MINERAL NUTRITION AND GROWTH OF EUCALYPTUS SEEDLINGS

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ABSTRACT

Seedlings of **Eucalyptus delegatensis** R.T. Bak., **E. maculata** Hook, and **E. brockwayi** C.A. Gardn. were grown for up to 10 weeks in a solid rooting medium and flushed daily with one of five different nutrient solutions. Stable relative growth rates and internal nitrogen concentrations were established after an initial nutrient adjustment (lag) phase. Relative growth rate and relative nutrient uptake rate were directly related after the lag phase. Partitioning of assimilate to roots decreased from 0.35 (root weight/total weight) at low concentrations of nutrients, to 0.10 at high concentrations.

INTRODUCTION

The use of fertilisers in the establishment of commercial eucalypt plantations is widespread (e.g., Cremer *et al.* 1978). However, there is a dearth of basic information regarding the relationship between rate of nutrient supply, nutrient uptake, and growth of *Eucalyptus*. Several workers have examined the effect of different nutrient regimes on growth under controlled conditions as discussed below, but nutrient supply has not generally been related to nutrient uptake or growth of the plant.

Ingestad (1982) proposed that nutrient supply be treated as a dynamic variable which is related to relative growth rate. The nutritional factor can be expressed as a "flux density" or the amount of nutrient available per unit of time and unit of area. Nutrients must be supplied in exponentially increasing amounts (or at a constant relative addition rate) equivalent to relative growth rate, to maintain constant relative growth rates. A series of experiments with birch (*Betula pendula* Roth), alder (*Alnus incana* Moench), and several conifers demonstrated that seedlings do maintain constant rates of exponential growth at constant internal nutrient concentrations, when relative nutrient addition rate is constant (Ingestad 1979a, b, 1981).

In none of the experiments on eucalypts discussed below was there an attempt to maintain constant relative nutrient addition rates; therefore, comparison of results is difficult. A hydroponic system was used by Lacey *et al.* (1966) to study the growth of *Eucalyptus grandis* W. Hill ex Maiden but nutrient solutions were changed only once during the 8-week growth period. Growth increased with increasing (initial) phosphorus concentration, with a concomitant decrease in root/shoot ratio and an increase in the concentrations of nitrogen, phosphorus, and potassium in the foliage.

Experiments with 12 species of *Eucalyptus* indicated a wide variation in growth response and nitrogen content with the form of nitrogen applied (Moore & Keraitis 1971). Barrow (1977) found that eucalypt seedlings grown in forest soil responded to a single application of phosphorus and the response appeared earlier in species with small seeds and thus smaller initial reserves. High levels of phosphorus led to poor growth and survival, but there is some indication from the data presented that seedlings with high phosphorus levels may have been deficient in nitrogen.

The experiments described in this paper were designed to determine whether stable exponential growth rates and stable internal nutrient concentrations could be sustained by applying nutrient solutions once each day to *Eucalyptus* seedlings in pots. The three species chosen for the experiment occur in widely different ecological niches and represent different sub-groups within the genus *Eucalyptus* (*E. delegatensis* R.T. Bak., Monocalyptus; *E. brockwayi* C.A. Gardn., Symphyomyrtus; *E. maculata* Hook, Corymbia; after Pryor & Johnson 1971). Any similarity in the response to nutrients of these three species would thus have broader implications for other species of the genus.

Abbreviations

N_c : Internal plant nitrogen concentration (mg/g)

R_g : Relative growth rate (g/g/day)

R_a : Relative nutrient addition rate (g/g/day)

R_u : Relative nutrient uptake rate (g/g/day)

R_p : Partitioning coefficient (Root mass/total plant mass)

MATERIALS AND METHODS

Plant Culture

The growth technique described by Ingestad & Lund (1979) required sophisticated root spray chambers with facilities for automatic addition of nutrient solutions. Such equipment was not available to us and, in the present experiments, pots were flushed daily with nutrient solutions in the expectation that nutrients would become available in exponentially increasing amounts to root systems growing at exponential rates.

Plant Material

Seeds of the three *Eucalyptus* species described in Table 1 were sown in trays of vermiculite on 5 July 1982. Seed of *E. delegatensis* had been stratified for 6 weeks at 4°C prior to this but *E. maculata* and *E. brockwayi* did not require stratification. Twenty-one days after sowing, single seedlings were transplanted to pots 10 cm in diameter containing 450 ml of a 50 : 50 mixture of sand and vermiculite. Seedlings which subsequently became too large for these pots were transferred to 15-cm pots containing 850 ml of sand and vermiculite. The pots were placed in a controlled environment chamber with day/night temperatures of 24°/19°C held for 10 and 14 hours respectively. The light source was natural sunlight but the photoperiod was

TABLE 1—Description of species used in nutrient experiments (seedlot number according to CSIRO Division of Forest Research Seed Centre)

Species	Seedlot No.	Location	Lat. (S)	Long. (E)	Alt. (m)
E. delegatensis	S13104	Maydena, Tas.	44°33′	146°34′	650
E. delegatensis	S13167	Batlow, N.S.W.	35°37′	148°09′	1100
E. maculata	S13569	Buladelah, N.S.W.	32°27′	152°8′	160
E. brockwayi	S12266	Norseman, W.A.	32°20′	121°43′	300

extended to 12 hours with low-intensity incandescent lights. Duplicate samples of 12 seedlings of *E. delegatensis,* eight of *E. maculata,* and 15 of *E. brockwayi* were removed 31 days after germination for dry weight and nutrient determination. Seedlings were watered only with de-ionized water until nutrient treatments were initiated 36 days after germination, hereafter referred to as day 0.

The five nutrient treatments were based on a modified Hoagland's solution (Hoagland & Arnon 1938) and varied from full strength to a dilution of 1 in 5000 (Table 2). Nutrient treatments were applied once each day, in the morning. Each pot was flushed with the solution and the excess allowed to drain away. The proportions of nutrients in the solutions were the same for all treatments but only the effects of nitrogen have been examined in this paper.

The experiment with *E. delegatensis* from Maydena (S13104) contained 120 seedlings (five treatments, four blocks, and five harvests). Each block was placed on a separate tray and treatments were allocated randomly to rows within each tray. Twenty seedlings were harvested every 14 days.

Seedlings of *E. delegatensis* from Batlow (S13167) and the other two species were allocated to two blocks but only one harvest of seedlings from these seedlots was made because of the workload involved in the more intensively sampled *E. delegatensis*

TABLE 2—Characteristics of the modified Hoagland's nutrient solutions used to irrigate seedlings

Treatment No.	Nitrogen concentration (mol/m^3)	Electrical conductivity (μS/cm)	Proportion of Hoagland's solution to deionised water
1	0.003	0.4	1 : 4999
2	0.03	4.0	1 : 499
3	0.30	40.0	1 : 49
4	3.0	400	1 : 4
5	15	2000	1 : 0

Proportions (by weight) of elements present in all treatment solutions;
N 100, K 112, Ca 78, S 31, Mg 23, P 16, Fe 2.4, Cl 0.055, Mn 0.054, B 0.050, Zn 0.010, Cu 0.006, Mo 0.004, Co 0.002

(S13104). Growth periods were 58 days for *E. maculata,* 59 days for *E. delegatensis,* and 64 days for *E. brockwayi.*

At each harvest, roots were carefully washed and fresh weights of roots, stems, and leaves determined. Leaf area was obtained by tracing around photocopies of each leaf on a graphics tablet attached to an Apple computer. Plant parts (leaf, root, and stem) were dried to constant weight at 70°C and reweighed. Individual fractions were analysed for nitrogen. Samples had to be combined where plants weighed less than 25 mg as this was the minimum amount of material which could be assayed satisfactorily. To avoid loss of the small amount of material available, samples were digested without grinding. Whilst many of the plant fractions were analysed separately, only seedling totals have been presented in this paper.

Chemical Analysis

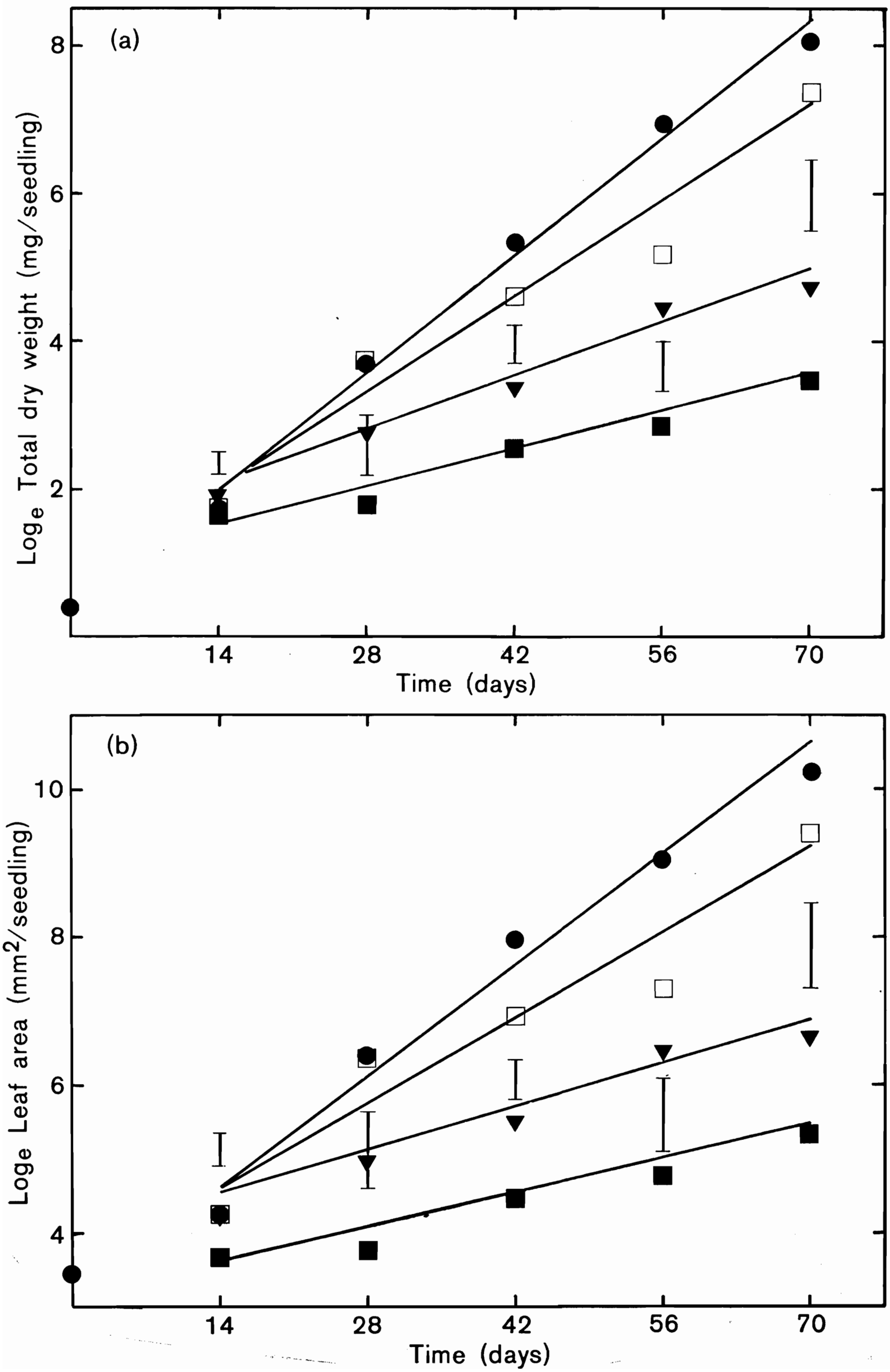
Plant samples were digested with sulphuric acid and hydrogen peroxide (Lowther 1980). Ammonia in the digest was reacted with phenol and sodium hypochlorite to form the indophenol blue complex, and nitrogen was determined by an automated spectrophotometric method.

RESULTS

Eucalyptus delegatensis (S13104) was the only seedlot harvested at 14-day intervals so data on relative growth rate over time are restricted to this seedlot. There was considerable variation in growth between individual seedlings within treatments, presumably due to inbreeding effects which are common in *Eucalyptus* spp. (Eldridge & Griffin 1983). Despite this variation, analysis of variance of the data from each harvest date showed clear and significant differences in most of the characteristics between Treatments 1, 3, 4, and 5 from day 28 onwards. Data points and bars for least significant differences ($p = 0.05$) between treatments at each harvest date are shown in Fig. 1a (total dry weight) and Fig. 1b (leaf area). Differences between Treatments 1 and 2 were rarely significant so data for Treatment 2 have been omitted for clarity. Exponential curves relating the growth of seedlings in each treatment to time were also fitted (Fig. 1a and 1b) and are explained in the Discussion section.

Nitrogen concentration (N_c) in seedlings at day 0 was 33 mg/g (total dry weight). During the first 14 days N_c in Treatments 1, 3, and 4 fell sharply; from day 14 to 28, concentrations in seedlings from Treatments 1 and 3 continued to fall but had stabilised by day 42 (Fig. 2). These data could not be subjected to analysis of variance as components and seedlings from Treatments 1 and 2 had to be combined to create a sample large enough for chemical analysis.

FIG. 1 (Right)—
(a) Total dry weight ($\log_e$ mg) and
(b) Leaf area ($\log_e$ mm^2) against time for **E. delegatensis** (S13104) seedlings irrigated with nutrient treatments 1 ■, 3 ▼, 4 □, and 5 ● (Table 2), and harvested at 14-day intervals. Vertical bars indicate least significant differences between treatments for each harvest date ($p = 0.05$). Lines indicate exponential curves calculated from day 14 to day 70.


(a)
Loge Total dry weight (mg/seedling)
8
6
4
2
14
28
42
56
70
Time (days)
(b)
Loge Leaf area (mm2/seedling)
10
8
6
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14
28
42
56
70
Time (days)

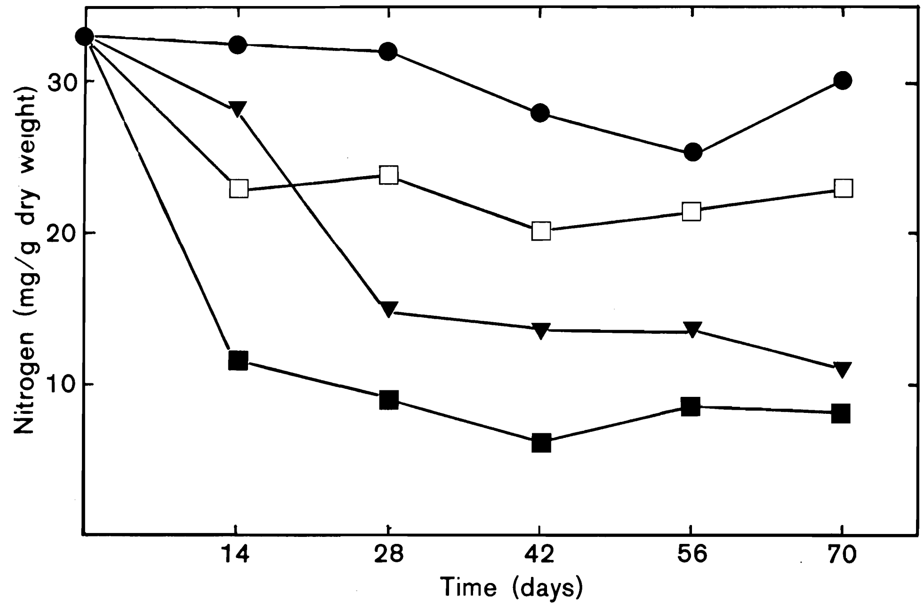

FIG. 2—Nitrogen concentration (mg/g) against time for **E. delegatensis** (S13104) seedlings irrigated with nutrient treatments 1 ■, 3 ▼, 4 □, and 5 ● (Table 2).

Assimilate partitioning differed markedly between plants in high and low nutrient treatments but also took about 42 days to stabilise (Fig. 3). After this time the ratio of root weight/total weight ranged from greater than 0.3 in Treatment 1 to 0.1 in Treatment 5.

Only one harvest was made of *E. maculata, E. brockwayi,* and the *E. delegatensis* from Batlow (S13167). The data confirmed the observations made on *E. delegatensis* from Maydena (S13104), with significant differences in dry weight, leaf area, assimilate partitioning, and nutrient concentration between low-nutrient (1, 2, and 3) and high-nutrient (4 and 5) treatments. Total dry weight of seedlings, R_p, and R_g values are given in Table 3. Differences in absolute mass, R_p, and R_g for the three species reflect, to some extent, the differences in seedling mass at the start of the experiment (3.15, 1.47, and 0.49 mg dry weight per seedling for *E. maculata, E. delegatensis,* and *E. brockwayi* respectively). *Eucalyptus delegatensis* clearly had a lower over-all growth rate than the other two species but the reasons for this have not been investigated.

DISCUSSION

Experiments with *Betula pendula* have demonstrated that germinating seedlings have an optimum nitrogen concentration and that when non-optimum nitrogen additions are made, the nitrogen concentration in the seedlings changes over a period of time, recognised as a lag phase, to a new level (Ingestad 1979a). Once constant internal nitrogen concentrations have been established, Ingestad (1982) observed that

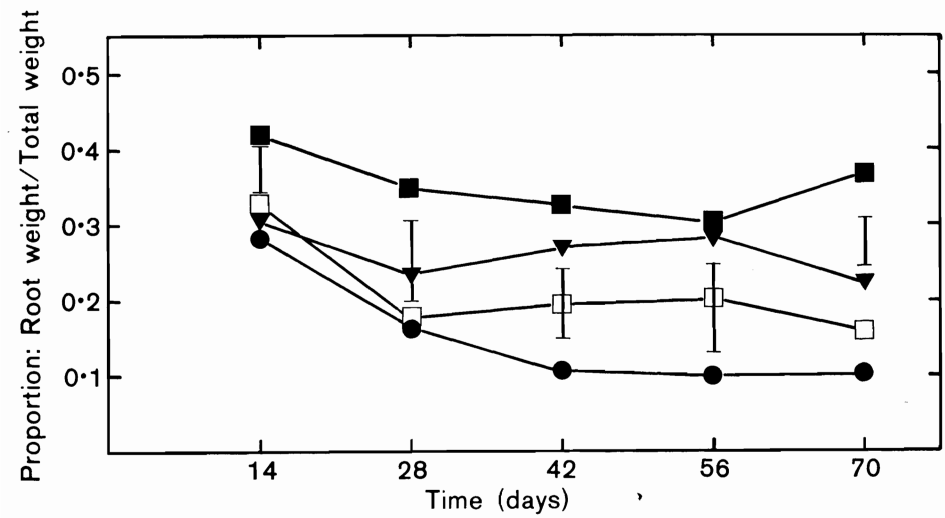

FIG. 3—Ratio of root weight to total weight (partitioning) of **E. delegatensis** (S13104) irrigated with nutrient treatments 1 ■, 3 ▼, 4 □, and 5 ● (Table 2). Vertical bars indicate least significant differences between treatments for each harvest date (p = 0.05).

TABLE 3—Total mass (oven dry, g) R_p (Root mass/total mass ratio), and R_g (g/g/day) of the three species which were harvested only once. The duration of the growth period for each species is indicated

Treatment No.	**E. delegatensis** (S13167) 59 days			**E. maculata** 58 days			**E. brockwayi** 64 days			Species means	
	Mass	R_p	R_g	Mass	R_p	R_g	Mass	R_p	R_g	Mass	R_p
1	0.01	0.38	0.04	1.01	0.21	0.10	0.19	0.22	0.09	0.40	0.27
2	0.02	0.33	0.04	0.42	0.35	0.08	0.52	0.22	0.11	0.32	0.30
3	0.10	0.24	0.07	1.66	0.27	0.11	0.14	0.19	0.09	0.63	0.23
4	1.38	0.11	0.12	3.82	0.13	0.12	2.78	0.15	0.15	2.66	0.13
5	1.30	0.11	0.11	5.65	0.13	0.13	3.46	0.14	0.14	3.47	0.13

L.S.D. between treatments (species means, p = 0.05)
Mass 0.84
R_p 0.06

a direct relationship exists between R_g and R_a up to the point of nutrient saturation. Beyond the point of nutrient saturation, R_g becomes constant and then decreases rapidly to the point where toxicity or osmotic effects result in plant mortality.

These relations will hold only when nutrients other than nitrogen are also supplied in the correct proportions for the requirements of the plant and are thus not limiting or toxic. There is a close relationship between nitrogen status and photosynthesis and, as nitrogen is required in larger amounts than other nutrients, only the effects of variations in this element are presented here. It was assumed that the nutrient solution applied to seedlings contained adequate supplies of all other nutrients, relative to nitrogen. To fully test this assumption would require a series of complex experiments and was not the point under test. Phosphorus concentrations were also determined in the harvested seedlings and the pattern of uptake closely resembled that of nitrogen.

The form of nitrogen supplied to seedlings influences growth rate and nitrogen uptake in *Eucalyptus* spp. (Moore & Keraitis 1971). Species vary considerably in their response, however, and this aspect was not examined in the present study. The ratio of nitrate to ammonium nitrogen supplied in all treatments was 93 : 7. The ratio of nitrogen to phosphorus is also important and high levels of either, in isolation, are detrimental (Groves & Keraitis 1976). The ratio used in the present study of 100 : 16 may not have been optimum but this aspect also requires further study.

The data presented in Fig. 2 demonstrate the lag effect for nitrogen concentration in *E. delegatensis*. Seedlings in Treatments 1 and 3 which were treated with low concentrations of nutrients took up to 42 days to adjust and reach a stable N_c and R_p, whereas seedlings in Treatments 4 and 5 which were treated with high concentrations of nutrients reached stable values of N_c and R_p more rapidly. Whilst N_c in seedlings from Treatment 5 fell slightly over the period of the experiment, this was probably caused by the increasing proportion of stem, in which values of N_c were lower than in leaves and roots. Stem weight/total weight ratios at day 70 were 0.18 for seedlings in Treatment 5 compared with 0.04 for seedlings in Treatment 1. Internal nutrient adjustment occurred most rapidly during the first 14 days (Fig. 2) and growth in Treatments 1 and 3 was more rapid during this period than in subsequent periods (Fig. 1a). Exponential curves were fitted to the total dry weight and leaf area data against time from day 14 to day 70. The equation took the form:

$$Y = ae^{bx}$$

where Y was total dry weight (mg) or leaf area (mm^2)
x was time (days)
a, e, and b were constants.

The exponential curves for total dry weight (Fig. 1a) all had correlation coefficients (r^2) of 0.95 or greater and those for leaf area (Fig. 1b) of 0.91 or greater. The constant "b" represents relative growth rate R_g, and varied from 0.037 in Treatment 1 to 0.113 in Treatment 5 (Fig. 1b).

Seedlings from all treatments partitioned a high proportion of total weight to roots during the first 14 days (Fig. 3). This is also evident in Fig. 1b, as the increase in leaf area was initially lower in Treatments 3, 4, and 5 than that observed subsequently. Relative growth in leaf area varied from 0.034 in Treatment 1 to 0.107 in Treatment 5 (Fig. 1b). The proportion of assimilate allocated to roots gradually declined in treatments receiving high nutrient levels.

Data for *E. grandis* presented by Lacey *et al.* (1966) were recalculated and showed a similar trend in partitioning. Seedlings with a N_c of 28.2 mg/g had a R_p of 0.18 whereas those with a N_c of 17.7 mg/g had a R_p of 0.36.

Seedlings of *E. delegatensis* (S13167), *E. maculata*, and *E. brockwayi* were harvested only once so the values of R_g in Table 3 include a variable period of internal nutrient adjustment and thus represent non-stable conditions. The magnitude of the error caused by this factor is greater in Treatments 1 and 3 than in Treatment 5 (Fig. 2). R_g and R_u in *E. delegatensis* were calculated for the period from day 42 to day 70, when N_c in all treatments had become relatively stable. The data points in Fig. 4 support the relationship proposed by Ingestad (1982) that, under stable conditions, R_g and R_a are directly related with a slope of 45°. In our experiments, R_u was calculated from seedling mass and N_c at each harvest and is equivalent to the relative nutrient addition rate R_a of Ingestad (1982). On the other hand, data for *E. maculata* over the 58-day period lie well above the 45° line. Seed of *E. maculata* is larger than *E. delegatensis* and contains more nitrogen. Seedlings harvested at the start of the experiments contained 50, 160, and 15 μg N for *E. delegatensis, E. maculata,* and *E. brockwayi* respectively. Seedlings subject to low external nutrient concentrations would take longer to reach stable levels of N_c and R_g than those subject to high concentrations, as evidenced by the slope of the line for *E. maculata*.

Our data indicate that under stable conditions of N_c, R_g of *E. delegatensis* varied from 0.02 to 0.10 g/g/day and was directly related to external nutrient status. Under non-stable conditions, R_g in *E. maculata* and *E. brockwayi* ranged from 0.08 to 0.13

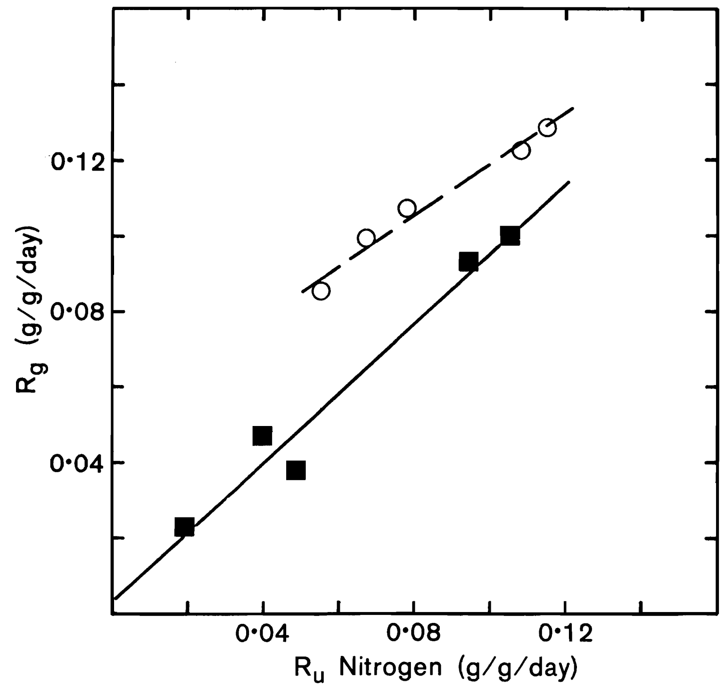

FIG. 4—The relationship between relative growth rate (R_g) and relative uptake of nitrogen (R_u) in **E. delegatensis** ■ seedlings from day 42 to day 70 and **E. maculata** ○ seedlings from day 0 to day 58.

and 0.09 to 0.14 g/g/day respectively. There have been few comparable estimates of R_g in *Eucalyptus* spp. reported. Seedlings of *E. diversicolor* F. Muell. (karri), *E. marginata* Donn ex Sm. (jarrah), and *E. calophylla* R. Br. (marri), grown for 9 weeks in soil which had received phosphate applications, had R_g values of 0.055, 0.042, and 0.036 g/g/day respectively. These are relatively low values and it is possible that plants which received high levels of phosphate suffered from a lack of nitrogen but data on foliage nitrogen concentrations were not presented (Barrow 1977).

High values of R_g were obtained for *E. grandis* during the first 7 to 14 days after germination (0.20 and 0.15 g/g/day for high and low light conditions respectively) but R_g fell rapidly to 0.12 and 0.05 g/g/day after 3 weeks (Doley 1978). We may assume that nutrient concentrations were falling during this period but no data were presented on this aspect.

Values of R_g from 0.116 g/g/day for *E. socialis* F. Muell ex Mig. to 0.178 for *E. blakelyi* Maiden were obtained for five species over the growth period 3 to 6 weeks by Mooney *et al.* (1978). These high values of R_g were associated with leaf nitrogen concentrations in excess of 40 mg/g and it is likely that seedling N_c was stable during the period of measurement.

Declining relative growth rates have frequently been reported giving the impression that changes in the basic physiological characteristics result from the aging process. The results obtained in the present study suggest that declining values of R_g are indicative of declining values of R_u and thus declining nutrient status, and do not necessarily reflect a change in basic physiological characteristics. Our observations support the suggestion (Linder 1982) that many of the low values of R_g that are frequently published for tree seedlings result from an inadequate supply of mineral nutrients.

In many reported studies, nutrients have been applied only once or with long intervals between applications. Such methods lead to a gradual decline in the concentration of the soil solution and a reduction in the rate of supply to the plants. The method described here maintained a constant relative rate of nutrient supply which enabled the plants to maintain contant relative rates of growth. Preliminary gas-exchange data from these seedlings indicated that there was a strong correlation between nutrient status and photosynthetic capacity (Cromer 1984).

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