protea, Leucadendron, Hakea and Banksia in particular, are able to establish successfully in post fire environments where the light intensity is high and the soils are bare (Stock et al. 1990).

Members of the Proteaceae growing in acid, nutrient poor sands in this region produce few, large seeds which are rich in phosphorus and nitrogen in particular (Esler et al. 1989, Stock et al. 1989, Stock et al. 1990, Mustart & Cowling 1993). In contrast, Proteaceae species growing in more nutrient rich adjacent limestone sands in the Fynbos, produce large numbers of small seeds which are not as nutrient rich (Esler et al. 1989, Mustart & Cowling 1993). Therefore, a compromise between seed size and number exists in plants that are constrained by nutrient availability.

It has been shown that the large seed size and high nutritional quality of Proteaceae seeds favours their successful establishment and survival in the nutrient poor soils of their natural environment (Stock et al. 1990, Mustart & Cowling 1993). In contrast, small seeded Proteaceae from limestone soils are unable to survive in adjacent, hostile, acid sands (Mustart & Cowling 1993) indicating that the nutritional content and quality of these seeds are not adequate for survival in lower nutrient environments. In adition it has been found that the dependance of seedlings on nutrient seed stores varies in relation to the particular soils to which they have become adapted to (Mustart & Cowling 1993).

This study is designed to test the nutritional requirements of seedlings from different seed sizes in members of the

Proteaceae, their dependance on seed nutrient stores and whether seeds from differing edaphic environments have different nutritional requirements and dependance on seed nutrient stores. Four acid sand species (calcifuges) and two limestone species (calcicoles) of the Protea family with a range of seed sizes were tested. The requirement of an essential element in a particular plant can be determined by growing the plants in nutrient solutions with all essential elements except one (Fenner 1986b). Performance of the seedlings under these nutrient treatments gives an indication of the requirement for particular elements. Plants with low requirements for particular elements in nutrient solution are likely to be dependant on the nutrient stores of the seeds. Differential requirements of elements for species from different edaphic environments is also measured by seedling performance.

Methods and Materials

Germination

The seeds of *P.compacta* (calcifuge), *L.meridianum* (calcicole) and *L.xanthoconus* (calcifuge) were collected on the Agulhas Plain, South Western Cape Province, South Africa, while the seeds of *P.obtusifolia* (calcicole), *P.cynaroides* (calcifuge) and *L.laureolum* (calcifuge) were obtained from a commercial supplier (Silverhill seeds, Kenilworth, Cape Town). One hundred and twenty seeds of each species were selected randomly and placed in petri dishes on filter paper moistened with a few millilitres of distilled water and a drop of benylate solution to prevent fungal infection of the seeds. The petri dishes were covered and sealed in plastic bags and placed in a germination chamber on a 5°C/20°C temeperature regime. The seeds were checked at weekly intervals and germination percentages recorded.

Growth Room and planting

Germinated seeds were allowed to grow in the petri dishes until the cotyledons were green and had opened (a period which took 31 days). Twelve germlings for each species were collected at this stage and dried for 48 hours at 80°C. However, *P.obtusifolia*, *P.cynaroides* and *L.xanthoconus* had poor germination success and therefore a second germination run had to be set up to obtain germlings for initial weighing and nutrient analyses. Germination rates for the six species were unequal and consequently, the germlings were placed under low intensity light for four days to allow for greening. Following

this, the germlings were planted in acid washed sand in 250 ml styrofoam cups with holes punched in the bottom to allow for drainage. The cups were arranged on metal trays with plants for each of the five nutrient treatments (-Nitrogen (-N), -Potassium (-K), -Calcium (-Ca), - Phosphorus (-P) and Control) so that no mixing of solutions could occur. In each nutrient treatment, 12 individuals of each of the six species were planted (72 plants per treatment, 360 plants in total).

The trays were placed under two types of lights which had a light intensity of ca. 400 $umol m^{-2} s^{-1}$. To ensure that all plants were equally illuminated, the treatment trays were rotated in the growth room at weekly intervals. Plants were grown at 25°C and 30% RH and were exposed to a 14L:10D photoperiod

Nutrient Solutions

Experimental nutrient solutions were made up as shown in Table 1. The various nutrient elements were selected so as to ensure an overall neutral charge in the solution and this was achieved by balancing the solutions with H^+ ions.

TABLE 1. Nutrient composition of the five nutrient treatment solutions.

MACRONUTRIENTS	$g.l^{-1}$	**STOCK**	**-N**	**-P**	**-Ca**	**-K**	**CTRL**
KH_2PO_4	136.09	1M	*		*		*
KNO_3	101.11	1M		*			*
$MgSO_4.7H_2O$	246.50	1M	*	*	*	*	*
$NH_4H_2PO_4$	115.03	1M				*	
$(NH_4)_2SO_4$	66.07	0.5M		*	*		
NaCl	58.44	1M	*	*	*	*	*
$NaNO_3$	84.99	1M			*	*	
$CaCl_2.2H_2O$	147.02	1M	*	*		*	
$CaNO_3$							*
MICRONUTRIENTS	$g.l^{-1}$	**STOCK**	**-N**	**-P**	**-Ca**	**-K**	**CTRL**
H_3BO_3	2.86	50mM	*	*	*	*	*
$MnCl_2.4H_2O$	1.81	20mM	*	*	*	*	*
$ZnSO_4.7H_2O$	0.22	1mM	*	*	*	*	*
$CuSO_4.5H_2O$	0.08	0.5mM	*	*	*	*	*
$H_2MoO_4.H_2O$	0.02	0.1mM	*	*	*	*	*
FeEDTA	500*ppm*		*	*	*	*	*

One molar (1M) stock solutions were made up for each nutrient by adding the molecular weight of the relevant nutrient to 1 litre of distilled water. The stock solutions were then kept in the fridge for the duration of the growth experiment. For each experimental nutrient solution, 20 ml of the relevant stock solution for the five treatments (see Table 1) were added to a 20 l bucket and made up to 20 l with distilled water. The buckets were kept sealed in the growth room, and bucket nutrient solutions were remade every two weeks. The pH of each of the experimental solutions was recorded.

Plants were fed the relative experimental nutrient solution

every fifth day, comencing one week after planting. The experimental nutrient solutions were administered in 20 ml aliquots using 20 ml syringes. Plants were watered with ca. 20 ml of distilled water. For the first two weeks after planting the plants required water daily, since the top layer of soil dried out very quickly in this period. Once roots had established, the plants were watered every second day for the first month and a half and then every third day thereafter.

Growth and Harvesting

(1) Growth

During the growth period, death of plants as well as any visible deficiency symptoms were recorded. The plants were grown for a period of 70 days, after which they were harvested. The cotyledons had turned yellow and had dried up by this stage. Harvesting was terminated on July 29.

(2) Harvetsing

Each plant was removed from the styrofoam cups and the excess sand and nutrients were washed off in three rinsing beakers containing distilled water. Any broken roots were collected out of the rinses for each plant. The sand from the styrofoam cups was also searched for broken root material. The rinsing beakers were changed after every five plants, and also for each nutrient treatment.

Each plant was separated into roots, cotyledons and shoots (stem plus leaves) and each portion of plant was placed in a separate paper envelope, stapled together, labelled and numbered. The envelopes were placed into an oven at 80°C and the samples

allowed to dry for 48 hours.

Dry weights for roots, cotyledons, shoots, germlings and embryos were determined for each plant using a 4 decimal place Mettler balance. Embryos were obtained by removing seed coats and weights were determined.

Nutrient analyses

To determine the total inorganic phosphorus content of two acid sand and two limestone sand species, acid digestions were conducted on material of *L.laureolum*, *L.meridianum*, *P.cynaroides*, and *P.obtusifolia*. Four whole plants and five germlings for each species were digested, according to the method of Jackson (1958).

(1) Predigestion

Whole plants were placed in thick walled boiling tubes for the predigestion stage. Initially, 3 ml of concentrated HNO_3 was added to the boiling tubes, however, at the digestion stage several samples of the samples ignited and were blown out of the test tubes. Consequently, 5 ml of HNO_3 were added to the subsequent samples and replacement predigestions were conducted for the exploded samples. Predigestion was carried out at 150-180°C on an aluminium heating block until the sample had dried, but was not charred. The addition of 5 ml of acid made this stage longer approx. 90 minutes, after which the samples were allowed to cool for 15 minutes. Blanks were made for both 3 ml and 5 ml acid.

(2) Digestion

A 1 ml aliquot of triacid mixture (10 HNO_3 : 1 H_2SO4 : 4 HClO) was added to the predigested samples. Samples were then

digested at 180°C until they were dry and clear (which was either white or yellowish,) and all the white perchloric acid fumes had dissipated. This took approx. 60 minutes, after which, the samples were allowed to cool for 15 minutes.

(3) Dilution

The cooled samples were diluted to 25 ml with distilled water and mixed. The samples were left until the precipitate had settled to the bottom of the boiling tubes. These diluted samples were used for colorimetric phosphorus analysis.

Murphy and Riley Colorimetric Determination of Phosphate

An amount of 500 ml of Murphy and Riley reagent was made up by adding 250 ml of 2.5M sulphuric acid, 2.64 g of ascorbic acid dissolved in 150 ml of distilled water, 75 ml of ammonium molybdate and 25 ml of antimony potassium tartrate in this order. After addition of each constituent, the solution was mixed.

Colour development

Test runs for each species and nutrient treatment were carried out before the final aliquot was determined. Initially, 1 ml of each sample was added to a 50 ml volumetric flask for each of the 4 species and the 5 treatments. The colour was too dark for -Ca, -N, -K and CTRL, therefore, 0.2 ml of sample was added for each of these treatments and 1 ml for the -P treatment. To this, 25 ml of distilled water was added to the volumetric flasks to dilute the acidity of the sample and 8 ml of Murphy and Riley reagent were added and the volumetric flask was made up to 50 ml with distilled water. The flasks were shaken and allowed to stand for 60 minutes. Blanks were made both for 3 ml and 5

ml acid and for 1 ml and 0.2 ml samples.

The absorbance of each sample solution was measured at 882 *nm* on a Spectronic 20 spectrophotometer (Bausch and Laumb) which was zeroed with distilled water.

Standard curve

Ten ml of a stock solution containing 0.4394 g KH_2PO_4 made up to 1 l was added to a 500 ml volumetric flask and made up to 500 ml. One ml of this solution was added to a 50 ml volumetric flask and so on to a total of 15 ml for each flask. One ml of solution contained 2 ug P ml^{-1}. Therefore, the standard curve was constructed for amounts of P in the range 2 ug-30 ug. Each flask was made up as for the samples and absorbance measured at 882 nm. Absorbance values were used to construct the standard curve and the equation of the regression line was used for the sample calculations. Two standard curves had to be constructed because two spectrophotometers were used to measure the absorbance of the samples. The absorbance values of the samples were converted to known amounts of P by the following equation:

$$P = \alpha \cdot \frac{25ml}{10ml}$$

α = absorbance value (amount of P in 10 ml) and

P = total amount of P in digested sample

Figures 1 and 2 (Appendix 1) show standard curves and the equations obtained were :

$$y = 0.016404x - 0.00775 \ldots\ldots\ldots (1)$$

$$y = 0.00924x - 0.00376 \ldots\ldots\ldots \quad (2)$$

for the Spectronic 20 digital (equation 1) and Spectronic 20 analogue (equation 2) spectrophotometers.

Atomic absorption spectrophotometry for calcium

Ten or 100 times dilutions of digested plant extract in 0.1% Lanthium chloride in 0.2N Nitric acid were read on an atomic absorption spectrophotometer for 0,1,2,3 ug Ca ml^{-1}. Calcium content of plants were determined.

Data analysis

Relative growth rate (RGR), total plant mass, root to shoot ratios, phosphorus concentration and phosphorus use efficiency were determined for the six species grown in the five nutrient treatments.

(1) Relative growth rates (RGR)

The measure of growth given in Table 6. is the mean value of R over a period of time and is calculated using the following equation:

$$R = \frac{(\ln W_2 - \ln W_1)}{(t_2 - t_1)}$$

where R = a mean value of growth over a period of time and
W2 = the final dry mass (total plant mass) in grams and
W1 = the initial dry mass (germling mass) in grams and
t2 = the growth period in days (growth time to harvesting) and
t1 = the start of the growth period in days (planting out of germlings ie. t=0)

This equation assumes that growth of a plant is in some way related to its size and is useful when comparing the growth of different sized plants or plants subjected to different environmental conditions (Fitter & Hay 1989). However, it is not an instantaneous measure of growth and nor does the equation assume that growth is exponential (Fitter & Hay 1989) such as the equation describing the relationship between plant weight and time ie.

$$R = \frac{1}{W} \cdot \frac{dW}{dt}$$

Therefore, the equation used to calculate RGR in Table 6. is best fitted to the conditions of the experiment because the species of plants used had different seed sizes and were exposed to different sets of nutrient treatments.

(2) Analysis of variance and regression analysis

Two way analysis of variance Type I sums of squares was used to determine significant differences between species and treatments for the data. Where there was a significant interaction between species and treatments, one way analysis of variance was performed and Tukey multiple range tests were used for both, being the most accurate range test available (Zar 1974).

The statgraphics computer package (1989) was used to perform analysis of variance and regression analyses were performed in Quattro Pro (1992).

Results

(1) Germination

There was considerable interspecific variation in the germination success of the six species under investigation (Table 2). *L.meridianum* exhibited the highest germination success (92%), while *P.obtusifolia* had the lowest, (45%).

TABLE 2. Germination percentages for each species (average for 120 seeds for each species expressed as a percentage)

SPECIES	GERMINATION %
Protea compacta	70.8
Protea cynaroides	53.6
Protea obtusifolia	45.0
Leucadendron laureolum	82.5
Leucadendron meridianum	91.7
Leucadendron xanthoconus	56.7

(2) Nutrient solutions

All the nutrient solutions had a pH close to five (Table 3), indicating that nutrient solution pH was not a causal factor in the differential growth responses and nutrient deficiency symptoms exhibited by the plants under the nutrient various nutrient treatments.

TABLE 3. pH of nutrient solutions used from 50 days after sowing.

NUTRIENT SOLUTIONS	pH OF SOLUTIONS
Control	4.98
- Calcium	4.95
- Nitrogen	4.90
- Potassium	4.93
- Phosphorus	5.43

(3) Seedling growth

(i) General observations

Thirty five days after sowing, several plants, under the various nutrient treatments exhibited signs of nutrient deprivation (Table 5). In addition, several plants died during the course of the experiment. After thirty five days from sowing, in the CTRL treatment, two *P.obtusifolia* plants had died, while, in the - Ca treatment, one *L.xanthoconus* plant had died and in the - K treatment one *P.cynaroides* plant had died and in the - N treatment two *P.cynaroides* plants had died. At sixty days after sowing, two more *P.obtusifolia* plants had died in the CTRL treatment, one *P.obtusifolia* in the -Ca treatment, six *P.obtusifolia* plants in the -K treatment and one *P.obtusifolia* plant in the -N treatment. The only treatment that had no deaths up to day 60 from sowing was -P. The total number of deaths of

each species in each treatment at harvesting is shown in Table 4. These results suggest that in the the control treatment, there was some sort of toxity effect which affected all the species.

Table 4. Total number of deaths in each species for each nutrient treatment at harvesting (day 73 for *P.compacta*, 74 for *P.cynaroides*, 79 for *P.obtusifolia*, 79 for *L.laureolum*, 77 for *L.meridianum*, 80 for *L.xanthoconus*).

	Total number of deaths				
	CTRL	-Ca	-K	-N	-P
P.compacta	2	0	0	0	0
P.cynaroides	3	0	0	2	0
P.obtusifolia	3	2	7	1	1
L.laureolum	4	0	0	0	0
L.meridianum	1	1	0	1	0
L.xanthoconus	5	1	0	0	0
Total deaths	18	4	7	4	1

TABLE 5. Nutrient deficiency symptoms of all the species for each treatment after 35 days of growth

	TREATMENTS AND SYMPTOMS				
SPECIES	- Ca	CTRL	- N	- P	- K
P.compacta	yellow leaf tips	brown leaf tips	healthy	brown leaf tips	healthy
P.cynaroides	red leaf edges & red leaf blotch	red leaf blotch	red leaf tips	red leaf blotch	red leaf blotch
P.obtusifolia	brown leaf tips & edges	brown leaf tips	red leaf tips	healthy	brown leaf blotch
L.laureolum	healthy	brown leaf blotch	healthy	healthy	brown leaf blotch
L.meridianum	healthy	leaf tip necrosis	red leaf blotch	healthy	healthy
L.xanthoconus	brown leaf tips	brown leaf blotch	red leaf tips	healthy	brown leaf blotch

(ii) Relative growth rates

Relative growth rates (RGR) for each species under each nutrient treatment is presented in Table 6.

Table 6. Average relative growth rates (RGR) (g dry mass per day) for the 6 Proteaceae species and 5 nutrient treatments. RGR is expressed as ln final dry mass in g, minus ln initial dry mass in g, divided by the length of the gowth period in days.

(* Where S.D. = Standard deviation for (n=12)) and (# where y to x represent the significant differences of RGR between treatments for Tukey range analysis, two way analysis of variance) and ($ where a to b represent significant differences of RGR between species for Tukey range analysis, two way analysis of variance) and (+ where a to b represent significant difference in RGR of each species for each nutrient treatment for Tukey range analysis, one way analysis of variance, ns = not significant). Alphabetical ascending order represent smallest to largest differences.

		RGR (relative growth rate (g dry matter per day)				
		y#	x	x	x	x
		-Ca	-K	-N	-P	CTRL
		b+	a	a	ab	a
a$	*P.compacta*	0.043	0.034	0.033	0.038	0.035
	S.D.*	0.005	0.006	0.006	0.005	0.006
		b	ab	ab	a	a
b	*P.cynaroides*	0.051	0.045	0.049	0.043	0.036
	S.D.	0.005	0.007	0.004	0.007	0.006
		b	a	a	a	a
ab	*P.obtusifolia*	0.051	0.036	0.004	0.044	0.040
	S.D.	0.003	0.007	0.004	0.009	0.009
		b	a	ab	ab	a
b	*L.laureolum*	0.039	0.038	0.038	0.031	0.040
	S.D.	0.009	0.008	0.005	0.013	0.013
		ns	ns	ns	ns	ns
a	*L.meridianum*	0.052	0.035	0.037	0.041	0.041
	S.D.	0.007	0.004	0.009	0.003	0.005
		ab	a	ab	b	ab
a	*L.xanthoconus*	0.037	0.030	0.038	0.046	0.035
	S.D.	0.007	0.008	0.005	0.004	0.004

Two way analysis of variance showed that there are significant differences ($P<0.01$), $F_{5,301}=11.789$ in the relative growth rates of species (Table 6). Nutrient treatment has a significant effect ($P<0.01$), $F_{4,301}=10.800$ on relative growth rates where plants in the -Ca treatment had RGR's that were significantly higher than all the other treatments (Table 6). The interaction between species and treatments was significant, $F_{20,301}=2.996$, therefore each species in each treatment was tested separately in a one way analysis of variance (Table 6). F ratios for each species were as follows: *P.compacta* $F_{4,49}=4.814$, *P.cynaroides* $F_{4,48}=4.798$, *P.obtusifolia* $F_{4,37}=7.083$, *L.laureolum* $F_{4,53}=6.270$, *L.meridianum* $F_{4,52}=1.416$ and *L.xanthoconus* $F_{4,53}=5.131$.

There is a negative relationship ($P<0.05$, $r^2=0.09$, $n=61$) between germling mass and the RGR of all of the six Proteaceae species in the -N treatment only . In all the other treatments there was not a significant relationship ($r^2=0.03$, $n=48$ for CTRL, $r^2=0.03$, $n=62$ for -P, $r^2=0.04$, $n=57$ for -K and $r^2=0.06$, $n=63$ for -Ca). This suggests that nutrient treatment affects the relationship between initial seed mass and RGR.

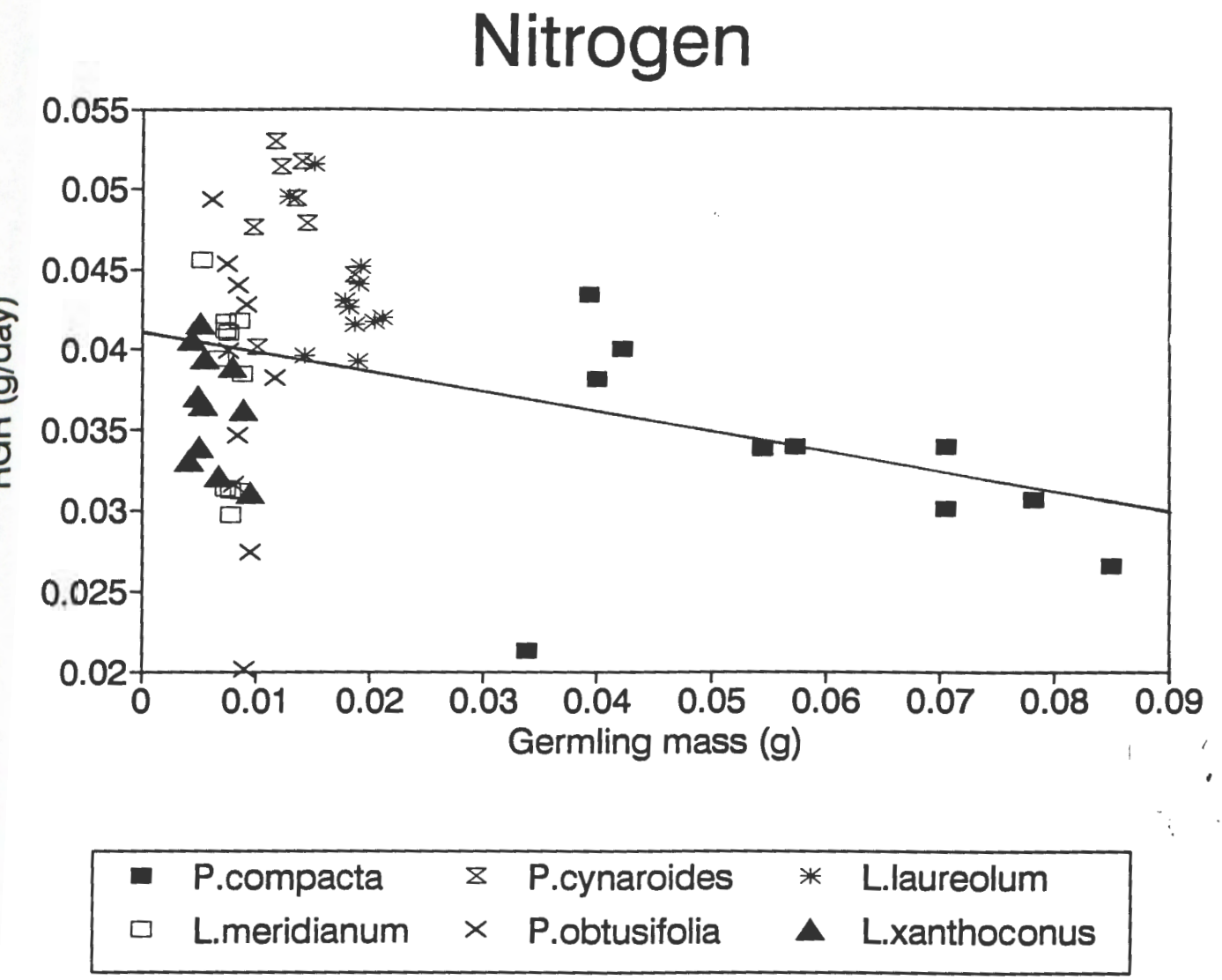

Figure 3. The relationship between initial plant size (germling mass in g) and relative growth rate (RGR g dry matter day^{-1}) for the six Proteaceae species in the -Nitrogen treatment. (r^2=0.09, constant=0.04, x coefficent=-0.11, S.E.=0.05).

(iii) Total plant mass

The initial (germling) and final (total plant) dry mass for each of the six species and five treatments are represented in Figure 4. *P.compacta* germlings had the largest initial mass and *L.xanthoconus* the smallest. The -Ca treatment had the largest plant biomass in all species except for *L.meridianum* and *L.xanthoconus* which the control had the largest total mass and -P the largest total mass respectively.

Two way analysis of variance showed that there were significant differences ($P<0.05$) in total plant masses between species ($F_{5,309}=184.291$) and treatments ($F_{4,309}=36.131$) (Figure 4) with a significant interaction ($F_{20,309}=7.245$). F ratios for one way analysis of variance for each species for each of the treatments are as follows: *P.compacta* $F_{4,57}=17.731$, *P.cynaroides* $F_{4,48}=6.75$, *P.obtusifolia* $F_{4,37}=4.634$, *L.laureolum* $F_{4,53}=16.499$, *L.meridianum* $F_{4,56}=1.44$ (ns) and *L.xanthoconus* $F_{4,53}=10.726$ represented in Figure 4.

Figure 5 shows total plant mass of the six species for each nutrient treatment in relation to the CTRL treatment plant mass which is set to zero with other masses represented as a difference from the control treatment. In the -Ca treatment all species had a greater total mass than the control except for *L.meridianum* and *L.xanthoconus*. In the -K treatment, only *P.cynaroides* and *L.xanthoconus* had a total mass greater than the control. In the -N treatment only *P.cynaroides* and *L.laureolum* had a total mass greater than the control. In the -P treatment all species except for *P.obtusifolia* and *L.meridianum* had a total mass greater than the control.

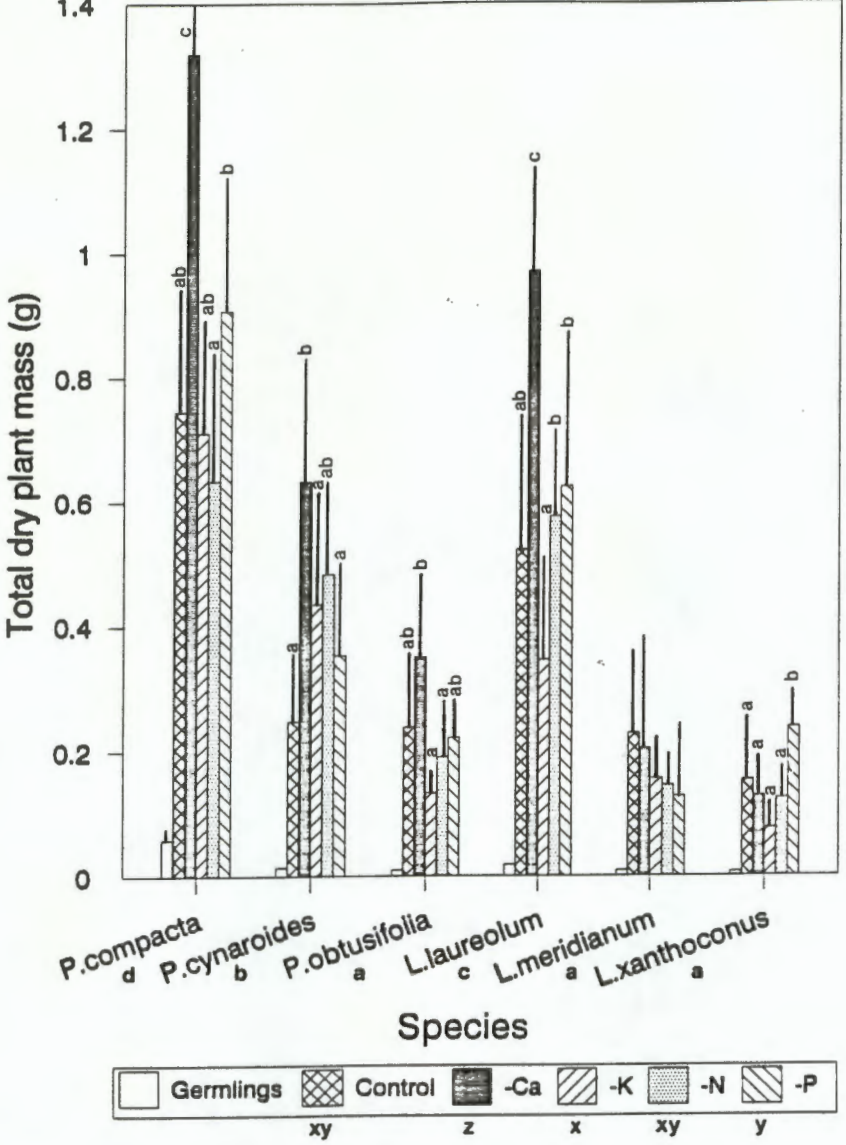

FIGURE 4. Total plant dry mass in g for initial mass (germlings) and final mass for the six Proteaceae species for the five nutrient treatments. Total plant mass is the dry mass of the roots, cotyledons and shoots (stem plus leaves) after harvesting. Values are an average value of 12 plants for each species, error bars indicate S.E.'s. Alphabetical representation a to d under species legends indicate significant differences in mass between species for Tukey range analysis, two way analysis of variance, where a is the smallest mass and d the largest. x to z under treatment legends indicate significant differences in mass for each treatment for Tukey range analysis, two way analysis of variance, where x is the smallest mass and z the largest. a to c above bars represent significant differences in mass for each species for each treatment for Tukey range analysis, one way anlysis of variance, where a is the smallest mass and c the largest.

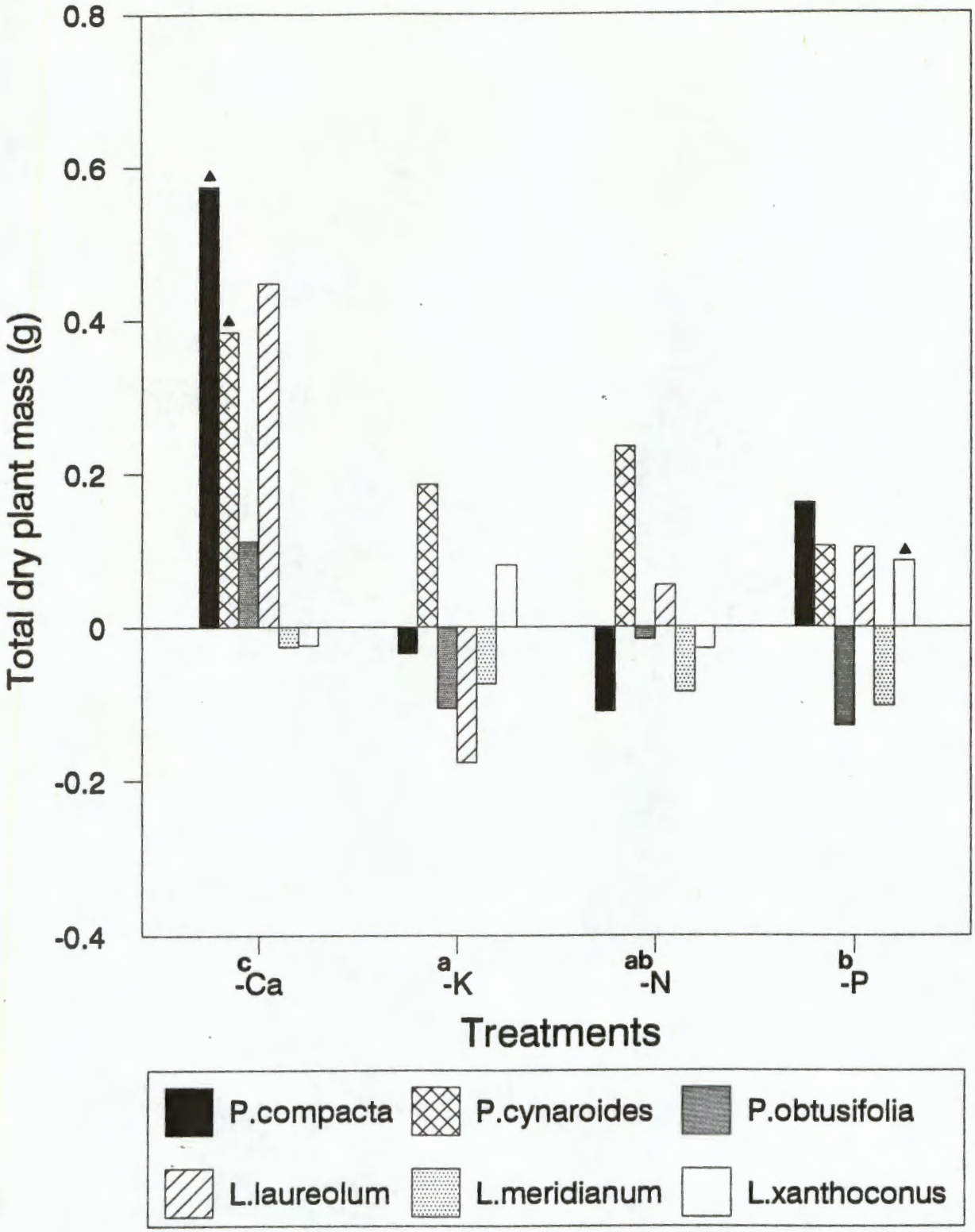

FIGURE 5. Total plant dry mass in g for each nutrient treatment and the six Proteaceae species in relation to the control treatment (mass set to 0). Only masses that are significantly different from the control are shown by triangles.

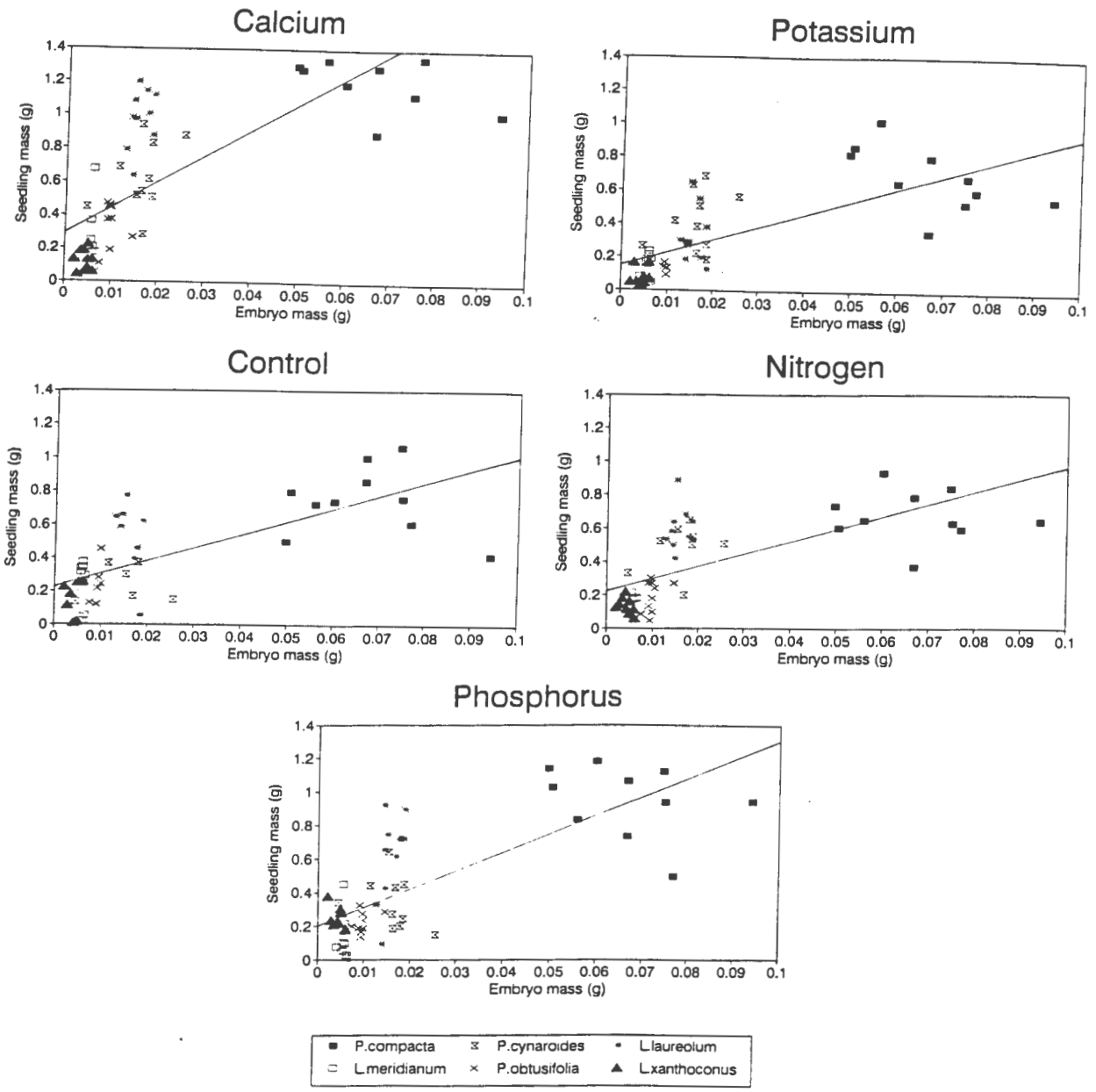

FIGURE 6. The relationship between seed mass (g) and final seedling mass (g) for each of the six Proteaceae species for each of the nutrient treatments. (-K c=0.15 x coefficient=7.83, CTRL c=0.21 x coefficient=7.73, -P c=0.20 x coefficient=10.81, -Ca c=0.30 x coefficient=15.32)

There is a strong positive relationship ($P < 0.001$) between initial seed mass and total plant mass for all of the six Proteaceae species in each nutrient treatment (-K $r^2=0.53$ for n=54, -P $r^2=0.60$ for n=59, CTRL $r^2=0.50$ for n=47, -Ca $r^2=0.60$ for n=58, -N $r^2=0.45$ for n=58) (Figure 6). This suggests that initial seed size determines the final plant size, irrespective of nutrient treatment.

(v) Root to Shoot ratios

Root to shoot ratios for each species under the five nutrient treatments are shown in Figure 7. In the -K treatment root to shoot ratios are higher than 2 for *P.compacta*, P.cynaroides and *L.laureolum*, all the large seeded species, while the root:shoot ratios are less than 1 in *P.obtusifolia*, *L.meridianum* and *L.xanthoconus*, the small seeded species. In the control treatment the root:shoot ratio for *L.xanthoconus* was highest and the only species to have a value greater than 1, in the -Ca treatment *L.meridianum* was the only species with a root to shoot ratio higher than 1. In the -N treatment, all root:shoot ratios were less than 1 and in the -P treatment *L.meridianum* was the only species with a root:shoot ratio greater than 1.

Two way analysis of variance showed treatment root to shoot ratios to be significantly different ($F_{4,310}$=7.815) (Figure 7). There was no difference in root to shoot ratios between species ($F_{5,310}$=1.498) and the interaction between species and treatments was significant ($F_{20,310}$=2.172).

F ratios for root to shoot ratios, one way analysis of variance for each treatment for each species were: *P.compacta* $F_{4,57}$=41.73, *P.cynaroides* $F_{4,48}$=25.992, *P.obtusifolia* $F_{4,37}$=9.953, *L.laureolum* $F_{4,54}$=3.909, *L.meridianum* $F_{4,56}$=0.424 (ns), *L.xanthoconus* $F_{4,53}$=0.762 (ns) (Figure 7).These results suggest that root to shoot ratios are a response to nutrient treatment.

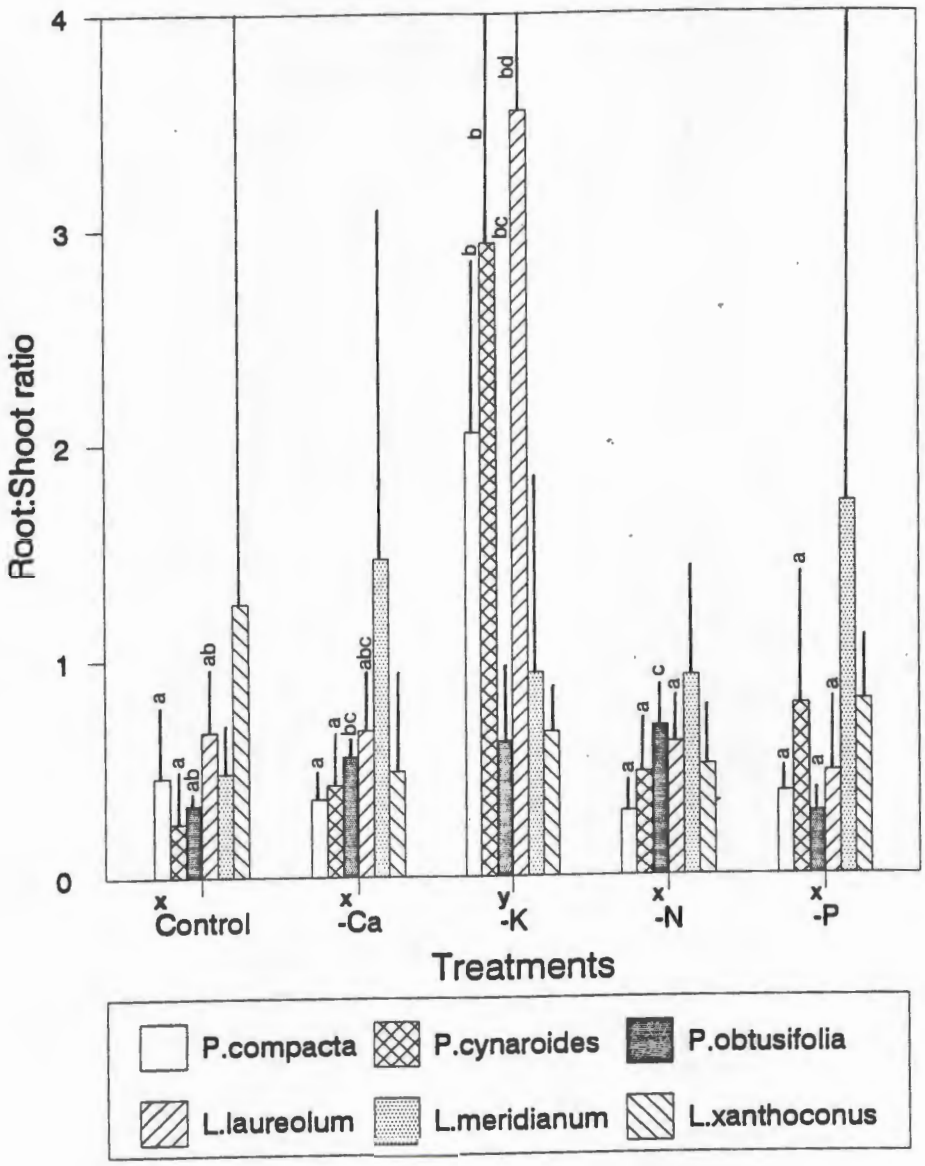

FIGURE 7. Root to shoot ratios of the six Proteaceae species in the five nutrient treatments. Root to shoot ratios are the ratio of dry root mass in g and dry shoot mass in g and are an average for 12 plants for each species, where error bars represent S.E's). x to y indicates significant differences in root to shoot ratios between treatments for Tukey range analysis, two way analysis of variance. a to d above bars represents significant differences for each species between each nutrient treatment for tukey range analysis, one way anlysis of variance. Smallest to largest differences are in ascending alphabetical order.

(4) Nutrient analysis (Phosphorus)

(i) Phosphorus concentration

The total phosphorus content in ug in the digested plant sample was divided by the mass in g of the plant to yield a plant phosphorus concentration for germlings and whole plants for *P.cynaroides*, *P.obtusifolia*, *L.laureolum* and *L.meridianum* under the five nutrient treatments (Figure 8a and 8b). The germlings, regardless of species, had the highest phosphorus concentration per gram of plant material. Both *P.obtusifolia* and *L.laureolum* had the highest phosphorus concentration in the -K treatment. *P.cynaroides* had the highest P concentration in the control treatment, while the P concentration for *L.meridianum* in the -N treatment was highest.

In a one way analysis of variance, there was a significant difference ($F^{3,19}$=14.112) between the phosphorus content in the germlings for species (Figure 8a) and between the phosphorus content in seedlings for each species (Figure 8a).

A significant (r^2=0.72 for -Ca, r^2=0.27 for -K, r^2=0.52 for -N, r^2=0.40 for -P and r^2=0.52 for CTRL all for n=16) positive relationship between the total P in the germling and final plant mass exists for all the nutrient treatments (Figure 9). These results suggest that cotyledonary phosphorus reserves play an important role in determining the final mass of the plants.

The phosphorus content in the germlings is positively correlated (r^2=0.42 for n=20) with the size of the germling in all four species, indicating that larger seeds have more phosphorus in the cotyledonary reserves than small seeds (Figure 10).

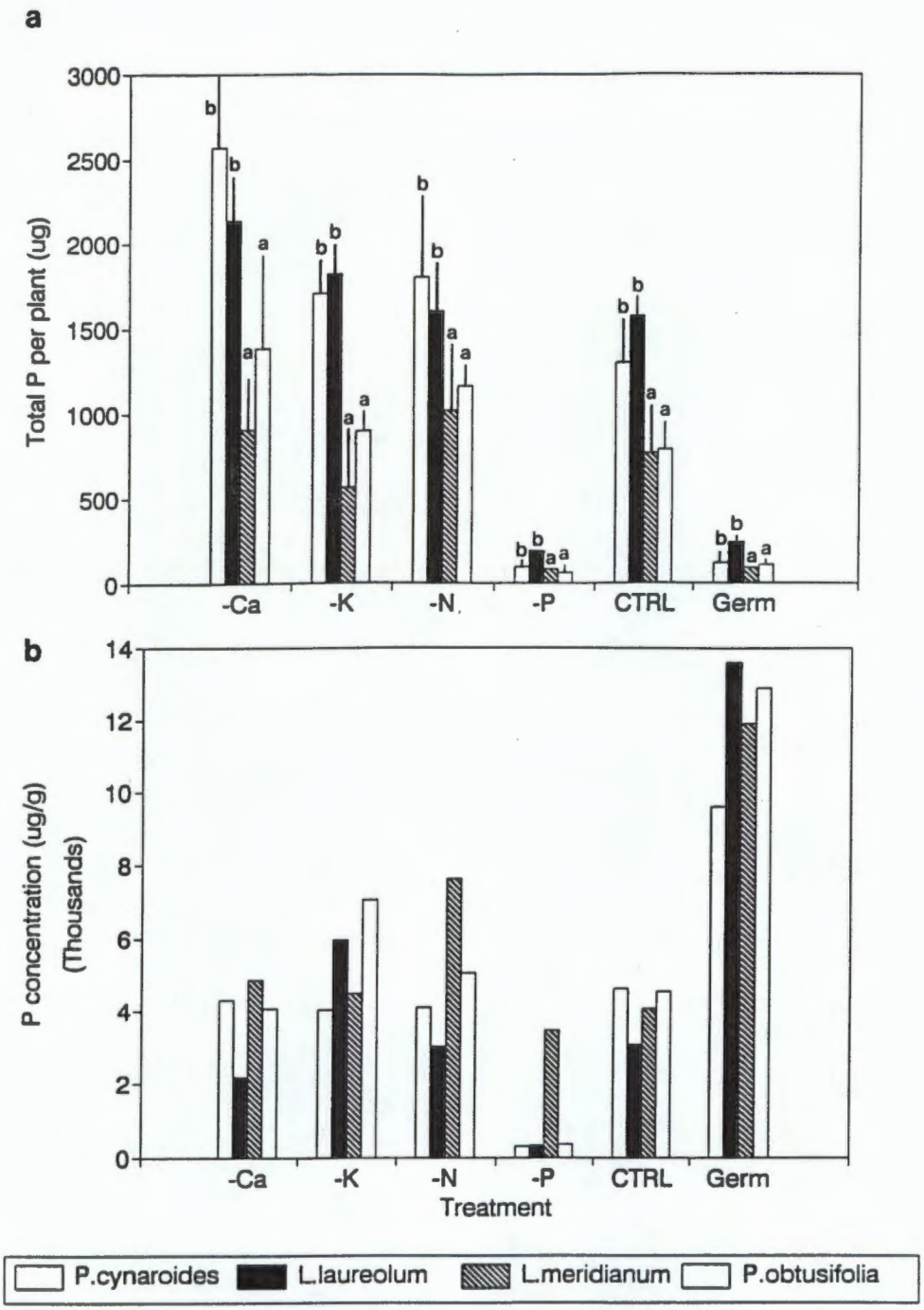

FIGURE 8a and b. Phosphorus content plant^{-1} in ug (a) and phosphorus concentration (ug.g-1) (b) of germlings and whole plants for *P.cynaroides*, *L.laureolum*, *P.obtusifolia* and *L.meridianum*. Alphabetical representation indicates significant differences for Tukey range analysis, one way anlysis of variance. Smallest to largest differences are in ascending alphabetical order.

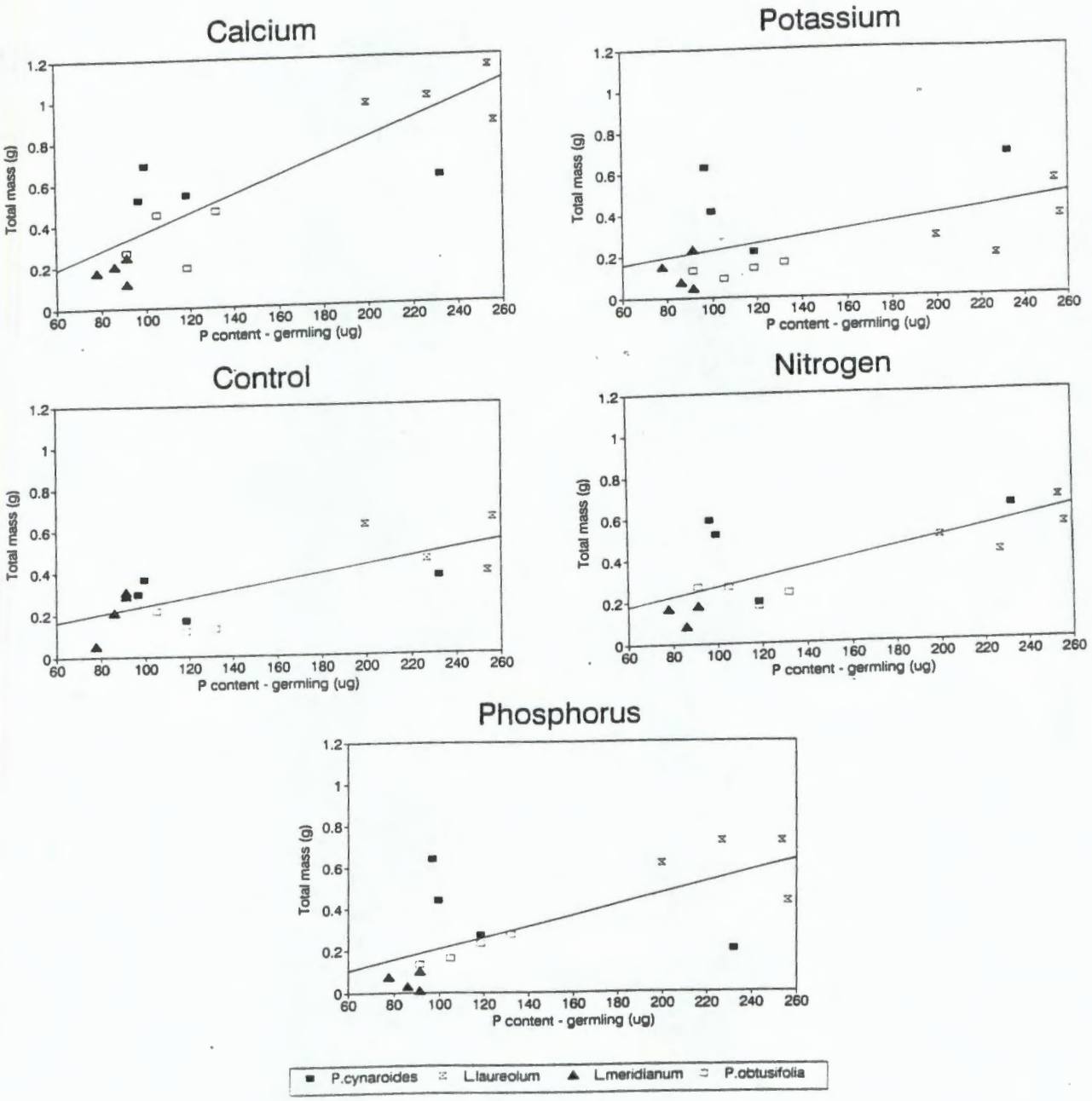

FIGURE 9. The relationship between initial phosphorus content in ug (germling p content) and total plant mass in g for the five nutrient treatments for the four species. (-K c=0.150 x coefficient=7.83, -Ca c=0.294 x coefficient=15.317, -N c=0.220 x coefficient=7.317, -P c=0.201 x coefficient=10.808 and CTRL c=0.210 x coefficient=7.727)

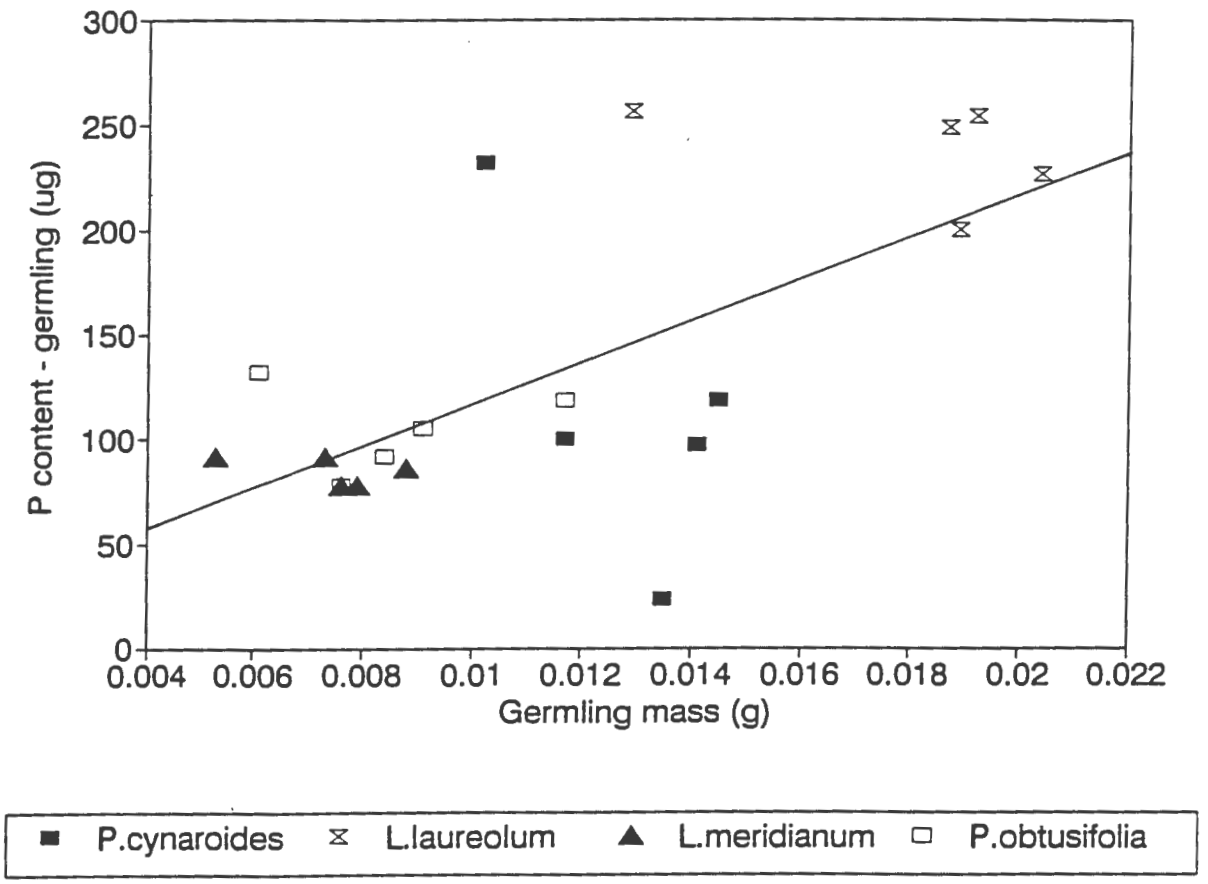

FIGURE 10. The relationship between germling mass in g and germling phophorus content for the four species. (c=19.47 x coefficient=9890.639)

(iii) Nutrient use efficiency

The nutrient use efficiency of phosphorus was calculated by taking the final plant dry mass (g) and dividing by the phosphorus content of the plants (mg). NUE was very high for all species in the -P treatment, except for *L.meridianum*. In the other treatments NUE was similar for all species.

Table 7. Nutrient use efficiency (NUE) for Phosphorus (g dry matter per mg nutrient) of the four Proteaceae species under the five nutrient treatments. NUE of phosphorus is expressed as final plant dry mass after harvesting divided by phosphorus element content of the dry plants.

	Phosphorus use efficiency (g dry matter per mg phosphorus)				
	-Ca	CTRL	-K	-N	-P
P.cynaroides	0.24	0.24	0.28	0.28	6.96
P.obtusifolia	0.28	0.23	0.15	0.21	5.66
L.laureolum	0.48	0.33	0.19	0.34	3.26
L.meridianum	0.22	0.27	0.29	0.19	0.71

(5) Ion chromatography

Figure 11, an ion chromatogram shows the content of cations in the digested samples of *L.laureolum* only. The germlings have very low concentrations of Na^{+}, $NH4^{+}$, Mg^{++} and Ca^{++}. The chromatogram for the -Ca treatment shows that *L.laureolum* plants must have taken up Ca^{++} because the concentration peak is much higher than for the germlings. This means that the -Ca treatment

(6) Atomic absorption spectrophotometry for Calcium

Figure 12a shows that the -Ca treatment plants definately were obtaining calcium from a source other than cotyledonary reserves. The calcium content of the germlings of all four species is low. *L.laureolum* has the highest calcium content (Figure 12a) in all the treatments, but this appears to be because it has the largest mass (Figure 12b). The control treatment plants have the highest calcium content (Figure 12a).

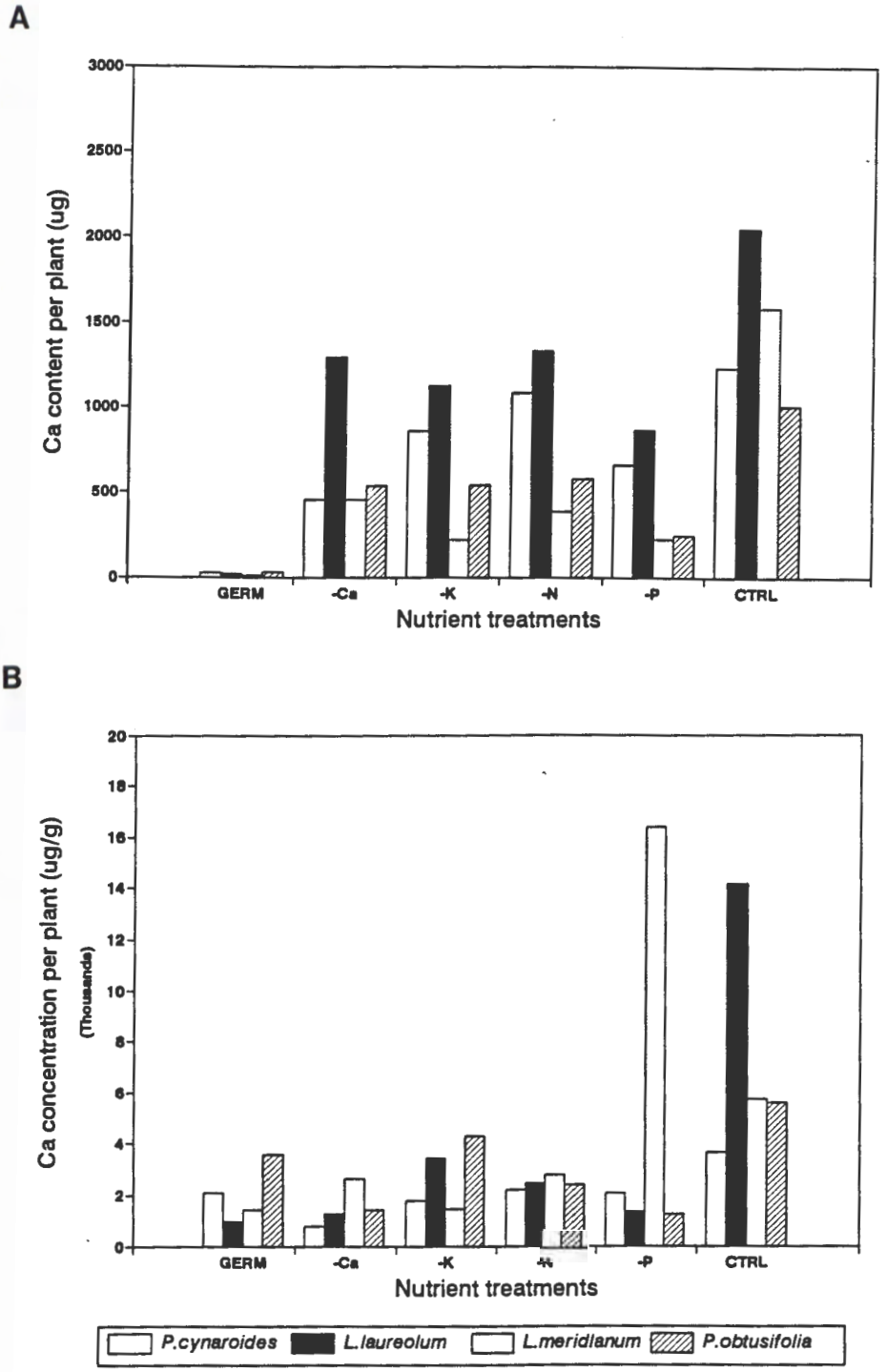

FIGURE 12a and b. Calcium content per plant ug (a) and concentration ugg^{-1} (b) for the four species for germlings and for the five nutrient treatments.

Discussion

Seedlings of the six Proteaceae species under investigation were able to establish and survive, despite the lack of essential nutrient elements in the various nutrient treatments. The low germination success (Table 2) and the large number of deaths (Table 4) of *P.obtusifolia* in all the treatments would appear to be related to the quality of the seed, rather than a response to the effect of nutrient treatment. Since, the seed of this species was obtained commercially, it is possible that the seed stock was old and unable to establish and survive as well as the other species did.

Early nutrient deficiency symptoms in some of the species after thirty five days of growth were observable. Stock, et al. (1990) grew Proteaceae species for 120 days with no added nutrients and these plants relied soley on what was present in the sand and in the cotyledons appearing to have no sign of nutrient deficiencies nor any deaths. However, a possible reason for these deficiency symptoms being evident so early in growth were likely to be a result of a forced response to grow rapidly, since nutrient additions were begun soon after planting. Every other macro and micro nutrient was in plentiful supply and therefore the need for the missing element would be far greater and would be determined by the rate of supply of the other nutrients and a rapid growth rate. In addition, it is likely that internal nutrient imbalances in the plants may have accounted for these observed deficiencies since the balance of ratios of absolute amounts of nutrients as been shown to affect

other protea species adversely (Witkowski 1989).

The control treatment seemed to have an adverse effect on each species with a large number of deaths (Table 4) and differing deficiency symptoms (Table 5). It appears as though there was some sort of toxicity effect in the control treatment. Claassens (1986) found that high levels of phosphorus in combination with high levels of salts were harmful to several Proteaceae, often resulting in deaths of plants and additionally, that high levels of $NH4^+$ were also detrimental. Similarly, Groves & Keraitis (1976) found in *Banksia serrata*, an Australian protea adapted to low nutrient soils, that high levels of nitrogen and phosphorus in combination were detrimental to the survival of this species. Witkowski (1989) found that the addition of high levels of nitrogen in *Protea repens* resulted in nutrient imbalances in the plants and high levels of mortality. It seems, therefore, that if Proteaceae seedlings are adapted to low nutrient conditions and have large resources of nitrogen and phosphorus in their cotyledons, that they may be intolerant of high levels of nitrogen and phosphorus in the soil.

Relative growth rates are usually negatively correlated with initial seed mass (Fenner 1983, Fenner & Lee 1989, Jurado & Westoby 1992, Fenner 1986b, Fenner 1986a). Large seeded species have lower relative growth rates than small seeded species. However, this relationship was evident in the -N treatment only (Figure 3). Therefore, in general, it appears that this relationship is not true for these particular species. This is in agreement with the idea of Stock et al. (1990), that seed size and relative growth rates have evolved independently as a

solution to establishing in hostile environments. The lack of correlation between seed size and relative growth rate found by Stock et. al. (1990) and Choe et al. (1988) would suggest that in a nutrient poor environment, there is more adaptive significance to having a seed that responds to the specific environment. In contrast, it appears that the negative relationship between seed size and relative growth rate is an observational one within a particular size class of plants. In other words, the relationship may well be mass specific but, in an environment that is adverse, such a relationship has no adaptive significance. There is a problem of this relationship being significant in the -N treatment. A possible explanation is that all Fynbos Proteaceae species have a sufficient nitrogen content in the cotyledonary reserves (Esler et al. 1989, Stock et al. 1990, Stock et al. 1989, Mustart & Cowling 1993). Therefore, the final mass that the plant attains may be dependant on this nitrogen supply, with a lack of external nitrogen not having an effect. Thus, plants in the -N treatment were able to get large and when the mass of these plants was taken into consideration with the growth rate, ie. in relative terms, this negative correlation was apparent. However, one may have expected a similar trend in the -P plants because Fynbos Proteaceae seeds also have high phosphorus contents (Esler et al. 1989, Stock et al. 1990, Stock et al. 1989, Mustart & Cowling 1993).

Significant differences in relative growth rates between species shows no clear relationship (Table 6) ie. no seed size relationship, no genus relationship and no calcicole vs.

calcifuge relationship. This contrasts with the findings of Stock et al. (1990), where RGR was conservative among species. Furthermore, relative growth rates seem to be affected by nutrient treatments (Table 6). These results seem to suggest that a factor such as relative growth rate is not phylogenetically constrained, and is however, a response that is determined by environmental factors such as nutrient scarcity.

The initial seed size of all the species shows a positive correlation with the final mass attained by the plant, such that large seeded species are likely to be larger plants and vice versa for small seeded species (Figure 6). Seed size seems to be the most important determinant of plant size (Chapin et al. 1989, Zhang & Maun 1993, Jurado & Westoby 1992, Fenner 1985, Stock et al. 1990). This relationship was significant in all the nutrient treatments, which means that environmental control, like the abscence of particular nutrients does not affect this relationship.

The bioassay technique proposed by Fenner (1986a) and (1986b) is a way of determining how much of a crucial element a plant requires. The final size that plants attain that are subjected to nutrient solutions with one crucial element missing, is an indication of how much of that particular element the plant requires. In the -Ca treatment, all species except for *L.meridianum* and *L.xanthoconus* showed enhanced growth (Figure 4). This would seem to suggest that the plants require very little calcium in their natural environment. However, plants that are subjected to acute calcium deficiency should exhibited stunted growth (Marschner 1986).

Ion chromatography (Figure 11) and calcium absorption spectrophotometry (Figure 12a and b) showed that there was a certain amount of calcium in the plant tissue, more than was initially present in the germling. Therefore, it seems that the -Ca treatment was a low calcium treatment, rather than a treatment where it was totally lacking. Since calcium is a very immobile cation and can often form complexes (Marschner 1986, Hanson 1984, Fitter & Hay 1989), it is possible that there was still calcium in the sand, which the acid washing could not remove. Potassium was the generally the most limiting nutrient, while phosphorus was the least limiting nutrient. This suggests that the mineral requirement for phosphorus of these species is not high.

The total plant mass of the calcicole species (*P.obtusifolia* and *L.meridianum*) was always less than the control except in the -Ca treatment (Figure 5). It has been postulated that small seeded species have faster growth rates than large seeded species because the mineral concentration of the small seeds are greater than for large seeds (Fenner 1983, Jurado & Westoby 1992). However, there are no clear relationships like this for these two species which have small seeds. The only other reason for such a trend may be that the calicole species are adapted (Mustart & Cowling 1993, Esler et al. 1989) to soils which are more nutrient rich (Thwaites & Cowling) than the soils which calcifuge species are adapted to. Therefore, the growth response of the calcicole species may be adversely affected by the lack of essential elements if the plants are preadapted to higher soil nutrient levels. It appears therefore, that the calcicole species rely

on external nutrient sources more than the calcifuge species do.

In all the treatments most of the contribution to total plant mass was by shoots, except in the -K treatment, where roots contributed more (Figure 7). There is no significant difference in the root to shoot ratios between species, however, in the -K treatment root to shoot ratios are significantly larger than the other treatments. This suggest that a large proportion of these plants nutrient reserves are allocated to the roots, indicating that potassium was an extremely limiting nutrient. Plants in low nutrient environments often allocate nutrients away from the shoots to the roots, to increase the rooting surface area thereby, maximising the opportunity to increase nutrient absorption (Chapin 1980). Furthermore, since there is no difference in the root to shoot ratios between the species (Figure 7), this suggests that this factor is consisitent among species of this family and Stock et al. (1990) have shown that nutrient mobilisation from cotyledonary reserves occurs at a similar rate in all species and therefore, the control of the root to shoot ratios is environmentally affected.

Phosphorus concentrations and contents are high in germlings (Figure 8a and b) in all of the species tested, whereas concentrations and contents of calcium are low (Figure 12a and b). It appears that cotyledonary supplies of phosphorus are sufficient to sustain growth for a long period of time in these species and that the reliance of the plant on external sources of phosphorus only become evident later. Similar results were found by Stock et al (1990). However, there is no significant difference in germling phosphorus content between the calcifuge

species and calcicole species (cf. Esler et al. 1989), rather that phosphorus content is significantly greater in *L.laureolum* a large seeded species. The reliance of the plants on external calcium supplies (Figure 12a and b) are far greater than for phosphorus which is due to the low initial content of calcium in the cotyledonary reserves.

There is a significant, positive relationship between initial phosphorus content and final plant mass (Figure 9) and the efficiency of phosphorus use of the four species were high in the -P treatment, except for *L.meridianum* (Table 7), suggesting that the other species are adapted to conditions of low phosphorus availability. *L.meridianum*, on the other hand, has evolved to adapt to soils that are more rich in phosphorus and therefore, has a lower phosphorus use efficiency. Phosphorus content of the seeds is positively correlated to seed size in the four species tested (Figure 10).

These relationships indicate that the rich cotyledonary nutrient reserves are an adaptation for successful establishment and survival of Fynbos Proteaceae in environments where soil phosphorus contents are low. In addition, a regeneration strategy like this would favour the establishment of fire adapted species, since, fire can act as a mineralsing agent and ash depostions often increase the soil nutrient status for the first year after fire (Stock & Lewis 1986). Plants with seeds rich in essential elements, particularly N and P, have a greater liklihood of establishing and surving for their first year as seedlings. Stock & Lewis (1986) suggest that the minerals released into soil after fire, move slowly down the soil profile.

Plants that establish from seeds rich in phosphorus, will not need to use soil minerals in their first few months of growth since they appear to be able to survive on cotyledonary reserves. The requirement for phosphorus may only occur after 70 days of growth and therefore, soil mineral elements are probably available to the seedlings lower in the soil profile. However, fire may also have an adverse effect on soil mineral status with certain elements being volatalised by very hot fires (Stock & Lewis 1986). Therefore, it would be of great adaptive significance to have a stategy that allows for regeneration from stored nutrients than from external nutrient sources in environments that have low nutrient soils and are subject to regular fire events.

Conclusions

The total mass of a plant is related to its seed size and this relationship is not affected by nutrient treatments. Therefore, in a plant's natural environment, the size of the plant will be determined, to a large extent, by the size of its seed. Seed mass is not negatively related to relative growth rate in these seedlings.

Nutrient treatment, or more specifically, lack of a particular essential element can however, affect the rate and accumulation of plant dry matter. Differential growth responses such as root and shoot production are affected by nutrient treatments and lack of soil potassium in particular, increases root to shoot ratios.

Potassium is the most limiting nutrient to plant growth, while phosphorus is the least limiting. High concentrations and imbalances of all essential elements in combination can adversely affect the survival of these species.

The amount of phosphorus present in cotyledons is positvely related to seed size and the total mass that a plant can attain depends on the initial phosphorus content of the seed.

The dependance on cotyledonary mineral resources differs between calcicoles and calcifuges. Calcicole plants require external phosphorus supplement more than calcifuge plants do.

Internal cotyledonary resources are a mechanism of adaptation to regenerate in soils low in phosphorus.

Acknowledgements

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Appendix 1. Standard curves for Murphy and Riley colorimetric phosphorus determination.

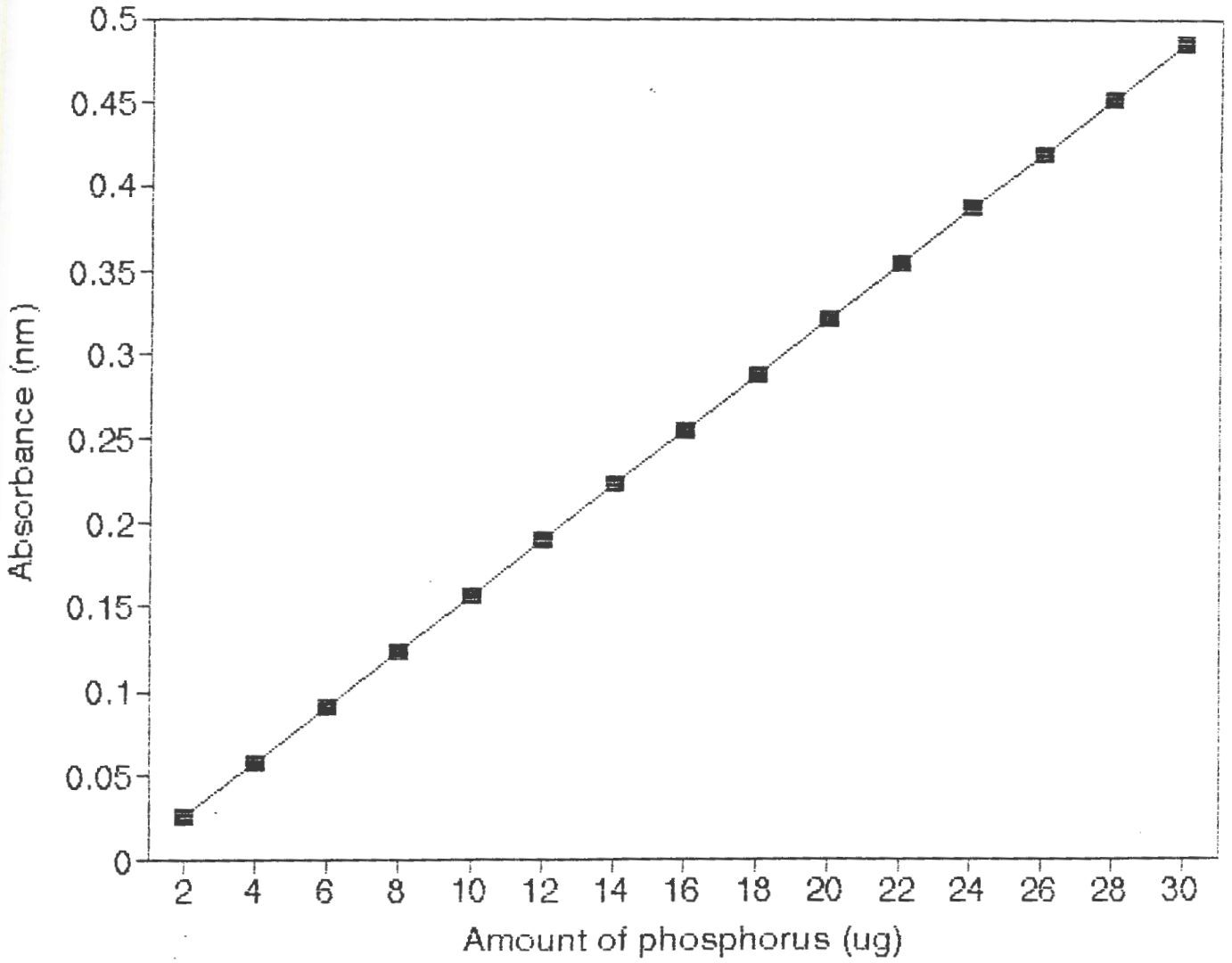

FIgure 1. Standard curve for digital Spectronic 20 spectrophotometer.

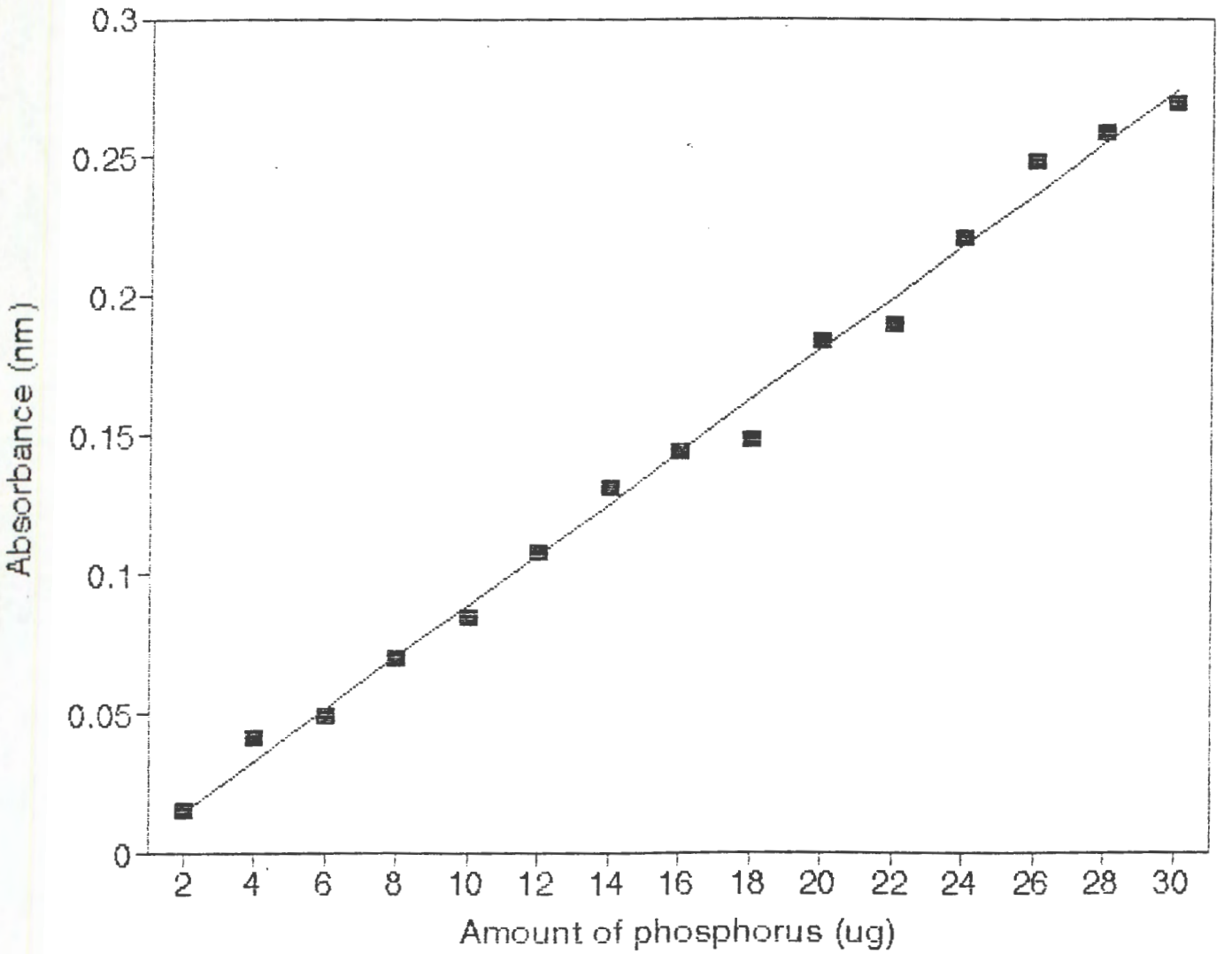

Figure 2. Standard curve for Spectronic 20 analogue spectrophotometer (r^2=0.9956)