STORAGE AND MOBILIZATION OF NITROGEN IN THE PEACH TREE

by

B. K. Taylor, B.Ag.Sc. (Hons.)

Department of Plant Physiology
Waite Agricultural Research Institute
University of Adelaide, South Australia

Thesis submitted for the Degree of
Doctor of Philosophy

April, 1966

TABLE OF CONTENTS

Page No.

PART I

SUMMARY

The accumulation, chemical composition, and distribution of storage nitrogen in young peach trees (Golden Queen scion on Elberta rootstock), and the importance of this stored nitrogen for new growth, were investigated in this thesis. In addition, the metabolic stability of constituents of the storage nitrogen of dormant peach trees was studied.

Results of an experiment, in which 1 year old peach trees were grown in sand culture over a 2 year period, showed that the trees accumulated nitrogen in proportion to supply during the first year, and suggested that this stored nitrogen was utilized for new growth during the second growing season irrespective of the external nitrogen supply. Tree growth in early spring was significantly correlated with the level of storage nitrogen in tree tissues, but after November, tree growth was markedly dependent upon the external nitrogen supply. If fertilizer nitrogen was not applied, the supply of storage nitrogen in tree tissues was exhausted by the end of November. Reaccumulation of storage nitrogen in tree tissues began in December and was rapid if the external nitrogen supply was high. Analysis of growth data recorded at the end of the second growing

season indicated that there was a significant negative interaction between the first and second year treatments; i.e. the lower the nitrogen application in the first year of the experiment, the greater the effect of applied nitrogen on tree growth in the second year, and vice versa.

Storage nitrogen in dormant trees consisted mainly of soluble organic nitrogen and free arginine was the principal constituent of this fraction. The level of arginine in storage tissues of the dormant trees was the most sensitive indicator of the nitrogen status of the trees. Approximately 70 to 80% of the storage nitrogen in dormant, 2 year old trees was found in the roots, irrespective of the nitrogen treatment.

In an attempt to corroborate these results, a range of nitrogenous compounds, including constituents of the storage nitrogen of dormant trees, were injected into 1 year old peach trees. However, no significant growth responses were observed.

Isotopes of 2 compounds which are known to be constituents of the storage nitrogen of dormant peach trees, viz. L-arginine-<u>guanido</u>-^{14}C-hydrochloride and L-asparagine-^{14}C (U), were injected into 2 year old peach trees in winter using a cut-shoot technique. Both compounds were metabolised by the trees and it is suggested that they are 'turned over' in dormant tissues. Aspartic acid was the primary breakdown product of ^{14}C-asparagine, but the breakdown products of ^{14}C-arginine hydrochloride were not identified. The latter isotope underwent considerable decomposition during paper chromatography. Most of the injected ^{14}C remained in the treated shoot or neighbouring tissues, but small amounts were translocated both upwards and downwards from the point of application.

PART II

STATEMENT

I hereby declare that the thesis here presented is my own work, that it contains no material previously published, except where due reference is made in the text, and that no part of it has been submitted for any other degree.

PART III

ACKNOWLEDGEMENTS

The author is indebted to the counsel and guidance of his supervisors, the late Professor L. H. May and Professor L. G. Paleg.

He expresses thanks to Mrs. F. M. Randles for assistance in the preparation of the diagrams, to Mr. D. L. Messent for advice and assistance with the statistical analyses, and to Mr. B. Falk for the photographic work.

The financial assistance given by the Commonwealth Government (Commonwealth Postgraduate Award) and by the Victorian Department of Agriculture is gratefully acknowledged. In addition, the author expresses thanks to the Victorian Department of Agriculture for provision of 3 years' leave, without which this course of study could not have been carried out.

PART IV

INTRODUCTION

Because of their large bulk and perennial type of growth trees normally accumulate large quantities of nutrients, including nitrogen, in wood and bark of roots, trunks and branches (Murneek 1930; Gardner et al. 1952; Lindner et al. 1954). It is generally accepted that this nutrient supply acts as a buffer against unfavourable seasons, or parts of a season, when uptake of nutrients from the soil is slow or limited. For example, the above-mentioned authors have suggested that the production of new growth by trees in early spring occurs at the expense of, and in proportion to, the supply of stored nutrients within tree tissues until uptake commences from the soil. In deciduous trees it is usual to observe a sharp decline in the nutrient content of woody tissues in spring, which is attributable to mobilization of reserves and translocation of nutrients to growing points. This loss is not made up until the following autumn when an increase in concentration of nutrients occurs after shoot elongation ceases (Murneek 1930).

The utilization of stored nutrients by the tree is clearly dependent upon their mobility within the tree. According to Gardner et al. (1952) there is evidence that N, P, K, S, Mg and Fe are readily mobile within tree tissues but that Ca is not. Therefore although Ca may accumulate in tree tissues it is not reused in the formation of new tissue.

The importance of storage nitrogen for the growth of fruit trees was first demonstrated conclusively by Roberts in 1921. He grew young apple trees in pot culture under a high or low nitrogen supply during a first year, and then supplied either a further high nitrogen application or tapwater only to each group of trees during a second growing season. He found that the amount of tree growth made during the second growing season was markedly dependent upon the level of nitrogen supplied during the first year. Following this work, several investigations were carried out to determine what seasonal changes occurred in the concentration of nitrogenous constituents in tissues of fruit trees (Thomas 1927<u>a</u>, 1927<u>b</u>; Mulay 1931, 1932; Karmarkar 1934). These studies, together with those relating to the application of nitrogenous fertilizers to trees at various times of the year (Aldrich 1931; Weinberger and Cullinan 1934; Williams 1939; Masharty 1944), established the importance of storage nitrogen in the nitrogen economy of both young and bearing fruit trees. Recent work has been directed towards an understanding of the chemical nature of the compounds involved in the storage of nitrogen in fruit trees (Oland 1954, 1959).

It is now clear that in dormant apple trees soluble nitrogen constitutes the major component of the storage nitrogen, and this appears to be the case in other tree species as well (McKee

1962; Baxter 1965). As suggested by Baxter (1965), this information could be used as the basis of a new method for the assessment of the nitrogen status of orchard trees. He found that the concentration of total α-amino nitrogen in root or shoot bark tissues of orchard trees, which were growing under different nitrogen regimes, gave a more sensitive indication of the nitrogen status of the trees than did the concentration of total nitrogen in the leaves. An added advantage of such a method would be that analysis of tree tissue is carried out in winter in advance of the normal spring application of fertilizers.

Since most of the recent studies relating to storage of nitrogen have been made on apple trees, it was decided to investigate aspects of the storage and mobilization of nitrogen in peach trees in this thesis. The following general aims were formulated:

(a) To determine to what extent the growth of peach trees is dependent upon nitrogen accumulated in tree tissues.

(b) To identify the constituents of the storage nitrogen of peach trees.

(c) To determine in which tree part the major portion of the nitrogen is stored.

PART V

REVIEW OF LITERATURE

A. EVIDENCE FOR STORAGE AND MOBILIZATION OF NITROGEN IN TREES

(a) Seasonal Changes in Levels of Nitrogenous Constituents in Tree Tissues

(i) Overall Trends

Pronounced seasonal rhythms have been found in the concentration of total N and soluble nitrogenous constituents in tissues of all of the deciduous fruit trees examined. Data covering changes in one or more of these constituents throughout a whole year have been published for apple (Thomas 1926, 1927a, 1927b; Karmarkar 1934; Kench 1938; Murneek 1942; Mochizuki and Hanada 1956; Mason and Whitfield 1960; Radu 1961; Romanovskaya 1963), peach (Williams 1931; Schneider 1958; Radu 1961), pear (Mulay 1931, 1932; Radu 1961), apricot (Radu 1961), cherry and plum (Romanovskaya 1963). Except for minor differences the findings of these workers conform to the following general pattern.

Over the period from bud swell to flowering the concentration of total and soluble nitrogenous constituents, especially nonprotein N, increases markedly in developing buds and the youngest shoots. This increase is accompanied by a sharp decline in the concentration of total nitrogen in the older shoots and branches, suggesting that protein hydrolysis occurs and that soluble nitrogenous compounds are translocated from these tissues to the developing meristems. Subsequently, as shoot growth commences and the growing season advances, the concentration of all forms of nitrogen in leaves, shoots, branches and roots declines until a minimum is observed at about the time of cessation of shoot elongation in summer. During late summer and autumn, however, the concentration of nitrogenous substances in woody tissues increases. Following leaf fall and onset of dormancy, the concentration of total N remains high in the woody tissues, especially in the bark, but the concentration of soluble nitrogenous constituents may increase or decrease, depending upon the compound or fraction concerned and the tree tissue analysed.

Seasonal trends in the concentration of total nitrogen in tissues of evergreen trees are somewhat similar to those reported for deciduous trees. Thus Cameron and Appleman (1933) found that each flush of shoot growth in citrus trees is accompanied by a sharp drop in the concentration of total nitrogen in adjacent woody tissues, indicating that new growth is probably made at the expense of nitrogen accumulated within these tissues.

The above observations suggest that stored nitrogen is mobilized for new growth and that nitrogen reaccumulates in woody tree tissues after shoot growth ceases.

(ii) Mobilization of Nitrogen in Woody Tissues in Spring

Aldrich (1931) and Nasharty (1944) observed a pronounced increase in the concentration of total nitrogen in young shoots of mature apple and peach trees prior to flowering, even in trees to which no nitrogenous fertilizer had been applied. Since the increase was approximately of the same order of magnitude in shoots of both fertilized and unfertilized trees, the results can be attributed to a mobilization of reserves rather than to uptake of nitrogen by the roots. In agreement with this finding the rate of uptake of nitrogen by roots of apple trees in spring (Pearse 1939) appears to be insufficient to account for the observed increases in concentration of nitrogen in young tree tissues at flowering. In circumstances where uptake of nitrogen from the soil is inhibited by unfavourable environmental conditions, for example low moisture content of the soil, new tree growth may be entirely at the expense of stored reserves (Murneek 1936). According to Kramer and Kozlowski (1960), forest trees invariably make a large part of their spring growth at the expense of storage nitrogen since the average forest soil is low in available nitrogen and therefore the rate of absorption is slow.

Few investigators have attempted to follow the redistribution of nitrogen within tree parts in spring on an absolute rather than concentration basis. One such study has been made recently by Yokomizo et al. (1964). During the first year of the experiment five year old apple trees were supplied with a high or low level of nitrogen, but in the second year water only was supplied to both groups of trees. The trees which received the high nitrogen treatment contained more nitrogen per tree at the end of the first year and produced more growth in the spring of the second year than did trees in the other group. In the high nitrogen trees most of the nitrogen which was mobilized for new growth came from the roots, but in the low nitrogen trees most of the nitrogen which was incorporated into new growth came from the old shoot tissues.

The rooting of shoot cuttings and the production of new shoots by tree stumps in spring are two situations in which the mobilization of storage nitrogen within tissues is undoubtedly important. (Wallace et al. 1954; Kramer and Kozlowski 1960). For example, Wallace et al. found that if no nitrogen was supplied during the rooting of citrus cuttings the nitrogen content of the foliage fell by 20% and that of the stem by 30%.

Ringing experiments have also provided evidence for the mobilization of nitrogen (Harley et al. 1958). In early spring, these workers removed bark rings from selected parts of 2 and 3 year old branches of mature apple trees in such a way that various amounts of branch tissue were isolated between rings. The amount of growth made by the spur left on each branch segment was dependent upon the amount of nitrogen stored within each segment.

The evidence for mobilization of nitrogen in woody tissues in spring agrees well with the observations made by Bollard (1953) on the seasonal changes in concentration of nitrogenous compounds in xylem sap of apple trees. He found that the level of total soluble nitrogen in xylem sap of shoots rose from a low level in winter to a pronounced peak at flowering. This was followed by a gradual decline through the growing season, except for a second smaller peak in December. Bollard (1957a) suggested that the sudden increase in the nitrogen concentration of the sap at flowering is probably due to mobilization of reserves in the roots, rather than to rapid uptake via the roots, since the nitrogen present in the sap was almost entirely organic in character consisting largely of aspartic acid, asparagine and glutamine.

(iii) Reabsorption of Nitrogen from Abscissing Tissues

Since flowers are rich in nitrogen and only a small proportion of the total number per tree develop into fruits, it would be expected that the tree would lose a considerable amount of nitrogen when undeveloped flowers abscissed. However, one third to one half of this nitrogen is translocated back into woody parts of the tree before abscission occurs (Murneek 1930). Immediately prior to abscission, protein hydrolysis predominates over protein synthesis in the flower parts (McKee 1958b).

A large part of the nitrogen present in leaves of deciduous trees also migrates to woody parts of the tree, including the roots, prior to leaf fall (Combes 1926a, 1926b; Thomas 1927a; Mulay 1931; Murneek and Logan 1932; Karmarkar 1934; McKee 1958a; Oland 1963b). Since the foliage of a mature apple tree, for example, contains up to one-half of the nitrogen content of the tree at the end of the growing season (Murneek 1942), it is clear that migration of this nitrogen is a most important factor in the efficient use of nitrogen by the tree. In apple trees the translocation of nitrogen from leaves to woody tissues commences 3 to 4 weeks prior to abscission, and continues until defoliation is complete. In forest trees, however, the migration process may take place over a period of 2 months (Combes 1926b).

During leaf yellowing in autumn, protein hydrolysis predominates over protein synthesis in leaf tissue (McKee 1958a) and therefore it is probable that the nitrogen which migrates from leaves to shoots is largely in the form of nonprotein N. Karmarkar (1934), for example, found that the decrease which occurred in the concentration of total N and protein N in apple leaves in autumn was accompanied by concomitant rises in the concentration of nonprotein N in the leaves and in the concentration of nonprotein N, total N and protein N in the bark of young shoots.

Estimates of the loss of nitrogen from the foliage during leaf yellowing in different tree species have ranged from 16 to 80% of the nitrogen present on an absolute basis. The extent of loss of nitrogen from the foliage is dependent upon the environmental conditions prevailing during the migration process and upon the overall nitrogen status of the tree. Thus, if warm, calm weather prevails during late autumn a high proportion of the nitrogen present in the leaves is translocated to woody tissues, but if the air temperature decreases early, or if windy conditions prevail, then leaf drop may occur before the migration process is complete (Murneek and Logan 1932; Oland 1963b). Further, according to Cullinan (1931) and Murneek and Logan (1932), the higher the nitrogen content of the tree as a whole, the smaller will be the proportion of the nitrogen content of the foliage which is translocated to the woody tissues.

Combes (1924, cited by McKee 1962) showed that the loss of nitrogen by leaves of deciduous trees in autumn was not due to leaching of leaves by rain.

Oland (1960, 1963a) has recently suggested that use could be made of these migration processes to increase the storage of nitrogen in apple trees in autumn through the application of post-harvest urea sprays. He found that strong urea foliar sprays applied after harvest rapidly increased the concentration of nitrogen in the leaves, and that at least part of this nitrogen was later translocated to buds.

Migration of nitrogen from leaves of evergreen trees prior to leaf abscission has also been recorded. For example, Wallace et al. (1951) calculated that leaves of 14 year old Valencia orange trees lost 8.5% of their nitrogen content prior to abscission.

(iv) Uptake of Nitrogen During Autumn and Winter

In autumn, roots of trees are highly active and make a good deal of growth (Heinicke 1935; Maggs 1965). This activity may be related to uptake of nutrients since Pearse (1959) showed that the rate of uptake of nitrogen by roots of apple trees in autumn is high. With the onset of winter, however, the rate of uptake declined sharply.

Results of a large number of field trials and pot experiments have shown conclusively that fruit trees, including both deciduous and evergreen species, are able to take up and accumulate nitrogen during autumn and winter, provided that the soil temperature is above freezing point. Thus uptake of nitrogen at this time of the year has been observed in apple trees (Sullivan and Kraybill 1930; Aldrich 1931; Murneek 1936; Smith 1936; Smith and Murneek 1938; Batjer et al. 1943; Oland 1959), peach trees (Weinberger and Cullinan 1934; Williams 1939), tung trees (Loustalot and Lagasse 1946) and citrus trees (Chapman and Parker 1942). This information has become the basis for recommending application of nitrogenous fertilizers to trees in autumn and winter.

The nitrogen which is taken up by trees during autumn and winter is stored in the larger roots (Aldrich 1931; Weinberger and Cullinan 1934; Murneek 1936; Smith 1936; Smith and Murneek 1938) and is not translocated to tree tops if the air temperature is less than 40 to 45°F (Williams 1939; Batjer et al. 1943). Since Eckerson (1931) was able to detect a high level of nitrate reductase activity in roots of apple trees in winter, it might be expected that most of the nitrogen stored in tree roots would be in organic compounds and this has been confirmed by analysis (Nightingale 1934; Oland 1959).

According to Batjer et al. (1943), the degree of soil aeration and the initial concentration of nitrogen in the roots influences the amount of nitrogen taken up by dormant trees. They found that absorption was increased if trees were grown in a well aerated medium compared with a poorly aerated medium. In contrast, the rate of absorption of nitrogen was inversely proportional to the initial concentration of nitrogen in tree roots. This latter result was taken to mean that there may be a limit to the amount of nitrogen which can be absorbed and held in the roots of dormant trees.

(b) Residual Effects of Nitrogenous Fertilizers on Tree Growth

Results of field experiments have revealed that there is a considerable time lag, e.g. 1 or more years, before growth and cropping capacity are affected if nitrogenous fertilizers are withheld from trees which were previously heavily fertilized (Burrell 1928; Rogers et al. 1955). However, such observations cannot be meaningfully interpreted as evidence for storage and mobilization of nitrogen within tree tissues, since it is possible that the residual effect resulted from utilization of nitrogen accumulated within the soil.

Pot-culture techniques have also been used to study the importance of storage nitrogen in the growth of trees (Roberts 1921; Harley et al. 1949; Oland 1959; Yokomizo et al. 1964). Each of these workers has used essentially the same procedure and in each case the experiments have been carried out on young apple trees. During the first year several levels of nitrogen were supplied to the trees, but during a second year plus or minus nitrogen treatments were applied.

In all cases the amount of new shoot growth at the end of the second growing season was markedly dependent upon the nitrogen treatment given the first year. It was concluded that the amount of shoot growth made by young apple trees during a growing season is strongly influenced by the level of storage nitrogen within the trees. However, this conclusion may be criticized in the case of the experiments carried out by Roberts (1921) and Harley et al. (1949) since these workers did not repot the trees into a nitrogen free medium at the end of the first growing season. It is therefore possible that in these experiments sufficient nitrogen accumulated in the growing medium during the first year to influence tree growth during the second year.

Oland (1959) concluded that the amount of new shoot growth made by young apple trees is dependent upon the level of storage nitrogen within the trees and the external nitrogen supply, providing, of course, that no other factor is limiting. His experiments showed that storage nitrogen is always used for new growth irrespective of the external nitrogen supply. If trees contained a high level of storage nitrogen at the beginning of the growing season then the influence of the current supply of nitrogen on growth was small and only noticeable at the end of the growing season. He also found that the storage volume of trees, as indicated by stem diameter measurements at the beginning of the experiment, influenced the amount of shoot growth made within a given treatment.

B. CHEMICAL COMPOSITION OF STORAGE NITROGEN

(a) Relative Importance of Soluble and Insoluble Nitrogen

Characteristically, storage organs and dormant tissues contain a high proportion of their nitrogen in soluble compounds and a correspondingly low proportion in insoluble compounds (Nightingale 1937; Steward, Thompson and Pollard 1958; McKee 1958c, 1962). According to McKee (1958c), soluble nitrogen usually accounts for 30 to 70% of the total N content of underground storage organs. Similar values have been reported for tissues of dormant fruit trees. For example, Davidson and Shive (1935) found that soluble organic N constituted 42% of the total N content of stems of dormant peach trees.

Oland (1954, 1959) compared the influence of nitrogen supply on levels of soluble N and insoluble N, and also followed changes in these fractions during the growing season, in woody storage tissues of young apple trees. He found that levels of soluble N were markedly influenced by both of these factors whereas only small changes were observed in levels of insoluble N. He concluded that the soluble N fraction constitutes the main form in which nitrogen is stored in apple trees. Since levels of soluble N decreased in storage tissues as the growing season progressed even when nitrogen was supplied to the trees, Oland suggested that this nitrogen was translocated to the growing points and utilized in growth.

In addition, however, Oland (1959) showed that at least part of the insoluble N present in stems and roots of apple trees may be mobilized for growth. He found that the concentration of soluble N increased at the expense of the insoluble N content of the tissues prior to the initiation of growth in spring. This observation confirms the earlier work of Loomis (1934, 1935) who demonstrated that much of the soluble nitrogen which built up beneath bark rings on stems of apple trees was derived from storage protein.

(b) Composition of the Soluble Nitrogen Fraction

As discussed earlier, the soluble nitrogen present in woody tissues has been shown to be mainly organic in character. While this has been known for over 30 years, a study of the individual compounds present in this fraction has only been possible since the development of chromatography and allied techniques.

Reuter (1957) identified the major constituents of the soluble nitrogen in vegetative storage organs of 166 different plant species. In the majority of species, glutamic acid, aspartic acid, asparagine and glutamine were the chief compounds detected, but in some species and family groups other nitrogenous compounds predominated. For example, arginine predominated in the Rosaceae and Saxifragaceae. Since most fruit tree species belong to the family Rosaceae it would be expected that arginine would be an important constituent of the storage nitrogen in these species. This has been confirmed for apple (Oland 1954, 1959; Romanovskaya 1963), peach (Schneider 1958), plum and cherry (Romanovskaya 1963). Each of these workers found that arginine was the predominant compound in the soluble nitrogen fraction of woody tissues during late autumn and winter when storage nitrogen is known to accumulate. Furthermore, they found that the concentration of arginine gradually declined to a low level during the growing season but then rose after the cessation of shoot growth. Thus the concentration of arginine in woody tissues of the trees undergoes a seasonal rhythm which is consistent with the hypothesis that arginine is the main form in which nitrogen is stored in these species.

Oland (1954, 1959) found that the level of arginine in woody tissues of apple trees was dependent upon the nitrogen supply. Thus he reported that arginine comprised 70% or more of the soluble nitrogen present in storage tissue of nitrogen-rich trees in spring, but the corresponding value in nitrogen-deficient trees was much less. His results also indicated that glutamine and asparagine are important constituents of the storage nitrogen of apple trees especially if these are nitrogen deficient. In contrast, the available evidence suggests that the amides are not important in the storage of nitrogen in peach, plum and cherry trees, since they may be undetectable in these trees during the dormant season (Schneider 1958; Romanovskaya 1963).

Since it is known that nitrogen is mainly translocated as aspartic acid, asparagine and glutamine in woody species of the family Rosaceae (Bollard 1957<u>a</u>; 1957<u>b</u>), it is clear that the utilization of arginine nitrogen in growth processes in these species does not involve translocation of arginine per se to the developing meristems. Therefore arginine must be degraded before its nitrogen becomes available for growth, and presumably the nitrogen which is thus released is used in the synthesis of aspartic acid and the amides. However, when growth ceases and accumulation of arginine begins in the woody tissues, arginine is found in the xylem sap and gradually replaces asparagine as the major constituent (Bollard 1957a).

C. COMMENTS ON THE INFORMATION AVAILABLE IN THE LITERATURE

Much of the information available in the literature has been presented on a concentration rather than an absolute basis. Since a change in the concentration of nitrogen in a tissue may be brought about by either a change in the nitrogen or dry matter contents of the tissue, it is clear that concentration changes cannot be accepted as conclusive evidence for the storage and mobilization of nitrogen within tree tissues.

It is also apparent from the literature that most of the detailed investigations relating to the importance of storage nitrogen for tree growth, the chemical composition of storage nitrogen, and the distribution of storage nitrogen within tree tissues have been carried out on apple trees. Similar investigations need to be carried out on other tree species as well.

In some areas of the subject no information is available. For instance, the metabolic pathways by which arginine is synthesised and degraded in tree tissues have not been investigated, nor are the factors known, apart from nitrogen supply, which control the synthesis and degradation of arginine in tree tissues. Environmental factors such as daylength and air-temperature may be involved in the latter. It is known that peach trees cease growth and become dormant if exposed to short days and low temperatures (Anon 1964), and it is possible that accumulation of arginine also occurs in direct response to these environmental factors rather than as a result of cessation of growth. It is pertinent in this respect that Boynton *et al.* (1961) found that arginine accumulated in leaves of strawberry plants which had been exposed to short days or to low night temperatures.

PART VI

EXPERIMENTAL WORK

A. STORAGE AND MOBILIZATION OF NITROGEN IN YOUNG PEACH TREES GROWN IN SAND CULTURE FOR TWO GROWING SEASONS

(a) Introduction

The aims of the experiment were as follows:

(i) to determine whether young peach trees accumulate nitrogen in proportion to nitrogen supply, and if so,

(ii) to determine whether this accumulated nitrogen is mobilized for new growth during the next growing season,

(iii) to determine the chemical nature of this storage nitrogen, and

(iv) to determine in which tree parts the nitrogen is accumulated.

It was thought that if the trees accumulated nitrogen during the first year of the experiment in proportion to nitrogen supply, then this stored nitrogen might be mobilized and used in new tree growth during the second year, especially in the early part of the growing season when uptake of nitrogen via the roots might be slow. The experiment was carried out on young rather than mature trees for the following reasons. With young trees, results are more easily expressed on an absolute basis, i.e. on a per organ or per tree basis, and this is important when the mobilization of nutrients is being studied. Young trees bear little or no fruit and therefore growth responses are not dependent upon crop load, and, as well, they are more amenable to treatment and manipulation than are large orchard trees. It was realized, however, that because of their small size and therefore small storage volume, the growth of young trees would probably not be as dependent upon levels of storage nitrogen as would the growth of large, mature trees.

(b) Methods

(i) Experimental Design

During the first year 3 levels of nitrogen were supplied to the trees. Further nitrogen treatments were superimposed on these during the second growing season, viz:

First Year	Second Year
N1	N1N0
	N1N3
	N1N9
N3	N3N0
	N3N3
	N3N9
N9	N9N0
	N9N3
	N9N9

Where, N0 = nil nitrate N
N1 = 20 p.p.m. "
N3 = 60 " "
N9 = 180 " "

Overall, there were 108 trees in the experiment, i.e.

9 treatments x 4 replicates x 3 harvests = 108

The experiment was laid out according to a randomised block design, where each block constituted a replicate and was a factorial of treatments x harvests.

(ii) Source of Trees and the Planting Procedure

In June 1963, 135 one-year old peach trees (Golden Queen scion on Elberta rootstock) were selected for uniformity of size, i.e., overall height, stem diameter and evenness of the root system, for the presence of leaf buds on the stem, and for freedom from disease and damage, at Balhannah Nurseries. These trees were 'heeled' in moist sand until planting.

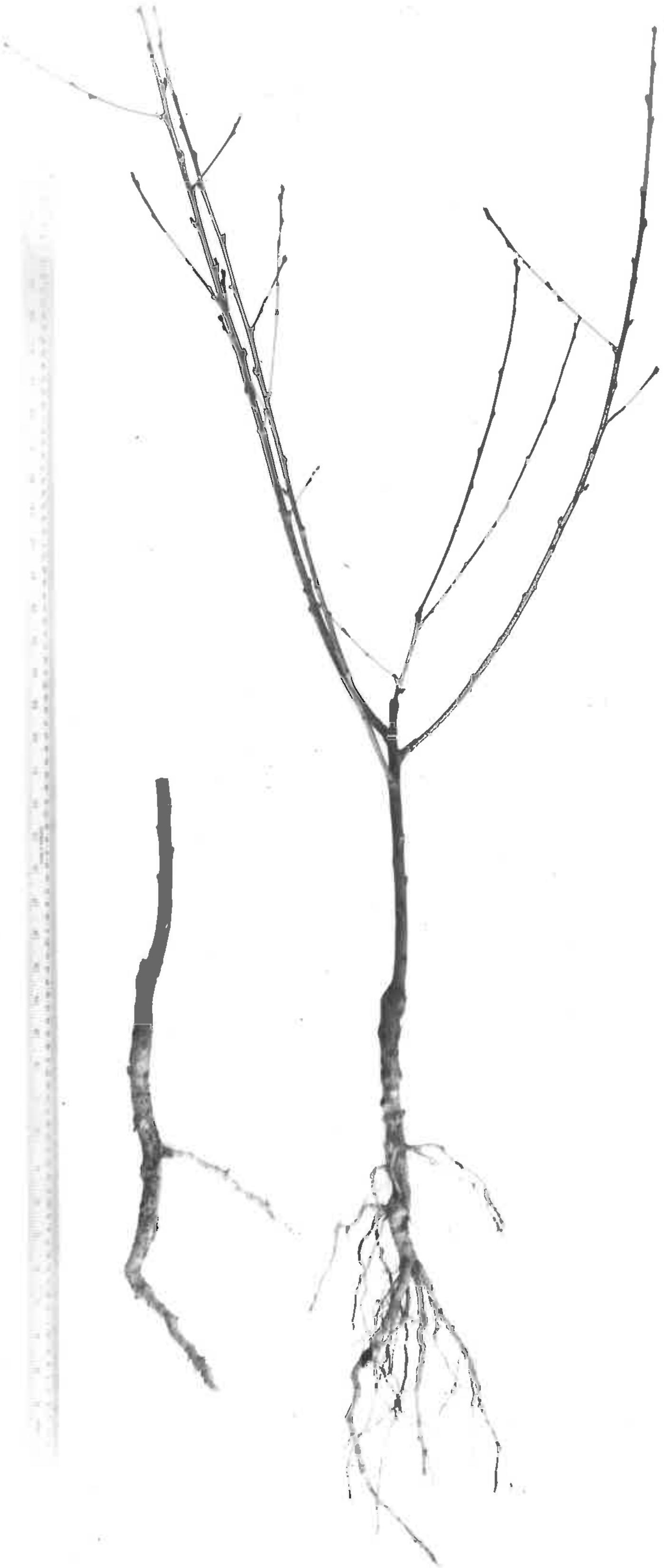
At planting, tree roots were washed clean under a jet of water, dried between sheets of blotting paper, and the trees were weighed. The roots and tops of each tree were then pruned severely to remove as much storage tissue as possible (Plate 1), thereby possibly increasing the influence of the applied treatments on tree growth in the first year. Tree tops were cut back to within 6 inches of the graft union. Since all prunings were retained and weighed it was possible to calculate the fresh weight of the pruned trees. After pruning, the root system of each tree was dipped in the following aqueous solutions:

(1) 5.25% NaOCl for 2 minutes

(2) 200 p.p.m. terramycin hydrochloride for 30 minutes

(3) 5.25% NaOCl for 2 minutes

PLATE 1 Trees before and after pruning.



This procedure was adopted in an attempt to eliminate possible infection of pruned roots by the wound parasite Agrobacterium tumefaciens, which produces galls on roots (crown gall). According to Deep (1958), terramycin hydrochloride is capable of penetrating dormant tree tissues and killing any crown gall bacteria present, while the NaOCl was expected to act as a surface-sterilizing compound. However, the treatment was not effective and root galls (presumably crown gall) were subsequently found on about 20% of the trees.

Trees were planted in numbered metal pots of 4 gallon capacity (lined internally with epoxy resin), each of which had been filled with 26 Kg sieved (passing 10 mesh per inch), steam-sterilized sand. The characteristics of the sand used in the experiment are given in Table 1. Each pot was provided with a central drainage hole which was covered with fine-meshed polythene gauze to prevent loss of sand. Potted trees were transferred to an unheated glasshouse and placed on benches.

Immediately after planting, the sand in each pot was leached thoroughly with distilled water to remove as much clay and soluble material as possible. The surface of the sand in each pot was then covered with a mulch of 3 Kg sterilized quartzite gravel to reduce water loss and to prevent algal growth. Prior to commencement of the nitrogen treatments, the sand in each pot was kept moist by frequent additions of distilled water.

Since the fresh weight of the pruned trees varied widely

TABLE 1

CHARACTERISTICS OF THE SAND USED FOR SAND CULTURE PURPOSES

Year	Type of Sand	Source of Sand	pH	pH After Acid Washing	Total N Content (µgN/mg d.wt.)	Dry Matter Content (%)
1963	coarse river sand	Waikerie, S. A.	7.6±0.05	-	26.8±3.7	97.4±0.03
1964	coarse river sand	Waikerie, S. A.	8.7±0.05	6.8±0.03	19.0±1.8	99.7±0.003

(as much as 3:1) they were stratified according to weight prior to allocation of treatments. The resultant grouping was split into 4 equal parts, where each part constituted a particular range of tree sizes and was designated a 'replicate'. Within each of these replicates the nine nitrogen treatments were allocated to trees at random and trees within each replicate were also placed in a random position in the glasshouse.

Apart from weight measurements, stem diameters, at a point 3 inches above the graft union, and the total length of pruned tops per tree, were recorded at planting.

On 26/7/63, prior to commencement of the first year treatments, 2 trees were chosen at random from each replicate and all 8 trees were harvested (harvest 0) according to the techniques described later. Trees were divided into root, stock plus stem, and bud fractions, and, after oven-drying, samples were analysed for nitrate N, total N and dry matter content.

(iii) Sand Culture Techniques

(1) Composition and Preparation of Nutrient Solutions

The composition of the nutrient solutions which were supplied to the trees is given in Tables 2A and 2B. Nitrogen was supplied as nitrate only in both years of the experiment. The solution used during the first year was adapted from that described by Batjer and Degman (1940) in which $NaNO_3$ is the nitrogen source. However, its use resulted in marginal chlorosis and marginal and tip

TABLE 2A

COMPOSITION OF NUTRIENT SOLUTIONS USED IN THE SAND-CULTURE EXPERIMENT

Major Elements (m.e./L.)

Ion	Solution I (1963-64 Growing Season)			Solution II (1964-65 Growing Season)		
	Treatment			Treatment		
	N1	N3	N9	N0	N3	N9
NO_3^-	1.3	4.0	12.0	nil	4.0	12.0
Na^+	1.3	4.0	12.0	1.5	1.5	1.5
K^+	3.0	3.0	3.0	3.0	3.0	3.0
PO_4^{-3}	9.0	9.0	9.0	4.5	4.5	4.5
Mg^{+2}	4.0	4.0	4.0	4.0	4.0	4.0
SO_4^{-2}	4.0	4.0	4.0	7.0	7.0	4.0
Ca^{+2}	9.0	9.0	9.0	9.0	9.0	9.0
Cl^-	9.0	9.0	9.0	9.0	5.0	nil
Salts Used	$NaNO_3$ KH_2PO_4 $MgSO_4 \cdot 7H_2O$ $CaCl_2 \cdot xH_2O$	$NaNO_3$ KH_2PO_4 $MgSO_4 \cdot 7H_2O$ $CaCl_2 \cdot xH_2O$	$NaNO_3$ KH_2PO_4 $MgSO_4 \cdot 7H_2O$ $CaCl_2 \cdot xH_2O$	K_2SO_4 $NaH_2PO_4 \cdot 2H_2O$ $MgSO_4 \cdot 7H_2O$ $CaCl_2 \cdot xH_2O$	$Ca(NO_3)_2$ K_2SO_4 $NaH_2PO_4 \cdot 2H_2O$ $MgSO_4 \cdot 7H_2O$ $CaCl_2 \cdot xH_2O$	$Ca(NO_3)_2$ KNO_3 $MgSO_4 \cdot 7H_2O$ $NaH_2PO_4 \cdot 2H_2O$

TABLE 2B

COMPOSITION OF NUTRIENT SOLUTIONS USED IN THE SAND CULTURE EXPERIMENT

Trace Elements (p.p.m.), After Hewitt (1952)

Ion or Element	Solution I (1963-64 Growing Season)			Solution II (1964-65 Growing Season)		
	Treatment			Treatment		
	N1	N3	N9	N0	N3	N9
Fe^{+3}	5.00	5.00	5.00	5.60	5.60	5.60
Mn^{+2}	0.55	0.55	0.55	0.55	0.55	0.55
Cu^{+2}	0.06	0.06	0.06	0.06	0.06	0.06
Zn^{+2}	0.07	0.07	0.07	0.07	0.07	0.07
B	0.37	0.37	0.37	0.37	0.37	0.37
Mo	0.02	0.02	0.02	0.02	0.02	0.02
Salts Used	FeK E.D.T.A.* $MnSO_4 \cdot 4H_2O$ $CuSO_4 \cdot 5H_2O$ $ZnSO_4 \cdot 7H_2O$ H_3BO_3 $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	As for N1	As for N1	Ferric citrate $MnSO_4 \cdot 4H_2O$ $CuSO_4 \cdot 5H_2O$ $ZnSO_4 \cdot 7H_2O$ H_3BO_3 $(Na)_2MoO_4$	As for N0	As for N0

* prepared according to Jacobson (1951)

leaf scorch of the older leaves on most trees in the N9 treatment during spring and autumn. Leaf analysis indicated that these symptoms were associated with an accumulation of Na^+ in the leaves. During the second year of the experiment $NaNO_3$ was replaced with KNO_3 and $Ca(NO_3)_2$. Resulting differences in the K^+ and Ca^{+2} content of the nutrient solutions were overcome by the addition of appropriate amounts of K_2SO_4 and $CaCl_2$. Ferric potassium ethylenediamine tetra-acetate and ammonium molybdate were also replaced by ferric citrate and sodium molybdate respectively, in order to avoid addition of traces of nitrogen to trees in the N0 treatment. Phosphorous was supplied as $NaH_2PO_4.2H_2O$ and the levels in the nutrient solutions were reduced to conform with those recommended by Hewitt (1952). Apart from those relating to nitrogen treatment, no abnormal leaf symptoms were observed in any treatment during the second year.

Nutrient solutions were prepared by diluting appropriate quantities of stock solutions, usually of molar concentration, with nitrogen-free distilled water. A.R. grade chemicals were used wherever possible. Solutions for each treatment were first prepared at ten times the required concentration in black polythene containers of 20 litre capacity. These solutions were then thoroughly mixed and suitable aliquots were diluted with distilled water prior to addition to the trees. The pH of the diluted nutrient solutions was approximately 5.6.

(2) Application of Treatments and Tree Management

First year nitrogen treatments were commenced on 27/7/63 and were continued until the end of June 1964. Nutrient applications were continued after cessation of shoot growth in March 1964 because it was thought that accumulation of nitrogen might occur in tree tissues in autumn and early winter in proportion to nitrogen supply. Two litres of nutrient solution were applied to each tree every second day, or daily during heat-wave conditions. The nutrient was poured onto the surface of each pot, allowed to percolate through the sand and the excess solution drained via a length of rubber tubing into a half-gallon polythene container (painted black except for a vertical strip which acted as a volume gauge) placed underneath each pot. When required, the leachate was remade to volume with distilled water and recycled. However, once a fortnight, the old nutrient solutions were discarded, the sand in each pot was thoroughly leached with distilled water and fresh nutrient solutions were applied.

Tree position within each replicate was changed in a random manner at monthly intervals during the growing season, but the position of replicates within the glasshouse was not altered during the experiment.

After bud burst in the first year, all shoots were removed except one which became the new stem of the tree. Buds failed to develop on 6.3% of the trees, and therefore these trees were discarded and spare trees, which had received the same nitrogen treatment, were inserted in their place. Root suckers were pinched off, but no further pruning or shaping of the tree was carried out during the experiment. The leaves which abscissed in autumn of the first year were discarded. Normal cultural practices were carried out in both years, e.g. trees were sprayed with bordeaux mixture at bud-burst and insecticides were applied when necessary.

At the end of the first year the dormant trees were allowed to over-winter in the glasshouse. During July and August 1964, distilled water only was supplied to the trees at a rate of 2 litres per tree per week. One-third of the trees were harvested in July 1964 (see later), and, in August 1964, the remainder were re-potted into fresh sand which had been sieved, acid-washed and steam-sterilised (see Table 1). Trees were repotted so that their growth response in the second year of the experiment would not be influenced by the level of nutrients which had accumulated in the sand during the first year. The sand was acid-washed according to the procedure described by Hewitt (1952). Excess acid was removed from the sand by leaching with water and with nitrogen-free nutrient solution containing 0.003$\underline{M}$ KH_2PO_4, 0.002$\underline{M}$ $MgSO_4$ and 0.045$\underline{M}$ $CaCl_2$. This latter treatment was continued until the pH of solution which passed through the sand was no longer acidic when tested with brom-cresol green indicator. It was assumed that this acid-washing treatment would remove all readily available nitrogen from the sand.

Basal portions of the root systems of many of the trees harvested at the end of the first year of the experiment were blackened. This damage was attributed to water-logging and anaerobic conditions at the base of each pot. The following steps were taken to prevent a recurrence of this problem during the second growing season. Prior to repotting, the bottom of each pot was sloped downwards towards the central drainage outlet, a layer of spongy polyurethane foam (½in. thick) was placed in the bottom of each pot in contact with a wick of the same material inserted through the drainage outlet, and a 1 inch layer of steam-sterilised, quartz gravel (3 Kg) was placed over the foam. No evidence of waterlogging was observed during the second year of the experiment. After repotting the trees, the surface of the sand in each pot was covered with a mulch of steam-sterilised quartz gravel (3 Kg).

Second year treatments were commenced on 5/9/64, when the trees were at the 'pink-tip' stage of bud burst, and were continued until all trees had been harvested in March 1965. Flower buds were removed at bud burst, and root suckers were pinched off as they developed, but this tissue was added back to the pots in an attempt to maintain the nitrogen balance. In addition, the leaves which abscissed from each tree during the second growing season were collected and placed in paper bags attached to each pot.

(iv) Seasonal Growth Measurements

Measurements were made of shoot length and stem diameter growth at 3 to 4 week intervals during both the first and second years of the experiment. During the first year, the total length of tops per tree (each shoot was measured from its base to the last node at its tip) and the stem diameter per tree at a fixed point (2 readings were made at right angles to one another at the centre of the fourth internode) were measured, while, in the second year, the total length of new shoots, the total number of new shoots, the average length of new shoots, and the stem diameter at the same fixed point were recorded for each tree.

(v) Harvesting Procedure

Apart from the trees harvested at planting (harvest 0), the following harvests were carried out during the experiment:

Harvest No.	Harvest Date	No. of Trees Harvested	Remarks
1	13/7/64 to 18/7/64	36 (3 treatments x 12 reps.)	end of 1st year; dormant, leafless trees
2	23/11/64 to 26/11/64	36 (9 treatments x 4 reps.)	midway through 2nd growing season
3	1/3/65 to 5/3/65	36 (9 treatments x 4 reps.)	end of 2nd growing season

At each harvest, trees were removed from the pots under a jet of water, the roots were thoroughly cleaned, and the trees were transferred to a cold room set at 2°C. Any root fragments which broke off were collected. Trees were only removed from the pots between 8 a.m. and noon on each day of the harvest in order to minimise possible diurnal variations in levels of nitrogenous constituents in tree tissues.

When required, each tree was removed from the cold room, weighed and quickly subdivided into the following parts:

(1) roots

(2) stock + stem + 1 year old shoots

(3) leaf + flower buds (bulked from all replicates per treatment), or new shoots

Tree tissues were wiped clean with a damp cloth when necessary, they were weighed, and the woody tissues were chopped into pieces 1 to 2 cm long with a pair of secateurs. Whenever possible, these operations were carried out on sheets of aluminium foil spread over trays of dry ice. Thus the tissue was kept cold but did not increase in fresh weight. Subdivided tissues were placed in labelled muslin bags and were stored at -20°C. If root galls were present on a tree, they were cut off and ovendried at 103°C for 16 hours. Abscissed leaf tissue was similarly treated.

At harvest, the fresh weight of each tree and its parts, and the number of shoots, leaves (including abscissed leaves), buds and flowers per tree were recorded whenever possible.

(vi) Preparation of Tree Tissues for Analysis

As soon as possible after each harvest, the frozen tissues were freezedried, weighed and chopped finely with scissors or secateurs. Any sand present in the root samples was removed by hand, weighed and appropriate corrections were made to the weight of the freeze-dried tissues. The dried tissues were then ground in a Casella grain mill to pass a sieve with pores 1 mm in diameter. No significant losses of tissue occurred during grinding. After mixing well, duplicate samples of each tissue were taken for dry matter analysis and the dry weight of each tree and its parts was calculated. The remaining tissue was placed in screw-lid jars and stored at -20°C until required. Oven-dried tissues were ground in the same way, but were stored at room temperature.

(vii) Determination of the Dry Matter Content of Tree Tissues

Duplicate samples of tissues were ovendried at 103°C for 16 hours. After cooling in a desiccator over silica gel for 1 hour, the samples were reweighed and the percentage dry matter content of the tissues was calculated.

(viii) Determination of Levels of Nitrogenous Constituents in Tree Tissues

Tree tissues were analysed in duplicate for their content of the following nitrogenous constituents (concomitant measurements were also made of the dry matter content of the tissues):

total N
nitrate N
soluble N
insoluble N
arginine N

total α-amino N

ammonium N

amide N

Results were expressed on both a concentration basis (mg N/g d.wt.) and on an absolute basis (mg N/tree part or per tree).

Abscissed leaves and root gall tissues were only analysed for total N. However, in calculating the content of nitrogenous constituents in root tissues, it was assumed, except in the case of total N, that the root gall tissues would contain the same concentration of nitrogen as the healthy root tissue. Even if this was not the case, little error is involved since the dry weight of the gall tissue per tree was usually a very small part of the total dry weight of the roots.

(1) Total N

Levels of total N in tree tissues were estimated by the micro-kjeldahl procedure recommended by McKenzie and Wallace (1954), except that the quantity of concentrated H_2SO_4 used in the digestion step was increased to 2 ml, and the distillations were carried out in an apparatus described by Jennings (1962). Determinations were carried out as follows: 25 to 50 mg dried, finely-ground plant material were weighed into a 30 ml micro-kjeldahl flask, 0.5 ml 10% HgO in $4\underline{N}$ H_2SO_4, 1.5 g K_2SO_4 and 2 ml conc. H_2SO_4 were added and the sample was digested till clear. The flask contents were then heated for a further 15 minutes to ensure that digestion was complete. After cooling and diluting with 10 ml distilled water, the digest was poured into the steam distillation apparatus (the flask was rinsed with 10 ml distilled water and this was also added to the distillation apparatus), 15 ml conc. $NaOH + Na_2S_2O_3$ reagent were added, and the ammonia was distilled into 5 ml 1% H_3BO_3 solution (containing methyl red and brom cresol green indicators). The ammonium N content of the distillate was determined by titration with 0.01N $KH(IO_3)_2$.

The standard deviation from the mean (calculated from the harvest 1 data) was 1.65% and the recovery of added $(NH_4)_2SO_4$, with or without plant material, was of the order of 98 to 99%. It should be noted that this procedure accounts for the nitrogen present in organic compounds and in ammonia but does not account for nitrogen as nitrate (Boynton *et al.* 1953).

(2) Nitrate Nitrogen

Analyses were carried out according to the phenol-disulphonic acid procedure of Humphries (1955). Low recoveries (of the order of 70%) of added nitrate were obtained in the presence of plant extracts, probably because of incomplete removal of organic matter from extracts prior to colour development (Humphries, personal communication). The standard deviation from the mean was ±2.26% (7 d.f.).

Nitrates were extracted from 0.1g samples of tree tissues with 10 ml NaH_2PO_4-$AgSO_4$ reagent and 1 to 2 ml aliquots of the extracts were analysed. After colour development, the optical density of the solutions was read against distilled water in a Unicam spectrophotometer set at 400 mμ. Standard nitrate solutions were included in each analysis to cover the range 0 to 20 μg N as nitrate.

(3) Soluble Nitrogen

A preliminary experiment was carried out to determine whether the amount of soluble N extracted from freezedried peach tissue would be dependent upon the extractant used. This experiment is described in detail in Appendix A.

In agreement with other workers (Stuart 1935; Oland and Yemm 1956; Oland 1959), it was found that aqueous solutions extracted significantly greater quantities of soluble N from the tissue (one-year old peach shoots) than did 80% aqueous ethanol. Phosphate buffer (0.1M, pH 7.0) and citrate buffer (0.05M, pH 5.0) were equally efficient at extracting soluble N from the tissue, but citrate buffer was chosen for routine extractions since the final extracts were not as highly coloured as were those carried out with phosphate buffer. The procedure was as follows:

½ to 1 g lots of freezedried ground tissue were weighed accurately into 100 ml centrifuge tubes fitted with rubber bungs. 54 ml cold (2°C) buffer were added to each tube, and after shaking vigorously, the extraction was allowed to proceed for 24 hours at 2°C (the extracts were occasionally shaken during this time). The extract was centrifuged and the supernatant was poured through a glass wool filter into a 250 ml volumetric flask. This procedure was repeated 3 times except that the extraction time was reduced to 1 hour. Successive extracts were bulked and made to volume with citrate buffer. After mixing, 75 to 100 ml aliquots of each extract were taken to dryness in vacuo in a rotery evaporator (water-bath temperature 35°C) and each residue was taken up in distilled water and made to volume in a 10 ml volumetric flask. The level of soluble N in 2 ml aliquots was determined by micro-kjeldahl analysis using the same method and equipment as described for total N analyses. However, a preliminary heating step was necessary before digestion (extract acidified first with 0.5 ml conc. H_2SO_4) to reduce the volume of the liquid in the flask, otherwise the extracts frothed badly during digestion and nitrogen was lost. The standard deviation from the mean was ±1.06% (calculated on the harvest 1 data).

(4) Insoluble Nitrogen

The concentration of insoluble N in the tissues was found by calculating the difference between the concentrations of total N and soluble N in each tissue.

(5) Arginine Nitrogen

The concentration of arginine N in the citrate buffer extracts was estimated by the method of Gilboe and Williams (1956). A straight-line relationship between optical density and the amount of arginine N in the solution only held over the range 0 to 10 µg N, and it was therefore necessary to adjust the concentration of arginine N in the test solution to fall within this range. Satisfactory readings were usually obtained if 0.25 to 1.0 ml aliquots of the extracts were diluted to 5 ml with distilled water.

Colour development was carried out in a cold room at 2°C and the optical density of the solutions was read versus distilled water at 506 mμ. These readings were made at room temperature as soon as possible after colour development. In some cases it was necessary to further dilute the extracts with distilled water before analysis otherwise pigments present in the extracts interfered with the test. In these circumstances blank values were high and the sensitivity of the method was reduced. Attempts were made to remove the pigments from the extracts with organic solvents or by treating them with cation exchange resin (Amberlite IR-120, H^+, 20 to 50 mesh) or decolorising charcoal, but without success. The optical density of extract blanks (8-hydroxyquinoline was replaced with an equivalent volume of distilled water) was used to correct the readings for any colour interference.

The standard deviation from the mean was ±5.57% (calculated on the harvest 1 data).

(6) Total α-Amino Nitrogen

The concentration of total α-amino N in the citrate buffer extracts was determined by the method of Rosen (1957). 0.5 to 2.0 ml aliquots of each extract, containing approximately 2 μg N, were pipetted out, made to a volume of 2.0 ml with citrate buffer pH 5.0, and the concentration of total α-amino N in these solutions was measured. Interference from coloured pigments in the citrate extracts was negligible. A series of standards, which covered the range 0 to 4.2 µg N per 8 ml solution, were included in each analysis. These standards were made by diluting a 2×10^{-4} M solution of D-α-alanine (B.D.H. laboratory reagent) with citrate buffer pH 5.0.

In order to achieve good replication and low blank values, the following procedure was followed during colour development. Each test tube was placed in, and removed from, the boiling water bath at 15 second intervals. On removal of each test tube from the water-bath, the tube contents were immediately diluted with 5 ml 50% aqueous isopropanol and shaken vigorously. The solutions were allowed to cool for 2 hours, and then their optical density was read at 570 mµ versus distilled water in a Unicam spectrophotometer.

The methyl cellosolve was redistilled and tested for clarity and peroxide content before use (Yemm and Cocking 1955), and, since the acetate-cyanide reagent is unstable (Grant 1963), it was freshly prepared before each analysis.

The standard deviation from the mean was ±1.59% (calculated on the harvest 1 data).

(7) Ammonium Nitrogen

Cold (2°C), 80% aqueous ethanol was used to extract ammonium N from freezedried tissues. Anomalous results were obtained if the tissues were extracted with cold 0.01N HCl or distilled water probably because the tissue 'broke down' if extracted with these solvents, thereby releasing previously bound ammonium N. Extractions were carried out in the same way as described earlier for soluble N except that the initial extraction period was for 4 hours and a total of 200 ml 80% ethanol was used per extraction. The ethanol was redistilled before use. Each extract was acidified with a few drops of 0.1N HCl (tested with bromphenol blue indicator) and taken to dryness in vacuo in a rotary evaporator (water-bath set at 35°C). A few drops of A.R. n-octyl alcohol were added to each extract to prevent 'bumping' during concentration. The residue was taken up in distilled water and made to volume in a 25 ml volumetric flask. The concentration of ammonium N in each extract was determined on a 10 ml aliquot by the method of Pucher et al. (1935), except that the Nessler's reagent was prepared and used as described by Jennings (1962). The standard deviation from the mean was ±0.93% (calculated on the harvest 1 data) and the recovery of added ammonium N in the presence of plant extracts was 102.1%.

(8) Amide Nitrogen

The concentration of amide N in tree tissues was estimated by analysis of the same extracts as were used for the ammonium N determinations. Analyses were carried out on 5 ml aliquots of the concentrated extracts by the method of Pucher et al. (1935), except that the Nessler's reagent was prepared and used as described by

Jennings (1962). The standard deviation from the mean was ±8.32% (calculated on the harvest 1 data).

(c) Results

Since the results recorded for trees infected with root galls generally fell within the range of values for healthy trees, the data from the 2 groups was bulked prior to statistical analysis.

Statistical analyses were usually carried out with the aid of a computer and examples of the computer printouts are given in Appendix C, section (a)(iii).

(i) The Nitrogen Content per Tree at Planting and the Influence of Tree Size at Planting on Subsequent Tree Growth

As expected, a significant positive correlation ($r = 0.78$) was found between the nitrogen content per tree at planting and the fresh weight per tree at planting (Fig.1), i.e. the nitrogen content per tree at planting increased with increasing tree size. No nitrate N was detected in the tree tissues.

Since it was thought that tree size at planting would influence subsequent tree growth, irrespective of the nitrogen treatment applied, the relationship between tree size at planting and tree size at the end of the first year of the experiment was examined statistically by simple regression and covariance analysis. The results are summarized in Table 3. The covariance analyses did not result in any significant adjustment to either the treatment

FIGURE 1 Relationship between the fresh weight per tree and the total nitrogen content per tree at planting.

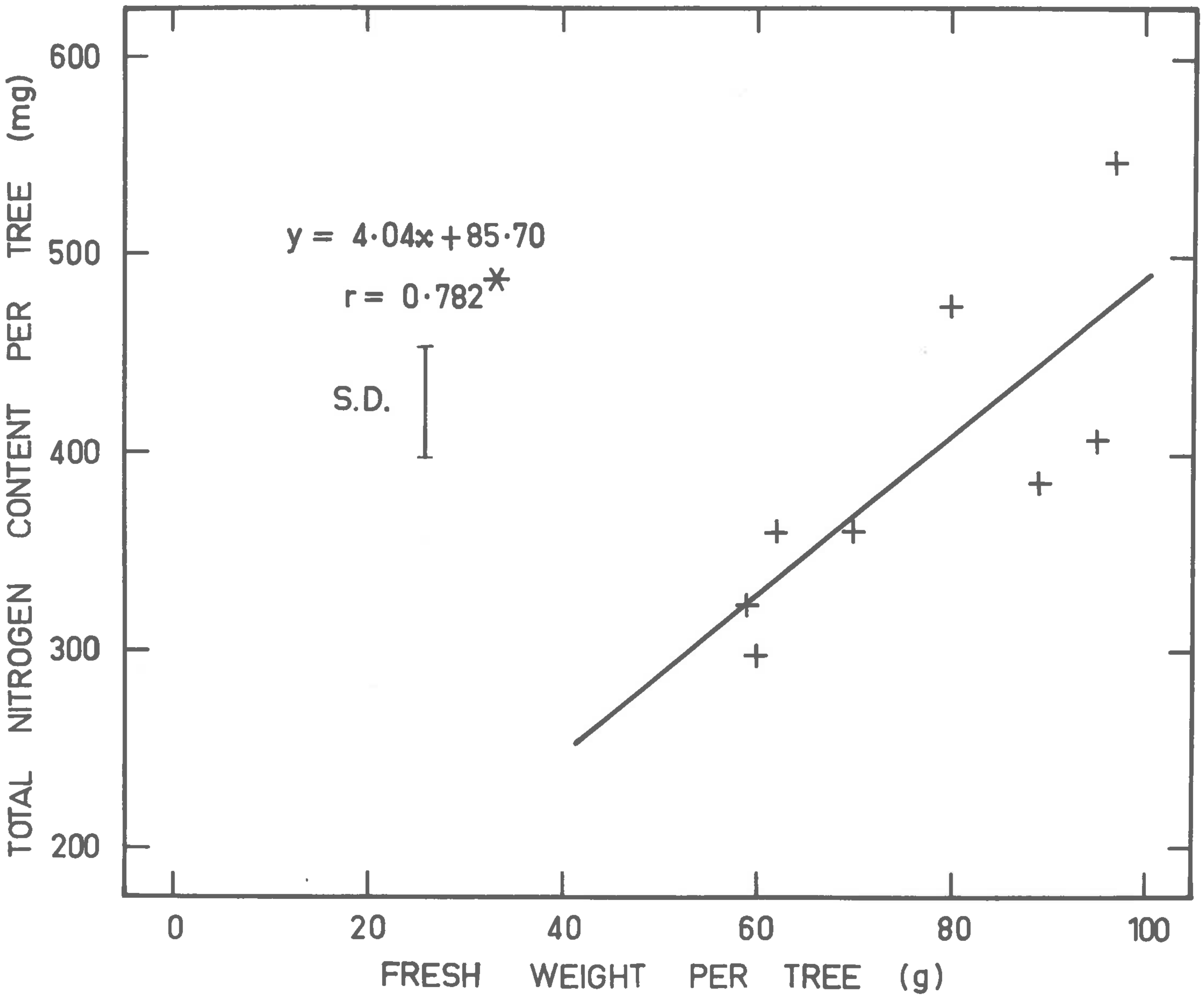

y = 4·04x + 85·70
r = 0·782*
S.D.
TOTAL NITROGEN CONTENT PER TREE (mg)
200
300
400
500
600
0
20
40
60
80
100
FRESH WEIGHT PER TREE (g)

TABLE 3

SUMMARY OF RESULTS OF SIMPLE REGRESSION AND COVARIANCE ANALYSES OF THE RELATIONSHIP BETWEEN TREE SIZE AFTER 1 YEAR AND TREE SIZE AT PLANTING

Log_{10} Data

Parameter of Tree Size After 1 Yr <u>V.</u> Parameter of Tree Size at Planting	Correlation Coefficient (34 d.f.) for Trees Receiving:			Pooled Correlation Coefficient	Parallel Correlation Coefficient*	Significance of Covariance Analysis
	N1	N3	N9	(106 d.f.)	(104 d.f.)	
Fresh weight/tree after 1 yr V. fresh weight/tree after pruning at planting	0.454^{xx}	0.433^{xx}	0.122^{NS}	0.092^{NS}	0.301^{NS}	No adjustment to treatment variance or error variance
Total length of tops/tree after 1yr V. total length of top prunings/tree at planting	-	-	-	-	0.431^{xxx}	No adjustment to treatment variance; slight adjustment to error variance
Stem diameter/tree after 1yr V. stem diameter/tree at planting	-	-	-	-	0.232^{x}	No adjustment to treatment variance or error variance
Total length of tops/tree after 1yr V. fresh weight/tree at planting	0.611^{xxx}	0.366^{x}	-0.078^{NS}	0.133^{NS}	0.359^{xxx}	Not tested
Stem diameter/tree after 1 yr V. fresh weight/tree at planting	0.379^{x}	0.168^{NS}	-0.034^{NS}	0.020^{NS}	0.203^{x}	Not tested
Fresh weight/tree after 1yr V. total length of top prunings/tree at planting	0.293^{NS}	0.502^{xx}	0.167^{NS}	0.177^{NS}	0.293^{xx}	Not tested
Fresh weight/tree after 1yr V. stem diameter/tree at planting	0.401^{x}	0.425^{xx}	0.103^{NS}	0.104^{NS}	0.275^{xx}	Not tested
Fresh weight/tree after 1yr V. fresh weight/tree before pruning at planting	0.476^{xx}	0.453^{xx}	0.179^{NS}	0.123^{NS}	0.342^{xxx}	Not tested
Fresh weight/tree after 1yr V. fresh weight top prunings/tree at planting	0.438^{xx}	0.489^{xx}	0.159^{NS}	0.117^{NS}	0.336^{xxx}	Not tested

* Slopes of lines adjusted parallel (influence of treatments removed): indicative of possible success of covariance analysis.

variance or to the error variance, and the pooled correlation coefficients were not significant for any of the pairs of parameters tested. However, when the data was examined separately for each nitrogen treatment, significant correlations were found between tree size at planting and tree size after 1 year in the N1 and N3 treatments but not in the N9 treatment. That is, the influence of initial tree size on subsequent growth was only important if small quantities of nitrogen were applied to the trees during the growing season. It is possible that the initial tree size influenced growth in the N9 treatment as well but that its effect was small and was masked by the large growth response to the external nitrogen application. Presumably the influence of initial tree size on subsequent tree growth is a function of the nitrogen content per tree at planting. However, since the trees had been stratified according to their fresh weight at planting, prior to application of the nitrogen treatments, the influence of initial tree size on subsequent growth was minimised.

(ii) Tree Growth During the First Year of the Experiment

Nitrogen deficiency symptoms were first observed on trees in the N1 and N3 treatments in November 1963, and, by the end of the growing season, the symptoms were pronounced. As would be expected, the symptoms were more severe on trees in the N1 treatment than on trees in the N3 treatment. No deficiency symptoms were observed on trees in the N9 treatment. Deficient trees were small, with few side shoots (these were slender and often reddish in colour), the foliage was sparse, and the leaves were small and greenish-yellow in colour.

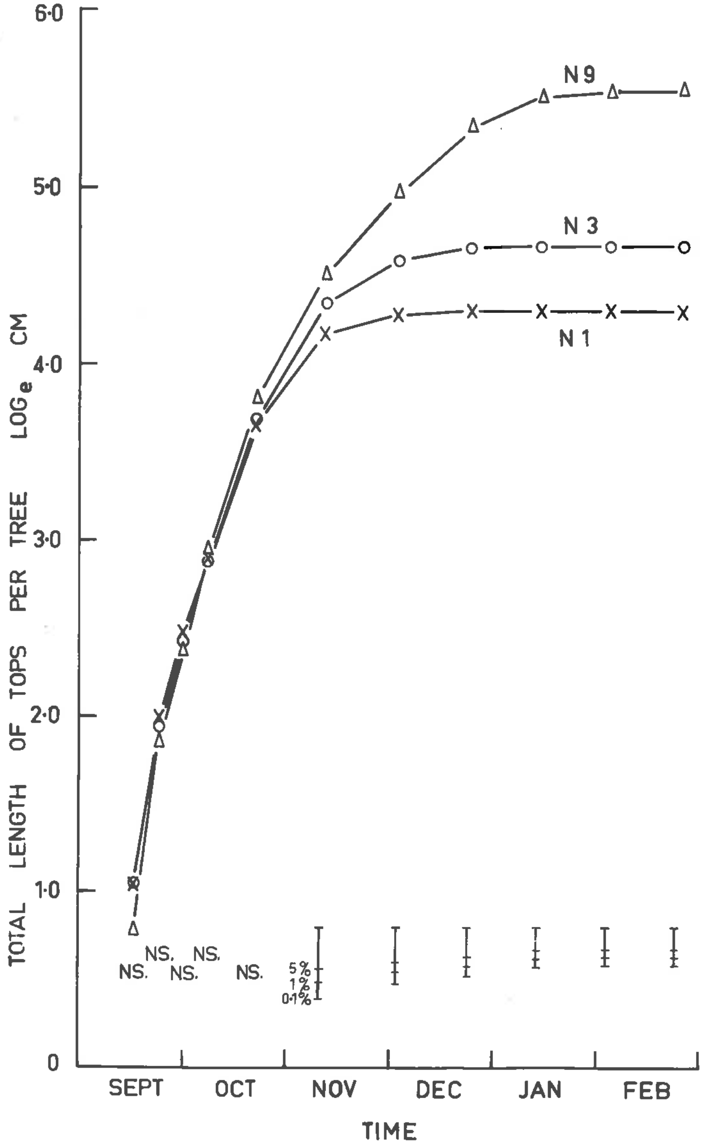
(1) Seasonal Changes in Tree Growth

The changes which occurred in the total length of tops per tree and the stem diameter per tree during the first year of the experiment are shown in Fig.2. The graphs were not extrapolated to zero at the beginning of the growing season because the data is expressed on a logarithmic basis. Significant differences between treatments were first observed for both types of growth in early November. Subsequently, as the growing season progressed, the amount of tree growth made was in proportion to nitrogen supply, and, by the end of the growing season, all treatments were significantly different at the 0.1% level. It is of interest to note that little increase occurred in the stem diameter per tree during late autumn and winter.

(2) Harvest 1 Data

The appearance and overall size of the trees at harvest 1 is shown in Plate 2, while data illustrating the influence of nitrogen treatment on tree growth at harvest 1 is given in Tables 4A and 4B. It is evident that growth of the trees, or their parts, when expressed in terms of fresh weight or dry weight, was in proportion to the nitrogen supply and that differences between all treatments

FIGURE 2 Influence of nitrogen treatment on the total length of tops per tree and the stem diameter per tree during the first growing season.


TOTAL LENGTH OF TOPS PER TREE LOG$_e$ CM
6·0
5·0
4·0
3·0
2·0
1·0
0
N 9
N 3
N 1
NS.
NS.
NS.
NS.
NS.
5%
1%
0·1%
SEPT
OCT
NOV
DEC
JAN
FEB
TIME

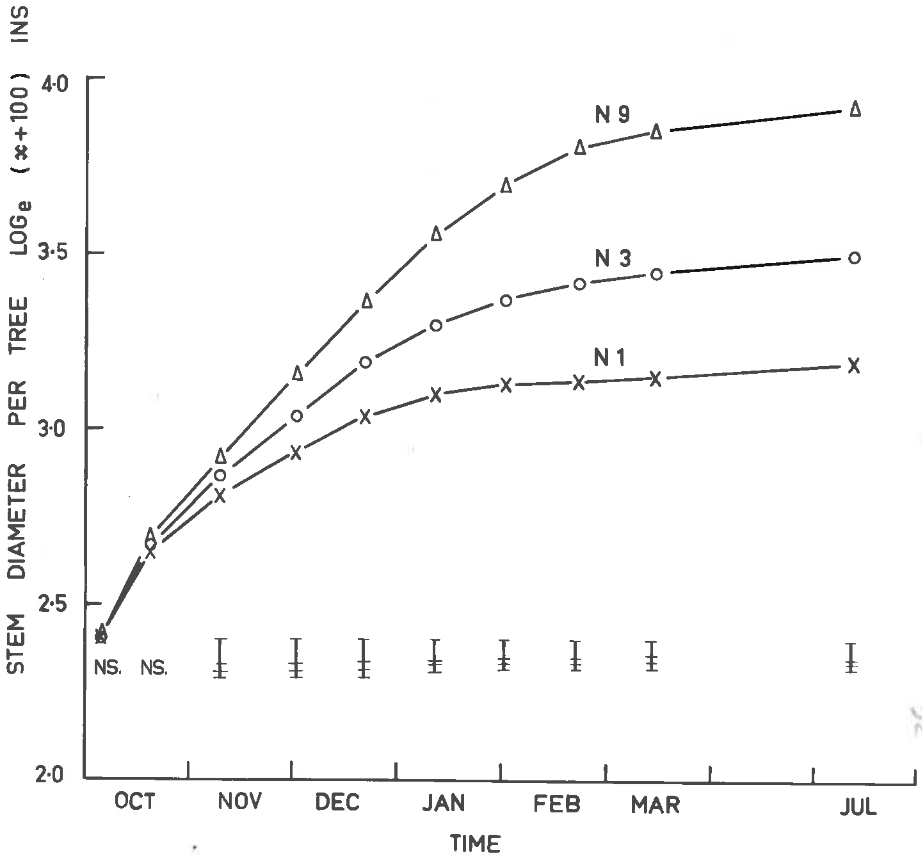

STEM DIAMETER PER TREE LOG$_e$ (x+100) INS
4·0
3·5
3·0
2·5
2·0
N 9
N 3
N 1
NS.
NS.
OCT
NOV
DEC
JAN
FEB
MAR
JUL
TIME

PLATE 2 Overall size and appearance of trees at each harvest:

top picture - harvest 1 (July 1964)
middle horizontal row - harvest 2 (November 1964)
bottom horizontal row - harvest 3 (March 1965)

Nitrogen treatments are indicated on cards at the base of the pots: first year treatment, left of oblique stroke; second treatment, right of oblique stroke. The vertical measure is 2 feet high.


N1/-
N3/-
N9/-


N1/N0
N1/N3
N1/N9
N3/N0
N3/N3
N3/N9
N9 N0
N9 N3
N9 N9
N1 N0
N1 N3
N1/N9
N3/N0
N3 N3
N3 N9
N9 N0
N9 N3
N9 N9

TABLE 4A

INFLUENCE OF NITROGEN TREATMENT ON TREE GROWTH AT HARVEST 1

N Treatment	F.Wt. Roots ($\log_e$ g)	F.Wt. Stock + Stem + Side Shoots* ($\log_e$ g)	F.Wt. Buds ($\log_e$ g)	F.Wt. Tree ($\log_e$ g)	D.Wt. Roots ($\log_{10}$ g)	D.Wt. Stock + Stem + Side Shoots* ($\log_{10}$ g)	D.Wt. Buds ($\log_{10}$ mg)	D.Wt. Tree ($\log_{10}$ g)	D.Wt. Tops / D.Wt. Roots	No. Shoots ($\log_{10}$)	No. Leaves^{+} ($\log_{10}(x+1)$)	No. Flower Buds ‡ ($\log_{10}$)	No. Leaf Buds ‡ ($\log_{10}$)	No. Flowers § ($\log_{10}$)
N1	5.334	3.826	0.012	5.540	1.828	1.397	2.731	1.968	0.38	0.354	1.818	1.475	1.515	0.537
N3	5.684	4.271	0.495	5.907	1.994	1.589	2.922	2.142	0.41	0.502	1.965	1.549	1.639	0.536
N9	6.054	5.123	1.261	6.393	2.196	1.941	3.249	2.392	0.57	0.555	2.313	1.884	1.984	0.599
L.S.D. 5%	0.109	0.121	0.209	0.101	0.046	0.059	0.095	0.045	0.050	0.077	0.032	0.065	0.041	NS
1%	0.148	0.164	0.283	0.137	0.062	0.079	0.128	0.060	0.068	0.102	0.043	0.086	0.054	
0.1%	0.198	0.220	0.379	0.183	0.083	0.106	0.172	0.081	0.091	0.131	0.055	0.111	0.070	

* Weight of buds excluded

\+ Abscissed prior to Harvest 1

‡ Counted in July 1964

§ Counted at 'pink-tip' stage of bud-burst (early September 1964)

TABLE 4B

INFLUENCE OF NITROGEN TREATMENT ON THE DRY MATTER CONTENT OF TREE TISSUES AT HARVEST 1

Values relative to the N1 Treatment (%).

N Treatment	D. wt. Roots	D. wt. Stock + Stem + Side Shoots	D. wt. Leaf + Flower Buds	D. wt Tree
N1	100.0	100.0	100.0	100.0
N3	145.1	153.3	158.9	147.4
N9	230.9	342.8	323.2	261.6

were significant. As the nitrogen supply was increased, the top/root ratio on a dry weight basis was also increased, i.e., the growth of the tops increased at a faster rate than the growth of the roots (see Table 4B). Furthermore, the number of shoots, leaves, flower buds and leaf buds per tree were all significantly increased with increasing nitrogen supply. However, there was no significant difference between the number of flowers per tree in September 1964. This result is surprising in view of the dependence of the flower-bud numbers per tree on the nitrogen supply, but little importance can be attributed to it because the trees were repotted subsequent to the date on which the flower-bud numbers were recorded but prior to the date on which the flower numbers were recorded. It is possible that many flower buds were damaged during repotting, despite the care taken, and that these buds later abscissed. Alternatively, the flower buds could have abscissed because of inadequate chilling in winter (the trees were left in the glasshouse over winter), or because $NaNO_3$ was used as the nitrate source, thus resulting in a possible toxic accumulation of Na^+ in the buds.

(iii) Tree Growth During the Second Year of the Experiment

(1) Seasonal Changes in Tree Growth

The seasonal changes which occurred in the total length of new shoots, the stem diameter, the total number of new shoots, and the average length of the new shoots, all on a per tree basis, are shown in Figs.3 to 6. In each case, the interaction means and

FIGURE 3 Influence of nitrogen treatment on the total length of new shoots per tree during the second growing season.

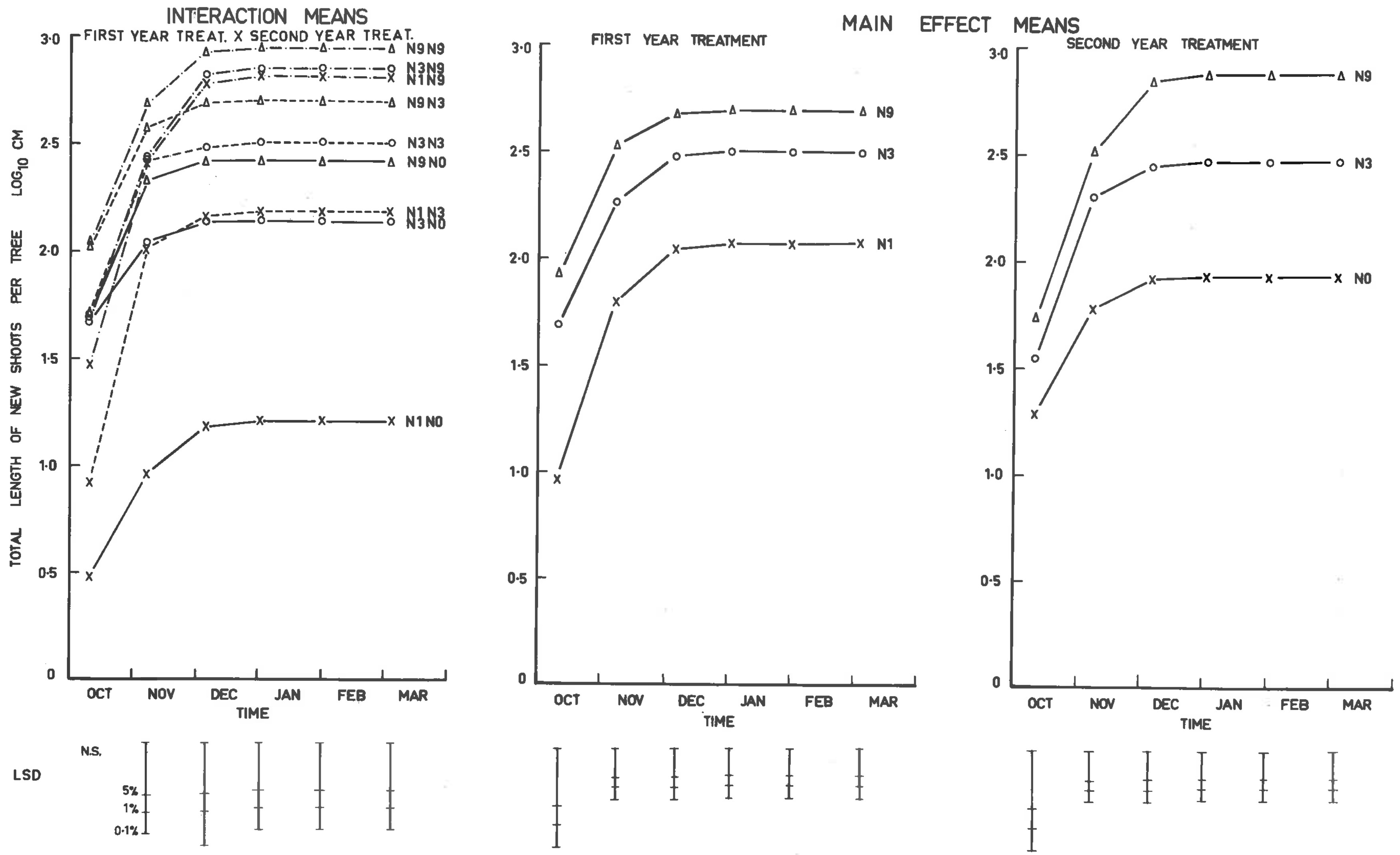

INTERACTION MEANS
FIRST YEAR TREAT. X SECOND YEAR TREAT.
TOTAL LENGTH OF NEW SHOOTS PER TREE LOG10 CM
N9 N9
N3 N9
N1 N9
N9 N3
N3 N3
N9 N0
N1 N3
N3 N0
N1 N0
MAIN EFFECT MEANS
FIRST YEAR TREATMENT
N9
N3
N1
SECOND YEAR TREATMENT
N9
N3
N0
3·0
2·5
2·0
1·5
1·0
0·5
0
OCT
NOV
DEC
JAN
FEB
MAR
TIME
LSD
N.S.
5%
1%
0·1%

FIGURE 4 Influence of nitrogen treatment on the stem diameter per tree during the second growing season.

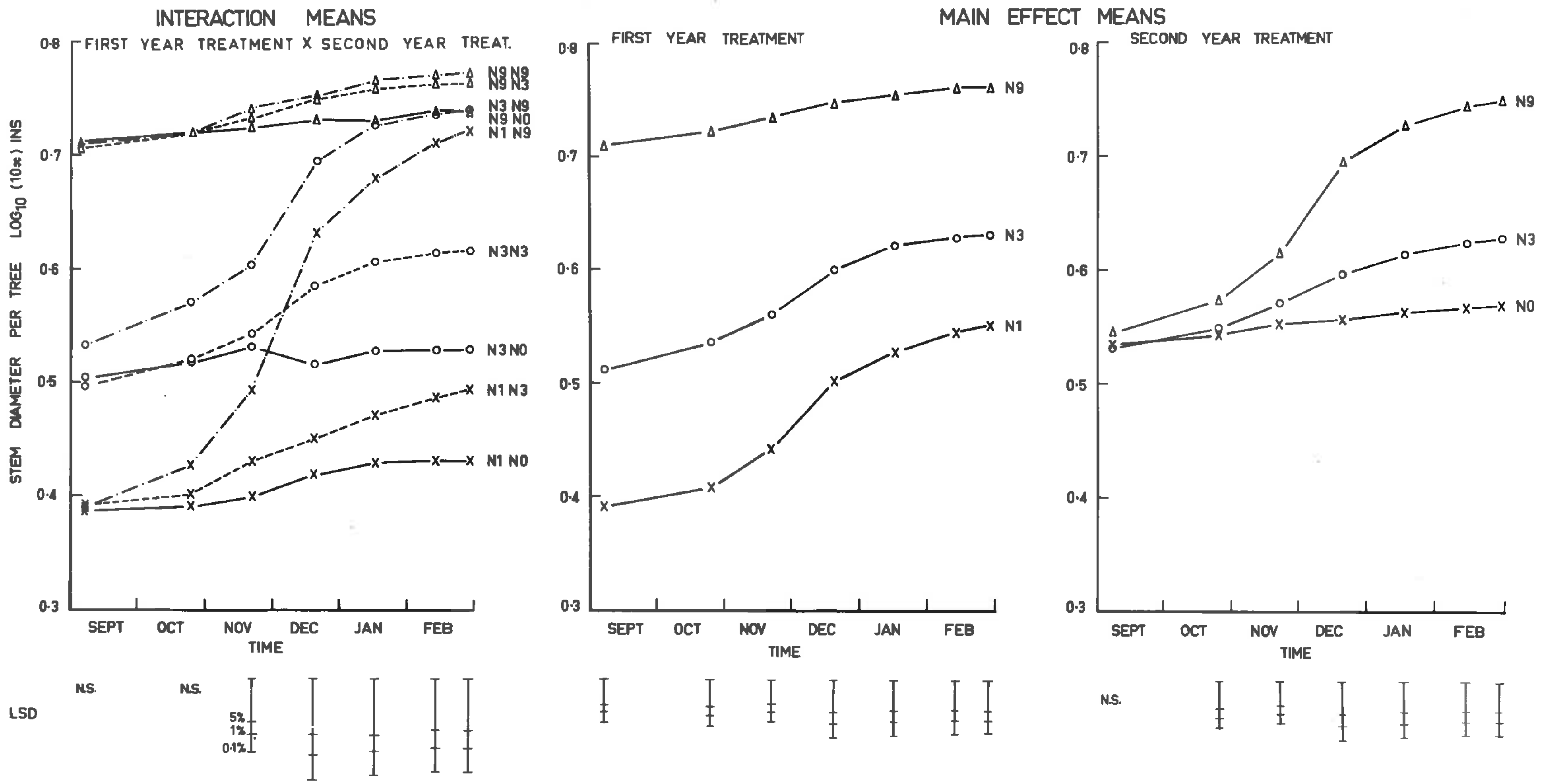

INTERACTION MEANS
FIRST YEAR TREATMENT X SECOND YEAR TREAT.
MAIN EFFECT MEANS
FIRST YEAR TREATMENT
SECOND YEAR TREATMENT
STEM DIAMETER PER TREE LOG10 (10cc) INS
0·8
0·7
0·6
0·5
0·4
0·3
N9 N9
N9 N3
N3 N9
N9 N0
N1 N9
N3N3
N3 N0
N1 N3
N1 N0
N9
N3
N1
N9
N3
N0
SEPT
OCT
NOV
DEC
JAN
FEB
TIME
LSD
N.S.
N.S.
5%
1%
0·1%
N.S.

FIGURE 5 Influence of nitrogen treatment on the total number of new shoots per tree during the second growing season.

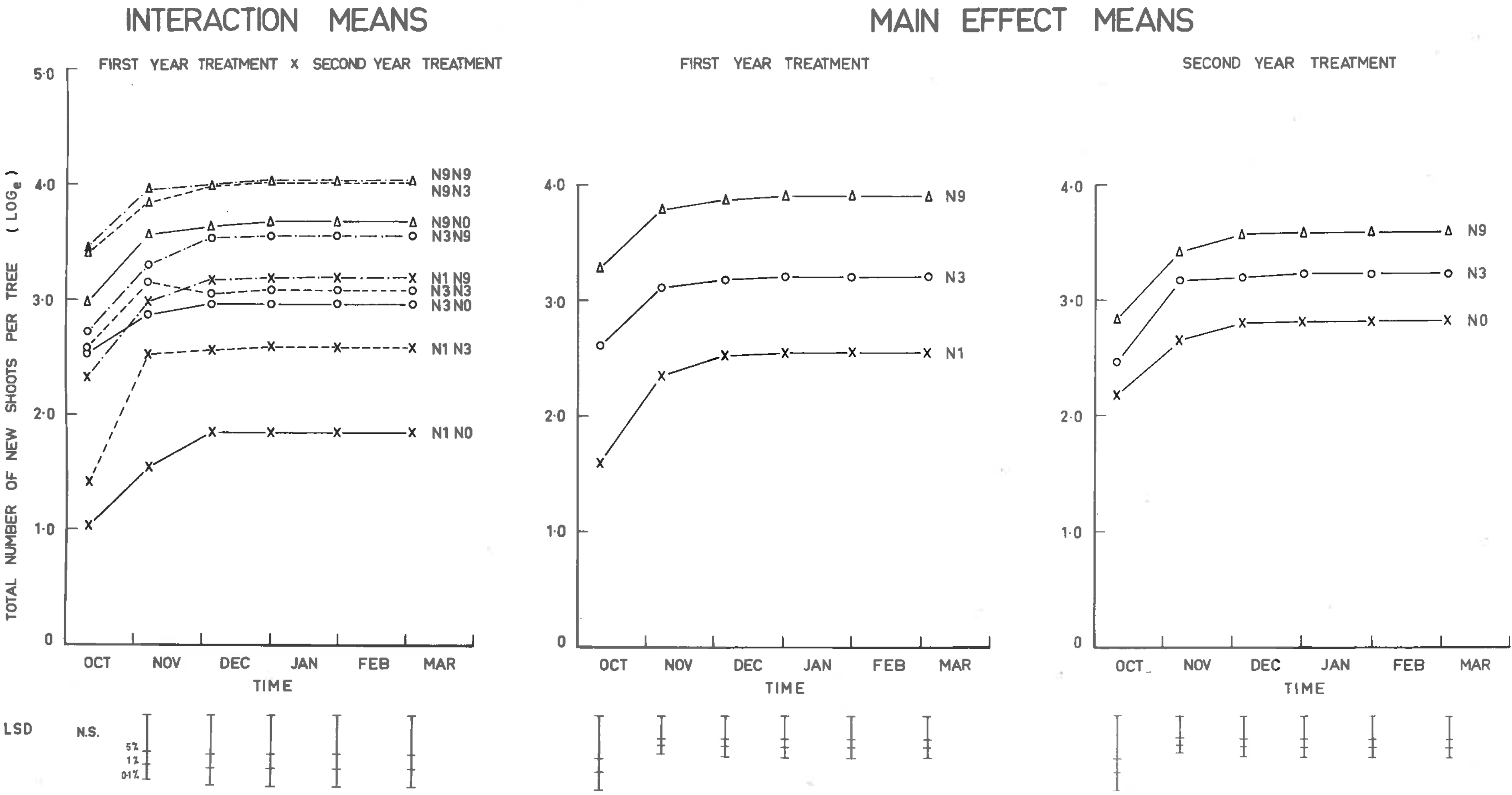
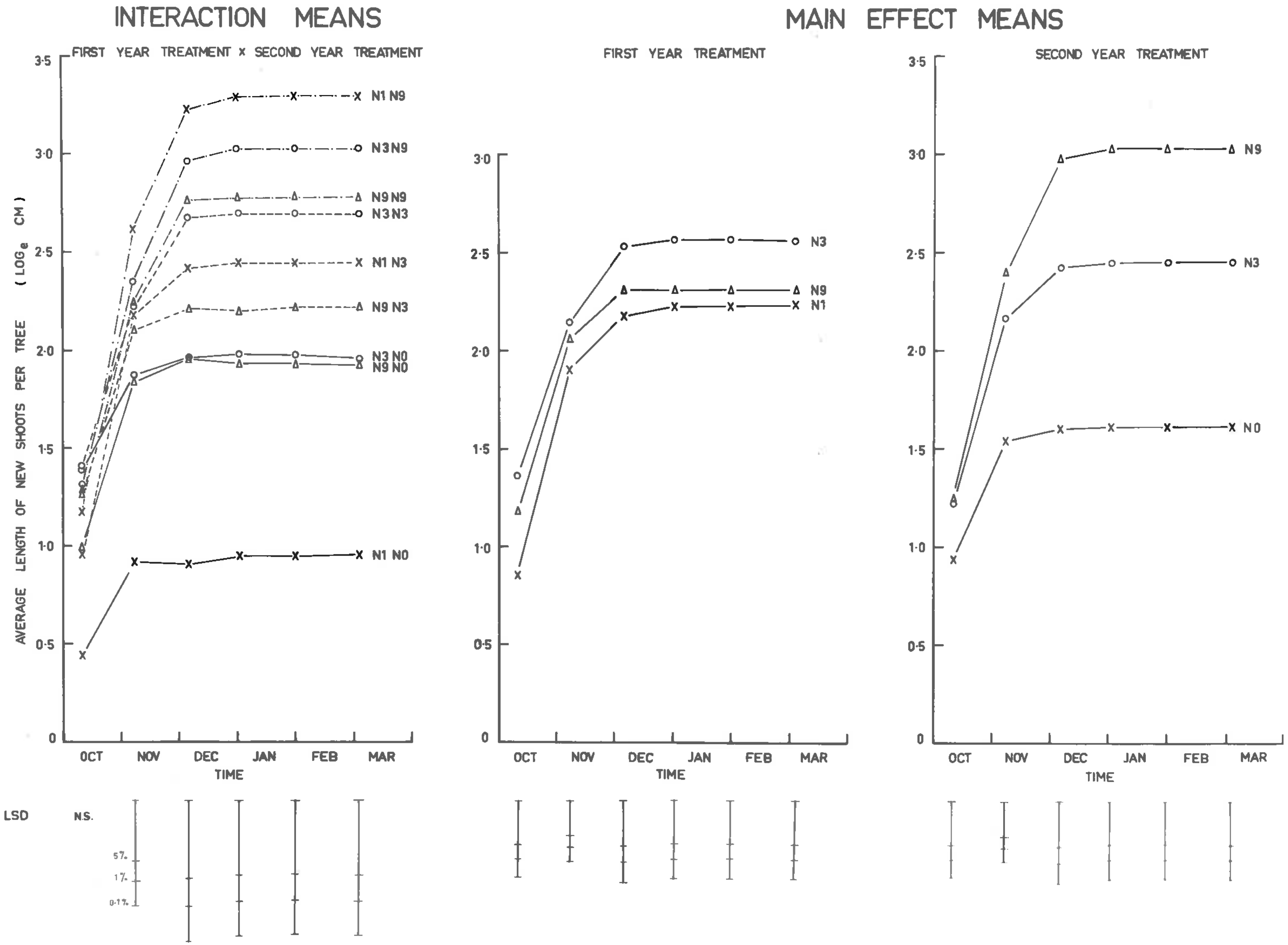

INTERACTION MEANS
FIRST YEAR TREATMENT x SECOND YEAR TREATMENT
TOTAL NUMBER OF NEW SHOOTS PER TREE (LOG$_e$)
5·0
4·0
3·0
2·0
1·0
0
N9 N9
N9 N3
N9 N0
N3 N9
N1 N9
N3 N3
N3 N0
N1 N3
N1 N0
OCT
NOV
DEC
JAN
FEB
MAR
TIME
LSD
N.S.
5%
1%
0·1%
MAIN EFFECT MEANS
FIRST YEAR TREATMENT
4·0
3·0
2·0
1·0
0
N9
N3
N1
OCT
NOV
DEC
JAN
FEB
MAR
TIME
SECOND YEAR TREATMENT
4·0
3·0
2·0
1·0
0
N9
N3
N0
OCT
NOV
DEC
JAN
FEB
MAR
TIME

FIGURE 6 Influence of nitrogen treatment on the average length of new shoots per tree during the second growing season.


INTERACTION MEANS
FIRST YEAR TREATMENT x SECOND YEAR TREATMENT
MAIN EFFECT MEANS
FIRST YEAR TREATMENT
SECOND YEAR TREATMENT
AVERAGE LENGTH OF NEW SHOOTS PER TREE (LOGe CM)
0
0·5
1·0
1·5
2·0
2·5
3·0
3·5
OCT
NOV
DEC
JAN
FEB
MAR
TIME
N1 N9
N3 N9
N9 N9
N3 N3
N1 N3
N9 N3
N3 N0
N9 N0
N1 N0
N3
N9
N1
N9
N3
N0
LSD
N.S.
5%
1%
0·1%

the main effects means, for both the first and second year treatments, have been plotted versus time for all nitrogen treatments. The main effect means, which indicate how important the first and second year treatments are for tree growth during the second year of the experiment, should only be referred to when there are no significant differences between the interaction means of the treatments, since, theoretically, there should be no main effects if there are significant differences between the interaction means. The graphs which illustrate the seasonal changes in the length and number of new shoots per tree for each treatment (Figs. 3, 5, 6) were not extrapolated to zero at the beginning of the growing season because the data is expressed on a logarithmic basis.

It is evident that the total length of new shoots per tree at the beginning of, and at the end of, the second growing season was dependent upon the level of the current nitrogen application and upon the nitrogen treatment applied in the first year of the experiment (Fig. 3). Thus maximum growth during the second year was in proportion to the level of nitrogen supplied to the trees in the first year if the level of nitrogen supplied during the second year was constant. This result supports the hypothesis that young peach trees accumulate nitrogen within their tissues according to nitrogen supply and that this nitrogen is used in tree growth during the next growing season. Analysis of data recorded at the end of the growing season showed that there was a significant negative interaction between the first and second year treatments, i.e., the greater the nitrogen supply in the first year the smaller was the effect of nitrogen application in the second year on the total length of new shoots produced per tree and vice versa. At the beginning of the second growing season, the influence of the first year treatment on the amount of new shoot growth made per tree was more pronounced than that of the second year treatment (c.f. the main effect means Fig.3). However, since the application of the second year treatments resulted in significant differences in tree growth at this time, e.g. trees in the N9 treatment made significantly more growth than those in the N0 treatment, it is clear that nitrogen was taken up by tree roots in early spring and that tree growth made early in the growing season was not entirely at the expense of storage nitrogen.

Several of the growth curves, which illustrate how the interaction means of the treatments change with time, crossed over during November (see Fig.3), e.g. the curve for trees in the N3N9 treatment crossed over and rose above that for trees in the N9N3 treatment. These crossovers were taken to indicate that there was a rapid uptake of external nitrogen during this month and that this nitrogen had a pronounced effect on tree growth. Prior to this time, much of the new growth would have been made at the expense of the nitrogen which had accumulated within tree tissue during the first year of the experiment. It is interesting to note, however, that the nitrogen treatments did not influence the length of the growing season, i.e., the time at which the growth curves reached their maximum value.

The seasonal changes which occurred in stem diameter and total number of new shoots per tree (Figs.4 and 5) also provided evidence to support the contention that tree growth made during the second year of the experiment was dependent upon, and in proportion to, levels of both storage nitrogen and external nitrogen supply. As in the case of the shoot length data reported above, the number of new shoots per tree and the stem diameter early in the growing season were dependent upon both first and second year treatments, especially the former. However, at the end of the growing season, the values obtained were in proportion to the level of nitrogen supplied in both the first and second year treatments. There was also a significant negative interaction between the first and second year treatments for both groups of data at the end of the growing season. The values recorded for stem diameter in September were dependent upon the first year treatments only. This is probably due to the fact that second year treatments had been started only 2 days before the measurements were made.

In comparison with the above results, the seasonal trends in the average length of new shoots per tree were somewhat different (Fig.6). Throughout the growing season, the average shoot length per tree was dependent upon the nitrogen treatment applied in both first and second years of the experiment but was not always proportional to the level of nitrogen supplied. For example, at the end of the growing season, the average shoot length per tree in the N1N9 treatment was significantly greater than in the N9N9 treatment. Thus the average shoot length per tree at the end of the growing season was proportional to the nitrogen treatment applied in the second year but was not proportional to the nitrogen treatment applied in the first year. Since it would be expected that the number of shoots produced per tree during the second growing season would be dependent upon the number of buds present on the tree at the end of the first year, it was thought that the average shoot length per tree during the second year would be dependent upon inter-shoot competition for the available nitrogen. The relationship between the average shoot length per tree, the total number of shoots per tree, and the nitrogen treatment, at the end of the second growing season, is shown in Fig.7. It is evident that when the nitrogen supply was high and constant during the second year, the average shoot length per tree declined with increasing nitrogen supply during the first year (c.f. N1N9, N3N9 and N9N9). However, when the nitrogen supply in the first year was increased, this resulted in an increased number of shoots per tree at the end of the second year (c.f. the same treatments). If the level of nitrogen supplied during the second year was constant but of a lower magnitude (N0 series or N3 series), then the average shoot length per tree either increased or decreased with increasing nitrogen supply in the first year,

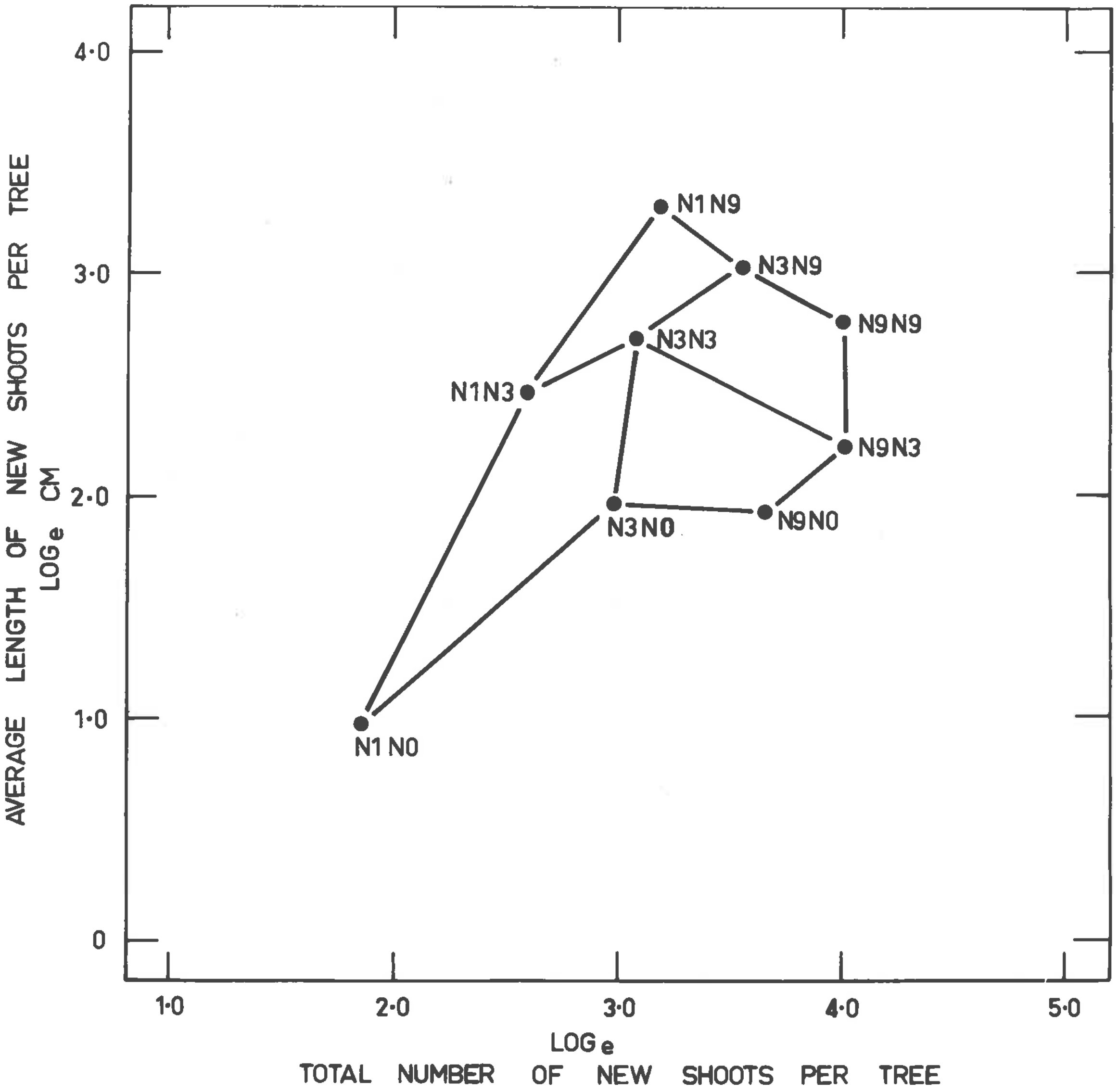


FIGURE 7 The relationship between average shoot length per tree, total number of new shoots per tree, and nitrogen treatment, at the end of the second growing season (harvest 3).

depending on whether the nitrogen supply to the tree was limiting or whether there was severe internal competition for the available nitrogen. Thus the average shoot length per tree at the end of the second year was dependent upon the level of nitrogen supplied to the trees and the number of shoots present on the trees.

(2) Harvest 2 Data

The appearance and overall size of the trees at harvest 2 is shown in Plate 2. It will be noted that the size of tree tops was dependent on, and in proportion to, the level of nitrogen supplied in both first and second years of the experiment. The top growth made by the trees in the N1N9 and N3N9 treatments was especially extensive, indicating a rapid recovery from nitrogen deficiency. Foliage colour also differed between treatments and was clearly dependent upon the external nitrogen supply. It ranged from dark green for trees in the N9 treatment to yellowish-green on trees in the N0 treatment. This result indicated that trees, which had received little or no external supply of nitrogen since the beginning of the second growing season, were under nitrogen stress in November, irrespective of the nitrogen treatment applied in the first year of the experiment.

Data illustrating the influence of the nitrogen treatments on tree growth at harvest 2 is set out in Tables 5A, 5B, 5C and 5D. Interaction means for all treatments, and main effect

TABLE 5A

INFLUENCE OF NITROGEN TREATMENT ON TREE GROWTH AT HARVEST 2

Log$_e$ Data (g)

N Treatment	F.Wt. Roots			F.Wt. Stock + Stem + 1 Yr Old Shoots			F.Wt. Woody Tissue of New Shoots			F.Wt. Leaves + Petioles (excluding abscissed leaves)			F.Wt. Tree		
	Inter-action Mean	1st Yr Treat-ment Mean	2nd Yr Treat-ment Mean	Inter-action Mean	1st Yr Treat-ment Mean	2nd Yr Treat-ment Mean	Inter-action Mean	1st Yr Treat-ment Mean	2nd Yr Treat-ment Mean	Inter-action Mean	1st Yr Treat-ment Mean	2nd Yr Treat-ment Mean	Inter-action Mean	1st Yr Treat-ment Mean	2nd Yr Treat-ment Mean
N1NO	5.406			3.831			-0.210			3.201			5.690		
N1N3	5.735	5.807 (N1)	5.645 (NO)	4.162	4.040 (N1)	4.435 (NO)	2.419	1.930 (N1)	1.693 (NO)	4.208	4.100 (N1)	4.135 (NO)	6.115	6.045 (N1)	6.195 (NO)
N1N9	5.794			4.127			3.581			4.891			6.329		
N3NO	5.814			4.249			2.238			4.223			6.184		
N3N3	6.076	5.980 (N3)	5.971 (N3)	4.397	4.396 (N3)	4.580 (N3)	2.918	2.894 (N3)	2.968 (N3)	4.679	4.667 (N3)	4.712 (N3)	6.470	6.404 (N3)	6.450 (N3)
N3N9	6.021			4.541			3.527			5.099			6.559		
N9NO	6.201			5.225			3.050			4.980			6.711		
N9N3	6.128	6.023 (N9)	6.194 (N9)	5.182	5.222 (N9)	4.643 (N9)	3.567	3.441 (N9)	3.605 (N9)	5.250	5.241 (N9)	5.162 (N9)	6.765	6.795 (N9)	6.599 (N9)
N9N9	6.254			5.260			3.706			5.494			6.909		
L.S.D. 5%	NS	0.125	0.125	0.167	0.096	0.096	0.738	0.426	0.426	0.175	0.101	0.101	0.162	0.093	0.093
1%		0.170	0.170	0.226	0.131	0.131	1.000	0.578	0.578	0.237	0.137	0.137	0.219	0.126	0.126
0.1%		0.227	0.227	0.303	0.175	0.175	1.340	0.773	0.773	0.318	0.183	0.183	0.293	0.169	0.169
Inter-action	nil			neg.			neg.			neg.			neg.		

TABLE 5B

INFLUENCE OF NITROGEN TREATMENT ON TREE GROWTH AT HARVEST 2

Log$_e$ Data

N Treatment	D. Wt. Roots (g)			D. Wt. Stock + Stem + 1 Yr Old Shoots (g)			D. Wt. New Shoots (excluding abscissed leaves) (g)			D. Wt. Abscissed Leaves (mg)			D. Wt. Tree (g)		
	Inter-action Mean	1st Yr Treat-ment Mean	2nd Yr Treat-ment Mean	Inter-action Mean	1st Yr Treat-ment Mean	2nd Yr Treat-ment Mean	Inter-action Mean	1st Yr Treat-ment Mean	2nd Yr Treat-ment Mean	Inter-action Mean	1st Yr Treat-ment Mean	2nd Yr Treat-ment Mean	Inter-action Mean	1st Yr Treat-ment Mean	2nd Yr Treat-ment Mean
N1N0	4.137			3.201			2.082			4.461			4.561		
N1N3	4.229	4.190 (N1)	4.523 (N0)	3.456	3.332 (N1)	3.773 (N0)	3.116	3.023 (N1)	3.103 (N0)	4.461	4.624 (N1)	5.999 (N0)	4.814	4.732 (N1)	5.083 (N0)
N1N9	4.204			3.341			3.872			4.948			4.970		
N3N0	4.497			3.506			3.261			6.146			5.034		
N3N3	4.665	4.578 (N3)	4.582 (N3)	3.709	3.699 (N3)	3.872 (N3)	3.623	3.647 (N3)	3.658 (N3)	5.837	6.008 (N3)	5.910 (N3)	5.224	5.184 (N3)	5.231 (N3)
N3N9	4.572			3.782			4.051			6.042			5.295		
N9N0	4.934			4.514			3.965			7.388			5.656		
N9N3	4.853	4.922 (N9)	4.585 (N9)	4.450	4.489 (N9)	3.875 (N9)	4.231	4.207 (N9)	4.116 (N9)	7.432	7.406 (N9)	6.129 (N9)	5.654	5.696 (N9)	5.347 (N9)
N9N9	4.980			4.504			4.426			7.398			5.777		
L. S. D. 5%	NS	0.144	NS	NS	0.099	NS	0.225	0.130	0.130	NS	0.907	NS	NS	0.102	0.102
1%		0.195			0.134		0.304	0.176	0.176		1.230			0.139	0.139
0.1%		0.261			0.180		0.407	0.235	0.235		1.646			0.136	0.136
Inter-action	nil			nil			neg.			nil			nil		

TABLE 5C

INFLUENCE OF NITROGEN TREATMENT ON TREE GROWTH AT HARVEST 2

Log_2 Data

N Treatment	D.Wt. Tops / D.Wt. Roots			No. Leaves (including abscissed leaves)			No. Abscissed Leaves			Percentage Leaf Abscission (natural scale)			Mean F.Wt./Leaf (excluding abscissed leaves) (mg)		
	Inter-action Mean	1st Yr Treat-ment Mean	2nd Yr Treat-ment Mean	Inter-action Mean	1st Yr Treat-ment Mean	2nd Yr Treat-ment Mean	Inter-action Mean	1st Yr Treat-ment Mean	2nd Yr Treat-ment Mean	Inter-action Mean	1st Yr Treat-ment Mean	2nd Yr Treat-ment Mean	Inter-action Mean	1st Yr Treat-ment Mean	2nd Yr Treat-ment Mean
N1No	-0.650			4.962			2.334			7.7			5.471		
N1N3	-0.233	-0.251 (N1)	-0.322 (NO)	5.465	5.494 (N1)	5.657 (NO)	2.158	2.367 (N1)	3.336 (NO)	3.9	5.0 (N1)	10.5 (NO)	5.848	5.748 (N1)	5.691 (NO)
N1N9	0.131			6.054			2.611			3.5			5.926		
N3NO	-0.357			5.629			3.283			10.0			5.731		
N3N3	-0.292	-0.198 (N3)	-0.113 (N3)	5.952	5.948 (N3)	5.972 (N3)	3.338	3.288 (N3)	3.307 (N3)	7.4	7.4 (N3)	8.1 (N3)	5.861	5.859 (N3)	5.885 (N3)
N3N9	0.055			6.262			3.242			4.9			5.935		
N9NO	0.043			6.380			4.391			13.9			5.821		
N9N3	0.187	0.138 (N9)	0.123 (N9)	6.498	6.551 (N9)	6.364 (N9)	4.426	4.427 (N9)	3.439 (N9)	13.0	12.3 (N9)	6.2 (N9)	5.946	5.884 (N9)	5.915 (N9)
N9N9	0.134			6.775			4.464			10.1			5.885		
L.S.D. 5%	0.242	0.140	0.140	0.203	0.117	0.117	NS	0.308	NS	NS	2.2	2.2	0.129	0.075	0.075
1%	0.328	0.189	0.189	0.275	0.159	0.159		0.418			3.0	3.0	0.175	0.101	0.101
0.1%	0.439	0.253	0.253	0.368	0.213	0.213		0.559			4.0	4.0	0.235	0.136	0.136
Inter-action	neg.			neg.			nil			nil			neg.		

TABLE 5D

INFLUENCE OF NITROGEN TREATMENT ON THE DRY MATTER CONTENT OF TREE TISSUES AT HARVEST 2

Values Relative to the N1N0 Treatment (%)

N Treatment	D.wt Roots	D.wt. Stock + Stem + 1 Yr Old Shoots	D.wt. New Shoots	D.wt. Abscissed Leaves	D.wt. Tree
N1N0	100.0	100.0	100.0	100.0	100.0
N1N3	109.3	126.1	275.6	100.0	127.6
N1N9	107.8	111.9	587.8	100.0	149.3
N3N0	144.6	146.6	320.7	500.0	160.2
N3N3	168.2	162.5	463.4	300.0	191.6
N3N9	153.3	173.9	709.8	400.0	205.9
N9N0	223.4	364.4	647.6	1700.0	297.3
N9N3	211.7	342.7	845.1	1800.0	300.3
N9N9	232.8	359.7	1037.8	1700.0	335.4

means for both the first and second year treatments, are given for each set of data, together with the type of interaction found, if any, between the first and second year treatments. It is evident that tree growth, when expressed as fresh weight or dry weight per tree, was dependent on, and in proportion to, the nitrogen treatments applied in both first and second years of the experiment. In most cases the differences between treatments were significant. A similar situation was found in regard to the growth of each tree part, except that the second year treatment did not significantly influence the growth of any tree part, on a dry weight basis, apart from the new shoot tissues. As shown in Tables 5C and 5D, if the level of nitrogen supplied to trees in the first year was constant, but that supplied in the second year was varied, then the top/root ratio increased on a dry weight basis with increasing nitrogen supply. However, dry weight per tree at harvest 2 was clearly more dependent upon the nitrogen treatment applied in the first year than it was upon the external nitrogen supply in the second year of the experiment (see Table 5B).

The top/root ratio on a dry weight basis, the number of leaves per tree, percentage leaf abscission and the mean fresh weight per leaf per tree were also found to be significantly affected by both first and second year treatments, while the number of abscissed leaves per tree was significantly dependent upon the first year treatment only. However, percentage leaf abscission was inversely proportional to the external nitrogen supply in the second year. That is, a greater proportion of the total number of leaves abscissed from trees receiving the N0 treatment than abscissed from trees receiving the N9 treatment, irrespective of the first year treatment. Percentage leaf abscission was therefore indicative of the severity of the nitrogen stress within the trees.

(3) Harvest 3 Data

The appearance and overall size of the trees at harvest 3 is shown in Plate 2. As in the case of the trees harvested in November 1964 (harvest 2), the size of tree tops was dependent on, and in proportion to, the level of nitrogen supplied in both first and second years of the experiment, while the foliage colour was dependent upon the level of nitrogen supplied in the second year. The foliage was dark green on trees in the N9 treatment, green on trees in the N3 treatment, except for N9N3 trees whose foliage was yellowish-green, and yellowish-green on trees in the N0 treatment.

Data illustrating the influence of the nitrogen treatments on tree growth at harvest 3 is set out in Tables 6A, 6B, 6C and 6D. These results were expressed in the same way as were those of harvest 2, and, generally, the results obtained were similar to those obtained at harvest 2. Thus the growth of trees and their parts at the end of the second growing season was significantly dependent upon, and in proportion to, the level of

TABLE 6A

INFLUENCE OF NITROGEN TREATMENT ON TREE GROWTH AT HARVEST 3

Log_e Data (g)

N Treatment	F.Wt. Roots			F.Wt. Stock + Stem + 1 Yr Old Shoots			F.Wt. Woody Tissue of New Shoots			F.Wt. Leaves + Petioles (excluding abscissed leaves)			F.Wt. Tree		
	Inter-action Mean	1st Yr Treat-ment Mean	2nd Yr Treat-ment Mean	Inter-action Mean	1st Yr Treat-ment Mean	2nd Yr Treat-ment Mean	Inter-action Mean	1st Yr Treat-ment Mean	2nd Yr Treat-ment Mean	Inter-action Mean	1st Yr Treat-ment Mean	2nd Yr Treat-ment Mean	Inter-action Mean	1st Yr Treat-ment Mean	2nd Yr Treat-ment Mean
N1N0	5.484			4.054			0.830			2.669			5.758		
N1N3	6.224	6.032 (N1)	5.960 (N0)	4.261	4.368 (N1)	4.567 (N0)	2.974	2.808 (N1)	2.188 (N0)	4.545	4.220 (N1)	3.550 (N0)	6.536	6.417 (N1)	6.285 (N0)
N1N9	6.388			4.790			4.620			5.445			6.958		
N3N0	6.086			4.363			2.442			3.551			6.338		
N3N3	6.178	6.239 (N3)	6.399 (N3)	4.323	4.547 (N3)	4.665 (N3)	3.695	3.562 (N3)	3.530 (N3)	4.853	4.652 (N3)	4.898 (N3)	6.592	6.682 (N3)	6.784 (N3)
N3N9	6.603			4.955			4.549			5.553			7.118		
N9N0	6.310			5.284			3.291			4.429			6.758		
N9N3	6.795	6.612 (N9)	6.574 (N9)	5.412	5.359 (N9)	5.042 (N9)	3.920	3.925 (N9)	4.577 (N9)	5.297	5.144 (N9)	5.568 (N9)	7.223	7.088 (N9)	7.120 (N9)
N9N9	6.730			5.383			4.563			5.707			7.284		
L.S.D. 5%	0.188	0.109	0.109	0.236	0.136	0.136	0.282	0.163	0.163	0.195	0.113	0.113	0.136	0.079	0.079
1%	0.255	0.147	0.147	0.320	0.185	0.185	0.382	0.221	0.221	0.265	0.153	0.153	0.185	0.107	0.107
0.1%	0.341	0.197	0.197	0.429	0.248	0.248	0.512	0.296	0.296	0.354	0.205	0.205	0.247	0.143	0.143
Inter-action	neg.			neg.			neg.			neg.			neg.		

TABLE 6B

INFLUENCE OF NITROGEN TREATMENT ON TREE GROWTH AT HARVEST 3

Log_e Data

N Treatment	D. Wt. Roots (g)			D. Wt. Stock + Stem + 1 Yr Old Shoots (g)			D. Wt. New Shoots (excluding abscissed leaves) (g)			D. Wt. Abscissed Leaves (mg)			D. Wt. Tree (g)		
	Inter-action Mean	1st Yr Treat-ment Mean	2nd Yr Treat-ment Mean	Inter-action Mean	1st Yr Treat-ment Mean	2nd Yr Treat-ment Mean	Inter-action Mean	1st Yr Treat-ment Mean	2nd Yr Treat-ment Mean	Inter-action Mean	1st Yr Treat-ment Mean	2nd Yr Treat-ment Mean	Inter-action Mean	1st Yr Treat-ment Mean	2nd Yr Treat-ment Mean
N1N0	4.214			3.520			1.771			8.066			4.712		
N1N3	4.778	4.704 (N1)	4.692 (N0)	3.687	3.731 (N1)	4.013 (N0)	3.694	3.454 (N1)	2.788 (N0)	7.615	8.045 (N1)	9.229 (N0)	5.305	5.311 (N1)	5.273 (N0)
N1N9	5.121			4.137			4.897			8.454			5.914		
N3N0	4.798			3.804			2.871			9.437			5.237		
N3N3	4.913	5.015 (N3)	5.044 (N3)	3.935	4.032 (N3)	4.144 (N3)	4.143	3.976 (N3)	4.114 (N3)	8.962	9.106 (N3)	8.797 (N3)	5.559	5.636 (N3)	5.668 (N3)
N3N9	5.333			4.356			4.913			8.868			6.062		
N9N0	5.063			4.714			3.722			10.133			5.820		
N9N3	5.442	5.335 (N9)	5.318 (N9)	4.810	4.773 (N9)	4.429 (N9)	4.505	4.419 (N9)	4.947 (N9)	9.314	9.893 (N9)	9.018 (N9)	6.139	6.082 (N9)	6.088 (N9)
N9N9	5.501			4.796			5.032			9.733			6.288		
L.S.D. 5%	0.168	0.097	0.097	0.225	0.130	0.130	0.172	0.099	0.099	0.365	0.211	0.211	0.120	0.069	0.069
1%	0.227	0.131	0.131	0.305	0.176	0.176	0.233	0.134	0.134	0.495	0.286	0.286	0.163	0.094	0.094
0.1%	0.304	0.176	0.176	0.408	0.236	0.236	0.312	0.180	0.180	0.663	0.383	0.383	0.218	0.126	0.126
Inter-action	neg.			neg.			neg.			neg.			neg.		

TABLE 6C

INFLUENCE OF NITROGEN TREATMENT ON TREE GROWTH AT HARVEST 3

Log$_e$ Data

N Treatment	D.Wt. Tops / D.Wt. Roots			No. Leaves (including abscissed leaves)			No. Abscissed Leaves			Percentage Leaf Abscission (natural scale)			Mean F. Wt./Leaf (excluding abscissed leaves) (mg)		
	Inter-action Mean	1st Yr Treat-ment Mean	2nd Yr Treat-ment Mean	Inter-action Mean	1st Yr Treat-ment Mean	2nd Yr Treat-ment Mean	Inter-action Mean	1st Yr Treat-ment Mean	2nd Yr Treat-ment Mean	Inter-action Mean	1st Yr Treat-ment Mean	2nd Yr Treat-ment Mean	Inter-action Mean	1st Yr Treat-ment Mean	2nd Yr Treat-ment Mean
N1N0	-0.529			4.927			4.511			66.3			5.754		
N1N3	-0.393	-0.253 (N1)	-0.408 (N0)	5.600	5.580 (N1)	5.558 (N0)	4.097	4.385 (N1)	5.139 (N0)	22.3	35.9 (N1)	66.2 (N0)	6.111	6.072 (N1)	6.018 (N0)
N1N9	0.163			6.213			4.547			19.2			6.353		
N3N0	-0.661			5.582			5.249			72.0			6.187		
N3N3	-0.165	-0.263 (N3)	-0.212 (N3)	5.994	5.992 (N3)	6.071 (N3)	5.104	5.072 (N3)	5.005 (N3)	41.7	45.2 (N3)	36.3 (N3)	6.320	6.272 (N3)	6.208 (N3)
N3N9	0.036			6.401			4.863			21.9			6.309		
N9N0	-0.034			6.166			5.658			60.4			6.113		
N9N3	-0.078	0.001 (N9)	0.105 (N9)	6.619	6.522 (N9)	6.465 (N9)	5.815	5.714 (N9)	5.027 (N9)	44.9	46.1 (N9)	24.7 (N9)	6.193	6.179 (N9)	6.298 (N9)
N9N9	0.116			6.780			5.671			33.1			6.232		
L.S.D. 5%	0.200	0.116	0.116	0.185	0.107	0.107	0.310	0.179	NS	8.9	5.1	5.1	0.197	0.114	0.114
1%	0.271	0.157	0.157	0.251	0.145	0.145	0.420	0.242		12.0	6.9	6.9	0.267	0.154	0.154
0.1%	0.363	0.210	0.210	0.336	0.194	0.194	0.562	0.324		16.1	9.3	9.3	0.358	0.206	0.206
Inter-action	neg.			neg.			neg.			neg.			neg.		

TABLE 6D

INFLUENCE OF NITROGEN TREATMENT ON THE DRY MATTER CONTENT OF TREE TISSUES AT HARVEST 3

Values Relative to the N1N0 Treatment (%)

N Treatment	D. wt. Roots	D. wt. Stock + Stem + 1 Yr Old Shoots	D. wt. New Shoots	D. wt. Abscissed Leaves	D. wt. Tree
N1N0	100.0	100.0	100.0	100.0	100.0
N1N3	175.4	114.2	671.7	61.8	179.4
N1N9	247.8	179.2	2236.7	144.1	329.5
N3N0	179.2	128.8	300.0	394.1	176.6
N3N3	200.6	143.1	1056.7	232.4	231.0
N3N9	303.8	224.5	2266.7	217.6	331.3
N9N0	233.4	318.8	690.0	741.2	299.9
N9N3	339.3	351.0	1511.7	541.2	411.7
N9N9	363.0	346.4	2555.0	502.9	479.2

nitrogen supplied in both first and second years of the experiment. However, in contrast with harvest 2 results, the dry matter content of all parts of the trees was found to be significantly influenced by both first and second year treatments. In addition, significant negative interactions were found between first and second year treatments for all of the data recorded at harvest 3. Further evidence was also obtained which showed that if the level of nitrogen supplied to trees in the first year was kept constant, the top/root ratio, when expressed on a dry weight basis, increased with increasing nitrogen supply in the second year of the experiment. Percentage leaf abscission values were much higher than were those found at harvest 2, but the same trends were apparent, i.e. the proportion of the total number of leaves per tree which abscissed (expressed as a %) was inversely proportional to external nitrogen supply in the second year. Basal leaves on the shoots were always the first to absciss.

(4) Seasonal Changes in the Dry Matter Content of Tree Tissues

The seasonal changes which occurred in the dry matter content of whole trees and their parts during the second growing season are shown in Fig.8. This figure was constructed by combining relevant data from harvests 1, 2 and 3. For this purpose harvest 1 was considered as being at the beginning of the second growing season. It is clear that the dry matter content

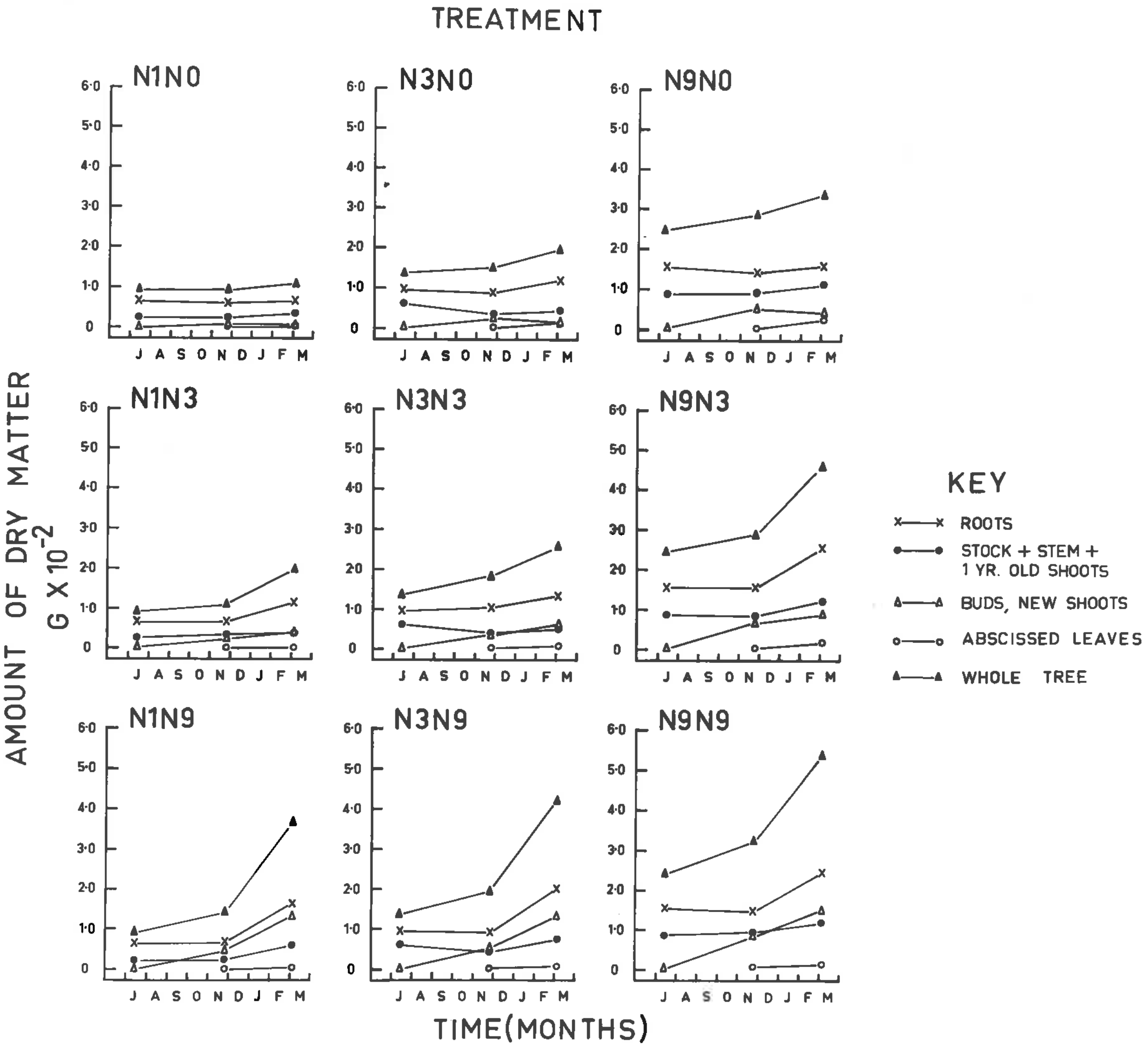


FIGURE 8 Influence of nitrogen treatment on the dry matter content of tree tissues during the second growing season.

during the second growing season was markedly dependent upon, and in proportion to, the level of nitrogen supplied in both first and second years of the experiment. This pronounced residual effect of the first year treatment on tree growth in the second year is consistent with the hypothesis that peach trees accumulate nitrogen in the first year in proportion to nitrogen supply, and that this nitrogen is mobilized for growth in the second year even if a high level of nitrogen is supplied. Fig.8 also shows that the increase in dry matter content of tree tissues was much more rapid in the latter half of the growing season than in the former half. In fact, during the first half of the growing season, the dry matter content of stock + stem + 1 year old shoot and root fractions declined in several treatments, especially those which had received the N3 treatment in the first year, indicating that redistribution of 'dry matter' had taken place in the trees. In most cases there was evidence to show that 'dry matter' had moved into the new shoot fraction. The dry matter content of the new shoot fraction of trees receiving the N0 treatment in the second year declined during the latter half of the growing season, and, since this decline appeared to be equivalent to the increase in dry matter in the abscissed leaf fraction, it is thought that the loss of dry matter from the new shoots was due to leaf abscission rather than to movement of dry matter into woody parts of the trees.

(5) Correlation Between the Amount of Shoot Growth Made per Tree in the Second Growing Season and the Total Nitrogen Content per Tree at Harvest 1

The relationship between the amount of new shoot growth made per tree at harvests 2 and 3 and the total N content per tree at harvest 1 was investigated by simple regression analysis. The results are set out in Table 7. As expected, a highly significant, positive correlation was found between the amount of new shoot growth made per tree at both harvests and the nitrogen content per tree at harvest 1 if the external nitrogen supply in the second year was low. In those treatments in which the level of nitrogen supplied to the trees in the second year was high, the level of correlation was reduced and in one case was not significant.

(iv) The Nitrogen Content of Tree Tissues at the End of the First Year of the Experiment

(1) Influence of Nitrogen Supply on the Accumulation of Nitrogenous Constituents in Tree Tissues

The concentration and absolute amounts of the nitrogenous constituents in tree tissues at harvest 1 are tabulated in Appendix C, section (a)(ii), together with calculated values for least significant differences between treatments. However, the data has also been expressed on a relative basis (see Tables 8A and 8B). No values are included for nitrate N since nitrate was not detected in any tissue at harvest 1.

TABLE 7

CORRELATION BETWEEN GROWTH OF NEW SHOOTS IN THE SECOND GROWING SEASON AND TOTAL NITROGEN CONTENT PER TREE PRIOR TO COMMENCEMENT OF GROWTH.

Log_{10} Data

Items in Regression	Treatments Pooled in Regression	r	d.f.	Significance
D.wt. new shoots at harvest 2 V. N/tree at harvest 1	N1N0, N3N0, N9N0	0.966	10	xxx
	N1N3, N3N3, N9N3	0.970	10	xxx
	N1N9, N3N9, N9N9	0.823	10	xxx
Length new shoots at harvest 2 V. N/tree at harvest 1	N1N0, N3N0, N9N0	0.843	10	xxx
	N1N3, N3N3, N9N3	0.946	10	xxx
	N1N9, N3N9, N9N9	0.784	10	xx
D.wt. new shoots at harvest 3 V. N/tree at harvest 1	N1N0, N3N0, N9N0	0.979	10	xxx
	N1N3, N3N3, N9N3	0.987	10	xxx
	N1N9, N3N9, N9N9	0.834	10	xxx
Length new shoots at harvest 3 V. N/tree at harvest 1	N1N0, N3N0, N9N0	0.823	10	xxx
	N1N3, N3N3, N9N3	0.714	10	xx
	N1N9, N3N9, N9N9	0.328	10	NS

TABLE 8A

INFLUENCE OF NITROGEN TREATMENT ON THE CONCENTRATION OF NITROGENOUS CONSTITUENTS IN TREE TISSUES AT HARVEST 1

Values relative to the N1 Treatment (%)

Tree Tissue	Nitrogen Treatment	Total N	Insoluble N	Soluble N	Ammonium N	Arginine N	Amide N	Total α-Amino N
Roots	N1	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	N3	134.1	110.3	187.6	134.4	195.7	136.4	193.4
	N9	173.4	106.3	324.2	121.9	334.8	155.8	304.6
Stock + stem + side shoots	N1	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	N3	152.7	118.4	258.1	107.0	224.0	209.7	276.3
	N9	186.1	128.6	362.5	77.2	480.4	237.3	379.4
Leaf + flower buds	N1	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	N3	127.3	105.2	240.6	93.7	328.6	241.4	225.4
	N9	154.7	120.5	331.0	81.1	452.3	251.4	301.8

TABLE 8B

INFLUENCE OF NITROGEN TREATMENT ON THE AMOUNTS OF NITROGENOUS CONSTITUENTS IN TREE TISSUES AT HARVEST 1

Values relative to the N1 Treatment (%)

Tree Tissue	Nitrogen Treatment	Total N	Insoluble N	Soluble N	Ammonium N	Arginine N	Amide N	Total α - Amino N
Roots	N1	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	N3	197.1	162.5	280.2	190.9	293.9	204.7	291.8
	N9	403.1	246.0	780.9	277.3	937.1	379.4	741.2
Stock + stem + side shoots	N1	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	N3	236.0	182.2	410.5	153.3	464.6	330.3	447.3
	N9	646.5	442.9	1307.2	253.3	1796.3	827.3	1394.5
Leaf + flower buds	N1	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	N3	203.5	166.7	400.0	100.0	500.0	882.4	366.7
	N9	503.5	339.6	1111.1	200.0	1383.3	1764.7	1000.0
Whole tree	N1	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	N3	206.0	167.3	305.8	178.4	300.1	235.7	317.5
	N9	459.8	295.8	882.8	273.0	1006.3	487.1	847.6

It will be observed from Tables 8A and 8B that the concentration and amount of each nitrogenous constituent in the tree tissues was usually increased, relative to the values in the N1 treatment, with increasing nitrogen supply, but the relative increase was much more pronounced for some constituents than for others. For example, the relative increase in the concentration and amount of soluble N was much greater than the corresponding increase in levels of insoluble N. It is concluded that the nitrogen which accumulated in tree tissues in response to increasing nitrogen supply during the first year of the experiment consisted mainly of soluble nitrogenous compounds. Furthermore, since the concentration and amount of arginine N in the tree tissues showed the greatest relative increase with increasing nitrogen supply of any of the nitrogenous constituents, with the exception of the amount of amide N in leaf + flower bud tissues, it is concluded that the estimation of levels of arginine N in the dormant peach trees gave the most sensitive indication of the nitrogen status of the trees. Estimation of levels of soluble N in the tissues gave the next best indication of the nitrogen status of the trees. Since the greatest relative increase in the levels of arginine N, on either a concentration or absolute basis, was found in the stock + stem + side shoot fraction, it appears that this would be the most suitable type of tissue to use in any assessment of the nitrogen status of dormant trees.

Ratios of the amounts of nitrogenous constituents in tree tissues at harvest 1 are shown in Table 9. The data indicates that levels of soluble N, as a proportion of the total N content of the tissues, increased, while levels of insoluble N, as a proportion of the total N content, decreased, with increasing nitrogen supply. These findings support the hypothesis that the storage nitrogen of peach trees consists mainly of soluble nitrogenous compounds. Furthermore, it is clear that almost all of the soluble N in the tissues was organic in character and that it consisted mainly of arginine N. Since the levels of arginine N, when expressed as a proportion of the soluble N content, were significantly increased in the tree tissues with increased nitrogen supply, it is evident that the composition of the soluble N fraction of the peach tissues was dependent upon the nitrogen supply. In circumstances where the nitrogen supply was low, a greater proportion of the soluble N which accumulated consisted of amide N. Overall, however, the levels of amide N constituted a very small part of the soluble N fraction. The levels of total α-amino N, when expressed as a proportion of the soluble N content, were not influenced by the nitrogen treatment. Irrespective of the nitrogen treatment applied, almost all of the soluble N in the peach tissues was accounted for as arginine, the amides and ammonia.

(2) Distribution of Nitrogenous Constituents in Tree Tissues at Harvest 1

The distribution of each nitrogenous constituent in tree

TABLE 9

RATIO OF AMOUNTS OF NITROGENOUS CONSTITUENTS IN TREE TISSUES AT HARVEST 1

(Expressed as percentages)

N Treatment	$\frac{\text{Soluble N}}{\text{Total N}}$				$\frac{\text{Insoluble N}}{\text{Total N}}$			
	Roots	Stock + Stem + Side Shoots	Leaf + Flower Buds	Whole Tree	Roots	Stock + Stem + Side Shoots	Leaf + Flower Buds	Whole Tree
N1	30.4	24.1	16.3	28.3	69.6	75.9	83.7	71.7
N3	42.6	41.2	30.8	41.5	57.4	58.8	69.2	58.5
N9	57.4	47.8	34.8	53.7	42.6	52.2	65.2	46.4
L.S.D. 5%	3.6	3.3	4.0	3.1	3.6	3.3	-	3.0
1%	4.9	4.4	7.3	4.2	4.9	4.4	-	4.1
0.1%	6.6	6.0	16.2	5.6	6.6	6.0	-	5.5

N Treatment	$\frac{\text{Arginine N}}{\text{Soluble N}}$				$\frac{\text{Total } \alpha\text{-Amino N}}{\text{Soluble N}}$			
	Roots	Stock + Stem + Side Shoots	Leaf + Flower Buds	Whole Tree	Roots	Stock + Stem + Side Shoots	Leaf + Flower Buds	Whole Tree
N1	78.1	66.0	60.8	75.7	31.4	25.1	33.1	30.2
N3	81.6	76.2	83.0	80.3	33.0	27.3	31.0	31.4
N9	94.6	93.0	83.0	94.1	30.0	26.8	30.1	29.0
L.S.D. 5%	6.6	9.1	6.7	6.0	1.5	1.7	2.2	1.4
1%	9.0	12.4	12.4	8.2	2.1	2.3	4.0	1.9
0.1%	12.1	16.6	27.4	10.9	2.8	3.1	8.8	2.5

TABLE 9 (Contd.)

RATIO OF AMOUNTS OF NITROGENOUS CONSTITUENTS IN TREE TISSUES AT HARVEST 1

(Expressed as percentages)

N Treatment	Ammonium N / Soluble N				Amide N / Soluble N			
	Roots	Stock + Stem + Side Shoots	Leaf + Flower Buds	Whole Tree	Roots	Stock + Stem + Side Shoots	Leaf + Flower Buds	Whole Tree
N1	1.4	4.3	8.6	2.0	7.1	9.7	4.2	7.5
N3	1.0	1.6	3.3	1.2	5.3	7.4	4.2	5.8
N9	0.5	0.8	2.1	0.6	3.6	5.9	3.2	4.2
L.S.D.								
5%	0.3	1.2	0.8	0.4	1.1	2.7	2.7	1.1
1%	0.5	1.6	1.5	0.5	1.5	3.7	5.0	1.5
0.1%	0.6	2.1	3.3	0.7	2.0	5.0	11.0	2.0

N Treatment	Arginine N + Ammonium + Amide N / Soluble N			
	Roots	Stock + Stem + Side Shoots	Leaf + Flower Buds	Whole Tree
N1	86.7	80.0	73.6	85.2
N3	87.9	85.2	90.5	87.3
N9	98.7	99.7	88.3	98.9
L.S.D.				
5%	6.1	7.5	5.0	5.4
1%	8.2	10.2	9.3	7.4
0.1%	11.0	13.6	20.5	9.8

tissues at harvest 1 is shown in Table 10. The results indicate that approximately 60 to 80% of the amount of nitrogen in the dormant trees was present in the roots, irrespective of the nitrogenous constituent considered and the nitrogen treatment applied. However, as the level of nitrogen supplied to the trees was increased, a smaller proportion of the nitrogen content of the tree was found in the roots and a correspondingly greater proportion was found in the stock + stem + side shoot fraction. This result is consistent with the known increase in the top/root ratio of the trees with increased nitrogen supply (see growth data).

(3) Nitrogen Uptake per Tree During the First Year

The nitrogen uptake per tree during the first year of the experiment was calculated from the difference between the total N content per tree at harvest 1 and the total N content per tree at planting. The results, which are shown in Table 11, indicate that the nitrogen uptake per tree during the first year was approximately directly proportional to the nitrogen supply.

(v) The Nitrogen Content of Tree Tissues During the Second Growing Season

(1) Levels of Nitrogenous Constituents in Tree Tissues at Harvests 2 and 3

The concentration and absolute amounts of the nitrogenous constituents in tree tissues at harvests 2 and 3 are tabulated in

TABLE 10

DISTRIBUTION OF NITROGENOUS CONSTITUENTS IN TREE TISSUES AT HARVEST 1

Amount of Nitrogen in Each Tree Part as a Percentage of the Total per Tree

N Treatment	Total N			Soluble N			Insoluble N			Arginine N		
	Roots	Stock + Stem + Side Shoots	Leaf + Flower Buds	Roots	Stock + Stem + Side Shoots	Leaf + Flower Buds	Roots	Stock + Stem + Side Shoots	Leaf + Flower Buds	Roots	Stock + Stem + Side Shoots	Leaf + Flower Buds
N1	76.3	22.9	0.8	80.2	19.3	0.5	74.8	24.3	0.9	82.9	16.6	0.4
N3	72.8	26.3	0.9	73.0	26.4	0.6	72.5	26.5	1.0	74.2	25.1	0.7
N9	66.7	32.4	0.9	70.5	28.8	0.7	61.9	36.6	1.4	70.8	28.6	0.6
L.S.D. 5%	2.6	2.6	0.2	3.0	3.0	0.2	3.5	3.5	0.5	4.0	3.9	0.1
1%	3.5	3.5	0.2	4.1	4.0	0.2	4.7	4.7	0.7	5.4	5.4	0.2
0.1%	4.7	4.7	0.3	5.4	5.4	0.3	6.3	6.3	0.9	7.3	7.2	0.2

N Treatment	Ammonium N			Amide N			Total α-Amino N		
	Roots	Stock + Stem + Side Shoots	Leaf + Flower Buds	Roots	Stock + Stem + Side Shoots	Leaf + Flower Buds	Roots	Stock + Stem + Side Shoots	Leaf + Flower Buds
N1	57.5	39.7	2.8	76.5	23.4	0.1	83.4	16.1	0.5
N3	63.4	34.6	2.0	66.5	33.0	0.5	76.5	22.9	0.6
N9	59.6	37.7	2.7	59.7	39.8	0.5	72.8	26.6	0.6
L.S.D. 5%	10.5	10.1	1.4	8.9	8.9	0.1	2.8	2.8	0.1
1%	14.3	13.7	1.9	12.1	12.0	0.2	3.8	3.7	0.2
0.1%	19.1	18.3	2.5	16.1	16.1	0.3	5.0	5.0	0.2

TABLE 11

NITROGEN UPTAKE PER TREE DURING THE FIRST YEAR OF THE EXPERIMENT

N Treatment	N uptake ($\log_e$ mg N)	Uptake Relative to N1 Treatment %
N1	5.586	100.0
N3	6.892	358.0
N9	7.394	957.6
LSD 5%	0.105	-
1%	0.143	-
0.1%	0.191	-

Appendix C section (a)(ii), together with calculated values for least significant differences between treatments. No values are included for nitrate N since nitrate was not detected in any tissue at harvests 2 and 3. In addition , tissues were not analysed for ammonium N or amide N at harvests 2 and 3 since these constituents did not accumulate to any marked extent during the first year of the experiment. The aim of the analyses which were carried out on tissues harvested during the second growing season was twofold, viz:

(a) to determine the fate of the nitrogenous constituents which had accumulated during the first year of the experiment, and

(b) to determine from what parts of the tree nitrogen is mobilized for new shoot growth if little or no external nitrogen is supplied.

Ratios of the amounts of nitrogenous constituents in tree tissues at harvests 2 and 3 are given in Tables 12 and 13. It is evident, in contrast with the harvest 1 data (Table 9), that the soluble N fraction constituted only a small and relatively constant proportion of the total N content of the tree tissues at both harvests 2 and 3, i.e. most of the nitrogen present in the tissue was present in insoluble form, presumably protein. Furthermore, the composition of the soluble N fraction in tree tissues during the second growing season was markedly different from that at harvest 1. For example, the importance of arginine as a constituent of the soluble N fraction was much reduced c.f. harvest 1, and, of the con-

TABLE 12

RATIO OF AMOUNTS OF NITROGENOUS CONSTITUENTS IN TREE TISSUES AT HARVEST 2

(Expressed as Percentages)

N Treatment	Soluble N / Total N				Insoluble N / Total N				Arginine N / Soluble N				Total α -Amino N / Soluble N			
	Roots	Stock + Stem + 1Yr Old Shoots	New Shoots	Whole Tree	Roots	Stock + Stem + 1Yr Old Shoots	New Shoots	Whole Tree	Roots	Stock + Stem + 1Yr Old Shoots	New Shoots	Whole Tree	Roots	Stock + Stem + 1Yr Old Shoots	New Shoots	Whole Tree
N1N0	13.8	14.2	9.3	12.7	86.2	85.8	90.7	87.3	7.4	3.1	0	5.4	26.7	19.0	15.4	23.6
N1N3	10.7	14.5	10.1	10.8	89.3	85.5	89.9	89.2	11.0	0	1.4	5.6	31.0	14.5	19.1	23.8
N1N9	10.2	13.6	11.2	11.0	89.8	86.4	88.8	89.0	13.7	0	1.5	4.7	20.7	6.8	15.7	16.5
N3N0	16.7	20.6	9.2	14.0	83.3	79.4	90.8	86.0	6.4	1.3	0	4.0	24.6	17.4	14.0	20.9
N3N3	13.8	19.9	10.4	12.8	86.2	80.1	89.6	87.2	22.8	0	1.5	11.6	25.8	14.3	15.7	20.1
N3N9	19.8	17.6	11.2	14.1	80.2	82.4	88.8	85.9	20.9	1.8	0.6	10.0	26.4	18.8	15.9	20.7
N9N0	26.5	30.7	10.1	20.4	73.5	69.3	89.9	79.6	16.6	11.1	1.0	12.5	26.5	21.3	12.0	22.3
N9N3	25.2	30.1	11.4	18.7	74.8	69.9	88.5	81.3	31.5	11.6	3.0	18.9	25.5	20.2	17.1	21.8
N9N9	27.5	30.1	13.0	19.6	72.5	69.9	87.0	80.4	21.8	6.2	2.1	12.3	18.4	18.4	9.7	15.3
Interaction L.S.D. 5%	NS	NS	NS	NS	NS	NS	NS	NS	NS	-* - -	- - -	NS	NS	5.3 7.2 9.6	NS	3.9 5.3 7.1
V.R. Test 1st year treatment	xxx	xxx	xx	xxx	xxx	xxx	xx	xxx	xxx	-	-	xxx	NS	xxx	xx	NS
2nd year treatment	NS	NS	xxx	NS	NS	NS	xxx	NS	xx	-	-	x	xx	x	xx	xxx

* Insufficient data for analysis

TABLE 13

RATIO OF AMOUNTS OF NITROGENOUS CONSTITUENTS IN TREE TISSUES AT HARVEST 3

(Expressed as Percentages)

N Treatment	Soluble N / Total N				Insoluble N / Total N				Arginine N / Soluble N				Total α-Amino N / Soluble N			
	Roots	Stock + Stem + 1Yr Old Shoots	New Shoots	Whole Tree	Roots	Stock + Stem + 1Yr Old Shoots	New Shoots	Whole Tree	Roots	Stock + Stem + 1Yr Old Shoots	New Shoots	Whole Tree	Roots	Stock + Stem + 1Yr Old Shoots	New Shoots	Whole Tree
N1N0	15.6	16.7	12.7	17.4	84.4	83.3	87.3	82.6	6.2	1.5	2.4	4.8	18.4	14.9	16.7	17.5
N1N3	20.8	27.0	10.1	17.3	79.2	73.0	89.9	82.7	16.9	19.9	4.7	14.8	22.6	22.8	15.2	21.0
N1N9	22.1	22.6	11.8	16.5	77.9	77.4	88.2	83.5	17.9	16.5	6.4	13.2	29.8	30.8	21.8	26.6
N3N0	18.5	21.2	12.8	17.7	81.5	78.8	87.2	82.3	11.7	3.9	4.0	9.4	19.8	14.5	18.1	18.7
N3N3	20.0	22.3	10.6	16.2	80.0	77.7	89.4	83.8	14.3	15.3	6.1	12.3	22.0	18.5	15.8	19.7
N3N9	20.2	24.5	12.6	16.6	79.8	75.5	87.4	83.4	15.1	18.9	6.2	12.8	29.2	28.0	23.1	26.7
N9N0	27.1	27.0	15.2	23.9	72.9	73.0	84.8	76.1	18.9	11.8	6.6	15.1	23.1	20.2	18.4	21.6
N9N3	17.3	24.8	13.2	16.5	82.7	75.2	86.9	83.5	9.3	7.7	5.0	7.8	14.4	11.6	11.6	13.1
N9N9	20.6	28.0	12.9	17.6	79.4	72.0	87.1	82.4	16.6	18.2	6.8	13.3	29.5	27.0	19.0	25.4
Interaction L.S.D. 5%	4.9	4.2	NS	NS	4.9	4.2	NS	NS	6.4	4.6	1.6	4.1	5.0	3.5	NS	4.2
1%	6.7	5.7			6.6	5.7			8.7	6.2	2.2	5.5	6.8	4.8		5.7
0.1%	9.0	7.7			8.9	7.7			11.6	8.3	2.9	7.4	9.1	6.4		7.7
V.R. Test 1st year treatment	NS	xx	xxx	x	NS	xx	xxx	x	NS	NS	xx	NS	NS	xx	NS	NS
2nd year treatment	NS	x	xxx	x	NS	x	xxx	x	NS	xxx	xxx	x	xxx	xxx	xxx	xxx

stituents analysed, total α-amino N was the most important constituent of the soluble N fraction, especially in the above ground parts of the tree.

(2) Seasonal Changes in Levels of Nitrogenous Constituents in Tree Tissues During the Second Growing Season

The seasonal changes which occurred in the concentration and absolute amounts of nitrogenous constituents of whole trees and tree parts during the second growing season are shown in Figs.9 to 13. Each figure was constructed by combining the relevant data from harvests 1, 2 and 3, and, for these purposes, harvest 1 was considered as being at the beginning of the second growing season.

Total N (Figs. 9, 10.)

It is evident from Fig.9 that the amount of total N in the trees, and in their parts, at each harvest is dependent upon, and in proportion to, the level of nitrogen which was supplied in both first and second years of the experiment. Furthermore, it will be observed that during the first half of the growing season nitrogen is exported from the roots and the old tree tops (stock + stem + 1 year old shoots) to the new shoots, even if a high level of external nitrogen is supplied to the trees. However, roots of trees in the N1N9 treatment were an exception in that they increased in nitrogen content over this period. Presumably nitrogen was also exported from

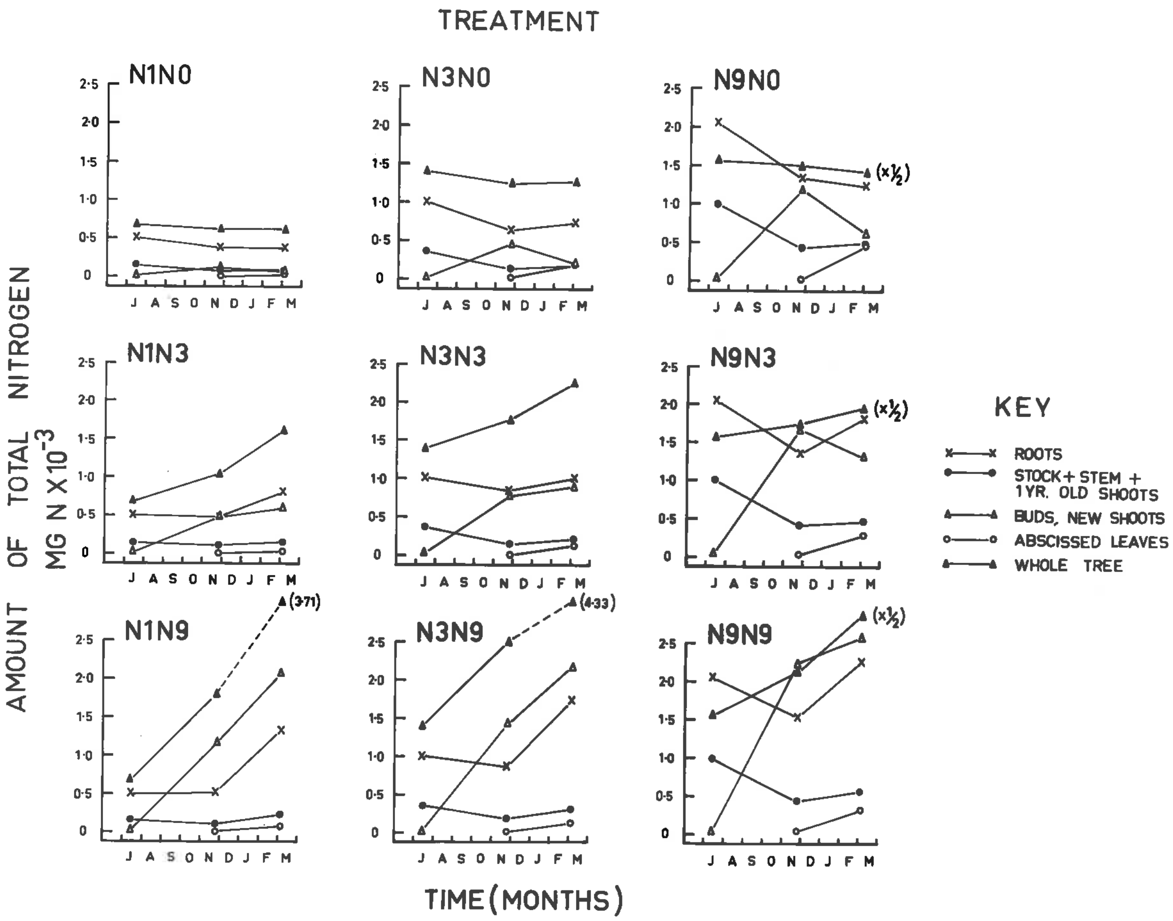


FIGURE 9 Influence of nitrogen treatment on the amount of total nitrogen in tree tissues during the second growing season.

(x½) indicates that the value for the whole tree was halved at each harvest.

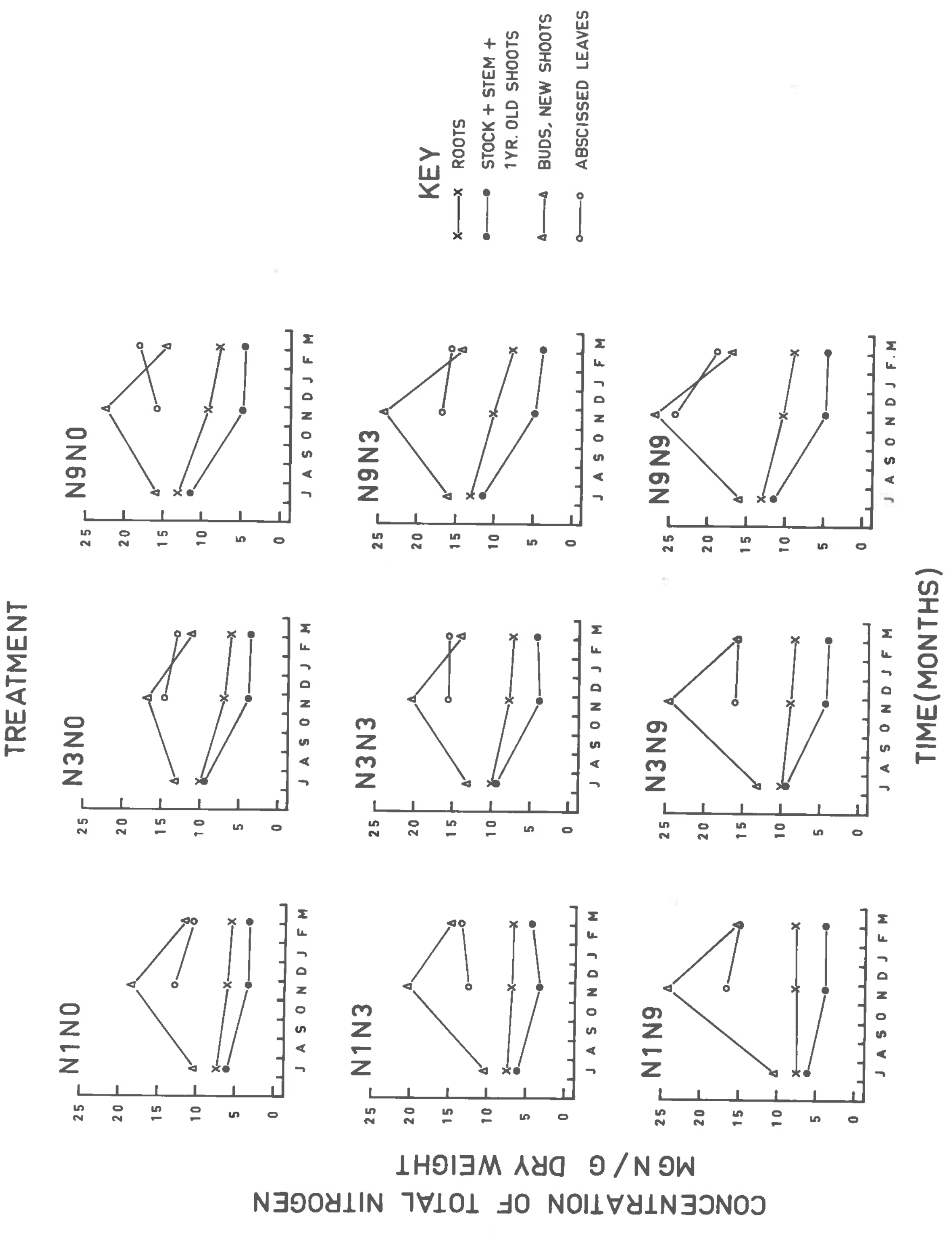


FIGURE 10 Influence of nitrogen treatment on the concentration of total nitrogen in tree tissues during the second growing season.

these tissues but was not detected because uptake of nitrogen exceeded loss of nitrogen. Where no external supply of nitrogen was given to the trees, the loss of nitrogen from the roots exceeded the loss from the old tree tops, but this situation was reversed if the trees received a high nitrogen application. Actual gains or losses of total N in each tree part during the second growing season have been calculated for each treatment and the results are shown in Table 14. Theoretically, it would be expected that if no nitrogen was supplied during the second growing season (N0 treatment) then the trees would not gain or lose nitrogen, but it is clear from Table 14 that this was not the case. However, since the apparent gains or losses of nitrogen by trees in these treatments represents only about 10% or less of the total N in the trees, these changes are thought to be due to experimental error arising from small differences in tree size, and therefore nitrogen content, at each harvest. Nevertheless, the results clearly indicate that storage nitrogen present in tree tissues was mobilized for new growth in all treatments.

During the second half of the growing season, the nitrogen content of the roots and old tops of the trees increased in all treatments, except in roots of trees in the N9N0 treatment where a further decrease occurred, and, in most cases, the increase in the nitrogen content of the roots exceeded that in the old tree tops. Since increases were found in tissues of trees which had not re-

TABLE 14

GAIN OR LOSS OF TOTAL NITROGEN BY EACH TREE PART AND TREE DURING THE SECOND GROWING SEASON

mgN

N Treat-ment	At Harvest 2 (harvest 2 minus harvest 1 values)					At Harvest 3 (harvest 3 minus harvest 2 values)				
	Roots	Stock + Stem + 1Yr Old Shoots	New Shoots	Abscissed Leaves	Whole Tree	Roots	Stock + Stem + 1Yr Old Shoots	New Shoots	Abscissed Leaves	Whole Tree
N1N0	-132.9	- 57.7	- 142.5	+ 1.3	- 56.8	+ 5.1	+ 30.9	- 78.5	+ 34.3	- 8.2
N1N3	- 32.2	- 49.1	+ 449.4	+ 0.6	+ 368.7	+335.0	+ 69.1	+135.9	+ 27.9	+ 567.9
N1N9	+ 13.1	- 46.1	+1158.3	+ 2.1	+1127.4	+805.0	+135.9	+900.6	+ 73.7	+1915.2
N3N0	-361.0	-226.0	+ 425.6	+ 6.6	- 154.8	+ 95.3	+ 22.0	-239.7	+169.3	+ 46.9
N3N3	-171.8	-209.3	+ 757.6	+ 4.7	+ 331.2	+156.3	+ 58.7	+111.5	+119.0	+ 445.5
N3N9	-139.0	-171.3	+1402.8	+ 6.8	+1099.3	+870.3	+121.7	+738.4	+113.0	+1843.4
N9N0	-722.2	-560.5	+1145.1	+26.7	- 110.9	- 92.5	+ 65.3	-565.8	+428.3	- 164.7
N9N3	-711.1	-581.2	+1624.3	+30.6	+ 362.6	+470.8	+ 56.4	-371.5	+257.2	+ 412.9
N9N9	-523.9	-556.3	+2206.5	+44.2	+1170.5	+728.3	+129.2	+331.2	+278.6	+1467.3

ceived any external supply of nitrogen, it is concluded that redistribution of nitrogen occurred in these trees, and it appears that nitrogen moved from the new shoots into the storage tissues (see Table 14). At the same time, the amount of nitrogen in the abscissed leaf fraction increased and largely accounted for the loss of nitrogen from the new shoot tissues in the N1N0, N3N0, N9N0 and N9N3 treatments. The nitrogen content of trees, which received an external supply of nitrogen, increased in proportion to the nitrogen supply at harvests 2 and 3.

Seasonal trends in the concentration of nitrogen in tree tissues during the second growing season were similar in all treatments and values were dependent upon, and in proportion to, the level of nitrogen supplied in both first and second years of the experiment (Fig.10). During the first half of the growing season the concentration of nitrogen in the woody tissues decreased in trees of all treatments, except in roots of trees in the N1N9 treatment, while the concentration of nitrogen increased in the new shoot tissues of all treatments. During the second half of the growing season, however, the concentration of nitrogen declined sharply in the new shoots, and a further decline in concentration was also observed in the woody tissues of most treatments. Since the rate of dry matter accumulation (Fig.8) exceeded the rate of accumulation of total N (Fig.9) during the latter half of the growing season, the decline in concentration of total N was usually due to growth dilution.

Soluble N (Figs. 11, 12)

It will be observed from Figs. 11 and 12 that the concentrations and amounts of soluble N in most tissues at each harvest were dependent upon, and in proportion to, the level of nitrogen supplied in both first and second years of the experiment. During the first half of the growing season there was a sharp decline in the concentration and amount of soluble N in all tree parts, except the new shoots, of all treatments. Since the amount of soluble N per tree fell over this period (except in the N1N9 treatment), it is evident that soluble N is utilized in growth processes as would be expected if it is the main form in which nitrogen is stored in peach trees. The increase in the amount of soluble N in the new shoot tissue at this time is consistent with this view and indicates that soluble N is translocated from the roots and old tree tops to the growing points. During the second half of the growing season, the concentration of soluble N in most treatments declined further due to growth dilution (see Figs. 8, 11, 12), but the amount of soluble N rose in most treatments in proportion to the external nitrogen supply. That is, nitrogen storage began in the trees during the latter half of the growing season. It is interesting to note that soluble N did not accumulate at this time of the year in the N9N3 treatment although it did in the N1N3 and N3N3 treatments. It was apparent from leaf symptoms that the trees in the N9N3 treatment were nitrogen deficient at harvest 3 and yet these trees made excellent growth during the growing season (Figs. 3, 4, 5). It is thought that soluble N did

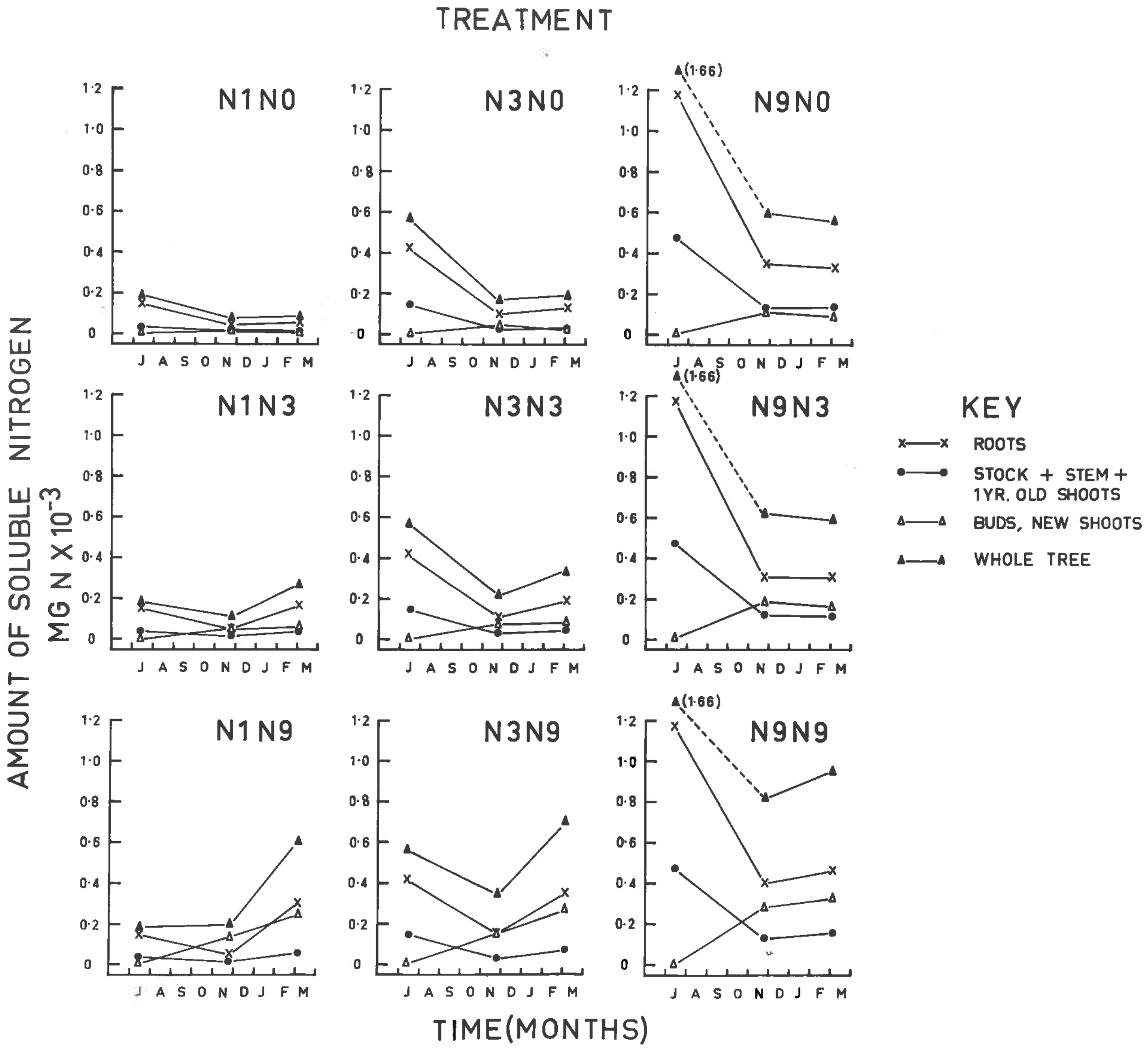
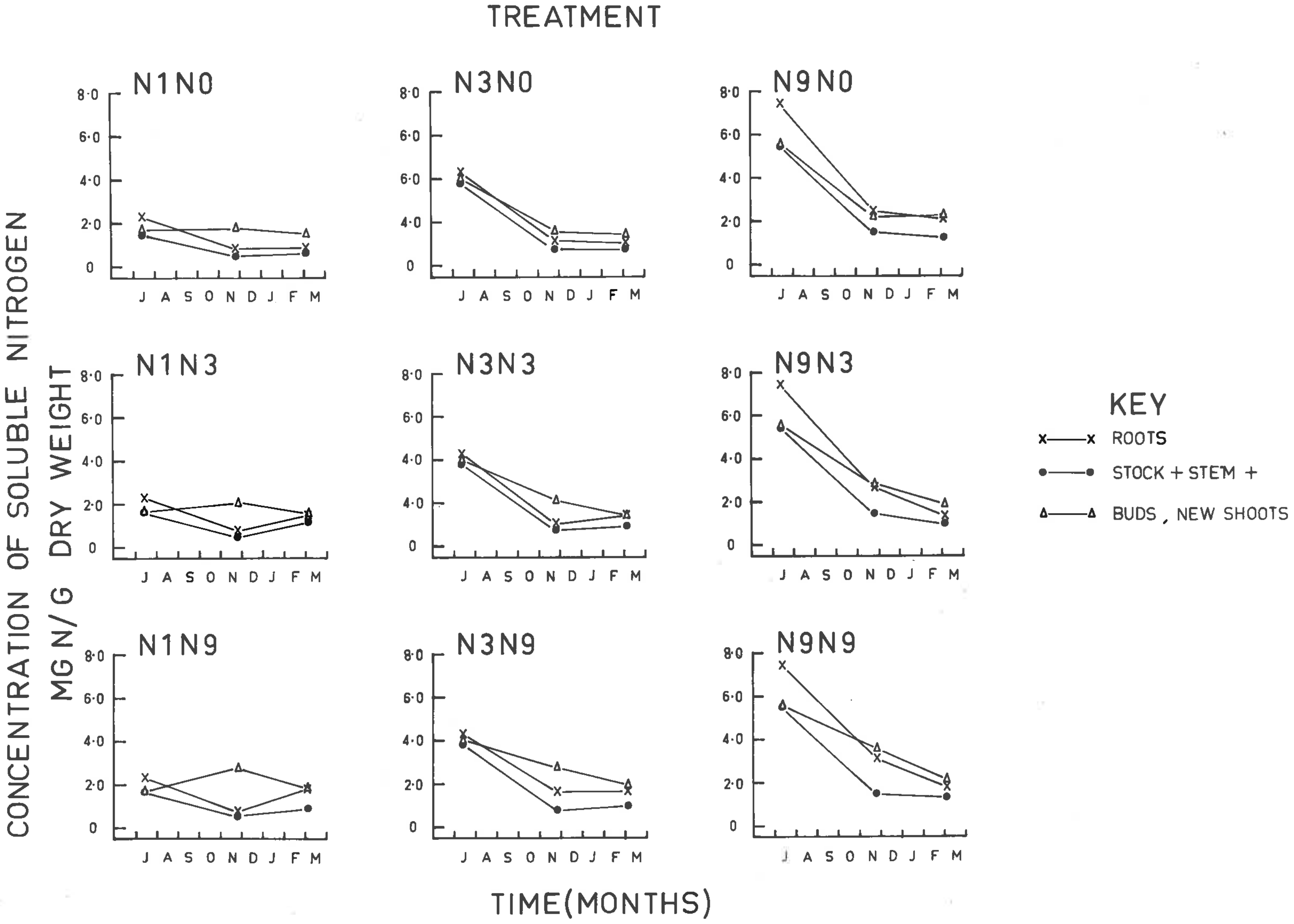


FIGURE 11 Influence of nitrogen treatment on the amount of soluble nitrogen in tree tissues during the second growing season.

FIGURE 12 Influence of nitrogen treatment on the concentration of soluble nitrogen in tree tissues during the second growing season.


TREATMENT
N1N0
N3N0
N9N0
N1N3
N3N3
N9N3
N1N9
N3N9
N9N9
8·0
6·0
4·0
2·0
0
J A S O N D J F M
CONCENTRATION OF SOLUBLE NITROGEN
MG N/G DRY WEIGHT
TIME(MONTHS)
KEY
x——x ROOTS
●——● STOCK + STEM +
Δ——Δ BUDS , NEW SHOOTS

not accumulate in these trees in proportion to nitrogen supply because the demand for nitrogen was greater than the supply.

Insoluble N (Figs.13, 14)

It is evident that the amount of insoluble N during the second growing season was dependent on, and in proportion to, the level of nitrogen supplied in both first and second year treatments (Fig.13). However, the influence of the first and second year treatments on the concentration of insoluble N in tree tissues was small and not apparent in all tissues and treatments (Fig.14). Since the amount of insoluble N increased throughout the growing season in most tree tissues and treatments (the rate of increase was dependent upon the current nitrogen supply), it is apparent that insoluble N was not utilised in growth processes; i.e., insoluble N did not act as storage nitrogen in the peach trees. However, there is one exception to this conclusion. There is evidence that small amounts of insoluble N in the stock + stem + 1 year old shoots of all treatments were mobilized during the first half of the growing season, and presumably this nitrogen was translocated to the growing points.

In the latter half of the growing season the amount of insoluble N in the new shoots of trees in the N1N0, N3N0, N9N0 and N9N3 treatments fell, presumably due to loss of insoluble N in abscissed leaves (the extent of this loss was not measured). Such a loss would also explain why the amount of insoluble N in trees of

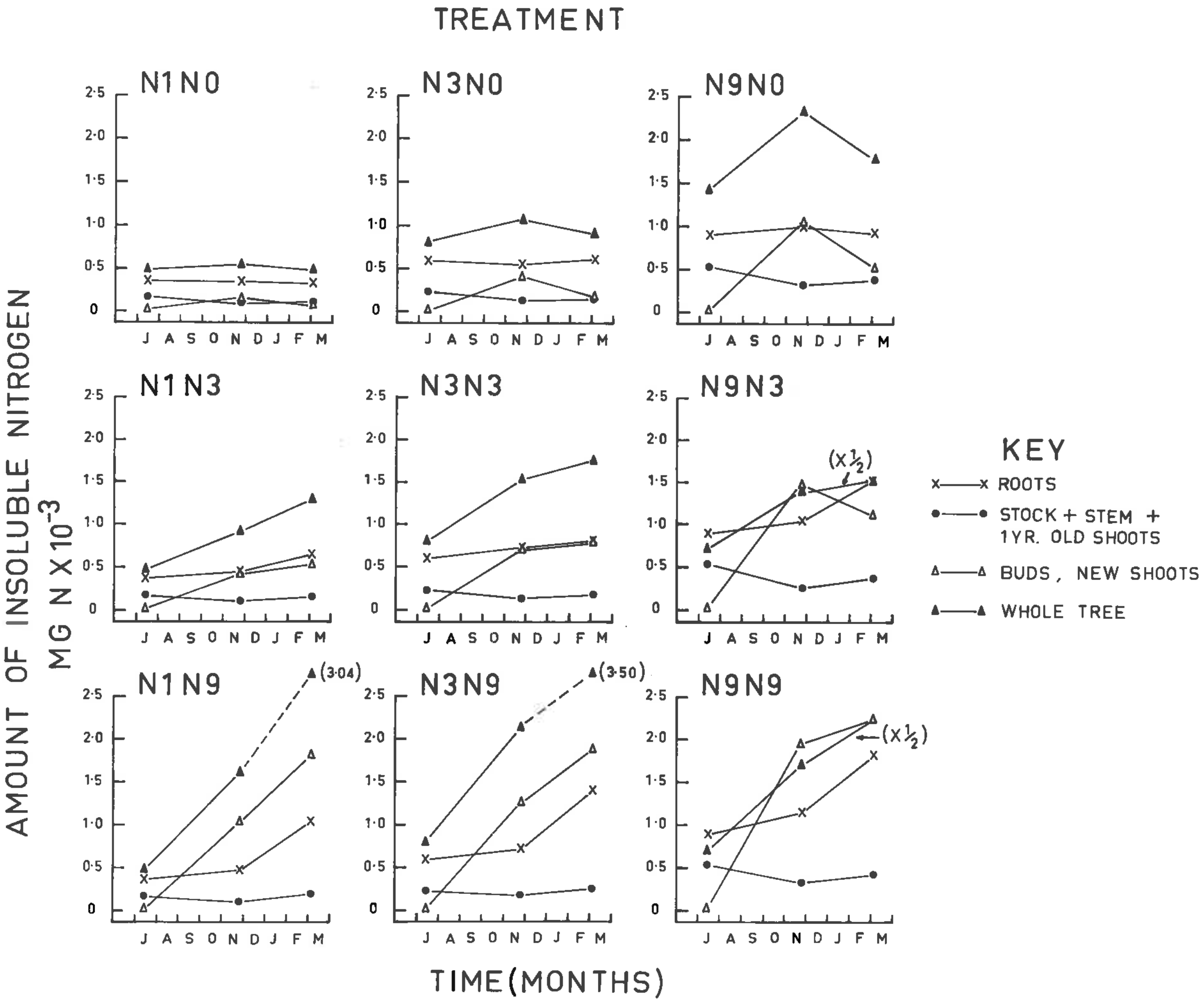


FIGURE 13 Influence of nitrogen treatment on the amount of insoluble nitrogen in tree tissues during the second growing season.

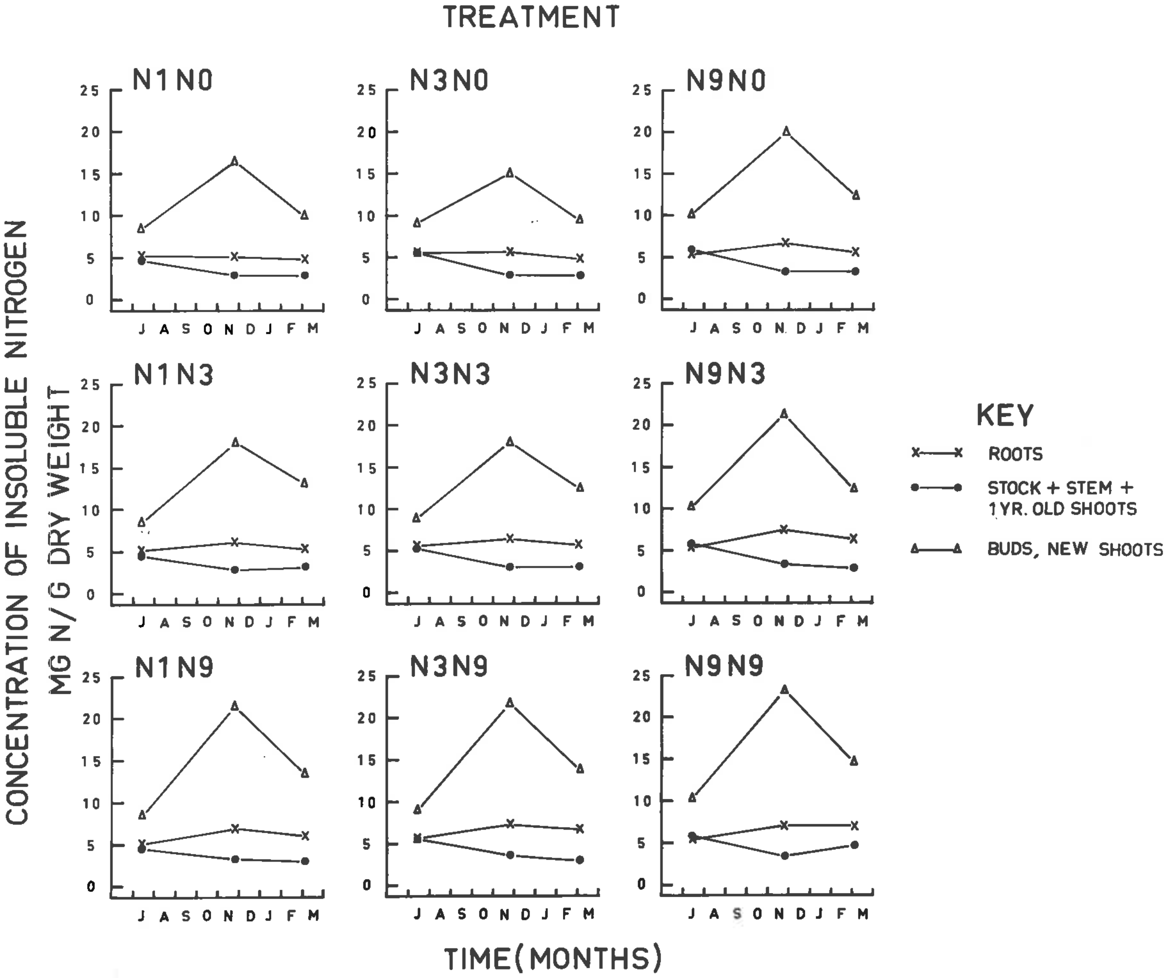


FIGURE 14 Influence of nitrogen treatment on the concentration of insoluble nitrogen in tree tissues during the second growing season.

the N9N0 treatment fell, without a concomitant rise in the amount of soluble N in the trees (Fig.11), in the latter half of the growing season.

There was little change in the concentration of insoluble N in the roots and old tops of the trees during the second growing season, but the concentration rose sharply and then fell in the new shoots during the season (Fig.14). This latter result was attributed to growth dilution during the latter half of the growing season in all treatments except N1N0, N3N0, N9N0 and N9N3 (c.f. Figs.8, 13, 14).

Arginine N (Figs. 15, 16)

The concentration and amount of arginine N in all tissues fell sharply to a very low level during the first half of the growing season and remained low during the remainder of the growing season. This pattern of change would be expected if arginine is utilized in growth processes. The first year treatments did not influence the concentration and amount of arginine N except at harvest 1.

Total α-Amino N (Figs. 17, 18).

The amount of total α-amino N in tree tissues during the second growing season was dependent upon, and in proportion to, the level of nitrogen supplied during the first and second years of the experiment (Fig.17). However, apart from the harvest 1 data, these treatments did not markedly influence the concentration of total α-amino N during the growing season (Fig.18). Overall, the con-

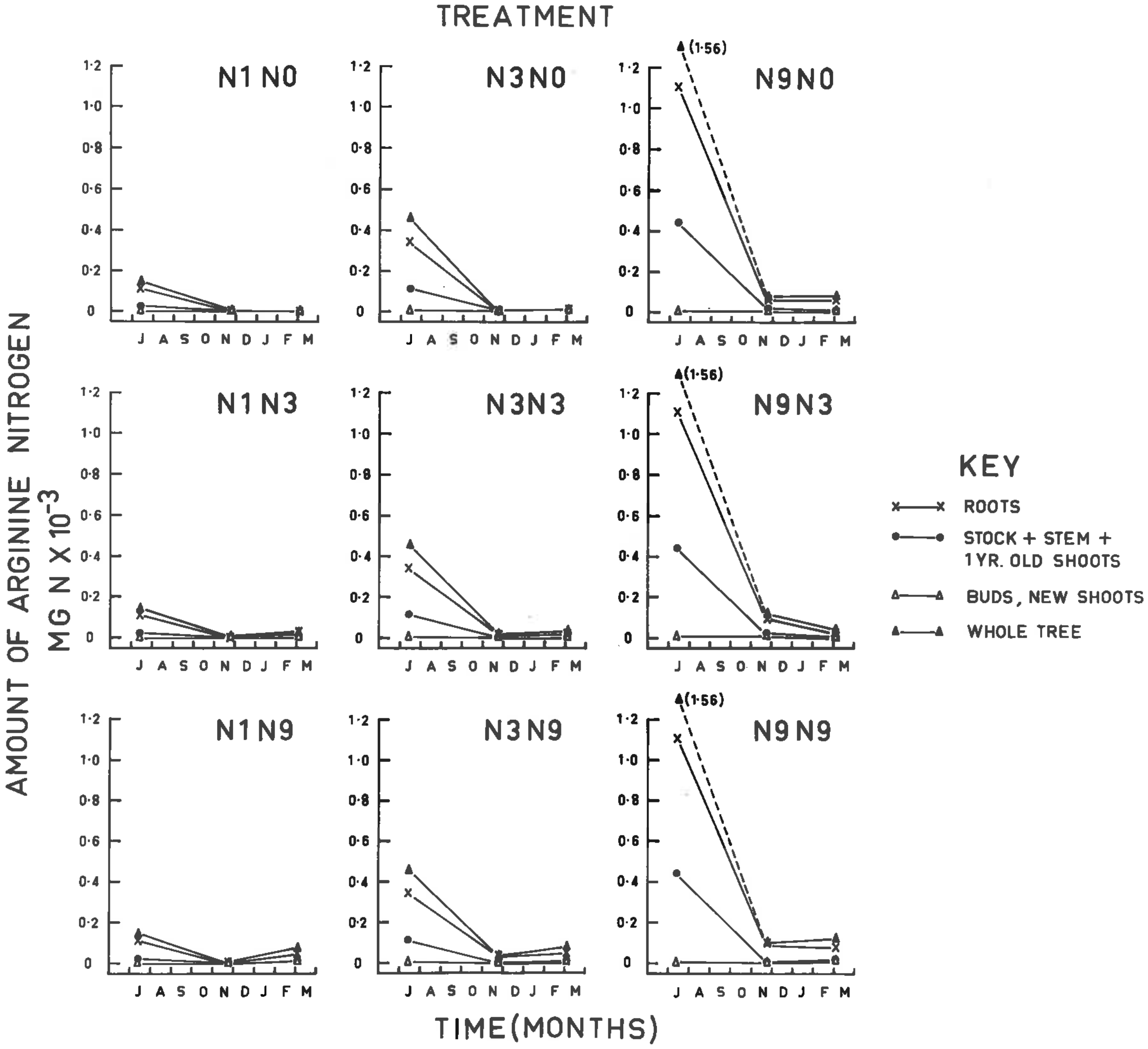


FIGURE 15 Influence of nitrogen treatment on the amount of arginine nitrogen in tree tissues during the second growing season.

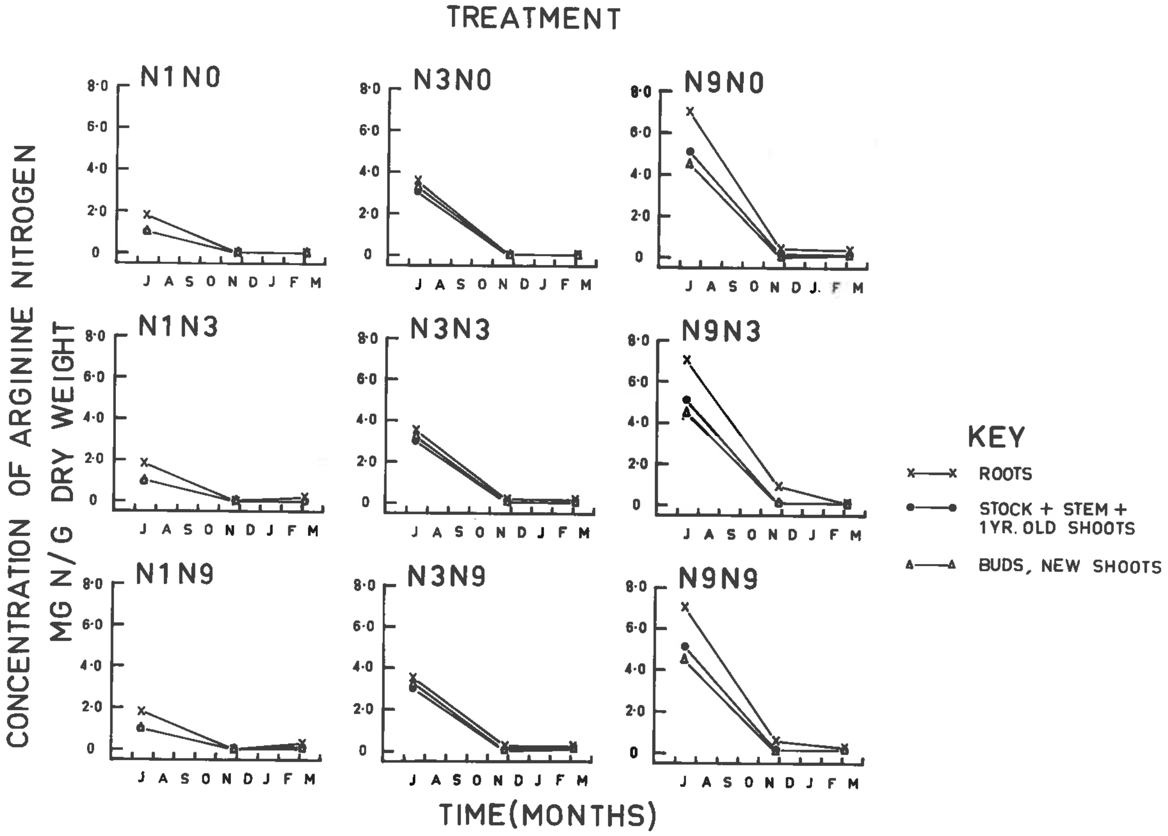


FIGURE 16 Influence of nitrogen treatment on the concentration of arginine nitrogen in tree tissuesduring the second growing season.

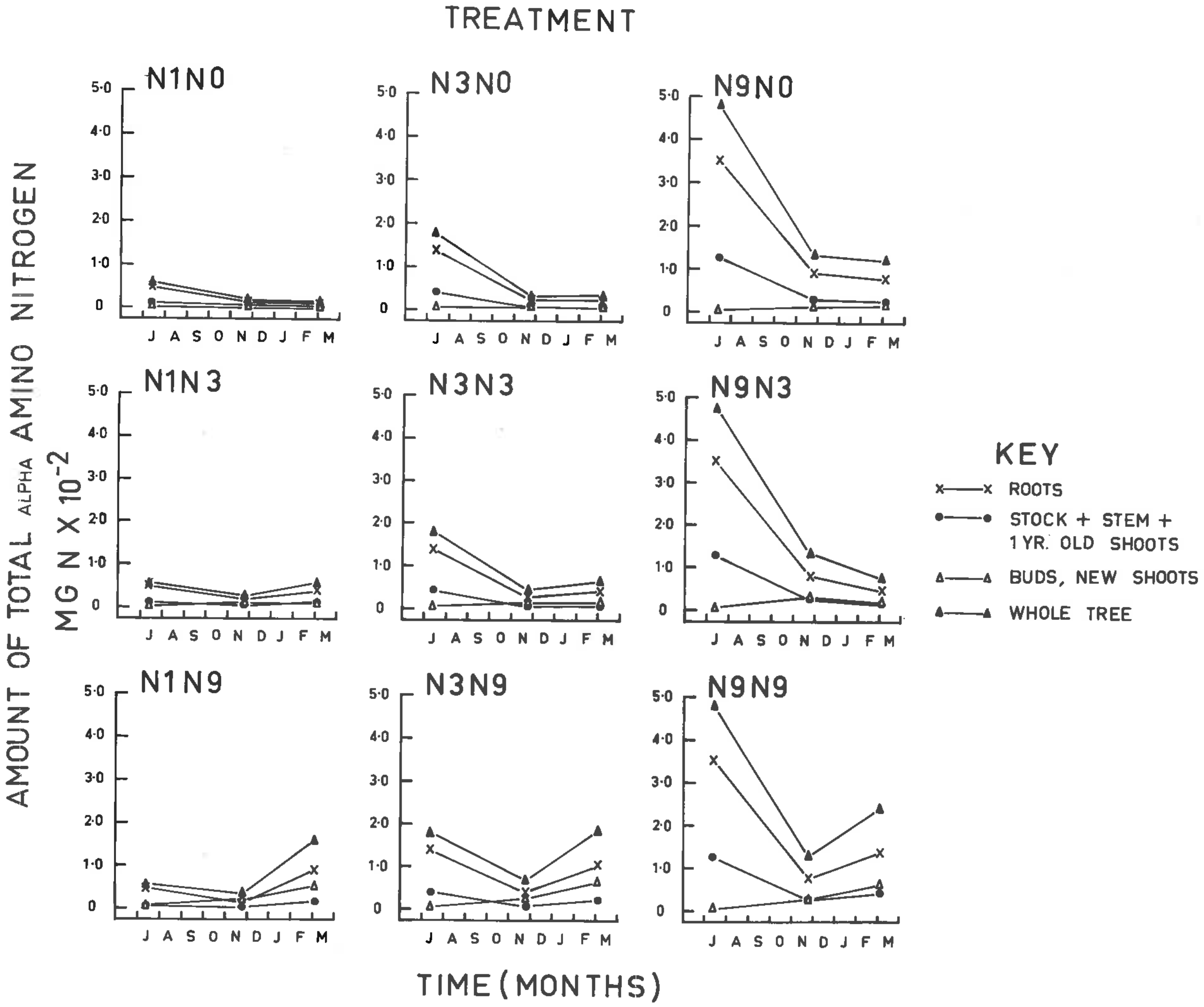


FIGURE 17 Influence of nitrogen treatment on the amount of total ∝-amino nitrogen in tree tissues during the second growing season.

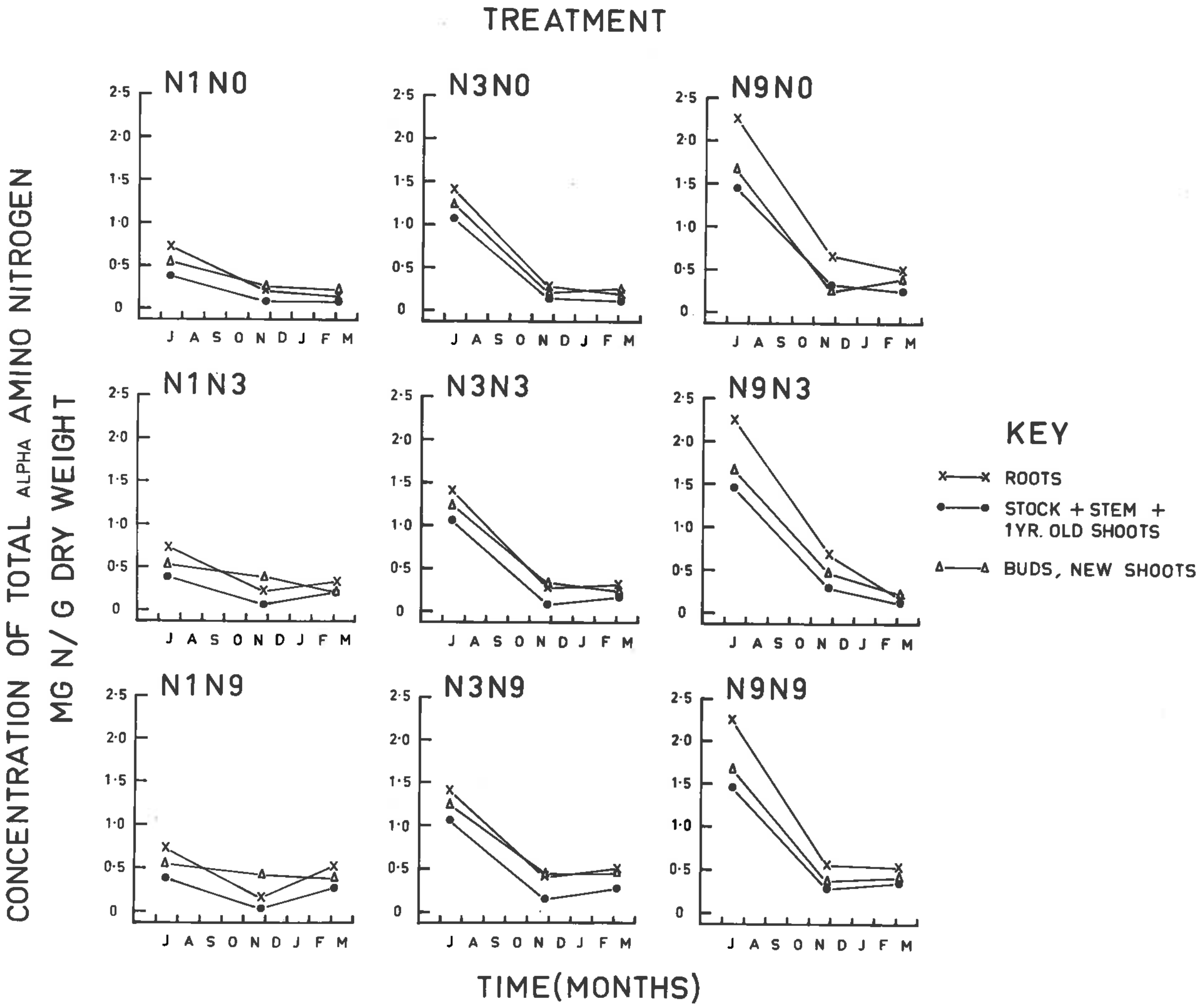


FIGURE 18 Influence of nitrogen treatment on the concentration of total α-amino nitrogen in tree tissues during the second growing season.

centration of total α-amino N in all treatments declined throughout the growing season, although small increases in concentration did take place at the end of the season in some treatments (Fig.18). However, while the amount of total α-amino N declined in all treatments during the first half of the growing season, except for small increases in the new shoot tissues, the amount of total α-amino N increased during the second half of the growing season in proportion to the external nitrogen supply (except in the N9N3 treatment).

It is apparent that seasonal trends in amounts of soluble N (Fig.11) and total α-amino N (Fig.17) were similar. While the amounts of both of these constituents rose in proportion to the external nitrogen supply during the latter half of the growing season, the amount of arginine did not increase to any marked extent at this time of the year (Fig.15). Therefore it is concluded that the soluble N (the main form of storage nitrogen in the trees), which built up in tree tissues during the summer months, contained little arginine but probably consisted largely of other free amino acids and amides (see Table 13 also). Unfortunately, the actual amount of nitrogen in the free amino acid and amide fraction of the extracts could not be calculated from the results of analyses for total α-amino N because the test is only valid for α-amino groups.

Summary of Seasonal Changes

Evidence has been presented which indicates that the concentrations and amounts of total N, soluble N, insoluble N, arginine N and total α-amino N in tissues of peach trees during the second growing season were usually dependent upon, and in proportion to, the level of nitrogen supplied to the trees in both first and second years of the experiment. Residual effects of the first year treatment on the composition of the tissues in the second growing season were found irrespective of the level of nitrogen supplied during the second year. Thus the nitrogen content during the second growing season was dependent upon the level of storage nitrogen at the beginning of the growing season and upon the external nitrogen supply. Furthermore, it was found that the nitrogen which was stored in the roots and old tree tops was translocated to the growing points of the trees during the first half of the growing season. However, during the latter half of the growing season, total N and soluble N reaccumulated in woody tissues of the trees of most treatments in proportion to the external supply of nitrogen. Small increases were also observed in the amount of total N and soluble N in some of the woody tissues of trees which did not receive any nitrogen during the second year, and it is concluded that in these cases nitrogen was translocated into the tissues prior to leaf abscission (percentage leaf abscission in these treatments at harvest 3 was high). Although the amounts of several of the nitrogenous constituents increased in some treatments during the latter half of the growing season, the concentration of these constituents did not usually increase because of growth dilution, i.e. the rate of accumulation of dry matter exceeded the rate of accumulation of nitrogen.

The soluble N which reaccumulated during the latter half of the second growing season probably consisted largely of free amino acids and amides, but arginine was not an important constituent of this fraction (c.f. harvest 1 data). The seasonal changes which were observed in the levels of soluble N and insoluble N were consistent with the hypothesis that soluble N, and not insoluble N, is the main form in which nitrogen is stored in woody tissues of peach trees. However, it was also found that a small part of the insoluble N content of stock + stem + 1 year old shoot tissues acted as a nitrogenous reserve in the trees.

(3) Distribution of Nitrogenous Constituents in Tree Tissues at Harvests 2 and 3

The distribution of each nitrogenous constituent in trees at harvests 2 and 3 is shown in Tables 15 and 16. The results indicate that, in those treatments where no nitrogen was supplied during the second growing season, the bulk of each nitrogenous constituent was found in the roots at both harvests. However, as the level of nitrogen supplied during the second year was increased, less nitrogen was found in the roots and old tree tops (stock + stem + 1 year old shoots), with the exception of arginine N at harvest 2, and more nitrogen was found in the new shoots of the trees. Apart from the arginine N at harvest 2, it is evident that the distribution of nitrogen in tree tissues during the second growing season was dependent upon the nitrogen treatments applied in both years of

TABLE 15

DISTRIBUTION OF NITROGENOUS CONSTITUENTS IN TREE TISSUES AT HARVEST 2

Amount of Nitrogen in Each Tree Part as a Percentage of the Total per Tree

N Treatment	Total N				Soluble N			Insoluble N			Arginine N			Total α-Amino N		
	Roots	Stock + Stem + 1Yr Old Shoots	New Shoots	Abscissed Leaves	Roots	Stock + Stem + 1Yr Old Shoots	New Shoots	Roots	Stock + Stem + 1Yr Old Shoots	New Shoots	Roots	Stock + Stem + 1Yr Old Shoots	New Shoots	Roots	Stock + Stem + 1Yr Old Shoots	New Shoots
N1N0	61.8	14.0	24.0	0.2	66.9	15.5	17.6	61.1	13.8	25.1	94.3	5.7	0	75.9	12.6	11.5
N1N3	46.0	10.1	43.8	0.1	45.3	13.6	41.1	49.3	9.7	44.2	91.9	0	8.1	59.0	8.2	32.8
N1N9	29.1	6.0	64.8	0.1	26.8	7.4	65.8	29.4	5.9	64.7	82.4	0	17.6	34.6	3.2	62.2
N3N0	52.5	11.2	35.7	0.5	59.8	16.6	23.6	51.5	10.4	38.1	96.4	0	3.6	70.1	14.1	15.8
N3N3	47.5	8.8	43.5	0.3	50.2	13.9	35.9	47.1	8.1	44.8	95.4	0	4.6	64.5	6.9	28.6
N3N9	35.1	7.8	56.9	0.3	44.5	9.7	45.8	33.5	7.5	59.0	96.3	1.4	2.3	55.7	8.9	35.4
N9N0	44.8	14.6	39.7	0.9	56.9	22.5	20.6	41.9	12.8	45.3	76.1	5.4	18.5	67.2	21.4	11.4
N9N3	38.8	12.1	48.2	0.9	49.6	20.0	30.4	36.3	10.5	53.2	85.1	11.1	3.8	57.7	20.5	21.8
N9N9	36.2	10.3	52.5	1.0	48.1	16.0	35.9	33.4	9.0	57.6	87.0	7.1	5.9	58.0	19.6	22.4
Interaction L.S.D.																
5%	6.2	2.1	5.3	NS	7.8	3.2	7.5	8.3	1.9	6.5	NS	-*	-	9.4	NS	9.7
1%	8.5	2.8	7.2		10.5	4.4	10.1	11.2	2.6	8.8		-	-	12.8		13.2
0.1%	11.3	3.7	9.7		14.1	5.9	13.5	15.0	3.4	11.8		-	-	17.1		17.6
V.R. Test 1st yr treatment	xx	xxx	NS	xxx	x	xxx	xxx	xx	xx	xx	NS	-	-	x	xxx	xxx
2nd yr treatment	xxx	xxx	xxx	NS	xxx	xxx	xxx	xxx	xxx	xxx	NS	-	-	xxx	xx	xxx

* Insufficient data for analysis

TABLE 16

DISTRIBUTION OF NITROGENOUS CONSTITUENTS IN TREE TISSUES AT HARVEST 3

Amount of Nitrogen in Each Tree Part as a Percentage of the Total per Tree

N Treatment	Total N				Soluble N			Insoluble N			Arginine N			Total α-Amino N		
	Roots	Stock + Stem + 1Yr Old Shoots	New Shoots	Abscissed Leaves	Roots	Stock + Stem + 1Yr Old Shoots	New Shoots	Roots	Stock + Stem + 1Yr Old Shoots	New Shoots	Roots	Stock + Stem + 1Yr Old Shoots	New Shoots	Roots	Stock + Stem + 1Yr Old Shoots	New Shoots
N1N0	63.5	19.0	11.4	6.1	68.5	21.6	9.9	67.5	20.0	12.5	91.6	3.9	4.5	71.7	19.0	9.3
N1N3	50.6	10.8	36.8	1.8	61.1	16.6	22.3	49.4	9.9	40.7	69.4	22.7	7.9	65.3	18.0	16.1
N1N9	35.3	6.6	55.6	2.0	50.3	9.1	40.5	33.8	6.2	60.0	68.1	11.3	20.5	56.3	10.5	33.2
N3N0	58.1	12.5	15.5	13.9	69.7	17.3	13.0	67.0	13.9	19.0	85.1	8.3	6.6	73.2	14.0	12.8
N3N3	44.9	9.7	39.8	5.6	58.2	13.9	27.9	45.5	9.6	44.9	67.3	17.6	15.1	64.3	13.2	22.5
N3N9	40.2	7.2	49.8	2.8	50.0	11.2	38.8	39.6	6.7	53.7	63.8	17.2	19.0	55.0	12.1	32.9
N9N0	44.2	17.9	21.7	16.2	59.3	24.0	16.7	50.6	20.5	28.9	73.6	18.8	7.6	63.4	22.4	14.2
N9N3	47.2	12.2	33.2	7.4	51.8	19.7	28.5	50.8	11.9	37.3	60.6	20.1	19.3	57.2	17.5	25.3
N9N9	39.2	10.0	45.0	5.8	47.6	16.9	35.5	40.2	9.3	50.5	59.3	23.7	17.0	55.2	17.9	26.8
Interaction L.S.D. 5%	5.2	2.6	4.2	3.1	NS	NS	NS	5.4	2.7	4.3	NS	8.3	NS	NS	NS	6.6
1%	7.0	3.6	5.7	4.2				7.3	3.7	5.9		11.3				8.9
0.1%	9.4	4.8	7.6	5.6				9.8	4.9	7.9		15.2				12.0
V.R. Test 1st yr treatment	xxx	xxx	NS	xxx	xx	xxx	NS	NS	xxx	NS	x	xx	NS	x	xxx	NS
2nd yr treatment	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xx	xxx	xxx	xx	xxx

the experiment. At harvest 3, the bulk of the arginine N was also found in the roots, but, with increasing nitrogen supply in the second year, less arginine N was found in the roots and more was found in the old tops and the new shoots of the trees.

(4) Nitrogen Uptake per Tree During the Second Growing Season

The nitrogen uptake per tree from the beginning of the growing season to harvest 2 or harvest 3 was calculated from the difference between the total N content per tree at each respective date. The results are shown in Table 17. It is evident that the nitrogen uptake per tree at both harvests was approximately directly proportional to the external nitrogen supply.

(d) Discussion

The young peach trees accumulated nitrogen in proportion to supply during the first year and the results suggest that this stored nitrogen exerted a powerful influence on the growth and chemical composition of the trees in the next growing season irrespective of the external nitrogen supply. For example, in agreement with the results of Roberts (1921) and Oland (1959) for apple, the amount of extension growth made by the trees was proportional to both the level of storage nitrogen in tree tissues at the beginning of the growing season and the current nitrogen supply (Fig.3). The amount of shoot growth made was highly correlated with the level of storage

TABLE 17

NITROGEN UPTAKE PER TREE DURING THE SECOND GROWING SEASON

Log$_e$ mgN

N Treatment	(Harvest 2)				(Harvest 3)			
	Interaction mean	Uptake Rel. to N1N3 Treat.(%)	1st Year Treatment Mean	2nd Year Treatment Mean	Interaction Mean	Uptake Rel. to N1N3 Treat.(%)	1st Year Treatment Mean	2nd Year Treatment Mean
N1N0	-	0.5			-	0		
N1N3	5.907	100.0	6.464 (N1)	- (N0)	6.840	100.0	7.430 (N1)	- (N0)
N1N9	7.021	306.1			8.020	324.9		
N3N0	-	0			-	0		
N3N3	5.890	103.5	6.444 (N3)	5.627 (N3)	6.709	88.3	7.348 (N3)	6.684 (N3)
N3N9	6.998	298.5			7.987	314.2		
N9N0	-	13.9			-	0		
N9N3	5.086	98.5	6.074 (N9)	7.027 (N9)	6.502	82.8	7.181 (N9)	7.955 (N9)
N9N9	7.062	317.8			7.859	281.7		
L.S.D. 5%	NS	-	NS	0.764	NS	-	NS	0.239
1%		-		1.057		-		0.331
0.1%		-		1.461		-		0.457

nitrogen in the trees prior to commencement of growth, provided that the current nitrogen supply was low (Table 7). In addition, however, results were also obtained which indicated that the growth of roots and old tree tops (stock + stem + 1 year old shoots) was likewise dependent upon the level of storage nitrogen and the current nitrogen supply, irrespective of how growth was measured (Fig.8; Tables 5A, 5B, 6A, 6B).

Statistical analysis of the growth data indicated that there was a significant negative interaction between the first and second year treatments at the end of the second growing season; i.e., the smaller the nitrogen application in the first year of the experiment, the greater was the influence of added nitrogen on tree growth during the second growing season, and vice versa. For instance, a comparison of the total length of the new shoots per tree at the end of the second growing season in treatments N1N9 and N9N9 indicates that the influence of the first year treatment was relatively unimportant, and that the growth of trees in the N1N9 treatment closely approached that of trees in the N9N9 treatment. This was not brought about by a greater uptake of nitrogen during the second growing season by trees in the N1N9 treatment c.f. trees in the N9N9 treatment (Table 17), and the reason for the relatively greater increase in tree size in the N1N9 treatment is unknown. However, either of the following possibilities could have been responsible:

(1) light interception by the foliage was more efficient in trees in the N1N9 treatment c.f. trees in the N9N9

treatment; the foliage was more dense in the latter treatment (see Plate 2), and therefore light intensity could have limited the rate of photosynthesis in leaves in the innermost parts of the foliage.

(2) individual leaves were more efficient and lived longer on trees in the N1N9 treatment c.f. the N9N9 treatment; it was observed that very few leaves yellowed and abscissed from trees in the N1N9 treatment c.f. trees in other treatments (see Table 50).

Although tree growth in early spring of the second year was also proportional to both the level of storage nitrogen in tree tissues and the external nitrogen supply (Figs.3, 4, 5), the data suggested that prior to November tree growth was mainly dependent upon the level of storage nitrogen, presumably because the rate of uptake of nitrogen via the roots was slow until then. After November, however, tree growth was mainly dependent upon the current nitrogen supply, and, since nitrogen deficiency symptoms were first seen in November on trees which did not receive any nitrogen during the second growing season, it is concluded that the supply of storage nitrogen in these trees was exhausted by this time of the year. In agreement with this, Mochizuki and Hanada (1956) concluded that the supply of storage nitrogen in young apple trees was exhausted by late November or early December and that subsequent growth was made at the expense of the current nitrogen supply. In contrast, however, Oland (1959) did not detect a growth response in young apple trees to the current nitrogen supply until January or February, providing that the level of storage nitrogen in the trees was high. He concluded that up to that time storage nitrogen was used for new growth, irrespective of the current nitrogen supply.

The capacity of a tree to store nitrogen (storage volume) probably influences the time of the year at which the tree will respond to current nitrogen supply. For example, a large mature tree may respond to current nitrogen supply much later in the season than a young tree even though both trees may have received the same nitrogen treatment for a number of years.

The residual effect of the first year nitrogen treatments on tree growth during the second year supports the hypothesis that stored nitrogen is utilised for new growth. However, since tree size at the beginning of the second growing season was also proportional to the nitrogen treatments applied in the first year, it is possible that at least part of this residual effect was due to an influence of tree size on subsequent growth. In an attempt to clarify the importance of storage nitrogen for new growth a range of nitrogenous compounds, including compounds known to be constituents of the storage nitrogen of dormant peach trees, were injected into one-year old peach trees of approximately the same size, but no significant stimulation of growth occurred (see Appendix B).

Results of analyses for a range of nitrogenous constituents and the calculated uptake of nitrogen per tree indicated that the peach trees accumulated nitrogen in proportion to nitrogen supply during the first year of the experiment (Tables 8B, 11). The evidence suggested that the storage nitrogen in dormant tissues consisted mainly of soluble organic nitrogen, although at least portion of the insoluble N of the stock + stem + 1 year old shoot tissues also acted as a nitrogenous reserve (see Fig.13). Oland (1954, 1959) came to the same conclusion regarding the composition of the storage nitrogen in young apple trees, except that he found that part of the insoluble N in the roots, as well as in the above-ground parts of the tree, acted as a nitrogenous reserve. The composition of the soluble nitrogen in young peach trees was similar to that found in apple trees (Oland 1954, 1959) and in mature peach trees (Schneider 1958; Taylor, unpublished data). Thus arginine was the chief compound detected in the soluble N fraction. However, contrary to Oland's results, which showed that the amides were also important constituents of the soluble N of apple tissues, the amides comprised only a small portion of the soluble N in young peach trees (Table 9). This result agrees with the observations made by Schneider (1958) and Taylor (unpublished data) on seasonal changes in the concentration of the amides in mature peach trees.

An assessment of the concentration or amount of arginine in dormant tissues of young peach trees gave the most sensitive indication of the nitrogen status of the trees (Tables 8A, 8B). Since arginine is also the major constituent of the soluble N in dormant tissues of mature trees (Schneider 1958; Taylor, unpublished data), it is apparent that this finding is applicable to either young or mature trees. Conceivably this information could be used as the basis of a new method for the assessment of the nitrogen status of orchard trees. In terms of sensitivity, the method would be superior to that proposed by Baxter (1965), since the latter method is based on an estimation of the level of α-amino N in dormant tree tissues. Thus, when the external nitrogen supply was increased, the increase in arginine N in tissues of the dormant trees usually exceeded the concomitant increase in total α-amino N in the tissues (Tables 8A, 8B).

During the first half of the second growing season stored nitrogen was translocated from woody tissues to developing shoots, irrespective of the current nitrogen supply (Fig.9). If no external nitrogen was given to the trees (N0 treatment) most of the storage nitrogen which was mobilized for growth came from the root tissues, but if a large nitrogen application was given to the trees (N9 treatment) most of the nitrogen which was mobilized came from the aboveground parts of the tree (see Table 14). In the latter treatment, nitrogen uptake by the trees would have partially masked the loss of nitrogen from the woody storage tissues. Since about two-thirds of the total amount of each nitrogenous constituent in the dormant peach trees was found in the roots, it is clear that this tissue contains the bulk of the storage nitrogen of the tree (see Table 10). Yokomizo _et al._ (1964) came to a similar conclusion after studying the storage and mobilization of nitrogen in 5 year old apple trees.

The amounts of soluble N and arginine N in the trees declined sharply during spring but the levels of insoluble N per tree increased; these results support the conclusion that the storage nitrogen of the trees consisted mainly of soluble N (c.f. Figs.11, 13, 15).

Total N and soluble N reaccumulated during the latter half of the second growing season in woody tissues of the peach trees in proportion to the external nitrogen supply (Figs.9, 11). Small increases in amounts of total N and soluble N were also noted in some of the woody tissues of trees which did not receive any external nitrogen supply during the second year. It is thought that in these cases nitrogen was translocated from the leaves into the storage tissues immediately prior to leaf abscission. There are two reasons for this suggestion. Firstly, such a pathway of translocation of nitrogen has been well documented for trees (see Literature Review), and secondly, it was observed that a large proportion of the leaves on the trees in this treatment abscissed during the latter half of the growing season (see Table 60). Accumulation of soluble N during late summer was unexpected because previous workers (Williams 1939; Schneider 1953; Radu 1961) have shown that the storage of nitrogen in peach trees does not take place to any marked extent until extension growth has ceased (February to March). In each case, however, they based their conclusions on a study of seasonal changes in concentration, rather than absolute amounts, of nitrogenous constituents. It is evident from the present study that the concentration of the nitrogenous constituents in peach trees usually declines during the latter half of the growing season as a result of growth dilution.

Although soluble N accumulated in storage tissues of peach trees during the latter half of the second growing season, arginine was not a major constituent of the fraction (see Table 13). Other free amino acids and amides, however, accumulated to a greater extent than arginine. It is known that the rate of accumulation of dry matter (presumably mainly carbohydrate) exceeded the rate of accumulation of soluble N during the latter half of the growing season (c.f. Figs.8, 11). This may be the reason why compounds such as arginine, which have a low C/N ratio, were not synthesised to any extent at this time of the year, while compounds which have higher C/N ratios, e.g. the amides and certain amino acids, accumulated. However, if this is so, it might also be expected that if the rate of accumulation of soluble N by the trees exceeds the rate of accumulation of carbohydrate, e.g. at leaf fall in autumn, then nitrogenous compounds, which have low C/N ratios, would accumulate in tree tissues. This hypothesis would therefore explain why arginine accumulates in peach tissues in autumn and winter (Schneider 1958; Taylor, unpublished data), but does not accumulate during the growing season, even if the amount of soluble N increases in tissues. The finding that the concentration of free amino acid N and amide N in the above-ground tissues of mature peach trees usually reached a peak in late summer and then declined in autumn and winter, while the concentration of arginine N increased at this time (Taylor, unpublished data), could be interpreted as supporting this hypothesis, and may indicate that the amides are degraded and that the nitrogen is incorporated into arginine. Alternatively, however, arginine may accumulate in storage tissues of peach trees during autumn and winter in response to the prevailing environmental conditions, such as short days and low temperatures (see Literature Review).

B. UPTAKE, TRANSLOCATION AND METABOLISM OF L-ARGININE-guanido-^{14}C-MONOHYDROCHLORIDE AND L-ASPARAGINE-^{14}C (U) BY TWO YEAR OLD PEACH TREES IN AUTUMN AND WINTER

(a) Introduction

As a basis for the study the following hypotheses were set up:

(1) storage nitrogen of dormant peach trees consists mainly of arginine and the amides.

(2) since arginine and the amides accumulate in peach trees during autumn and winter, these compounds are relatively inert in storage tissues at this time of the year.

(3) arginine and the amides, if injected into peach trees during autumn or winter, move to, and accumulate in, storage tissues.

At the time of inception of this study, the results described in section A of the Experimental Work were not available and the formulation of the first two hypotheses listed above was based on the studies of Schneider (1958) and Taylor (unpublished data) with mature peach trees. The aim of the experiments was to determine whether the latter two hypotheses are correct.

(b) Methods

(i) Source of Trees and Tree Management

One year old peach trees (Golden Queen scion on Elberta rootstock) were selected for uniformity of size and freedom from disease and damage at Balhannah Nurseries in June 1963. At planting, the roots of the trees were lightly trimmed and were then dipped in solutions of NaOCl and terramycin hydrochloride (see p.26) in an attempt to prevent infection of the trees with crown gall. Following this, the trees were planted in porous clay pots (15 inch diameter), each of which contained 12.36 Kg of steam-sterilised soil overlying 2 Kg of sterilised, quartzite gravel. After tamping down the soil, 2 Kg of sterilised gravel were added to each pot as a surface mulch. The soil-mix consisted of 3 parts soil, 2 parts sand and 1 part gravel ([illegible] inch diameter). After planting, tree tops were severely pruned; each side shoot was cut back to leave 2 buds only and in some cases the stem was also shortened. The pruned trees were then placed on a bench in the open and a watering line was attached (pipe-line fitted with sprinkler jets).

At bud burst, the trees were sprayed with freshly prepared bordeaux mixture to control 'curl-leaf' fungus. Later, as shoot growth commenced, the number of new shoots per tree was reduced to about 4 and these were allowed to develop as side-shoots. All pruning cuts were sealed with 'Tree Guard' mastick. Trees were watered as required (usually every second day), and solutions of complete fertilizer were added to the trees at approximately monthly intervals during the last 3 months of the growing season.

(ii) Source and Storage of Isotopes

0.1 mc of L-arginine-guanido-^{14}C-monohydrochloride (specific activity 13.6 mc/mM) and 0.1 mc of L-asparagine-^{14}C (uniformly labelled; specific activity 22.5 mc/mM) were obtained from The Radiochemical Centre, Amersham, Buckinghamshire, England. Each freezedried compound was taken up in 5.0 ml distilled water and deep-frozen until required.

(iii) Application of Isotopes to Trees

(1) Injection of Amino Acid Solutions into Woody Tissues of Young Peach Trees

Preliminary experiments were carried out in June 1963 with aqueous, non-radioactive solutions of L-arginine hydrochloride and L-asparagine (50 to 5000 μg amino acid/ml) to determine whether the solutions could be successfully injected into shoots and roots of dormant trees, whether the concentration of the injected solutions influenced the amount of liquid taken up by the trees, and whether uptake of liquid was influenced by the time of day at which the injection was commenced. These experiments are described below:

One year old peach trees were planted in 'second soil' in glazed earthenware pots of 3 gallon capacity. Major roots (approx. 0.5 cm in diameter) were tagged at planting with a length of polythene extrusion so that they could be readily located later if required for root injections. Potted trees were placed on benches in a glasshouse and were watered as required with tap water. Any leaves which remained on the trees were picked off prior to injection.

The injection technique used was similar to that described by Roach (1938) for the injection of solutions into shoots of woody plants. The tip of a shoot or major root was cut off, a polythene tube was pushed over the cut end of the shoot or root until it was firmly attached, and then the other end of the tube was connected to a burette. The test solution was immediately poured into the burette, air bubbles were removed from the column of liquid by tapping the burette or tapping and squeezing the tubing, and then the initial volume of liquid in the burette was read off. Subsequently, burette readings were recorded at hourly intervals and the difference between two successive readings for any one tree was a measure of the uptake of liquid per hour. Readings were also made of the volume of liquid in a control burette, i.e. one not connected to a tree, and this data indicated that the loss of liquid from the burettes as a result of evaporation was negligible. Two injection experiments were carried out and each was approximately of 2 days duration.

During the first experiment the temperature of a flask of distilled water (500 ml volume), which had been placed on the bench in the glasshouse alongside the potted trees, was recorded whenever the uptake measurements were made. However, since the rate of uptake of the liquid was significantly correlated with this water temperature (see Table 20), more detailed records of temperature fluctuations were made during the second experiment. Records were kept of water temperature (500 and 50 ml volumes), air temperature (thermometer suspended on a tree) and soil temperature (thermometer bulb 3 inches below the soil level in a pot).

The results of the 2 experiments are shown in Tables 18, 19 and 20. It is evident from Tables 18 and 19 that the uptake of injected liquid was not dependent upon the time of day at which the injection was commenced, nor was it dependent upon the compound injected or the way in which the solutions were injected into the trees (except at certain concentrations), but it was dependent upon the concentration of the injected solution. Thus it was found that the intermediate concentration of the injected solutions (500 µg amino acid/ml) resulted in the greatest uptake as a result of root injection (Table 19). It appears that the uptake of the solutions increased with increasing solution concentration (see shoot injection data also, Table 19), except at high concentrations (5000 µg amino acid/ml) which may have been toxic.

The data in Table 20 indicates that there was a highly significant correlation between the volume of amino acid solution taken up by the dormant trees and the soil and water temperature (500 ml volume) in both experiments. In contrast, however, the correlation between solution uptake and air or water temperature (50 ml volume) was either of a low order of significance or was non-significant.

TABLE 18

TOTAL UPTAKE (ml) OF SOLUTIONS OF L-ARGININE HYDROCHLORIDE (50 µg/ml) AND L-ASPARAGINE (50 µg/ml) BY 1 YEAR OLD ELBERTA PEACH TREES AS A RESULT OF ROOT INJECTION OVER A 44 HOUR PERIOD

(Experiment 1)

Treatments were replicated twice

Injected Compound	Time of Day Injection Commenced		
	9-10 A.M.	12-1 P.M.	4-5 P.M.
Arginine-HCl (B.D.H., L.R.)	8.45	8.03	4.45
Asparagine (Merck, A.R.)	6.80	7.98	7.48

ANALYSIS OF VARIANCE

		d.f.	M.S.	V.R.	
Total SS	128.167	11			
Time SS	9.408	2	4.704	0.264	N.S.
Compd. SS	0.585	1	0.585	0.033	N.S.
Time x Compd. SS	11.291	2	5.645	0.317	N.S.
Error SS	106.884	6	17.814		

TABLE 19

TOTAL UPTAKE (ml) OF SOLUTIONS OF L-ARGININE HYDROCHLORIDE AND L-ASPARAGINE BY 1 YEAR OLD ELBERTA PEACH TREES AS A RESULT OF SHOOT AND ROOT INJECTION OVER A 31 HOUR PERIOD

(Experiment 2)

Treatments were replicated twice

Type of Injection	Concentration of Arginine HCl Solutions (μg/ml)			Concentration of Asparagine Solutions (μg/ml)		
	50	500	5000	50	500	5000
Shoot Injection	5.93	7.75	8.07	5.50	4.60	6.45
Root Injection	2.65	3.80	4.45	3.08	3.78	3.15

ANALYSIS OF VARIANCE

		d.f.	M.S.	V.R.	
Total SS	147.633	23			
Injection SS (I)	9.127	1	9.127	2.903	N.S.
Compd. SS (N)	6.202	1	6.202	1.973	N.S.
I x N SS	3.082	1	3.082	0.980	N.S.
Concn. SS (C)	41.465	2	20.733	6.594	x
C x I SS	44.747	2	22.373	7.116	xx
C x N SS	3.117	2	1.558	0.496	N.S.
C x I x N SS	2.215	2	1.108	0.352	N.S.
Error SS	37.730	12	3.144		

Type of Injection	Concentration Means				
	50		500		5000 (μg/ml)
Shoot Inj.	↓ 5.71	N.S.	6.17	N.S.	7.26 ↓
Root Inj.	2.86	⟸	3.79	⟹	3.80

L.S.D. 5% 2.73
1% 3.83
0.1% 5.41

TABLE 20

THE RELATIONSHIP BETWEEN AIR, SOIL AND WATER TEMPERATURE (°C) AND THE UPTAKE (ml) OF INJECTED SOLUTIONS OF L-ARGININE HYDROCHLORIDE AND L-ASPARAGINE BY 1 YEAR OLD ELBERTA PEACH TREES OVER A 31 HOUR PERIOD

Expt. No.	Type of Injection	Compound Injected							
		Arginine HCl (50-5000 µg/ml)				Asparagine (50-5000 µg/ml)			
		Items in Regression	r	d.f.	Significance	Items in Regression	r	d.f.	Significance
1	Root Injection	Root uptake V. water temp. (500 ml vol)	0.774	16	xxx	Root uptake V. water temp. (500 ml vol.)	0.792	16	xxx
2	Root Injection	Root uptake V. air temp.	0.425	15	N.S.	Root uptake V. air temp.	0.398	15	N.S.
		Root uptake V. soil temp.	0.827	15	xxx	Root uptake V. soil temp.	0.737	15	xxx
		Root uptake V. water temp. (500 ml vol.)	0.727	15	xxx	Root uptake V. water temp. (500 ml vol.)	0.639	15	xxx
		Root uptake V. water temp. (50 ml vol.)	0.168	15	N.S.	Root uptake V. water temp. (50 ml vol.)	0.153	15	N.S.
2	Shoot Injection	Shoot uptake V. air temp.	0.620	15	xx	Shoot uptake V. air temp.	0.601	15	x
		Shoot uptake V. soil temp.	0.821	15	xxx	Shoot uptake V. soil temp.	0.786	15	xxx
		Shoot uptake V. water temp. (500 ml vol.)	0.826	15	xxx	Shoot uptake V. water temp. (500 ml vol.)	0.807	15	xxx
		Shoot uptake V. water temp. (50 ml vol.)	0.292	15	N.S.	Shoot uptake V. water temp. (50 ml vol.)	0.352	15	N.S.

Since it was thought that the soil temperature and the water temperature (500 ml volume) would be dependent on, but would lag behind, the air temperature, the appropriate data was plotted and is shown in Fig.19. It is clear that, while the soil temperature and water temperature (500 ml volume) data is apparently unrelated to the air temperature data, when corrections were made for a lag period of 1 to 2 hours then a linear relationship between each of the items is apparent. Therefore it was concluded that solution uptake by the dormant trees was positively correlated with air temperature; i.e. the higher the air temperature (which influences water and soil temperatures after a lag period), the faster the uptake of injected solutions by the dormant trees, probably because of increased transpiration rates.

As a consequence of this work, it was concluded that amino acid solutions could be readily injected into dormant peach trees via shoots or roots, and that the rate of uptake was temperature dependent. The rate of uptake per tree ranged from 0 to 0.90 ml/hour.

In experiments in which solutions of ^{14}C-labelled arginine hydrochloride and asparagine were injected into shoots of young peach trees during autumn and winter, the following technique was used. The trees which were to be injected with the isotopes were placed in a small glasshouse compartment (except for a control tree which was placed in a separate compartment to avoid possible $^{14}CO_2$ contamination) which was heated during the experiment so that up-

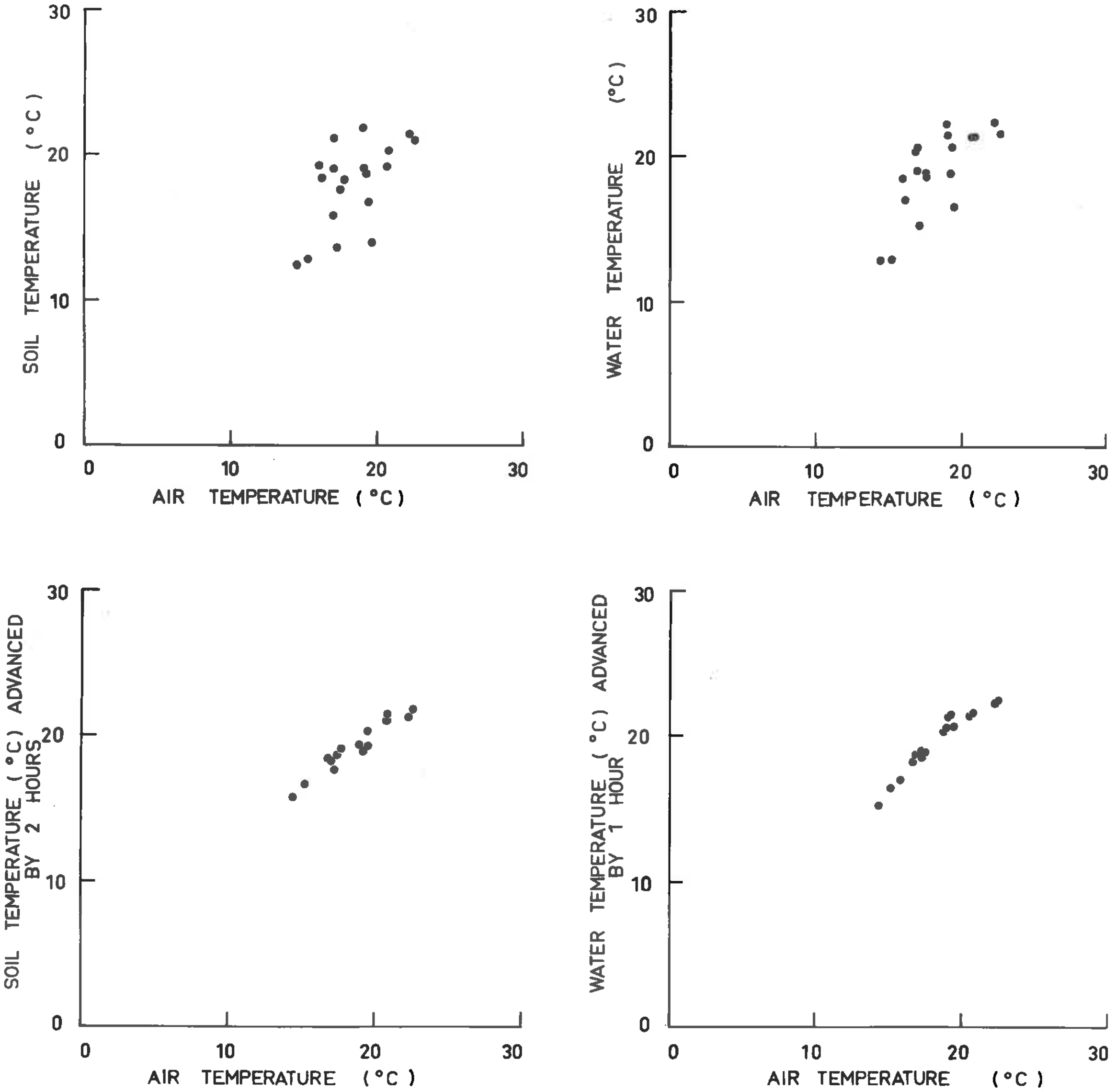


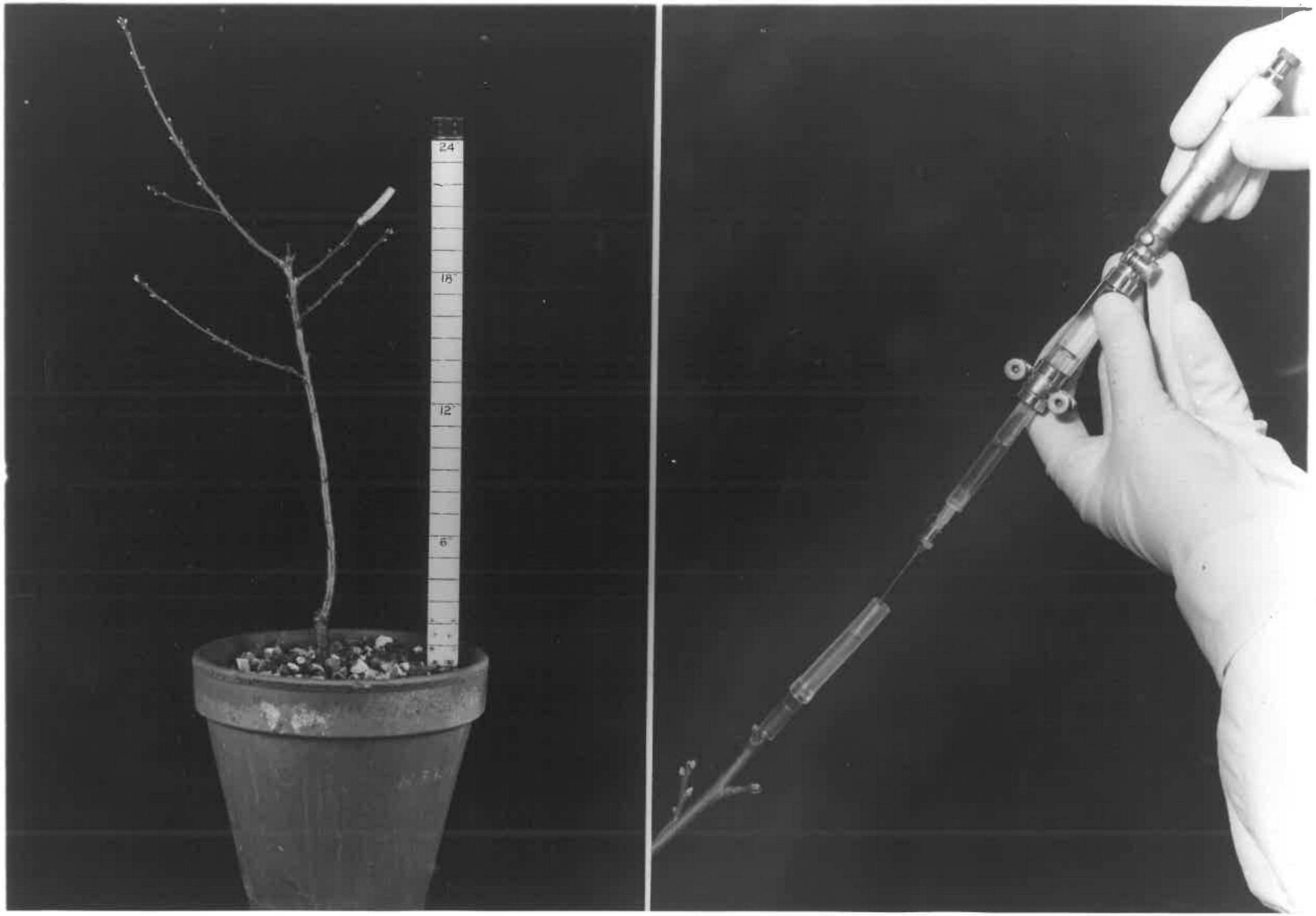
FIGURE 19 Relationship between soil, water (500 ml vol.) and air temperature during an injection experiment carried out in June 1963.

take would be as rapid as possible. The distal end of a shoot was cut off, a short length of polythene tubing was pushed over the cut end of the shoot, and a few drops of distilled water were squirted into the tube to keep the cut end moist. After the seal had been checked for leakage, the appropriate volume of ^{14}C-labelled solution (e.g. 1 ml) was pipetted into the tube by means of an Agla syringe (see Plate 3). When all of the liquid had been taken up by the tree (1 ml was taken up in 7 to 10 hours by dormant, leafless trees), the tube was disconnected and placed in a beaker of distilled water. The amount of radioactivity in this water was later measured (after concentration in vacuo and making to volume) and this value was taken into account when the amount of radioactivity taken up by the tree was calculated.

(2) Application of Isotope Solutions to Leaves

In March 1964, 250 µl of ^{14}C-labelled arginine hydrochloride were applied to the upper surface of a mid-shoot leaf on a young peach tree. This experiment was carried out in an unheated glasshouse compartment. Apical and basal portions of the leaf were first taped with adhesive material in such a way that liquid could be held in the central portion of the leaf surface without loss, and the leaf was also firmly taped to a stake to prevent movement. The isotope solution was then applied dropwise to the leaf surface with the aid of an Agla syringe. After 7 hours all of the applied liquid

PLATE 3 Technique used to inject radioactive solutions into shoots of young peach trees: left, treated tree with injection tube in place; right, pipetting the solution into the injection tube.


24"
18"
12"
6"

had evaporated or had been absorbed by the leaf, but a deposit, presumably arginine hydrochloride, was observed on the leaf surface. At this stage the treated leaf was washed well by squirting its upper surface with a jet of distilled water. This water was collected in a beaker, concentrated <u>in vacuo</u>, made to volume, and the amount of radioactivity in it was taken into account when the uptake of isotope by the tree was calculated.

(iv) Harvesting techniques and Preparation of Tissue for Autoradiography and Analysis

At harvest, the trees were washed out of the pots under a jet of water and the roots were thoroughly cleaned and blotted dry. The shape of each tree was then sketched and representative portions of each part of the tree were selected and labelled for subsequent autoradiography. Each tree was then quickly divided into the following parts: root, stock + stem + side shoots, and the injected shoot. In one experiment, leaves and buds were also separated from the other tissues. Tissue pieces were immediately plunged into dry ice to avoid possible chemical changes during harvesting. Each tree part was chopped into small pieces (excepting tissue selected for autoradiography), wrapped in muslin bags and freeze-dried. Dried tissues were weighed and ground in a Casella mill to pass a sieve with pores 1 mm in diameter. The ground tissues were placed in labelled screw-lid jars and deep-frozen until required for analysis.

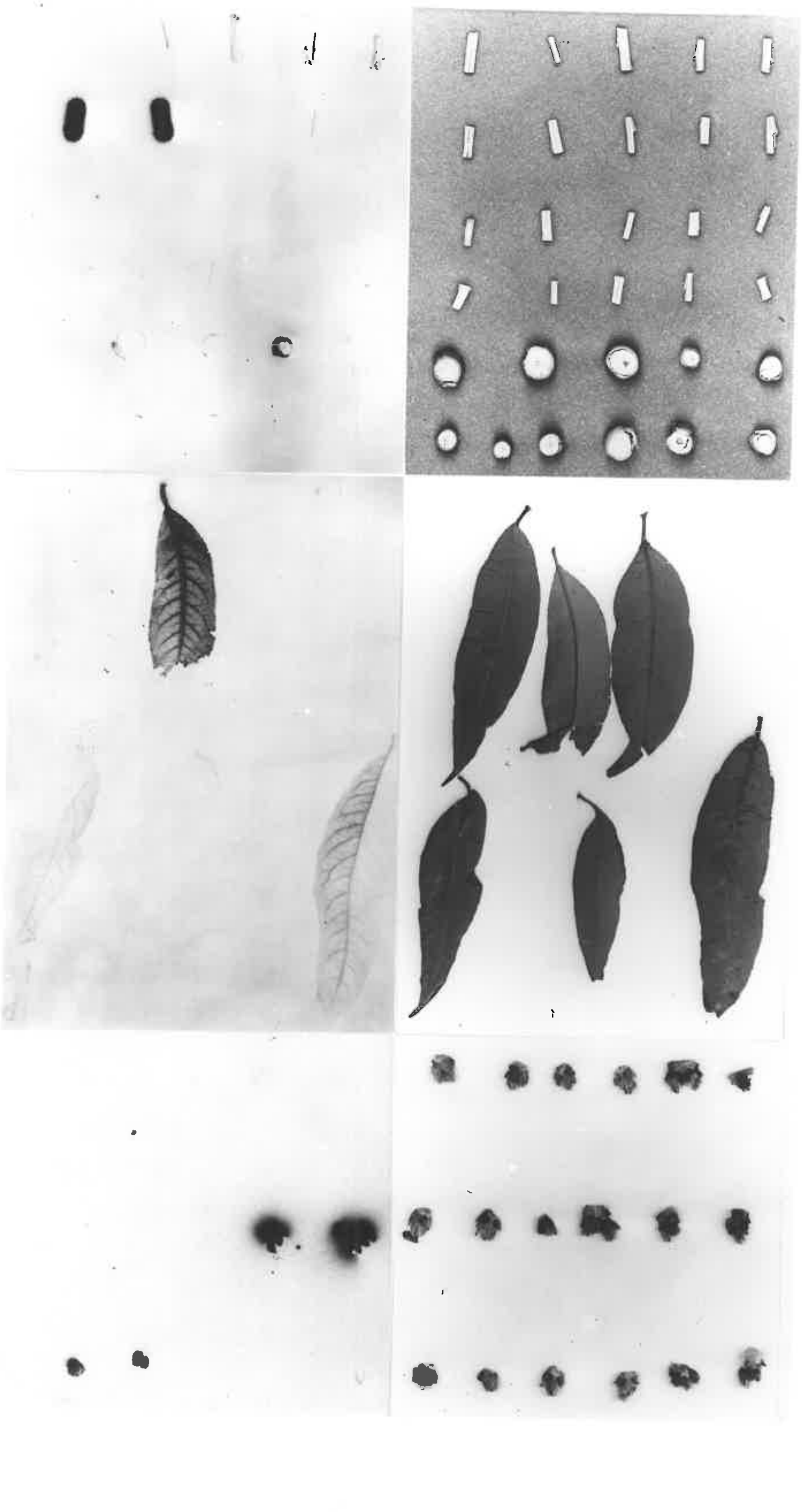
Samples which had been selected for tissue autoradiography, including samples from a control tree, were cut into small pieces if necessary (L.S. and T.S. sections) so that they would present a flat surface to X-ray film. Tissue pieces were then placed in labelled vials or a press (fibrous roots and leaves) and freeze-dried. Dried tissues were deep-frozen until required for autoradiography.

(v) Tissue and Chromatogram Autoradiography

Tissue pieces were placed in contact with a sheet of Kodirex (no-screen) X-ray film (Kodak, Australasia Ltd.). After separating each sheet of film with at least 4 thicknesses of blotting paper, a bundle of the loaded films was tightly clamped in a wooden press fitted with G-clamps. The press was wrapped in 2 thicknesses of black cloth and placed in a cold room at 2°C for a period of 1 month. After this period, the films were developed in a dark room using standard Kodak X-ray developer and fixer. Since the intensity of the image on the developed films is a rough measure of the amount of radioactivity present in the tissues (see Plate 4), it was possible to approximately measure the amount of radioactivity in the tissues by reference to an arbitrary set of standard images.

The presence of radioactive compounds on paper chromatograms was detected in a similar manner.

PLATE 4 Example of results of tissue autoradiography: left vertical column, autoradiographs; right vertical column, normal photographs. Pieces of stem, stock, side shoots, leaves and buds of a treated tree are shown.



(vi) Extraction, Separation, and Identification of ^{14}C-labelled Compounds

Duplicate amounts of freezedried finely-ground tissues (1 to 5 g) were extracted with 75 ml cold (2°C), 80% aqueous ethanol and then with 60 ml lots of cold distilled water until only negligible amounts of radioactivity could be extracted with fresh extractant (an extract was evaporated to a small volume and tested for radioactivity). The tissue was first placed in a glass centrifuge tube (100 ml capacity), extractant was added and after thorough mixing, the stoppered tubes were placed in a refrigerator for 24 hours. After centrifuging and filtering the supernatant through a Whatman No.4 filter paper, fresh extractant was added to the tube. All extractions except the first were of 1 hour duration. After the extraction was complete, successive extracts were bulked and either evaporated to dryness _in vacuo_ in a rotary evaporator (water-bath temperature 35°C), or passed directly through an 8x1.5 cm column of Dowex 50W X-8 resin (200 to 400 mesh) in order to separate amino acids and related compounds from acidic and neutral compounds (organic acids and sugars). Where an extract was evaporated to dryness without prior fractionation, the residue was taken up in distilled water and made to a volume of 25 ml in a volumetric flask. The level of radioactivity in these concentrated extracts was then measured (see later). All extracts were stored at 2°C prior to analysis. Tissue residues, which remained after extraction of the soluble radioactive compounds, were collected, airdried, weighed, reground and analysed for radioactivity.

Separation of acidic and neutral compounds from the free amino acid fraction was carried out according to the method of Plaisted (1958). No radioactivity was irreversibly sorbed onto the resin during this operation as tests showed that the radioactivity in the free amino acid fraction plus that in the resin eluate equalled the level of radioactivity in the crude extract. After concentration in vacuo, the free amino acid fraction was taken up in 10% isopropanol (a preservative for amino acids, see Block et al. 1958) and made to a volume of 5 ml in a volumetric flask, while the sugar and organic acid fraction was taken up in distilled water and made to volume in a 25 ml volumetric flask. Aliquots of these fractions were assayed for radioactivity.

^{14}C-labelled compounds in the free amino acid fraction were separated by paper chromatography. Aliquots of the concentrated extracts were spotted onto large sheets (18½ x 15½ inches) of Whatman No.1 paper with a Misco safety micro-pipette. A stream of warm air was directed onto the origin from beneath the paper during application in order to hasten the drying process. Occasionally acetic acid-washed paper (Whatman No.1) was also used. This paper was prepared in the following way: the paper sheets were first soaked in 2N acetic acid overnight, and then, on the next day, the acetic acid was sucked off and the paper was washed repeatedly with distilled water until the pH of the eluate from the paper was approximately pH 6.0. Chromatograms were developed in both directions in solvent systems which are commonly used to separate amino acid mixtures (Block *et al.* 1958; Smith 1960), using the descending method in the dark at 27°C. After development, the dried, solvent-free chromatograms were placed in contact with X-ray film to locate the ^{14}C-labelled spots. The length of this exposure was dependent upon the level of radioactivity applied to the chromatogram. Where possible at least 0.5 μc was applied to each chromatogram and a suitable exposure time for this level of radioactivity was found to be about 14 days. Aliquots of the standard ^{14}C-labelled amino acid solutions, which were obtained from Amersham, were tested for purity in the same way.

When ^{14}C-labelled spots were found on chromatograms, attempts were made to identify them from Rf characteristics and their specific colour reactions, using the data and tests outlined by Smith (1960) as a guide. In addition, the level of radioactivity in each ^{14}C-labelled spot was measured by liquid scintillation counting.

(vii) Protein Hydrolysis and Separation of Protein Amino Acids

Subsamples of the tissue residues, which remained after extraction, were weighed into 250 ml round-bottomed flasks. 100 ml 6*N* HCl/g tissue, 6 drops of octyl alcohol (to prevent frothing) and 6 glass beads were added to each flask, and these were then connected in series under water-cooled, reflux condensers in a fumehood. The flask contents were gently boiled for 16 hours to hydrolyse tissue proteins. At the end of this time, the hydrolyzates were cooled and the black humin was filtered off. Tests, in which samples of dried humin were exposed to X-ray film, indicated that the humin was not radioactive. Each filtered hydrolyzate was taken to dryness <u>in vacuo</u> in a rotary evaporator (water-bath temperature 35°C) to remove the HCl, and the residue was taken up in water and either made to volume in a 10 ml volumetric flask for subsequent determination of radioactivity, or else the protein amino acids were separated out on a column of Dowex 50W X-8 resin in the same way as described earlier for the free amino acid fraction. Aliquots of the concentrated protein amino acid fraction were chromatographed and the ^{14}C-labelled protein amino acids were identified as described above for the free amino acids. The radioactivity present in each ^{14}C-labelled protein amino acid was also measured by liquid scintillation counting. Tests indicated that substantial amounts of radioactivity were lost when the tissues were hydrolyzed in this way. Thus lower levels of radioactivity were found in tissue hydrolyzates c.f. tissue homogenates. It is thought that this loss of radioactivity was due to the production of $^{14}CO_2$ during hydrolysis.

(viii) Determination of Levels of Radioactivity in Extracts, Hydrolyzates, Injection-tube Washings, Leaf Washings, Homogenates and in Compounds Separated by Paper Chromatography.

(1) Extracts, Hydrolyzates, Injection-tube Washings and Leaf Washings

0.2 aliquots of the concentrated solutions were pipetted

(Misco safety pipette) onto weighed aluminium planchets (flat type AERE Harwell, drg. no. A36688) and a drop of teepol:distilled water (1:1 v/v) was added to aid spreading. The added liquid was confined to an area of 2.27 cm^2 on each planchet by means of a wax ring (applied with a chinagraph pencil). The liquid was evaporated off under an infra-red lamp set 10 inches above the planchets: a hairdrier also blew warm air over the planchets (occasionally rotated) from one side thus reducing the tendency for radioactivity to collect at the outer rim of the planchets (Cook and Duncan 1952). Planchets were cooled in a desiccator over anhydrous $CaCl_2$, re-weighed, and the radioactivity of the dried films was measured by placing each planchet on the top shelf of a sample oven (type N619, Ekco Electronics Ltd., England) fitted with an end-window Geiger-Müller tube. This tube was coupled to an automatic scaler (type N530F, Ekco Electronics Ltd.) via a probe unit (type N558, Ekco Electronics Ltd.) which was set to give a 'dead' time of 400 μ seconds. The G/M tube was operated at 1600 volts D.C. All measurements of radioactivity were made in duplicate and readings were corrected for coincidence (standard tables), background (measured and subtracted) and self-absorption (estimated). Where possible at least 1000 counts were recorded and when this was achieved the counting error was equal to or less than $\pm 3.2\%$.

In order to correct for self-absorption of ^{14}C, a self-absorption curve was constructed in the following way. A constant amount of ^{14}C-labelled asparagine in 10 μl distilled water was plated out on a series of planchets (area 2.27 cm^2) with increasing volumes (50 to 250 µl) of a non-radioactive plant extract. The liquid was evaporated off, the planchets were cooled, weighed, and the level of radioactivity on each was measured in the G/M apparatus. The resulting graph of radioactivity versus the weight of tissue extract is shown in Fig.20A. In order to use this curve to correct for self-absorption, the weight of the dried film on the planchet is measured and the corresponding Y axis value (a) is read off the graph. Then, knowing the Y axis value (b) of a standard reference point on the X axis, the self-absorption correction factor is equal to $^b/a$.

A typical calculation of the level of soluble radioactivity in a tree part is as follows:

$$\text{Amount of radioactivity } (\mu c) = (x-x^1) \times \frac{b}{a} \times \frac{100}{5.36} \times \frac{V}{v} \times \frac{W_2}{W_1} \times \frac{1}{3.7\times10^4}$$

where, x = sample count (cps)) corrected for coincidence

x^1 = background count (cps))

$^b/a$ = self-absorption factor

V = total volume of extract (ml)

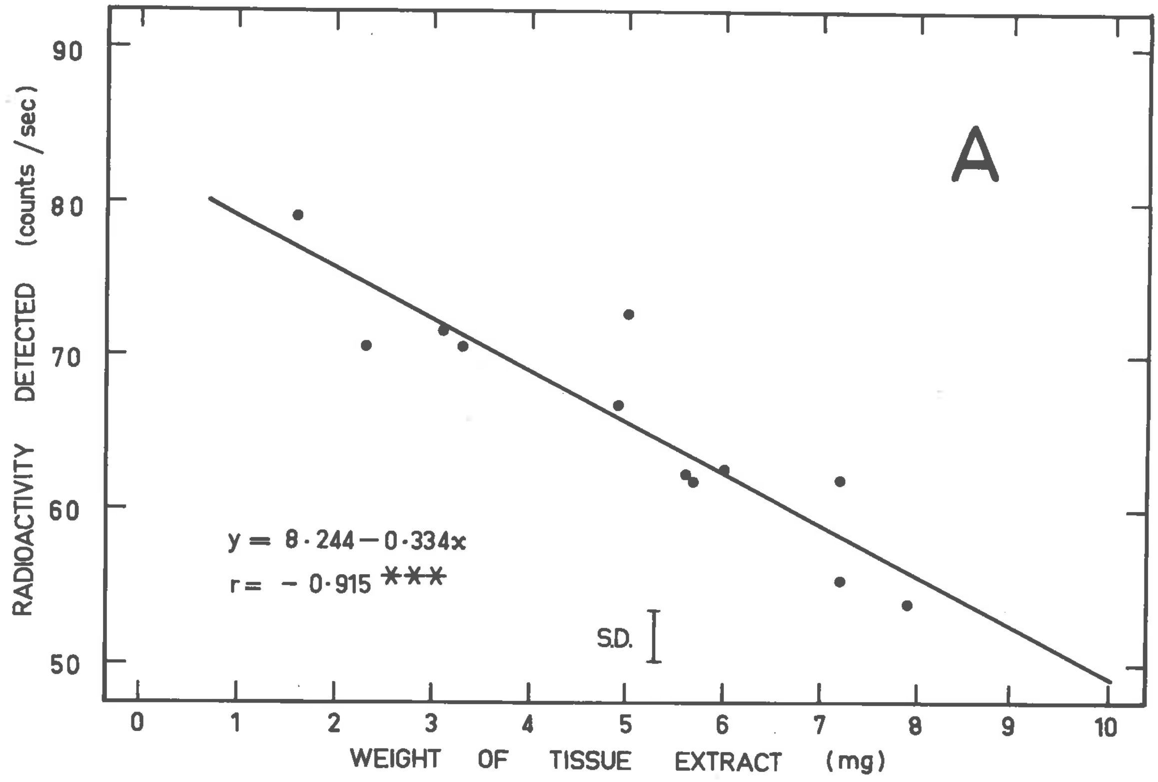
v = volume of extract analysed (ml)

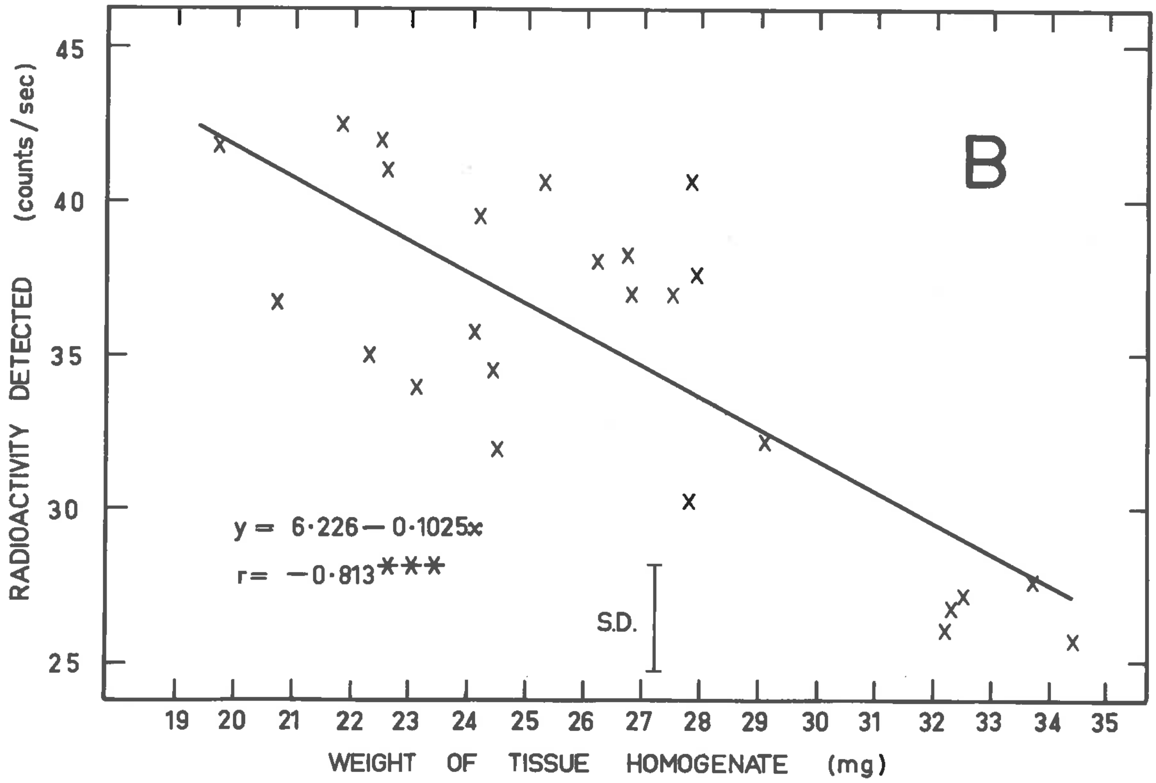
$\frac{5.36}{100}$ = counting efficiency of apparatus

$\frac{W_2}{W_1} = \frac{\text{d.wt. of tree part}}{\text{d.wt. of sample extracted}}$

$1\mu c = 3.7\times10^4$ cps

FIGURE 20 Graphs illustrating self-absorption of ^{14}C in tissue extracts (A) and tissue homogenates (B).


A
RADIOACTIVITY DETECTED (counts / sec)
90
80
70
60
50
0
1
2
3
4
5
6
7
8
9
10
WEIGHT OF TISSUE EXTRACT (mg)
y = 8·244 − 0·334x
r = − 0·915 ***
S.D.


B
RADIOACTIVITY DETECTED (counts / sec)
45
40
35
30
25
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
WEIGHT OF TISSUE HOMOGENATE (mg)
y = 6·226 − 0·1025x
r = −0·813 ***
S.D.

Since the distribution of ^{14}C in tree tissues was not uniform, it was necessary to analyse the tissue set aside for autoradiography, as well as the remaining tissue, before the level of radioactivity in the tree parts and the whole tree could be calculated.

(2) Homogenates

In a few cases, the level of radioactivity in the insoluble fraction was determined on tissue homogenates rather than on tissue hydrolyzates. Despite the fact that loss of ^{14}C occurred during hydrolysis of tissue residues, the level of radioactivity in the insoluble fraction was usually determined on tissue hydrolyzates because the method was less time-consuming.

Homogenates were prepared in the following way: 50 mg samples of air-dried, finely-ground tissue were accurately weighed into a large glass homogenizer, 1.2 ml of distilled water wereadded and the tissue was homogenized by hand until all particles would pass through the tip of a 1 ml graduated pipette. 0.7 ml of the homogenate was then plated onto a weighed aluminium planchet (rinsed in petroleum ether and etched for 3 to 4 minutes in hot N KOH before use), the surface of which had been previously coated with a layer of concentrated gum (Stephens commercial gum). This gum was applied with a small brush and the water which was present was subsequently driven off under an infra-red lamp. The use of the gum resulted in much improved adherence between the dried homogenate film and the planchet surface. The homogenates, which were spread over an area of 3.63 cm^2, were each dried to a film under an infra-red lamp, and the level of radioactivity present in each was measured in a G/M system as described earlier for extracts and hydrolyzates.

Since the weight of the homogenates, which were tested for radioactivity, varied, it was necessary to correct for self-absorption. Thus a self-absorption curve was constructed in the following way. A constant, known amount of ^{14}C-asparagine in 10 µl water was mixed with increasing amounts of non-radioactive homogenate on a series of weighed planchets. Each homogenate-asparagine mixture was dried to a thin film, the planchet was cooled and weighed, and the level of radioactivity emitted from the planchet was measured. Values for the radioactivity detected were then plotted versus the weight of the tissue homogenate (Fig. 20B). This graph was used in the same way as described earlier for the determination of the self-absorption factors of extracts etc. The amount of radioactivity in tissue homogenates was calculated as follows:

Amount of radioactivity
in the insoluble
fraction of a tree part
(µc)

$$= (x - x^1) \times \frac{b}{a} \times \frac{100}{5.36} \times \frac{W_2}{W_1} \times \frac{1}{3.7 \times 10^4}$$

where, x = sample count (cps))
)corrected for coincidence
x^1 = background count (cps))

$\frac{b}{a}$ = self-absorption factor

$\frac{5.36}{100}$ = counting efficiency of apparatus

$$\frac{W_2}{W_1} = \frac{\text{calculated d.wt. of the tissue residue in a tree part}}{\text{d.wt. of tissue residue sample on planchet}}$$

$1 \mu c = 3.7 \times 10^4$ cps

As in the case of the soluble radioactivity, it was necessary to analyse tissue set aside for autoradiography, as well as the remaining tissue, before the level of radioactivity in the insoluble fraction of a tree part could be calculated.

(3) Compounds Separated by Paper Chromatography

After locating ^{14}C-labelled compounds on paper chromatograms by autoradiography, the level of radioactivity in each compound was determined by the liquid scintillation technique of Wells (1963). In this procedure the ^{14}C-labelled spots were cut out of the chromatograms, placed face down in 5.0 ml of scintillator solution in counting pots (Quickfit, of 50 ml capacity, and fitted with ground glass tops) 4.5 cm in diameter. These pots were placed in a scintillation counter (type N664A Ekco Electronics Ltd., England), which was connected to an automatic scaler (type N530F Ekco Electronics Ltd.), and the radioactivity emitted was recorded. The equipment was operated at room temperature at 1150 volts D.C. with a discriminating bias of 10 volts D.C. and a linear amplifier gain of 100. Optical contact between the base of the pot and the top of the photomultiplier tube was maintained with silicone oil (100 centistokes, Swift and Co., Sydney, N.S.W.). The scintillator solution consisted of 2, 5-diphenyloxyazole (3g) and p-bis-2, 5-diphenyloxyazylbenzene (0.2g) dissolved in 1 litre of toluene (A.R.).

In contrast to the results of Pocchiori and Rossi (1962), it was found that there was no significant difference between the amount of radioactivity emitted from top or bottom surfaces of the paper spots. This may have been brought about by the fact that large volumes of extracts etc. were applied to the chromatograms (up to 1 ml) to ensure that readily detectable levels of radioactivity were present. Since paper spots which were larger than 9 cm^2 would not fit into the counting pots, these spots were cut into pieces, each piece was counted separately, and the level of radioactivity in the whole spot was taken as the summation of the radioactivity in the pieces. Recorded counts were corrected for background and for the efficiency of counting.

Wells (1963) claimed that count rate was independent of the size of spot being assayed. However, this was found to be incorrect since the counting efficiency significantly declined with increasing spot area (Fig.21). The data shown in this figure was obtained in the following way:

Paper squares, 1.0, 2.25, 4.00, 6.25 and 9.00 cm^2 in area, were cut from a sheet of Whatman No.1 chromatography paper. Using an Agla syringe, 0.04 μc ^{14}C-asparagine in 10 μl distilled

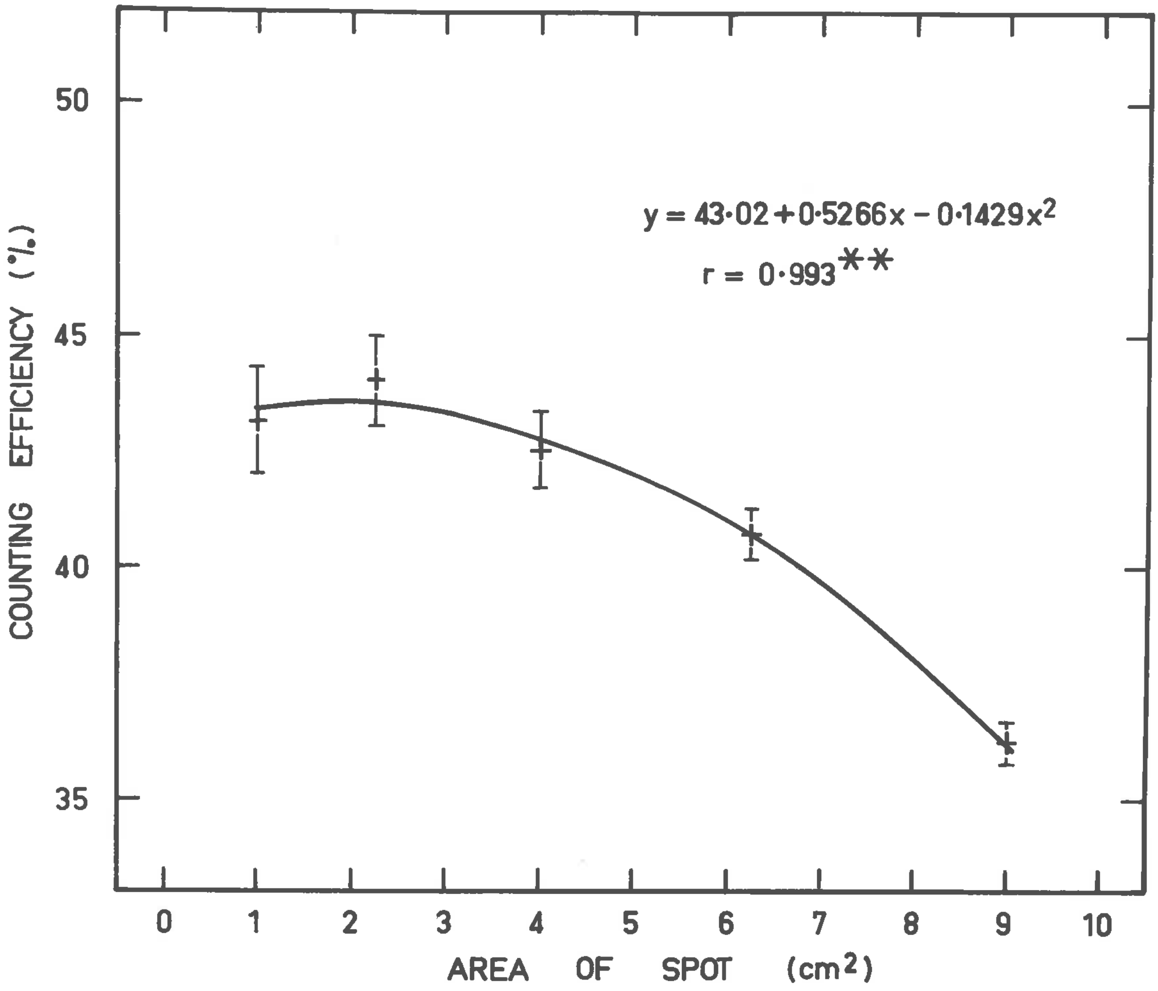


FIGURE 21 Influence of spot area on the efficiency of counting of ^{14}C on paper chromatograms, using the liquid scintillation technique of Wells (1963).

water was spotted onto the centre of each square (held in a horizontal position by a paper clip). Droplets of water were added to the centre of the spot as required until the liquid had spread uniformly over the surface of the paper square. The liquid was evaporated off under a stream of warm air from a hair-drier, and the radioactivity emitted from each square was measured in the liquid scintillation system. After correction for background, the counting efficiency was calculated for each area.

As a consequence, the area of each radioactive spot on the chromatograms was measured with a planimeter prior to counting so that the counting efficiency could be calculated.

(c) Results

(i) Experiment 1.- Uptake, Translocation and Fate of L-Arginine-guanido-^{14}C-Monohydrochloride After Application to Young Peach Trees in Autumn

The aim of this experiment was to determine which method of applying isotopes (shoot injection or leaf application) would result in greater uptake of ^{14}C by the trees. Preliminary information regarding the translocation and metabolic fate of the isotope in tree tissues was also sought.

(1) Uptake of Applied Isotopes

In March 1964, 5 μc of the ^{14}C-labelled isotope were applied to 2 year old peach trees, either as a leaf application or as a shoot injection, and the trees were harvested after a 24 hour period. Results indicated that only 37.9% of the ^{14}C which had been applied to a leaf surface entered the tree, whereas 99.9% of the ^{14}C which had been applied to a shoot entered the tree. In addition, 50.5% of the ^{14}C which had been applied to the leaf surface was recovered from tree tissues, c.f. 81.6% recovery from tissues of the shoot injected tree. In subsequent experiments, isotopes were always applied to trees by means of the shoot injection technique.

(2) Distribution and Translocation of ^{14}C in Tree Tissues

Data relating to the distribution and translocation of ^{14}C in tree tissues over the 24 hour period is set out in Tables 21A, 21B and in Fig.22. It is evident from this data that almost all of the ^{14}C recovered from tree tissues was found in the above-ground parts of the trees, irrespective of the way in which the isotopes were applied. Further, it is apparent that most of the ^{14}C activity remained in the treated organ.

However, while the amounts of ^{14}C which were translocated from the injected organs appeared to be small, results of autoradiographic studies (Fig.22) indicated that movement of ^{14}C

TABLE 21A

AMOUNTS OF ^{14}C RECOVERED (μc) IN TREE TISSUES 24 HOURS AFTER INJECTING 5μc L-ARGININE-*guanido*-^{14}C-HYDROCHLORIDE INTO A SHOOT ON A 2 YEAR OLD PEACH TREE

Tree Tissue	Soluble Activity		Insoluble Activity		Total Activity	
	^{14}C	% of ^{14}C in Tree	^{14}C	% of ^{14}C in Tree	^{14}C	% of ^{14}C in Tree
Stem + Stock + side shoots	1.99	48.74	1.92	47.27	3.91	96.00
Roots	0.01	0.32	0	0	0.01	0.32
Leaves	0.06	1.40	0.04	0.95	0.10	2.35
Buds	0.05	1.18	0.01	0.15	0.06	1.33
Total	2.11	51.64	1.97	48.37	4.08*	100.0

* = 81.58% of the ^{14}C taken up by the tree

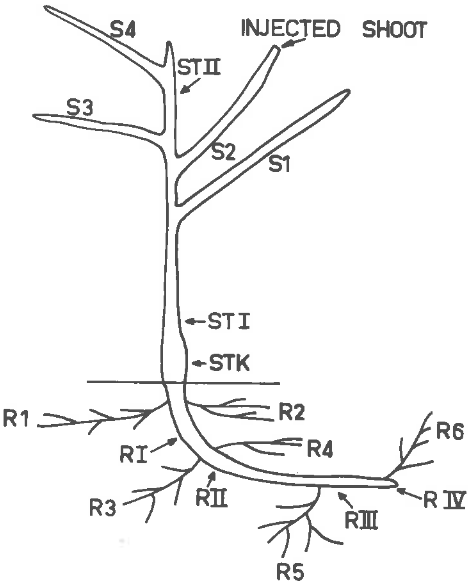
TABLE 21B

AMOUNTS OF ^{14}C RECOVERED (µc) IN TREE TISSUES 24 HOURS AFTER APPLYING 5µc L-ARGININE-*guanido*-^{14}C-HYDROCHLORIDE TO A LEAF ON A 2 YEAR OLD PEACH TREE

Tree Tissue	Soluble Activity		Insoluble Activity		Total Activity	
	^{14}C	% of ^{14}C in Tree	^{14}C	% of ^{14}C in Tree	^{14}C	% of ^{14}C in Tree
Stem + Stock + side-shoots	0.02	2.19	0	0	0.02	21.9
Roots	0.04	3.96	0	0	0.04	3.96
Leaves	0.53	55.63	0.37	38.22	0.90	93.85
Buds	0	0	0	0	0	0
Total	0.59	61.78	0.37	38.22	0.96*	100.0

* = 50.53% of the ^{14}C taken up by the tree

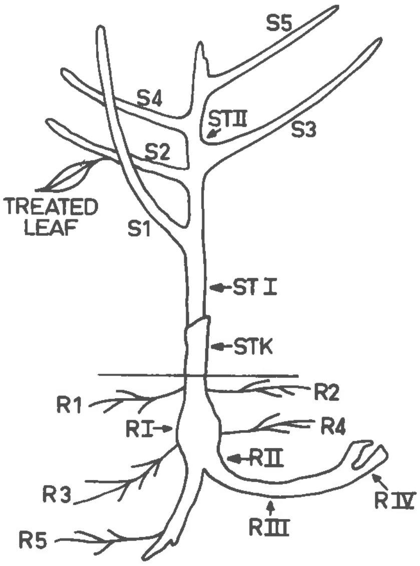
FIGURE 22 Distribution and amount (arbitrary units) of ^{14}C in tissues of 2 year old peach trees 24 hours after application of ^{14}C-labelled arginine HCl to a shoot (A) or a leaf (B).



SHOOTS, LEAVES AND BUDS						
SHOOT	BASE	TIP	BASAL LEAVES	TIP LEAVES	BASAL BUDS	TIP BUDS
S1	++	nil	nil	nil	nil	nil
S2	++++	++++	++++	—	++++	++++
S3	++	++	nil	++	nil	nil
S4	++	++	nil	++	nil	nil

ROOTS, STEM AND STOCK						
FIBROUS ROOTS	R1	R2	R3	R4	R5	R6
	nil	nil	nil	nil	nil	+
MAJOR ROOTS	RI	RII	RIII	RIV		
	++	++	+	+		
STEM	ST I	ST II				
	++	+++				
STOCK	STK					
	++					

A



SHOOTS, LEAVES AND BUDS						
SHOOT	BASE	TIP	BASAL LEAVES	TIP LEAVES	BASAL BUDS	TIP BUDS
S1	nil	nil	nil	nil	nil	nil
S2	+++	nil	nil	nil	nil	nil
S3	nil	nil	nil	nil	nil	nil
S4	nil	nil	nil	nil	nil	nil
S5	nil	nil	nil	nil	nil	nil

ROOTS, STEM AND STOCK					
FIBROUS ROOTS	R1	R2	R3	R4	R5
	+	+	nil	nil	+
MAJOR ROOTS	RI	RII	RIII	RIV	
	++	++	nil	nil	
STEM	ST I	ST II			
	++	nil			
STOCK	STK				
	++				

B

throughout tree tissues was nonetheless extensive. Furthermore, the pattern of movement of ^{14}C was dependent upon the way in which the isotope was applied to the trees. Thus, if the isotope was injected into a shoot ^{14}C moved both upwards and downwards from the point of application, but if the isotope was applied to the upper surface of a midshoot leaf ^{14}C moved downwards only (Fig.22). Since most of the applied ^{14}C remained in the treated organ, it was concluded that there is no evidence to support the hypothesis that the applied arginine would be translocated to, and would accumulate in, storage tissues during autumn.

(3) Metabolic Fate of L-Arginine-guanido-^{14}C-HCl

Since ^{14}C activity was found in tissue residues after extraction (insoluble activity, Tables 21A, 21B), and, since ^{14}C activity was found in the sugar plus organic acid fraction (extracts were fractionated on columns of ion-exchange resin), it was concluded that ^{14}C-labelled arginine HCl was metabolised in tree tissues during autumn. No attempt was made to identify the ^{14}C-labelled products, but chromatographic analysis of the free amino acid and protein amino acid fractions in the stock + stem + side shoot tissues of the shoot-injected tree was carried out. The results, which are shown in Table 22, indicate that ^{14}C arginine was the predominant component of both the free amino acid and the protein amino acid fractions.

TABLE 22

DISTRIBUTION OF ^{14}C AMONGST FREE AMINO ACIDS AND PROTEIN AMINO ACIDS OF THE STOCK + STEM + SIDE-SHOOT TISSUES OF THE SHOOT-INJECTED TREE

Spot	Activity in Free Amino Acid Fraction*		Activity in Protein Amino Acid Fraction*	
	^{14}C (cps)	% Total Activity per Compound	^{14}C (cps)	% Total Activity per Compound
Origin	3.5	1.5	0.4	0.2
Arginine	120.6	51.9	222.9	95.2
All others	108.2	46.6	10.8	4.6
Total	232.3	100.0	234.1	100.0

* Solvent systems used during paper chromatography:

(1) n-butanol:acetic acid:water (12:3:5 v/v)

(2) phenol:water (4:1 v/v)

(ii) Experiment 2.- Translocation and Metabolic Fate of L-Arginine-guanido-^{14}C-Monohydrochloride and L-Asparagine-^{14}C (U) in Dormant Peach Trees

In this experiment, 20 μc of the ^{14}C-labelled amino acids were fed to 2 year old peach trees (dormant and leafless) in June 1964 by the shoot injection technique. Trees were harvested at approximately 10 or 24 hours after treatment. All treatments were carried out in duplicate.

(1) Distribution and Translocation of ^{14}C in Tree Tissues

Results relating to the distribution and translocation of ^{14}C approximately 10 and 24 hours after treatment are set out in Tables 23A, 23B and Fig.23. It will be observed that, in all trees except trees 4 and 8 (Table 23A), most of the ^{14}C remained in the injected shoot irrespective of the compound injected or the treatment time. In trees 4 and 8, however, the bulk of the ^{14}C activity was found in shoot and stem tissues which were close to the injected shoot. Since it was known that the injected shoots of trees 4 and 8 were shorter than those of the other trees, it is thought that greater quantities of the injected isotopes were able to move out of the injected shoots of these two trees before the negative tension in the xylem was equalised by the injected liquid (Rosch 1938); i.e. a spill-over mechanism operated.

TABLE 23A

AMOUNTS OF ^{14}C (μc) IN TREE TISSUES AND AMONGST FRACTIONS CONTAINING FREE AMINO ACIDS, SOLUBLE SUGARS AND ACIDS, AND PROTEIN AMINO ACIDS AFTER INJECTING ^{14}C-LABELLED ARGININE HYDROCHLORIDE AND ASPARAGINE INTO TREE SHOOTS

(Figures in brackets = % of ^{14}C recovered in tree)

Tree No. and Treatment	Uptake ^{14}C per Tree (μc)	Treatment Time (hr)	Injected Shoot				Stock + Stem + Side Shoots			
			Free Amino Acids	Soluble Sugars and Acids	Protein Amino Acids	Total	Free Amino Acids	Soluble Sugars and Acids	Protein Amino Acids	Total
1-^{14}C Arginine HCl	19.87	9.25	10.75 (61.29)	2.36 (13.46)	1.30 (7.41)	14.41 (82.16)	2.10 (11.97)	0.30 (1.71)	0.44 (2.51)	2.84 (16.19)
2-^{14}C Arginine HCl	19.68	7.50	12.56 (69.01)	3.49 (19.18)	1.83 (10.05)	17.88 (98.24)	0.18 (0.99)	0.03 (0.16)	0.02 (0.11)	0.23 (1.26)
3-^{14}C Arginine HCl	19.66	24.50	4.19 (43.51)	2.68 (27.83)	1.69 (17.55)	8.56 (88.89)	0.63 (6.54)	0.19 (1.97)	0.21 (2.18)	1.03 (10.69)
4-^{14}C Arginine HCl	19.90	25.25	2.62 (28.95)	1.18 (13.04)	1.56 (17.24)	5.36 (59.23)	1.62 (17.90)	0.93 (10.28)	1.07 (11.82)	3.62 (40.00)
5-^{14}C Asparagine	19.74	10.00	11.28 (67.30)	4.73 (28.22)	0.45 (2.69)	16.46 (98.21)	0.19 (1.13)	0.04 (0.24)	0.03 (0.18)	0.26 (1.55)
6-^{14}C Asparagine	19.83	9.25	11.93 (62.86)	3.41 (17.97)	0.35 (1.84)	15.69 (82.67)	2.73 (14.38)	0.31 (1.63)	0.18 (0.95)	3.22 (16.96)
7-^{14}C Asparagine	19.96	25.00	1.89 (25.68)	3.79 (51.49)	1.00 (13.59)	6.68 (90.76)	0.30 (4.08)	0.22 (2.99)	0.11 (1.49)	0.63 (8.56)
8-^{14}C Asparagine	17.38	25.00	0.85 (7.62)	1.89 (16.93)	0.47 (4.21)	3.21 (28.76)	3.55 (31.81)	1.80 (16.13)	2.55 (22.85)	7.90 (70.79)

TABLE 23B

AMOUNTS OF ^{14}C (µc) IN TREE TISSUES AND AMONGST FRACTIONS CONTAINING FREE AMINO ACIDS, SOLUBLE SUGARS AND ACIDS, AND PROTEIN AMINO ACIDS AFTER INJECTING ^{14}C-LABELLED ARGININE HYDROCHLORIDE AND ASPARAGINE INTO TREE SHOOTS

(Figures in brackets = % of ^{14}C recovered in tree; figures in brackets* = % recovery of ^{14}C taken up by tree)

Tree No. and Treatment	Uptake ^{14}C per Tree (µc)	Treatment Time (hr)	Roots				Whole Tree			
			Free Amino Acids	Soluble Sugars and Acids	Protein Amino Acids	Total	Free Amino Acids	Soluble Sugars and Acids	Protein Amino Acids	Total
1-^{14}C Arginine HCl	19.87	9.25	0.05 (0.28)	0 (0)	0.24 (1.37)	0.29 (1.65)	12.90 (73.55)	2.66 (15.16)	1.98 (11.29)	17.54 (88.27)*
2-^{14}C Arginine HCl	19.68	7.50	0.03 (0.16)	0 (0)	0.06 (0.33)	0.09 (0.49)	12.77 (70.16)	3.52 (19.34)	1.91 (10.50)	18.20 (92.48)*
3-^{14}C Arginine HCl	19.66	24.50	0.02 (0.21)	0 (0)	0.02 (0.21)	0.04 (0.42)	4.84 (50.26)	2.87 (29.80)	1.92 (19.94)	9.63 (48.98)*
4-^{14}C Arginine HCl	19.90	25.25	0.06 (0.66)	0 (0)	0.01 (0.11)	0.07 (0.77)	4.30 (47.51)	2.11 (23.32)	2.64 (29.17)	9.05 (45.48)*
5-^{14}C Asparagine	19.74	10.00	0.02 (0.12)	0 (0)	0.02 (0.12)	0.04 (0.24)	11.49 (68.56)	4.77 (28.46)	0.50 (2.98)	16.76 (84.90)*
6-^{14}C Asparagine	19.83	9.25	0.03 (0.16)	0 (0)	0.04 (0.21)	0.07 (0.37)	14.69 (77.40)	3.72 (19.60)	0.57 (3.00)	18.98 (95.71)*
7-^{14}C Asparagine	19.96	25.00	0.03 (0.41)	0 (0)	0.02 (0.27)	0.05 (0.68)	2.22 (30.16)	4.01 (54.48)	1.13 (15.35)	7.36 (36.87)*
8-^{14}C Asparagine	17.38	25.00	0.04 (0.36)	0 (0)	0.01 (0.09)	0.05 (0.45)	4.44 (39.78)	3.69 (33.07)	3.03 (27.15)	11.16 (64.21)*

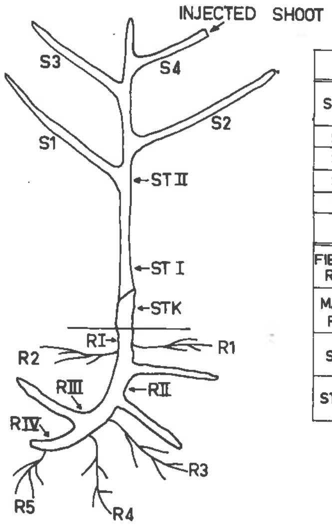
FIGURE 23 Distribution and amount (arbitrary units) of ^{14}C in tissues of dormant peach trees (2 years old) subsequent to shoot injection with ^{14}C-labelled amino acids:

Tree A.- harvested 7.5 hours after treatment with ^{14}C-labelled arginine HCl.

Tree B.- harvested 24.5 hours after treatment with ^{14}C-labelled arginine HCl.

Tree C.- harvested 10.0 hours after treatment with ^{14}C-labelled asparagine.

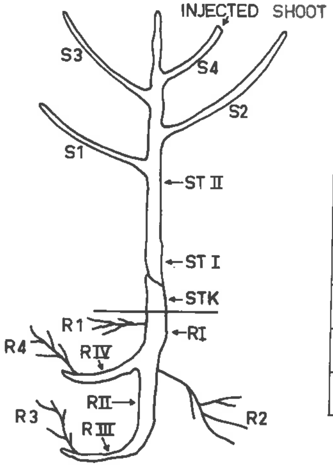
Tree D.- harvested 25.0 hours after treatment with ^{14}C-labelled asparagine.



SHOOTS AND BUDS				
SHOOT	BASE	TIP	BASAL BUDS	TIP BUDS
S1	+	nil	+	+
S2	+	nil	+	+
S3	+	nil	+	+
S4	++++	++++++	+++	+++

ROOTS, STEM AND STOCK					
FIBROUS ROOTS	R1	R2	R3	R4	R5
	+	+	+	+	+
MAJOR ROOTS	R I	R II	R III	R IV	
	+	+	+	+	
STEM	ST I	ST II			
	+	++++			
STOCK	STK				
	+				

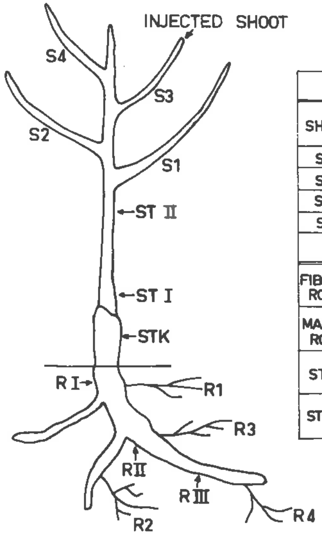
A



SHOOTS AND BUDS				
SHOOT	BASE	TIP	BASAL BUDS	TIP BUDS
S1	+	nil	+	nil
S2	+	nil	+	nil
S3	+	nil	nil	nil
S4	++++	++++++	+++	+++

ROOTS, STEM AND STOCK				
FIBROUS ROOTS	R1	R2	R3	R4
	+	+	+	+
MAJOR ROOTS	R I	R II	R III	R IV
	+	nil	nil	+
STEM	ST I	ST II		
	++	++++		
STOCK	STK			
	+			

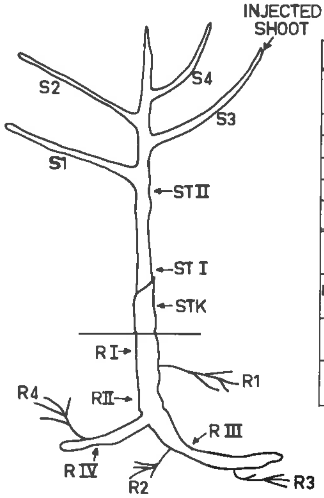
B



SHOOTS AND BUDS				
SHOOT	BASE	TIP	BASAL BUDS	TIP BUDS
S1	+	+	+	nil
S2	+	nil	nil	nil
S3	++++	++++++	+++	+++
S4	+	nil	+	nil

ROOTS, STEM AND STOCK				
FIBROUS ROOTS	R1	R2	R3	R4
	+	+	+	+
MAJOR ROOTS	R I	R II	R III	
	+	+	+	
STEM	ST I	ST II		
	+	++		
STOCK	STK			
	+			

C



SHOOTS AND BUDS				
SHOOT	BASE	TIP	BASAL BUDS	TIP BUDS
S1	+	nil	+	+
S2	+	nil	+	+
S3	++++	++++++	+++	+++
S4	+	+	nil	nil

ROOTS STEM AND STOCK				
FIBROUS ROOTS	R1	R2	R3	R4
	+	nil	+	+
MAJOR ROOTS	R I	R II	R III	R IV
	+	+	+	+
STEM	ST I	ST II		
	++	++		
STOCK	STK			
	++			

D

As in Experiment 1, although most of the injected ^{14}C remained in the injected shoot or nearby tissues, the evidence presented in Fig.23 indicates that small amounts of ^{14}C were translocated both upwards and downwards and that the movement of ^{14}C throughout the tree tissues was quite extensive. In addition, it is interesting to note that there was no evidence of further movement of ^{14}C through tree tissues when the treatment time was extended from approximately 10 to 24 hours. This implies that if movement did take place it was not on a sufficient scale to be detected by autoradiography, or else that uptake and movement of ^{14}C through tree tissues were concomitant and interdependent. Since autoradiography is known to be a very sensitive process it is thought that the latter possibility was operative. Roach (1938) considered that a shoot of a dormant plant behaves like a partially evacuated vessel and that injected liquid moves in to equalise this tension. However, as the injected liquid moved along the injected shoot, a stage would be reached where the negative tension in the xylem would be balanced by the frictional force created when the liquid moved through the fine xylem spaces. Thus an equilibrium would be established which would be maintained until the negative tension increased through tranpiration loss. Since the rate of transpiration of water in dormant tissues is very slow (Roach 1938; preliminary injection experiments), this hypothesis would explain why the bulk of the ^{14}C remained in the injected shoot, and why, if the equilibrium had been established by the end of the injection period (i.e. after 7 to 10 hours), extensive movement of ^{14}C did not take place thereafter.

(2) Metabolic Fate of ^{14}C-labelled Arginine and Asparagine in Dormant Peach Trees

The data shown in Tables 23A and 23B clearly indicates that both ^{14}C-labelled amino acids were metabolised in the dormant peach trees. After approximately 10 hours treatment time, considerable amounts of ^{14}C were found in soluble sugars and acids and protein amino acids. This latter result suggested that protein was being synthesised at a slow rate in the tissues. The level of ^{14}C in the free amino acid fraction decreased sharply with increased treatment time (the rate of breakdown was approximately constant) as would be expected if the compounds were metabolised. However, the amount of ^{14}C in the other fractions did not increase to any marked extent with increasing treatment time, and it is concluded that the ^{14}C which was not accounted for in the analyses (see % recovery figures, Table 23B) was incorporated into insoluble compounds other than protein or else was lost as $^{14}CO_2$. Since the amount of ^{14}C present in soluble sugars and acids remained practically constant with increasing treatment time, it is suggested that production of this labelled fraction was associated with the entry of the isotopes into the trees.

In an attempt to identify the primary breakdown products of the ^{14}C-labelled amino acids, components of the free amino acid fraction of the injected shoot tissues were separated by paper chromatography. The results are shown in Table 24. It is apparent that aspartic acid was the primary breakdown product of ^{14}C-asparagine as might be expected. However, the breakdown products of arginine-guanido-^{14}C-hydrochloride did not appear to be amino acids, since ^{14}C-arginine remained the predominant compound in the free amino acid fraction after both 10 and 24 hours. No attempt was made to identify the other ^{14}C-labelled amino acids in the shoot tissues which had been injected with ^{14}C-labelled arginine hydrochloride, because it was found that the isotope decomposed during chromatography.

When standard solutions of the isotopes (ex Amersham) were chromatographed by the two-dimensional technique, a large number of ^{14}C-labelled impurities were found on the chromatograms (Tables 25A, 25B). There appeared to be 3 possible explanations for this:

(A) the impurities were present in the standards when despatched from Amersham.

(B) the impurities arose during the storage of the isotopes at Waite Institute (storage time of 3 months).

(C) the impurities arose as a result of decomposition during paper chromatography.

The first possibility was ruled out because the proportion

TABLE 24

DISTRIBUTION OF ^{14}C AMONGST FREE AMINO ACIDS IN INJECTED SHOOT TISSUES APPROXIMATELY 10 AND 24 HOURS AFTER TREATMENT OF 2 YEAR OLD PEACH TREES WITH ^{14}C-LABELLED ARGININE HYDROCHLORIDE AND ASPARAGINE

Spot	Injected Compound							
	^{14}C-Arginine HCl*				^{14}C-Asparagine+			
	^{14}C (cps)		% Total Activity per Compd.		^{14}C (cps)		% Total Activity per Compd.	
	10hr	24hr	10hr	24hr	10hr	24hr	10hr	24hr
Origin	135.6	27.4	0.9	0.5	60.6	17.2	0.4	0.8
Cystine					4.9	5.0	0	0.2
Aspartic					1504.0	346.1	9.3	15.2
Glutamic					485.9	366.2	3.0	16.0
Asparagine					12645.1	900.3	78.1	39.4
Threonine					25.5	9.2	0.1	0.4
Glutamine					365.1	71.6	2.3	3.1
α -Alanine					47.1	40.9	0.2	1.8
β -Alanine + Tyrosine					175.0	47.4	1.1	2.1
Lysine					43.5	29.7	0.3	1.3
Arginine	12664.6	4291.3	86.3	76.4	291.0	125.0	1.8	5.5
Proline					91.9	108.0	0.6	4.7
γ-NH$_2$-Butyric					253.4	178.9	1.6	7.8
Histidine					52.6	0	0.3	0
Serine					0	16.5	0	0.7
Glycine					0	11.7	0	0.5
Unknowns	1868.0	1296.8	12.8	23.1	153.4	9.1	0.9	0.4
Total	14668.2	5615.5	100.0	100.0	16199.0	2282.8	100.0	99.9

Solvent systems used during paper chromatography

*(1) n-butanol:acetic acid:water
(12:3:5 v/v)
(2) phenol:water
(4:1:trace w/v)

+(1) n-butanol:acetic acid:water
(12:3:5 v/v)
(2) phenol:water:ammonia
(4:1:trace w/v)

TABLE 25A

DETECTION OF ^{14}C-LABELLED IMPURITIES IN A STANDARD SOLUTION OF L-ASPARAGINE-^{14}C (U) AND THE DECOMPOSITION OF THIS ISOTOPE DURING PAPER CHROMATOGRAPHY

Compound Chromatographed	Chromat. Load (μc)	Use of Acid-washed Paper	Solvent Systems	Propn. Total ^{14}C in Compd. (%)	Propn. Total ^{14}C in Impurities (%)	No. Impurities	Separation of Spots
^{14}C Asparagine (standard soln.)	0.43	-	(1) n-butanol:acetic acid:water (12:3:5 v/v) (2) phenol:water:NH_3 (4:1:trace w/v)	95.9	4.1	14	good
^{14}C Asparagine (rechromatographed)	0.05	-	As above	95.4	4.6	11	good
As above	0.05	+	As above	93.4	6.6	5	poor
As above	0.06	-	(1) n-butanol:acetic acid:water (12:3:5 v/v) (2) phenol:water (4:1 w/v)	97.0	3.0	8	good
As above	0.06	-	(1) n-butanol:5% aq.ammonium acetate:acetic acid (37:25:10 v/v) (2) phenol:water:ammonium acetate (80:20:1 w/v/w)	97.2	2.8	9	good

TABLE 25B

DETECTION OF ^{14}C-LABELLED IMPURITIES IN A STANDARD SOLUTION OF L-ARGININE-*guanido*-^{14}C-HYDROCHLORIDE AND THE DECOMPOSITION OF THIS ISOTOPE DURING PAPER CHROMATOGRAPHY

Compound Chromatographed	Chromat. Load (µc)	Use of Acid-washed Paper	Solvent Systems	Propn. Total ^{14}C in Compd. (%)	Propn. Total ^{14}C in Impurities (%)	No. Impurities	Separation of Spots
^{14}C-Arginine HCl (standard soln)	0.21	-	(1) n-butanol:acetic acid:water (12:3:5 v/v) (2) phenol:water:NH_3 (4:1:trace w/v)	89.5	10.5	14	good
^{14}C-Arginine (rechromatographed)	0.19	-	As above	71.6	28.4	14	good
As above	0.11	+	As above	77.8	22.2	12	good
As above	0.15	-	(1) n-butanol:acetic acid:water (12:3:5 v/v) (2) phenol:water (4:1 w/v)	36.7	63.3	18	good
As above	0.16	-	(1) n-butanol:water (85:15 v/v) (2) phenol:water (4:1 w/v)	91.6	8.4	6	poor
As above	0.20	-	(1) n-butanol:5% aq.ammonium acetate: acetic acid (37:25:10 v/v) (2) phenol:water:ammonium acetate (80:20:1 w/v/w)	95.2	4.8	9	poor

of the total ^{14}C content which was present in the impurities far exceeded the maximum limit set by the Amersham authorities (1 to 2%). The second possibility was not checked experimentally because the supply of the standard isotope solutions ran out. However the third possibility was checked in the following way. ^{14}C-labelled arginine and asparagine were separated on paper chromatograms, the authentic compounds were located by autoradiography and specific colour tests, and the paper spots were cut out. After each of the ^{14}C-labelled amino acids had been extracted from the paper pieces with 15 ml of distilled water (soaked for 30 minutes with occasional shaking), the extracts were concentrated in vacuo in a rotary evaporator (water-bath temperature 35°C) and the concentrated solutions were respotted on chromatograms. The chromatograms were developed and the ^{14}C-labelled compounds were located by autoradiography. The results, which are shown in Plate 5 and in Tables 25A and 25B clearly indicate that both ^{14}C-labelled amino acids decomposed during chromatography. While the extent of breakdown of ^{14}C-labelled asparagine under these conditions may be considered as negligible, the breakdown of ^{14}C-labelled arginine was pronounced. Since closely similar patterns of ^{14}C-labelled impurities were obtained on autoradiographs of standard isotope solutions and rechromatographed isotope solutions, providing the same solvent systems were used during chromstography, it is thought that the impurities arose as a result of the chromatographic techniques used rather than as a result of lengthy storage before

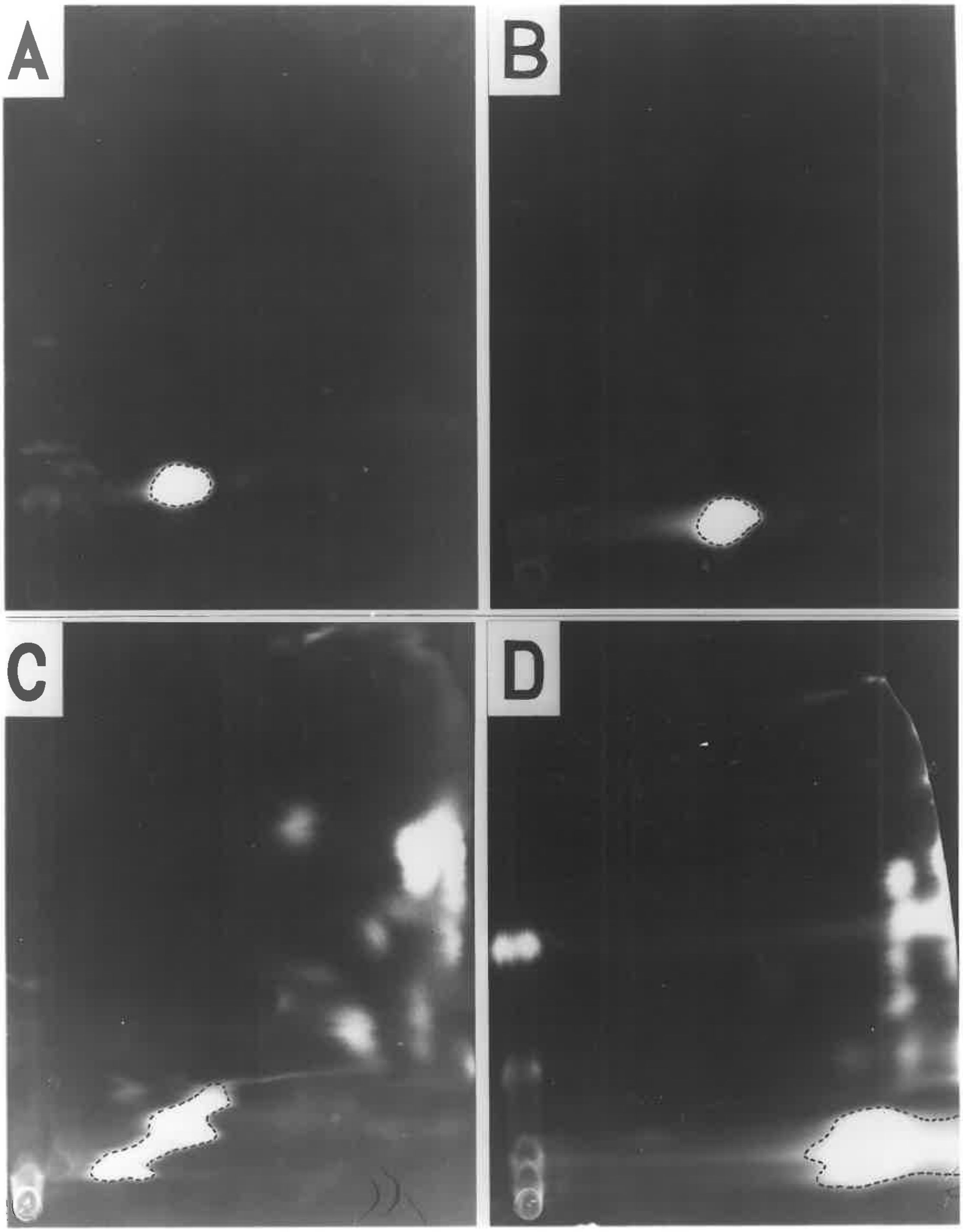
PLATE 5 Autoradiographs showing the extent of decomposition of L-arginine-guanido-^{14}C-hydrochloride and L-asparagine-^{14}C (U) during paper chromatography. Authentic compounds are outlined.

A. L-asparagine-^{14}C, solvent system I.
B. L-asparagine-^{14}C, solvent system II.
C. L-arginine-guanido-^{14}C-HCl, solvent system I.
D. L-arginine-guanido-^{14}C-HCl, solvent system II.

Solvent system I: (1st) n-butanol:acetic acid:water
(12:3:5 v/v)
(2nd) phenol:water (4:1 w/v)

Solvent system II:(1st) n-butanol:acetic acid:water
(12:3:5 v/v)
(2nd) phenol:water:ammonia
(4:1:trace w/v)

The first component of each solvent system was run in the vertical direction, while the second was run in the horizontal direction.


A
B
C
D

use. Unfortunately, this could not be confirmed by chromatographing the standard isotope solutions under conditions whereby no breakdown occurred, since tests with a range of solvent systems indicated that breakdown of the compounds occurred to a greater or lesser extent in each of them (Tables 25A, 25B).

(d) Discussion

It is clear that arginine and asparagine, which are known to be constituents of the storage nitrogen of dormant peach trees (Experimental Work, Section A), are metabolized in tissues of dormant peach trees. This result was unexpected since it was thought that, in order for these compounds to accumulate in concentration and amount during autumn and winter, they would be metabolically inert in the tree tissues. It is possible, of course, that the method used to inject the isotopes into the trees may have influenced the results, e.g. enzymes may have been released from the cut tissue, but the fact that further metabolism occurred after uptake was complete (c.f. results after 10 and 24 hour treatment times, Tables 23A, 23B, 24) suggests that this possibility could not completely account for the observed results. Furthermore, Blommaert (1963a, 1963b) has recently reported that ^{14}C-labelled thiourea and urea are readily metabolised in dormant peach shoots. It is therefore apparent that dormant peach tissues are able to metabolise a range of organic compounds.

Although evidence was obtained which indicated that arginine was metabolised in dormant peach tissues, chromatographic analysis of extracts showed that free arginine was present in very high concentration in the trees over the experimental period. This was expected in view of the results reported earlier (Experimental Work, Section A). It is suggested that the apparent incongruity relating to the accumulation and degradation of arginine in tissues of dormant trees is due to a turnover of the arginine in the tissues. The fact that arginine was one of the compounds synthesised when ^{14}C-asparagine was metabolised in tissues of the dormant trees (Table 24) supports this suggestion.

Little information was obtained to indicate by what metabolic pathway arginine is degraded in peach tissues. Results of chromatographic analyses suggested that the primary breakdown products of arginine do not include other free amino acids (Table 24). Chromogenic tests failed to reveal the presence of ^{14}C-urea in tissue extracts, and this may mean that arginine is not degraded to ornithine and urea by the enzyme arginase. However, in contrast, the results indicate that asparagine is first degraded to aspartic acid, following which glutamic acid and a range of other amino acids are formed (Table 24). These findings are in agreement with the hypothesis that asparagine is deaminated in the tissues under the influence of the enzyme asparagine synthetase (Webster 1959).

Most of the injected ^{14}C remained in the injected shoot or treated leaf (Tables 21A, 21B, 23A, 23B; Figs. 22, 23), and no evidence was found for the movement of large amounts of ^{14}C through tree tissues even if the treatment time was increased (Fig. 23). It was therefore concluded that injected amino acids do not accumulate in the woody storage tissues of the trees. An hypothesis, which was put forward to explain why most of the ^{14}C remains in injected shoot tissues, was discussed earlier (see Results), but this would not explain why most of the ^{14}C which had been applied to the upper surface of a treated leaf remained in the leaf. In this latter case, about half of the ^{14}C-arginine was converted into insoluble, presumably immobile, compounds (Table 21B), and it is possible that the remaining soluble activity was not readily translocated to leaf xylem and phloem, from whence ^{14}C would be translocated to other tree parts.

Although it is known that a number of amino acids decompose during paper chromatography (Lawrence *et al.* 1960; Moses 1962), the breakdown of ^{14}C-labelled arginine and asparagine during chromatographic analysis apparently has not been reported previously. Since the breakdown of ^{14}C-labelled arginine was extensive in several of the solvent systems (Table 25B), it is clear that the stability of ^{14}C-labelled isotopes should always be investigated before results, which relate to the metabolic fate of the isotopes in plant tissues, can be interpreted.

C. CONCLUSIONS

In this section the findings of each of the separate experiments have been interrelated as far as possible, and areas in which further research is needed have been indicated.

Young peach trees accumulated nitrogen in proportion to the external nitrogen supply. Analysis of dormant trees showed that, in response to an increasing supply of nitrogen, soluble nitrogenous compounds accumulated in woody storage tissues of the trees to a much greater extent than did insoluble nitrogenous compounds. Furthermore, the amounts of soluble N in storage tissues declined during spring (utilized for growth) while the amounts of insoluble N in the tissues usually increased at this time (not utilized for growth). It is concluded that the storage nitrogen of the young peach trees consisted mainly of soluble nitrogen. Evidence was also presented which showed that at least part of the insoluble N content of the stock + stem + 1 year old shoot tissues acted as a nitrogenous reserve.

Analysis of dormant trees showed that most of the storage nitrogen of the trees was present in the roots. Considerable quantities of this nitrogen were transported to the growing points during the following spring. However, if the trees received a high nitrogen application during the growing season, most of the storage nitrogen which was mobilized for new shoot growth was obtained from the above-ground tissues rather than from the roots, presumably because uptake of nitrogen partially offsets the loss of storage nitrogen from the roots. In this regard, nitrogen uptake per tree was not influenced by the level of storage nitrogen in the tree.

Reaccumulation of soluble N in storage tissues of the trees commenced in December and was especially rapid if the external nitrogen supply was high. In trees supplied with ample nitrogen, storage of nitrogen took place because nitrogen supply exceeded nitrogen demand. However, nitrogen accumulation also occurred on a small scale during the latter half of the growing season in trees which did not receive fertilizer nitrogen during the growing season. This is interpreted to mean that nitrogen was translocated from leaves to woody tissues prior to leaf fall. The soluble nitrogen in young dormant trees largely consisted of free arginine, and the determination of the amount of arginine in the storage tissues gave the most sensitive indication of the nitrogen status of the trees. Furthermore, the amount of soluble N in the trees which was accounted for as arginine increased with increasing nitrogen supply, indicating that accumulation of arginine was restricted if the nitrogen supply was low. Where arginine accumulation was restricted, the importance of amide N as a component of the soluble N increased. Since arginine also accumulates in mature peach trees during autumn and winter in proportion to the nitrogen supply (Taylor, unpublished data), it is concluded that the storage nitrogen of both young and mature peach trees largely consists of arginine. It is thought that this information could be used as a basis for the development of a very sensitive method of assessing the nitrogen status of orchard trees.

Two hypotheses were put forward in an attempt to explain why arginine does not accumulate in tree tissues except in autumn and winter, even though soluble N may accumulate in tree tissues earlier than this. In the first hypothesis it is suggested that arginine, which has a low $^{C}/_{N}$ ratio, could only be synthesised if the available nitrogen supply in tree tissues exceeds the supply of available carbon. These conditions would be satisfied in autumn when carbohydrate accumulation ceases (photosynthesis would cease at leaf fall) but nitrogen absorption does not. Alternatively, it is suggested that arginine accumulates in direct response to the environmental factors prevailing during autumn and winter. Further work is required to determine whether either of these hypotheses is correct.

The results suggested that the amount of new growth made by young peach trees was significantly dependent upon, and in proportion to, the level of storage nitrogen in the trees and the external nitrogen supply, irrespective of how tree growth was measured. Tree growth in early spring was significantly correlated with the level of storage nitrogen in the tree tissues. After November, however, tree growth was mainly dependent upon the external nitrogen supply, and it is concluded that the supply of storage nitrogen was exhausted or at a low level by this time.

Analysis of growth data indicated that there was a significant negative interaction at the end of the growing season between the current nitrogen supply and the nitrogen treatment applied in the previous year; i.e. the smaller the nitrogen application in the previous year the greater the effect of the current nitrogen application on tree growth, and vice versa. It is concluded that deficient trees respond more markedly to applied nitrogen than nitrogen sufficient trees, but the reason for this is not known. Additional work on the subject is required.

An attempt was made to corroborate the above results by injecting a range of nitrogenous compounds, including constituents of the storage nitrogen of dormant peach trees, into young peach trees, but no significant growth response occurred. It is concluded that either insufficient quantities of the substances were injected into the trees to influence growth, or else that little or none of the injected solutions were translocated to the growing points.

Experiments showed that L-arginine-*guanido*-^{14}C-hydrochloride and L-asparagine-^{14}C (U) were metebolised in dormant tissues of young peach trees. This result was unexpected in view of the fact that both of these compounds (especially arginine) accumulate in storage tissues during autumn and winter. It is suggested that arginine, and possibly asparagine as well, are constantly being 'turned over' in dormant tissues, and this suggestion warrants further investigation. The primary breakdown product of ^{14}C-labelled asparagine was identified as aspartic acid, but the breakdown products of ^{14}C-labelled arginine were not identified. It is therefore clear that further work is required to determine by what metabolic pathways arginine is degraded and synthesised in tissues of peach trees. Considerable amounts of ^{14}C-arginine (but not ^{14}C-asparagine) decomposed during paper chromatography, and it is concluded that the stability of ^{14}C-labelled compounds should always be checked before results relating to their metabolism in plant tissues can be interpreted.

The distribution of ^{14}C in tree tissues subsequent to application of the isotopes to the trees in autumn and winter indicated that most of the applied ^{14}C remained in the treated organ or in neighbouring tissues. It is concluded that the bulk of the injected amino acids did not move to, or accumulate in, the storage tissues of the trees.

PART VII

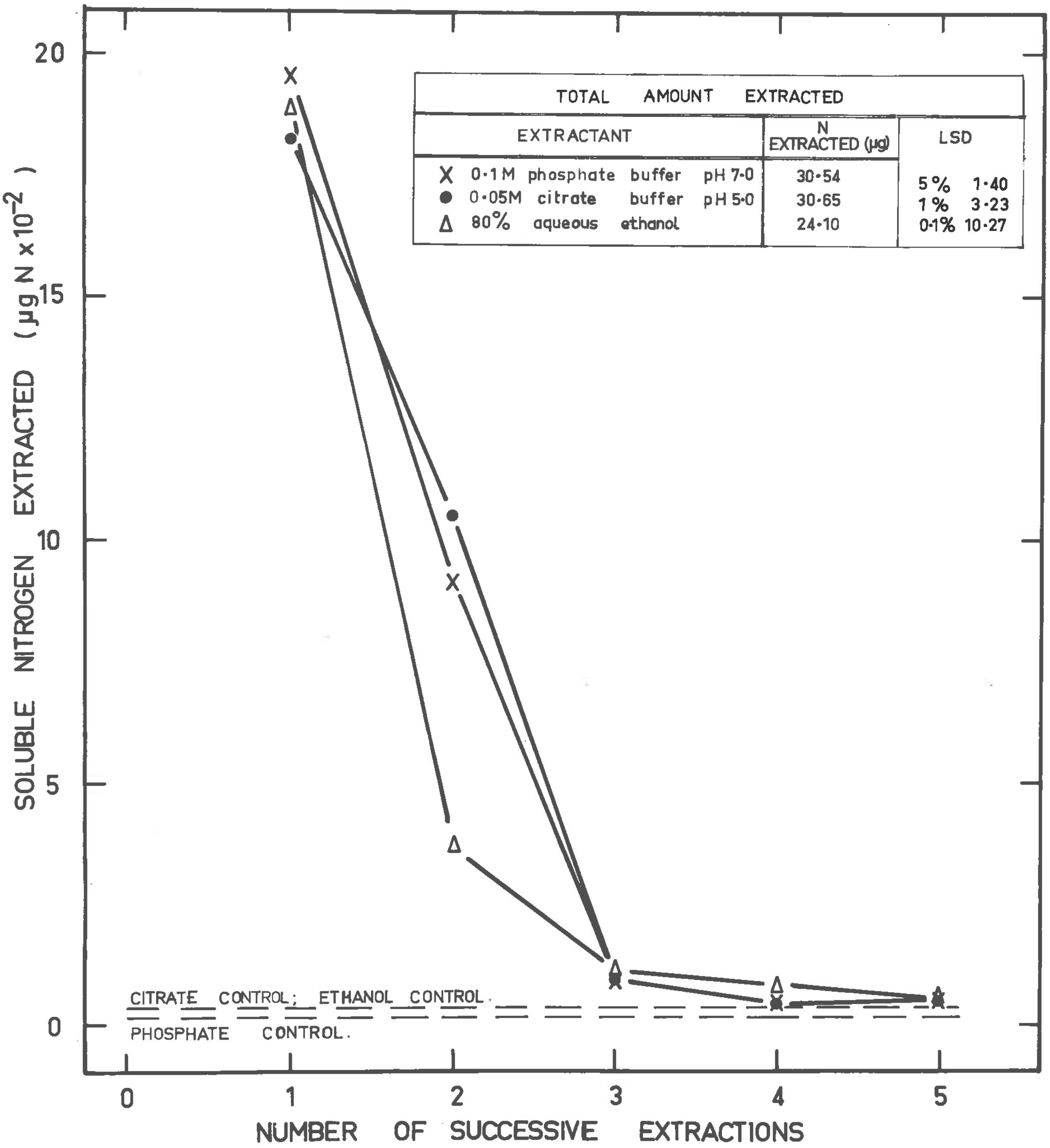
APPENDICES

APPENDIX A. Extraction of Soluble Nitrogen from Peach Tissues

An experiment was carried out to compare the amount of soluble N extracted from freezedried peach shoot tissue by 0.05M citrate buffer pH 5.0, 0.1M phosphate buffer pH 7.0 and 80% aqueous ethanol.

1.000g lots of dried, finely-ground tissue were successively extracted (5 times) with cold (2°C) solvent and the level of soluble N in aliquots of concentrated extracts was determined by micro-kjeldahl analysis. Extraction, concentration, and micro-kjeldahl procedures were carried out as described in the main text (Soluble N determinations, Experimental Work, Section A).

Results of the experiment are shown in Fig.24. The total amount of soluble N extracted by each solvent and the amounts of soluble N in each successive extract are shown. It is evident that the citrate and phosphate buffers extracted significantly more nitrogen from the tissue than did 80% ethanol. The difference in extracting power was almost entirely due to the small amount of nitrogen extracted by 80% ethanol during the second successive extraction of the tissue. Only small amounts of nitrogen were released



TOTAL AMOUNT EXTRACTED

EXTRACTANT	N EXTRACTED (µg)	LSD
X 0·1M phosphate buffer pH 7·0	30·54	5% 1·40
● 0·05M citrate buffer pH 5·0	30·65	1% 3·23
Δ 80% aqueous ethanol	24·10	0·1% 10·27

FIGURE 24. Influence of extractant on the amount of soluble nitrogen successively extracted from freeze-dried shoot tissue of peach trees.

from the tissue after the third successive extraction, irrespective of the extractant used.

The results do not give any indication why the aqueous solutions extracted more soluble N from the tissue than did 80% ethanol. However, the following explanations are possible:

(a) protein breakdown occurs when the tissue is extracted with aqueous solutions, but does not occur when the tissue is extracted with 80% ethanol.

(b) the nitrogenous constituents of peach tissues are more soluble in aqueous solvents than in ethanolic solvents.

(c) the tissue extracted is more permeable to aqueous solvents than it is to 80% ethanol.

APPENDIX B.- The influence of Injected Nitrogenous Compounds on the Growth of One Year Old Peach Trees During a Single Growing Season

(a) Introduction

An attempt was made to corroborate the results outlined in Experimental Work, section A. A range of nitrogenous compounds, including compounds which are known to be constituents of the storage nitrogen of dormant peach trees, were injected into young peach trees and the growth response was measured.

(b) Methods

(i) The Trees, the Planting Procedure and Tree Management

27 one-year old peach trees (Golden Queen scion on Elberta rootstock) were selected for uniformity of size and shape, and freedom from damage and disease, at Balhannah Nurseries in July 1964. The trees were 'heeled' into moist sand until planting. Immediately prior to planting, the roots of the trees were washed well and any broken roots present were removed. The trees were weighed and their roots were surface sterilised by dipping them in a solution of 5.25% NaOCl for 5 minutes. Each tree was then planted in a wooden box (painted with bituminous paint inside and silver-frost outside) 11½ inches square and 12½ inches deep (external dimensions), which contained 23.5 Kg of steam-sterilised soil-mix of low nitrogen content (0.19% on a dry weight basis)

overlying a 2" layer of sterilised quartzite gravel (8 Kg). The following quantities of commercial fertilizers were mixed into each half-ton lot of soil-mix before use:- 'hoof and horn' 190g, superphosphate 560g, sulphate of potash 300g, and ground limestone 140g. The soil-mix consisted of 3 parts soil, 2 parts sand and 1 part gravel ($\frac{1}{8}$ inch diam.). 'Normal' soil was not used because of possible drainage problems. Each box was provided with 7 drainage holes in its base and was so constructed that one side could be unscrewed and removed. Since it was planned to inject the solutions into the major roots of the trees, these roots were forcibly directed towards the removeable side of the box at planting. The surface of the soil in each pot was covered with 3Kg of sterilised quartzite gravel. After planting, the top of each tree was severely cut back to remove as much storage material as possible; only 5 or 6 leaf buds were left on each stem. The prunings were weighed and the fresh weight of the pruned trees was calculated. The pruned trees were then transferred to the pot-culture area in Alvestoke orchard, Waite Institute.

The soil in each box was kept moist by frequent additions of tap water; these were usually applied every alternate day. In addition, 200 ml of nitrogen-free 'complete' nutrient solution were applied to the surface of each box at two-monthly intervals throughout the growing season. The composition of the nutrient solution was the same as that given to the trees in the N0 treatment in section A (see Tables 2A and 2B). All trees were sprayed with freshly prepared bordeaux mixture at the 'pink tip' stage of budburst. After bud burst, the number of new shoots per tree was reduced to 4 or 5 and all root suckers were pinched off as well.

(ii) The Treatments and Their Application

A list of the compounds which were injected into trees, the concentration of the aqueous injection solutions, the total amount of nitrogen injected into each tree during the growing season, and the injection dates, are given in Table 26. The compounds were chosen for the following reasons: arginine and asparagine were chosen because they were known to be constituents of the storage nitrogen of dormant peach trees, whereas serine and KNO_3 were included for comparative purposes only, since they were not important constituents of the storage nitrogen. It was thought that a comparison of the growth response brought about by the injection of these compounds into trees would indicate whether the growth response was specific for each compound, or whether it was merely the result of an increase in the nitrogen content of the tree.

The concentration of nitrogen in the injection solutions was limited to no more than 6 mgN/ml, since, at higher concentrations, asparagine precipitated out of solution. Each of the 9 treatments was replicated 3 times. However, prior to carrying out the first injection, the trees were grouped in order of ascending fresh weight at planting and then this group was divided into 3 equal parts, each of which was made a replicate and represented a particular range of tree sizes. Treatments were allocated at random within each repli-

TABLE 26

AMOUNTS OF EACH NITROGENOUS COMPOUND INJECTED INTO YOUNG PEACH TREES

Injected Compound	Chemical Grade	Relative N Level	Injection Date				
			24/9/64		4/12/64		Total Amount of N Injected into Each Tree
			N Conc. of Injected Solutions (mg N/ml)	Vol. Injected per Tree (ml)	N Conc. of Injected Solutions (mg N/ml)	Vol. Injected per Tree (ml)	(mg N)
Distilled Water Control	-	-	0	20	0	20	0
L-Arginine HCl, B.D.H.	L.R.	1	0.2	20	0.6	20	16
L-Asparagine, Merck	A.R.	1	0.2	20	0.6	20	16
L-Serine, B.D.H.	L.R.	1	0.2	20	0.6	20	16
KNO_3, B.D.H.	A.R.	1	0.2	20	0.6	20	16
L-Arginine HCl, B.D.H.	L.R.	10	2.0	20	6.0	20	160
L-Asparagine, Merck	A.R.	10	2.0	20	6.0	20	160
L-Serine, B.D.H.	L.R.	10	2.0	20	6.0	20	160
KNO_3, B.D.H.	A.R.	10	2.0	20	6.0	20	160

cate, and the position of the treated trees was fully randomised in the area allocated for the experiment. It was thought that stratification of the trees, and application of the treatments in this way, would minimize any influence of initial tree size on subsequent tree growth.

The first series of injections were made on 24/9/64 just after the commencement of shoot growth. The injection method used was similar to that worked out for the injection of radio-active solutions into young peach trees. Thus the following procedure was carried out. The removeable side of each box was taken off, a suitable major root was located, and the terminal 2 to 3 inches of the root were exposed and cleaned. The tip of the root was cut off and a polythene tube (0.5 to 1.0 cm internal diameter, 15 to 24 inches long) was attached to the freshly cut end of the major root. A small amount of distilled water was immediately squirted into the tube to keep the cut end moist and to test the connection for leakage. The upper end of the injection tube was attached to a wooden stake, and the test solution was then pipetted into the polythene tube. Air bubbles were removed by tapping and squeezing the tube. During the injection period the exposed side of the box was covered with a piece of black polythene sheeting. After all of the solution had been taken up by the tree, the tube was disconnected, the cut end of the root was daubed with pruning mastick, and the side of the box was replaced.

Unfortunately, use of this injection method resulted in very variable rates of uptake of solutions from tree to tree; some trees took up all of the liquid in one day whereas others took up the liquid over a 3-week period. Furthermore, in some cases uptake of the injected liquid stopped before all of the liquid had been absorbed. In these cases, re-injection of the same root did not result in further uptake and the injection could only be completed by attaching the injection tube to a different root on the same tree. This injection method was therefore considered unsatisfactory and a different method was used for the second series of injections which were carried out on 4/12/64.

On this date, a pressure apparatus was used to force the solutions into the tree stems under a pressure of 50lb/square inch. The apparatus used was designed by Dr. B.G. Coombe, Department of Plant Physiology, Waite Institute, and was similar in construction and operation to the pressure injection apparatus described by Schubert (1963). The required pressure for the injections was supplied by a small cylinder of carbon dioxide gas. However, this method did not prove to be entirely satisfactory either because when the injection head was screwed into the tree stems it often caused them to split, and, as a consequence, some leakage of the test solutions usually resulted. Initially a series of 6 injections were planned during the growing season, but because of the problem of injecting the solutions into the trees only 2 injections were carried out.

(iii) Measurement of Tree Growth and Harvesting Techniques

The total length of new shoots per tree and the stem diameter of each tree (2 readings were made at right angles 4 inches above the graft union) were measured at monthly intervals throughout the growing season.

All trees were harvested on 8/3/65. They were removed from the boxes under a jet of water, the roots were thoroughly cleaned (any root fragments which broke off during this operation were collected) and blotted dry between sheets of blotting paper. The following data was recorded for each harvested tree.- fresh weight, total length of new shoots, shoot numbers, leaf numbers, average length of new shoots and the stem diameter. When this information had been recorded, the tissues of each tree were chopped into small pieces and these were placed in paper bags and ovendried at 103°C for 16 hours. Dried tissues were removed from the oven, allowed to cool and were then weighed. From these readings, the dry weight of each tree was calculated.

(c) Results

The trees which were injected with the high concentration of KNO_3 on 24/9/64, and which rapidly took up the injected solution, showed leaf damage 4 days later. Thus the basal leaves of these trees were misshapen, curled inwards, and were blackened and scorched on margins and tips. It was concluded that the leaves were damaged by the introduction of toxic amounts of KNO_3 into the trees. However, the observation also indicated that the injected KNO_3, and presumably the other compounds as well, was rapidly translocated from roots to shoots. Leaf damage was not observed in any other treatment.

The results obtained at the end of the growing season are given in Table 27. The seasonal growth data has not been included because none of the treatments resulted in significant differences in growth during the growing season. It is evident from Table 27 that none of the treatments resulted in significantly greater tree growth c.f. the control, except in regard to the number of leaves per tree. However, the differences observed in the number of leaves per tree are of doubtful significance because the values obtained for the control treatment exceeded those obtained in some of the other treatments. Leaf symptoms of nitrogen deficiency were noted on trees of all treatments at the end of the growing season, but they were less severe on trees which had received the higher nitrogen applications.

(d) Discussion

The attempt to stimulate the growth of young peach trees by injecting them with nitrogenous compounds, including compounds which are known to be constituents of the storage nitrogen of dormant peach trees, was not successful. In view of the fact that the trees in all treatments were nitrogen deficient at the end of the growing season, there are 2 possible reasons for this lack of response:

TABLE 27

INFLUENCE OF INJECTED NITROGENOUS COMPOUNDS ON TREE GROWTH

Log_{10} Data

Compound Injected	Relative N Level	Fresh Weight per Tree (g)	Dry Weight per Tree (g)	No. Leaves per Tree	No. Shoots per Tree	Total Length of Shoots per Tree (cm)	Avg. Shoot Length per Tree (cm)	Stem Diameter per Tree x10 (ins)
Distilled water control	-	2.514	2.230	2.132	0.795	2.233	1.439	0.652
Arginine HCl	1	2.446	2.139	1.981	0.852	2.249	1.388	0.617
Asparagine	1	2.466	2.161	1.961	0.719	2.195	1.475	0.641
Serine	1	2.506	2.162	2.098	0.833	2.254	1.420	0.651
KNO_3	1	2.484	2.158	2.056	0.861	2.272	1.411	0.652
Arginine HCl	10	2.514	2.189	2.096	0.725	2.176	1.451	0.646
Asparagine	10	2.479	2.178	2.117	0.852	2.237	1.384	0.673
Serine	10	2.518	2.230	2.134	0.859	2.239	1.380	0.655
KNO_3	10	2.500	2.175	2.093	0.966	2.257	1.291	0.650
Level of Significance in V.R. Test		NS	NS	P=0.01	NS	NS	NS	NS
L.S.D. 5% 1% 0.1%		-	-	0.094 0.130 0.179	-	-	-	-

(1) sufficient nitrogen was injected into the trees to stimulate growth, but not all of it was translocated to the growing points, or,

(2) insufficient nitrogen was injected into the trees to stimulate growth.

Unfortunately, the available data does not indicate which possibility was operative; in both cases the lack of a growth response would be due to an insufficient supply of nitrogen for new shoot growth. Since differences in leaf colour were noted at the end of the growing season on trees which had received 160 versus 16 mg N during the season, it would be expected that most, if not all, of the injected nitrogen had been translocated into the new shoots, and that the lack of a growth response was due to the fact that insufficient nitrogen was injected into the trees. Nevertheless, incomplete translocation of the injected solutions to the growing points of the trees is feasible. For example, Roach (1938) states that an injected solution will move through the vascular system of a woody plant until the negative tension of the xylem is balanced by the frictional force generated when a column of liquid moves through the fine spaces in the xylem, i.e. the injected liquid may not reach the growing points of the plant. Furthermore, it was found that, in experiments in which ^{14}C-labelled amino acids were injected into woody shoots of 2 year-old peach trees in autumn and winter (see Experimental Work, section B), a large proportion of the injected radioactivity stayed in the injected shoot, even when extensive translocation of the remaining radioactivity took place in the tree tissues.

(e) Primary Data

The primary data for Appendix B is given on pp. 194 to 197.

HARVEST DATA

* Trees with root galls

Fresh Weight per Tree (g)

Treatment	Replicate		
	1	2	3
Water	321.9	289.6	374.3
Arg. 1	257.3	226.3	374.8
Asp. 1	271.5	279.3	330.3
Ser. 1	296.7	320.4	346.9
KNO_3 1	271.4	348.8*	298.3
Arg. 10	293.8	347.7	341.2
Asp. 10	281.8	311.4*	312.2
Ser. 10	306.4	343.7	340.6
KNO_3 10	306.3*	328.9	313.8

Dry Weight per Tree (g)

Treatment	Replicate		
	1	2	3
Water	155.9	183.0	171.7
Arg. 1	122.2	119.3	179.1
Asp. 1	139.1	138.4	158.5
Ser. 1	137.2	142.4	156.4
KNO_3 1	133.9	151.8*	146.8
Arg. 10	139.9	161.9	163.3
Asp. 10	136.5	159.6*	157.1
Ser. 10	182.0	159.5	168.6
KNO_3 10	141.9*	154.2	153.0

HARVEST DATA

* Trees with root galls

Total Length of New Shoots per Tree (cm)

Treatment	Replicate		
	1	2	3
Water	160.8	191.9	161.7
Arg. 1	154.7	185.9	194.1
Asp. 1	134.5	198.4	143.7
Ser. 1	196.1	147.5	199.2
KNO_3 1	218.2	170.0*	176.2
Arg. 10	137.4	147.4	166.7
Asp. 10	131.2	213.6*	183.5
Ser. 10	163.3	162.5	196.4
KNO_3 10	179.1*	197.7	166.3

Stem Diameter per Tree (10x) in.

Treatment	Replicate		
	1	2	3
Water	0.463	0.445	0.438
Arg. 1	0.378	0.426	0.441
Asp. 1	0.406	0.440	0.468
Ser. 1	0.450	0.411	0.484
KNO_3 1	0.418	0.464*	0.465
Arg. 10	0.455	0.414	0.461
Asp. 10	0.452	0.464*	0.497
Ser. 10	0.441	0.454	0.462
KNO_3 10	0.430*	0.454	0.455

HARVEST DATA

* Trees with root galls

Leaf No. per Tree

Treatment	Replicate		
	1	2	3
Water	149	133	126
Arg. 1	103	88	97
Asp. 1	79	102	95
Ser. 1	139	104	136
KNO_3 1	124	119*	100
Arg. 10	118	112	147
Asp. 10	106	154*	137
Ser. 10	139	131	139
KNO_3 10	122*	122	128

Shoot No. per Tree

Treatment	Replicate		
	1	2	3
Water	5	6	8
Arg. 1	5	8	9
Asp. 1	4	6	6
Ser. 1	7	5	9
KNO_3 1	8	8*	6
Arg. 10	5	5	6
Asp. 10	5	9*	8
Ser. 10	6	7	9
KNO_3 10	11*	8	9

HARVEST DATA

* Trees with root galls

Average Length of New Shoots per Tree (cm)

Treatment	Replicate		
	1	2	3
Water	32.2	32.0	20.2
Arg. 1	30.9	23.2	20.3
Asp. 1	33.6	33.1	24.0
Ser. 1	28.0	29.5	22.1
KNO_3 1	27.3	21.3*	29.4
Arg. 10	27.5	29.5	27.8
Asp. 10	26.2	23.7*	22.9
Ser. 10	27.2	23.2	21.8
KNO_3 10	16.3*	24.7	18.5

APPENDIX C. (a)(i).- Sand Culture Experiment;

Experimental Data

FRESH WEIGHT PER TREE AT PLANTING (BEFORE PRUNING) AND FRESH WEIGHT OF TOP PRUNINGS PER TREE AT PLANTING

g

* Trees with root galls

Treatment											
N1				N3				N9			
Tree No.	Rep. No.	F.Wt./Tree at Ptg.	F.Wt. Prunings	Tree No.	Rep. No.	F.Wt./Tree at Ptg.	F.Wt. Prunings	Tree No.	Rep. No.	F.Wt./Tree at Ptg.	F.Wt. Prunings
126	1	119.2	53.8	109	1	129.3	53.2	71	1	164.6	84.1
122		125.2	62.0	32		117.9	52.7	2		122.7	53.1
50		143.7	65.0	58		146.3	66.0	135		111.1	49.2
111		110.2	56.0	47		144.3	60.9	131		141.1	64.4
26		151.9	65.9	120		141.2	59.0	39		177.2	90.8
132		134.3	60.4	48		147.9	68.0	113		112.1	53.0
61		121.3	52.8	63		135.0	59.1	77		115.3	57.5
134		121.2	50.1	130		133.0	55.0	80		131.8	60.9
84		125.8	63.9	133		128.9	48.9	78		151.9	67.1
107	2	150.5	63.0	64	2	194.2	92.6	128	2	164.8	79.5
83		153.8	65.6	82		167.7	77.3	90		133.3	52.2
117		137.7	52.7	108*		161.8	67.0	27*		160.2	72.1
112		148.4	52.9	103		168.7	76.0	89		133.5	51.8
86		162.5	73.4	127		154.6	70.7	105		157.4	66.8
102		144.9	60.8	24		163.9	73.0	99		166.8	74.1
11		179.8	83.1	87*		150.7	66.0	23		176.0	75.8
70		167.4	68.7	52		142.1	56.6	106		139.1	52.9
15		219.0	117.0	79		148.2	65.4	95*		163.0	73.0
51	3	197.0	97.1	62	3	200.7	92.5	121*	3	172.0	71.3
56		240.7	113.2	18*		182.4	68.0	118		165.9	55.2
55		238.2	115.9	34		194.0	90.1	125*		216.5	116.2
67		206.2	105.6	88		207.7	109.8	57		195.1	89.4
35		179.8	73.5	38		206.7	101.8	30*		193.0	93.2
68		176.3	62.7	43		178.5	80.8	98		207.9	98.0
20		162.9	70.0	13		218.5	106.8	36		196.0	100.3
81		173.0	75.0	72		226.9	103.4	115*		161.9	61.9
116		148.8	52.8	85		176.3	73.6	94		178.3	80.1
101	4	196.2	76.0	40	4	217.9	84.8	49	4	224.8	87.5
60		220.8	91.7	1		242.0	92.3	129		249.8	112.0
42		238.6	104.1	28		236.1	111.4	9*		210.1	90.2
96		206.6	92.4	22		200.9	82.9	12		223.9	89.9
66		187.9	58.4	97		227.2	92.0	91		160.6	72.0
46		203.2	89.3	114		238.9	113.5	54		202.8	79.2
65		237.1	100.0	45		204.1	84.1	16		184.3	74.0
100*		223.5	105.9	74*		243.0	99.9	92		170.6	42.7
110		229.8	100.6	69		198.8	93.0	21		209.1	91.1

FRESH WEIGHT PER TREE AT PLANTING (AFTER PRUNING) AND AFTER 1 YEAR

g

* Trees with root galls

Treatment											
N1				N3				N9			
Tree No.	Rep. No.	F.Wt./Tree at Ptg.	F.Wt./Tree 1st Year	Tree No.	Rep. No.	F.Wt./Tree at Ptg.	F.Wt./Tree 1st Year	Tree No.	Rep. No.	F.Wt./Tree at Ptg.	F.Wt./Tree 1st Year
126	1	54.8	259.4	109	1	64.6	273.1	71	1	64.5	593.1
122		54.8	320.0	32		52.4	398.0	2		59.0	712.5
50		62.8	255.5	58		67.4	353.5	135		51.5	584.2
111		47.1	256.1	47		59.7	389.0	131		64.0	593.0
26		67.0	281.0	120		66.0	470.9	39		66.2	510.2
132		59.5	259.6	48		66.9	412.8	113		52.5	886.0
61		64.1	216.7	63		63.4	356.3	77		49.0	636.6
134		59.7	229.1	130		60.2	342.2	80		52.6	727.4
84		52.1	221.0	133		63.3	302.1	78		66.8	546.3
107	2	76.2	270.0	64	2	77.4	464.0	128	2	76.7	608.7
83		72.2	322.7	82		73.9	537.1	90		72.5	414.3
117		68.8	255.8	108*		74.3	490.4	27*		67.7	860.9
112		73.3	245.0	103		77.9	440.6	89		73.0	603.9
86		71.5	296.0	127		73.6	402.9	105		76.3	1131.0
102		68.0	246.1	24		69.1	332.4	99		71.8	843.7
11		67.7	272.7	87*		72.0	287.8	23		78.0	738.2
70		77.4	282.9	52		72.4	405.1	106		73.3	537.4
15		75.3	253.2	79		68.5	317.9	95*		72.4	554.9
51	3	80.0	232.0	62	3	85.7	368.5	121*	3	83.9	786.0
56		91.8	258.8	18*		90.5	366.4	118		82.7	750.0
55		89.3	327.8	34		86.5	379.9	125*		78.2	676.9
67		86.8	273.4	88		82.0	394.1	57		78.7	582.0
35		85.6	280.9	38		85.7	419.5	30*		83.6	690.5
68		91.3	277.1	43		82.7	424.7	98		90.0	613.9
20		80.6	301.8	13		91.2	421.2	36		82.5	545.2
81		79.2	229.4	72		93.2	369.1	115*		86.0	545.7
116		84.8	202.4	85		88.4	386.3	94		81.7	522.1
101	4	104.8	322.1	40	4	95.8	459.1	49	4	95.7	879.9
60		102.3	296.3	1		131.4	564.0	129		112.2	842.0
42		101.0	302.4	28		103.3	518.1	9*		96.6	708.9
96		99.5	345.3	22		94.3	307.3	12		104.5	692.7
66		95.2	288.3	97		107.1	412.4	91		75.4	929.9
46		93.9	331.9	114		101.6	467.9	54		97.0	1044.5
65		105.9	274.9	45		100.0	448.3	16		78.2	709.5
100*		95.3	299.8	74*		112.2	410.8	92		102.5	527.0
110		103.5	301.8	69		100.0	387.7	21		96.4	655.6

TOTAL LENGTH OF TOPS PER TREE AT PLANTING (BEFORE PRUNING) AND AFTER 1 YEAR

cm

* Trees with root galls

Treatment											
N1				N3				N9			
Tree No.	Rep. No.	T.L.T./Tree at Ptg.	T.L.T./Tree 1st Year	Tree No.	Rep. No.	T.L.T./Tree at Ptg.	T.L.T./Tree 1st Year	Tree No.	Rep. No.	T.L.T./Tree at Ptg.	T.L.T./Tree 1st Year
126	1	365.6	38.7	109	1	363.5	72.3	71	1	663.4	248.5
122		545.0	76.5	32		543.0	86.7	2		436.6	313.3
50		605.4	84.9	58		535.5	118.9	135		359.9	217.6
111		399.6	48.8	47		550.3	83.9	131		570.9	234.4
26		603.5	57.1	120		505.7	103.7	39		751.4	264.7
132		443.4	51.6	48		640.8	105.5	113		502.4	291.4
61		562.3	89.1	63		500.1	101.4	77		709.8	265.0
134		331.8	33.1	130		510.1	122.9	80		458.6	350.9
84		473.3	69.9	133		320.8	74.0	78		657.3	249.5
107	2	507.1	51.3	64	2	739.3	126.1	128	2	660.9	272.2
83		427.2	61.4	82		573.1	119.8	90		316.4	172.5
117		341.7	64.4	108*		473.3	151.3	27*		672.9	314.9
112		332.7	54.7	103		425.4	108.1	89		335.1	273.5
86		573.1	90.9	127		543.3	138.3	105		549.6	310.7
102		517.6	59.7	24		625.3	122.2	99		589.9	274.8
11		650.9	67.6	87*		433.7	55.8	23		559.2	360.0
70		569.2	117.0	52		513.3	126.0	106		368.9	280.9
15		757.3	102.5	79		311.2	43.9	95*		624.9	317.1
51	3	771.4	55.3	62	3	565.8	136.7	121*	3	575.9	336.2
56		881.3	82.0	18*		437.3	94.1	118		296.4	276.7
55		676.1	88.3	34		757.4	144.6	125*		815.2	242.7
67		776.5	103.4	88		810.9	123.4	57		704.5	153.5
35		653.2	81.6	38		732.0	140.0	30*		726.3	238.8
68		538.5	117.7	43		604.8	105.5	98		852.9	181.4
20		482.6	80.9	13		736.4	139.1	36		820.3	183.4
81		503.2	57.5	72		789.4	112.4	115*		422.8	185.8
116		303.0	37.3	85		460.4	123.9	94		591.5	252.8
101	4	338.3	97.3	40	4	693.7	114.9	49	4	674.3	296.2
60		599.0	92.3	1		565.4	144.9	129		881.3	413.1
42		739.6	119.5	28		735.1	138.2	9*		691.6	282.3
96		508.6	82.4	22		492.1	60.9	12		464.2	285.9
66		446.5	55.6	97		650.7	89.5	91		526.7	245.5
46		719.9	109.6	114		891.4	98.4	54		534.1	245.0
65		651.1	148.0	45		535.6	133.1	16		594.4	321.5
100*		780.6	139.3	74*		780.8	161.2	92		321.2	175.1
110		491.1	115.0	69		617.0	130.2	21		663.0	240.8

STEM DIAMETER PER TREE AT PLANTING AND AFTER 1 YEAR

in.

* Trees with root galls

Treatment											
N1				N3				N9			
Tree No.	Rep. No.	S. D./Tree at Ptg.	S. D./Tree 1st Year	Tree No.	Rep. No.	S. D./Tree at Ptg.	S. D./Tree 1st Year	Tree No.	Rep. No.	S. D./Tree at Ptg.	S. D./Tree 1st Year
126	1	0.358	0.245	109	1	0.395	0.330	71	1	0.446	0.500
122		0.386	0.253	32		0.366	0.348	2		0.392	0.543
50		0.390	0.231	58		0.411	0.248	135		0.348	0.495
111		0.390	0.233	47		0.396	0.341	131		0.394	0.519
26		0.402	0.239	120		0.420	0.311	39		0.450	0.350
132		0.393	0.243	48		0.406	0.314	113		0.375	0.512
61		0.399	0.201	63		0.404	0.320	77		0.379	0.528
134		0.391	0.223	130		0.388	0.363	80		0.396	0.541
84		0.415	0.242	133		0.398	0.279	78		0.417	0.523
107	2	0.419	0.255	64	2	0.459	0.250	128	2	0.446	0.518
83		0.412	0.256	82		0.423	0.404	90		0.362	0.429
117		0.380	0.231	108*		0.411	0.342	27*		0.423	0.489
112		0.385	0.246	103		0.422	0.355	89		0.401	0.508
86		0.411	0.229	127		0.416	0.359	105		0.420	0.534
102		0.360	0.226	24		0.399	0.370	99		0.448	0.507
11		0.424	0.218	87*		0.429	0.310	23		0.453	0.529
70		0.405	0.245	52		0.414	0.375	106		0.409	0.475
15		0.498	0.288	79		0.413	0.282	95*		0.442	0.512
51	3	0.473	0.225	62	3	0.459	0.324	121*	3	0.393	0.535
56		0.491	0.272	18*		0.419	0.321	118		0.376	0.543
55		0.500	0.280	34		0.455	0.348	125*		0.502	0.506
67		0.438	0.272	88		0.482	0.321	57		0.480	0.487
35		0.405	0.254	38		0.481	0.323	30*		0.446	0.500
68		0.395	0.275	43		0.435	0.353	98		0.484	0.489
20		0.415	0.221	13		0.470	0.327	36		0.479	0.499
81		0.440	0.215	72		0.489	0.402	115*		0.399	0.485
116		0.406	0.213	85		0.421	0.376	94		0.439	0.491
101	4	0.485	0.229	40	4	0.427	0.351	49	4	0.449	0.500
60		0.476	0.250	1		0.477	0.351	129		0.514	0.547
42		0.468	0.256	28		0.503	0.299	9*		0.483	0.549
96		0.486	0.236	22		0.472	0.290	12		0.474	0.497
66		0.398	0.221	97		0.469	0.308	91		0.410	0.498
46		0.483	0.260	114		0.498	0.353	54		0.432	0.526
65		0.483	0.273	45		0.450	0.361	16		0.420	0.552
100*		0.494	0.287	74*		0.472	0.361	92		0.396	0.497
110		0.508	0.303	69		0.481	0.354	21		0.478	0.492

SEASONAL CHANGES IN THE TOTAL LENGTH OF TOPS PER TREE DURING THE FIRST GROWING SEASON

cm

* Trees with root galls

N Treatment	Tree No.	Date										
		1963							1964			
		16/9	23/9	30/9	7/10	21/10	11/11	2/12	23/12	13/1	3/2	24/2
N1 (rep.1)	126	0	1.6	2.7	4.5	16.1	33.5	38.1	38.7	38.7	38.7	38.7
	122	1.7	1.7	2.3	3.3	13.0	42.2	70.5	76.5	76.5	76.5	76.5
	50	2.5	5.9	10.0	18.7	48.9	81.7	84.9	84.9	84.9	84.9	84.9
	111	0.9	1.1	4.7	9.3	22.5	38.4	46.3	48.8	48.8	48.8	48.8
	26	3.3	6.9	9.7	14.1	28.2	50.7	56.3	56.7	57.1	57.1	57.1
	132	3.8	6.5	8.1	9.3	17.5	39.8	47.6	51.0	51.6	51.6	51.6
	61	2.9	10.9	19.9	33.1	70.5	88.4	89.1	89.1	89.1	89.1	89.1
	134	0	1.9	3.6	5.8	13.4	30.8	33.1	33.1	33.1	33.1	33.1
	84	1.7	7.7	16.3	31.7	60.9	69.4	69.9	69.9	69.9	69.9	69.9
N1 (rep.2)	107	1.5	4.1	6.9	10.2	24.0	47.9	50.2	51.3	51.3	51.3	51.3
	83	4.8	10.0	16.1	24.6	43.9	60.9	61.2	61.4	61.4	61.4	61.4
	117	3.1	6.8	10.4	17.0	39.8	63.6	64.0	64.4	64.4	64.4	64.4
	112	3.2	5.9	10.0	15.1	40.1	54.4	54.7	54.7	54.7	54.7	54.7
	86	6.5	13.0	18.5	29.4	66.5	90.0	90.9	90.9	90.9	90.9	90.9
	102	6.5	12.3	20.5	31.1	49.4	59.4	59.7	59.7	59.7	59.7	59.7
	11	5.0	9.6	13.4	18.5	35.0	61.4	67.6	67.6	67.6	67.6	67.6
	70	10.2	22.0	52.1	78.7	111.2	116.4	117.0	117.0	117.0	117.0	117.0
	15	7.3	16.0	25.3	44.1	76.5	100.5	102.5	102.5	102.5	102.5	102.5
N1 (rep.3)	51	6.4	10.0	13.0	15.1	17.3	29.3	50.7	55.3	55.3	55.3	55.3
	56	7.9	13.8	16.3	18.8	26.0	58.2	80.1	82.0	82.0	82.0	82.0
	55	4.7	8.4	11.5	17.4	50.0	84.9	88.3	88.3	88.3	88.3	88.3
	67	2.5	8.2	11.0	13.6	19.0	45.8	85.8	102.6	103.1	103.2	103.4
	35	6.0	10.8	14.3	19.1	42.1	77.2	81.6	81.6	81.6	81.6	81.6
	68	4.6	12.6	25.6	52.1	99.9	117.7	117.7	117.7	117.7	117.7	117.7
	20	7.4	14.2	22.9	34.5	64.0	80.5	80.9	80.9	80.9	80.9	80.9
	81	3.0	5.0	6.7	9.6	22.4	53.8	57.5	57.5	57.5	57.5	57.5
	116	0	2.5	3.9	6.1	13.4	22.3	27.5	37.3	37.3	37.3	37.3
N1 (rep.4)	101	1.6	4.5	8.6	14.6	46.4	93.8	96.9	97.3	97.3	97.3	97.3
	60	3.4	8.0	15.2	20.8	38.1	81.7	92.0	92.3	92.3	92.3	92.3
	42	6.6	14.3	23.8	40.8	83.5	118.9	119.5	119.5	119.5	119.5	119.5
	96	2.0	5.0	8.1	12.7	34.2	74.7	81.4	81.4	82.4	82.4	82.4
	66	5.2	10.2	13.4	15.8	22.5	39.6	54.4	55.6	55.6	55.6	55.6
	46	2.3	8.2	14.5	27.5	69.2	106.0	109.6	109.6	109.6	109.6	139.6
	65	5.1	11.3	20.3	40.1	107.7	146.9	148.0	148.0	148.0	148.0	148.0
	100*	5.0	12.8	22.8	46.5	109.2	137.9	139.3	139.3	139.3	139.3	139.3
	110	3.9	10.0	14.4	17.7	48.0	105.8	115.0	115.0	115.0	115.0	115.0

TOTAL LENGTH OF TOPS PER TREE (Contd.)

cm

* Trees with root galls

N Treatment	Tree No.	Date										
		1963								1964		
		16/9	23/9	30/9	7/10	21/10	11/11	2/12	23/12	13/1	3/2	24/2
N3 (rep.1)	109	1.1	2.1	2.9	3.4	5.1	17.6	50.5	70.2	70.9	71.5	72.3
	32	0	0.9	4.3	10.2	27.8	50.3	62.9	85.3	86.7	86.7	86.7
	58	6.2	10.5	14.3	18.2	40.1	77.6	109.1	118.1	118.9	118.9	118.9
	47	2.2	4.9	7.3	10.2	23.4	48.1	73.8	82.8	83.7	83.9	83.9
	120	4.9	12.1	20.7	39.2	81.8	101.1	101.9	102.1	102.7	103.6	103.7
	43	6.1	11.8	18.0	32.9	80.3	101.6	103.1	103.3	104.1	104.6	105.5
	63	2.3	10.4	16.7	25.0	47.2	76.7	90.4	101.1	101.4	101.4	101.4
	130	2.0	5.2	7.6	9.7	13.9	33.0	99.1	121.4	122.0	122.1	122.9
	133	2.0	8.0	15.8	29.2	60.9	72.9	74.0	74.0	74.0	74.0	74.0
N3 (rep.2)	64	3.1	7.2	13.1	23.9	57.2	88.9	117.0	125.1	125.3	125.5	126.1
	82	3.4	8.2	12.7	20.3	55.9	96.2	106.2	115.3	118.9	119.4	119.8
	108*	8.6	17.0	29.3	48.5	117.0	144.5	147.1	150.8	151.3	151.3	151.3
	103	4.5	7.5	10.2	13.4	32.5	80.8	101.4	107.8	108.1	108.1	108.1
	127	4.5	14.0	26.3	51.2	105.0	133.1	135.8	137.5	137.6	137.8	138.3
	24	3.9	5.5	5.9	6.8	13.8	51.8	101.1	120.5	121.4	122.2	122.2
	87*	1.8	4.6	9.1	16.8	31.7	51.9	53.9	53.9	54.7	55.8	55.8
	52	2.8	8.2	14.5	23.7	61.7	103.4	117.4	125.4	125.6	125.9	126.0
	79	1.5	1.8	2.6	5.0	15.9	34.0	37.2	37.2	42.9	43.5	43.9
N3 (rep.3)	62	2.7	9.3	17.0	32.8	86.0	127.1	132.9	136.7	136.7	136.7	136.7
	18*	4.6	7.6	10.1	13.7	28.0	69.2	91.8	93.8	94.1	94.1	94.1
	34	2.8	8.4	15.1	25.5	65.8	124.2	140.4	143.9	144.6	144.5	144.6
	88	7.8	13.8	18.6	29.9	77.2	114.3	118.5	121.8	123.4	123.4	123.4
	38	6.8	15.1	24.5	44.3	96.8	128.9	135.8	140.0	140.0	140.0	140.0
	43	1.2	2.4	4.1	7.6	25.3	76.4	94.5	98.8	103.4	105.0	105.5
	13	5.3	10.6	14.3	19.6	44.3	95.4	121.7	137.3	138.3	138.7	139.1
	72	7.5	11.8	13.3	14.1	19.1	54.7	95.7	108.3	110.7	112.0	112.4
	85	3.5	5.0	6.4	8.0	18.4	68.6	111.6	123.8	123.9	123.9	123.9
N3 (rep.4)	40	7.0	12.3	17.9	26.6	61.1	102.0	110.2	114.4	114.9	114.9	114.9
	1	0	1.5	5.8	11.5	48.5	115.6	139.5	144.3	144.9	144.9	144.9
	28	3.6	10.1	18.4	36.8	87.9	134.3	138.2	138.2	138.2	138.2	138.2
	22	0.8	3.3	5.4	8.7	16.7	33.5	52.4	60.7	60.9	60.9	60.9
	97	3.2	7.2	11.6	17.9	45.9	77.9	84.5	89.5	89.5	89.5	89.5
	114	2.8	8.7	14.6	18.1	36.6	69.6	90.5	97.4	98.4	98.4	98.4
	45	6.2	10.8	16.0	22.1	42.1	88.9	127.1	133.1	133.1	133.1	133.1
	74*	6.9	12.2	16.0	19.6	35.2	105.1	150.2	159.3	160.0	160.5	161.2
	69	1.4	6.0	10.8	17.0	36.7	94.9	115.9	127.6	128.2	128.4	130.2

TOTAL LENGTH OF TOPS PER TREE (Contd.)

cm

* Trees with root galls

N Treatment	Tree No.	Date										
		1963							1964			
		16/9	23/9	30/9	7/10	21/10	11/11	2/12	23/12	13/1	3/2	24/2
N9 (rep.1)	71	8.7	15.0	20.5	27.6	58.3	138.0	204.1	242.9	247.2	247.5	248.5
	2	6.9	13.0	17.6	24.0	51.0	142.1	264.7	310.9	313.2	313.2	313.3
	135	0	0	0	1.7	5.3	19.0	37.2	137.4	207.0	215.8	217.6
	131	0	3.6	8.5	17.7	65.0	105.5	142.7	207.2	230.0	232.8	234.4
	39	2.8	8.2	16.6	35.5	85.9	128.3	181.1	248.1	261.5	264.1	264.7
	113	3.0	6.7	10.0	14.0	27.6	105.0	224.6	274.2	288.9	289.4	291.4
	77	1.0	6.0	15.2	32.7	71.2	106.6	140.8	241.8	261.7	263.5	265.0
	80	2.7	5.3	8.4	12.9	27.5	87.6	282.3	340.6	350.9	350.9	350.9
	78	2.5	6.2	10.2	17.0	42.8	81.6	172.4	215.2	243.9	247.7	249.5
N9 (rep.2)	128	7.6	13.7	18.2	25.3	59.4	118.3	174.8	234.6	266.1	271.5	272.2
	90	0	1.0	3.2	7.4	26.2	61.6	96.7	132.6	166.7	171.6	172.5
	27*	8.8	15.3	24.5	44.8	112.1	160.2	206.2	286.0	312.2	314.2	314.9
	89	1.9	3.5	6.1	9.8	25.9	92.4	200.6	258.4	271.1	273.2	273.5
	105	4.5	11.2	17.3	29.2	68.9	117.0	191.3	291.5	307.3	308.5	310.7
	99	1.6	4.8	8.9	16.0	50.8	131.7	192.4	244.4	268.7	272.5	274.8
	23	2.8	9.0	19.6	45.2	114.9	188.8	256.6	346.4	356.3	358.9	360.0
	106	0	2.2	6.7	17.9	78.5	169.8	210.9	252.8	277.7	280.6	280.9
	95*	4.5	5.7	6.3	6.4	7.4	20.2	105.0	193.7	235.4	315.9	317.1
N9 (rep.3)	121*	6.0	15.4	25.8	51.0	122.6	202.8	262.2	329.4	334.1	335.1	336.2
	118	2.1	4.7	7.2	11.7	26.5	79.5	155.0	212.1	269.3	275.8	276.7
	125*	1.1	6.5	11.9	17.6	29.8	44.3	50.8	124.3	233.2	241.9	242.7
	57	1.7	4.7	7.3	11.0	17.7	32.9	50.8	93.0	136.8	151.0	153.5
	30*	7.2	11.0	14.0	19.8	50.3	83.8	107.8	202.9	234.4	237.9	238.8
	98	1.2	4.3	7.4	10.8	14.7	19.7	21.6	34.2	131.5	174.5	181.4
	36	7.6	12.6	15.8	18.3	24.1	41.0	122.9	165.0	178.7	182.1	183.4
	115*	1.4	5.0	10.3	21.3	73.2	129.3	154.3	176.7	184.9	185.7	185.8
	94	3.8	9.3	12.3	15.8	19.0	25.6	36.7	53.4	185.9	251.2	252.8
N9 (rep.4)	49	1.6	9.9	23.1	50.7	124.8	183.1	222.7	276.2	294.4	295.4	296.2
	129	6.5	13.6	23.0	42.6	136.2	268.5	351.7	412.3	413.1	413.1	413.1
	9*	5.3	11.3	15.9	21.5	33.6	66.3	134.3	244.6	274.8	280.8	282.3
	12	0	2.4	5.6	10.3	41.1	126.6	215.3	272.0	282.6	283.4	285.9
	91	5.0	9.8	14.3	22.4	64.2	130.5	175.9	239.0	245.5	245.5	245.5
	54	3.7	7.5	11.1	18.9	45.4	108.0	185.9	241.3	243.0	243.2	245.0
	16	6.8	14.5	24.1	40.0	89.8	146.2	221.7	306.9	318.2	319.4	321.5
	92	2.6	6.7	12.0	20.9	74.5	135.3	153.7	172.1	173.2	173.6	175.1
	21	4.8	9.8	16.8	28.3	70.2	146.1	202.1	238.7	240.8	240.8	240.8

SEASONAL CHANGES IN THE STEM DIAMETER PER TREE DURING THE FIRST GROWING SEASON

10x in.

* Trees with root galls

N Treatment	Tree No.	Date									
		1963					1964				
		5/10	19/10	9/11	1/12	21/12	11/1	1/2	22/2	14/3	11/7
N1 (rep.1)	126	0	1.08	1.48	1.87	2.09	2.22	2.29	2.33	2.40	2.45
	122	0	1.18	1.59	1.72	1.98	2.21	2.38	2.46	2.50	2.53
	50	1.00	1.34	1.51	1.67	1.89	1.93	2.03	1.99	2.26	2.31
	111	0.78	1.14	1.44	1.69	1.98	2.10	2.17	2.15	2.17	2.33
	26	0.85	1.27	1.52	1.76	1.94	2.15	2.21	2.30	2.30	2.39
	132	0.77	1.04	1.30	1.51	1.78	1.98	2.26	2.38	2.37	2.43
	61	1.09	1.42	1.54	1.78	1.85	2.01	2.06	1.91	1.94	2.01
	134	0.96	1.28	1.58	1.82	1.98	2.22	2.25	2.27	2.22	2.23
	84	1.16	1.50	1.72	2.02	2.29	2.32	2.41	2.48	2.47	2.42
N1 (rep.2)	107	1.19	1.55	1.71	1.98	2.07	2.14	2.22	2.45	2.46	2.55
	83	1.20	1.72	1.93	2.17	2.39	2.45	2.57	2.52	2.57	2.56
	117	0.98	1.26	1.47	1.70	1.85	1.91	2.09	2.06	2.08	2.31
	112	1.18	1.66	1.87	1.99	2.24	2.24	2.38	2.42	2.48	2.46
	86	1.14	1.51	1.70	1.87	2.08	2.28	2.36	2.30	2.28	2.29
	102	1.30	1.55	1.71	1.84	2.02	2.06	2.13	2.18	2.13	2.26
	11	1.11	1.20	1.40	1.62	1.79	1.90	2.07	2.05	2.14	2.18
	70	1.44	1.71	1.83	2.02	2.12	2.25	2.26	2.25	2.30	2.45
	15	1.45	1.80	2.05	2.25	2.51	2.60	2.67	2.65	2.67	2.38
N1 (rep.3)	51	1.01	1.17	1.33	1.62	1.82	1.98	2.00	2.12	2.39	2.25
	56	1.26	1.46	1.82	1.97	2.22	2.36	2.41	2.41	2.49	2.72
	55	1.06	1.45	1.72	1.97	2.32	2.53	2.57	2.64	2.74	2.80
	67	0.86	1.10	1.36	1.71	2.06	2.37	2.43	2.52	2.67	2.72
	35	1.04	1.45	1.74	1.97	2.14	2.36	2.36	2.36	2.43	2.54
	68	1.26	1.63	1.76	1.93	2.20	2.24	2.25	2.27	2.17	2.75
	20	1.25	1.54	1.73	1.93	2.07	2.08	2.17	2.18	2.16	2.21
	81	0.85	1.19	1.49	1.66	1.85	2.00	2.00	2.01	1.99	2.15
	116	0	1.07	1.20	1.40	1.59	1.72	1.85	1.88	1.95	2.13
N1 (rep.4)	101	1.15	1.57	1.77	1.94	2.14	2.19	2.23	2.27	2.26	2.29
	60	1.22	1.47	1.86	2.10	2.26	2.43	2.38	2.40	2.46	2.50
	42	1.36	1.76	1.99	2.14	2.32	2.45	2.53	2.53	2.51	2.56
	96	1.15	1.55	1.81	1.97	2.09	2.18	2.18	2.21	2.29	2.36
	66	1.03	1.17	1.40	1.62	1.87	2.07	2.08	2.13	2.17	2.21
	46	1.23	1.62	1.96	2.13	2.30	2.47	2.47	2.50	2.47	2.80
	65	1.38	1.73	2.06	2.30	2.46	2.55	2.60	2.62	2.60	2.73
	100*	1.40	1.78	2.04	2.38	2.59	2.66	2.58	2.68	2.79	2.87
	110	1.42	1.84	2.17	2.31	2.56	2.72	2.75	2.69	2.73	3.03

STEM DIAMETER PER TREE (Contd.)

10x in.

* Trees with root galls

N Treatment	Tree No.	Date									
		1963					1964				
		5/10	19/10	9/11	1/12	21/12	11/1	1/2	22/2	14/3	11/7
N3 (rep.1)	109	0	1.12	1.43	1.72	2.18	2.43	2.65	2.89	3.14	3.30
	32	0.83	1.21	1.62	1.98	2.27	2.56	2.89	3.10	3.29	3.48
	58	1.11	1.31	1.49	1.64	1.98	2.16	2.31	2.35	2.32	2.48
	47	0.89	1.27	1.60	1.98	2.41	2.70	3.10	3.38	3.30	3.41
	120	1.24	1.67	1.92	2.21	2.55	2.80	2.91	3.08	3.03	3.11
	48	1.29	1.60	1.79	2.12	2.40	2.71	2.82	2.91	2.92	3.14
	63	1.10	1.36	1.62	1.96	2.37	2.64	2.82	2.92	3.10	3.20
	130	0.86	1.05	1.32	1.69	2.04	2.43	2.84	3.18	3.26	3.63
	133	1.17	1.42	1.57	1.89	2.14	2.34	2.56	2.55	2.66	2.79
N3 (rep.2)	64	1.07	1.38	1.46	1.63	1.85	1.97	2.18	2.22	2.43	2.50
	82	1.06	1.64	2.05	2.30	2.65	3.08	3.43	3.74	3.78	4.04
	108*	1.59	1.81	2.10	2.50	2.77	2.91	3.17	3.30	3.29	3.42
	103	1.17	1.53	1.95	2.26	2.71	3.04	3.17	3.26	3.44	3.55
	127	1.54	1.84	2.16	2.62	2.93	3.11	3.33	3.42	3.47	3.59
	24	0.48	1.03	1.53	1.93	2.44	2.74	3.08	3.31	3.49	3.70
	87*	1.11	1.58	1.77	2.02	2.27	2.52	2.69	2.89	2.91	3.10
	52	1.02	1.47	1.84	2.27	2.70	3.00	3.25	3.40	3.57	3.75
	79	0.81	1.31	1.49	1.74	2.05	2.17	2.48	2.59	2.94	2.82
N3 (rep.3)	62	1.17	1.74	2.09	2.39	2.80	3.01	3.12	3.18	3.20	3.24
	18*	1.00	1.30	1.69	2.00	2.30	2.52	2.72	2.77	2.77	3.21
	34	1.16	1.53	1.94	2.30	2.71	3.03	3.25	3.23	3.27	3.48
	88	1.20	1.56	1.78	2.04	2.38	2.65	2.82	2.91	3.00	3.21
	38	1.49	1.84	2.01	2.28	2.59	2.85	2.94	3.09	3.21	3.23
	43	0	1.26	1.77	2.09	2.48	2.81	3.14	3.36	3.49	3.53
	13	1.03	1.39	1.66	2.02	2.35	2.67	2.91	3.11	3.12	3.27
	72	1.18	1.32	1.68	2.05	2.44	2.81	3.07	3.30	3.53	4.02
	85	0.95	1.37	1.80	2.06	2.55	2.88	3.20	3.47	3.49	3.76
N3 (rep.4)	40	1.23	1.55	1.86	2.23	2.59	2.96	3.10	3.24	3.31	3.51
	1	1.43	1.91	2.16	2.62	2.86	3.23	3.28	3.34	3.35	3.51
	28	1.28	1.58	1.81	2.12	2.47	2.69	2.79	2.92	2.96	2.99
	22	0	1.28	1.67	1.92	2.23	2.51	2.56	2.69	2.79	2.90
	97	1.03	1.40	1.67	2.00	2.34	2.58	2.79	2.95	3.00	3.08
	114	1.18	1.30	1.77	2.21	2.65	2.85	3.10	3.46	3.52	3.53
	45	1.28	1.59	1.97	2.24	2.57	2.85	2.96	3.02	3.15	3.61
	74*	1.27	1.39	1.88	2.42	2.73	3.07	3.17	3.37	3.37	3.61
	69	1.10	1.48	1.82	2.21	2.55	2.86	2.98	3.09	3.36	3.54

STEM DIAMETER PER TREE (Contd.)

10x in.

* Trees with root galls

N Treatment	Tree No.	Date									
		1963						1964			
		5/10	19/10	9/11	1/12	21/12	11/1	1/2	22/2	14/3	11/7
N9 (rep.1)	71	1.25	1.51	2.02	2.72	3.36	3.90	4.34	4.75	4.81	5.00
	2	1.18	1.44	1.96	2.74	3.36	4.01	4.49	4.90	5.07	5.43
	135	0	0.92	1.29	1.69	2.16	2.88	3.64	4.11	4.54	4.95
	131	1.15	1.90	2.25	2.81	3.40	3.98	4.52	4.69	5.26	5.19
	39	1.09	1.39	1.70	2.07	2.43	2.80	3.12	3.18	3.49	3.50
	113	0.99	1.34	1.85	2.37	3.00	3.54	3.99	4.35	4.62	5.12
	77	1.20	1.53	1.88	2.40	3.06	3.60	4.23	4.49	4.88	5.28
	80	0.84	1.12	1.55	2.31	3.08	3.88	4.42	4.75	4.95	5.41
	78	1.10	1.48	1.88	2.37	2.94	3.43	4.20	4.44	4.78	5.23
N9 (rep.2)	128	1.32	1.63	1.96	2.36	3.02	3.60	4.13	4.58	4.87	5.18
	90	0.77	1.35	1.71	2.19	2.73	3.18	3.59	3.82	4.17	4.29
	27*	1.22	1.67	2.06	2.49	3.13	3.73	4.33	4.49	4.83	4.89
	89	0.88	1.32	1.79	2.17	2.78	3.34	3.99	4.36	4.61	5.08
	105	1.11	1.56	1.96	2.66	3.28	3.83	4.37	4.85	5.02	5.34
	99	1.13	1.61	1.90	2.44	3.22	3.77	4.20	4.49	4.89	5.07
	23	1.15	1.70	2.12	2.73	3.29	3.88	4.55	4.74	5.07	5.29
	106	1.14	1.62	1.97	2.46	2.86	3.43	3.90	4.01	4.32	4.75
	95*	0.85	0.99	1.21	1.64	2.29	2.93	3.73	4.17	4.61	5.12
N9 (rep.3)	121*	1.42	1.94	2.34	2.93	3.59	4.15	4.64	4.85	5.11	5.35
	118	1.12	1.39	1.94	2.44	3.14	3.58	4.30	4.70	5.01	5.43
	125*	1.05	1.31	1.46	1.67	2.15	2.91	3.56	4.11	4.42	5.06
	57	0.80	1.09	1.36	1.64	2.17	2.85	3.41	3.95	4.35	4.87
	30*	1.04	1.40	1.77	2.22	2.84	3.56	4.14	4.49	4.63	5.00
	98	0.89	1.11	1.15	1.24	1.42	1.98	2.69	3.41	3.98	4.89
	36	1.10	1.27	1.61	2.06	2.66	3.22	3.78	4.23	4.49	4.99
	115*	1.20	1.71	2.01	2.49	3.01	3.50	3.93	4.03	4.26	4.85
	94	1.03	1.18	1.33	1.45	1.68	2.51	3.09	3.84	4.18	4.91
N9 (rep.4)	49	1.23	1.76	2.19	2.89	3.33	3.88	4.26	4.62	4.73	5.00
	129	1.35	2.11	2.68	3.30	3.84	4.23	4.62	4.98	5.21	5.47
	9*	1.18	1.36	1.75	2.32	2.78	3.46	4.16	4.57	4.99	5.49
	12	1.00	1.49	1.94	2.61	3.13	3.65	4.14	4.36	4.75	4.97
	91	1.14	1.52	1.97	2.64	3.17	3.78	4.19	4.48	4.60	4.98
	54	1.29	1.73	2.18	2.78	3.41	3.90	4.21	4.68	4.93	5.26
	16	1.39	1.93	2.40	2.92	3.51	4.11	4.53	4.78	5.16	5.52
	92	1.55	2.10	2.55	2.99	3.47	4.08	4.49	4.59	5.14	4.97
	21	1.36	1.78	2.30	2.97	3.43	3.89	4.30	4.57	4.67	4.92

DRY WEIGHT OF TREE PARTS AT HARVEST 1

g

* Trees with root galls

Tree No.	N Treatment	Rep. No.	D. Wt. Roots	D. Wt. Tops	D. Wt. Buds	D. Wt. per Tree	$\frac{\text{D. Wt. Tops}}{\text{D. Wt. Roots}}$
61	N1	1	57.9	21.9	0.56	80.4	0.39
134			52.8	16.8	0.31	70.0	0.32
84			55.9	22.1	0.43	78.4	0.40
11		2	59.8	23.0	0.45	83.2	0.39
70			85.2	29.5	0.68	115.4	0.35
15			66.8	29.6	0.59	97.0	0.45
20		3	83.9	27.8	0.66	112.4	0.34
81			55.8	19.7	0.36	76.0	0.36
116			55.6	17.6	0.47	73.7	0.33
65		4	78.5	35.5	0.75	114.8	0.46
100*			84.9	30.6	0.64	116.1	0.37
110			84.7	34.0	0.81	119.6	0.41
63	N3	1	90.8	37.1	0.68	128.6	0.42
130			86.9	36.8	0.52	124.3	0.43
133			92.9	27.8	0.58	121.3	0.31
24		2	96.2	31.2	0.55	128.0	0.33
87*			77.5	39.4	0.86	117.9	0.52
52			111.0	48.6	1.29	160.9	0.45
13		3	98.4	50.8	0.94	150.1	0.53
72			101.8	38.2	0.56	140.7	0.38
85			98.5	38.4	1.34	138.3	0.40
45		4	111.9	46.8	0.99	159.7	0.43
74*			127.5	42.8	1.00	171.3	0.34
69			99.0	34.8	1.35	135.2	0.37
77	N9	1	166.3	98.4	1.81	266.5	0.60
80			197.9	91.6	1.64	291.1	0.47
78			151.4	86.1	1.73	239.2	0.58
23		2	169.5	115.6	1.93	287.0	0.69
106			143.5	74.2	2.09	219.8	0.53
95*			137.3	82.5	1.22	221.0	0.61
36		3	132.4	78.6	1.30	212.4	0.60
115*			159.8	81.3	1.94	243.0	0.52
94			139.4	76.9	1.42	217.7	0.56
16		4	175.6	95.5	2.30	273.4	0.56
92			152.3	78.5	2.39	233.0	0.53
21			172.8	97.5	1.97	272.3	0.58

FRESH WEIGHT OF TREE PARTS AT HARVEST 1

g

* Trees with root galls

Tree No.	N Treatment	Rep. No.	F.Wt. Roots	F.Wt. Tops	F.Wt. Buds	F.Wt. per Tree
61	N1	1	177.9	37.7	1.1	216.7
134			197.1	31.4	0.6	229.1
84			180.0	40.2	0.8	221.0
11		2	228.8	43.1	0.8	272.7
70			230.0	51.7	1.2	282.9
15			196.9	55.2	1.1	253.2
20		3	251.0	49.5	1.3	301.8
81			189.3	39.4	0.7	229.4
116			167.9	33.6	0.9	202.4
65		4	207.4	66.1	1.4	274.9
100*			242.8	55.8	1.2	299.8
110			238.5	61.8	1.5	301.8
63	N3	1	289.4	65.7	1.2	356.3
130			275.3	65.8	1.1	342.2
133			251.1	49.9	1.1	302.1
24		2	272.4	58.7	1.3	332.4
87*			218.9	67.2	1.7	287.8
52			312.0	90.5	2.6	405.1
13		3	326.9	92.5	1.8	421.2
72			299.0	69.0	1.1	369.1
85			310.0	73.6	2.7	386.3
45		4	360.1	86.3	1.9	448.3
74*			328.8	80.2	1.8	410.8
69			313.2	72.0	2.5	387.7
77	N9	1	455.7	177.3	3.6	636.6
80			553.5	170.8	3.1	727.4
78			373.9	168.8	3.6	546.3
23		2	507.1	227.4	3.7	738.2
106			384.1	149.1	4.2	537.4
95*			378.9	164.4	2.6	545.9
36		3	393.1	149.5	2.6	545.2
115*			391.9	149.8	4.0	545.7
94			369.9	149.3	2.9	522.1
16		4	521.8	183.2	4.5	709.5
92			371.0	151.4	4.6	527.0
21			462.1	189.8	3.7	655.6

NO. LEAVES, SHOOTS, FLOWER-BUDS, LEAF-BUDS AND FLOWERS AT THE END OF THE FIRST GROWING SEASON

* Trees with root galls

N1 (rep.1)						N1 (rep.2)					
Tree No.	No. Leaves	No. Shoots	No. Flower Buds	No. Leaf Buds	No. Flowers	Tree No.	No. Leaves	No. Shoots	No. Flower Buds	No. Leaf Buds	No. Flowers
126	48	0	13	20	2	107	62	1	23	34	0
122	63	2	12	29	5	83	64	0	23	28	9
50	65	1	46	31	10	117	52	1	34	31	8
111	50	0	19	20	2	112	64	2	29	32	5
26	52	0	21	26	8	86	64	1	34	40	14
132	45	0	14	24	3	102	61	1	25	30	12
61	70	2	30	36	0	11	50	1	21	21	0
134	45	0	13	21	0	70	79	3	48	42	0
84	62	1	23	28	0	15	73	2	39	37	0

N1 (rep.3)						N1 (rep.4)					
Tree No.	No. Leaves	No. Shoots	No. Flower Buds	No. Leaf Buds	No. Flowers	Tree No.	No. Leaves	No. Shoots	No. Flower Buds	No. Leaf Buds	No. Flowers
51	46	1	21	25	4	101	86	3	43	42	4
56	65	2	25	33	4	60	83	3	47	34	9
55	66	1	14	37	9	42	85	3	45	38	4
67	64	2	39	33	8	96	75	1	50	42	7
35	65	1	40	33	14	66	52	0	26	22	11
68	87	3	44	42	1	46	74	2	52	39	5
20	62	1	50	30	0	65	95	4	53	44	0
81	51	1	21	23	0	100*	92	3	44	46	0
116	51	2	19	27	0	110	109	4	35	60	0

NO. LEAVES, SHOOTS, FLOWER-BUDS, LEAF-BUDS AND FLOWERS AT THE END OF THE FIRST GROWING SEASON

* Trees with root galls

N3 (rep.1)						N3 (rep.2)					
Tree No.	No. Leaves	No. Shoots	No. Flower Buds	No. Leaf Buds	No. Flowers	Tree No.	No. Leaves	No. Shoots	No. Flower Buds	No. Leaf Buds	No. Flowers
109	75	3	25	41	10	64	87	1	36	50	2
32	80	2	23	30	1	82	106	1	21	52	3
58	83	2	54	46	3	108*	104	3	26	47	1
47	77	1	20	38	0	103	97	3	27	54	7
120	81	2	36	37	13	127	102	3	30	49	7
48	95	3	41	40	16	24	90	5	21	43	0
63	76	1	32	37	0	87*	85	1	31	28	0
130	89	4	20	43	0	52	101	1	38	46	0
133	65	1	28	33	0	79	61	1	12	29	7

N3 (rep.3)						N3 (rep.4)					
Tree No.	No. Leaves	No. Shoots	No. Flower Buds	No. Leaf Buds	No. Flowers	Tree No.	No. Leaves	No. Shoots	No. Flower Buds	No. Leaf Buds	No. Flowers
62	96	2	45	49	14	40	90	2	48	39	18
18*	77	2	41	38	7	1	134	4	72	63	19
34	115	2	66	59	1	28	95	4	33	52	30
88	97	2	55	46	6	22	82	1	39	33	14
38	99	3	43	46	6	97	77	1	39	31	1
43	90	3	26	39	2	114	77	1	35	42	5
13	93	2	61	49	0	45	119	4	41	49	0
72	86	2	20	38	0	74*	115	4	55	52	0
85	123	7	40	51	0	69	109	3	50	40	0

NO. LEAVES, SHOOTS, FLOWER-BUDS, LEAF-BUDS AND FLOWERS AT THE END OF THE FIRST GROWING SEASON

* Trees with root galls

N9 (rep.1)						N9 (rep.2)					
Tree No.	No. Leaves	No. Shoots	No. Flower Buds	No. Leaf Buds	No. Flowers	Tree No.	No. Leaves	No. Shoots	No. Flower Buds	No. Leaf Buds	No. Flowers
71	135	5	101	93	6	128	183	6	84	74	12
2	196	8	106	113	13	90	185	6	59	67	6
135	156	8	71	94	4	27*	210	8	103	100	21
131	199	4	68	84	1	89	240	8	66	126	7
39	221	5	105	105	9	105	231	7	91	101	9
113	184	6	83	83	0	99	242	6	61	117	7
77	208	9	78	91	0	23	271	6	78	129	0
80	229	10	50	123	0	106	267	9	83	129	0
78	178	5	1	62	87	95*	215	9	53	96	0

N9 (rep.3)						N9 (rep.4)					
Tree No.	No. Leaves	No. Shoots	No. Flower Buds	No. Leaf Buds	No. Flowers	Tree No.	No. Leaves	No. Shoots	No. Flower Buds	No. Leaf Buds	No. Flowers
121*	228	7	99	121	4	49	212	7	83	99	15
118	237	9	92	115	5	129	259	11	89	131	10
125*	194	8	79	91	3	9*	211	6	94	94	13
57	142	4	60	67	4	12	228	8	79	108	12
30*	190	6	84	65	27	91	204	5	68	103	11
98	171	7	26	69	3	54	211	8	87	102	10
36	145	5	62	62	0	16	221	8	103	116	0
115*	194	3	72	89	0	92	179	3	97	82	0
94	206	10	59	89	0	21	209	7	73	90	0

DRY WEIGHT OF TREE PARTS AT HARVEST 2 (g)

* Trees with root galls; ** Tree numbers

Roots

N Treatment	Replicate 1	2	3	4
N_1N_0	52.3 (126)**	57.1 (117)	72.5 (35)	71.0 (101)
N_1N_3	58.4 (122)	71.9 (83)	66.9 (67)	79.1 (60)
N_1N_9	60.9 (132)	52.0 (102)	78.0 (55)	81.5 (42)
N_3N_0	63.6 (109)	100.5 (127)	97.9* (18)	103.5 (40)
N_3N_3	100.5 (48)	113.7* (108)	102.3 (38)	108.7 (28)
N_3N_9	87.5 (58)	96.1 (103)	100.7 (62)	103.2 (1)
N_9N_0	114.1 (135)	151.3 (99)	121.1* (125)	178.1 (129)
N_9N_3	182.7 (2)	81.6 (90)	120.1 (98)	150.6* (9)
N_9N_9	142.6 (131)	118.3 (89)	147.6* (30)	180.0* (49)

New Shoots

N Treatment	Replicate 1	2	3	4
N_1N_0	8.4 (126)	5.7 (117)	8.4 (35)	10.3 (101)
N_1N_3	24.3 (122)	24.5 (83)	20.9 (67)	20.8 (60)
N_1N_9	54.3 (132)	44.8 (102)	49.2 (55)	44.4 (42)
N_3N_0	25.4 (109)	28.0 (127)	21.9* (18)	29.7 (40)
N_3N_3	41.8 (48)	31.7* (108)	43.4 (38)	34.9 (28)
N_3N_9	63.6 (58)	62.9 (103)	62.7 (62)	43.5 (1)
N_9N_0	55.7 (135)	42.7 (99)	58.7* (125)	55.4 (129)
N_9N_3	76.8 (2)	58.9 (90)	62.8 (98)	78.7* (9)
N_9N_9	104.8 (131)	75.5 (89)	65.2* (30)	94.8* (49)

DRY WEIGHT OF TREE PARTS AT HARVEST 2 (g)

* Trees with root galls; ** Tree numbers

Abscissed Leaves

N Treatment	Replicate 1	2	3	4
N_1N_0	0 (126)**	0 (117)	0.4 (35)	0 (101)
N_1N_3	0 (122)	0.1 (83)	0.1 (67)	0 (60)
N_1N_9	0.3 (132)	0 (102)	0.1 (55)	0.1 (42)
N_3N_0	0.6 (109)	0.2 (127)	0.4* (18)	0.6 (40)
N_3N_3	0.4 (48)	0.2* (108)	0.3 (38)	0.3 (28)
N_3N_9	0.3 (58)	0.5 (103)	0.6 (62)	0.2 (1)
N_9N_0	2.3 (135)	1.3 (99)	1.0* (125)	2.0 (129)
N_9N_3	1.4 (2)	2.7 (90)	0.9 (98)	2.1* (9)
N_9N_9	2.9 (131)	1.7 (89)	0.9* (30)	1.4* (49)

Top/Root Ratio

N Treatment	Replicates 1	2	3	4
N_1N_0	0.52 (126)	0.48 (117)	0.45 (35)	0.66 (101)
N_1N_3	0.89 (122)	0.75 (83)	0.83 (67)	0.71 (60)
N_1N_9	1.36 (132)	1.34 (102)	1.03 (55)	0.90 (42)
N_3N_0	0.88 (109)	0.69 (127)	0.58* (18)	0.68 (40)
N_3N_3	0.75 (48)	0.67* (108)	0.87 (38)	0.71 (28)
N_3N_9	1.18 (58)	1.11 (103)	1.07 (62)	0.89 (1)
N_9N_0	1.13 (135)	0.91 (99)	1.24* (125)	0.93 (129)
N_9N_3	0.91 (2)	1.63 (90)	1.15 (98)	1.24* (9)
N_9N_9	1.31 (131)	1.45 (89)	0.98* (30)	1.12* (49)

DRY WEIGHT OF TREE PARTS AT HARVEST 2 (g)

* Trees with root galls; ** Tree numbers

Stock + Stem + 1 Year Old Shoots

N Treatment	Replicate 1	2	3	4
N_1N_0	18.9 (126)**	21.9 (117)	24.1 (35)	36.4 (101)
N_1N_3	27.6 (122)	23.7 (83)	34.5 (67)	35.6 (60)
N_1N_9	28.7 (132)	24.9 (102)	30.9 (55)	28.8 (42)
N_3N_0	30.8 (109)	41.3 (127)	35.3* (18)	40.9 (40)
N_3N_3	33.2 (48)	44.4* (108)	45.1 (38)	41.8 (28)
N_3N_9	39.5 (58)	43.7 (103)	44.7 (62)	48.1 (1)
N_9N_0	73.7 (135)	94.5 (99)	91.1* (125)	109.4 (129)
N_9N_3	89.5 (2)	73.7 (90)	75.7 (98)	107.8* (9)
N_9N_9	81.8 (131)	96.5 (89)	79.2* (30)	106.6* (49)

Whole Tree (Includes abscissed leaves)

N Treatment	Replicate 1	2	3	4
N_1N_0	79.6 (126)	84.7 (117)	105.4 (35)	117.7 (101)
N_1N_3	110.3 (122)	126.2 (83)	122.4 (67)	135.5 (60)
N_1N_9	144.2 (132)	121.7 (102)	158.2 (55)	154.8 (42)
N_3N_0	129.4 (109)	170.0 (127)	155.5* (18)	174.7 (40)
N_3N_3	175.9 (48)	190.0* (108)	191.1 (38)	185.7 (28)
N_3N_9	190.9 (58)	203.2 (103)	208.7 (62)	195.0 (1)
N_9N_0	245.8 (135)	239.8 (99)	271.9* (125)	344.9 (129)
N_9N_3	350.4 (2)	216.9 (90)	257.5 (98)	339.2* (9)
N_9N_9	332.1 (131)	292.0 (89)	292.9* (30)	382.8* (49)

FRESH WEIGHT OF TREE PARTS AT HARVEST 2 (g)

* Trees with root galls; ** Tree numbers

Roots

N Treatment	Replicate 1	2	3	4
N_1N_0	212.4 (126)**	205.0 (117)	246.2 (35)	229.7 (101)
N_1N_3	307.9 (122)	356.9 (83)	284.4 (67)	293.1 (60)
N_1N_9	320.0 (132)	270.1 (102)	375.0 (55)	358.2 (42)
N_3N_0	246.0 (109)	360.2 (127)	383.0* (18)	371.0 (40)
N_3N_3	415.4 (48)	448.0* (108)	402.9 (38)	479.6 (28)
N_3N_9	404.2 (58)	396.9 (103)	431.0 (62)	416.5 (1)
N_9N_0	413.0 (135)	532.2 (99)	466.7* (125)	576.7 (129)
N_9N_3	558.8 (2)	299.0 (90)	516.1 (98)	512.3* (9)
N_9N_9	517.1 (131)	419.1 (89)	522.5* (30)	646.9* (49)

Stock + Stem + 1 Year Old Shoots

N Treatment	Replicate 1	2	3	4
N_1N_0	35.1 (126)	41.8 (117)	45.3 (35)	68.0 (101)
N_1N_3	57.9 (122)	60.9 (83)	69.6 (67)	69.1 (60)
N_1N_9	62.8 (132)	57.4 (102)	67.0 (55)	61.1 (42)
N_3N_0	69.1 (109)	79.0 (127)	64.5* (18)	78.5 (40)
N_3N_3	67.3 (48)	88.6* (108)	89.0 (38)	81.8 (28)
N_3N_9	86.4 (58)	90.2 (103)	97.7 (62)	101.7 (1)
N_9N_0	154.7 (135)	193.0 (99)	183.8* (125)	217.2 (129)
N_9N_3	184.5 (2)	155.9 (90)	159.7 (98)	216.3* (9)
N_9N_9	176.8 (131)	208.0 (89)	170.7* (30)	218.3* (49)

FRESH WEIGHT OF TREE PARTS AT HARVEST 2 (g)

* Trees with root galls; ** Tree numbers

Leaves

N Treatment	Replicate 1	2	3	4
N_1N_0	25.3 (126)**	19.1 (117)	24.4 (35)	30.8 (101)
N_1N_3	71.9 (122)	71.0 (83)	62.4 (67)	64.0 (60)
N_1N_9	146.0 (132)	126.8 (102)	138.9 (55)	122.1 (42)
N_3N_0	68.1 (109)	72.0 (127)	58.1* (18)	76.2 (40)
N_3N_3	120.6 (48)	92.3* (108)	120.9 (38)	100.0 (23)
N_3N_9	173.6 (58)	173.5 (103)	180.0 (62)	133.1 (1)
N_9N_0	146.4 (135)	125.0 (99)	154.8* (125)	157.9 (129)
N_9N_3	219.9 (2)	169.9 (90)	175.2 (98)	201.4* (9)
N_9N_9	274.8 (131)	241.8 (89)	205.7* (30)	256.3* (49)

Woody Tissue of New Shoots

N Treatment	Replicate 1	2	3	4
N_1N_0	1.8 (126)	0.1 (117)	2.0 (35)	1.2 (101)
N_1N_3	13.1 (122)	14.0 (83)	8.7 (67)	10.0 (60)
N_1N_9	39.1 (132)	38.3 (102)	36.0 (55)	30.8 (42)
N_3N_0	11.9 (109)	9.0 (127)	6.8* (18)	10.6 (40)
N_3N_3	24.2 (48)	14.5* (108)	24.0 (38)	13.9 (28)
N_3N_9	33.9 (58)	40.3 (103)	45.0 (62)	21.8 (1)
N_9N_0	25.6 (135)	17.9 (99)	25.4* (125)	17.1 (129)
N_9N_3	35.6 (2)	30.1 (90)	40.2 (98)	36.5* (9)
N_9N_9	58.4 (131)	36.3 (89)	29.5* (30)	43.9 (49)

FRESH WEIGHT OF TREE PARTS AT HARVEST 2 (g)

* Trees with root galls; ** Tree numbers

Whole Tree

N Treatment	Replicate 1	2	3	4
N_1N_0	274.6 (126)**	266.0 (117)	317.9 (35)	329.7 (101)
N_1N_3	450.8 (122)	502.8 (83)	425.1 (67)	436.2 (60)
N_1N_9	567.9 (132)	492.6 (102)	616.9 (55)	572.2 (42)
N_3N_0	386.1 (109)	520.2 (127)	512.4* (18)	536.3 (40)
N_3N_3	627.5 (48)	643.4* (108)	636.8 (38)	675.3 (28)
N_3N_9	698.1 (58)	700.9 (103)	753.7 (62)	673.1 (1)
N_9N_0	739.7 (135)	868.1 (99)	730.7* (125)	968.9 (129)
N_9N_3	998.8 (2)	654.9 (90)	891.2 (98)	963.5* (9)
N_9N_9	1027.1 (131)	905.2 (89)	928.4* (30)	1165.4* (49)

Mean Weight per Leaf

N Treatment	Replicate 1	2	3	4
N_1N_0	0.28 (126)	0.15 (117)	0.17 (35)	0.17 (101)
N_1N_3	0.32 (122)	0.33 (83)	0.27 (67)	0.27 (60)
N_1N_9	0.35 (132)	0.31 (102)	0.31 (55)	0.33 (42)
N_3N_0	0.31 (109)	0.24 (127)	0.28* (18)	0.27 (40)
N_3N_3	0.32 (48)	0.29* (108)	0.34 (38)	0.26 (23)
N_3N_9	0.38 (58)	0.34 (103)	0.33 (62)	0.27 (1)
N_9N_0	0.30 (135)	0.27 (99)	0.30* (125)	0.28 (129)
N_9N_3	0.34 (2)	0.35 (90)	0.31 (98)	0.33* (9)
N_9N_9	0.33 (131)	0.28 (89)	0.31* (30)	0.32* (49)

HARVEST 2 GROWTH DATA

* Trees with root galls; ** Tree numbers

Leaf No. per Tree (excluding abscissed leaves)

N Treatment	Replicate 1	2	3	4
N_1N_0	91 (126)**	127 (117)	140 (35)	186 (101)
N_1N_3	227 (122)	214 (83)	230 (67)	237 (60)
N_1N_9	419 (132)	410 (102)	446 (55)	373 (42)
N_3N_0	219 (109)	302 (127)	211* (18)	282 (40)
N_3N_3	373 (48)	318* (108)	353 (38)	385 (28)
N_3N_9	452 (58)	512 (103)	546 (62)	488 (1)
N_9N_0	495 (135)	457 (99)	517* (125)	566 (129)
N_9N_3	652 (2)	486 (90)	572 (98)	613* (9)
N_9N_9	839 (131)	863 (89)	656* (30)	809* (49)

No. Abscissed Leaves per Tree and % Leaf Abscission per Tree

N Treatment	Replicate and % Leaf Abscission 1	%	2	%	3	%	4	%
N_1N_0	7 (126)	7.1	7 (117)	5.2	21 (35)	13.0	11 (101)	5.6
N_1N_3	10 (122)	4.2	14 (83)	6.1	5 (67)	2.1	8 (60)	3.3
N_1N_9	24 (132)	5.4	7 (102)	1.7	17 (55)	3.7	12 (42)	3.1
N_3N_0	40 (109)	15.4	24 (127)	7.4	21* (18)	9.1	25 (40)	8.1
N_3N_3	27 (48)	6.8	28 (108)	8.1	32 (38)	8.3	26 (28)	6.3
N_3N_9	25 (58)	5.2	24 (103)	4.5	34 (62)	5.9	21 (1)	4.1
N_9N_0	106 (135)	17.6	62 (99)	11.9	64* (125)	11.0	101 (129)	15.1
N_9N_3	78 (2)	10.7	102 (90)	17.3	59 (98)	9.4	104* (9)	14.5
N_9N_9	108 (131)	11.4	102 (89)	10.6	51* (30)	7.2	101* (49)	11.1

DRY WEIGHT OF TREE PARTS AT HARVEST 3 (g)

* Trees with root galls; ** Tree numbers

Roots

N Treatment	Replicate 1	2	3	4
N_1N_0	58.1 (111)**	70.7 (107)	62.3 (56)	81.7 (96)
N_1N_3	109.7 (50)	106.4 (86)	122.5 (68)	139.6* (46)
N_1N_9	191.2 (26)	144.4* (112)	148.4 (51)	192.1* (66)
N_3N_0	109.8 (47)	114.7* (64)	116.4 (83)	147.7* (97)
N_3N_3	151.3* (120)	136.3 (79)	144.4 (34)	115.0 (22)
N_3N_9	210.9 (32)	193.7 (82)	206.3 (43)	217.8* (114)
N_9N_0	144.3 (71)	139.8 (128)	165.1 (118)	187.7* (12)
N_9N_3	209.9 (113)	237.4 (105)	232.4* (121)	245.7 (54)
N_9N_9	192.5 (39)	281.3* (27)	240.7 (57)	275.8* (91)

New Shoots

N Treatment	Replicate 1	2	3	4
N_1N_0	4.7 (111)	6.1 (107)	5.7 (56)	7.3 (96)
N_1N_3	37.4 (50)	42.3 (86)	38.1 (68)	43.4* (46)
N_1N_9	127.2 (26)	126.3* (112)	148.6 (51)	134.6* (66)
N_3N_0	14.5 (47)	17.7* (64)	15.5 (83)	24.4* (97)
N_3N_3	56.6* (120)	75.9 (79)	61.9 (34)	59.3 (22)
N_3N_9	139.7 (32)	136.4 (82)	137.3 (43)	139.7* (114)
N_9N_0	37.7 (71)	43.8 (128)	44.5 (118)	39.7* (12)
N_9N_3	101.0 (113)	82.2 (105)	90.1* (121)	89.4 (54)
N_9N_9	161.6 (39)	143.4* (27)	154.2 (57)	154.1* (91)

DRY WEIGHT OF TREE PARTS AT HARVEST 3 (g)

* Trees with root galls; ** Tree numbers

Abscissed Leaves

N Treatment	Replicate 1	Replicate 2	Replicate 3	Replicate 4
N_1N_0	4.9 (111)**	2.4 (107)	3.8 (56)	2.3 (96)
N_1N_3	2.3 (50)	2.3 (86)	2.0 (68)	1.6* (46)
N_1N_9	3.3 (26)	7.2 (112)	5.1 (51)	4.0* (66)
N_3N_0	17.2 (47)	10.5* (64)	13.1 (88)	12.8* (97)
N_3N_3	10.4* (120)	6.5 (79)	7.7 (34)	7.1 (22)
N_3N_9	6.1 (32)	6.7 (82)	5.4 (43)	11.5* (114)
N_9N_0	27.1 (71)	22.2 (128)	24.9 (118)	25.7* (12)
N_9N_3	14.9 (113)	20.6 (105)	18.4* (121)	19.8 (54)
N_9N_9	21.2 (39)	14.2* (27)	16.4 (57)	16.4* (91)

Top/Root Ratio

N Treatment	Replicate 1	Replicate 2	Replicate 3	Replicate 4
N_1N_0	0.45 (111)	0.58 (107)	0.71 (56)	0.65 (96)
N_1N_3	0.67 (50)	0.77 (86)	0.67 (68)	0.60* (46)
N_1N_9	1.02 (26)	1.27* (112)	1.48 (51)	1.00* (66)
N_3N_0	0.47 (47)	0.56* (64)	0.54 (88)	0.50* (97)
N_3N_3	0.68* (120)	0.87 (79)	0.90 (34)	0.97 (22)
N_3N_9	1.01 (32)	1.22 (82)	1.02 (43)	0.92* (114)
N_9N_0	0.97 (71)	1.09 (128)	1.03 (118)	0.80* (12)
N_9N_3	1.06 (113)	0.89 (105)	0.97* (121)	0.80 (54)
N_9N_9	1.43 (39)	0.99* (27)	1.08 (57)	1.04* (91)

DRY WEIGHT OF TREE PARTS AT HARVEST 3 (g)

* Trees with root galls; ** Tree numbers

Stock + Stem + 1 Year Old Shoots

N Treatment	Replicate 1	2	3	4
N_1N_0	21.2 (111)**	35.2 (107)	38.3 (56)	45.6 (96)
N_1N_3	35.8 (50)	39.7 (86)	44.1 (68)	40.6* (46)
N_1N_9	67.5 (26)	56.4* (112)	70.6 (51)	57.1* (66)
N_3N_0	36.6 (47)	47.0* (64)	47.3 (88)	49.9* (97)
N_3N_3	45.8* (120)	42.3 (79)	67.5 (34)	52.4 (22)
N_3N_9	72.9 (32)	99.9 (82)	73.3 (43)	69.1* (114)
N_9N_0	101.8 (71)	108.6 (128)	126.3 (118)	110.7* (12)
N_9N_3	121.5 (113)	129.7 (105)	134.2* (121)	107.2 (54)
N_9N_9	114.2 (39)	135.1* (27)	105.3 (57)	131.9* (91)

Whole Tree (Includes abscissed leaves)

N Treatment	Replicate 1	2	3	4
N_1N_0	88.9 (111)	114.4 (107)	110.1 (56)	136.9 (96)
N_1N_3	185.2 (50)	190.7 (86)	206.7 (68)	225.2* (46)
N_1N_9	389.2 (26)	334.3* (112)	372.7 (51)	387.8* (66)
N_3N_0	178.1 (47)	189.9* (64)	192.3 (83)	234.8* (97)
N_3N_3	264.1* (120)	261.0 (79)	281.5 (34)	233.8 (22)
N_3N_9	429.6 (32)	436.7 (82)	422.3 (43)	429.1* (114)
N_9N_0	310.9 (71)	314.4 (128)	360.8 (118)	364.8* (12)
N_9N_3	447.3 (113)	469.9 (105)	475.1* (121)	468.1 (54)
N_9N_9	489.5 (39)	574.0* (27)	516.6 (57)	578.2* (91)

FRESH WEIGHT OF TREE PARTS AT HARVEST 3 (g)

* Trees with root galls; ** Tree numbers

Roots

N Treatment	Replicate			
	1	2	3	4
N_1N_0	237.5 (111)**	247.8 (107)	214.9 (56)	265.5 (96)
N_1N_3	462.0 (50)	543.1 (86)	470.1 (68)	550.0* (46)
N_1N_9	719.1 (26)	536.1* (112)	530.7 (51)	611.1* (66)
N_3N_0	418.1 (47)	409.2* (64)	422.1 (88)	516.7* (97)
N_3N_3	524.3* (120)	516.1 (79)	490.1 (34)	407.1 (22)
N_3N_9	802.4 (32)	728.1 (82)	710.4 (43)	711.6* (114)
N_9N_0	508.0 (71)	415.0 (128)	646.9 (118)	670.9* (12)
N_9N_3	827.6 (113)	893.2 (105)	849.0* (121)	1015.0 (54)
N_9N_9	689.3 (39)	992.6* (27)	839.6 (57)	855.2* (91)

Stock + Stem + 1 Year Old Shoots

N Treatment	Replicate			
	1	2	3	4
N_1N_0	37.6 (111)	58.6 (107)	64.7 (56)	77.2 (96)
N_1N_3	63.9 (50)	71.7 (86)	77.6 (68)	70.9* (46)
N_1N_9	132.1 (26)	110.7* (112)	132.3 (51)	108.1* (66)
N_3N_0	64.8 (47)	81.6* (64)	83.6 (88)	86.0* (97)
N_3N_3	82.0* (120)	76.4 (79)	55.9 (34)	92.3 (22)
N_3N_9	132.4 (32)	184.7 (82)	134.6 (43)	122.9* (114)
N_9N_0	180.9 (71)	159.1 (128)	227.3 (118)	194.0* (12)
N_9N_3	222.4 (113)	233.0 (105)	239.7* (121)	203.0 (54)
N_9N_9	209.1 (39)	242.1* (27)	184.5 (57)	240.0* (91)

FRESH WEIGHT OF TREE PARTS AT HARVEST 3 (g)

* Trees with root galls; ** Tree numbers

Leaves

N Treatment	Replicate			
	1	2	3	4
N_1N_0	10.8 (111)**	16.9 (107)	13.1 (56)	18.1 (96)
N_1N_3	89.0 (50)	101.8 (86)	90.9 (68)	95.6* (46)
N_1N_9	216.9 (26)	214.7* (112)	253.2 (51)	244.0 (66)
N_3N_0	23.9 (47)	37.2* (64)	33.1 (88)	50.2* (97)
N_3N_3	114.1* (120)	150.0 (79)	127.3 (34)	123.6 (22)
N_3N_9	258.9 (32)	256.7 (82)	263.0 (43)	253.0* (114)
N_9N_0	75.8 (71)	90.0 (128)	90.1 (118)	80.3* (12)
N_9N_3	222.2 (113)	182.0 (105)	200.9* (121)	195.9 (54)
N_9N_9	299.8 (39)	297.0* (27)	301.8 (57)	305.0 (91)

Woody Tissue of New Shoots

N Treatment	Replicate			
	1	2	3	4
N_1N_0	3.2 (111)	1.6 (107)	2.7 (56)	2.0 (96)
N_1N_3	18.0 (50)	22.2 (86)	17.9 (68)	20.5* (46)
N_1N_9	101.5 (26)	98.2* (112)	110.9 (51)	95.8* (66)
N_3N_0	13.2 (47)	9.3* (64)	9.0 (88)	15.8* (97)
N_3N_3	34.9 (120)	58.9 (79)	35.1 (34)	36.4 (22)
N_3N_9	101.8 (32)	97.2 (82)	87.9 (43)	92.0* (114)
N_9N_0	21.9 (71)	26.6 (128)	33.2 (118)	27.0* (12)
N_9N_3	56.2 (113)	45.0 (105)	50.1* (121)	51.0 (54)
N_9N_9	113.1 (39)	84.0 (27)	99.7 (57)	89.0* (91)

FRESH WEIGHT OF TREE PARTS AT HARVEST 3 (g)

* Trees with root galls; ** Tree numbers

Whole Tree

N Treatment	Replicate 1	2	3	4
N_1N_0	239.1 (111)**	324.9 (107)	295.4 (56)	362.8 (96)
N_1N_3	632.9 (50)	738.8 (86)	656.5 (68)	737.0* (46)
N_1N_9	1169.6 (26)	959.7* (112)	1027.1 (51)	1059.0* (66)
N_3N_0	520.0 (47)	537.3* (64)	547.8 (88)	668.7* (97)
N_3N_3	755.3* (120)	801.4 (79)	708.4 (34)	659.4 (22)
N_3N_9	1295.5 (32)	1266.7 (82)	1195.9 (43)	1179.5* (114)
N_9N_0	786.6 (71)	720.7 (128)	997.5 (118)	972.2* (12)
N_9N_3	1328.4 (113)	1353.2 (105)	1339.7* (121)	1464.9 (54)
N_9N_9	1311.3 (39)	1615.7* (27)	1425.6 (57)	1489.2* (91)

Mean Weight per Leaf

N Treatment	Replicate 1	2	3	4
N_1N_0	0.32 (111)	0.46 (107)	0.32 (56)	0.21 (96)
N_1N_3	0.50 (50)	0.48 (86)	0.40 (68)	0.43* (46)
N_1N_9	0.54 (26)	0.54* (112)	0.61 (51)	0.61* (66)
N_3N_0	0.54 (47)	0.46* (64)	0.46 (88)	0.49* (97)
N_3N_3	0.51* (120)	0.60 (79)	0.51 (34)	0.61 (22)
N_3N_9	0.53 (32)	0.52 (82)	0.56 (43)	0.59* (114)
N_9N_0	0.44 (71)	0.50 (128)	0.42 (118)	0.45* (12)
N_9N_3	0.50 (113)	0.51 (105)	0.44* (121)	0.51 (54)
N_9N_9	0.48 (39)	0.47* (27)	0.57 (57)	0.52* (91)

HARVEST 3 GROWTH DATA

* Trees with root galls; ** Tree numbers

Leaf No. per Tree (excluding abscissed leaves)

N Treatment	Replicate			
	1	2	3	4
N_1N_0	34 (111)**	37 (107)	41 (56)	85 (96)
N_1N_3	179 (50)	214 (86)	230 (68)	221* (46)
N_1N_9	402 (26)	396* (112)	415 (51)	400* (66)
N_3N_0	44 (47)	81* (64)	72 (88)	103* (97)
N_3N_3	225* (120)	252 (79)	250 (34)	204 (22)
N_3N_9	492 (32)	489 (82)	469 (43)	431* (114)
N_9N_0	174 (71)	180 (128)	216 (118)	278* (12)
N_9N_3	442 (113)	360 (105)	456* (121)	398 (54)
N_9N_9	621 (39)	628* (27)	529 (57)	582* (91)

No. Abscissed Leaves per Tree and % Leaf Abscission per Tree

N Treatment	Replicate							
	1	%	2	%	3	%	4	%
N_1N_0	88 (111)	72.1	75 (107)	67.0	97 (56)	70.3	107 (96)	55.7
N_1N_3	53 (50)	22.8	56 (86)	20.7	69 (68)	23.1	64* (46)	22.5
N_1N_9	83 (26)	17.1	128* (112)	24.4	98 (51)	19.1	76* (66)	16.0
N_3N_0	204 (47)	82.3	177* (64)	68.6	210 (88)	74.5	173* (97)	62.7
N_3N_3	266* (120)	54.2	146 (79)	36.7	139 (34)	35.7	136 (22)	40.0
N_3N_9	99 (32)	16.8	125 (82)	20.4	128 (43)	21.4	177* (114)	29.1
N_9N_0	206 (71)	54.2	276 (128)	60.5	292 (118)	57.5	406* (12)	69.5
N_9N_3	297 (113)	40.2	333 (105)	48.1	344* (121)	43.0	371 (54)	48.2
N_9N_9	365 (39)	37.0	280* (27)	30.8	268 (57)	33.6	259* (91)	30.8

SEASONAL CHANGES IN THE TOTAL LENGTH OF NEW SHOOTS PER TREE DURING THE SECOND GROWING SEASON

cm

* Trees with root galls

N Treatment	Tree No.	Harvest No.	Date					
			1964		1965			
			10/10	7/11	5/12	1/1	30/1	3/3
N1N0	126	2	5.5	19.9				
	117	2	0	0				
	35	2	15.9	24.2				
	101	2	0	5.3				
	111	3	29.2	48.5	48.5	48.5	48.5	48.5
	107	3	1.4	7.4	7.4	7.7	7.7	7.9
	56	3	10.4	24.1	26.8	27.0	26.9	28.0
	96	3	0	3.5	6.4	7.4	7.4	7.4
N1N3	122	2	23.8	154.8				
	83	2	42.8	155.2				
	67	2	5.5	90.0				
	60	2	6.5	91.3				
	50	3	14.0	104.2	137.2	141.7	140.7	141.8
	86	3	0	69.2	175.3	191.5	190.2	191.2
	68	3	6.3	93.8	134.4	139.1	139.8	141.6
	46*	3	10.9	91.0	144.1	156.7	156.2	156.8
N1N9	132	2	76.2	330.3				
	102	2	7.1	175.7				
	55	2	17.2	280.9				
	42	2	32.1	173.2				
	26	3	32.4	281.0	638.2	721.1	727.7	723.9
	112*	3	17.2	227.1	572.2	622.8	626.7	627.7
	51	3	86.5	425.9	673.1	718.0	719.4	721.3
	66*	3	37.5	256.0	538.3	583.4	581.8	583.5

TOTAL LENGTH OF NEW SHOOTS PER TREE (Contd.)

cm

* Trees with root galls

N Treatment	Tree No.	Harvest No.	Date					
				1964		1965		
			10/10	7/11	5/12	1/1	30/1	3/3
N3N0	109	2	18.0	79.7				
	127	2	33.9	107.5				
	18*	2	72.4	95.4				
	40	2	69.1	115.8				
	47	3	78.2	147.1	166.6	169.3	169.8	168.8
	64*	3	25.4	93.9	106.6	108.3	108.3	107.8
	88	3	63.8	97.5	112.7	115.4	114.9	117.3
	97*	3	55.0	159.7	181.5	183.0	183.6	183.5
N3N3	48	2	41.4	229.9				
	108*	2	71.9	194.8				
	38	2	130.7	310.6				
	28	2	81.3	175.1				
	120*	3	93.7	291.0	353.5	355.8	357.0	356.3
	79	3	54.9	289.8	417.0	424.8	425.5	424.8
	34	3	38.6	217.0	308.0	313.7	316.3	313.2
	22	3	5.6	79.1	198.6	237.8	235.5	238.4
N3N9	58	2	62.1	332.0				
	103	2	75.6	327.6				
	62	2	80.9	373.1				
	1	2	0	93.2				
	32	3	99.1	147.0	703.9	754.9	755.1	755.6
	82	3	155.6	421.6	721.8	766.8	767.9	766.8
	43	3	77.4	289.9	563.3	620.7	623.1	627.9
	114*	3	160.4	499.0	686.0	762.3	762.9	763.4

TOTAL LENGTH OF NEW SHOOTS PER TREE (Contd.)

cm

* Trees with root galls

N Treatment	Tree No.	Harvest No.	Date					
			1964			1965		
			10/10	7/11	5/12	1/1	30/1	3/3
N9N0	135	2	26.9	278.2				
	99	2	19.2	154.8				
	125*	2	84.4	311.1				
	129	2	63.7	172.9				
	71	3	40.2	148.1	197.1	197.7	200.3	200.0
	128	3	54.3	195.6	254.5	259.1	258.1	258.1
	118	3	132.1	340.1	356.8	357.3	355.2	359.3
	12*	3	48.0	192.9	278.1	281.6	280.9	277.9
N9N3	2	2	114.6	362.3				
	90	2	95.8	282.4				
	98	2	93.4	482.1				
	9*	2	82.8	435.1				
	113	3	120.8	395.8	547.7	564.1	564.7	560.9
	105	3	114.6	391.7	483.2	490.0	493.3	493.3
	121*	3	106.6	351.7	467.4	488.4	486.0	486.0
	54	3	115.7	336.6	454.5	488.8	487.7	488.8
N9N9	131	2	151.8	620.9				
	89	2	22.0	306.6				
	30*	2	62.7	294.2				
	49*	2	176.1	452.8				
	39	3	124.6	665.9	988.6	1034.6	1032.5	1031.1
	27*	3	148.6	526.1	761.5	802.7	813.1	811.4
	57	3	123.7	549.9	825.3	873.4	872.3	874.2
	91*	3	238.6	611.7	827.0	847.4	848.2	850.9

SEASONAL CHANGES IN STEM DIAMETER PER TREE DURING THE SECOND GROWING SEASON

(10x) in.

* Trees with root galls

N Treatment	Tree No.	Harvest No.	Date						
				1964				1965	
			7/9	24/10	21/11	19/12	16/1	13/2	28/2
N1N0	126	2	2.41	2.41	2.52				
	117	2	2.31	2.27	2.29				
	35	2	2.47	2.53	2.63				
	101	2	2.34	2.43	2.52				
	111	3	2.38	2.38	2.44	2.47	2.63	2.60	2.59
	107	3	2.55	2.62	2.62	2.80	2.80	2.82	2.82
	56	3	2.72	2.72	2.70	2.76	2.82	2.88	2.88
	96	3	2.39	2.44	2.41	2.51	2.52	2.56	2.57
N1N3	122	2	2.57	2.78	2.89				
	83	2	2.59	2.70	2.83				
	67	2	2.64	2.64	2.82				
	60	2	2.51	2.62	2.82				
	50	3	2.10	2.10	2.27	2.47	2.63	2.74	2.77
	86	3	2.43	2.47	2.62	2.92	3.12	3.24	3.25
	68	3	2.27	2.30	2.54	2.86	2.89	3.04	3.09
	46*	3	2.68	2.68	2.89	3.13	3.28	3.35	3.47
N1N9	132	2	2.48	2.71	3.10				
	102	2	2.27	2.29	2.65				
	55	2	2.75	3.00	3.36				
	42	2	2.56	2.73	2.98				
	26	3	2.34	2.56	3.12	4.18	4.94	5.14	5.24
	112*	3	2.61	2.89	3.24	4.13	4.76	5.04	5.07
	51	3	2.27	2.66	3.25	4.39	4.65	5.27	5.42
	66*	3	2.40	2.69	3.39	4.50	4.85	5.21	5.40

STEM DIAMETER PER TREE (Contd.)

(10x) in.

* Trees with root galls

N Treatment	Tree No.	Harvest No.	Date						
			1964				1965		
			7/9	24/10	21/11	19/12	16/1	13/2	28/2
N3N0	109	2	3.45	3.48	3.56				
	127	2	3.54	3.62	3.78				
	18*	2	3.00	3.00	3.22				
	40	2	3.40	3.63	3.63				
	47	3	3.55	3.73	3.73	3.73	3.87	3.90	3.90
	64*	3	2.57	2.57	2.66	2.63	2.76	2.76	2.76
	88	3	3.09	3.24	3.32	3.40	3.45	3.47	3.50
	97*	3	3.08	3.25	3.46	3.51	3.53	3.53	3.52
N3N3	48	2	3.14	3.14	3.64				
	108*	2	3.50	3.62	3.59				
	38	2	3.25	3.55	3.60				
	28	2	2.95	3.06	3.18				
	120*	3	3.15	3.42	3.63	3.75	3.97	4.06	4.07
	79	3	2.79	3.25	3.37	3.98	3.98	3.99	3.99
	34	3	3.52	3.66	3.84	4.23	4.47	4.52	4.51
	22	3	2.93	2.94	3.18	3.50	3.81	3.97	4.04
N3N9	58	2	2.49	2.61	2.99				
	103	2	3.50	4.02	4.36				
	62	2	3.41	3.75	3.87				
	1	2	3.50	3.61	3.86				
	32	3	3.45	3.96	4.36	5.09	5.47	5.53	5.55
	82	3	4.14	4.32	4.53	5.08	5.67	5.71	5.71
	43	3	3.43	3.65	4.15	4.84	5.00	5.33	5.34
	114*	3	3.59	4.14	4.29	4.86	5.28	5.37	5.54

STEM DIAMETER PER TREE (Contd.)

(10x) in.

* Trees with root galls

N Treatment	Tree No.	Harvest No.	Date						
			1964				1965		
			7/9	24/10	21/11	19/12	16/1	13/2	28/2
N9N0	135	2	4.86	5.30	5.31				
	99	2	5.10	5.16	5.18				
	125*	2	4.98	5.00	5.16				
	129	2	5.42	5.51	5.60				
	71	3	3.00	5.00	5.19	5.19	5.19	5.21	5.20
	128	3	5.13	5.19	5.19	5.37	5.39	5.59	5.59
	118	3	5.55	5.53	5.53	5.61	5.63	5.75	5.73
	12*	3	5.04	5.19	5.22	5.37	5.35	5.40	5.45
N9N3	2	2	5.46	5.64	5.71				
	90	2	4.28	4.39	4.56				
	98	2	4.71	4.96	5.21				
	9*	2	5.48	5.58	5.81				
	113	3	5.12	5.28	5.71	5.61	5.78	5.77	5.76
	105	3	5.15	5.35	5.50	5.58	5.79	5.78	5.78
	121*	3	5.41	5.44	5.52	5.63	5.70	5.86	5.88
	54	3	5.19	5.40	5.39	5.67	5.70	5.76	5.79
N9N9	131	2	5.54	5.61	5.91				
	89	2	5.02	5.25	5.51				
	30*	2	5.03	5.08	5.13				
	49*	2	5.04	5.20	5.62				
	39	3	5.20	5.45	5.68	5.99	6.16	6.32	6.35
	27*	3	4.94	5.18	5.48	5.72	5.87	5.93	5.94
	57	3	5.05	5.05	5.23	5.27	5.55	5.65	5.66
	91*	3	5.04	5.28	5.55	5.70	5.78	5.80	5.80

SEASONAL CHANGES IN THE TOTAL NO. NEW SHOOTS PER TREE DURING THE SECOND GROWING SEASON

* Trees with root galls

N Treatment	Tree No.	Harvest No.	Date					
			1964			1965		
			10/10	7/11	5/12	1/1	30/1	3/3
N1N0	126	2	3	4				
	117	2	0	0				
	35	2	7	8				
	101	2	0	3				
	111	3	10	10	10	10	10	10
	107	3	1	3	3	3	3	3
	56	3	5	9	11	11	11	11
	96	3	0	2	5	5	5	5
N1N3	122	2	8	14				
	83	2	9	14				
	67	2	2	11				
	60	2	3	12				
	50	3	4	11	11	11	11	11
	86	3	0	9	12	12	12	12
	68	3	3	9	12	12	12	12
	46*	3	3	13	18	20	20	20
N1N9	132	2	16	21				
	102	2	3	15				
	55	2	6	22				
	42	2	10	17				
	26	3	11	16	27	27	27	27
	112*	3	9	19	22	22	22	22
	51	3	15	20	26	27	27	27
	66*	3	11	21	21	22	22	22

TOTAL NO. NEW SHOOTS PER TREE (Contd.)

*Trees with root galls

N Treatment	Tree No.	Harvest No.	Date					
				1964			1965	
			10/10	7/11	5/12	1/1	30/1	3/3
N3N0	109	2	6	11				
	127	2	7	15				
	18*	2	15	17				
	40	2	17	20				
	47	3	16	16	19	19	19	19
	64*	3	9	19	20	20	20	20
	88	3	17	19	20	20	20	22
	97*	3	12	18	18	18	18	18
N3N3	48	2	13	26				
	108*	2	11	19				
	38	2	21	23				
	28	2	25	31				
	120*	3	16	22	23	24	24	24
	79	3	10	17	17	17	17	17
	34	3	12	27	27	28	28	28
	22	3	3	18	19	20	20	20
N3N9	58	2	19	31				
	103	2	21	32				
	62	2	20	36				
	1	2	0	13				
	32	3	17	17	33	34	34	34
	82	3	26	30	33	33	33	33
	43	3	22	31	36	37	37	37
	114*	3	26	30	35	36	36	36

TOTAL NO. NEW SHOOTS PER TREE (Contd.)

* Trees with root galls

N Treatment	Tree No.	Harvest No.	Date					
			1964			1965		
			10/10	7/11	5/12	1/1	30/1	3/3
N9N0	135	2	13	37				
	99	2	9	23				
	125*	2	17	37				
	129	2	24	41				
	71	3	18	37	37	38	38	38
	128	3	19	28	32	32	32	32
	118	3	41	49	49	49	49	49
	12*	3	19	26	34	38	38	38
N9N3	2	2	29	48				
	90	2	25	39				
	98	2	16	33				
	9*	2	32	47				
	113	3	35	49	53	53	53	53
	105	3	29	49	52	53	53	53
	121*	3	40	56	60	61	61	61
	54	3	33	48	49	53	53	53
N9N9	131	2	38	58				
	89	2	13	56				
	30*	2	26	41				
	49*	2	47	55				
	39	3	32	54	57	59	59	59
	27*	3	35	50	55	56	56	56
	57	3	25	43	44	46	46	46
	91*	3	44	55	58	60	60	60

SEASONAL CHANGES IN THE AVERAGE LENGTH OF NEW SHOOTS PER TREE DURING THE SECOND GROWING SEASON

cm

* Trees with root galls

N Treatment	Tree No.	Harvest No.	Date					
			1964			1965		
			10/10	7/11	5/12	1/1	30/1	3/3
N1N0	126	2	1.8	5.0				
	117	2	1.0	1.0				
	35	2	2.3	3.0				
	101	2	1.0	1.8				
	111	3	2.9	4.9	4.9	4.9	4.9	4.9
	107	3	1.4	2.5	2.5	2.6	2.6	2.6
	56	3	2.1	2.7	2.4	2.4	2.4	2.5
	96	3	1.8	1.3	1.5	1.5	1.5	1.5
N1N3	122	2	3.0	11.1				
	83	2	4.8	11.1				
	67	2	2.8	8.2				
	60	2	2.2	7.6				
	50	3	3.5	9.5	12.5	12.9	12.8	12.9
	86	3	1.0	7.7	14.6	16.0	15.9	15.9
	68	3	2.1	10.4	11.2	11.6	11.7	11.8
	46*	3	3.6	7.0	8.0	7.9	7.8	7.8
N1N9	132	2	4.8	15.7				
	102	2	2.4	11.7				
	55	2	2.9	12.8				
	42	2	3.2	10.2				
	26	3	2.9	17.6	23.6	26.7	27.0	26.8
	112*	3	1.9	12.0	26.0	28.3	28.5	28.5
	51	3	5.8	21.3	25.9	26.6	26.6	26.7
	66*	3	3.4	12.2	25.6	26.5	26.4	26.5

AVERAGE LENGTH OF NEW SHOOTS PER TREE (Contd.)

cm

* Trees with root galls

N Treatment	Tree No.	Harvest No.	Date					
			1964			1965		
			10/10	7/11	5/12	1/1	30/1	3/3
N3N0	109	2	3.0	7.2				
	127	2	4.8	7.2				
	18*	2	4.8	5.6				
	40	2	4.1	5.8				
	47	3	4.9	9.2	8.8	8.9	8.9	8.9
	64*	3	2.8	4.9	5.3	5.4	5.4	5.4
	88	3	3.8	5.1	5.6	5.8	5.7	5.3
	97*	3	4.6	8.9	10.1	10.2	10.2	10.2
N3N3	48	2	3.2	8.8				
	108*	2	6.5	10.3				
	38	2	6.2	13.5				
	28	2	3.3	5.6				
	120*	3	5.9	13.2	15.4	14.8	14.9	14.8
	79	3	5.5	17.0	24.5	25.0	25.0	25.0
	34	3	3.2	8.0	11.4	11.2	11.3	11.2
	22	3	1.9	4.4	10.5	11.9	11.8	11.9
N3N9	58	2	3.3	10.7				
	103	2	3.6	10.2				
	62	2	4.0	10.4				
	1	2	1.0	7.2				
	32	3	5.8	8.6	21.3	22.2	22.2	22.2
	82	3	6.0	14.1	21.9	23.2	23.3	23.2
	43	3	3.5	9.4	15.6	16.8	16.8	17.0
	114*	3	6.2	16.6	19.6	21.2	21.2	21.2

AVERAGE LENGTH OF NEW SHOOTS PER TREE (Contd.)

cm

* Trees with root galls

N Treatment	Tree No.	Harvest No.	Date					
			1964			1965		
			10/10	7/11	5/12	1/1	30/1	3/3
N9N0	135	2	2.1	7.5				
	99	2	2.1	6.7				
	125*	2	5.0	8.4				
	129	2	2.7	4.2				
	71	3	2.2	4.0	5.3	5.2	5.3	5.3
	128	3	2.9	7.0	8.0	8.1	8.1	8.1
	118	3	3.2	6.9	7.3	7.3	7.2	7.3
	12*	3	2.5	7.4	8.2	7.4	7.4	7.3
N9N3	2	2	4.0	7.5				
	90	2	3.8	7.2				
	98	2	5.8	14.6				
	9*	2	2.6	9.3				
	113	3	3.5	8.1	10.3	10.6	10.6	10.6
	105	3	4.0	8.0	9.3	9.2	9.3	9.3
	121*	3	2.7	6.3	7.8	8.0	8.0	8.0
	54	3	3.5	7.0	9.3	9.2	9.2	9.2
N9N9	131	2	4.0	10.7				
	89	2	1.7	5.5				
	30*	2	2.4	7.2				
	49*	2	3.7	8.2				
	39	3	3.9	12.3	17.3	17.5	17.5	17.5
	27*	3	4.2	10.5	13.8	14.3	14.5	14.5
	57	3	4.9	12.8	18.8	19.0	19.0	19.0
	91*	3	5.4	11.1	14.3	14.1	14.1	14.2

CONCENTRATION AND AMOUNT OF TOTAL N IN TREE TISSUES AT HARVEST 1

* Trees with root galls
** Tissue from all replicates bulked before analysis

Tree No.	N Treat-ment	Rep. No.	Concn. in Roots (mgN/g d.wt)	Concn. in Stock + Stem + Side Shoots (mgN/g d.wt)	Concn. in Buds ** (mgN/g d.wt)	Amount in Roots (mgN)	Amount in Stock + Stem + Side Shoots (mgN)	Amount in Buds (mgN)	Amount in Tree (mgN)
61	N1	1	7.783	6.570	10.253	460.3	143.9	5.7	609.9
134		"	9.167	7.553		483.0	126.9	3.2	613.1
84		"	8.334	6.148		470.3	135.9	4.4	610.6
11		2	8.250	6.280		502.3	144.4	4.6	651.3
70		"	6.698	6.310		581.2	186.1	7.0	774.3
15		"	7.191	5.762		487.6	170.6	6.1	664.3
20		3	6.646	5.421		564.2	150.7	6.8	721.7
81		"	8.065	6.858		455.1	135.1	3.7	593.9
116		"	8.799	6.380		486.8	112.3	4.8	603.9
65		4	5.939	5.823		499.7	206.7	7.7	714.1
100*		"	6.220	4.840		575.4	148.1	6.6	730.1
110		"	6.621	5.551		570.2	188.7	8.3	767.2
63	N3	1	10.799	9.733	13.049	985.7	361.1	8.9	1355.7
130		"	10.154	9.588		913.9	352.8	6.8	1273.5
133		"	12.271	10.051		1142.8	279.4	7.6	1429.8
24		2	10.230	10.952		989.8	341.7	7.2	1338.7
87*		"	12.344	10.371		973.1	408.6	11.2	1392.9
52		"	9.259	8.424		1039.0	409.4	16.8	1465.2
13		3	8.900	7.969		891.4	404.8	12.3	1308.5
72		"	9.545	9.504		989.7	363.1	7.3	1360.1
85		"	10.153	9.417		1013.4	361.6	17.5	1392.5
45		4	9.133	8.679		1057.4	406.2	12.9	1476.2
74*		"	7.846	7.914		1081.5	338.7	13.1	1433.3
69		"	9.764	9.660		1013.9	336.2	17.6	1367.7
77	N9	1	10.915	12.820	15.866	1843.0	[illegible]	28.7	3133.2
80		"	12.308	10.519		2474.0	963.5	26.0	3463.5
78		"	13.897	11.283		2109.4	971.5	27.5	3108.4
23		2	11.465	9.647		2000.8	1115.2	30.6	3146.6
106		"	14.251	13.261		2058.2	984.0	33.2	3075.4
95*		"	12.668	11.403		1767.9	940.7	19.4	2728.0
36		3	14.501	11.576		1941.1	909.9	20.6	2871.6
115*		"	14.235	11.932		2232.8	970.1	30.7	3233.7
94		"	12.164	11.359		1726.6	873.5	22.5	2622.6
16		4	11.725	10.306		2086.6	984.2	36.5	3107.5
92		"	15.198	12.243		2340.6	961.1	37.9	3339.6
21		"	12.348	10.450		2153.3	1018.9	31.3	3203.5

CONCENTRATION AND AMOUNT OF SOLUBLE N IN TREE TISSUES AT HARVEST 1

* Trees with root galls
** Tissue from all replicates bulked before analysis

Tree No.	N Treatment	Rep. No.	Concn. in Roots (mgN/g d.wt)	Concn. in Stock + Stem + Side Shoots (mgN/g d.wt)	Concn. in Buds** (mgN/g d.wt)	Amount in Roots (mgN)	Amount in Stock + Stem + Side Shoots (mgN)	Amount in New Shoots (mgN)	Amount in Tree (mgN)
61	N1	1	2.817	1.751	1.669	163.1	38.4	0.9	202.4
134		"	3.173	2.790		167.5	46.9	0.5	214.9
84		"	2.738	1.536		153.1	35.1	0.7	188.9
11		2	2.454	1.490		146.7	34.2	0.7	181.6
70		"	1.698	1.445		144.6	42.6	1.2	188.4
15		"	2.070	1.297		138.3	38.4	1.0	177.7
20		3	1.861	1.313		156.1	36.5	1.1	193.7
81		"	2.877	1.533		160.5	30.2	0.6	191.3
116		"	3.145	1.915		174.9	33.7	0.8	209.4
65		4	1.792	1.230		140.7	43.6	1.3	185.6
100*		"	1.550	0.625		131.6	19.1	1.1	151.8
110		"	1.434	1.102		125.7	37.4	1.3	164.4
63	N3	1	4.802	4.482	4.015	436.0	166.3	2.8	605.1
130		"	4.379	4.164		380.5	153.2	2.1	535.8
133		"	6.623	4.364		615.3	121.3	2.4	739.0
24		2	5.045	5.041		485.3	157.3	2.2	644.8
87*		"	5.770	4.644		447.2	183.0	3.4	633.6
52		"	3.774	2.992		418.9	145.4	5.1	569.4
13		3	3.374	2.773		332.0	140.8	3.8	476.6
72		"	3.967	3.669		403.8	140.2	2.2	546.2
85		"	4.413	3.905		434.7	149.9	5.4	590.0
45		4	3.467	3.202		388.0	149.9	4.0	541.9
74*		"	3.038	2.939		387.3	125.8	4.1	517.2
69		"	3.241	4.467		320.9	155.5	5.4	481.8
77	N9	1	7.272	5.618	5.525	1209.3	552.8	10.0	1772.1
80		"	6.734	4.929		1342.6	451.5	9.0	1803.1
78		"	8.131	5.675		1231.0	488.7	9.6	1729.3
23		2	5.195	4.027		880.6	465.5	10.6	1356.7
106		"	8.509	6.527		1221.0	484.3	11.6	1716.9
95*		"	6.388	5.377		945.0	443.6	6.8	1396.1
36		3	8.442	5.776		1117.7	454.0	7.2	1578.9
115*		"	8.874	5.873		1418.1	477.5	10.6	1921.4
94		"	6.856	5.383		955.7	413.9	7.8	1377.4
16		4	6.441	5.040		1131.0	481.3	12.7	1625.0
92		"	9.348	6.368		1423.7	499.9	13.2	1936.8
21		"	6.932	4.949		1197.8	480.6	10.9	1689.3

CONCENTRATION AND AMOUNT OF INSOLUBLE N IN TREE TISSUES AT HARVEST 1

* Trees with root galls
** Tissue from all replicates bulked before analysis

Tree No.	N Treat-ment	Rep. No.	Concn. in Roots (mgN/g d.wt)	Concn. in Stock + Stem + Side Shoots (mgN/g d.wt)	Concn. in Buds** (mgN/g d.wt)	Amount in Roots (mgN)	Amount in Stock + Stem + Side Shoots (mgN)	Amount in Buds (mgN)	Amount in Tree (mgN)
61	N1	1	4.966	4.819	8.584	297.2	105.5	4.8	407.5
134		"	5.994	4.763		315.5	80.0	2.7	398.2
84		"	5.646	4.562		317.2	100.8	3.7	421.7
11		2	5.796	4.790		355.6	110.2	3.9	469.7
70		"	5.000	4.865		436.6	143.5	5.8	585.9
15		"	5.121	4.465		349.3	132.2	5.1	486.6
20		3	4.785	4.108		408.1	114.2	5.7	528.0
31		"	5.188	5.325		294.6	104.9	3.1	402.6
116		"	5.654	4.465		311.9	78.6	4.0	394.5
65		4	4.147	4.593		359.0	163.1	6.4	528.5
100*		"	4.670	4.215		443.8	129.0	5.5	578.3
110		"	5.137	4.449		444.5	151.3	7.0	602.8
63	N3	1	5.997	5.251	9.934	549.7	194.8	6.1	750.6
130		"	5.775	5.424		533.4	199.6	4.7	737.7
133		"	5.648	5.687		527.5	158.1	5.2	690.8
24		2	5.185	5.911		504.5	184.4	5.0	693.9
87*		"	6.574	5.727		525.9	225.6	7.8	759.3
52		"	5.485	5.432		620.1	264.0	11.7	895.8
13		3	5.526	5.196		559.4	264.0	8.5	831.9
72		"	5.578	5.835		585.9	222.9	5.1	813.9
85		"	5.740	5.512		578.7	211.7	12.1	802.5
45		4	5.666	5.477		669.1	256.3	8.9	934.3
74*		"	4.808	4.975		694.2	212.9	9.0	916.1
69		"	6.523	5.193		693.0	180.7	12.2	885.9
77	N9	1	5.643	7.202	10.344	633.7	708.7	18.7	1316.1
80		"	5.524	5.590		1131.4	512.0	17.0	1660.4
78		"	5.766	5.608		878.4	482.8	17.9	1379.1
23		2	6.270	5.628		1120.2	649.7	20.0	1789.9
106		"	5.742	6.734		837.2	499.7	21.6	1358.5
95*		"	5.780	6.026		822.2	497.1	12.6	1331.9
36		3	6.059	5.800		823.4	455.9	13.4	1292.7
115*		"	5.361	6.059		814.7	492.6	20.1	1312.3
94		"	5.308	5.976		770.9	459.6	14.7	1245.2
16		4	5.284	5.266		955.8	502.9	23.8	1482.5
92		"	5.850	5.875		916.9	461.2	24.7	1441.5
21		"	5.416	5.521		955.5	538.3	20.4	1514.2

CONCENTRATION AND AMOUNT OF ARGININE N IN TREE TISSUES AT HARVEST 1

*Trees with root galls
**Tissue from all replicates bulked before analysis

Tree No.	N Treat-ment	Rep. No.	Concn. in Roots (mgN/g d.wt)	Concn. in Stock + Stem + Side Shoots (mgN/g d.wt)	Concn. in Buds** (mgN/g d.wt)	Amount in Roots (mgN)	Amount In Stock + Stem + Side Shoots (mgN)	Amount in Buds (mgN)	Amount in Tree (mgN)
61	N1	1	2.550	1.635	1.014	147.6	35.8	0.6	184.0
134		"	2.619	2.275		138.3	38.2	0.3	176.8
84		"	2.106	1.187		117.7	26.2	0.4	144.3
11		2	2.168	1.028		129.6	23.6	0.5	153.7
70		"	1.332	0.798		113.5	23.5	0.7	137.7
15		"	1.677	0.853		112.0	25.2	0.6	137.8
20		3	1.594	0.892		133.7	24.8	0.7	159.2
81		"	2.038	1.186		113.7	23.4	0.4	137.5
116		"	2.795	1.555		155.4	27.4	0.5	183.3
65		4	1.268	0.624		99.5	22.2	0.8	122.5
100*		"	0.837	0.121		71.1	3.7	0.6	75.4
110		"	1.034	0.609		87.6	20.7	0.8	109.1
63	N3	1	4.363	3.631	3.332	396.2	134.7	2.3	533.2
130		"	3.631	3.291		315.5	121.1	1.7	438.3
133		"	5.977	3.823		555.3	106.3	1.9	663.5
24		2	4.501	4.249		433.0	132.6	1.8	567.4
87*		"	4.797	4.119		371.8	162.3	2.9	537.0
52		"	3.057	2.109		339.3	102.5	4.3	446.1
13		3	2.641	1.620		259.9	82.3	3.1	345.3
72		"	3.430	2.173		354.3	83.0	1.9	439.2
85		"	3.955	3.464		389.6	133.0	4.5	527.1
45		4	2.616	2.227		292.7	104.2	3.3	400.2
74*		"	2.169	1.986		276.5	85.0	3.3	364.8
69		"	1.905	3.572		188.6	124.3	4.5	317.4
77	N9	1	7.489	5.495	4.586	1245.4	540.7	8.3	1794.4
80		"	6.316	3.463		1249.9	317.2	7.5	1574.6
78		"	7.928	5.598		1200.3	482.0	7.9	1690.2
23		2	4.716	3.799		799.4	439.2	8.9	1247.5
106		"	9.185	6.890		1318.0	511.2	9.6	1838.8
95*		"	6.387	4.786		876.9	394.8	5.6	1277.3
36		3	7.360	5.589		974.5	439.3	6.0	1419.8
115*		"	9.351	5.982		1494.3	486.3	8.9	1989.5
94		"	6.502	5.311		906.4	408.4	6.5	1321.3
16		4	6.454	4.522		1133.3	431.9	10.5	1575.7
92		"	7.336	5.955		1117.3	467.5	11.0	1595.8
21		"	5.711	3.947		986.9	384.8	9.0	1380.7

CONCENTRATION AND AMOUNT OF TOTAL α-AMINO N IN TREE TISSUES AT HARVEST 1

* Trees with root galls
** Tissue from all replicates bulked before analysis

Tree No.	N Treat- ment	Rep. No.	Concn. in Roots (mgN/g d.wt)	Concn. in Stock + Stem + Side Shoots (mgN/g d.wt)	Concn. in Buds ** (mgN/g d.wt)	Amount in Roots (mgN)	Amount in Stock + Stem + Side Shoots (mgN)	Amount in Buds (mgN)	Amount in Tree (mgN)
61	N1	1	0.821	0.329	0.552	47.5	7.2	0.3	55.0
134		"	1.111	0.761		58.7	12.8	0.2	71.7
84		"	0.896	0.388		50.1	8.6	0.2	58.9
11		2	0.790	0.431		47.2	9.9	0.2	57.3
70		"	0,534	0.356		45.5	10.5	0.4	56.4
15		"	0.684	0.339		45.7	10.0	0.3	56.0
20		3	0.563	0.320		47.2	8.9	0.4	56.5
81		"	0.989	0.466		55.2	9.2	0.2	64.6
116		"	1.016	0.550		56.5	9.7	0.3	66.5
65		4	0.493	0.261		38.7	9.3	0.4	48.4
100*		"	0.479	0.133		40.7	4.1	0.4	45.2
110		"	0.413	0.268		35.0	9.1	0.4	44.5
63	N3	1	1.627	1.174	1.244	147.7	43.6	0.8	192.1
130		"	1.347	1.086		117.1	40.0	0.6	157.7
133		"	2.073	1.233		192.6	34.3	0.7	227.6
24		2	1.504	1.286		144.7	40.1	0.7	185.5
87*		"	1.857	1.279		143.9	50.4	1.1	195.4
52		"	1.227	0.830		136.2	40.3	1.6	178.1
13		3	1.130	0.761		111.2	38.7	1.2	151.1
72		"	1.318	1.068		134.2	40.8	0.7	175.7
85		"	1.545	1.100		152.2	42.2	1.7	196.1
45		4	1.117	0.883		125.0	41.3	1.2	167.5
74*		"	1.026	0.761		130.8	32.6	1.2	164.6
69		"	1.220	1.271		120.8	44.2	1.7	166.7
77	N9	1	2.169	1.533	1.666	360.7	150.8	3.0	514.5
80		"	2.150	1.275		425.5	116.8	2.7	545.0
78		"	2.288	1.456		346.4	125.4	2.9	474.7
23		2	1.740	1.223		294.9	141.4	3.2	439.5
106		"	2.540	1.719		364.5	127.5	3.5	495.5
95*		"	2.071	1.460		284.3	120.5	2.0	406.8
36		3	2.498	1.493		330.7	117.3	2.2	450.2
115*		"	2.573	1.582		411.2	128.6	3.2	543.0
94		"	2.006	1.436		279.6	110.4	2.4	392.4
16		4	1.923	1.266		337.7	120.9	3.8	462.4
92		"	2.801	1.753		426.6	137.6	4.0	568.2
21		"	2.000	1.292		345.6	126.0	3.3	474.9

CONCENTRATION AND AMOUNT OF AMMONIUM N IN TREE TISSUES AT HARVEST 1

* Trees with root galls
** Tissue from all replicates bulked before analysis

Tree No.	N Treat- ment	Rep. No.	Concn. in Roots (mgN/g d.wt)	Concn. in Stock + Stem + Side Shoots (mgN/g d.wt)	Concn. in Buds** (mgN/g d.wt)	Amount in Roots (mgN)	Amount in Stock + Stem + Side Shoots (mgN)	Amount in Bud (mgN)	Amount in Tree (mgN)
61	N1	1	0.010	0.037	0.143	0.6	0.8	0.1	1.5
134		"	0.055	0.038		2.9	0.6	0	3.5
84		"	0.032	0.069		1.8	1.5	0.1	3.4
11		2	0.038	0.098		2.3	2.3	0.1	4.7
70		"	0.038	0.100		3.2	3.0	0.1	6.3
15		"	0.022	0.040		1.5	1.2	0.1	2.8
20		3	0.046	0.019		3.9	0.5	0.1	4.5
81		"	0.041	0.039		2.3	0.8	0.1	3.2
116		"	0.042	0.079		2.3	1.4	0.1	3.8
65		4	0.030	0.039		2.4	1.4	0.1	3.9
100*		"	0.019	0.060		1.6	1.8	0.1	3.5
110		"	0.015	0.069		1.3	2.3	0.1	3.7
63	N3	1	0.062	0.049	0.134	5.6	1.8	0.1	7.5
130		"	0.028	0.060		2.4	2.2	0.1	4.7
133		"	0.049	0.076		4.6	2.1	0.1	6.8
24		2	0.042	0.075		4.0	2.3	0.1	6.4
87*		"	0.050	0.050		3.9	2.0	0.1	6.0
52		"	0.050	0.039		5.6	1.9	0.2	7.7
13		3	0.037	0.033		3.6	1.7	0.1	5.4
72		"	0.049	0.135		5.0	5.2	0.1	10.3
85		"	0.039	0.037		3.8	1.4	0.2	5.4
45		4	0.030	0.053		3.4	2.5	0.1	6.0
74*		"	0.036	0.060		4.6	3.0	0.1	7.7
69		"	0.038	0.051		3.8	1.8	0.2	5.8
77	N9	1	0.013	0.013	0.116	2.2	1.3	0.2	3.7
80		"	0.040	0.041		7.9	3.8	0.2	11.9
78		"	0.043	0.043		6.5	3.7	0.2	10.4
23		2	0.013	0.006		2.2	0.7	0.2	3.1
106		"	0.059	0.058		8.5	4.3	0.2	13.0
95*		"	0.054	0.045		7.4	3.7	0.1	11.2
36		3	0.056	0.042		7.4	3.3	0.2	10.9
115*		"	0.013	0.029		2.1	2.4	0.2	4.7
94		"	0.050	0.062		7.0	4.8	0.2	12.0
16		4	0.040	0.042		7.0	3.8	0.3	11.1
92		"	0.015	0.087		2.3	6.8	0.3	9.4
21		"	0.075	0.066		13.0	6.4	0.2	19.6

CONCENTRATION AND AMOUNT OF AMIDE N IN TREE TISSUES AT HARVEST 1

* Trees with root galls
** Tissue from all replicates bulked before analysis

Tree No.	N Treatment	Rep. No.	Concn. in Roots (mgN/g d.wt)	Concn. in Stock + Stem + Side Shoots (mgN/g d.wt)	Concn. in Buds** (mgN/g d.wt)	Amount in Roots (mgN)	Amount in Stock + Stem + Side Shoots (mgN)	Amount in Buds (mgN)	Amount in Tree (mgN)
61	N1	1	0.189	0.061	0.070	10.9	1.3	0	12.2
134		"	0.258	0.240		13.6	4.0	0	17.6
84		"	0.199	0.123		11.1	2.7	0	13.8
11		2	0.118	0.012		7.1	0.3	0	7.4
70		"	0.094	0.140		8.0	4.1	0	12.1
15		"	0.152	0.133		10.2	3.9	0	14.1
20		3	0.156	0.167		13.1	4.6	0	17.7
81		"	0.288	0.164		16.1	3.2	0	19.3
116		"	0.182	0.180		10.1	3.2	0	13.3
65		4	0.088	0.131		6.9	4.7	0.1	11.7
100*		"	0.145	0.141		12.3	4.3	0	16.6
110		"	0.106	0.111		9.0	3.8	0.1	12.9
63	N3	1	0.218	0.320	0.169	19.8	11.9	0.1	31.8
130		"	0.197	0.137		17.1	5.0	0.1	22.2
133		"	0.302	0.361		28.1	10.0	0.1	38.2
24		2	0.216	0.276		20.8	8.6	0.1	29.5
87*		"	0.342	0.221		26.5	8.7	0.1	35.3
52		"	0.183	0.272		20.3	13.2	0.2	33.7
13		3	0.129	0.221		12.7	11.2	0.2	24.1
72		"	0.210	0.320		21.4	12.5	0.1	34.0
85		"	0.254	0.271		25.0	10.4	0.2	35.6
45		4	0.198	0.280		22.2	13.1	0.2	35.5
74*		"	0.161	0.243		20.5	10.4	0.2	31.1
69		"	0.292	0.446		28.9	15.5	0.2	44.6
77	N9	1	0.296	0.151	0.176	49.2	14.9	0.3	64.4
80		"	0.249	0.257		49.3	23.5	0.3	73.1
78		"	0.211	0.364		31.9	31.3	0.3	63.5
23		2	0.299	0.139		50.7	16.1	0.3	67.1
106		"	0.278	0.444		37.9	32.9	0.4	73.2
95*		"	0.314	0.444		43.1	36.6	0.2	79.9
36		3	0.195	0.369		25.8	29.0	0.2	55.0
115*		"	0.214	0.221		34.2	18.0	0.3	52.5
94		"	0.265	0.455		36.9	35.0	0.2	72.1
16		4	0.187	0.443		32.8	42.3	0.4	75.5
92		"	0.349	0.171		53.2	13.4	0.4	67.0
21		"	0.229	0.360		39.6	35.1	0.3	75.0

CONCENTRATION AND AMOUNT OF TOTAL N IN TREE TISSUES AT HARVEST 2

* Trees with root galls

Tree No.	N Treatment	Rep. No.	Concn. in Roots (mgN/g d.wt)	Concn. in Stock + Stem + 1Yr Old Shoots (mgN/g d.wt)	Concn. in New Shoots (mgN/g d.wt)	Concn. in Abscissed Leaves (mgN/g d.wt)	Amount in Roots (mgN)	Amount in Stock + Stem + 1Yr Old Shoots (mgN)	Amount in New Shoots (mgN)	Amount in Abscissed Leaves (mgN)	Amount in Tree (mgN)
126	N_1N_0	1	6.910	3.585	18.699	-	361.4	67.8	157.1	0	586.3
117		2	6.159	3.614	20.600	-	351.7	79.1	117.4	0	548.2
35		3	5.844	3.222	16.654	12.745	423.7	77.7	139.9	5.1	646.4
101		4	5.307	3.318	17.308	-	376.8	120.8	178.3	0	675.9
122	N_1N_3	1	7.691	3.366	19.682	-	449.2	92.9	478.3	0	1020.4
83		2	6.951	3.232	19.323	12.490	499.8	96.0	473.4	1.2	1069.2
67		3	6.753	3.248	20.211	12.359	451.8	112.1	422.4	1.2	987.5
60		4	6.517	3.344	21.460	-	515.5	119.0	446.4	0	1080.9
132	N_1N_9	1	8.704	3.670	23.165	17.272	530.1	105.3	1257.9	5.2	1898.5
102		2	7.856	4.117	25.445	-	408.5	102.5	1139.9	0	1650.9
55		3	7.126	3.552	23.902	16.020	555.8	109.8	1176.0	1.6	1843.2
42		4	7.400	3.975	24.379	16.482	603.1	114.5	1082.4	1.6	1801.6
109	N_3N_0	1	8.027	4.628	19.061	19.789	510.5	142.5	484.1	11.9	1149.0
127		2	6.628	3.518	15.766	14.071	661.1	145.3	441.4	2.8	1250.6
18*		3	6.595	3.521	16.194	12.694	756.6	124.3	354.6	5.1	1240.6
40		4	6.359	3.379	15.783	11.341	658.2	138.2	468.8	6.8	1272.0
48	N_3N_3	1	7.987	4.139	21.590	14.802	802.7	137.4	902.5	5.9	1848.5
108*		2	7.855	3.493	21.107	14.506	962.4	155.1	669.1	2.9	1789.5
33		3	7.555	3.830	19.350	16.390	772.9	172.7	839.8	4.9	1790.3
28		4	7.408	3.635	19.967	16.205	805.2	151.9	665.4	4.9	1627.4
58	N_3N_9	1	9.449	4.407	22.402	13.923	826.8	174.1	1424.8	4.2	2429.9
103		2	9.225	4.401	24.279	21.569	886.5	192.3	1527.1	10.8	2616.7
62		3	8.193	4.073	23.901	16.322	825.5	182.1	1498.6	9.8	2516.0
1		4	9.066	4.585	27.744	12.583	935.6	220.5	1206.9	2.5	2365.5
135	N_9N_0	1	9.896	5.108	22.673	18.279	1129.1	376.5	1262.9	42.0	2810.5
99		2	9.708	5.622	24.223	14.869	1468.8	531.3	1034.3	19.3	3053.7
125*		3	8.674	4.045	19.601	14.090	1152.3	368.5	1150.6	14.1	2685.5
129		4	9.017	4.263	22.513	15.732	1605.9	466.4	1247.2	31.5	3351.0
2	N_9N_3	1	9.319	4.699	23.275	15.445	1702.6	420.6	1787.5	21.6	3932.3
90		2	12.113	5.341	26.376	20.341	988.4	393.6	1553.5	54.9	2990.4
98		3	10.836	4.481	25.398	16.170	1301.4	339.2	1595.0	14.6	3250.2
9*		4	8.400	4.700	21.296	14.943	1407.9	506.7	1676.0	31.4	3622.0
131	N_9N_9	1	9.717	5.109	24.209	30.550	1385.6	417.9	2537.1	88.6	4429.2
89		2	11.696	5.670	29.891	21.429	1383.6	547.2	2256.8	36.4	4224.0
30*		3	10.210	4.552	30.193	21.626	1585.9	359.5	1969.6	19.5	3934.5
49*		4	9.497	4.072	22.979	25.088	1793.9	434.1	2178.4	32.3	4438.7

CONCENTRATION AND AMOUNT OF SOLUBLE N IN TREE TISSUES AT HARVEST 2

* Trees with root galls

Tree No.	N Treatment	Rep. No.	Concn. in Roots (mgN/g d.wt.)	Concn. in Stock + Stem + 1Yr Old Shoots (mgN/g d.wt.)	Concn. in New Shoots (mgN/g d.wt.)	Amount in Roots (mg N)	Amount in Stock + Stem + 1Yr Old Shoots (mg N)	Amount in New Shoots (mg N)	Amount in Tree (mg N)
126	N_1N_0	1	1.068	0.572	1.807	55.8	10.8	15.2	81.8
117		2	0.959	0.531	2.009	54.7	11.6	11.5	77.8
35		3	0.632	0.451	1.468	45.8	10.9	12.3	69.0
101		4	0.715	0.406	1.521	50.8	14.8	15.7	81.3
122	N_1N_3	1	0.717	0.470	2.031	41.9	13.0	49.4	104.3
83		2	0.654	0.444	2.099	47.0	13.2	51.4	111.6
67		3	0.760	0.521	1.933	50.8	13.0	40.4	103.2
60		4	0.836	0.478	2.045	66.1	17.0	42.5	125.6
132	N_1N_9	1	0.957	0.542	2.466	58.3	15.6	133.9	207.8
102		2	0.791	0.483	3.072	41.1	12.2	137.6	190.9
55		3	0.755	0.549	2.608	58.9	17.0	128.3	204.2
42		4	0.663	0.494	2.721	54.0	14.2	120.8	189.0
109	N_3N_0	1	1.793	1.082	1.696	114.0	33.3	43.1	190.4
127		2	1.020	0.722	1.385	102.5	29.8	38.8	171.1
18*		3	0.985	0.650	1.546	96.4	22.9	33.9	153.2
40		4	0.909	0.675	1.519	94.1	27.6	45.1	166.8
48	N_3N_3	1	1.168	0.791	2.472	117.4	26.3	103.3	247.0
108*		2	1.245	0.700	2.136	141.6	31.1	67.7	240.4
38		3	1.151	0.810	1.865	117.7	36.5	80.9	235.1
28		4	0.728	0.713	2.008	79.1	29.8	70.1	179.0
58	N_3N_9	1	1.410	0.681	2.288	123.4	26.9	145.5	295.8
103		2	1.855	0.967	2.935	178.3	42.3	184.6	405.2
62		3	1.140	0.645	2.561	114.8	28.8	160.6	304.2
1		4	2.082	0.795	3.234	214.9	38.2	140.7	393.8
135	N_9N_0	1	3.307	1.691	2.265	377.3	124.6	126.2	628.1
99		2	2.387	2.024	2.441	436.8	191.3	104.2	732.3
125*		3	1.515	1.093	1.916	183.5	99.6	112.5	395.6
129		4	2.277	1.133	2.345	405.5	124.0	129.9	659.4
2	N_9N_3	1	1.938	1.373	2.483	354.1	122.9	190.7	667.7
90		2	5.051	1.862	3.309	412.2	137.2	194.9	744.3
98		3	2.243	1.360	3.068	269.4	103.0	192.7	565.1
9*		4	1.476	1.215	2.201	222.3	131.0	173.2	526.5
131	N_9N_9	1	2.726	1.599	2.717	388.7	130.8	284.7	804.2
89		2	4.887	2.046	4.320	578.1	197.4	326.2	1101.7
30*		3	2.324	1.435	4.505	343.0	113.7	293.7	750.4
49*		4	1.638	0.834	2.676	294.8	94.2	253.7	642.7

CONCENTRATION AND AMOUNT OF INSOLUBLE N IN TREE TISSUES AT HARVEST 2

* Trees with root galls

Tree No.	N Treatment	Rep. No.	Concn. in Roots (mgN/g d.wt.)	Concn. in Stock + Stem + 1Yr Old Shoots (mgN/g d.wt.)	Concn. in New Shoots (mgN/g d.wt.)	Amount in Roots (mg N)	Amount in Stock + Stem + 1Yr Old Shoots (mg N)	Amount in New Shoots (mg N)	Amount in Tree (mg N)
126	N_1N_0	1	5.842	3.013	16.892	305.6	57.0	141.9	504.5
117		2	5.200	3.083	18.591	297.0	67.5	105.9	470.4
35		3	5.212	2.771	15.186	377.9	66.8	127.6	572.3
101		4	4.592	2.912	15.737	326.0	106.0	162.6	594.6
122	N_1N_3	1	6.974	2.896	17.651	407.3	79.9	428.9	916.1
83		2	6.297	2.738	17.224	452.8	82.8	422.0	957.6
67		3	5.993	2.727	18.278	401.0	94.1	382.0	877.1
60		4	5.681	2.866	19.415	449.4	102.0	403.9	955.3
132	N_1N_9	1	7.747	3.128	20.699	471.8	89.7	1124.0	1685.5
102		2	7.065	3.628	22.373	367.4	90.3	1002.3	1460.0
55		3	6.371	3.003	21.294	496.9	92.8	1047.7	1637.4
42		4	6.737	3.431	21.658	549.1	100.3	961.6	1611.0
109	N_3N_0	1	6.234	3.546	17.365	396.5	109.2	441.0	946.7
127		2	5.608	2.796	14.381	558.6	115.5	402.6	1076.7
18*		3	5.610	2.871	14.648	660.2	101.4	320.7	1082.3
40		4	5.450	2.704	14.264	564.1	110.6	423.7	1098.4
48	N_3N_3	1	6.819	3.348	19.118	685.3	111.1	799.2	1595.6
108*		2	6.610	2.793	18.971	820.8	124.0	601.4	1546.2
38		3	6.404	3.020	17.485	655.2	136.2	758.9	1550.3
28		4	6.680	2.922	17.059	726.1	122.1	595.3	1443.5
58	N_3N_9	1	7.363	3.726	20.114	703.4	147.2	1279.3	2129.9
103		2	7.370	3.434	21.344	708.2	150.0	1342.5	2200.7
62		3	7.058	3.428	21.340	710.7	153.3	1338.0	2202.0
1		4	6.984	3.790	24.510	720.7	182.3	1066.2	1969.2
135	N_9N_0	1	6.589	3.417	20.408	751.8	251.9	1136.7	2140.4
99		2	6.821	3.598	21.782	1032.0	340.0	930.1	2302.1
125*		3	7.159	2.952	17.685	968.8	268.9	1038.1	2275.8
129		4	6.740	3.130	20.168	1200.4	342.4	1117.3	2660.1
2	N_9N_3	1	7.381	3.326	20.792	1348.5	297.7	1596.8	3243.0
90		2	7.062	3.479	23.067	576.2	256.4	1358.6	2191.2
98		3	8.593	3.121	22.330	1032.0	236.2	1402.3	2670.5
9*		4	6.924	3.485	19.095	1185.6	375.7	1502.8	3064.1
131	N_9N_9	1	6.991	3.510	21.492	996.9	287.1	2252.4	3536.4
89		2	6.809	3.624	25.571	805.5	349.8	1930.6	3085.9
30*		3	7.886	3.117	25.638	1242.9	246.8	1674.9	3164.6
49*		4	7.859	3.188	20.303	1499.1	339.9	1924.7	3763.7

CONCENTRATION AND AMOUNT OF ARGININE N IN TREE TISSUES AT HARVEST 2

* Trees with root galls

Tree No.	N Treat-ment	Rep. No.	Concn. in Roots (mgN/g d.wt.)	Concn. in Stock + Stem + 1Yr Old Shoots (mgN/g d.wt.)	Concn. in New Shoots (mgN/g d.wt.)	Amount in Roots (mgN)	Amount in Stock + Stem + 1Yr Old Shoots (mgN)	Amount in New Shoots (mgN)	Amount in Tree (mgN)
126	N_1N_0	1	0.120	0.070	0	6.3	1.3	0	7.6
117		2	0.089	0	0	5.1	0	0	5.1
35		3	0	0	0	0	0	0	0
101		4	0.065	0	0	4.6	0	0	4.6
122	N_1N_3	1	0.065	0	0	3.8	0	0	3.8
83		2	0.056	0	0	4.0	0	0	4.0
67		3	0.069	0	0.105	4.6	0	2.2	6.8
60		4	0.142	0	0	11.2	0	0	11.2
132	N_1N_9	1	0.159	0	0.077	9.7	0	4.2	13.9
102		2	0.109	0	0.085	5.7	0	3.8	9.5
55		3	0.121	0	0	9.4	0	0	9.4
42		4	0.055	0	0	4.5	0	0	4.5
109	N_3N_0	1	0.220	0.055	0	14.0	1.7	0	15.7
127		2	0.049	0	0	4.9	0	0	4.9
13*		3	0	0	0	0	0	0	0
40		4	0.076	0	0	7.9	0	0	7.9
48	N_3N_3	1	0.273	0	0.148	27.4	0	6.2	33.6
108*		2	0.203	0	0	23.6	0	0	23.6
38		3	0.120	0	0	12.3	0	0	12.3
28		4	0.295	0	0	32.1	0	0	32.1
58	N_3N_9	1	0.239	0	0	20.9	0	0	20.9
103		2	0.457	0.068	0.074	43.9	3.0	4.7	51.6
62		3	0.192	0	0	19.3	0	0	19.3
1		4	0.524	0	0	54.1	0	0	54.1
135	N_9N_0	1	0.663	0.245	0	75.6	18.1	0	93.7
99		2	0.484	0.230	0	73.2	26.5	0	99.7
125*		3	0.153	0.069	0.075	18.5	6.3	4.4	29.2
129		4	0.449	0.111	0	80.0	12.1	0	92.1
2	N_9N_3	1	0.470	0.134	0.133	85.9	12.0	10.6	108.5
90		2	2.222	0.532	0.215	181.3	39.2	12.7	233.2
98		3	0.877	0.024	0	105.3	1.8	0	107.1
9*		4	0.271	0.066	0	40.8	7.1	0	47.9
131	N_9N_9	1	0.610	0	0.071	87.0	0	7.4	94.4
89		2	1.294	0.323	0.110	153.1	31.2	8.3	192.6
30*		3	0.424	0.128	0.144	62.6	10.1	9.4	82.1
49*		4	0.329	0	0	59.2	0	0	59.2

CONCENTRATION AND AMOUNT OF TOTAL α-AMINO N IN TREE TISSUES AT HARVEST 2

* Trees with root galls

Tree No.	N Treat-ment	Rep. No.	Concn. in Roots (mgN/g d.wt.)	Concn. in Stock + Stem + 1Yr Old Shoots (mgN/g d.wt.)	Concn. in New Shoots (mgN/g d.wt.)	Amount in Roots (mgN)	Amount in Stock + Stem + 1Yr Old Shoots (mgN)	Amount in New Shoots (mgN)	Amount in Tree (mgN)
126	N_1N_0	1	0.271	0.092	0.248	14.2	1.7	2.1	18.0
117		2	0.261	0.107	0.343	14.9	2.3	2.0	19.2
35		3	0.170	0.079	0.219	12.3	1.9	1.8	16.0
101		4	0.196	0.094	0.245	13.9	3.4	2.5	19.8
122	N_1N_3	1	0.207	0.080	0.340	12.1	2.2	8.3	22.6
83		2	0.207	0.060	0.379	14.9	1.8	9.3	26.0
67		3	0.237	0.061	0.376	15.9	2.1	7.9	25.9
60		4	0.268	0.077	0.446	21.2	2.7	9.3	33.2
132	N_1N_9	1	0.279	0.047	0.334	17.0	1.3	18.1	36.4
102		2	0.156	0.032	0.721	8.1	0.8	32.3	41.2
55		3	0.145	0.055	0.334	11.3	1.7	16.4	29.4
42		4	0.098	0.012	0.348	8.0	0.3	15.5	23.8
109	N_3N_0	1	0.361	0.238	0.218	23.0	7.3	5.5	35.8
127		2	0.262	0.122	0.188	26.3	5.0	5.3	36.6
18*		3	0.227	0.104	0.198	22.2	3.7	4.3	30.2
40		4	0.268	0.098	0.251	27.7	4.0	7.5	39.2
48	N_3N_3	1	0.347	0.132	0.400	34.9	4.4	16.7	56.0
108		2	0.295	0.091	0.309	33.5	4.0	9.8	47.3
38		3	0.329	0.116	0.337	33.7	5.2	14.6	53.5
28		4	0.153	0.010	0.280	16.6	0.4	9.8	26.8
58	N_3N_9	1	0.345	0.109	0.361	30.2	4.3	23.0	57.5
103		2	0.330	0.201	0.445	36.5	8.8	28.0	73.3
62		3	0.394	0.080	0.431	39.7	3.6	27.0	70.3
1		4	0.544	0.204	0.509	56.1	9.8	22.1	88.0
135	N_9N_0	1	0.859	0.387	0.221	98.0	28.5	12.3	138.8
99		2	0.750	0.523	0.307	113.5	49.4	13.1	176.0
125*		3	0.427	0.205	0.239	51.7	18.7	17.0	87.4
129		4	0.582	0.199	0.248	105.7	21.8	13.7	139.2
2	N_9N_3	1	0.412	0.226	0.372	75.3	20.2	28.6	124.1
90		2	1.366	0.448	0.553	111.5	33.0	32.6	177.1
98		3	0.639	0.319	0.560	76.7	24.1	35.2	136.0
9*		4	0.370	0.204	0.406	55.7	22.0	32.0	109.7
131	N_9N_9	1	0.570	0.263	0.237	81.3	21.5	24.8	127.6
89		2	1.133	0.480	0.555	134.0	46.3	41.9	222.2
30*		3	0.362	0.221	0.547	53.4	17.5	35.7	106.6
49*		4	0.226	0.162	0.131	40.7	17.3	12.4	70.4

CONCENTRATION AND AMOUNT OF TOTAL N IN TREE TISSUES AT HARVEST 3

* Trees with root galls

Tree No.	N Treat-ment	Rep. No.	Concn. in Roots (mgN/g d.wt.)	Concn. in Stock + Stem + 1Yr Old Shoots (mgN/g d.wt.)	Concn. in New Shoots (mgN/g d.wt.)	Concn. in Abscissed Leaves (mgN/g d.wt.)	Amount in Roots (mg N)	Amount in Stock + Stem + 1Yr Old Shoots (mg N)	Amount in New Shoots (mg N)	Amount in Abscissed Leaves (mg N)	Amount in Tree (mg N)
111	N_1N_0	1	5.978	3.435	10.513	10.803	347.3	72.8	49.4	52.9	522.4
107		2	5.903	3.069	10.913	8.429	417.3	108.0	66.6	20.2	612.1
56		3	5.842	3.574	12.532	11.948	364.0	136.9	71.4	45.4	617.7
96		4	4.961	3.323	12.514	10.397	405.3	151.5	91.4	23.9	672.1
50	N_1N_3	1	7.378	4.877	15.550	16.415	809.4	174.6	581.6	37.8	1603.4
86		2	6.858	4.227	14.726	14.424	729.7	167.8	622.9	33.2	1553.6
68		3	6.763	4.277	15.254	12.531	828.5	188.6	581.2	25.2	1623.5
46*		4	6.211	4.076	13.322	11.098	888.7	165.5	578.2	17.8	1650.2
26	N_1N_9	1	7.311	3.350	15.196	12.745	1397.9	226.1	1932.9	42.1	3599.0
112*		2	8.290	4.685	16.075	16.286	1220.4	264.2	2030.3	117.3	3632.2
51		3	7.998	3.748	14.962	17.313	1186.9	264.6	2223.4	88.3	3763.2
66*		4	7.751	3.863	15.393	13.906	1512.3	220.6	2071.9	55.6	3860.4
47	N_3N_0	1	6.043	3.700	11.299	15.146	663.5	135.4	163.8	260.5	1223.2
64		2	5.787	3.423	11.340	12.450	663.8	160.9	200.7	130.7	1156.1
88		3	6.753	3.553	11.645	12.439	786.0	168.1	180.5	163.0	1297.6
97*		4	5.563	3.482	10.046	11.665	854.4	173.8	245.1	149.3	1422.6
120*	N_3N_3	1	7.104	4.298	13.606	16.279	1090.3	196.8	770.1	169.3	2226.5
79		2	7.374	3.815	14.450	16.048	1005.1	161.4	1096.8	104.3	2367.6
34		3	6.712	3.908	12.646	13.825	969.2	263.8	782.8	106.5	2122.3
22		4	7.858	4.387	14.720	16.161	903.7	229.9	872.9	114.7	2121.2
32	N_3N_9	1	8.005	3.978	15.540	15.191	1688.3	290.0	2170.9	92.7	4241.9
82		2	8.393	3.962	16.036	15.895	1625.7	395.8	2187.3	106.5	4315.3
43		3	8.790	3.962	15.661	14.395	1813.4	290.4	2150.3	77.7	4331.8
114*		4	8.260	4.051	16.088	17.585	1828.3	279.9	2102.7	202.2	4413.1
71	N_9N_0	1	8.071	4.854	15.046	19.986	1164.6	494.1	567.2	541.6	2767.5
128		2	8.502	4.842	14.457	17.020	1188.6	525.8	633.2	377.8	2725.4
118		3	7.149	3.993	14.272	18.022	1180.3	504.3	635.1	448.7	2768.4
12*		4	7.648	4.332	15.030	16.930	1452.4	479.6	596.7	452.0	2980.7
113	N_9N_3	1	7.798	3.540	13.052	13.828	1636.8	430.1	1318.3	206.0	3591.2
105		2	7.793	3.813	14.596	16.462	1850.1	494.5	1199.8	339.1	3883.5
121*		3	7.823	4.173	15.999	17.598	1991.9	560.0	1441.5	323.8	4317.2
54		4	7.346	3.742	13.046	14.252	1804.9	401.1	1166.3	282.2	3654.5
39	N_9N_9	1	8.368	4.851	15.371	20.722	1610.8	554.0	2484.0	439.3	5088.1
27*		2	9.712	4.365	17.560	17.633	2782.9	589.7	2518.1	250.4	6141.1
57		3	9.135	4.859	17.131	17.484	2198.8	511.7	2649.3	286.7	5645.5
91*		4	8.356	4.709	16.963	19.190	2470.0	621.1	2614.0	314.7	6019.8

CONCENTRATION AND AMOUNT OF SOLUBLE N IN TREE TISSUES AT HARVEST 3

* Trees with root galls

Tree No.	N Treat-ment	Rep. No.	Conc. in Roots (mgN/g d.wt.)	Concn. in Stock + Stem + 1Yr Old Shoots (mgN/g d.wt.)	Concn. in New Shoots (mgN/g d.wt.)	Amount in Roots (mgN)	Amount in Stock + Stem + 1Yr Old Shoots (mgN)	Amount in New Shoots (mgN)	Amount in Tree (mgN)
111	N_1N_0	1	0.932	0.634	1.434	54.1	13.4	6.7	74.2
107		2	0.964	0.527	1.474	68.2	18.6	9.0	95.8
56		3	0.904	0.600	1.459	56.3	23.0	8.3	87.6
96		4	0.753	0.473	1.509	61.5	21.6	11.0	94.1
50	N_1N_3	1	2.011	1.561	1.620	220.6	55.9	60.6	337.1
86		2	1.355	1.116	1.534	144.2	44.3	64.9	253.4
68		3	1.265	1.080	1.483	155.0	47.6	56.5	259.1
46*		4	1.084	0.818	1.346	151.3	33.2	58.4	242.9
26	N_1N_9	1	1.327	0.530	1.530	253.7	35.8	194.6	484.1
112*		2	2.021	1.293	2.154	291.8	72.9	272.1	636.8
51		3	1.966	0.856	1.850	291.8	60.4	274.9	627.1
66*		4	1.943	0.941	1.724	373.3	53.7	232.1	659.1
47	N_3N_0	1	1.042	0.713	1.369	114.4	26.1	19.9	160.4
64		2	1.233	0.876	1.528	141.4	41.2	27.0	209.6
98		3	1.051	0.633	1.433	122.3	29.9	22.2	174.4
97*		4	1.111	0.770	1.353	164.1	38.4	33.0	235.5
120*	N_3N_3	1	1.574	0.993	1.432	238.1	45.5	81.1	364.7
79		2	1.138	0.860	1.543	155.1	36.4	117.1	308.6
34		3	1.315	0.743	1.280	189.9	50.2	79.2	319.3
22		4	1.792	1.079	1.615	206.1	56.5	95.8	358.4
32	N_3N_9	1	1.413	0.926	1.789	298.0	67.5	249.9	615.4
82		2	1.589	1.102	2.000	307.8	110.1	272.8	690.7
43		3	1.936	0.969	2.096	399.4	71.0	287.8	758.2
114*		4	1.848	0.911	2.103	402.5	63.0	274.9	740.4
71	N_9N_0	1	2.090	1.386	2.263	301.6	141.1	85.3	528.0
128		2	2.948	1.505	1.970	411.9	163.4	86.3	661.6
118		3	1.709	0.985	2.440	282.2	124.4	108.6	515.2
12*		4	1.858	1.026	2.270	345.0	113.6	90.1	548.7
113	N_9N_3	1	1.130	0.816	1.477	237.2	99.1	149.2	485.5
105		2	1.436	0.907	2.049	352.8	117.6	168.4	638.8
121*		3	1.595	1.088	2.104	370.7	146.0	189.6	706.3
54		4	1.114	0.984	1.837	273.7	105.5	164.2	543.4
39	N_9N_9	1	1.399	1.232	2.045	269.3	140.7	330.5	740.5
27*		2	2.391	1.241	2.156	672.6	167.7	309.2	1149.5
57		3	1.833	1.437	2.076	441.2	151.3	320.1	912.6
91*		4	1.749	1.354	2.353	482.4	178.6	362.6	1023.6

CONCENTRATION AND AMOUNT OF INSOLUBLE N IN TREE TISSUES AT HARVEST 3

* Trees with root galls

Tree No.	N Treatment	Rep. No.	Concn. in Roots (mgN/g d.wt)	Concn. in Stock + Stem + 1Yr Old Shoots (mgN/g d.wt)	Concn. in New Shoots (mgN/g d.wt)	Amount in Roots (mgN)	Amount in Stock + Stem + 1Yr Old Shoots (mgN)	Amount in New Shoots (mgN)	Amount in Tree (mgN)
111	N_1N_0	1	5.046	2.801	9.079	293.2	59.4	42.7	395.3
107		2	4.939	2.542	9.439	349.1	89.4	57.6	496.1
56		3	4.938	2.974	11.073	307.7	113.9	63.1	484.7
96		4	4.208	2.850	11.005	343.8	129.9	80.4	554.1
50	N_1N_3	1	5.367	3.316	13.930	588.8	118.7	521.0	1228.5
86		2	5.503	3.111	13.192	585.5	123.5	558.0	1267.0
68		3	5.498	3.197	13.771	673.5	141.0	524.7	1339.2
46*		4	5.127	3.258	11.976	737.4	132.3	519.8	1389.5
26	N_1N_9	1	5.984	2.820	13.666	1144.2	190.3	1738.3	3072.8
112*		2	6.269	3.392	13.921	923.6	191.3	1758.2	2878.1
51		3	6.032	2.892	13.112	895.1	204.2	1948.5	3047.8
66*		4	5.808	2.922	13.669	1139.0	166.9	1839.8	3145.7
47	N_3N_0	1	5.001	2.987	9.930	549.1	109.3	143.9	802.3
64		2	4.554	2.547	9.812	522.4	119.7	173.7	815.8
88		3	5.702	2.920	10.212	663.7	138.2	158.3	960.2
97*		4	4.452	2.712	8.693	690.3	135.4	212.1	1037.8
120*	N_3N_3	1	5.530	3.305	12.174	852.2	151.3	689.0	1692.5
79		2	6.236	2.955	12.907	850.0	125.0	979.7	1954.7
34		3	5.397	3.165	11.366	779.3	213.6	703.6	1696.5
22		4	6.066	3.308	13.105	697.6	173.4	777.1	1648.1
32	N_3N_9	1	6.592	3.052	13.751	1390.3	222.5	1921.0	3533.8
82		2	6.804	2.860	14.036	1317.9	285.7	1914.5	3518.1
43		3	6.354	2.993	13.565	1414.0	219.4	1862.5	3495.9
114*		4	6.412	3.140	13.985	1425.8	216.9	1827.8	3470.5
71	N_9N_0	1	5.981	3.468	12.783	863.0	353.0	481.9	1697.9
128		2	5.556	3.337	12.487	776.7	362.4	546.9	1686.0
118		3	5.440	3.008	14.028	898.1	379.9	526.5	1804.5
12*		4	5.810	3.306	12.760	1107.4	366.0	506.6	1980.0
113	N_9N_3	1	6.668	2.724	11.575	1399.6	331.0	1169.1	2899.7
105		2	6.307	2.906	12.547	1497.3	376.9	1031.4	2905.6
121*		3	6.228	3.085	13.895	1621.2	414.0	1251.9	3287.1
54		4	6.232	2.758	11.209	1531.2	295.6	1002.1	2828.9
39	N_9N_9	1	6.969	3.619	13.326	1341.5	413.3	2153.5	3908.3
27*		2	7.321	3.124	15.404	2110.3	422.0	2208.9	4741.2
57		3	7.302	3.422	15.105	1757.6	360.4	2329.2	4447.2
91*		4	6.607	3.355	14.610	1987.6	442.5	2251.4	4631.5

CONCENTRATION AND AMOUNT OF ARGININE N IN TREE TISSUES AT HARVEST 3

* Trees with root galls

Tree No.	N Treatment	Rep. No.	Concn. in Roots (mgN/g d.wt)	Concn. in Stock + Stem + 1Yr Old Shoots (mgN/g d.wt)	Concn. in New Shoots (mgN/g d.wt)	Amount in Roots (mgN)	Amount in Stock + Stem + 1Yr Old Shoots (mgN)	Amount in New Shoots (mgN)	Amount in Tree (mgN)
111	N_1N_0	1	0.071	0.037	0.062	4.1	0.8	0.3	5.2
107		2	0.056	0	0.032	4.0	0	0.2	4.2
56		3	0.060	0	0.045	3.7	0	0.3	4.0
96		4	0.037	0	0	3.0	0	0	3.0
50	N_1N_3	1	0.484	0.358	0.081	53.1	12.8	3.0	68.9
86		2	0.235	0.262	0.056	25.0	10.4	2.4	37.8
68		3	0.185	0.220	0.055	22.7	9.7	2.1	34.5
46*		4	0.124	0.103	0.088	17.3	4.2	3.8	25.3
26	N_1N_9	1	0.159	0.049	0.083	30.4	3.3	10.6	44.3
112*		2	0.419	0.246	0.167	60.5	13.9	21.1	95.5
51		3	0.350	0.142	0.138	51.9	10.0	20.5	82.4
66*		4	0.408	0.199	0.083	78.4	11.4	11.2	101.0
47	N_3N_0	1	0.167	0.026	0.063	18.3	1.0	0.9	20.2
64		2	0.137	0.016	0.063	15.7	0.8	1.1	17.6
88		3	0.155	0.031	0.043	18.0	1.5	0.7	20.2
97*		4	0.056	0.039	0.057	8.3	1.9	1.4	11.6
120*	N_3N_3	1	0.273	0.182	0.083	41.3	8.3	4.7	54.3
79		2	0.101	0.119	0.091	13.8	5.0	6.9	25.7
34		3	0.199	0.112	0.086	28.7	7.6	5.3	41.6
22		4	0.281	0.150	0.096	32.3	7.9	5.7	45.9
32	N_3N_9	1	0.241	0.168	0.101	50.8	12.2	14.1	77.1
82		2	0.193	0.200	0.125	37.4	20.0	17.1	74.5
43		3	0.305	0.219	0.134	62.9	16.1	18.4	97.4
114*		4	0.377	0.152	0.133	82.1	10.5	17.4	110.0
71	N_9N_0	1	0.524	0.151	0.181	75.6	15.4	6.8	97.8
128		2	0.572	0.226	0.105	80.0	24.5	4.6	109.1
118		3	0.235	0.098	0.167	47.1	12.4	7.4	66.9
12*		4	0.267	0.115	0.142	50.1	12.7	5.6	68.4
113	N_9N_3	1	0.082	0.057	0.076	17.2	6.9	7.7	31.8
105		2	0.209	0.078	0.110	49.6	10.1	9.0	68.7
121*		3	0.160	0.102	0.118	37.2	13.7	10.6	61.5
54		4	0.065	0.058	0.073	16.0	6.2	6.5	28.7
39	N_9N_9	1	0.172	0.203	0.126	33.1	23.2	20.4	76.7
27*		2	0.454	0.242	0.133	127.7	32.7	19.1	179.5
57		3	0.370	0.254	0.155	89.1	26.7	13.7	129.5
91*		4	0.259	0.259	0.173	71.4	34.2	26.7	132.3

CONCENTRATION AND AMOUNT OF TOTAL α-AMINO N IN TREE TISSUES AT HARVEST 3

* Trees with root galls

Tree No.	N Treatment	Rep. No.	Concn. in Roots (mgN/g d.wt)	Concn. in Stock + Stem + 1 Yr Old Shoots (mgN/g d.wt)	Concn. in New Shoots (mgN/g d.wt)	Amount in Roots (mg N)	Amount in Stock + Stem + 1Yr Old Shoots (mg N)	Amount in New Shoots (mg N)	Amount in Tree (mg N)
111	N_1N_0	1	0.211	0.089	0.295	12.3	1.9	1.4	15.6
107		2	0.151	0.072	0.232	10.7	2.5	1.4	14.6
56		3	0.148	0.098	0.224	9.2	3.8	1.3	14.3
96		4	0.143	0.073	0.225	11.7	3.3	1.6	16.6
50	N_1N_3	1	0.502	0.352	0.282	55.1	12.6	10.5	78.2
86		2	0.300	0.259	0.216	31.9	10.3	9.1	51.3
68		3	0.313	0.255	0.228	38.3	11.2	8.7	58.2
46*		4	0.203	0.179	0.187	28.3	7.3	8.1	43.7
26	N_1N_9	1	0.446	0.185	0.314	85.3	12.5	39.9	137.7
112*		2	0.602	0.378	0.446	86.9	21.3	56.3	164.5
51		3	0.573	0.257	0.452	85.0	18.1	67.2	170.3
66*		4	0.516	0.275	0.365	99.1	15.7	49.1	163.9
47	N_3N_0	1	0.283	0.116	0.271	31.1	4.2	3.9	39.2
64		2	0.211	0.129	0.222	24.2	6.1	3.9	34.2
88		3	0.215	0.082	0.314	25.0	3.9	4.9	33.8
97*		4	0.160	0.111	0.219	23.6	5.5	5.3	34.4
120*	N_3N_3	1	0.343	0.177	0.176	51.9	8.1	10.0	70.0
79		2	0.298	0.149	0.247	40.5	6.3	18.7	65.6
34		3	0.205	0.116	0.188	29.6	7.8	11.6	49.0
22		4	0.434	0.250	0.327	49.9	13.1	19.4	82.4
32	N_3N_9	1	0.333	0.237	0.326	80.8	17.3	45.5	143.6
82		2	0.401	0.280	0.337	77.7	28.0	46.0	151.7
43		3	0.678	0.330	0.482	139.9	24.2	66.2	230.3
114*		4	0.546	0.244	0.719	118.9	16.9	94.0	229.8
71	N_9N_0	1	0.526	0.250	0.437	75.9	25.5	16.5	117.9
128		2	0.696	0.346	0.392	97.3	37.6	17.2	152.1
118		3	0.380	0.194	0.445	62.7	24.5	19.8	107.0
12*		4	0.396	0.204	0.369	74.3	22.6	14.6	111.5
113	N_9N_3	1	0.134	0.080	0.135	28.1	9.7	13.6	51.4
105		2	0.258	0.110	0.245	61.2	14.3	20.1	95.6
121*		3	0.226	0.143	0.286	52.5	19.2	25.8	97.5
54		4	0.160	0.108	0.212	39.3	11.6	19.0	69.9
39	N_9N_9	1	0.417	0.333	0.426	80.3	38.0	68.8	187.1
27*		2	0.671	0.313	0.355	188.8	42.3	50.9	282.0
57		3	0.532	0.428	0.406	128.1	45.1	62.6	235.8
91*		4	0.547	0.349	0.450	150.9	46.0	69.3	266.2

APPENDIX C (a)(ii).- Sand Culture Experiment; Treatment Means and Least Significant Differences (Nitrogen Data)

AMOUNTS OF NITROGENOUS CONSTITUENTS IN TREE TISSUES AT HARVEST 1

Log$_e$ mg N

Tree Tissue	N Treatment	Total N	Soluble N	Insoluble N	Arginine N	Ammonium N	Amide N	Total α-Amino N
Roots	N1	6.233	5.008	5.878	4.752	0.681	2.339	3.847
	N3	6.913	6.028	6.369	5.817	1.409	3.066	4.917
	N9	7.626	7.056	6.778	6.995	1.641	3.681	5.850
L. S. D.	5%	0.075	0.107	0.106	0.158	0.460	0.189	0.109
	1%	0.101	0.145	0.144	0.214	0.624	0.256	0.148
	0.1%	0.136	0.195	0.193	0.286	0.835	0.343	0.198
Stock + stem + side shoots	N1	6.233	3.571	4.745	3.095	0.247	1.034	2.179
	N3	6.913	4.999	5.357	4.717	0.784	2.352	3.701
	N9	7.626	6.160	6.248	6.032	1.172	3.241	4.840
L. S. D.	5%	0.096	0.135	0.124	0.235	0.419	0.473	0.154
	1%	0.130	0.183	0.168	0.336	0.568	0.640	0.209
	0.1%	0.174	0.245	0.225	0.517	0.761	0.857	0.279

AMOUNTS OF NITROGENOUS CONSTITUENTS IN TREE TISSUES AT HARVEST 1

Log$_e$ mgN (except for bud data which is expressed as mgN)

Tree Tissue	N Treatment	Total N	Soluble N	Insoluble N	Arginine N	Ammonium N	Amide N	Total α-Amino N
Leaf + flower buds	N1	5.7	0.9	4.8	0.6	0.1	0	0.3
	N3	11.6	3.6	8.0	3.0	0.1	0.1	1.1
	N9	28.7	10.0	18.7	8.3	0.2	0.3	3.0
L.S.D. 5%		1.6	0.4	-*	1.1	0.006	0.1	0.0009
1%		2.8	0.6	-	2.1	0.01	0.2	0.002
0.1%		6.3	1.4	-	4.6	0.02	0.4	0.004
Whole tree	N1	6.505	5.230	6.169	4.940	1.269	2.615	4.029
	N3	7.231	6.344	6.691	6.120	0.872	3.480	5.186
	N9	8.032	7.407	7.261	7.341	2.190	4.215	6.169
L.S.D. 5%		0.024	0.083	0.079	0.140	0.354	0.159	0.090
1%		0.032	0.113	0.108	0.189	0.480	0.216	0.121
0.1%		0.044	0.151	0.144	0.253	0.642	0.289	0.162

* Insufficient data for analysis

CONCENTRATION OF NITROGENOUS CONSTITUENTS IN TREE TISSUES AT HARVEST 1

mg N/g d.wt.

Tree Tissue	N Treatment	Total N	Soluble N	Insoluble N	Arginine N	Ammonium N	Amide N	Total α-Amino N
Roots	N1	7.48	2.30	5.18	1.84	0.03	0.17	0.73
	N3	10.03	4.32	5.71	3.59	0.04	0.23	1.42
	N9	12.97	7.47	5.50	7.06	0.04	0.26	2.23
L.S.D. 5%		0.99	0.78	0.43	0.85	0.01	0.05	0.23
1%		1.35	1.05	0.65	1.15	0.02	0.06	0.31
0.1%		1.80	1.41	0.87	1.54	0.03	0.09	0.42
Stock + stem + side shoots	N1	6.13	1.51	4.62	1.06	0.06	0.13	0.38
	N3	9.36	3.89	5.47	3.02	0.06	0.28	1.06
	N9	11.40	5.46	5.94	5.11	0.04	0.32	1.46
L.S.D. 5%		0.79	0.56	0.35	0.72	0.02	0.08	0.16
1%		1.07	0.76	0.47	0.98	0.03	0.11	0.21
0.1%		1.44	1.02	0.63	1.31	0.04	0.15	0.28
Leaf + flower buds	N1	10.25	1.67	8.58	1.01	0.14	0.07	0.55
	N3	13.05	4.02	9.03	3.33	0.13	0.17	1.24
	N9	15.87	5.53	10.34	4.59	0.12	0.18	1.67
L.S.D. 5%		2.73	0.29	-*	1.00	0.11	0.31	0.002
1%		5.01	0.52	-	1.84	0.19	0.57	0.003
0.1%		11.08	1.16	-	4.08	0.43	1.27	0.007

* Insufficient data for analysis

AMOUNT OF TOTAL NITROGEN IN TREE TISSUES AT HARVEST 2

$\log_e$ mg N

N Treatment	Roots			Stock + Stem + 1 Yr Old Shoots			New Shoots			Abscissed Leaves			Whole Tree		
	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean
N1N0	5.856			4.434			4.987			0.452			6.417		
N1N3	6.170	6.093 (N1)	6.502 (N0)	4.649	4.588 (N1)	5.140 (N0)	6.119	6.055 (N1)	6.042 (N0)	0.394	0.593 (N1)	1.878 (N0)	6.946	6.952 (N1)	7.175 (N0)
N1N9	6.252			4.682			7.058			0.934			7.494		
N3N0	6.462			4.922			6.073			1.939			7.113		
N3N3	6.725	6.651 (N3)	6.695 (N3)	5.035	5.071 (N3)	5.234 (N3)	6.636	6.653 (N3)	6.721 (N3)	1.711	1.862 (N3)	1.816 (N3)	7.474	7.468 (N3)	7.520 (N3)
N3N9	6.766			5.255			7.250			1.937			7.816		
N9N0	7.183			6.065			7.065			3.242			7.895		
N9N3	7.189	7.236 (N9)	6.783 (N9)	6.018	6.053 (N9)	5.337 (N9)	7.409	7.394 (N9)	7.339 (N9)	3.342	3.415 (N9)	2.177 (N9)	8.140	8.163 (N9)	7.888 (N9)
N9N9	7.332			6.075			7.708			3.661			8.355		
L.S.D. 5%	NS	0.128	0.128	NS	0.105	0.105	0.151	0.087	0.087	NS	0.550	NS	0.107	0.062	0.062
1%		0.174	0.174		0.143	0.143	0.204	0.118	0.118		0.749		0.145	0.084	0.084
0.1%		0.233	0.233		0.191	0.191	0.273	0.158	0.158		1.010		0.195	0.112	0.112
Inter-action	nil			nil			neg.			nil			neg.		

AMOUNT OF SOLUBLE NITROGEN IN TREE TISSUES AT HARVEST 2

$\log_e$ mg N

N Treatment	Roots			Stock + Stem + 1 Yr Old Shoots			New Shoots			Whole Tree		
	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean
N1N0	3.944			2.479			2.607			4.348		
N1N3	3.926	3.944 (N1)	4.790 (N0)	2.717	2.627 (N1)	3.564 (N0)	3.822	3.765 (N1)	3.688 (N0)	4.722	4.786 (N1)	5.237 (N0)
N1N9	3.962			2.684			4.568			5.237		
N3N0	4.620			3.337			3.689			5.135		
N3N3	4.714	4.737 (N3)	4.788 (N3)	3.425	3.424 (N3)	3.651 (N3)	4.374	4.373 (N3)	4.477 (N3)	5.410	5.464 (N3)	5.521 (N3)
N3N9	5.028			3.510			5.056			5.847		
N9N0	5.308			4.875			4.769			6.378		
N9N3	5.723	5.831 (N9)	4.984 (N9)	4.811	4.848 (N9)	3.684 (N9)	5.235	5.223 (N9)	5.196 (N9)	6.430	6.501 (N9)	5.943 (N9)
N9N9	5.962			4.360			5.664			6.695		
L.S.D. 5%	NS	0.201	NS	NS	0.164	NS	0.174	0.101	0.101	0.207	0.119	0.119
1%		0.272			0.223		0.236	0.136	0.136	0.280	0.162	0.162
0.1%		0.364			0.298		0.316	0.183	0.183	0.375	0.217	0.217
Inter-action	nil			nil			neg.			neg.		

AMOUNT OF INSOLUBLE NITROGEN IN TREE TISSUES AT HARVEST 2

Log_e mg N

N Treatment	Roots			Stock + Stem + 1 Yr Old Shoots			New Shoots			Whole Tree		
	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean
N1N0	5.784			4.280			4.890			6.279		
N1N3	6.057	5.995 (N1)	6.317 (N0)	4.492	4.435 (N1)	4.890 (N0)	6.013	5.947 (N1)	5.942 (N0)	6.831	6.828 (N1)	6.997 (N0)
N1N9	6.145			4.535			6.939			7.375		
N3N0	6.284			4.692			5.977			6.956		
N3N3	6.578	6.476 (N3)	6.510 (N3)	4.812	4.855 (N3)	4.988 (N3)	6.526	6.545 (N3)	6.609 (N3)	7.335	7.317 (N3)	7.363 (N3)
N3N9	6.566			5.060			7.132			7.661		
N9N0	6.832			5.697			6.959			7.757		
N9N3	6.895	6.929 (N9)	6.573 (N9)	5.659	5.690 (N9)	5.103 (N9)	7.288	7.272 (N9)	7.213 (N9)	7.924	7.935 (N9)	7.720 (N9)
N9N9	7.009			5.714			7.568			8.125		
L.S.D. 5%	NS	0.155	0.155	0.171	0.098	0.098	0.153	0.088	0.088	0.127	0.074	0.074
1%		0.210	0.210	0.231	0.134	0.134	0.207	0.120	0.120	0.173	0.100	0.100
0.1%		0.281	0.281	0.309	0.179	0.179	0.278	0.160	0.160	0.231	0.134	0.134
Inter-action	nil			neg.			neg.			neg.		

AMOUNT OF ARGININE NITROGEN IN TREE TISSUES AT HARVEST 2

mg N

N Treatment	Roots $\log_e (x+1)$			Stock + Stem + 1Yr Old Shoots	New Shoots	Whole Tree $\log_e (x+1)$		
	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Inter-action Mean	Inter-action Mean	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean
N1N0	1.380			0.3	0	1.421		
N1N3	1.851	1.770 (N1)	2.350 (N0)	0	0.6	1.933	1.876 (N1)	2.464 (N0)
N1N9	2.080			0	2.0	2.275		
N3N0	1.667			0.4	0	1.694		
N3N3	3.159	2.768 (N3)	3.716 (N3)	0	1.6	3.209	2.807 (N3)	3.274 (N3)
N3N9	3.478			0.7	1.2	3.517		
N9N0	4.005			15.8	1.1	4.276		
N9N3	4.517	4.320 (N9)	3.333 (N9)	15.0	5.8	4.681	4.514 (N9)	3.452 (N9)
N9N9	4.441			10.3	6.3	4.585		
L.S.D. 5%	NS	0.487	0.487	-*	-	NS	0.505	0.505
1%		0.661	0.661	-	-		0.685	0.685
0.1%		0.855	0.855	-	-		0.917	0.917
Inter-action	nil			-	-	nil		

* Insufficient data for analysis

AMOUNT OF TOTAL α-AMINO NITROGEN IN TREE TISSUES AT HARVEST 2

Log$_e$ mgN

N Treatment	Roots			Stock + Stem + 1 Yr Old Shoots			New Shoots			Whole Tree		
	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean
N1NO	2.624			0.807			0.735			2.901		
N1N3	2.754	2.578 (N1)	3.436 (NO)	0.778	0.476 (N1)	1.898 (NO)	2.161	1.958 (N1)	1.693 (NO)	3.283	3.217 (N1)	3.781 (NO)
N1N9	2.357			-0.158			2.977			3.466		
N3NO	3.207			1.573			1.712			3.564		
N3N3	3.348	3.411 (N3)	3.483 (N3)	0.900	1.424 (N3)	1.624 (N3)	2.515	2.481 (N3)	2.714 (N3)	3.788	3.873 (N3)	3.991 (N3)
N3N9	3.678			1.799			3.215			4.269		
N9NO	4.476			3.315			2.633			4.878		
N9N3	4.349	4.357 (N9)	3.427 (N9)	3.194	3.221 (N9)	1.598 (N9)	3.466	3.120 (N9)	3.151 (N9)	4.902	4.858 (N9)	4.176 (N9)
N9N9	4.245			3.154			3.260			4.794		
L.S.D. 5%	NS	0.262	NS	0.820	0.474	NS	0.383	0.221	0.221	0.352	0.203	0.203
1%		0.355		1.112	0.642		0.519	0.300	0.300	0.476	0.275	0.275
0.1%		0.476		1.488	0.859		0.696	0.402	0.402	0.638	0.368	0.368
Inter-action	nil			neg.			neg.			neg.		

CONCENTRATION OF TOTAL NITROGEN IN TREE TISSUES AT HARVEST 2

Log$_e$ mg N/g d.wt.

N Treatment	Roots			Stock + Stem + 1 Yr Old Shoots			New Shoots			Abscissed Leaves		
	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean
N1N0	1.796			1.233			2.904			2.546		
N1N3	1.941	1.928 (N1)	1.985 (N0)	1.193	1.256 (N1)	1.367 (N0)	3.003	3.032 (N1)	2.939 (N0)	2.520	2.625 (N1)	2.649 (N0)
N1N9	2.048			1.341			3.187			2.808		
N3N0	1.928			1.317			2.812			2.650		
N3N3	2.041	2.054 (N3)	2.097 (N3)	1.326	1.372 (N3)	1.362 (N3)	3.008	3.006 (N3)	3.063 (N3)	2.738	2.715 (N3)	2.689 (N3)
N3N9	2.194			1.473			3.199			2.757		
N9N0	2.231			1.551			3.099			2.752		
N9N3	2.309	2.289 (N9)	2.190 (N9)	1.566	1.563 (N9)	1.462 (N9)	3.178	3.186 (N9)	3.222 (N9)	2.809	2.912 (N9)	2.913 (N9)
N9N9	2.327			1.572			3.282			2.174		
L.S.D. 5%	NS	0.063	0.063	NS	0.071	0.071	NS	0.074	0.074	NS	0.136	0.136
1%		0.086	0.086		0.096	0.096		0.100	0.100		0.186	0.186
0.1%		0.115	0.115		0.129	0.129		0.133	0.133		0.253	0.253
Inter-action	nil			nil			nil			nil		

CONCENTRATION OF SOLUBLE NITROGEN IN TREE TISSUES AT HARVEST 2

mg N/g d.wt. $\text{Log}_e\ (X \times 1000)$

N Treatment	Roots			Stock + Stem + 1 Yr Old Shoots			New Shoots		
	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Inter-action	1st Yr Treat. Mean	2nd Yr Treat. Mean	Inter-action	1st Yr Treat. Mean	2nd Yr Treat. Mean
N1N0	6.715			6.185			7.431		
N1N3	6.605	6.662 (N1)	7.176 (N0)	6.169	6.201 (N1)	6.698 (N0)	7.614	7.650 (N1)	7.492 (N0)
N1N9	6.665			6.250			7.904		
N3N0	7.031			6.640			7.335		
N3N3	6.957	7.117 (N3)	7.113 (N3)	6.623	6.633 (N3)	6.686 (N3)	7.654	7.634 (N3)	7.727 (N3)
N3N9	7.364			6.636			7.912		
N9N0	7.781			7.269			7.711		
N9N3	7.777	7.816 (N9)	7.306 (N9)	7.268	7.267 (N9)	6.717 (N9)	7.912	7.923 (N9)	7.937 (N9)
N9N9	7.889			7.264			8.146		
L.S.D. 5%	NS	0.235	NS	NS	0.149	NS	NS	0.122	0.122
1%		0.319			0.202			0.166	0.166
0.1%		0.427			0.270			0.222	0.222
Interaction	nil			nil			nil		

CONCENTRATION OF INSOLUBLE NITROGEN IN TREE TISSUES AT HARVEST 2

Log_e mg N/g d.wt.

N Treatment	Roots			Stock + Stem + 1 Yr Old Shoots			New Shoots		
	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean
N1N0	1.647			1.072			2.807		
N1N3	1.828	1.805 (N1)	1.770 (N0)	1.036	1.103 (N1)	1.116 (N0)	2.897	2.924 (N1)	2.839 (N0)
N1N9	1.941			1.194			3.068		
N3N0	1.744			1.086			2.716		
N3N3	1.891	1.869 (N3)	1.910 (N3)	1.103	1.156 (N3)	1.116 (N3)	2.898	2.898 (N3)	2.951 (N3)
N3N9	1.973			1.278			3.080		
N9N0	1.921			1.183			2.994		
N9N3	2.010	1.976 (N9)	1.970 (N9)	1.209	1.201 (N9)	1.227 (N9)	3.057	3.064 (N9)	3.097 (N9)
N9N9	1.997			1.210			3.141		
L.S.D. 5%	0.098	0.056	0.056	NS	0.055	0.055	NS	0.069	0.069
1%	0.132	0.076	0.076		0.074	0.074		0.093	0.093
0.1%	0.177	0.102	0.102		0.099	0.099		0.125	0.125
Inter-action	neg.			nil			nil		

CONCENTRATION OF ARGININE NITROGEN IN TREE TISSUES AT HARVEST 2

mg N/g d.wt.

N Treatment	Roots $\log_e \left[(X+0.01)\times1000\right]$			Stock + Stem + 1 Year Old Shoots	New Shoots
	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Interaction Mean	Interaction Mean
N1N0	4.021			0.012	0
N1N3	4.475	4.412 (N1)	4.691 (N0)	0	0.026
N1N9	4.740			0	0.041
N3N0	4.068			0.014	0
N3N3	5.404	5.095 (N3)	5.486 (N3)	0	0.037
N3N9	5.813			0.017	0.019
N9N0	5.984			0.176	0.019
N9N3	6.578	6.313 (N9)	5.643 (N9)	0.189	0.088
N9N9	6.376			0.113	0.081
L.S.D. 5%	NS	0.567	0.567	-*	-
1%		0.768	0.768	-	-
0.1%		1.029	1.029	-	-
Inter-action	nil			-	-

* Insufficient data for analysis

CONCENTRATION OF TOTAL α-AMINO NITROGEN IN TREE TISSUES AT HARVEST 2

mg N/g d.wt. Log$_e$ (X x 1000)

N Treatment	Roots			Stock + Stem + 1 Yr Old Shoots			New Shoots		
	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean
N1N0	5.395			4.527			5.560		
N1N3	5.431	5.296 (N1)	5.821 (N0)	4.233	4.071 (N1)	5.037 (N0)	5.949	5.841 (N1)	5.498 (N0)
N1N9	5.061			3.452			6.014		
N3N0	5.618			4.876			5.359		
N3N3	5.591	5.741 (N3)	5.808 (N3)	4.113	4.638 (N3)	4.666 (N3)	5.795	5.742 (N3)	5.962 (N3)
N3N9	6.015			4.924			6.071		
N9N0	6.450			5.709			5.576		
N9N3	6.404	6.342 (N9)	5.749 (N9)	5.652	5.640 (N9)	4.645 (N9)	6.142	5.820 (N9)	5.942 (N9)
N9N9	6.173			5.558			5.742		
L.S.D. 5%	NS	0.301	NS	0.746	0.431	NS	NS	NS	0.238
1%		0.408		1.011	0.584				0.323
0.1%		0.547		1.354	0.782				0.433
Interaction	nil			pos.			nil		

AMOUNT OF TOTAL NITROGEN IN TREE TISSUES AT HARVEST 3

Log$_e$ mgN

N Treatment	Roots			Stock + Stem + 1 Yr Old Shoots			New Shoots			Abscissed Leaves			Whole Tree		
	Interaction Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Interaction Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Interaction Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Interaction Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Interaction Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean
N1NO	5.946			4.727			4.221			3.491			6.403		
N1N3	6.700	6.611 (N1)	6.558 (NO)	5.159	5.126 (N1)	5.337 (NO)	6.381	6.078 (N1)	5.301 (NO)	3.310	3.684 (N1)	4.912 (NO)	7.382	7.335 (N1)	7.164 (NO)
N1N9	7.187			5.493			7.631			4.251			8.219		
N3NO	6.603			5.068			5.275			5.134			7.148		
N3N3	6.897	6.987 (N3)	7.034 (N3)	5.345	5.384 (N3)	5.550 (N3)	6.770	6.573 (N3)	6.768 (N3)	4.797	4.882 (N3)	4.584 (N3)	7.700	7.740 (N3)	7.779 (N3)
N3N9	7.460			5.739			7.674			4.715			8.372		
N9NO	7.124			6.216			6.409			6.112			7.940		
N9N3	7.505	7.445 (N9)	7.451 (N9)	6.147	6.235 (N9)	5.858 (N9)	7.152	7.137 (N9)	7.719 (N9)	5.644	5.837 (N9)	4.907 (N9)	8.256	8.282 (N9)	8.414 (N9)
N9N9	7.706			6.342			7.850			5.755			8.650		
L.S.D. 5%	0.161	0.093	0.093	0.221	0.128	0.128	0.170	0.098	0.098	0.497	0.287	0.287	0.087	0.050	0.050
1%	0.218	0.126	0.126	0.300	0.173	0.173	0.230	0.133	0.133	0.673	0.389	0.389	0.117	0.068	0.068
0.1%	0.293	0.169	0.169	0.404	0.232	0.232	0.308	0.178	0.178	0.902	0.521	0.521	0.157	0.091	0.091
Interaction	neg.			neg.			neg.			neg.			neg.		

AMOUNT OF SOLUBLE NITROGEN IN TREE TISSUES AT HARVEST 3

Log_e mgN

N Treatment	Roots			Stock + Stem + 1 Yr Old Shoots			New Shoots			Whole Tree		
	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean
N1N0	4.091			2.932			2.153			4.472		
N1N3	5.108	4.967 (N1)	4.951 (N0)	3.795	3.572 (N1)	3.780 (N0)	4.095	3.911 (N1)	3.299 (N0)	5.601	5.489 (N1)	5.354 (N0)
N1N9	5.703			3.988			5.485			6.393		
N3N0	4.900			3.507			3.221			5.262		
N3N3	5.273	5.342 (N3)	5.365 (N3)	3.841	3.892 (N3)	4.129 (N3)	4.523	4.449 (N3)	4.579 (N3)	5.828	5.877 (N3)	5.932 (N3)
N3N9	5.854			4.330			5.602			6.550		
N9N0	5.804			4.901			4.523			6.329		
N9N3	5.716	5.871 (N9)	5.883 (N9)	4.751	4.907 (N9)	4.462 (N9)	5.119	5.147 (N9)	5.629 (N9)	6.376	6.518 (N9)	6.598 (N9)
N9N9	6.094			5.068			5.799			6.850		
L.S.D. 5%	0.293	0.169	0.169	0.293	0.169	0.169	0.180	0.104	0.104	0.189	0.109	0.109
1%	0.396	0.229	0.229	0.397	0.229	0.229	0.244	0.141	0.141	0.256	0.148	0.148
0.1%	0.531	0.306	0.306	0.531	0.307	0.307	0.327	0.189	0.189	0.343	0.198	0.198
Inter-action	neg.			neg.			neg.			neg.		

AMOUNT OF INSOLUBLE NITROGEN IN TREE TISSUES AT HARVEST 3

Log_e mgN

N Treatment	Roots			Stock + Stem + 1 Yr Old Shoots			New Shoots			Whole Tree		
	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean
N1NO	5.776			4.545			4.085			6.172		
N1N3	6.467	6.390 (N1)	6.328 (NO)	4.857	4.879 (N1)	5.091 (NO)	6.274	5.955 (N1)	5.155 (NO)	7.174	7.121 (N1)	6.821 (NO)
N1N9	6.923			5.235			7.506			8.018		
N3NO	6.400			4.829			5.137			6.801		
N3N3	6.675	6.770 (N3)	6.820 (N3)	5.092	5.126 (N3)	5.270 (N3)	6.659	6.445 (N3)	6.648 (N3)	7.464	7.476 (N3)	7.545 (N3)
N3N9	7.234			5.458			7.540			8.162		
N9NO	6.806			5.900			6.244			7.489		
N9N3	7.320	7.202 (N9)	7.214 (N9)	5.862	5.925 (N9)	5.568 (N9)	7.011	6.989 (N9)	7.586 (N9)	7.998	7.961 (N9)	8.192 (N9)
N9N9	7.481			6.012			7.712			8.397		
L.S.D. 5%	0.167	0.096	0.096	0.226	0.130	0.130	0.175	0.101	0.101	0.108	0.062	0.062
1%	0.226	0.130	0.130	0.306	0.177	0.177	0.237	0.137	0.137	0.146	0.084	0.084
0.1%	0.303	0.175	0.175	0.410	0.236	0.236	0.317	0.183	0.183	0.195	0.113	0.113
Inter-action	neg.			neg.			neg.			neg.		

AMOUNT OF ARGININE IN TREE TISSUES AT HARVEST 3

$\log_e (X + 0.1)$ mg N

N Treatment	Roots			Stock + Stem + 1 Yr Old Shoots			New Shoots			Whole Tree		
	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean
N1N0	1.323			-1.753			-1.335			1.417		
N1N3	3.295	2.861 (N1)	2.708 (N0)	2.162	0.855 (N1)	4.299 (N0)	1.049	0.811 (N1)	0.138 (N0)	3.662	3.142 (N1)	2.894 (N0)
N1N9	3.959			2.154			2.719			4.345		
N3N0	2.674			2.883			0.091			2.839		
N3N3	3.298	3.333 (N3)	3.294 (N3)	1.970	1.641 (N3)	2.105 (N3)	1.740	1.550 (N3)	1.640 (N3)	3.702	3.675 (N3)	3.719 (N3)
N3N9	4.027			2.664			2.820			4.455		
N9N0	4.120			2.755			1.809			4.427		
N9N3	3.239	3.896 (N9)	4.088 (N9)	2.183	2.768 (N9)	2.723 (N9)	2.130	2.394 (N9)	2.837 (N9)	3.794	4.347 (N9)	4.550 (N9)
N9N9	4.278			3.366			2.972			4.821		
L.S.D. 5%	0.638	0.369	0.369	0.822	0.475	0.475	0.485	0.280	0.280	0.467	0.281	0.281
1%	0.865	0.499	0.499	1.117	0.645	0.645	0.657	0.379	0.379	0.660	0.381	0.381
0.1%	1.158	0.669	0.669	1.503	0.868	0.868	0.879	0.508	0.508	0.884	0.510	0.510
Inter-action	neg.			neg.			neg.			neg.		

AMOUNT OF TOTAL α-AMINO NITROGEN IN TREE TISSUES AT HARVEST 3

$\log_e$ mgN

N Treatment	Roots			Stock + Stem + 1 Yr Old Shoots			New Shoots			Whole Tree		
	Interaction Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Interaction Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Interaction Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Interaction Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean
N1NO	2.390			1.022			0.351			2.725		
N1N3	3.615	3.497 (N1)	3.326 (NO)	2.317	2.049 (N1)	1.965 (NO)	2.204	2.170 (N1)	1.558 (NO)	4.035	3.942 (N1)	3.695 (NO)
N1N9	4.487			2.809			3.955			5.066		
N3NO	3.251			1.577			1.495			3.565		
N3N3	3.738	3.868 (N3)	3.708 (N3)	2.140	2.255 (N3)	2.347 (N3)	2.662	2.751 (N3)	2.606 (N3)	4.184	4.322 (N3)	4.183 (N3)
N3N9	4.616			3.049			4.096			5.216		
N9NO	4.338			3.296			2.829			4.795		
N9N3	3.771	4.328 (N9)	4.659 (N9)	2.585	3.212 (N9)	3.204 (N9)	2.951	3.305 (N9)	4.061 (N9)	4.332	4.869 (N9)	5.254 (N9)
N9N9	4.874			3.755			4.134			5.480		
L.S.D. 5%	0.380	0.219	0.219	0.344	0.199	0.199	0.301	0.174	0.174	0.295	0.170	0.170
1%	0.514	0.297	0.297	0.467	0.269	0.269	0.408	0.236	0.236	0.399	0.231	0.231
0.1%	0.639	0.398	0.398	0.625	0.361	0.361	0.547	0.316	0.316	0.535	0.309	0.309
Interaction	neg.			neg.			neg.			neg.		

CONCENTRATION OF TOTAL NITROGEN IN TREE TISSUES AT HARVEST 3

Log_e mg N/g d.wt.

N Treatment	Roots			Stock + Stem + 1 Yr Old Shoots			New Shoots			Abscissed Leaves		
	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Inter-Action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean
N1N0	1.733			1.208			2.449			2.333		
N1N3	1.915	1.902 (N1)	1.862 (N0)	1.471	1.345 (N1)	1.324 (N0)	2.687	2.624 (N1)	2.514 (N0)	2.601	2.547 (N1)	2.592 (N0)
N1N9	2.058			1.357			2.734			2.705		
N3N0	1.795			1.264			2.404			2.554		
N3N3	1.931	1.966 (N3)	1.979 (N3)	1.410	1.352 (N3)	1.406 (N3)	2.627	2.598 (N3)	2.654 (N3)	2.744	2.684 (N3)	2.694 (N3)
N3N9	2.123			1.383			2.762			2.755		
N9N0	2.058			1.502			2.688			2.883		
N9N3	2.040	2.093 (N9)	2.121 (N9)	1.338	1.462 (N9)	1.429 (N9)	2.648	2.718 (N9)	2.772 (N9)	2.738	2.852 (N9)	2.796 (N9)
N9N9	2.183			1.546			2.818			2.929		
L.S.D. 5%	0.092	0.053	0.053	0.117	0.067	0.067	0.095	0.055	0.055	0.173	0.100	0.100
1%	0.125	0.072	0.072	0.158	0.091	0.091	0.129	0.074	0.074	0.235	0.136	0.136
0.1%	0.167	0.097	0.097	0.212	0.122	0.122	0.172	0.099	0.099	0.314	0.132	0.182
Inter-action	neg.			neg.			neg.			neg.		

CONCENTRATION OF SOLUBLE NITROGEN IN TREE TISSUES AT HARVEST 3

Log $_e$ (X x 1000) mg N/g d.wt.

N Treatment	Roots			Stock + Stem + 1 Yr Old Shoots			New Shoots		
	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean
N1N0	6.785			6.319			7.292		
N1N3	7.237	7.171 (N1)	7.147 (N0)	7.016	6.698 (N1)	6.675 (N0)	7.308	7.365 (N1)	7.420 (N0)
N1N9	7.489			6.759			7.496		
N3N0	7.009			6.610			7.258		
N3N3	7.268	7.235 (N3)	7.229 (N3)	6.813	6.768 (N3)	6.893 (N3)	7.288	7.381 (N3)	7.373 (N3)
N3N9	7.429			6.832			7.597		
N9N0	7.648			7.094			7.709		
N9N3	7.131	7.443 (N9)	7.473 (N9)	6.850	7.041 (N9)	6.940 (N9)	7.523	7.636 (N9)	7.590 (N9)
N9N9	7.501			7.180			7.675		
L.S.D. 5%	0.281	0.162	0.162	0.290	0.167	0.167	0.135	0.078	0.078
1%	0.381	0.220	0.220	0.393	0.227	0.227	0.183	0.106	0.106
0.1%	0.510	0.295	0.295	0.526	0.304	0.304	0.245	0.142	0.142
Interaction	neg.			neg.			neg.		

CONCENTRATION OF INSOLUBLE NITROGEN IN TREE TISSUES AT HARVEST 3

Log_e mg N/g d.wt.

N Treatment	Roots			Stem + Stock + 1 Yr Old Shoots			New Shoots		
	Interaction Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Interaction Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Interaction Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean
N1N0	1.562			1.025			2.314		
N1N3	1.681	1.680 (N1)	1.631 (N0)	1.169	1.098 (N1)	1.079 (N0)	2.580	2.501 (N1)	2.332 (N0)
N1N9	1.795			1.098			2.609		
N3N0	1.590			1.025			2.266		
N3N3	1.757	1.748 (N3)	1.763 (N3)	1.157	1.094 (N3)	1.126 (N3)	2.519	2.470 (N3)	2.534 (N3)
N3N9	1.897			1.109			2.627		
N9N0	1.739			1.186			2.565		
N9N3	1.849	1.847 (N9)	1.881 (N9)	1.053	1.152 (N9)	1.139 (N9)	2.507	2.584 (N9)	2.639 (N9)
N9N9	1.952			1.217			2.680		
L.S.D. 5%	NS	0.048	0.048	0.087	0.050	NS	0.098	0.056	0.056
1%		0.066	0.066	0.118	0.068		0.132	0.076	0.076
0.1%		0.088	0.088	0.158	0.091		0.177	0.102	0.102
Interaction	nil			neg.			neg.		

CONCENTRATION OF ARGININE NITROGEN IN TREE TISSUES AT HARVEST 3

$\mathrm{Log}_e \left[(\% + 0.01) \times 1000\right]$ mg N/g d.wt.

N Treatment	Roots			Stock + Stem + 1 Yr Old Shoots			New Shoots		
	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean
N1N0	4.171			2.690			3.581		
N1N3	5.469	5.139 (N1)	5.009 (N0)	5.420	4.369 (N1)	3.772 (N0)	4.365	4.252 (N1)	4.273 (N0)
N1N9	5.778			4.997			4.810		
N3N0	4.866			3.612			4.189		
N3N3	5.343	5.282 (N3)	5.218 (N3)	4.999	4.625 (N3)	4.941 (N3)	4.594	4.557 (N3)	4.529 (N3)
N3N9	5.637			5.263			4.887		
N9N0	5.990			5.014			5.049		
N9N3	4.841	5.517 (N9)	5.712 (N9)	4.405	4.978 (N9)	5.258 (N9)	4.628	4.908 (N9)	4.915 (N9)
N9N9	5.720			5.515			5.048		
L.S.D. 5%	0.624	NS	0.360	0.674	0.389	0.389	0.518	0.299	0.299
1%	0.846		0.488	0.916	0.529	0.529	0.702	0.405	0.405
0.1%	1.133		0.654	1.232	0.712	0.712	0.940	0.542	0.542
Interaction	neg.			neg.			neg.		

CONCENTRATION OF TOTAL α -AMINO NITROGEN IN TREE TISSUES AT HARVEST 3

Log$_e$ (X x 1000) mg N/g d.wt.

N Treatment	Roots			Stock + Stem + 1 Yr Old Shoots			New Shoots		
	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean	Inter-action Mean	1st Yr Treat. Mean	2nd Yr Treat. Mean
N1N0	5.082			4.410			5.490		
N1N3	5.745	5.701 (N1)	5.542 (N0)	5.537	5.176 (N1)	4.860 (N0)	5.419	5.625 (N1)	5.630 (N0)
N1N9	6.274			5.580			5.966		
N3N0	5.361			4.682			5.536		
N3N3	5.733	5.762 (N3)	5.572 (N3)	5.114	5.132 (N3)	5.111 (N3)	5.427	5.684 (N3)	5.400 (N3)
N3N9	6.191			5.600			6.091		
N9N0	6.183			5.489			6.015		
N9N3	5.237	5.900 (N9)	6.249 (N9)	4.682	5.346 (N9)	5.632 (N9)	5.355	5.793 (N9)	6.022 (N9)
N9N9	6.281			5.867			6.011		
L.S.D. 5%	0.393	NS	0.227	0.355	NS	0.205	NS	NS	0.191
1%	0.533		0.308	0.482		0.278			0.259
0.1%	0.714		0.412	0.645		0.372			0.347
Interaction	neg.			neg.			nil		

APPENDIX C (a)(iii).- Sand Culture Experiment; Examples of Computer Printouts and Analyses of Variance

(a) Examples of Computer Printouts

(1) 2 way A.O.V., type 1

```
371/A123/68 EXPT 1 H1 CONC NIT FRACT ROOTS AMIDE N (MGM/GM)

ROW MEANS    SED 2.6249E-02
 2.3544E-01 2.2178E-01  2.1033E-01  1.9500E-01

COL MEANS    SED 2.2732E-02
 1.6458E-01 2.2517E-01  2.5717E-01

R*C MEANS    SED 4.5464E-02
 2.1533E-01 1.2133E-01  2.0867E-01  1.1300E-01
 2.3900E-01 2.4700E-01  1.9767E-01  2.1700E-01
 2.5200E-01 2.9700E-01  2.2467E-01  2.5500E-01

SOURCE      DF      SS            MS                  VR
TOTAL       35      0.167122      1.674005(CF)
ROWS         3      0.007957      0.002652            0.855
COLS         2      0.053064      0.026532            8.557
R*C          6      0.031690      0.005282            1.704
ERROR       24      0.074411      0.003100      GM = 2.15639E-01
```

(2) 2 way A.O.V., type 2

```
449/F123/25  EXPT I H2N CONC STCK+ST+LYROSH MGM/GDWT LOGE

BLOCK MEANS   SED 3.9771E-02
 0.4484E 00  1.4445E 00  1.3372E 00  1.3577E 00

ROW MEANS     SED 3.4443E-02
 1.3669E 00  1.3622E 00  1.4617E 00

COL MEANS     SED 3.4443E-02
 1.2555E 00  1.3719E 00  1.5634E 00

R*C MEANS     SED 5.9657E-02
 1.2327E 00  1.1930E 00  1.3407E 00
 1.3166E 00  1.3262E 00  1.4730E 00
 1.5512E 00  1.5675E 00  1.5715E 00

SOURCE    DF        SS              MS          VR          OMISS VALS
TOTAL     35  9.50463243E-01 7.02525206E-01(CF) GM=1.3969E 00 CV=0.0604
BLOCKS     3  9.02506523E-02 3.00835508E-02  4.226E 00
ROWS       2  7.57076666E-02 3.78538333E-02  5.318E 00
COLS       2  5.801947.2E-01 2.90097356E-01  4.076E 01
R*C        4  3.34796123E-02 8.36990308E-03  1.176E 00
ERROR     24  1.70830600E-01 7.11794166E-03
```

(3) Simple Regression Analysis

```
425/123 EXPT I L NEW SHOOTS N2/TOT N N1 LOG10

ID     N     X MEAN         Y MEAN          XCSS             XYCSP          YCSS
ID     R          SLOPE           CONST       SE OF SLOPE, CONST            SD

1     12  .315177E+01  .193373E+01  .88654000E-00  .13341510E+01  .37383990E+01
 1  .8427         1.730492       -3.520337        .349605      1.105966        .329175
2     12  .315174E+01  .247087E+01  .88653000E-00  .67696000E-00  .57750600E-00
 2  .9461          .763606         .064175        .082660       .261493        .077829
3     12  .315174E+01  .237659E+01  .88653000E-00  .16859000E-00  .52197000E-01
 3  .7837          .190163        2.277235        0.47659       .150766        .044873

PARLL REG .26596000E+01 .23797010E+01 .43631020E+01 .89475898E-00 .16219172E-00

POOL   36 .31517583E+01 .24270691E+01 .26596600E+01 .23795600E+01 .97365600E+01
     .4676         .894685        -.392764        .290048        .917557        .473025

SOURCE          DF          SS          MS          VR
SLOPES           2.      1.074569     .537234     13.844
DISPLACEMENTS    2.      5.363758    2.684379     69.168
ERROR           30.      1.164273     .038809
```

(b) **Example of Covariance Analysis**

e.g. Stem diameter per tree at the end of the first year versus stem diameter per tree at planting

$\text{Log}_{10}(10x)$ in.

Source	X	XY	Y	df	Reg.SS	Res.SS	df	MS	VR
Total	0.178021	0.023687	2.008041	107					
Block	0.079143	0.022972	0.006814	3		1.84827			
Treatment	0.001337	-0.015612	1.845519	2		1.814990	2	0.907497	634.7
BXT	0.003918	0.002595	0.008346	6	0.001718	0.006627	5	0.001325	0.866
Error	0.093623	0.013732	0.147362	96	0.002014	0.145348	95	0.001530	
Pooled) BXT + Error) Error	0.097541	0.016327	0.155708	102	0.002733	0.152975	101	0.00514	1.81 (Reg.)
Treat. + Pooled Error	0.098878	0.000715	2.001227	104	0.000005	2.001222	103		
Adj. Treat.						1.848247	2	0.924123	610.39
Pooled Error + Blocks	0.176684	0.039299	0.162522	105	0.008741	0.153781	104	0.001479	5.91 (Reg.)
Treatment + Pooled Error + Blocks	0.178021	0.023687	2.008041	107	0.003152	2.004889	106		
Adj. Treat.				2		1.851108	2	0.925554	625.80
Means	0.3883	0.5211	0.7070		SED = 0.008790				
Adj. Means	0.3892	0.5179	0.7092		SED = 0.009284				

APPENDIX C (b)(i).- Isotope Experiment; Experimental Data

UPTAKE OF AMINO ACID SOLUTIONS (ml) BY 1 YEAR OLD ELBERTA PEACH TREES AS A RESULT OF ROOT INJECTION OVER A 31 HOUR PERIOD
(First injection expt.)

	Compd. Injected		L-Arginine (50 µg/ml)						L-Asparagine (50 µg/ml)					
Date	Water Temp. (500ml vol)	Time	Replicate No.						Replicate No.					
			1	2	3	4	5	6	1	2	3	4	5	6
5/6/63	13.4	10am	0.10	0.20	0.10	0.30	0.20	0	0.20	0	0.10	0.20	0.30	0.10
	14.0	11 "	0.10	0.20	0.10	0.30	0.15	0.05	0.20	0.05	0.20	0.20	0.30	0.10
	14.9	12 "	0.15	0.25	0.15	0.30	0.15	0	0.20	0.05	0.20	0.20	0.30	0.05
	16.5	1pm	0.25	0.35	0.15	0.30	0.25	0.10	0.30	0.05	0.30	0.35	0.35	0.15
	16.5	2 "	0.30	0.35	0.25	0.40	0.30	0.10	0.35	0.10	0.40	0.45	0.40	0.20
	16.8	3 "	0.20	0.25	0.20	0.30	0.25	0.05	0.30	0.10	0.30	0.35	0.30	0.10
	16.1	4 "	0.25	0.40	0.20	0.40	0.25	0.15	0.40	0.15	0.40	0.50	0.40	0.20
	15.2	5 "	0.15	0.30	0.20	0.35	0.25	0.05	0.30	0.15	0.30	0.40	0.30	0.20
	14.7	6 "	0.10	0.20	0.15	0.25	0.15	0.10	0.30	0.15	0.25	0.30	0.30	0.10
	13.7	7 "	0.10	0.20	0.10	0.25	0.15	0.05	0.20	0.10	0.10	0.20	0.30	0.10
	13.0	8 "	0.10	0.10	0.05	0.25	0.05	0.10	0.20	0.05	0.05	0.20	0.20	0.10
6/6/63	11.3	10am	0.05	0.05	0.05	0.05	0.05	0.05	0.10	0.05	0.10	0.10	0.15	0
	12.1	11 "	0.05	0.05	0.05	0.20	0.05	0	0.10	0	0.05	0.10	0.20	0.05
	13.3	12 "	0.05	0.10	0.05	0.15	0.10	0.05	0.10	0.05	0.10	0.10	0.20	0.05
	13.3	1 pm	0.20	0.20	0.15	0.25	0.15	0.05	0.15	0.05	0.25	0.25	0.20	0.05
	13.0	2 "	0.10	0.20	0.10	0.20	0.15	0.05	0.20	0.05	0.20	0.25	0.15	0.10
	12.8	3 "	0.15	0.15	0	0.20	0.15	0	0.15	0.05	0.20	0.20	0.25	0.10
	12.4	4 "	0.05	0.15	0.20	0.20	0.10	0.05	0.15	0.05	0.10	0.20	0.20	0.05

UPTAKE OF AMINO ACID SOLUTIONS (ml) BY 1 YEAR OLD ELBERTA PEACH TREES AS A RESULT OF ROOT INJECTION OVER A 31 HOUR PERIOD

(Second injection expt.)

Date and Time	Air Temp. (°C)	Soil Temp. (°C)	Water Temp. (500 ml vol.) (°C)	Water Temp. (50 ml vol.) (°C)	Concn. of L-Arginine in Injection Soln. (µg/ml)						Concn. of L-Asparagine in Injection Soln. (µg/ml)					
					50		500		5,000		50		500		5,000	
12/6/63 9 a.m.	15.2	12.8	12.9	14.0	-	-	-	-	-	-	-	-	-	-	-	-
10 "	19.5	14.0	16.5	19.5	0.05	0	0.10	0.15	0.05	0.10	0.05	0.10	0.10	0.20	0.20	0.05
11 "	19.3	16.7	20.7	26.0	0.05	0.10	0.25	0.50	0.25	0.20	0.05	0.25	0.35	0.40	0.10	0.05
12	20.6	19.2	21.5	24.3	0.15	0.05	0.40	0.45	0.35	0.20	0.05	0.25	0.55	0.50	0.25	0.05
1 p.m.	20.7	20.3	21.5	22.6	0.20	0.05	0.55	0.60	0.40	0.30	0.10	0.35	0.70	0.60	0.30	0.05
2 "	22.5	21.1	21.6	22.6	0.20	0.15	0.50	0.60	0.30	0.30	0.10	0.30	0.60	0.55	0.35	0.05
3 "	22.2	21.5	22.5	22.6	0.15	0.05	0.45	0.75	0.30	0.40	0.05	0.40	0.60	0.65	0.30	0.05
4 "	18.9	21.8	22.3	20.8	0.25	0.20	0.45	0.90	0.35	0.40	0.15	0.40	0.65	0.70	0.30	0.15
5 "	16.8	21.2	20.4	18.8	0.20	0.10	0.35	0.60	0.25	0.25	0.10	0.25	0.45	0.55	0.30	0.05
6 "	15.9	19.3	18.5	16.9	0.20	0.10	0.25	0.45	0.10	0.15	0.05	0.15	0.30	0.35	0.25	0.10
7 "	16.1	18.4	17.0	16.1	0.15	0.10	0.20	0.40	0.20	0.20	0.10	0.15	0.35	0.35	0.15	0.10
13/6/63 9 a.m.	14.4	12.4	12.9	14.3	1.20	0.70	1.65	3.10	0.90	1.20	0.60	1.05	2.20	2.45	1.50	0.60
10 "	17.1	13.7	15.3	17.8	0.05	0	0.05	0.15	0	0.05	0	0.05	0.05	0.10	0.05	0
11 "	16.9	15.8	19.0	24.1	0.05	0	0.10	0.25	0.05	0.05	0.05	0.10	0.15	0.10	0.05	0.05
12	17.4	17.6	18.9	20.9	0.05	0.05	0.25	0.25	0.10	0.10	0.05	0.10	0.30	0.20	0.10	0
1 p.m.	17.6	18.3	18.7	19.9	0.10	0.05	0.25	0.35	0.15	0.15	0.05	0.10	0.35	0.25	0.10	0.05
2 "	19.2	18.7	18.9	20.0	0.15	0.05	0.30	0.35	0.15	0.15	0.05	0.10	0.35	0.25	0.15	0.05
3 "	19.0	19.0	21.4	20.1	0.10	0.05	0.30	0.50	0.15	0.25	0.05	0.20	0.40	0.25	0.15	0.05
4 "	16.9	19.0	20.6	18.9	0.15	0.05	0.30	0.55	0.15	0.25	0.05	0.15	0.35	0.30	0.10	0.10

UPTAKE OF AMINO ACID SOLUTIONS (ml) BY 1 YEAR OLD PEACH TREES AS A RESULT OF SHOOT INJECTION OVER A 31 HOUR PERIOD

(Second injection expt.)

Date and Time		Air Temp. (°C)	Soil Temp. (°C)	Water Temp. (500 ml vol.) (°C)	Water Temp. (50 ml vol.) (°C)	Concn. of L-Arginine HCl in Injection Soln. (ug/ml)						Concn. of L-Asparagine in Injection Soln. (ug/ml)					
						50		500		5,000		50		500		5,000	
12/6/63	9 a.m.	15.2	12.8	12.9	14.0	-	-	-	-	-	-	-	-	-	-	-	-
	10 "	19.5	14.0	16.5	19.5	0.10	0.10	0.25	0.25	0.10	0.20	0.10	0.15	0.10	0.05	0.15	0.20
	11 "	19.3	16.7	20.7	26.0	0.05	0.30	0.30	0.30	0.25	0.40	0.15	0.30	0.25	0.10	0.35	0.35
	12	20.6	19.2	21.5	24.3	0.35	0.35	0.45	0.40	0.35	0.50	0.30	0.35	0.35	0.15	0.40	0.35
	1 p.m.	20.7	20.3	21.5	22.6	0.40	0.50	0.55	0.50	0.45	0.65	0.40	0.40	0.45	0.15	0.45	0.45
	2 "	22.5	21.1	21.6	22.6	0.30	0.45	0.50	0.50	0.35	0.55	0.35	0.40	0.45	0.15	0.45	0.45
	3 "	22.2	21.5	22.5	22.6	0.45	0.65	0.50	0.55	0.35	0.80	0.35	0.40	0.40	0.20	0.40	0.55
	4 "	18.9	21.8	22.3	20.8	0.30	0.55	0.50	0.65	0.35	0.80	0.40	0.45	0.40	0.15	0.45	0.60
	5 "	16.8	21.2	20.4	18.8	0.25	0.35	0.30	0.35	0.25	0.40	0.25	0.25	0.25	0.15	0.25	0.35
	6 "	15.9	19.3	18.5	16.9	0.20	0.20	0.30	0.30	0.25	0.35	0.20	0.10	0.20	0.15	0.25	0.25
	7 "	16.1	18.4	17.0	16.1	0.20	0.20	0.20	0.25	0.20	0.25	0.15	0.20	0.20	0.10	0.25	0.25
13/6/63	9 a.m.	14.4	12.4	12.9	14.3	1.30	1.45	1.65	2.05	1.75	2.50	1.20	1.15	1.60	0.80	1.55	1.95
	10 "	17.1	13.7	15.3	17.8	0.10	0.10	0.10	0.15	0.10	0.10	0.05	0.05	0.10	0.05	0.05	0.10
	11 "	16.9	15.8	19.0	24.1	0.05	0.15	0.15	0.15	0.10	0.25	0.10	0.20	0.20	0.05	0.05	0.15
	12	17.4	17.6	18.9	20.9	0.15	0.20	0.20	0.25	0.25	0.30	0.15	0.20	0.25	0.05	0.10	0.25
	1 p.m.	17.6	18.3	18.7	19.9	0.20	0.25	0.40	0.30	0.25	0.40	0.25	0.30	0.35	0.10	0.05	0.25
	2 "	19.2	18.7	18.9	20.0	0.20	0.30	0.35	0.30	0.25	0.40	0.20	0.25	0.35	0.05	0.10	0.25
	3 "	19.0	19.0	21.4	20.1	0.20	0.30	0.35	0.40	0.30	0.60	0.30	0.40	0.35	0.10	0.05	0.35
	4 "	16.9	19.0	20.6	18.9	0.25	0.40	0.35	0.45	0.25	0.55	0.20	0.35	0.30	0.10	0.15	0.30

PART VIII

REFERENCES

Aldrich, W.W. (1931).- Nitrogen intake and translocation in apple trees following fall, winter and spring sodium nitrate applications. *Proc. Amer. Soc. hort. Sci.* **28** : 532-538.

Anon (1964).- Effect of daylength and day and night temperatures on the induction of winter rest in peach seedlings. Annu. Rept Fruit Food Technol. Res. Inst. Stellenbosch, South Africa. pp.27-28.

Batjer, L.P. and Degman, E.S. (1940).- Effects of various amounts of nitrogen, potassium and phosphorous on growth and assimilation in young apple trees. *J. Agric. Res.* **60** : 101-116.

Batjer, L.P., Magness, J.R. and Regeimbal, L.O. (1943).- Nitrogen intake of dormant apple trees at low temperature . *Proc. Amer. Soc. hort. Sci.* **42** : 69-73.

Baxter, P. (1965).- A simple and rapid test, using the ninhydrin method, for the determination of the nitrogen status of fruit trees. *J. hort. Sci.* **40** : 1-12.

Block, R.J., Durrum, E.L. and Zweig, G. (1958).- "A Manual of Paper Chromatography and Paper Electrophoresis." 2nd Ed. 710 pp. (Academic Press Inc.: New York).

Blommaert, K.L.J. (1963a).- Some studies on the uptake of ^{14}C-labelled thiourea by dormant peach buds. *S. Afr. J. Agric. Sci.* **6** : 375-380 .

Blommaert, K.L.J. (1963b).- Some studies on the metabolism of ^{14}C-labelled thiourea by peach buds in relation to its rest-breaking effect. In "Application of Isotopes and Radiation", National Conference on Nuclear Energy, Pretoria, South Africa, pp.464-470.

Bollard, E.G. (1953).- The use of tracheal sap in the study of apple-tree nutrition. *J. exp. Bot.* **4** : 363-8 .

Bollard, E.G. (1957*a*).- Composition of the nitrogen fraction of apple tracheal sap. *Aust. J. Biol. Sci.* **10** : 279-287.

Bollard, E.G. (1957*b*).- Nitrogenous compounds in tracheal sap of woody members of the family Rosaceae. *Aust. J. Biol. Sci.* **10** : 288-291.

Boynton, D., Margolis, D. and Gross, C.R. (1953).- Exploratory studies on nitrogen metabolism by McIntosh apple leaves sprayed with urea. *Proc. Amer. Soc. hort. Sci.* **62** : 135-146.

Boynton, D., Yatsu, L. and Kwong, S.S. (1961).- Some factors influencing the intermediary nitrogenous compounds in leaves of the strawberry plant (*Fragaria chiloensis*. var. ananassa). In "Plant Analysis and Fertilizer Problems." pp. 301-313. (Ed. W. Reuther) (Amer. Inst. Biol. Sci. Publication No.8).

Burrell, A.B. (1928).- Immediate and residual effects of sodium nitrate upon yield and growth of McIntosh apple trees. *Proc. Amer. hort. Sci.* **25** : 258-262 .

Cameron, S.H. and Appleman, D. (1933).- The distribution of total nitrogen in the orange tree. *Proc. Amer. Soc. hort. Sci.* **30** : 341-348.

Chapman, H.D. and Parker, E.R. (1942).- Weekly absorption of nitrate by young bearing orange trees growing out of doors in solution cultures. *Pl. Physiol.* **17** : 366-376.

Combes, M.R. (1926*a*).- Étude de la migration automnale des substances azotées chez le chêne, par l'analyse de plantes entières. *C.r. Acad. Sci., Paris* **182** : 984-987.

Combes, M.R. (1926*b*).- La migration des substances azotées chez le Hêtre au cours du jaunissement automnal. *C.r. Acad. Sci., Paris* **182** : 1169-1171.

Cook, G.B. and Duncan, J.F. (1952).- "Modern Radiochemical Practice." 407 pp. (Clarendon Press: Oxford).

Cullinan, F.P. (1931).- Some relationships between tree response and internal composition of the shoots of the peach. *Proc. Amer. Soc. hort. Sci.* **28** : 1-5 .

Davidson, O.W. and Shive, J.W. (1935).- Determination of the nitrogenous fractions in vegetative tissue of the peach. *Pl. Physiol.* **10** : 73-92.

Deep, I. (1958).- Crown gall chemotherapy with terramycin. *Pl. Dis. Reptr* **42** : 1210-1213.

Eckerson, S.H. (1931).- Seasonal distribution of reductase in the various organs of the apple tree. Contr. Boyce Thompson Inst. 3 : 405-412.

Gardner, V.R., Bradford, F.C. and Hooker, H.D. (1952).- "The Fundamentals of Fruit Production." 3rd Ed. 739 pp. (McGraw Hill Book Inc.: New York).

Gilboe, D.D. and Williams, J.N. (1956).- Evaluation of the Sakaguchi reaction for the quantitative determination of arginine. Proc. Soc. exp. Biol. Med. 91 : 535-536.

Grant, D.R. (1963).- Reagent stability in Rosen's ninhydrin method of analysis for amino acids. Anal. Biochem. 6 : 109-110.

Harley, C.P., Moon, H.H. and Regeimbal, L.O. (1949).- A study of correlation between growth and certain nutrient reserves in young apple trees. Proc. Amer. Soc. hort. Sci. 53 : 1-5.

Harley, C.P. Regeimbal, L.O. and Moon, H.H. (1958).- The role of nitrogen reserves in new growth of apple and the transport of P^{32} from roots to leaves during early-spring growth. Proc. Amer. Soc. hort. Sci. 72 : 57-63.

Heinicke, A.J. (1935).- Root growth in young apple trees made shortly before and after defoliation. Proc. Amer. Soc. hort. Sci. 33 : 164-165.

Hewitt, E.J. (1952).- Sand and water culture methods used in the study of plant nutrition. Commonw. Bur. hort. Plantn Crops Tech. Bull. No.22, 240pp.

Humprhies, E.C. (1955).- Mineral components and plant analysis. In Modern Methods of Plant Analysis 1 : 482-483. (Ed. K. Paech and M.V. Tracy)(Springer-Verlag: Berlin).

Jacobson, L. (1951).- Maintenance of iron supply in nutrient solutions by a single addition of ferric potassium ethylenediamine tetra-acetate. Pl. Physiol. 26 : 411-413.

Jennings, A.C. (1962).- The chemistry of methods of estimation of nitrogen and sulphur in biological materials; studies to develop improved methods for these and other determinations. M. Sc. Thesis, University of Adelaide.

Karmarkar, D.V. (1934).- The seasonal cycles of nitrogenous and carbohydrate materials in fruit trees I. The seasonal cycles of total nitrogen and of soluble nitrogen compounds in the wood, bark and leaves portions of terminal shoots of apple trees under two cultural systems - grass plus annual spring nitrate and arable without nitrogenous fertilizer. J. Pomol. 12 : 177-221.

Kench, J.E. (1938).- The seasonal cycles of ash, carbohydrate and nitrogenous constituents in the terminal shoots of apple trees and the effects of five vegetatively propagated rootstocks on them III. Nitrogenous constituents. J. Pomol. 16 : 346-363.

Kramer, P.J. and Kozlowski, T.T. (1960).- "Physiology of Trees." 642pp. (McGraw Hill Book Co.: New York).

Lawrence, J.M., Miller, J.V. and Rasmussen, L.W. (1960).- Estimation of amino acids in plant tissue by paper chromatography. Wash. Agric. Exp. Sta. Tech. Bull. No.32, 12pp.

Lindner, A.C., Benson, N.R. and Bullock, R.M. (1954).- Plum, prune and apricot nutrition. IN "Mineral Nutrition of Fruit Crops". pp.666-683 (Ed. N.F. Childers) (Somerset Press: Somerville).

Loomis, W.E. (1934).- The translocation of nitrogen in woody plants. Proc. Amer. Soc. hort. Sci. 32 : 61-64.

Loomis, W.E. (1935).- Translocation and growth balance in woody plants. Ann. Bot. 49 : 247-272.

Laustalot, A.J. and Lagasse, F.S. (1946).- A comparison of winter and early spring applications of nitrogen to tung trees. Proc. Amer. Soc. hort. Sci. 48 : 51-58.

McKee, H.S. (1958a).- Nitrogen metabolism in leaves. In Handb. PflPhysiol. VIII : 516-553. (Ed. W. Ruhland) (Springer-Verlag: Berlin).

McKee, H.S. (1958b).- Protein metabolism in flowers. In Handb. PflPhysiol. VIII : 554-563. (Ed. W. Ruhland) (Springer-Verlag: Berlin).

McKee, H.S. (1958c).- Protein metabolism of vegetative storage organs. In Handb. PflPhysiol. VIII : 564-580. (Ed. W. Ruhland) (Springer-Verlag: Berlin).

McKee, H.S. (1962).-"Nitrogen Metabolism In Plants." 728pp. (Clarendon Press: Oxford).

McKenzie, H.A. and Wallace, H.S. (1954).- The kjeldahl determinations of nitrogen: a critical study of digestion conditions - temperature, catalyst and oxidising agent. *Aust. J. Chem.* **7** : 55-70.

Maggs, D.H. (1965).- Growth rates in relation to assimilate supply and demand II. The effect of particular leaves and growing regions in determining the dry matter distribution in young apple leaves. *J. exp. Bot.* **16** : 387-404.

Mason, A.C. and Whitfield, A.B. (1960).- Seasonal changes in the uptake and distribution of mineral elements in apple trees. *J. hort. Sci.* **35** : 35-55.

Mochizuki, T. and Hanada, S. (1956).- The seasonal changes of the constituents of young apple trees II. Nitrogen, phosphorous and potassium. Hirosaki Univ. Fac. Agric. Bull. No.2 pp.25-39 (Japanese).

Moses, V. (1962).- Decomposition of nine amino acids during chromatography on paper. *J. Chromatog.* **9** : 241-243.

Mulay, A.S. (1931).- Seasonal changes in total, soluble, soluble-protein, non-protein and insoluble nitrogen in current year's shoots of Bartlett pear. *Pl. Physiol.* **6** : 519-529.

Mulay, A.S. (1932).- Seasonal changes in the composition of the non-protein nitrogen in the current year's shoots of Bartlett pear. *Pl. Physiol.* **7** : 107-118

Murneek, A.E. (1930).- Quantitative distribution and seasonal fluctuations of nitrogen in apple trees. *Proc. Amer. Soc. hort. Sci.* **27** : 228-231.

Murneek, A.E. and Logan, J.C. (1932).- Autumnal migration of nitrogen and carbohydrates in the apple tree with special reference to leaves. Mo. Agric. Exp. Sta. Bull. No.171, 30pp.

Murneek, A.E. (1936).- Fertilizing fruit trees with nitrogen. Mo. Agric. Exp. Sta. Bull. No.363, 20pp.

Murneek, A.E. (1942).- Quantitative distribution of nitrogen and carbohydrates in apple trees. Mo. Agric. Exp. Sta. Res. Bull. No.348, 28pp.

Nasharty, A.H. (1944).- Accumulation of nitrogen from different sources by peach trees. *Proc. Amer. Soc. hort. Sci.* **45** : 5-8.

Nightingale, G.T. (1934).- Ammonium and nitrate nutrition of dormant Delicious apple trees at 48°F. Bot. Gaz. 95 : 437-452.

Nightingale, G.T. (1937).- The nitrogen nutrition of green plants. Bot. Rev. 3 : 85-174.

Oland, K. (1954).- Nitrogenous constituents of apple maidens grown under different nitrogen treatments. Physiol. Plant. 7 : 463-474.

Oland, K. and Yemm, E.W. (1956).- Nitrogenous reserves of apple trees. Nature 178 : 219.

Oland K. (1959).- Nitrogenous reserves of apple trees. Physiol. Plant. 12 : 594-648.

Oland, K. (1960).- Nitrogen feeding of apple trees by post-harvest urea sprays. Nature 185 : 857.

Oland, K. (1963a).- Responses of cropping apple trees to post-harvest urea sprays. Nature 198 : 1282-1283.

Oland, K. (1963b).- Changes in the content of dry matter and major nutrient elements of apple foliage during senescence and abscission. Physiol. Plant. 16 : 682-694.

Pearse, H.L. (1939).- Water culture studies with apple trees II. The seasonal absorption of nitrogen and potassium by Cox's Orange Pippin on Malling rootstocks numbers IX and XII. J. Pomol. 17 : 344-361.

Plaisted, P.H. (1958).- Clearing free amino acid solutions of plant extracts for paper chromatography. Contr. Boyce Thompson Inst. 19 : 231-244.

Pocchiori, F. and Rossi, C. (1962).- Quantitative radio paper chromatography. In Chromatographic Reviews 4 : 1-18. (Ed. M. Lederer) (Elsevier Publishing Co.: Amsterdam).

Possingham, J.V. (1956).- The effect of mineral nutrition on the content of free amino acids and amides in tomato plants I. A comparison of the effects of deficiencies of copper, zinc, manganese, iron and molybdenum. Aust. J. Biol. Sci. 9 : 539-551.

Pucher, G.W., Vickery, H.B. and Leavenworth, C.S. (1935).- Determination of ammonia and of amide nitrogen in plant tissue. Ind. Engng Chem. (analyt. Edn) 7 : 152-156.

Radu, J.F. (1961).- La dynamique de l'azote total au cours de la vegetation et de la periode de repos chez le pommier, le poirier, l'abricotier et le pêcher. Lucr. sti. Inst. Cerc. horti-vitic (Rumania) 1959-60, pp.97-113.

Reuter, G. (1957).- Die Hauptformen des löslichen Stickstoffs in vetativen pflanzlichen Speicherorganen und ihre systematische Bewertbarkeit. Flora 145 : 326-338.

Roach, W.A. (1938).- Plant injection for diagnostic and curative purposes. Imp. Bur. hort. Plantn Crops. Tech. Comm. No.10, 10pp.

Roberts, R.H. (1921).- Nitrogen reserves in apple trees. Proc. Amer. Soc. hort. Sci. 18 : 143-145.

Rogers, B.L., Batjer, L.P. and Billingsley, H.D. (1955).- Fertilizer applications as related to nitrogen, phosphorous, potassium, calcium and magnesium utilization by peach trees. Proc. Amer. Soc. hort. Sci. 66 : 7-12.

Romanovskaya, O.I. (1963).- Patterns of amino acid metabolism in shoots of orchard trees during their annual cycle of development. Soviet Pl. Physiol. 10 : 581-584.

Rosen, H. (1957).- A modified ninhydrin colorimetric analysis for amino acids. Archs Biochem. Biophys. 67 : 10-15.

Schneider, A. (1958).- Les variations annuelles des acides aminés libres du Pêcher. C.r. Acad. Sci. Paris 247 : 1034-1037.

Schubert, O.E. (1963).- A pressure apparatus designed for injection of liquids into tree limbs. Proc. Amer. Soc. hort. Sci. 83 : 270-275.

Smith, G.E. (1936).- Nitrogen content and growth response from fall and spring fertilizer applications to apple trees. Proc. Amer. Soc. hort. Sci. 34 : 133-136.

Smith, G.E. and Murneek, A.E. (1938).- Comparative value of cyanamid in fertilization of apple trees. Soil changes and tree response. Mo. Agric. Exp. Sta. Res. Bull. No.273, 52pp.

Smith, I. (1960).- "Chromatographic and Electrophoretic Techniques." vol.1, 2nd Ed. 617pp. (William Heinemann Ltd.: London).

Steward, F.C., Thompson, J.F. and Pollard, J.K. (1958).- Contrasts in the nitrogenous composition of rapidly growing and non-growing plant tissues. J. exp. Bot. 9 : 1-10.

Stuart, N.W. (1935).- Determination of amino nitrogen in plant extracts. *Pl. Physiol.* **10** : 135-148.

Sullivan, J.T. and Kraybill, H.R. (1930).- Seasonal changes in the composition of Stayman apple trees II. Forms of nitrogen. *Proc. Amer. Soc. hort. Sci.* **27** : 220.

Thomas, W. (1926).- The nitrogenous metabolism of *Pyrus malus*. *Proc. Amer. Soc. hort. Sci.* **23** : 73-80.

Thomas, W. (1927*a*).- Nitrogenous metabolism of *Pyrus malus* L. III The partition of nitrogen in the leaves, one and two year old branch growth and non-bearing spurs throughout a year's cycle. *Pl. Physiol.* **2** : 109-137.

Thomas, W. (1927*b*).- Nitrogenous metabolism of *Pyrus malus* L. IV The effect of sodium nitrate applications on the total nitrogen and its partition products in the leaves, new and one year old branch growth throughout a year's cycle. *Pl. Physiol.* **2** : 245-271.

Wallace, A., Cameron, S.H. and Mueller, R.T. (1951).- Seasonal use and loss of nutrients and dry matter in orange trees. *Proc. Amer. Soc. hort. Sci.* **58** : 5-10.

Wallace, A., Zidan, Z.I., Mueller, R.T. and North, C.P. (1954).- Translocation of nitrogen in citrus trees. *Proc. Amer. Soc. hort. Sci.* **64** : 87-104.

Webster, G.C. (1959).- The biosynthesis of amide and peptide bonds. In *Soc. Exptl. Biol. Symp.* **XIII**: 330-344. (Ed. H.K. Porter) (University Press: Cambridge).

Weinberger, J.H. and Cullinan, F.P. (1934).- Nitrogen intake and growth response in peach trees following fall and spring fertilizer applications. *Proc. Amer. Soc. hort. Sci.* **32** : 65-69.

Wells, J.R.E. (1963).- Oxalic acid synthesis in *Oxalis pes-caprae* (L.). Ph.D. Thesis, University of Adelaide.

Williams, C.F. (1931).- Seasonal variations in nitrogen concentration in twigs of peach trees under sandhill conditions. *Proc. Amer. Soc. hort. Sci.* **28**: 39-44.

Williams, C.F. (1939).- Fall fertilization of peach trees in the sandhills N.C. Agric. Exp. Sta. Bull. No.321, 15pp.

Yemm, E.W. Cocking, E.C. (1955).- The determination of amino acids with ninhydrin. *Analyst* **80**: 209-213.

Yokomizo, H., Yamazaki, T., Mori, M. and Fukuda, H. (1964).- The accumulation of minerals and carbohydrates of apple trees grown in sand culture and their translocation in the following spring. Morioka Hort. Res. Sta. Bull. No.2, series C, pp.55-67 (Japanese).