

NITROGEN AND CONIFER STUDIES

by

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ABSTRACT

Part 1

The vascular bundle of leaves with the C_4 -pathway of photosynthesis is usually surrounded by two concentric chlorophyllous cell layers: an outer mesophyll layer and an inner bundle sheath layer. The localization of the nitrate-assimilating enzymes, nitrate reductase, nitrite reductase, and glutamate dehydrogenase in Zea mays, Gomphrena globosa, and Sorghum sudanense was studied by differential grinding. Nitrate reduction to nitrite appears to occur primarily in mesophyll cells. The nitrate content of the mesophyll cells was much higher than the nitrate content of the bundle sheath cells. The distribution of nitrite reductase seemed to be related to the presence of chloroplasts with grana. Ammonia incorporation into organic compounds by glutamate dehydrogenase was localized in the bundle sheath cells.

Part 2

Four conifer species, Douglas-fir (Pseudotsuga menziesii var. menziesii) (Mirb.) Franco, Western redcedar, (Thuja plicata) Donn, Western hemlock (Tsuga heterophylla) (Raf.) Sarg, and Lodgepole pine (Pinus contorta var. contorta) Dougl., were grown on three different sources

of nitrogen (nitrate, ammonia, and a combination of nitrate and ammonia 7:1).

A linear relationship was found between leaf area and leaf dry weight for three species (Douglas-fir, Lodgepole pine and Western hemlock). Different nitrogen treatments had no effect on this relationship.

Part 2-A In this part the tree seedlings were grown for 18 weeks.

For Douglas-fir, Western redcedar and Western hemlock, survival and growth on the nitrate solution was similar to survival and growth on the combination solution. Ammonia was an unfavorable source of nitrogen for survival and growth of Douglas-fir and Western redcedar. For Western hemlock, ammonia was only detrimental to survival. Hemlock seedlings which survived ammonia treatment grew as well as trees growing on the other two sources of nitrogen. Lodgepole pine survived equally well under all treatments and was the only species that grew best on ammonia.

Part 2-B At 18 weeks the seedlings were transferred from sand culture to liquid nutrient solution. The solution was changed every two days for a period of six days. The pH of the solutions was measured when the solutions were changed. At the end of the six days, the starch content of

the plants was measured. The results indicate that these forest species differ in their tolerance to ammonia. Western hemlock and Lodgepole pine seem to be able to tolerate higher levels of ammonia than Douglas-fir and Western redcedar.

Part 2-C In this part the seedlings were grown for a period of approximately one year.

The trends shown in the 18 week experiment were confirmed in the longer experiment and some additional treatment effects appeared.

Part 2-D In this part the seedlings were grown for a period of approximately one year.

Within each species, an attempt was made to correlate differences in growth among nitrogen treatments with differences in gas exchange. It was found from calculations of stomatal resistance (r_s) that the entry of CO_2 was not limiting dry matter production. It is postulated that mesophyll resistance (r_m) may be a factor involved in controlling growth in these trees.

Part 3

Two-year old (2-0) Douglas-fir (Pseudotsuga menziesii) seedlings were lifted in the spring and mud-packed. These seedlings were tested for the effects of various storage

conditions.

Mud-packed seedlings, stored in the field for 19 days and subsequently planted, had higher survival and root growth than those having other storage conditions. The other storage conditions included higher and lower temperature than in the field and light versus dark. Treatment of the mud-packs with fertilizer and vermiculite had no effect on any of the parameters measured.

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PREFACE

This thesis differs from most theses in that it does not deal exclusively with one topic. This lack of exclusivity is partly a result of personal preference and partly a result of circumstance. However all studies reported in this thesis with the exception of Part I are concerned with the physiology of whole plants.

It was my intention on arriving at UBC to get as much research experience as possible.

The study with C_4 -plants allowed me to work on a project that required rapid publication to ensure the originality of discoveries and also allowed me more experience with enzymatic techniques. My discovery that the location of nitrite reductase (a $NADPH_2$ requiring enzyme) seems to be correlated with the presence of granal chloroplasts agrees with evidence that only granal chloroplasts are capable of NADPH production.

The study with mud-packed seedlings allowed me to work on a project which had the possibility of immediate applicability. My discovery that spring-lifted mud-packs should be stored outside may prove to be of significance from both a conservational and a financial point of view.

The project with forest species and nitrogen sources permitted me to gain some experience with sand culture techniques, some experience in growing forest species and some experience with psychrometric techniques. Evidence which I collected seems to indicate that forest species vary in their tolerance to NH_4^+ rather than their ability to preferentially metabolize NO_3^- or NH_4^+ . This is a significant finding in that it agrees with a new school of thought arising in the field of agriculture.

It was my initial intention to make the section on nitrogen nutrition and forest species more physiological and more cohesive than it turned out. This project was started as a dual project with Sara Madoc-Jones. It was our intention that she would look at the project from an ecological point of view and that I would look at the project from a physiological point of view. However due to the death of Sara, certain parts of the project had to be abandoned. I tried to salvage as much as I could. I attempted to study the criteria which I felt would give me the greatest insight into the growth responses we were observing.

PART 1. THE LOCALIZATION OF NITRATE-ASSIMILATING ENZYMES
IN LEAVES OF PLANTS WITH THE C_4 -PATHWAY OF PHOTOSYNTHESIS.¹

INTRODUCTION

The vascular bundle of leaves with the C_4 -pathway of photosynthesis is usually surrounded by two concentric cell layers, an inner bundle sheath layer and an outer mesophyll layer. Cells of the mesophyll layer contain granal chloroplasts distributed about a large central vacuole. Surveys indicate that the bundle sheath chloroplasts of dicotyledons, chloridoid grasses, and some panicoid grasses have well-developed grana, that sugar cane and sorghum lack grana entirely, and that corn and close relatives are intermediate, forming only rudimentary grana (6, 14, 21, 25, 27, 32, 43). Differences in the capacity for dye reduction (13) of agranal and granal chloroplasts of single leaves have led to the conclusion that the agranal chloroplasts are incapable of forming reductant by non-cyclic electron transport.

Considerable work has been done with the enzymes involved in the photosynthetic carbon metabolism of these C_4 -plants and their localization within the leaf (1, 5,

¹ This article by G.E. Mellor and E.B. Tregunna appeared in Canadian Journal of Botany, Vol. 49: 137-142 (1971). E.B. Tregunna supervised the study. Subsequent developments on this topic are discussed in addenda.

16, 17, 18, 31, 37, 39, 40, 41, 42). Although many studies have been carried out on nitrate reductase, nitrite reductase and glutamate dehydrogenase in corn, a C₄-plant (2, 3, 4, 8, 15, 24, 35, 37, 44), there is only one report (40) on the localization of such events in the leaves of C₄-plants.

In 1967 Ritenour et al. (35) studied the intracellular localization of nitrate reductase, nitrite reductase, and glutamate dehydrogenase in corn and foxtail leaves. They found that nitrite reductase was localized within the chloroplasts. This result agreed with the findings of Ramirez et al. (33). Nitrate reductase was thought to be localized in the cytoplasm, although the techniques used did not eliminate the possibility that nitrate reductase may be localized on the external chloroplast membrane. Glutamate dehydrogenase was found in the mitochondria. Several investigations (7, 10, 11, 34) with etiolated seedling tissue have also indicated that glutamic acid dehydrogenase is localized in the mitochondria. Recently Joy (23) reported that in pea roots both soluble and particulate (mostly mitochondria) fractions contain NADH₂ and NADPH₂ dependent glutamate dehydrogenase. In 1968 Leech and Kirk (28) reported that in leaves of Vicia faba, NADPH₂ glutamate dehydrogenase occurred in the chloroplast while NAD-dependent glutamate dehydrogenase

occurred in the mitochondria. The method of Joy (22) was used for extraction and assay of glutamate dehydrogenase in this paper.

In view of these observations I have investigated the tissue localization of nitrate-assimilating enzymes in C₄-plants with granal (Gomphrena globosa), agranal (Sorghum sudanense), and rudimentary granal (Zea mays) bundle sheath chloroplasts.

MATERIALS

Zea mays, Gomphrena globosa, and Sorghum sudanense were grown on vermiculite supplemented with nutrient solution containing 100 ppm NO₃⁻-N as Ca(NO₃)₂·4H₂O. Growth conditions were 16-h day, 27/21C (day/night) temperature and 1000 ft-c provided in a growth chamber. Leaves of 3-week-old Zea plants, leaves of 6-week-old Sorghum, and the youngest fully expanded leaves of 2- to 3-month-old Gomphrena plants were used. The age differences were necessary to obtain plants of similar size.

METHODS

Extraction of Enzymes from Mesophyll and Bundle Sheath Tissue

Zea and Sorghum extracts, enriched in either bundle sheath or mesophyll cell contents, were obtained with the use of an 'Osterizer' homogenizer. Leaves of Zea and

Sorghum (2 g) were cut into 1-cm pieces and were ground with the homogenizer in 40 ml of the appropriate buffer for 20 sec. Microscopic inspection revealed that, primarily, mesophyll cells were ruptured during this treatment while some mesophyll cells and most of the bundle sheath cells remained intact. This extract was therefore enriched in the contents of the mesophyll cells. The remaining leaf pieces were homogenized for 1 min. Microscopic inspection of the remaining pieces revealed that almost all of the intact cells were bundle sheath cells. These remaining pieces were subjected to a very vigorous grinding with a pestle in a mortar containing glass beads and grinding media. Sufficient grinding pressure was used to break the glass beads, creating a very sharp angular grinding material. Most, but not all, of the bundle sheath cells were ruptured by this treatment, yielding an extract enriched in the contents of bundle sheath cells. The homogenizer proved unsatisfactory for the differential grinding of Gomphrena. Gomphrena was ground as described by Berry et al. (5).

The grinding medium used for nitrate reductase was that described by Hageman and Flesher (15). The grinding medium of Joy and Hageman (24) was used for nitrite reductase and the grinding medium of Joy (22) was used for glutamate dehydrogenase. The extraction method for

glutamate dehydrogenase included the addition of a non-ionic detergent to aid the breakdown of particle membranes. This breakdown of particle membranes ensured that both particulate and cytoplasmic glutamate dehydrogenase was analyzed. Plant material was harvested immediately before use after 4 h of illumination. Protein precipitable by trichloroacetic acid was determined by the method of Lowry (29). A standard curve was constructed using bovine serum albumin.

Enzyme assay - Nitrate reductase (1.6.6.1) was assayed by measuring NADH₂-dependent production of nitrite (15). Nitrite reductase was assayed by measuring disappearance of nitrite using dithionite as electron donor, as described by Joy and Hageman (24). Glutamate dehydrogenase (1.4.1.2. and 1.4.1.4) was assayed by measuring, spectrophotometrically at 340 nm, the rate of oxidation of NAD(P)H₂ dependent on the presence of ammonia and α -ketoglutarate (22). Enzyme units: 1 milliunit of enzyme catalyzed the transformation of 1 nmole of substrate per minute.

Nitrate analysis - The method of Miller and Wideman (30) was used in the determination of nitrate.

RESULTS AND DISCUSSION

The specific activities of nitrate reductase, nitrite reductase, and glutamate dehydrogenase are presented in

Table 1. The total protein extracted from the leaf was about equally divided between the two extracts.

As is seen in Table 1 nitrate reductase activity was much higher in the mesophyll extracts of all three types of plants. Table 2 shows that in both Gomphrena and Zea most of the NO_3^- occurs in the mesophyll extract. Thus the mesophyll extract contains high amounts of nitrate reductase activity and high amounts of nitrate. The occurrence of nitrate in the bundle sheath extract may be due to (a) improper separation of bundle sheath and mesophyll, (b) the passage of nitrate from the vascular system to the mesophyll, (c) storage of some nitrate in the bundle sheath cells, or (d) a combination of all three.

In Zea and Sorghum nitrite reductase activity is confined primarily to mesophyll cells (Table 1). The nitrite reductase activity in Gomphrena seemed to occur in both the mesophyll and the bundle sheath cells. In Zea and Sorghum the mesophyll cells are capable of dye reduction and contain granal chloroplasts. The bundle sheath cells of Zea and Sorghum, however, are incapable of dye reduction and contain not more than rudimentary grana (13). In Gomphrena both the mesophyll and bundle sheath cells are capable of dye reduction (W. J. S. Downton, pers.

TABLE 1. Activities of enzymes, in milliunits per milligram of protein, in extracts of mesophyll and bundle sheath tissue of Zea mays, Gomphrena globosa, and Sorghum sudanense.

Enzyme	<u>Zea mays</u>		<u>Gomphrena globosa</u>		<u>Sorghum sudanense</u>	
	Mesophyll	Bundle sheath	Mesophyll	Bundle sheath	Mesophyll	Bundle sheath
Nitrate reductase	5.50*	0.60	6.40	1.53	2.67	1.52
	3.77*	0.68	3.56	0.60	3.84	1.21
Nitrite reductase	7.97	1.68	2.54	5.06	3.47	1.10
	6.03	1.87	3.83	3.24	2.55	0.53
Glutamate dehydrogenase						
NADH	58	284	71	153	64	178
	52	201	57	181	61	138
NADPH	18	35	35	72	18	34
	15	45	27	54	13	19

*Replicates.

TABLE 2. NO_3^- -N content, as nmoles NO_3^- -N per milligram of protein, in extracts of mesophyll and bundle sheath tissue of Zea mays and Gomphrena globosa.

Plant	Mesophyll	Bundle sheath
<u>Gomphrena globosa</u>	5956*	170
<u>Zea mays</u>	715	214

*Mean of two determinations.

commun.)* and both contain at least thylakoid overlaps (12) which are believed to be sufficient for non-cyclic electron flow (20). Thus the results indicate that only grana-containing chloroplasts are linked to nitrite reduction.

It has been suggested that grana are necessary for photosystem II activity (20, Addendum 2) and that chloroplasts deficient in grana would be incapable of non-cyclic electron flow and NADP reduction. It is believed that nitrite reductase receives electrons from ferredoxin when the latter has been reduced by illumination or by NADPH_2 and a diaphorase enzyme (9, 19, 24, 33, 38). This requirement for ferredoxin to donate electrons to nitrite reductase suggests that nitrite reductase should be associated with granal chloroplasts.

The rudimentary grana in bundle sheath cells of Zea seem to play little or no role in nitrite reduction. The occurrence of nitrite reductase primarily in the mesophyll of Zea agrees with the findings of Slack et al. (42). Using density fractionation in non-aqueous media to separate Zea mesophyll and bundle sheath chloroplasts, they found that about 80% of the nitrite reductase activity occurred in the mesophyll chloroplasts.

In all three types of plants the bulk of both NADH_2 and NADPH_2 glutamate dehydrogenase activity occurs in the bundle

*Research School of Biological Sciences, Australian National University, Canberra, A. C. T., Australia.

sheath (Table 1). The occurrence of glutamate dehydrogenase activity in the bundle sheath of Gomphrena suggests that as far as glutamate dehydrogenase activity is concerned, the mitochondria in the bundle sheath are more active than the mitochondria in the mesophyll. This result coincides with the findings of Laetsch (25) and Downton et al. (14) that in the dicotyledons which they examined the mitochondria in the bundle sheath are considerably larger and more developed. This differential development of mitochondria between the mesophyll and the bundle sheath in tropical grasses has never, to our knowledge, been reported. In Zea and Sorghum, however, the bulk of the glutamate dehydrogenase still occurred in the bundle sheath.

With the methods available for cell separation, the possibility of obtaining mesophyll extracts free of bundle sheath contamination or bundle sheath extracts free from mesophyll contamination is high. Thus for the purpose of this discussion, it is assumed that the occurrence of nitrate reductase in the bundle sheath extracts of Zea, Sorghum, and Gomphrena, the occurrence of nitrite reductase in the bundle sheath extracts of Zea and Sorghum and the occurrence of glutamate dehydrogenase in the mesophyll extracts of Zea, Sorghum, and Gomphrena is contamination. However, as more refined methods become available for cell separation, this assumption may prove incorrect and the

enzyme may be found to occur in both areas. Therefore on the basis of this assumption of contamination, it would seem that the steps in the assimilation of nitrate are separated.

Thus in Zea and Sorghum it seems that nitrate and nitrite are both reduced primarily in the mesophyll (Fig. 1) and the resulting ammonia is incorporated into organic form in the bundle sheath. Ammonia, however, is somewhat toxic to plants because it perhaps inhibits the production of ATP in the mitochondria and photosynthetic electron-transport system (36). Thus it seems probable that rapid transport of the ammonia from the mesophyll to the bundle sheath is required.

Laetsch has noted that in sugar cane the wall separating the mesophyll cells from the bundle sheath cells is traversed by many plasmodesmata (26). Plasmodesmata linking the mesophyll cells to the bundle sheath cells can be seen in published micrographs of several other plants which have this type of leaf and β -carboxylation (6, 25). Thus plasmodesmata provide a connection between the mesophyll and the bundle sheath cells. Whether the transport from the mesophyll to the bundle sheath is active or passive remains to be established.

In Gomphrena, nitrate is reduced primarily in the mesophyll (Fig. 2), while nitrite can be reduced in both

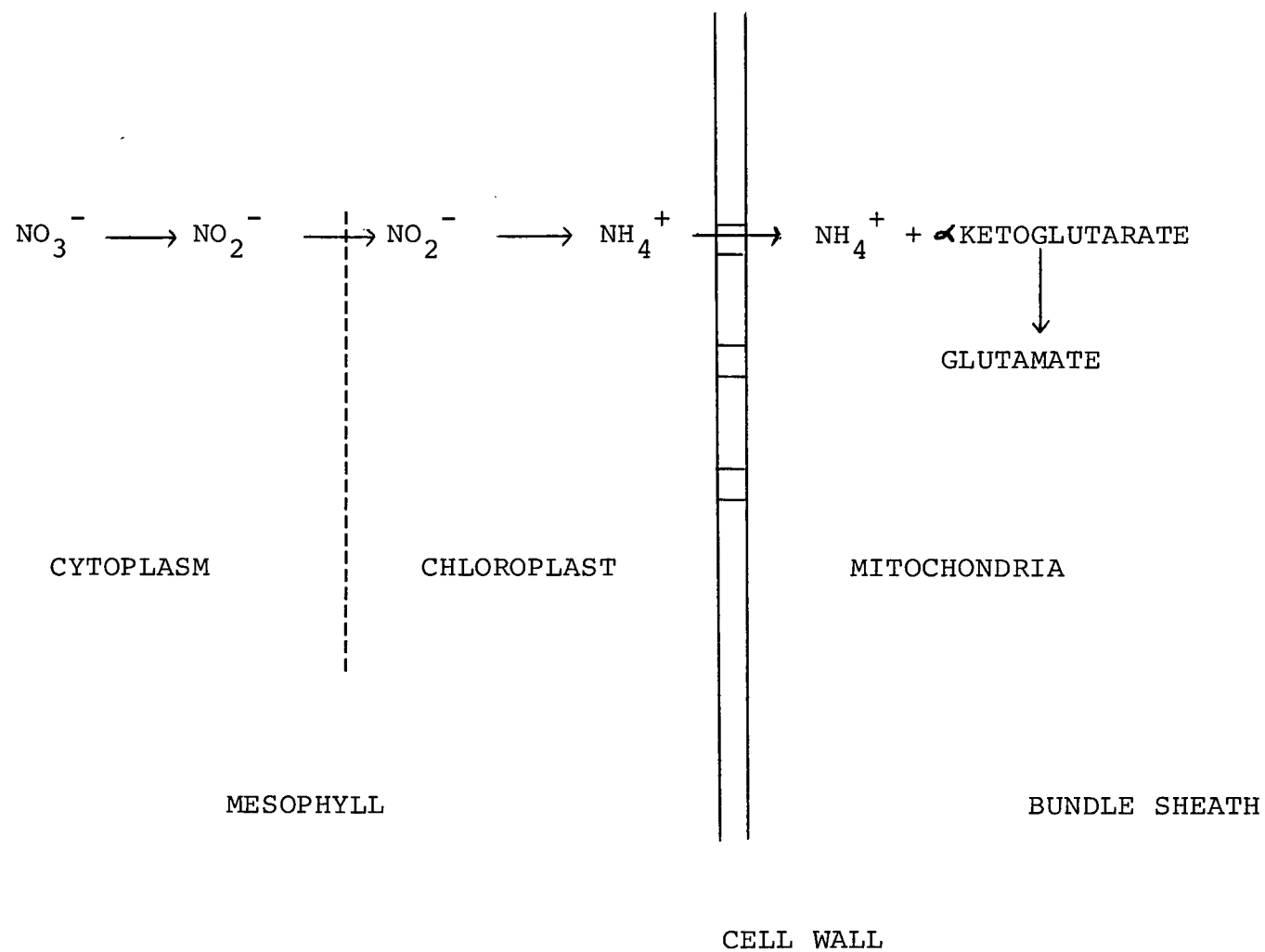


Figure 1. Schematic division of reactions between bundle sheath and mesophyll in Zea and Sorghum.

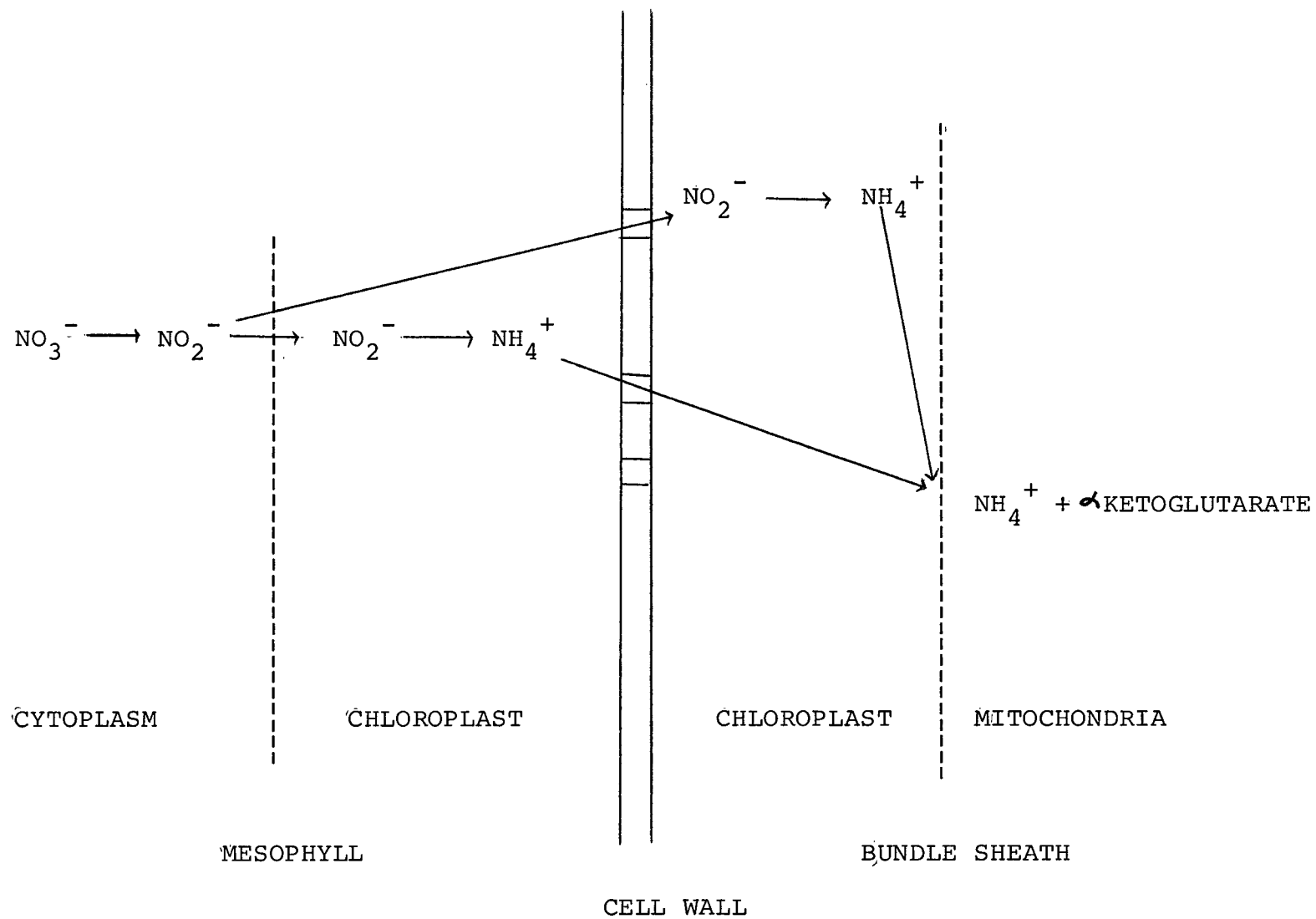


Figure 2. Schematic division of reactions between bundle sheath and mesophyll in Gomphrena.

the bundle sheath and the mesophyll. Ammonia incorporation takes place primarily in the bundle sheath. Again the transport of ammonia and nitrite from the mesophyll to the bundle sheath could occur via the plasmodesmata.

In conclusion it should be noted that when nitrate-assimilating enzymes in C_4 -plants are studied the location of the enzymes should be kept in mind when the tissues are ground. Enzymes localized in the mesophyll should pose no problem as far as complete isolation is concerned, since the mesophyll cells are easily ruptured by normal methods of grinding. The bundle sheath cells are partly resistant to normal methods of grinding. Thus for complete isolation of enzymes localized completely or partly in the bundle sheath a more vigorous grinding is required.

ADDENDA

1. While this paper was being reviewed for publication, a paper appeared by J. W. Maranville (Influence of nickel on the detection of nitrate reductase activity in sorghum extracts. Plant Physiol. 45: 591-593. 1970.) Maranville's paper indicates that nickel increases the nitrate reductase activity in sorghum extracts. Nickel was not used in the sorghum extracts of this paper. The addition of nickel to sorghum extracts may have increased the nitrate reductase activity reported in Table 1.

2. Sane et al. (P.V. Sane, D.J. Goodchild and R.B. Park. Biochim. Biophys. Acta 216: 162. 1970.) and Arntzen et al. (C.J. Arntzen, R.A. Dilley and J. Neumann. Biochim. Biophys. Acta 245:409-424. 1971.) have shown from chloroplast fragmentation studies that granal lamellae have both Photosystem I and II activities and that stromal lamellae have only Photosystem I activity i.e. only granal lamellae seem capable of reducing NADP.

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PART 2-A. A COMPARISON OF THE GROWTH AND SURVIVAL OF FOUR
CONIFER SPECIES SUPPLIED WITH DIFFERENT FORMS
OF NITROGEN.

INTRODUCTION

Few studies have been carried out to compare nitrate and ammonia as sources of nitrogen for conifers. The evidence collected to date is inconclusive as to which is the most favorable form of nitrogen for conifers. This section reports on the growth and survival of Douglas-fir (Pseudotsuga menziesii var. menziesii), Lodgepole pine (Pinus contorta var. contorta), Western hemlock (Tsuga heterophylla), and Western redcedar (Thuja placata) grown for 18 weeks on nitrate alone, ammonia alone or a combination of nitrate and ammonia (7:1).

Krajina (4) has carried out some studies of nitrogen nutrition with Douglas-fir, Western redcedar and Western hemlock. Swan (7) has carried out similar studies with Western hemlock. In both of these works the concentration of nitrogen in each of the treatments was different. This use of different nitrogen concentrations has led to difficulty in interpreting which was the more favorable form of nitrogen. Krajina concluded that nitrate was the more favorable form of nitrogen for Douglas-fir and Western redcedar, while ammonia was the more favorable source of

nitrogen for Western hemlock. Swan concluded that a combination of nitrate and ammonia (4:1) was more favorable to growth of Western hemlock than nitrate alone or ammonia alone. Van Den Driessche (8) found that for Douglas-fir, seedling dry weight was greatest when ammonia and nitrate were provided in equal amounts. Ammonia alone resulted in greater growth than nitrate alone. For Western hemlock, there was no difference in seedling dry weight between treatments using nitrate alone or ammonia alone or a combination of nitrate and ammonia.

To our knowledge, no work has been done to determine the most favorable source of nitrogen for Lodgepole pine.

MATERIALS AND METHODS

Seeds of Douglas-fir, Lodgepole pine, Western hemlock and Western redcedar were obtained from the University of British Columbia Research Forest (elevation of 1000 ft). Stratified seeds were grown in pots of sand (20-30 mesh sand purchased from the Ottawa Silica Co., Ottawa, Illinois). The sand was moistened with distilled water until one week after germination, at which time, nutrient solution containing 14 ppm of N (Table 1) was applied. Three different sources of inorganic nitrogen were used: ammonia only, nitrate only and a combination of nitrate and ammonia (7:1) (hereafter referred to as NH_4^+-N , NO_3^--N and $(\text{NO}_3^- + \text{NH}_4^+)-\text{N}$).

TABLE 1. Composition and concentration (ppm) of full-strength nutrient solution.

Element	Solution		
	$\text{NO}_3^- + \text{NH}_4^+ (7:1)$	NO_3^-	NH_4^+
N	12.2 + 1.8	14.0	14.0
P	11.8	11.8	11.8
K	22.0	22.0	22.0
Ca	20.0	20.0	20.0
Mg	9.0	9.0	9.0
S	15.0	15.0	15.0
Cu	12.6×10^{-4}	12.6×10^{-4}	12.6×10^{-4}
Zn	31.2×10^{-4}	31.2×10^{-4}	31.2×10^{-4}
Mo	31.2×10^{-4}	31.2×10^{-4}	31.2×10^{-4}
Fe	12.6×10^{-2}	12.6×10^{-2}	12.6×10^{-2}
Mn	31.2×10^{-3}	31.2×10^{-3}	31.2×10^{-3}
B	31.2×10^{-3}	31.2×10^{-3}	31.2×10^{-3}

pH = 5.5 ± 0.1

respectively). The composition of these nutrient solutions was similar to those solutions used by Krajina (4). However, the level of all nutrients except chloride was the same in all three solutions.

At the end of seven weeks, 50 live seedlings were chosen at random and transplanted into plastic bullets (9) containing sand. Each nitrogen treatment for each species consisted of 50 bullets. The 50 bullets were held in a plastic stand. Thus there were 12 stands of trees (4 species x 3 treatments).

The seedlings were irrigated with fresh nutrient three times per week and distilled water once a week. The seedlings were grown in a growth room with 90% relative humidity in the day and 65% relative humidity at night. Fluorescent and incandescent lamps provided 20,000 lux over a photoperiod of 16 hr (6 am to 10 pm). Dawn and twilight effects were produced by staggering the order in which the lights came on and went off. A 12 hr temperature cycle was used (8 am to 8 pm at 24C; 8 pm to 8 am at 20C).

Eighteen weeks after germination, seedling survival was determined, the surviving seedlings were harvested and total dry weight, root dry weight and shoot/root ratio (S/R) were recorded. Plant material was either oven dried at 80C or lyophilized; the methods gave equal

results. Leaf area (Appendix I) was determined for all species except for Western redcedar; it was impossible to determine accurately the area of the cedar scales. All results except survival were analyzed with one way analyses of variance followed by determination of the least significant difference (LSD). Survival data were analyzed by means of the Chi-square-2xn contingency table.

RESULTS

The data on plant survival are given in Table 2. The dry weights, leaf area and S/R for the survivors are given in Tables 3, 4, 5, and 6 for Douglas-fir, Western redcedar, Western hemlock and Lodgepole pine respectively.

Douglas-fir

The seedlings in the NO_3^- -N and $(\text{NO}_3^- + \text{NH}_4^+)$ -N treatments had similar percent survival and the trees in NH_4^+ -N had a significantly lower percent survival (Table 2). Seedlings receiving NH_4^+ -N produced significantly lower total dry weight and root dry weight and a significantly higher S/R than the other two treatments (Table 3). There was no significant difference in leaf area among the three treatments.

Western Redcedar

The seedlings in the $(\text{NO}_3^- + \text{NH}_4^+)$ -N and NO_3^- -N

TABLE 2. Survival (%) of Douglas-fir, Lodgepole pine, Western redcedar and Western hemlock supplied with different sources of nitrogen.

Species	Source of Nitrogen		
	$\text{NO}_3^- + \text{NH}_4^+$ (7:1)	NO_3^-	NH_4^+
Douglas-fir	98	90	64*
Western redcedar	100	100	20*
Western hemlock	100	92	64*
Lodgepole pine	92	86	88

*Difference among means within a species significant at the 5% level.

TABLE 3. Total dry weight, root dry weight, S/R and leaf area of Douglas-fir supplied with different sources of nitrogen.¹

	Source of Nitrogen		
	$\text{NO}_3^- + \text{NH}_4^+ (7:1)$	NO_3^-	NH_4^+
Total dry weight (gm/plant)	<u>.1019</u>	<u>.1192</u>	.0787
Root dry weight (gm/plant)	<u>.0498</u>	<u>.0554</u>	.0271
S/R	<u>1.12</u>	<u>1.32</u>	2.13
Total leaf surface area (cm ² /plant)	<u>12.86</u>	<u>13.19</u>	<u>10.74</u>

¹Values connected by the same line do not differ at the 5% level by LSD.

TABLE 4. Total dry weight, root dry weight, S/R and leaf dry weight of Western redcedar supplied with different sources of nitrogen.¹

	Source of Nitrogen		
	$\text{NO}_3^- + \text{NH}_4^+ (7:1)$	NO_3^-	NH_4^+
Total dry weight (gm/plant)	.1015	.1440	.0265
Root dry weight (gm/plant)	<u>.0358</u>	<u>.0429</u>	.0121
S/R	<u>2.19</u>	<u>2.48</u>	<u>2.06</u>
Leaf dry weight (gm/plant)	<u>.0792</u>	<u>.0939</u>	.0142

¹Values connected by the same line do not differ at the 5% level by LSD.

TABLE 5. Total dry weight, root dry weight, S/R and leaf area of Western hemlock supplied with different sources of nitrogen.¹

	Source of Nitrogen		
	$\text{NO}_3^- + \text{NH}_4^+ (7:1)$	NO_3^-	NH_4^+
Total dry weight (gm/plant)	<u>.0672</u>	<u>.0523</u>	<u>.0518</u>
Root dry weight (gm/plant)	<u>.0190</u>	<u>.0150</u>	<u>.0142</u>
S/R	<u>2.58</u>	<u>3.00</u>	<u>3.10</u>
Total leaf surface area (cm ² /plant)	15.28	<u>11.39</u>	<u>10.64</u>

¹Values connected by the same line do not differ at the 5% level by LSD.

TABLE 6. Total dry weight, root dry weight, S/R and leaf area of Lodgepole pine supplied with different sources of nitrogen.¹

	Source of Nitrogen		
	$\text{NO}_3^- + \text{NH}_4^+ (7:1)$	NO_3^-	NH_4^+
Total dry weight (gm/plant)	<u>.1214</u>	<u>.1119</u>	.1677
Root dry weight (gm/plant)	<u>.0580</u>	<u>.0526</u>	<u>.0653</u>
S/R	<u>1.14</u>	<u>1.34</u>	1.94
Total leaf surface area (cm ² /plant)	<u>12.10</u>	<u>10.97</u>	17.52

¹Values connected by the same line do not differ at the 5% level by LSD.

treatments had 100% survival, while those in the NH_4^+ -N treatment had only 20% survival (Table 2). The seedlings receiving NO_3^- -N treatment produced the largest total dry weight followed by the $(\text{NO}_3^- + \text{NH}_4^+)$ -N treatment (Table 4). Those on the NH_4^+ -N treatment produced by far the lowest total dry weight. Trees in NH_4^+ -N produced significantly lower amounts of leaf and root dry weight than the other two treatments. There was no significant difference in S/R among the three treatments.

Western Hemlock

Seedlings receiving $(\text{NO}_3^- + \text{NH}_4^+)$ -N and NO_3^- -N had 100% and 92% survival respectively (Table 2). Seedlings receiving NH_4^+ -N had only 64% survival. Total dry weight, root dry weight and S/R showed no significant difference among the three treatments (Table 5). The seedlings grown with $(\text{NO}_3^- + \text{NH}_4^+)$ -N produced a significantly larger leaf area than seedlings grown on the other two treatments.

Lodgepole Pine

There was little difference in survival among the three treatments (Table 2). Treatments with NH_4^+ -N resulted in significantly larger total dry weight, leaf area and S/R than the other two treatments (Table 6). There was no difference in root dry weight among the three treatments.

DISCUSSION

On the basis of growth and survival data it would seem that Douglas-fir, Western redcedar and Western hemlock survived and grew well with either NO_3^- -N or $(\text{NO}_3^- + \text{NH}_4^+)$ -N. The NH_4^+ -N treatment was detrimental to both the growth and survival of Douglas-fir and Western redcedar, but only to the survival of Western hemlock. Western hemlock seedlings which survived on the NH_4^+ -N treatment grew as well as trees growing on the other two sources of nitrogen. Lodgepole pine survived equally well with all nitrogen treatments and was the only species that grew best on NH_4^+ -N.

The lower survival of Western hemlock seedlings in the NH_4^+ -N treatment may not be very significant in the natural field environment if excessive seedling production occurs. With Douglas-fir and Western redcedar the small size of seedlings grown on NH_4^+ -N may lead to increased mortality in the field due to competition.

Many workers have proposed that a low S/R is preferable for survival of conifers (1, 3, 5, 6). However, Hermann (2) has shown that survival for Douglas-fir seedlings with a small root system was significantly lower than for seedlings with a large root system, regardless of the size of the shoot. A consideration of the data reported here for effect of nitrogen treatment on survival, root weight and S/R, would indicate that: 1) For Douglas-fir, a low S/R and a

large root system are both indicative of high survival (Tables 2 and 3). 2) For Western redcedar, development of a large root system by the survivors is more indicative of high survival than is a low S/R (Tables 2 and 4). 3) For Western hemlock, neither root development nor a low S/R of the survivors is indicative of survival (Tables 2 and 5). 4) For Lodgepole pine, development of a large root system is more indicative of high survival than development of a low S/R.

With Lodgepole pine, the high S/R of the NH_4^+-N treatment (Table 6) was not detrimental to survival (Table 2), possibly because this treatment produced seedlings with as large a root system as that produced by seedlings grown on the other treatments. Douglas-fir seedlings grown on NH_4^+-N also had a high S/R (Table 3), but had low survival (Table 2). In this case the increased S/R was a result of decreased root development.

The similar leaf area produced by Douglas-fir (Table 3) on the three treatments indicates that photosynthetic area was not the limiting factor in the low production of total dry weight on the NH_4^+-N treatment.

Western hemlock seedlings produced only 1/3 to 1/2 the dry matter produced by Douglas-fir or Lodgepole pine in spite of the fact that these three species had similar leaf areas (Tables 3, 5, and 6).

There seems to be a relationship between where the trees occur in the field and the ability of the trees to metabolize ammonia. Young Douglas-fir and Western redcedar, which occur on soils where nitrate is expected to be the predominant form of nitrogen (4), grew and survived poorly on NH_4^+ -N as compared to growth and survival on the other nitrogen treatments. Western hemlock and Lodgepole pine, which occur on soils where ammonia is expected to be the predominant form of nitrogen (4) grew as well or better on NH_4^+ -N than on the other two sources of nitrogen. Van Den Driessche recently reported (8) that Douglas-fir grew better on NH_4^+ -N alone than on NO_3^- -N alone. This observation is in contrast to the data reported here and the results of Krajina (4) and indicates that the relationship between where the trees occur in the field and the ability of the trees to metabolize NH_4^+ -N or NO_3^- -N is not as clear-cut as we have stated.

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PART 2-B. NITROGEN UPTAKE AND STARCH CONTENT OF CONIFERS
SUPPLIED WITH DIFFERENT SOURCES OF NITROGEN.

INTRODUCTION

Some plants appear to utilize ammonia in preference to nitrate and vice versa. Potatoes, rice, buckwheat (7), some conifer species (17) and low bushberry (16), are examples of plants that seem to utilize ammonia in preference to nitrate, while beets, wheat (7), tomatoes, mustard, rye, oats (10) and apple (6) trees are examples of plants that seem to utilize nitrate in preference to ammonia.

In previous work (Part 2-A) it was concluded that Western hemlock and Lodgepole pine survived and grew better when supplied with ammonia rather than nitrate. The opposite results were found for Douglas-fir and Western redcedar. However, two pieces of information prevented me from concluding that Western redcedar and Douglas-fir utilize nitrate preferentially and that Western hemlock and Lodgepole pine utilize ammonia preferentially: a) Van Den Driessche (17) concluded that Douglas-fir seedlings grow better when supplied with ammonia and b) the results of a 1 year study (Part 2-C) indicate that growth of Lodgepole pine and Western hemlock was less on nitrate than on either ammonia or a combination of nitrate and ammonia (7:1) where nitrate was the major source of nitrogen.

Recent work with other plants suggested a solution to this apparent contradiction of results. Harada et al. (8) and Blair et al. (2) have shown that with some plants, there is no difference between growth on nitrate or ammonia, when low concentrations of nitrogen are supplied. This suggests that plants may differ in their ability to tolerate various levels of ammonia rather than their ability to utilize either ammonia or nitrate preferentially. This tolerance may be expressed in several ways a) perhaps by regulating the amount of ammonia taken up and/or b) by a metabolic regulation once the ammonia is taken up.

It has been shown that wheat seedlings (15, 18), rye grass (13) and some conifers (17) take up more ammonia than nitrate when the two forms are present together. In conifers (17) ammonia was taken up exclusively when the two sources were present together.

Ammonia in toxic levels is thought to inhibit photophosphorylation (12) and subsequently to inhibit carbon dioxide fixation (5). Therefore if ammonia is present at an inhibitory concentration, the amount of starch would be expected to be low. Two reports (4, 9) have indicated that starch content of ammonia-treated Douglas-fir is lower than in nitrate-treated Douglas-fir. In addition Bassham (1) has shown that ammonia has a regulator effect on Chlorella,

influenced metabolism from sugar synthesis to protein synthesis.

An experiment was undertaken with Douglas-fir, Western redcedar, Lodgepole pine and Western hemlock to determine

- a) if ammonia was taken up preferentially over nitrate and
- b) if the starch content of the seedlings was related to the source of available nitrogen.

METHODS AND MATERIALS

Details of growing conditions, culture solutions, culture technique, dry weight and survival have been described previously (Part 2-A). The seedlings were grown in bullets and were supplied with 1 of 3 sources of nitrogen: nitrate only (NO_3^- -N), ammonia only (NH_4^+ -N) or a combination of nitrate and ammonia (7:1) ($(\text{NO}_3^- + \text{NH}_4^+)$ -N). At 4 months after germination, the seedlings were removed from the bullets, adhering sand was washed off and the roots were placed in jars (2 seedlings per jar) of nutrient solution containing the appropriate nitrogen treatment (eg. seedlings supplied with NO_3^- -N in the bullets were placed in NO_3^- -N nutrient solution). The jars were aerated constantly and the nutrient solution was changed every second day. The pH of the nutrient solutions was measured for six consecutive days.

After six days on the nutrient solution the seedlings

were lyophilized. Soluble carbohydrate was extracted by sonication of .1 gm lyophilized material in 20 ml ethanol. Starch and the solid residue were retained. Starch was extracted from the residue and hydrolyzed to glucose with perchloric acid according to McCready et al. (14). Glucose was determined using hydrogen peroxide and sulfuric acid according to Dubois et al. (3).

All data was analyzed by analyses of variance followed by determination of the LSD at 5% level.

RESULTS

Detailed descriptions of dry weight, leaf area and root dry weight were reported in Part 2-A.

pH Data

The initial pH of the nutrient solution was 5.5. The pH after 2 days exposure to the roots is given in Table 1. With Western redcedar and Western hemlock, the pHs of the NH_4^+ -N and $(\text{NO}_3^- + \text{NH}_4^+)$ -N treatment solutions were indistinguishable from each other and have a significantly lower pH than the NO_3^- -N treatment. With Douglas-fir and Lodgepole pine the pH of the $(\text{NO}_3^- + \text{NH}_4^+)$ -N treatment solution was significantly higher than the pH of the NH_4^+ -N treatment solution but was still much lower than the pH of the NO_3^- -N treatment solution.

TABLE 1. The mean pH of the nutrient solutions after treatment for 2 days.^{1,2}

Species	Source of Nitrogen		
	$\text{NH}_4^+ - \text{N}$	$(\text{NO}_3^- + \text{NH}_4^+) - \text{N}$	$\text{NO}_3^- - \text{N}$
Douglas-fir	4.20	4.69	6.17
Western redcedar	<u>4.15</u>	<u>4.27</u>	6.10
Western hemlock	<u>4.22</u>	<u>4.39</u>	5.79
Lodgepole pine	3.85	4.87	6.02

¹Initial pH of nutrient solutions 5.5.

²Values connected by the same line do not differ at the 5% level by LSD.

Starch Content

Douglas-fir and Western redcedar treated with NH_4^+ -N had the lowest starch content compared to the other nitrogen treatments (Table 2). The $(\text{NO}_3^- + \text{NH}_4^+)$ -N treatment produced levels of starch equal to that of the nitrate treatment. With Lodgepole pine and Western hemlock, there was no difference in starch production among the three treatments.

DISCUSSION

When NO_3^- -N is taken up, the pH of a nutrient solution rises, probably as a result of the expulsion of OH^- and/or HCO_3^- (2,10). When NH_4^+ -N is taken up the pH of the nutrient solution decreases, probably as a result of the expulsion of H^+ (2,10).

The difference in pH between the NH_4^+ -N treatment and the $(\text{NO}_3^- + \text{NH}_4^+)$ -N treatment of Douglas-fir and Lodgepole pine (Table 1) may be due to 1) less uptake of NH_4^+ -N from the $(\text{NO}_3^- + \text{NH}_4^+)$ -N treatment with concomitant lower expulsion of H^+ and/or 2) simultaneous uptake of small amounts of NO_3^- -N with NH_4^+ -N from the $(\text{NO}_3^- + \text{NH}_4^+)$ -N and thus release of both H^+ and OH^- . For all four species, it can be concluded that NH_4^+ -N was the predominant source of nitrogen when supplied as $(\text{NO}_3^- + \text{NH}_4^+)$ -N. These results agree with Van Den Driessche's work with conifers (2). The

TABLE 2. Starch content of conifer seedlings supplied with different sources of nitrogen for 4 months (g/gm dry weight).¹

Species	Source of Nitrogen		
	$\text{NH}_4^+ \text{-N}$	$(\text{NO}_3^- + \text{NH}_4^+) \text{-N}$	$\text{NO}_3^- \text{-N}$
Douglas-fir	52.1	<u>118.1</u>	<u>107.3</u>
Western redcedar	106.7	<u>143.7</u>	<u>131.0</u>
Western hemlock	<u>173.3</u>	<u>179.7</u>	<u>181.0</u>
Lodgepole pine	<u>117.0</u>	<u>114.4</u>	<u>103.4</u>

¹Values connected by the same line do not differ at the 5% level by LSD.

NH_4^+ -N content of the $(\text{NO}_3^- + \text{NH}_4^+)$ -N was 1.75 ppm N while 12.25 ppm of NO_3^- -N was present.

When NH_4^+ -N is present in toxic levels, NH_4^+ -N inhibits carbon dioxide fixation (5) and NH_4^+ -N has also been shown to switch sugar production to protein production (1). Both of these mechanisms would interfere with starch production. When Douglas-fir and Western redcedar were supplied with 14 ppm NH_4^+ -N, starch production was low (Table 2). On the other hand, when Douglas-fir and Western redcedar seedlings were supplied with $(\text{NO}_3^- + \text{NH}_4^+)$ -N, NH_4^+ -N (1.75 ppm N) was the predominant form of nitrogen taken up. Trees in this treatment produced amounts of starch equal to amounts produced with NO_3^- -N only. With Lodgepole pine and Western hemlock starch production was the same on both high (14 ppm N) and low levels (1.75 ppm N) of NH_4^+ -N and high levels (14 ppm N) of NO_3^- -N. These results would indicate that Lodgepole pine and Western hemlock have a higher tolerance to external supplies of NH_4^+ -N than Douglas-fir and Western redcedar. However Douglas-fir and Western redcedar are tolerant of low amounts of NH_4^+ -N. In addition, the high dry matter production (Part 2-A) of Lodgepole pine and Western hemlock on either NH_4^+ -N or $(\text{NO}_3^- + \text{NH}_4^+)$ -N is now easily explained. In fact, the seedlings supplied with

$(\text{NO}_3^- + \text{NH}_4^+)-\text{N}$ were utilizing NH_4^+-N .

The tolerance of Douglas-fir to low levels of NH_4^+-N may explain why Van Den Driessche (17) concluded that Douglas-fir grew better when supplied with NH_4^+-N rather than with NO_3^--N .

The variation in tolerance of the seedlings to ammonia may be easily explained in terms of selection by where the trees occur in nature. Douglas-fir and Western redcedar, which grow and survive poorly at high levels of NH_4^+-N (Part 2-A) occur on soils where NO_3^--N is thought to be the predominant form of nitrogen (11). In these species, selection for ammonia tolerance or protection against NH_4^+-N toxicity has not been necessary. Lodgepole pine and Western hemlock which grow and survive well (Part 2-A) at high levels of NH_4^+-N occur on soils where NH_4^+-N is considered to be the predominant form of nitrogen (11). In these species selection for ammonia tolerance has been necessary.

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PART 2-C. A COMPARISON OF THE GROWTH OF FOUR CONIFER
SPECIES SUPPLIED WITH DIFFERENT FORMS OF
NITROGEN FOR 10 TO 12 MONTHS.¹

INTRODUCTION

Previously (Part 2-A) it was shown that the survival and growth of conifer seedlings were affected by the source of nitrogen. For Douglas-fir and Western hemlock, survival and growth on a nitrate solution were similar to survival and growth on a combination of nitrate and ammonia (7:1). Ammonia was an unfavorable source of nitrogen for survival and growth of Douglas-fir and Western redcedar. For Western hemlock, ammonia treatment was only detrimental to survival. Hemlock trees which survived ammonia treatment grew as well as trees growing on the other two sources of nitrogen. Lodgepole pine survived equally well under all treatments and was the only species that grew best on ammonia.

Swan (2) reported that over a four month growing period, Jack pine (Pinus banksiana) and White spruce (Picea glauca) grew better when supplied with ammonia rather than

¹ This section deals with work initiated by S. Madoc-Jones but continued by the author after the untimely death of S. Madoc-Jones. Also the data in this section represents part of a paper presented at the XII Pacific Science Congress, Canberra, 1971. Ammonia and Nitrate in the Nitrogen Economy of Some Conifers Growing in Douglas-fir Communities of the Pacific North-West America by V.J. Krajina, S. Madoc-Jones and G.E. Mellor.

nitrate. Durzan and Steward (1) continued Swan's experiment for another 12 months and found that nitrate now gave the best results for White pine, while Jack pine still grew best when supplied with ammonia. The object of the work presented in this section was to determine the effect of supplying four conifer species with different sources of nitrogen for a period of 10 to 12 months.

METHODS AND MATERIALS

Seed source, growing conditions and nutrient solution were described previously (Part 2-A). Seeds of Douglas-fir, Western redcedar, Western hemlock and Lodgepole pine were sown (38 per 1 gallon crock) in sand and the crocks were irrigated initially seven times a day with demineralized water. Irrigation with nutrient solutions was begun one week after germination. Each crock was irrigated automatically and received 250 ml of nutrient four times a day. For the first seven weeks after germination the concentration of the nutrient solution was that used in Part 2-A (i.e. 14 ppm N). At seven weeks after germination the concentration of the nutrient solution was doubled (28 ppm N) and remained so until harvest. The same nitrogen treatments were used as in Part 3-B ($\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$ and $(\text{NO}_3^- + \text{NH}_4^+)\text{-N}$). The crocks were placed in the growth

room in a randomized complete block design (3 blocks).

Five weeks after germination the plants were thinned to five per crock. Thirteen weeks after germination Douglas-fir and Lodgepole pine were thinned to two plants per crock. Fifteen weeks after germination Western redcedar and Western hemlock were thinned to two plants per crock. The two largest Douglas-fir and Lodgepole pine seedlings were chosen to remain. This choice was justified on the grounds that since both species are shade intolerant in the area of their provenance the two largest seedlings would have the greatest chance of survival in nature. The Western redcedar and the Western hemlock seedlings chosen to remain were the largest and the smallest seedlings because both species are shade tolerant.

Western redcedar and Douglas-fir were harvested 10 months after germination and Western hemlock and Lodgepole pine, 12 months after germination. Shoot height was determined, then the trees were harvested and divided into roots, leaves and branches. The trees were oven dried at 80C to constant weight.

Leaf area of Douglas-fir, Western redcedar, and Western hemlock was determined by choosing 100 live needles at random from each tree. These needles were divided into 10 groups of 10 needles each. The area of these needles

was determined by multiplying together length times width times a correction factor for needles of this age as described in Appendix I. Necrotic and chlorotic needles or portions of needles were not used in these determinations. After leaf area had been determined for each group of needles, the groups were dried to constant weight. After drying the average area of 1 gm dry weight of leaves was determined for each tree (average of the 10 groups). Thus with a knowledge of total leaf dry weight, the total leaf area of a tree was determined. Leaf area of Western redcedar was determined by photocopying leaves from each tree and then using a planimeter to determine the area of the Xerox copies. The leaves were then dried to constant weight. Thus the area of 1 gm dry weight of leaves was determined and again with a knowledge of total leaf dry weight, total leaf area could be determined.

All data were analyzed with analyses of variance followed by determination of the LSD at the 5% level.

RESULTS

Douglas-fir

The $(\text{NO}_3^- + \text{NH}_4^+)$ -N treatment produced the tallest seedlings followed by the NO_3^- -N treatment and the NH_4^+ -N

treatment (Table 1). Seedlings supplied with NH_4^+ -N produced the shortest plants, and the lowest shoot dry weight, total dry weight and leaf area. The NO_3^- -N seedlings had a larger total dry weight than the $(\text{NO}_3^- + \text{NH}_4^+)$ -N seedlings. This difference in total dry weight was mostly attributable to differences in root dry weight. In spite of a difference in total dry weight there was no significant difference in leaf area between the NO_3^- -N and the $(\text{NO}_3^- + \text{NH}_4^+)$ -N seedlings. The difference in dry matter production between the NH_4^+ -N and $(\text{NO}_3^- + \text{NH}_4^+)$ -N treatments was a result of difference in shoot dry weight. The tips of the older needles were frequently chlorotic and necrotic. Dr. V.J. Krajina identified these characteristics as symptoms of magnesium and calcium deficiency. Several NH_4^+ -N seedlings lost many leaves before harvest.

Western Redcedar

Seedlings on the NO_3^- -N treatment had a larger dry matter production than those on the $(\text{NO}_3^- + \text{NH}_4^+)$ -N treatment and this difference was due to a difference in shoot dry weight (Table 2). The NH_4^+ -N treatment produced the shortest seedlings, the smallest leaf area and the smallest total, root and shoot dry weight. There was a direct relationship between leaf area and dry matter production (i.e. the larger the leaf area, the larger the dry matter production). The older leaves of the NH_4^+ -N treated

TABLE 1. Total, root, and shoot dry weight, leaf area and height of Douglas-fir supplied with different sources of nitrogen.¹

	Source of Nitrogen		
	NO_3^-	$\text{NO}_3^- + \text{NH}_4^+ (7:1)$	NH_4^+
Total dry weight (gm/crock)	167.4	133.4	68.8
Root dry weight (gm/crock)	64.0	<u>42.0</u>	<u>27.3</u>
Shoot dry weight (gm/crock)	<u>103.4</u>	<u>91.5</u>	41.5
Total surface area of leaves (cm^2/plant)	<u>5855.1</u>	<u>4280.9</u>	1621.8
Shoot height (cm)	74.0	90.2	43.2

¹Values connected by the same line do not differ at the 5% level by LSD.

TABLE 2. Total, root, and shoot dry weight, leaf area and height of Western redcedar supplied with different sources of nitrogen.¹

	Source of Nitrogen		
	NO_3^-	$\text{NO}_3^- + \text{NH}_4^+ (7:1)$	NH_4^+
Total dry weight (gm/crock)	201.5	160.7	37.6
Root dry weight (gm/crock)	<u>47.1</u>	<u>40.7</u>	8.2
Shoot dry weight (gm/crock)	154.4	120.0	29.6
Total surface area of leaves (cm^2 /plant)	6410.0	5060.0	1410.0
Shoot height (cm)	76.7	77.0	42.6

¹Values connected by the same line do not differ at the 5% level by LSD.

seedlings were necrotic and chlorotic (symptoms of calcium and magnesium deficiency according to Dr. Krajina).

Western Hemlock

There were no significant differences in height among any of the treatments (Table 3). The NO_3^- -N treatment resulted in the lowest leaf area and smallest total, root and shoot dry weight. With the exception of leaf area, there was no difference in any of the parameters measured between the NH_4^+ -N and $(\text{NO}_3^- + \text{NH}_4^+)$ -N treatments. One might have expected a difference in total dry matter production between the NH_4^+ -N and $(\text{NO}_3^- + \text{NH}_4^+)$ -N treatments considering the large difference in leaf area. The leaves of seedlings on the NH_4^+ -N and $(\text{NO}_3^- + \text{NH}_4^+)$ -N were dark green, however a few showed some necrotic and chlorotic areas. These areas were identified by Dr. V.J. Krajina as being indicative of magnesium deficiency. The seedlings supplied with NO_3^- -N produced yellowish green leaves.

Lodgepole Pine

There was no significant difference in height between the three treatments (Table 4). The seedlings on the NO_3^- -N treatment produced the lowest shoot dry weight, total dry weight and leaf area. The NO_3^- -N and $(\text{NO}_3^- + \text{NH}_4^+)$ -N seedlings produced similar amounts of root dry weight and thus differences in total dry weight between

TABLE 3. Total, root, and shoot dry weight leaf area and height of Western hemlock supplied with different sources of nitrogen.¹

	Source of Nitrogen		
	NO_3^-	$\text{NO}_3^- + \text{NH}_4^+ (7:1)$	NH_4^+
Total dry weight (gm/crock)	107.6	<u>235.4</u>	<u>212.2</u>
Root dry weight (gm/crock)	26.3	<u>51.4</u>	<u>49.6</u>
Shoot dry weight (gm/crock)	81.3	<u>184.0</u>	<u>162.5</u>
Total surface area of leaves (cm ² /plant)	9078.2	22496.5	12341.9
Shoot height (cm)	<u>49.6</u>	<u>47.6</u>	<u>44.3</u>

¹Values connected by the same line do not differ at the 5% level by LSD.

TABLE 4. Total, root, and shoot dry weight, leaf area and height of Lodgepole pine supplied with different sources of nitrogen.¹

	Source of Nitrogen		
	NO_3^-	$\text{NO}_3^- + \text{NH}_4^+ (7:1)$	NH_4^+
Total dry weight (gm/crock)	113.2	<u>210.0</u>	<u>229.7</u>
Root dry weight (gm/crock)	<u>48.6</u>	<u>67.6</u>	<u>82.2</u>
Shoot dry weight (gm/crock)	65.6	<u>142.4</u>	<u>147.5</u>
Total surface area of leaves (cm^2/plant)	2251.7	<u>6460.8</u>	<u>6789.8</u>
Shoot height (cm)	<u>36.7</u>	<u>29.0</u>	<u>33.7</u>

¹Values connected by the same line do not differ at the 5% level by LSD..

these two treatments were due to differences in shoot dry weight. There was no difference in any of the parameters measured between the NH_4^+ -N and $(\text{NO}_3^- + \text{NH}_4^+)$ -N treatments. The leaves of seedlings treated with NH_4^+ -N and $(\text{NO}_3^- + \text{NH}_4^+)$ -N were dark green while many leaves of the nitrate treatment were yellowish green over their entire length.

DISCUSSION

The experiment described in this part extends the growth period from the experiment described in Part 2-A. There were, however, certain differences in culture techniques between the two experiments: receptacles for growing, concentration of nitrogen, and the frequency of nitrogen application.

Both Douglas-fir and Western redcedar seedlings on the NH_4^+ -N treatment grew least over both the 4 month growing period and over the 10 month growing period.

At 4 months the NO_3^- -N and $(\text{NO}_3^- + \text{NH}_4^+)$ -N Douglas-fir seedlings were indistinguishable from each other, but after 10 months certain differences became noticeable. The NO_3^- -N treatment produced taller seedlings and a higher total dry weight. This difference in dry weight was due to differences in root dry weight.

At both 4 and 10 months, the trends among nitrogen treatments with Western redcedar were exactly the same with the exception that a difference in leaf area was

detectable between the NO_3^- -N and the $(\text{NO}_3^- + \text{NH}_4^+)$ -N treatments at the end of 10 months. Over both treatment periods, the NO_3^- -N treatment produced the largest dry weight.

At 4 months, the only effect of source of nitrogen for Western hemlock was on leaf area and survival. At the end of the 12 month treatment period, seedlings on the NO_3^- -N treatment produced by far the lowest dry matter and leaf area. Over both treatment periods, seedlings on the NH_4^+ -N and $(\text{NO}_3^- + \text{NH}_4^+)$ -N treatments were indistinguishable from one another except for leaf area. The $(\text{NO}_3^- + \text{NH}_4^+)$ -N treatment produced seedlings with the largest leaf area.

At the end of the 4 month treatment period, NH_4^+ -N was the most favorable source of nitrogen for Lodgepole pine. Seedlings from the NO_3^- -N and the $(\text{NO}_3^- + \text{NH}_4^+)$ -N treatment were indistinguishable from one another. At the end of the 12 month treatment period, the NO_3^- -N treatment produced the lowest dry weight and smallest leaf area. Seedlings on the NH_4^+ -N and $(\text{NO}_3^- + \text{NH}_4^+)$ -N treatments were now indistinguishable from one another.

In conclusion the trends shown in the 4 month experiments were confirmed in the longer experiment and some additional treatment effects appeared, particularly in separating the treatments with $(\text{NO}_3^- + \text{NH}_4^+)$ -N from treat-

ments in which only one form of nitrogen was present.

At the concentrations of nitrogen used (14 or 28 ppm), NO_3^- -N is the most favorable form of nitrogen for Douglas-fir and Western redcedar while NH_4^+ -N is the most favorable form of nitrogen for Lodgepole pine and Western hemlock.

In Part 2-B, it was shown that NH_4^+ -N was the predominant form of nitrogen taken up from the $(\text{NO}_3^- + \text{NH}_4^+)$ -N treatment. At this low concentration of NH_4^+ -N (1.75 ppm), Douglas-fir and Western redcedar survived and grew better than when supplied with a high rate of NH_4^+ -N (14.0 ppm). At the end of 10 months, Western redcedar in the lower concentration of NH_4^+ -N also gave better growth than in the higher concentration of NH_4^+ -N. However at both 4 and 10 months, the low NH_4^+ -N treatment produced less dry weight than the NO_3^- -N treatment. The quantity of NH_4^+ -N in the low NH_4^+ -N treatment may still have been too high to obtain the optimum growth rate of the seedlings.

At 4 months Douglas-fir seedlings on the low NH_4^+ -N and NO_3^- -N treatments were indistinguishable from each other; however at 10 months the NO_3^- -N treatment produced a greater amount of dry matter than the low NH_4^+ -N treatment. At the end of the 12 month period the quantity of NH_4^+ -N in the low NH_4^+ -N treatment may still have been too high to obtain the optimum growth rate of the seedlings.

With Lodgepole pine and Western hemlock at the end of 4 months, the NO_3^- -N treatment with one exception produced amounts of dry matter similar to those produced on the other two treatments. However, at the end of 12 months, the NO_3^- -N treatment produced less dry matter than the other two treatments. The seedlings may have been able to metabolize enough NO_3^- -N to supply their needs when they were small, but as the seedlings increased in size, they might not have been able to assimilate or convert enough NO_3^- -N to meet their needs.

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PART 2-D. GAS EXCHANGE OF CONIFER SEEDLINGS SUPPLIED WITH
DIFFERENT FORMS OF NITROGEN.

INTRODUCTION

In Part 2-C it was shown that Douglas-fir seedlings treated with NH_4^+ -N produced the lowest dry weight and leaf area (Tables 1 and 2). There was no difference in leaf area between the NO_3^- -N and $(\text{NO}_3^- + \text{NH}_4^+)$ -N seedlings although the NO_3^- -N seedlings produced a higher dry weight.

With Western redcedar, there was a direct relationship between total dry weight and leaf area (i.e. the larger the leaf area, the larger the dry matter production). The NO_3^- -N treated seedlings produced the largest leaf area and the largest amount of dry matter, followed by the $(\text{NO}_3^- + \text{NH}_4^+)$ -N treatment and then the NH_4^+ -N treatment.

NH_4^+ -N and $(\text{NO}_3^- + \text{NH}_4^+)$ -N treated Western hemlock seedlings produced similar amounts of dry matter, although there was a large difference in leaf area. The NO_3^- -N treated seedlings produced the least amount of dry matter and leaf area.

Lodgepole pine seedlings supplied with NO_3^- -N produced the lowest dry weight and leaf area. There was no difference in dry weight and leaf area between the NH_4^+ -N and $(\text{NO}_3^- + \text{NH}_4^+)$ -N treatments.

TABLE 1. Total dry weight of conifer seedlings supplied with different sources of nitrogen (gm.pot⁻¹).¹

Species	Nitrogen Treatment		
	NO ₃ ⁻	NO ₃ ⁻ + NH ₄ ⁺ (7:1)	NH ₄ ⁺
Douglas-fir	167.4	133.4	68.8
Western redcedar	201.5	160.7	37.6
Lodgepole pine	113.2	<u>210.0</u>	<u>229.7</u>
Western hemlock	107.6	<u>235.4</u>	<u>212.2</u>

¹Values connected by the same line do not differ at the 5% level by LSD.

TABLE 2. Leaf area of conifer seedlings supplied with different sources of nitrogen
(cm².pot⁻¹).¹

Species	Nitrogen Treatment		
	NO_3^-	$\text{NO}_3^- + \text{NH}_4^+ (7:1)$	NH_4^+
Douglas-fir	<u>11,710.2</u>	<u>8,561.8</u>	3,243.6
Western redcedar	12,820.0	10,120.0	2,820.0
Lodgepole pine	4,503.4	<u>12,921.6</u>	<u>13,579.6</u>
Western hemlock	18,156.4	44,993.0	24,683.8

¹Values connected by the same line do not differ at the 5% level by LSD.

The lack of correlation between leaf area and plant dry weight with Douglas-fir and Western hemlock suggested that the nitrogen treatment was affecting the gas exchange of the leaves. The results presented in this paper suggest trends in stomatal resistance (r_s) which would affect both water relations and gas exchange.

SYMBOLS AND UNITS

$(C)_2)_a$	External CO_2 concentration ($gm\ cm^{-3}$)
$(CO_2)_c$	Chloroplast CO_2 concentration ($gm\ cm^{-3}$)
T	Transpiration ($gm\ cm^{-2}\ sec^{-1}$)
P	CO_2 flux ($gm\ cm^{-2}\ min^{-1}$)
RH	Relative humidity
r_a	Boundary layer diffusion resistance ($sec\ cm^{-1}$)
r_m	Mesophyll diffusion resistance ($sec\ cm^{-1}$)
r_s	Stomatal diffusion resistance ($sec\ cm^{-1}$)
$(H_2O)_a$	Water vapor concentration of air ($gm\ cm^{-3}$)
$(H_2O)_s$	Water vapor concentration of stomatal cavity ($gm\ cm^{-3}$)
Ψ	Total water potential (bar)
Ψ_g	Gravitational potential (bar)
Ψ_m	Matric potential (bar)
Ψ_p	Pressure potential (bar)
Ψ_s	Solute potential (bar)

THEORY

Internal water stress may be described in terms of the energy status of the contained water, usually expressed as total water potential (Ψ) (1). In both plant and soil systems, Ψ is given by the equation

$$\Psi = \Psi_s + \Psi_p + \Psi_m + \Psi_g \quad (I)$$

The matric potential (Ψ_m) and gravitational potential (Ψ_g) are usually neglected for plant systems and equation (I) may be written as

$$\Psi = \Psi_s + \Psi_p \quad (II)$$

Transpiration is given by

$$T = \frac{(H_2O)_s - (H_2O)_a}{r_s + r_a} \quad (III)$$

For plants $(H_2O)_s - (H_2O)_a$ is calculated from measurements of relative humidity, assuming 100% RH inside the stomates. Likewise the flow of CO_2 is controlled by a similar relationship

$$P = \frac{(CO_2)_a - (CO_2)_c}{r_s + r_a + r_m} \quad (IV)$$

$(CO_2)_c$ is usually assumed to be zero. The resistances $r_s + r_a$ for P can be calculated from T, taking into account the different diffusion coefficients of water vapor and CO_2 in air.

METHODS AND MATERIALS

Four conifer species, Douglas-fir, Western hemlock, Lodgepole pine and Western redcedar were grown in pots of sand. After thinning, each pot contained 2 seedlings. The seedlings were supplied with complete nutrient solution in which only the source of nitrogen was varied. Nitrogen was supplied as ammonia only, nitrate only or as a combination of nitrate and ammonia (7:1). (Hereafter these treatments will be referred to as NH_4^+ -N, NO_3^- -N and $(\text{NO}_3^- + \text{NH}_4^+)$ -N). The nitrogen content of the solutions was 14 ppm N for the first 7 weeks and 28 ppm thereafter. Exact details of the culture technique are described elsewhere (Part 2-C).

The seedlings were grown in a growth room with 90% RH in the day and 65% at night. Fluorescent and incandescent lamps provided 20,000 lux over a photoperiod of 16 hr (6 am to 10 pm). Dawn and twilight effects were produced by staggering the order in which the lights came on and went off. A 12 hr temperature cycle was used (8 am to 8 pm at 24C; 8 pm to 8 am at 20C).

Douglas-fir and Western redcedar seedlings were harvested 10 months after germination while Lodgepole pine and Western hemlock were harvested 12 months after germination.

Leaf Ψ and Ψ_s were measured with a three wire thermocouple psychrometer (2). The techniques used are described by Ehlig (3). Ψ_p was determined from equation (II). Leaf samples were taken at 9 am from the growing point of the trees. Each tree was sampled 3 times. Transpiration was determined by covering the pot with a sheet of Parafilm and measuring the loss of water over a 24 hr period. After determination of transpiration, the trees were harvested and oven dried at 80C.

RESULTS AND DISCUSSION

Leaf Ψ , Ψ_s , Ψ_p

Sensitivity of the thermocouples ranged from .23 to .29 μ v per bar. For routine determinations with the psychrometer the standard error of observation of standard KCl solutions was about $\pm .5$ bar.

There was no difference in leaf Ψ , Ψ_s and Ψ_p among the different nitrogen treatments. Therefore one value of leaf Ψ , Ψ_s and Ψ_p was determined for each species (Table 3). The mean values of leaf Ψ for Douglas-fir, Western redcedar, Lodgepole pine and Western hemlock were -21.2, -11.8, -10.6 and -18.8 bars with standard errors of .8, .5, .3 and .7 bars. The standard error of

TABLE 3. Leaf Ψ , Ψ_s and Ψ_p of conifer seedlings supplied with different sources of nitrogen (bars).

Species	Ψ	Ψ_s	Ψ_p
Douglas-fir	-21.2	-34.0	+12.8
Western redcedar	-11.8	-21.4	+9.7
Lodgepole pine	-10.6	-24.5	+13.9
Western hemlock	-18.8	-29.4	+10.6
Nutrient Solution			
NH_4^+	-.77		
$\text{NO}_3^- + \text{NH}_4^+$	-.65		
NO_3^-	-.43		

leaf Ψ_s for Douglas-fir, Western redcedar, Lodgepole pine and Western hemlock were 1.1, .6, .4 and .6 respectively. Table 3 contains Ψ for the respective nutrient solutions. The values of Ψ reported in Table 3 are similar to values reported for other woody species (4, 5, 6). Values of Ψ_s of from -16 to -31 bars have been reported for ever-green conifers (8). Values of Ψ_s reported here fall close to this range. To our knowledge no value of Ψ_p for trees has been reported.

The similar leaf Ψ among the nitrogen treatments within species indicates that there was no difference in water deficit between the nitrogen treatments.

Water Uptake

The amounts of water lost from the pots at the time of leaf sampling for the leaf potential studies are given in Table 4.

With Douglas-fir and Western redcedar, the NH_4^+ -N treatment lost 1/3 to 1/4 the amount of water of the NO_3^- -N treatment and 1/2 to 1/3 the amount of water of the $(\text{NO}_3^- + \text{NH}_4^+)$ -N treatment (Table 4).

Lodgepole pine seedlings supplied with NO_3^- -N lost approximately 1/2 the amount of water of the other two treatments.

TABLE 4. Water loss of conifer seedlings supplied with different sources of nitrogen
(ml H₂O/24 hr.pot).

Species	Nitrogen Treatment		
	NO_3^-	$\text{NO}_3^- + \text{NH}_4^+ (7:1)$	NH_4^+
Douglas-fir	683.7	403.2	180.3
Western redcedar	627.2	554.2	203.2
Lodgepole pine	222.2	486.3	395.1
Western hemlock	482.4	553.6	324.7

Western hemlock seedlings treated with $(\text{NO}_3^- + \text{NH}_4^+)-\text{N}$ lost the most water followed by the $\text{NO}_3^- - \text{N}$ and $\text{NH}_4^+ - \text{N}$ treatments.

Since transpiration is considered an unavoidable evil (7) which often results in water deficits and because there was no difference in water deficit (Table 3) among the nitrogen treatments, we suggest that differences in growth among nitrogen treatments within species cannot be attributed directly to differences in water relations. However similar leaf Ψ may have been maintained by controlling T via r_s .

As previously stated stomatal resistance to flow of CO_2 is determined from T. Thus the water relations of these plants may have had an indirect effect on yield i.e. by limiting the rate of entry of CO_2 . Thus $r_s + r_a$ was calculated according to equation III. In this experiment r_a is considered to be constant within species and because of the small size of leaves and air circulation in the growth room, r_a is considered to be small. Thus calculation of $r_s + r_a$ gives an approximation of r_s and all variability within species between $r_s + r_a$ is attributable to r_s . A comparison of $r_s + r_a$ (Table 5) with dry weight (Table 1) shows that for Western redcedar, Douglas-fir and Lodgepole pine, there was little difference in $r_s + r_a$ among nitrogen treatments but a large difference in yield. With Western

TABLE 5. Transpiration resistance ($r_s + r_a$) of conifer seedlings supplied with different sources of nitrogen (sec.cm^{-1}).

Species	Nitrogen Treatment		
	NO_3^-	$\text{NO}_3^- + \text{NH}_4^+ (7:1)$	NH_4^+
Douglas-fir	3.3	4.0	3.3
Western redcedar	3.4	3.9	3.2
Lodgepole pine	3.9	5.0	6.5
Western hemlock	7.2	15.5	14.4

hemlock, there was a direct relationship between $r_s + r_a$ and yield i.e. the higher the value of $r_s + r_a$, the higher the yield. An inverse relationship would be needed to support causality. These results indicate that in no case is $r_s + r_a$ restricting dry matter production. These results suggest that the effect of nitrogen treatment on r_m had an effect on dry matter production. It has been reported for Pinus halpensis that light and temperature affected CO_2 uptake through r_m (9). Similarly, Wuenscher and Kozlowski (10) found that for three woody species at 3000 ft-c the differences in r_m were more important than differences in r_s in determining total CO_2 transfer resistance.

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APPENDIX I

The relationship between leaf area and leaf dry weight of three conifer species grown on three sources of nitrogen.¹

Determination of leaf area is a fundamental measurement for physiological experiments. Several methods of estimating conifer leaf area are available (1, 2, 4, 5, 6, 7), but the small size and three-dimensional shape of the leaves make this difficult. For experiments using a large number of trees, the simplest method is to correlate leaf area with leaf dry weight (4). For mature Ponderosa pine, Cable (4) found a linear relationship between fascicle dry weight and surface area of fascicles. Once this initial relationship has been established only the determination of fascicle dry weight is required to determine fascicle surface area. On the other hand, Brix (3) has shown that a linear relationship did not occur for Douglas-fir seedlings between ages 65 and 100 days grown at varying light intensities and varying temperatures. In our study, the leaf surface area of each seedling was measured to determine the relationship between leaf surface area and leaf dry weight of 18-week-old seedlings. An additional study was made on the effect of different sources of nitrogen on the relationship between leaf surface area and leaf dry weight of entire

¹ This article by G.E. Mellor and E.B. Tregunna will appear in Can. J. For. Res. in Press.

seedlings.

Three conifer species, Douglas-fir (Pseudotsuga menziesii var. menziesii), Lodgepole pine (Pinus contorta var. contorta) and Western hemlock (Tsuga heterophylla) were grown for 18 weeks in sand in a growth chamber under conditions described previously (8). The seedlings were given nutrient solution in which only the source of nitrogen and the concentration of chloride were varied. Three different sources of nitrogen (nitrate only, ammonia only, and a combination of nitrate and ammonia (7:1) were used. All treatments contained 14 ppm N. At 18 weeks after germination, five seedlings of each species from each nitrogen source were selected at random and the leaves were removed. The length and width of the leaves were measured by means of a micrometer. The total surface area of the leaves was determined by multiplying length times width times a correction factor. The correction factor accounts for longitudinal and crosssectional curvature. The correction factors for Douglas-fir and Western hemlock were taken from Barker (2) and the correction factor for Lodgepole pine was taken from Smith (9). Leaf dry weight of each seedling was determined by drying the leaves in an oven at 80 C to constant weight. The relationship between leaf dry weight and leaf surface area was determined by calculating the correlation coefficient (r). The

leaf surface area to leaf dry weight relationship among nitrogen treatments within species was analyzed by testing for homogeneity of r values.

With all three conifer species, no difference was found between r values determined for the three nitrogen sources. Thus a pooled r value was determined for each species. The pooled r values were: Lodgepole pine (.949), Western hemlock (.967) and Douglas-fir (.927). These values are significant at the 5% level and indicate a linear relationship between leaf dry weight and leaf area of entire seedlings. After the linear relationship was established a regression coefficient was determined and the regression line drawn using the formula $y = bx$ (Figs. 1, 2, 3).

This linear relationship between total leaf area and leaf dry weight provides an easy and quick method for determination of leaf area, once the initial relationship is found. A linear relationship indicates that in experiments comparing photosynthetic or transpiration rates of seedlings grown on different sources of nitrogen, the differences in results between nitrogen treatments will be of the same magnitude whether units of leaf dry weight or leaf area are used.

Figure 1. The relationship between leaf area and leaf dry weight for Douglas-fir seedlings supplied with different sources of nitrogen.

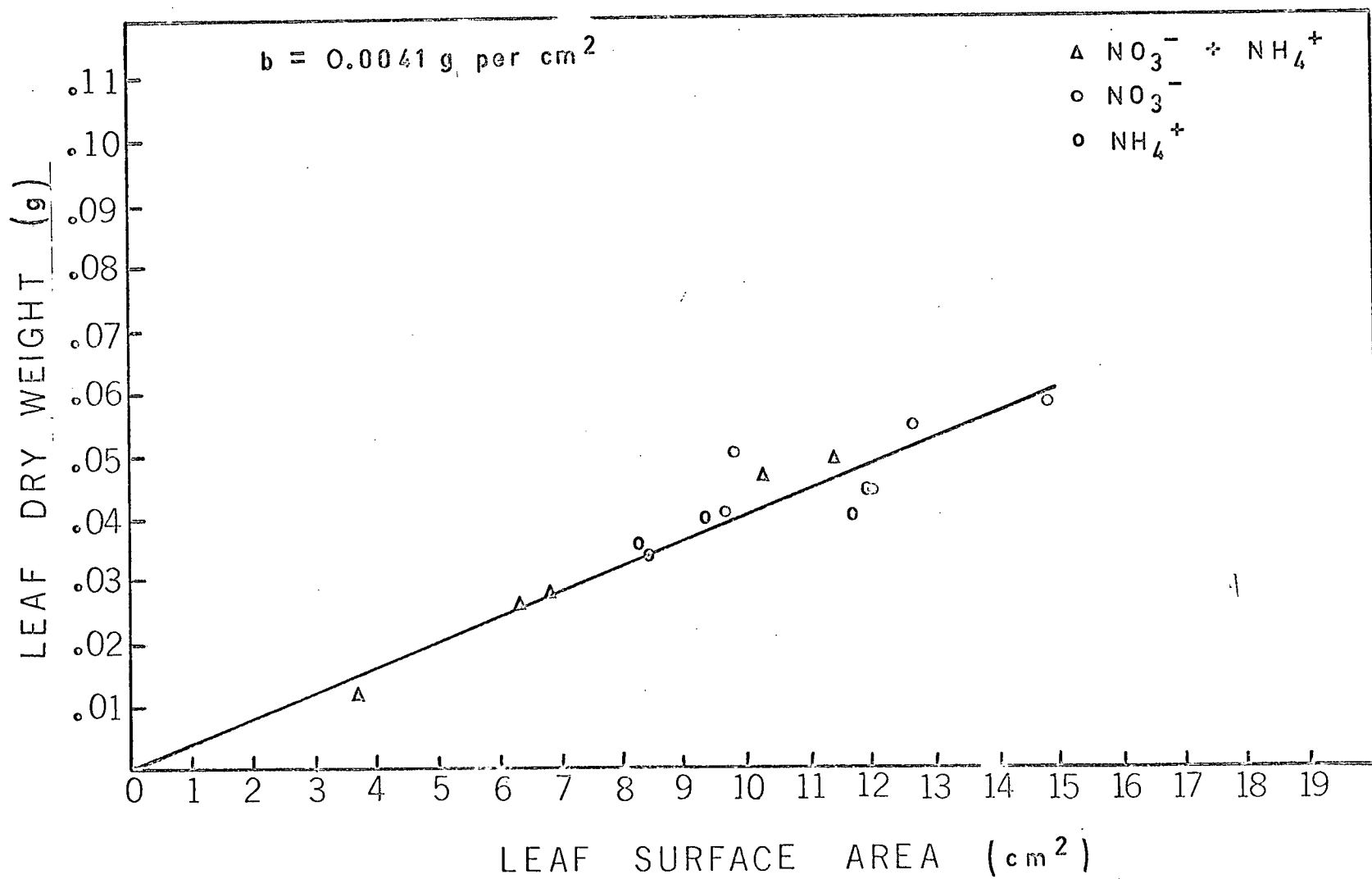


Figure 2. The relationship between leaf area and leaf dry weight for Lodgepole pine seedlings supplied with different sources of nitrogen.

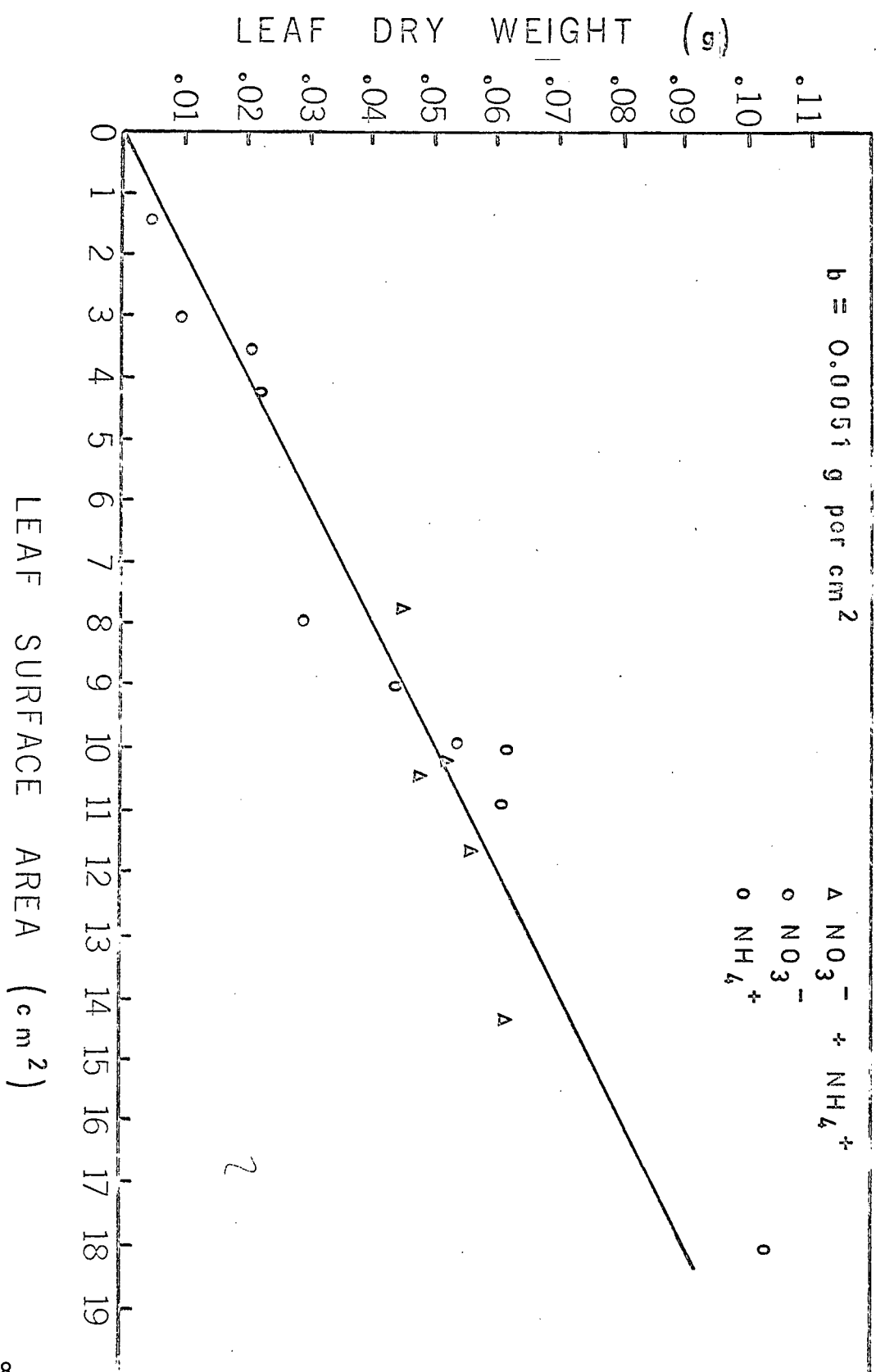
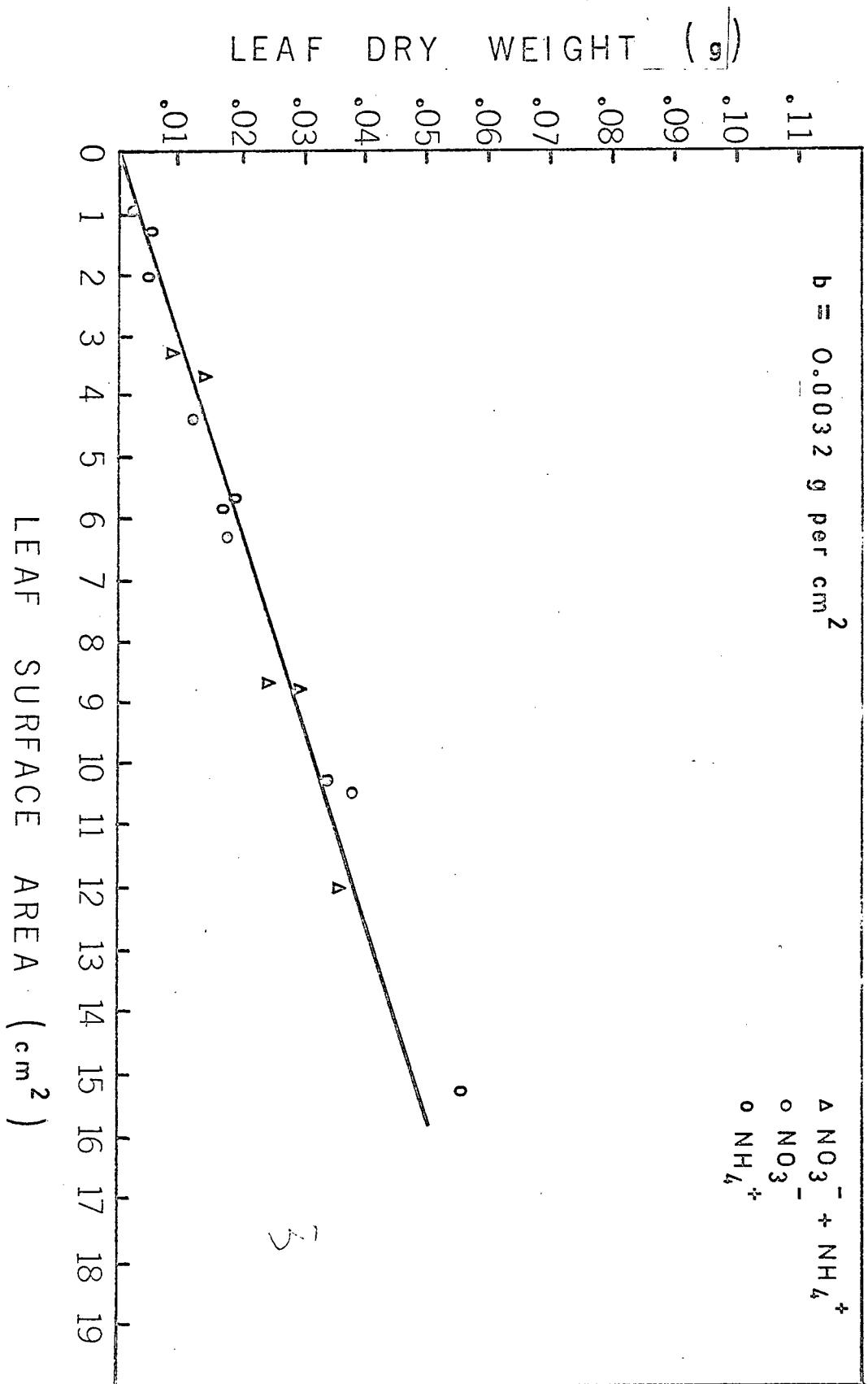


Figure 3. The relationship between leaf area and leaf dry weight for Western hemlock seedlings supplied with different sources of nitrogen.



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EPILOGUE

In Part 2, I studied the effects of NH_4^+ -N and NO_3^- -N on four species of conifers. I have concluded from these studies that Western hemlock and Lodgepole pine are more tolerant of NH_4^+ -N than Douglas-fir and Western redcedar. The exact level of NH_4^+ -N that these species will tolerate should be determined. This experiment should be carried out with a well-stirred liquid culture in which the level of nitrogen is monitored daily. In this manner the level of nitrogen in contact with the root surface would not vary to any great degree.

A study of the nitrate-reducing enzymes in these forest species should also be carried out. Such a study would indicate the ability of these species to metabolize NO_3^- -N.

A brief attempt was made to extract nitrate reductase. However it was not possible to extract protein precipitable by trichloroacetic acid from Douglas-fir, let alone nitrate reductase. One ml of a Douglas-fir extract was added to a very active nitrate reductase preparation from corn. The nitrate reductase from corn was completely inhibited by addition of the Douglas-fir extract.

In addition, a study of the amino acid pools in these species might give further insight into the ability of these species to tolerate various levels of ammonia.

PART 3. A STUDY OF STORAGE CONDITIONS FOR SPRING-LIFTED
MUD-PACKED DOUGLAS-FIR SEEDLINGS.¹

INTRODUCTION

In British Columbia, reforestation by planting makes use of 1-0 and 2-0 seedlings. The seedlings are grown in nurseries, lifted between November and April, stored at 2C in the dark and then planted in the field.

A current method of planting employs mud-packed seedlings. Mud-packing is done by surrounding the root system of the seedlings with a mixture of peat moss, clay and water formed into a cylinder. The mud is then dried in a stream of warm air until it is semi-rigid. In this condition the root system is thought to be protected from dessication and physical damage. The seedlings are planted by punching a hole in the ground with a dibble or steel rod to the same depth as the length of the mud-capsule. The rod is withdrawn and the mud-capsule is placed in the hole, leaving the stem and the needles of the seedling above the ground. Soil is tramped down tightly against the mud-pack.

A critical period in the establishment of seedlings is the time between lifting and subsequent regeneration of a

¹ This section which is part of an article by G.E. Mellor, R.A. Keller, and E.B. Tregunna will appear in Canadian Journal of Forest Research. E.B. Tregunna supervised this study.

new root system. Water stress due to root, shoot, or whole plant dessication may occur in the preparation and storage of seedlings for planting. Water stress may also occur after planting when, because of root injury or disruption of the soil-plant-air continuum, transpiration exceeds water uptake (1).

In this study, the effect of various storage conditions on survival and root regeneration of spring-lifted mud-packed Douglas-fir seedlings is examined. Winjum (4) has shown that Douglas-fir seedlings lifted after April 1st are damaged by four weeks of storage at 2C. He found that the field survival of stored seedlings was reduced by 30 to 90% when compared with the survival of unstored seedlings. During the last two years, seedlings lifted after April 1st have been used for mud-packing. In many instances these mud-packed seedlings have been placed in storage for a period of two to three weeks at 2C. The object of this study was to determine the best method of storing spring-lifted mud-packed Douglas-fir seedlings. As mentioned previously, a critical period in the establishment of seedlings is the time between lifting and regeneration of a new root system. Thus various storage conditions were chosen to determine if root regeneration could be enhanced.

MATERIALS AND METHODS

During the week of April 19th, 1970, 2-0 Douglas-fir trees grown at Green Timbers Forest Nursery from seed lot (92G14/B2/1020/201) were lifted and mud-packed at Pelton Reforestation Company. Immediately after mud-packing, 220 seedlings were divided into 11 storage treatments (2 replications x 10 trees) (Table 1). Before placing the trees in their respective storage treatments, the mud-packed portion of the seedling was soaked for 10 min in water or 1/2-strength Hoagland's solution. After soaking, the mud-packs were dried to their original semi-rigid condition. The seedlings stored in the dark were placed in plastic bags, as is done by the Pelton Reforestation Company. The seedlings stored in the light (25C and field) were placed in plastic bags which were then transferred to a rigid container in order to keep the trees in an upright position. The portion of each seedling above the mud-pack was completely exposed. For the vermiculite treatments, wet vermiculite was added to the bags. The trees stored in the field were placed in a shelter which had only the top covered with polyethylene plastic. The plastic was slightly opaque and provided shelter from both direct sun and rain.

The mud-packs kept in cartons in the dark retained their moisture during the storage period while the exposed mud-packs lost moisture. The exposed mud-packs at 25C had

TABLE 1. Storage treatments and conditions.

2C Room (dark)	10C Room (dark)	Field ^{a,b}	25C Room ^c
- fertilizer	- fertilizer	- vermiculite - fertilizer	- vermiculite - fertilizer + light
+ fertilizer	+ fertilizer	+ vermiculite - fertilizer	+ vermiculite - fertilizer + light
		+ vermiculite + fertilizer	+ vermiculite + fertilizer + light
			- vermiculite - fertilizer - light

^aField refers to the roof of the Bio-Sciences Building, University of British Columbia.

^bField conditions during storage

Ave. Maximum daily Temperature 12.6C Ave. Minimum daily Temperature 5.6C Ave. RH 75%

Range 9.5C-18.0C

Range 2.5C-9.0C

^c25C Room conditions during storage

1000 ft-candles, 16 hour day Ave. RH 65%

to be moistened daily and the mud-packs in the field had to be moistened daily or every other day depending on the weather conditions. The mud-packs were moistened with a fine spray of water. Holes for drainage were cut in the bottom of the bags. In spite of the fact that as little water as possible was used for moistening, water drainage from the bags was poor. This was particularly true of the vermiculite treatments.

During storage (8th, 11th, 14th and 18th days) root growth and survival were measured. A seedling was considered to be dead when its needles were brown and dropping and the stem was dry, brittle and easily broken. Root growth was evaluated by measuring the amount of root protruding from the mud-pack.

After 19 days storage, the seedlings were planted in the UBC Botanical Garden at 10 inch intervals in rows 2 ft apart. Each row consisted of one replicate. In order to minimize the effect of moisture stress, the soil was moistened periodically to prevent the mud-packs from drying out. After 44 days, the seedlings were dug up and were evaluated on the basis of survival and root growth.

In evaluating the resulting data, analyses of variance were used followed by Tukey's w test ($\alpha = .05$).

RESULTS AND DISCUSSION

Fertilizer and vermiculite treatment of the mud-packs

had no effect on any of the parameters measured. Therefore the individual treatments within each storage location were combined except in the 25C treatments. With the 25C treatments the (+) light treatments are reported separately from the (-) light treatments.

After 19 days in storage there were no roots visible on the outside of the mud-packs from any of the storage locations. Survival of the trees at the end of the storage treatment is shown in Table 2. Storage of the trees at 25C (+) light produced significantly lower storage survival than the other treatments. All other storage treatments were statistically indistinguishable.

The maximum daily temperature throughout the planted period ranged from 13.5C to 30.0C with an average of 19C. The minimum daily temperature ranged from 4.0C to 20C with an average of 9.0C.

Field survival (Table 2) was computed on the basis of the number of seedlings planted. The trees stored in the field had by far the highest field survival. All other storage treatments had a field survival of less than 53%. The low survival of spring-lifted seedlings at 2C, agrees with the findings of Winjum (4). In addition to studying field and storage survival individually the combined survival (total survival) should also be considered (Table 2). The field stored trees had the highest total survival.

TABLE 2. Survival and root growth of mud-packed Douglas-fir seedlings stored in various locations.

Storage location	Storage survival ¹ %	Field survival %	Total survival	Root growth ² cm
Field	96.6 a	75.5 a	75.0 a	36.2 a
2C room	100.0 a	50.0 b	50.0 b	22.2 b
10C room	100.0 a	47.5 b	47.5 b	25.0 b
25C room (+) light	63.3 b	53.2 b	41.6 b	26.8 b
25C room (-) light	100.0 a	10.0 c	10.0 c	0.8 c

¹Means marked by the same letter are not significantly different.

²Root growth is expressed as cm of root protruding from the mud-pack per surviving seedling.

All other treatments had a total survival of 55% or less.

The field stored trees had the greatest amount of root growth (Table 2). This length of root was made up of numerous roots and represents an extensive capacity for obtaining moisture and nutrients. These plants had a considerable area of contact with the soil beyond the surface of the mud-pack. Trees stored at 2C, 10C, and 25C (+) light produced intermediate amounts of root growth while the trees stored at 25 (-) light produced the lowest amount of root growth. There was a direct relationship between root growth and field survival. Seedlings which did not survive the planting period produced no root growth. This observation agrees with the findings of Stone (2). He noted that at 60 days after planting, bare-root Douglas-fir seedlings which had died, had produced no new roots.

Since an effort was made to reduce the effect of moisture stress in this study, survival as noted should not be extrapolated in an effort to predict actual field behaviour. On the other hand, Stone et al. (3) noted that the root regenerating potential is a measure of the physiological condition of the seedling and under many field conditions, one might reasonably expect it to be reflected in field survival.

In summary, it would seem from the parameters

measured in this study, that spring-lifted, mud-packed Douglas-fir seedlings should be stored in the field. Storage at 2C, 10C and 25C (+) light produced intermediate results. Storage at 25C (-) light was the least satisfactory.

ADDENDUM

On January 12th after publication of this paper in Can. J. For. Res., a letter was received from M. Crown, Forester with the Pacific Logging Company Limited. Mr. Crown supplied some unpublished survival data concerning spring-lifted mud-packs which had been stored at 2C. In addition, he added "We read with interest your paper on the moisture status of mud-packs. The results would appear to give the reason why operational experience with spring-lifted mud-packs has generally been less satisfactory than winter-lifted mud-packs."

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