NITROGEN STATUS OF PINUS RADIATA SEEDLINGS AFTER UNDERCUTTING:

CHANGES IN TOTAL, SOLUBLE, AND INSOLUBLE NITROGEN

A. COKER

Forest Research Institute, New Zealand Forest Service,
Private Bag, Rotorua, New Zealand

(Received for publication 31 January 1984; revision 27 November 1984)

ABSTRACT

Changes in total, soluble, and insoluble nitrogen and chlorophyll concentrations in **Pinus radiata** D. Don seedlings were monitored for 56 days after a single undercutting (root pruning). After undercutting there was a marked reduction in total soluble and insoluble shoot nitrogen concentration. Some of this mobilised nitrogen contributed towards lateral root growth while 15% was lost, possibly from the damaged root as exudate. Twenty-one days after undercutting, total seedling nitrogen content began to improve, coinciding with lateral root dry matter gains. By 56 days, undercut seedling nitrogen content approached that at Day 0, while control seedlings had acquired an additional 20.6 mg nitrogen.

Lateral root dry weights and nitrogen content of undercut seedlings were higher than those of control plants after 56 days. However, soluble nitrogen concentration in lateral roots of undercut seedlings was not significantly different from the controls, although total and insoluble nitrogen concentrations were significantly below control plant values.

There was no significant difference in chlorophyll concentration of needles between control and undercut seedlings.

INTRODUCTION

Successful survival of *Pinus radiata* seedlings after planting out depends on their ability to maintain favourable water relations, and to re-establish root and shoot growth (van Dorsser & Rook 1972). Conditioning of seedlings through undercutting and wrenching in the nursery is one means of improving survival after planting out (Cameron & Rook 1969; Chavasse 1980). The net morphological effect is a reduction in shoot growth, accompanied by rapid growth of fibrous roots, resulting in a higher root/shoot ratio than in unconditioned seedlings (Rook 1971; van Dorsser & Rook 1972).

According to Stupendick & Shepherd (1980) the sequence of physiological events after undercutting of *P. radiata* seedlings was as follows. After tap root severance, internal plant water deficits resulted in stomatal closure with reduced transpiration,

photosynthesis, and translocation of assimilates to the root system. Eight to 12 days later, leaf water potentials recovered and photosynthesis showed signs of recovery. By Day 32 photosynthesis was restored to 60% of the initial rate.

After wrenching, metabolic activity in the root increases, with the root acting as a preferential sink for assimilates. Rook (1971) indicated that within a month of undercutting over 30% of the current ^{14}C photosynthate was translocated to the roots, compared with less than 10% in controls.

One of the documented effects of wrenching is yellowing of needles because of a substantial reduction in chlorophyll (van Dorsser & Rook 1972). This chlorosis is believed to arise from the translocation of nitrogen to developing roots thereby reducing the proportion of total nitrogen in foliage. However, Rook (1969) was unable to find any reduction in total nitrogen levels of roots, stem, or foliage. In contrast, M. Colley (unpubl. data) and Court (1974) noted that after wrenching the percentage of total nitrogen increased in roots and decreased in foliage and stem. The role of nitrogen and mineral elements in the conditioning of pine seedlings is poorly understood (van Dorsser & Rook 1972) although guidelines indicating acceptable levels for raising healthy seedlings have been presented (Knight 1978).

Wrenching effects may also be explained by changes in hormonal levels caused by removal of hormone synthetic sites and the creation of new sources and hormonal sinks (Botlger 1978; Bacon 1978). Disruption and redistribution of nitrogen levels as well as cytokinin, gibberellin, and auxin synthesis are all interrelated in such a situation (Wareing 1980).

The study reported in this paper was confined to nutritional factors describing changes in nitrogen and chlorophyll levels between undercut and control seedlings. The nitrogen status (total, insoluble, and soluble nitrogen) of bud, foliage, stem, branches, tap root, and lateral root tissues was followed over a 2-month period after seedlings were undercut. Nitrogen content and concentration changes are presented in relation to dry matter changes in these tissues.

METHOD

Seedling Material and Treatment

Pinus radiata seedlings (spaced at 100 × 100 mm) were raised in the FRI nursery in a randomised block layout. Application of fertilisers ceased 1 month prior to undercutting.

Five-month-old seedlings, approximately 170 mm in height, were undercut at a nominal 80 mm in early March (i.e., late summer) using a reciprocating undercutter as described by van Dorsser & Rook (1972). Seedlings were harvested at 0 (immediately before undercutting), 1, 3, 5, 8, 10, 21, and 56 days after undercutting.

Sampling of Seedling Material

Ten "representative" seedlings were taken from each of two blocks for analysis (10 × 2 replicates) – for undercut and control treatments. Seedlings were selected with tap root length in the range 70–90 mm after undercutting since this was the average length found at Day 1. Similarly, the tap roots of control seedlings were cut to this length since it was impossible to remove the root completely intact.

Harvested seedlings were washed under running water. Shoot height and root collar diameter were measured. The 10 seedlings from each replicate were bulked, and the plants from the duplicate samples were divided into foliage, apical bud, branches, tap root, lateral roots, and stem.

Tissues were frozen in liquid nitrogen, freeze dried (48 hours at 760 mm Hg), and dry weights recorded. Dried material was ground in a Wiley mill to pass through a 40-mesh sieve and stored over silica gel *in vacuo* at 4°C. Prior to analysis tissue was further dried *in vacuo* (overnight at 260 mm, 30°C) to remove any traces of moisture.

Analysis of Total, Soluble, and Insoluble Nitrogen

Duplicate samples (100 mg) of ground tissue were weighed directly into plastic syringe barrels (10 ml capacity) with tightly fitted filter-paper discs (Whatman, Grade 17) at the base to retain the sample.

Soluble nitrogen was extracted with 4 × 5 ml acetone/0.1 M sodium chloride solution (80 : 20). The solution was acidified (0.1 ml conc. H_2SO_4) and concentrated. Then 2 ml H_2SO_4 and 1 ml chilled 50% H_2O_2 were added to each sample, and the samples were digested at 350°C for 20 min. on the Tecator Block Digestion unit.

Syringe barrels containing the residue (insoluble nitrogen) were dried and the dried residue and filter paper were digested in the presence of 2 ml chilled H_2SO_4, 2.4 mg selenium powder, 0.5 g K_2SO_4, and 1 ml chilled 50 % H_2O_2 at 350°C for 20 min. Total nitrogen was determined in the same way on dried unextracted tissue.

Standard solutions of $(NH_4)_2SO_4$ (0–80 mg/l for total and insoluble nitrogen; 0–10 mg/l for soluble nitrogen) were digested in the same way together with acetone/salt solution or filter paper as appropriate.

Ammonium nitrogen liberated was determined colorimetrically using the phenol hypochlorite reagent in a Technicon Auto Analyser unit (Hambraeus *et al.* 1976).

Chlorophyll (a + b) Foliar Determinations

Chlorophyll extraction was as outlined by Linder (1974) except that 1 g fresh needles was extracted with 80% acetone and 100 mg $MgCO_3$. Tissue was macerated and filtered, and the samples were made up to 100 ml. Fresh weight and oven-dry weight conversion factors were calculated and chlorophyll was quantified as outlined by Arnon (1949).

RESULTS

Selection of Seedling Numbers per Sample

Initially 15 randomly selected seedlings were collected from those growing in the nursery and 15 individual estimates were made of total nitrogen in the tap root and lateral root, current and 1-year-old stem, and current and 1-year-old foliage. Less variation was found within root and foliage samples – stem samples varied most. A minimum of five to 10 seedlings were required for these tissue categories before there was less than 10% error around the mean due to seedling variability. Consequently 10 seedlings were harvested for each replicated sample.

Visual Changes

In the experiment reported, the classical symptoms of wilting (due to water stress) and yellowing of foliage (due to nitrogen deficiency) were not observed. There were no significant differences in foliar chlorophyll between undercut and control seedlings throughout the 56-day period. Chlorophyll (a + b) content remained constant at 5.00 ± 0.21 mg chlorophyll/g dried tissue.

Although it has been recorded (Menzies 1980) that wilting does not occur after undercutting at a depth greater than 8 cm, other factors probably contributed to the absence of expected symptoms. Wilting due to drought stress was observed in all seedlings for about a week prior to undercutting and this probably reduced the severity of the undercutting effect.

Seedling Growth Characteristics

Fifty-six days after treatment, undercut seedlings had a mean height of 223 ± 6 mm whereas controls measured 277 ± 7 mm. Root collar diameter was 4.4 ± 0.1 mm compared with control seedlings which were 4.7 ± 0.01 mm. Undercutting had a small effect on sturdiness and root/shoot ratio, indicating a slight improvement in seedling quality (Table 1).

TABLE 1—Seedling sturdiness and root/shoot ratio of undercut and control seedlings at 56 days (mean ± s.e.)

Seedling quality	Undercut	Control
Sturdiness	51.00 ± 0.67	59.00 ± 0.69
Root/Shoot	0.163 ± 0.012	0.115 ± 0.019

Undercutting resulted in the loss of at least 22% of the root system (dry weight basis as estimated from the portion removed from control tap roots). By Day 21 and thereafter only lateral root dry weight showed substantial gains over control seedling values (Fig. 1). After 56 days all of the tissues in undercut seedlings, except for lateral roots, had a lower dry matter content than control seedlings. More lateral or fibrous root growth than tap root growth occurred in undercut seedlings compared with tap and lateral root growth in control seedlings. Similar patterns of growth after undercutting, e.g., reduction in height and dry matter changes, did follow previously reported trends (Rook 1971; Benson & Shepherd 1977) although stem thickening as indicated by root collar diameter was not statistically significant in the experiment reported here.

During its first year of growth *P. radiata* in New Zealand does not show a true dormant period (van Dorsser & Rook 1972). Consequently the purpose of drought conditioning and undercutting is to check shoot growth and to induce fibrous lateral root growth.

Total Seedling Nitrogen Content

Although photosynthate transport and utilisation have been studied after undercutting (Rook 1971; Stupendick & Shepherd 1980), the nitrogen balance throughout the seedling tissues has not been established unambiguously. Information to date

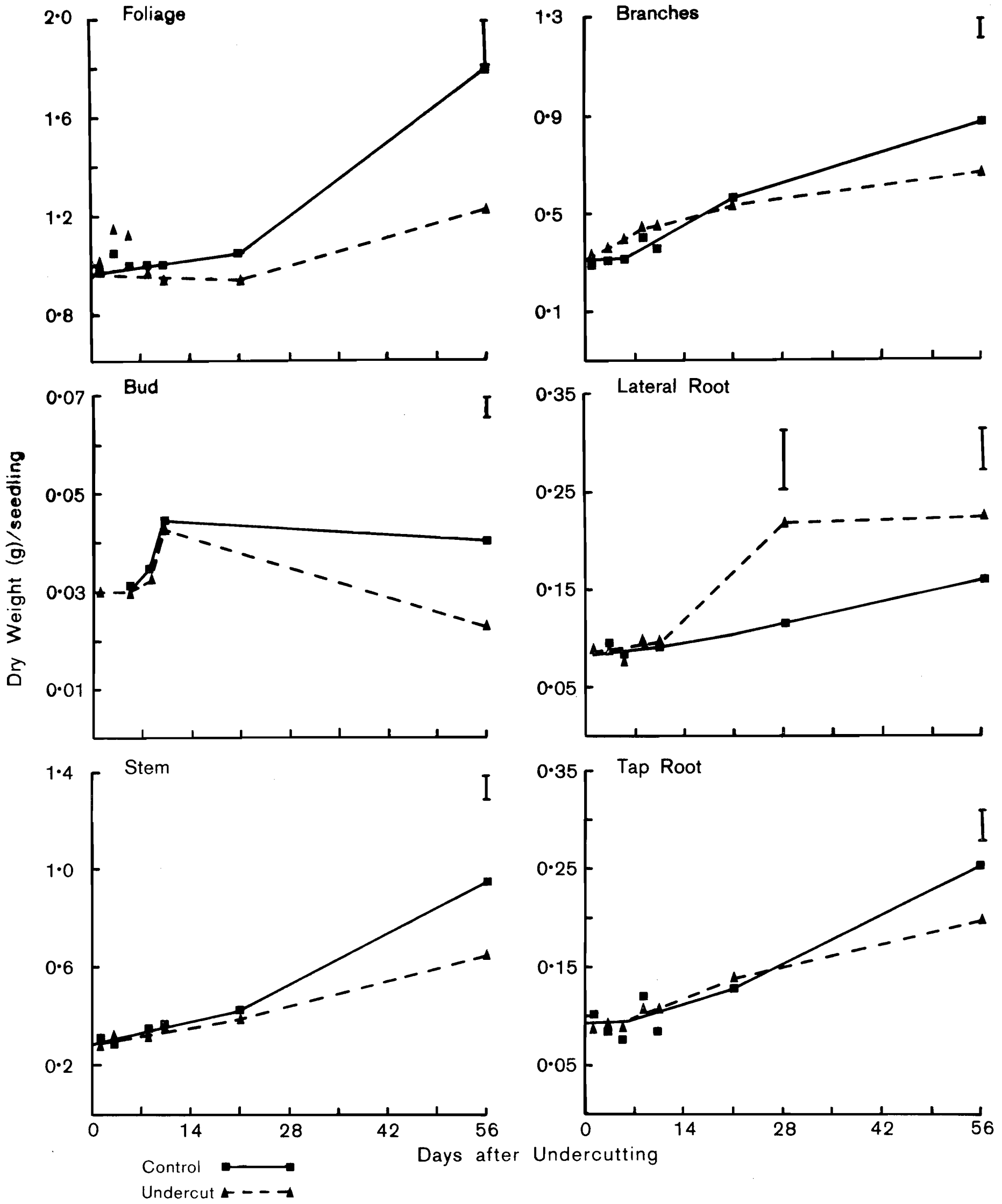

FIG. 1—Dry weight variation in foliage, branches, lateral root, tap root, stem, and bud tissues. Tap root dry weight is the 70–90 mm position, equivalent to undercut tap root.
I – indicates LSD between C and U at p = 0.05.

(Court 1974; Benson & Shepherd 1977) indicates that foliar nitrogen levels drop, and that this nitrogen is translocated and used for root regrowth.

In the current experiment there was an immediate loss of 2.5 mg N/seedling after undercutting followed by a steady loss of a further 3 mg over a 21-day period before nitrogen content started to increase (Fig. 2). After 56 days, undercut seedlings still

had 20.6 mg less nitrogen than control seedlings which at this stage had increased to 55.8 mg N in control seedlings. The nitrogen content of undercut seedlings by the end of 56 days was 35.2 mg compared with 36.8 mg N at Day 0.

Since undercutting removed 22% of the root system (dry weight basis), this subsequently resulted in a loss of 15% N (nitrogen content) per seedling remainder over the following 21-day period (Fig. 2). Such a loss was most likely by exudation of solubilised nitrogen components out of the damaged root system (Bowen 1969; Rovira 1969).

The nitrogen content in undercut lateral roots was approximately 75% of that in control seedlings at Day 21 but rose to 125% relative to controls after 56 days. In all other tissues nitrogen content stayed below control values at 56 days (Table 2). These lateral root results represent a typical plant "sink" situation.

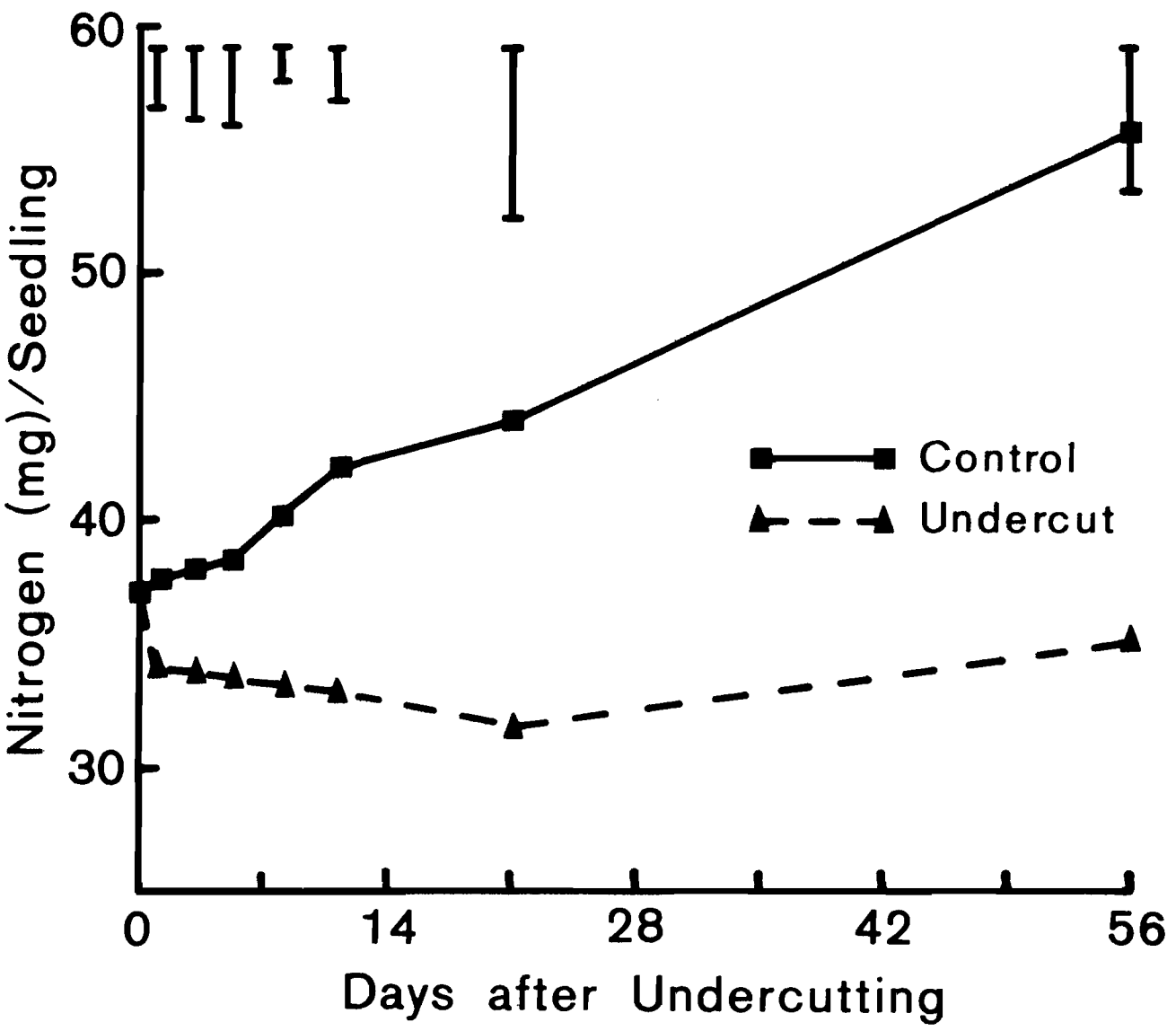

FIG. 2—Nitrogen content of control and undercut seedlings of **Pinus radiata**. After Day 0, comparisons between control and undercut seedlings are significant at $p = 0.05$.
I – indicates LSD between C and U at $p = 0.05$.

TABLE 2—Nitrogen content (mg) per seedling in seedling tissues at 56 days

	Undercut	Control
Foliage	18.67	31.56
Stem	4.54	7.62
Bud	0.43	0.96
Branches	8.34	12.43
Tap Root	0.96	1.41
Lateral Root	2.24	1.80

Treatment means across the table (within a plant part) differ significantly at $p = 0.05$.

Nitrogen Concentration

Total nitrogen concentrations within tissues changed both as a consequence of undercutting and seasonally.

After undercutting, nitrogen concentrations (mg N/g dry weight) declined rapidly in all tissues (Fig. 3). This drop continued until Days 5–8 when nitrogen concentration in lateral and tap roots rose substantially and that in other tissues levelled out. The

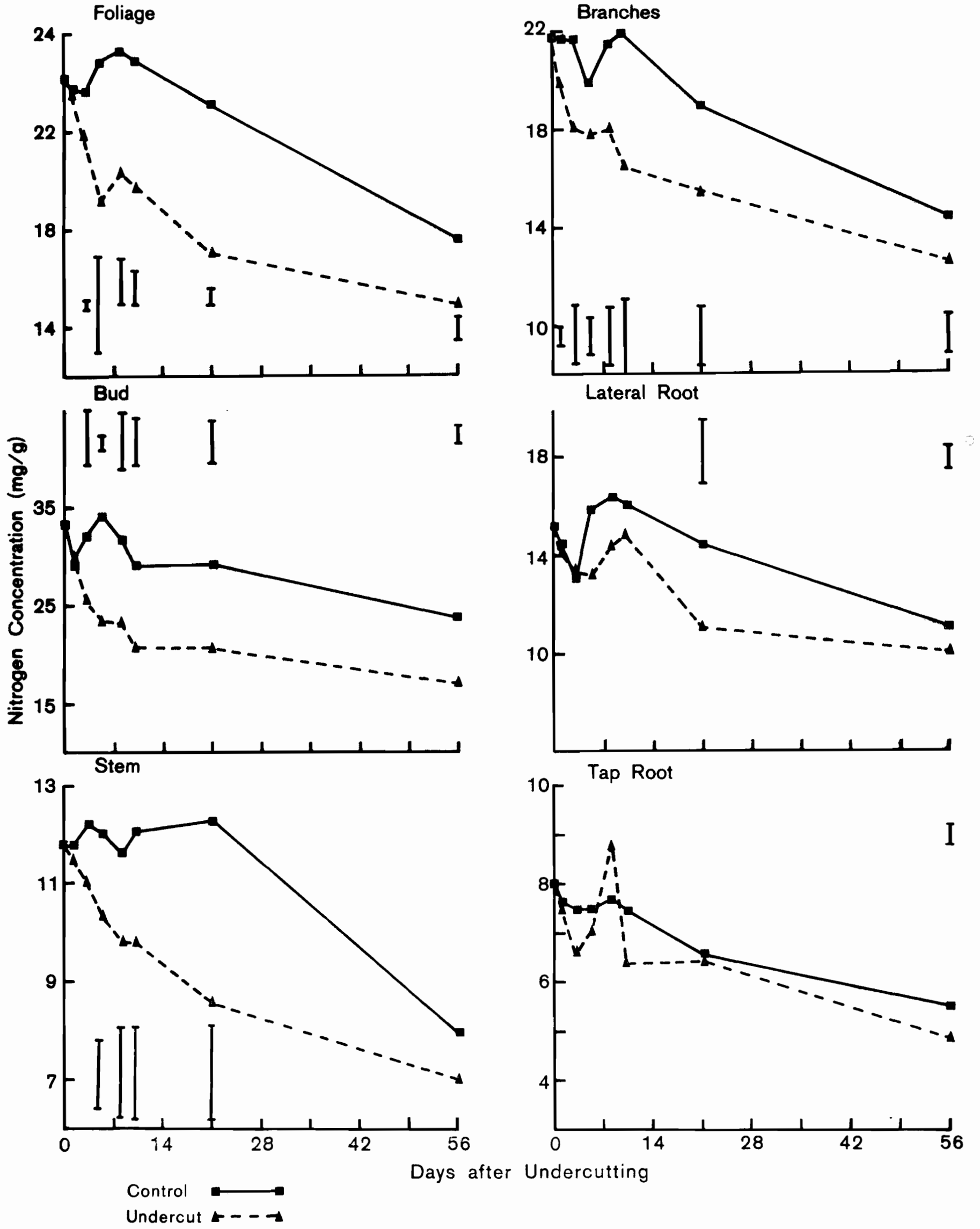

FIG. 3—Total nitrogen concentration changes for control and undercut seedlings over the 56-day sampling period.
I – indicates LSD between C and U at p = 0.05.

increased nitrogen concentration in lateral root tissues was not maintained – in contrast to the increased nitrogen content – because dry matter production in this tissue diluted the concentration. The importance of nitrogen content as opposed to comparisons based on nitrogen concentration has been noted by Zholkevich & Koretskaya (1959), Taylor (1967), and Tromp (1970).

After 21 and 10 days respectively, nitrogen concentrations in lateral and tap roots then declined but at a much slower rate. All other tissues showed a slow but steady decline for the duration of the survey period after approximately Day 10. Nitrogen concentrations in control seedlings either rose slightly or stayed relatively constant in all tissues over the initial 10 days. Thereafter controls also showed a slow but steady decline which presumably reflected a natural seasonal trend (Mead & Will 1976). Typical concentrations were foliage max. 25.29 mg N/g at Day 8, min. 15.10 mg N/g at Day 56: lateral root max. 16.46 mg N/g at Day 8, min. 11.17 mg N/g at Day 56.

Chlorosis of seedlings was not observed during the whole experiment, probably because foliar nitrogen was 2.4% initially and did not drop below 1.5% N after 56 days even though fertiliser application had ceased 1 month before undercutting.

Nitrogen concentration in undercut seedling tissues was always less than in control seedlings (except briefly in tap root tissues). In all tissues except tap root, maximum concentrations were at Day 0 and minimum concentrations at Day 56. Typical concentrations were foliage max. 24.17 mg N/g at Day 0, min. 15.10 mg N/g at Day 56; in lateral roots concentration was 14.38 mg N/g at Day 8 *versus* 15.08 mg N/g at Day 0 and 10.06 mg N/g at Day 56. In the tap root the maximum concentration was 8.86 mg N/g at Day 8 *versus* 7.05 mg N/g at Day 0 and 4.90 mg N/g at Day 56.

A rapid drop in nitrogen concentrations in all shoot tissues may occur before root growth commences. Levels of nitrogen components in foliage have also been reported to alter during senescence, moisture stress, salt stress, and generally anything which alters root health (Thimann 1980). Furthermore, moderate reductions in nitrogen favour apical dominance, induce dormancy or pseudo-dormancy, depress cytokinin levels, and can favour root growth (Bachelard 1980).

Insoluble and Soluble Nitrogen Concentrations

Insoluble nitrogen concentrations reflected the trends shown by total nitrogen concentration in all tissues of control and undercut seedlings (Fig. 4). All undercut seedling tissues except for lateral root and tap root showed an initial rapid decline followed by a slower decrease. Insoluble nitrogen concentrations in lateral and tap root also showed a rise at 10–14 days before a subsequent decrease. After 56 days insoluble nitrogen concentrations in most undercut seedlings were substantially lower than those in control seedlings. Only stem values coincided with those in control seedlings.

Soluble nitrogen concentrations were at all times only 10–15% of the total nitrogen concentration (Fig. 5). Immediately after undercutting there was a rapid drop in soluble nitrogen followed by a slower decline after Day 10. In fact after 56 days all tissues with the exception of tap root had soluble nitrogen concentrations which were not significantly different from control seedlings.

The soluble nitrogen in buds, branches, and stem tissue of undercut seedlings did not reflect control trends. However, foliage, tap root, and lateral root soluble nitrogen

in undercut seedlings maintained similar seasonal trends to control seedlings throughout the experiment. On the other hand, there was marked perturbation of seasonal trends in insoluble nitrogen levels in all tissues of undercut seedlings, with rapidity in recovery from undercutting depending on the tissue type (Fig. 4).

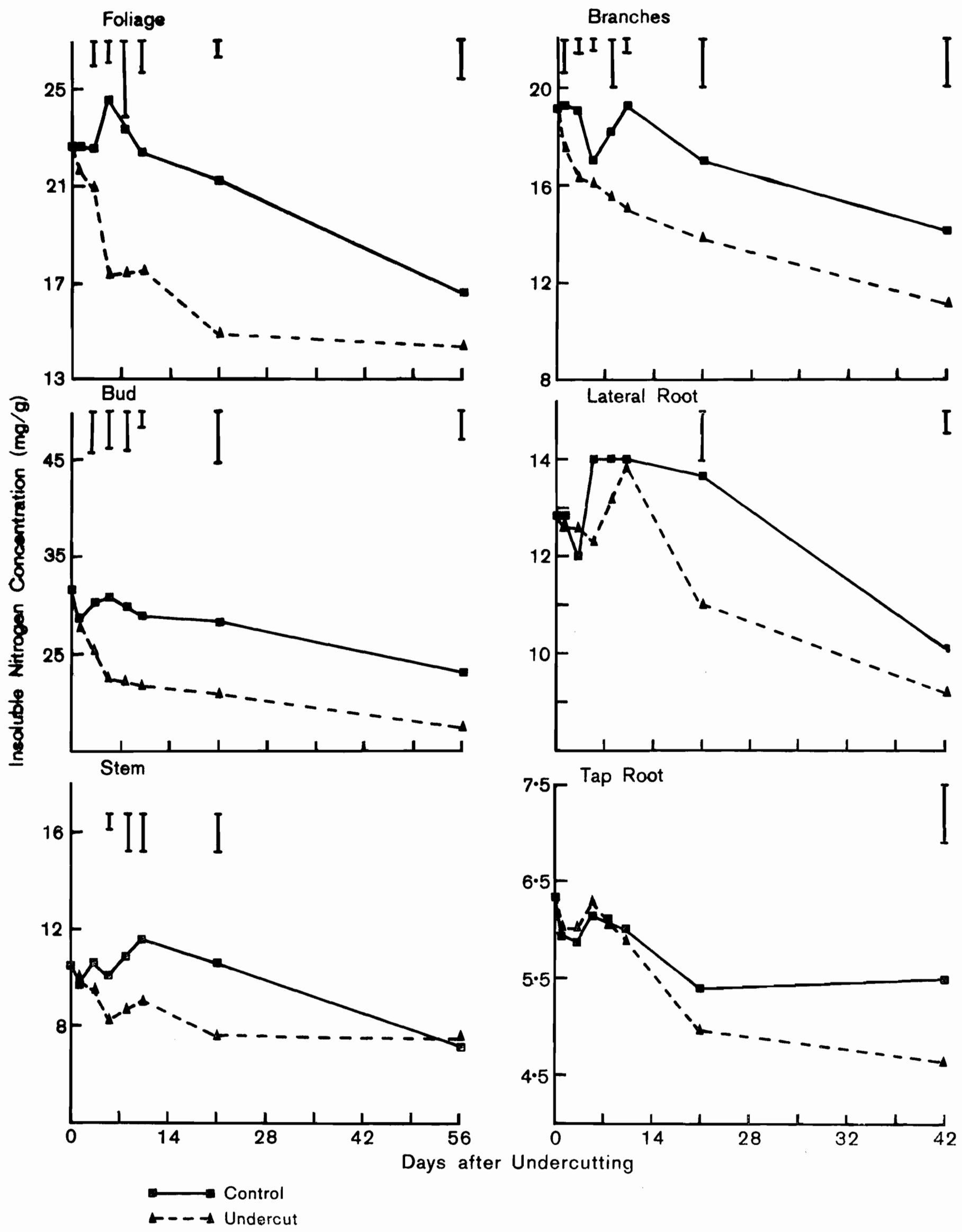

FIG. 4—Changes in insoluble nitrogen concentration of foliage, branch, bud, tap, and lateral root tissues after undercutting and in controls.
I – indicates LSD between C and U at p = 0.05.

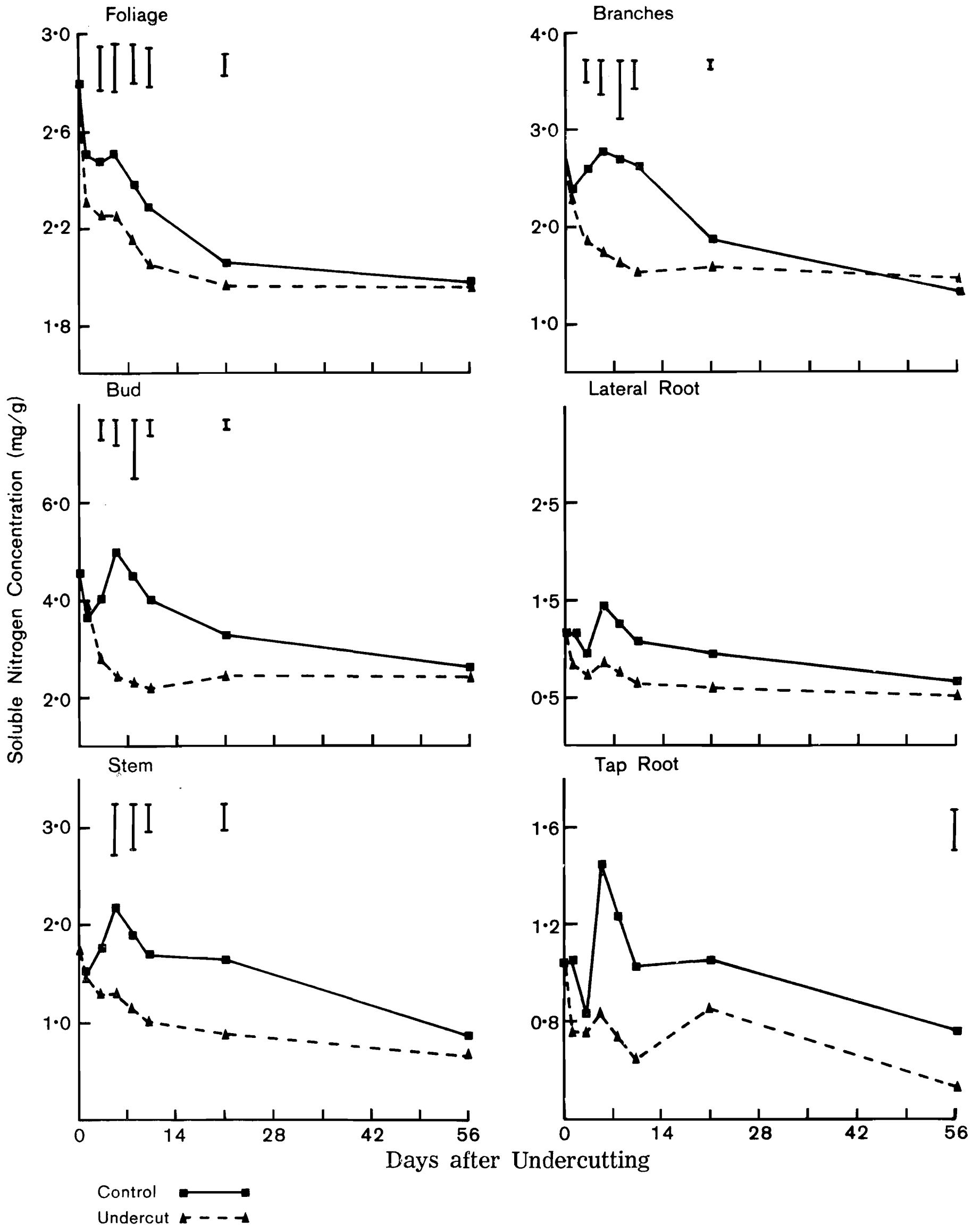

FIG. 5—Changes in soluble nitrogen concentration of foliage, branch, bud, tap, and lateral root tissues after undercutting and in controls.
I – indicates LSD between C and U at p = 0.05.

The best evidence for mobilisation of nitrogen (hydrolysis followed by subsequent translocation of the soluble nitrogen) in undercut seedlings in this study comes from insoluble nitrogen concentration results. Foliage, branch, bud, and stem concentrations dropped by 20–25% over the first 5 days in contrast to lateral and tap root values which decreased only by approximately 5% (Fig. 4). The reduction in soluble nitrogen from foliage, branch, and bud tissue resulted from translocation of soluble nitrogen

which was then either lost initially as root exudate through damaged roots, or was incorporated into insoluble nitrogen and utilised in the production of fibrous lateral roots. An increase in stem soluble nitrogen concomitant with an increase in nitrogen in lateral roots was not apparent (Fig. 5). Just after undercutting a rapid reduction of soluble nitrogen in the stem occurred and then soluble nitrogen levels between Days 3 and 5 remained constant. Reasons for not seeing a corresponding increase in this study around Days 3–5 include a slow but steady release of soluble nitrogen, particularly if nitrogen translocation is restricted to a few amino acids. The analytical method used may not be sensitive in picking up these small amounts at a time. Further resolution of nitrogen translocation requires the use of tracer studies.

From these results it would seem that undercutting does cause a redistribution of nitrogen within a seedling. Specifically, greatest changes occur in the insoluble nitrogen component which seems to be reduced in shoot tissues and increased in regenerating root tissues, substantiating earlier observations of nitrogen mobilisation (Court 1974).

CONCLUSIONS

Although the expected symptoms of wilting or chlorosis did not occur after undercutting in this trial, nitrogen levels in undercut seedlings were altered. Plant total nitrogen content fell but lateral root nitrogen content increased. Concentrations of insoluble nitrogen forms also dropped in other tissues but rose in lateral roots prior to increased dry weight. Soluble nitrogen forms showed a rapid initial drop but subsequently followed control seedling trends. A period of 10–21 days after undercutting was required before growth trends corresponded to seasonal trends shown by control seedlings.

Since both dry matter and nitrogen content values for lateral roots increased above control seedling values, it can be concluded that regenerating root systems form a strong "sink" for both photosynthate and mobilised nitrogen forms from other parts of the plant.

ACKNOWLEDGMENTS

I wish to thank Mr J. C. van Dorsser and Mr A. E. Summers for supplying and undercutting seedlings, and Miss C. Jones and Mrs J. M. Summers for assistance in the field and throughout the work. I am grateful to the Soils and Site Amendment Research Group (FRI) for use of their equipment for nitrogen analyses, Mr I. A. Andrew for help with statistical analyses, and Mr J. Hobbs for a computer program for nitrogen computations. I also wish to gratefully acknowledge the advice given by Dr D. A. Rook, Dr J. A. Zabkiewicz, and Mr N. Turner (PPD, DSIR, Mt Albert) at various stages during this investigation.

REFERENCES

ARNON, D. I. 1949: Copper enzymes in isolated chloroplasts. Polyphenol oxidase in **Beta vulgaris**. **Plant Physiology 24:** 1–15.

BACHELARD, E. P. 1980: Control of dormancy. Pp. 273–96 **in** Little, C. H. A. (Ed.) "Control of Shoot Growth In Trees", IUFRO Workshop on "Xylem Physiology and Shoot Growth Physiology", Fredericton, New Brunswick, Canada.

BACON, G. J. 1978: Physiological aspects of conditioning **Pinus caribaea** Mor. var. **hondurensis** B & G seedlings to withstand water stress. Ph.D. Thesis, Australian National University, Canberra.

BENSON, A. D.; SHEPHERD, K. R. 1977: Effects of nursery practice on **Pinus radiata** seedling characteristics and field performance. II. Nursery root wrenching. **New Zealand Journal of Forestry Science 7:** 68–76.

BOTLGER, M. 1978: Levels of endogenous indole-3-acetic acid and abscisic acid during the course of formation of lateral roots. **Zeitschrift für Pflanzenphysiologie 86:** 283–6.

BOWEN, G. D. 1969: Nutrient status effects on losses of amides and amino acids from pine roots. **Plant and Soil 30:** 139–41.

CAMERON, R. J.; ROOK, D. A. 1969: Wrenching as a means of conditioning planting stock. Pp. 111–7 **in** Chavasse, C. G. R.; Weston, G. C. (Comp. and Ed.) "Forest Nursery and Establishment Practice in New Zealand", **FRI Symposium No. 9.**

CHAVASSE, C. G. R., 1980: Planting stock quality: A review of factors affecting performance. **New Zealand Journal of Forestry 25(2):** 144–71.

COURT, D. J. 1974: Nitrogen mobilisation in **Pinus radiata** in response to undercutting and wrenching treatments. M.Sc. Thesis, University of Auckland.

HAMBRAEUS, L.; FORSUM, E.; ABRAHAMSSON, L.; LONNERDAL, B. 1976: Automatic total nitrogen analysis in nutritional evaluations using a block digestor. **Annals of Biochemistry 72:** 78–85.

KNIGHT, P. J. 1978: Fertiliser practice in New Zealand forest nurseries. **New Zealand Journal of Forestry Science 8:** 27–53.

LINDER, S. 1974: A proposal for the use of standardised methods for chlorophyll determination in ecological and eco-physiological investigations. **Physiologia Plantarum 32:** 154–6.

MEAD, D. J.; WILL, G. M. 1976: Seasonal and between-tree variation in the nutrient levels in **Pinus radiata** foliage. **New Zealand Journal of Forestry Science 6:** 3–13.

MENZIES, M. I. 1980: Effect of nursery conditioning on the water relations of two-year-old Douglas fir seedlings after lifting and outplanting. Ph.D. Thesis, University of Washington.

ROOK, D. A. 1969: Studies on the physiology of wrenched seedlings. Pp. 119–21 **in** Chavasse, C. G. R.; Weston, G. C. (Comp. and Ed.) "Forest Nursery and Establishment Practice in New Zealand", **FRI Symposium No. 9.**

——— 1971: Effect of undercutting and wrenching on growth of **Pinus radiata** D. Don seedlings. **Journal of Applied Ecology 8:** 477–90.

ROVIRA, A. D. 1969: Plat root exudates. **Botanical Review 35:** 35–7.

STUPENDICK, JO-ANNE T.; SHEPHERD, K. R. 1980: Root regeneration of root-pruned **Pinus radiata** seedlings. II. Effects of root pruning on photosynthesis and translocation. **New Zealand Journal of Forestry Science 10:** 148–58.

TAYLOR, B. K. 1967: Storage and mobilisation of nitrogen in fruit trees. A Review. **Journal of the Australian Institute of Agricultural Science 33:** 23–9.

THIMANN, K. V. 1980: The senescence of leaves. Pp. 85–115 **in** Thimann, K. V. Ed.) "Senescence in Plants", CRC Press.

TROMP, J. 1970: Storage and mobilisation of nitrogenous compounds in apple trees with special reference to arginine. Pp. 143–59 **in** Luckwill, L. C.; Cutting, C. V. (Ed.) "Physiology of Tree Crops", Academic Press, London and New York.

van DORSSER, J. C.; ROOK, D. A. 1972: Conditioning of radiata pine seedlings by undercutting and wrenching: Description of methods, equipment and seedling response. **New Zealand Journal of Forestry 17(1):** 61–73.

WAREING, P. F. 1980: Root hormones and shoot growth. Pp. 237–56 **in** Little, C. H. A. (Ed.) "Control of Shoot Growth In Trees", IUFRO Workshop on "Xylem Physiology and Shoot Growth Physiology", Fredericton, New Brunswick, Canada.

ZHOLKEVICH, V. N.; KORETSKAYA, T. F. 1959: Metabolism of pumpkin roots during soil drought. **Soviet Journal of Plant Physiology 6:** 690–700.