THE NITROGEN NUTRITION OF THE PEACH TREE

II.* STORAGE AND MOBILIZATION OF NITROGEN IN YOUNG TREES

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Summary

The chemical composition and distribution of storage nitrogen in young peach trees and the importance of this stored nitrogen for new growth were investigated. Young peach trees, which were grown in sand culture for two growing seasons, accumulated nitrogen in proportion to supply during the first year, and the results 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 began in tree tissues in December and was rapid if the external nitrogen supply was high.

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 woody tissues of the dormant trees was the most sensitive indicator of the nitrogen status of the trees.

Approximately 60–80% of the storage nitrogen in dormant 2-year-old trees was found in root tissues, irrespective of the nitrogen treatment.

I. Introduction

The amount of new shoot growth made by young apple trees is a function of the nitrogen content of the trees at the beginning of the growing season and the external nitrogen supply, providing that no other factor is limiting growth (Roberts 1921; Harley, Moon, and Regeimbal 1949; Oland 1959; Yokomizo *et al.* 1964). Similarly, it has been concluded from studies on the seasonal changes in concentration of total nitrogen and other nitrogenous constituents in tissues of mature peach trees that nitrogen accumulates in woody tissues in late summer, autumn, and winter in proportion to nitrogen supply, and that this stored nitrogen is mobilized for new growth during the next growing season (Schneider 1958; Radu 1961; Taylor 1967). However, this latter conclusion must be regarded as tentative as in each case the data were expressed on a concentration rather than absolute (per tree) basis.

The experiment described in Part I of this series (Taylor 1967) clearly indicated that large orchard trees were unsuitable for further investigation of the role of storage

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nitrogen in tree growth. When large trees are used as experimental material analytical data cannot usually be expressed on an absolute basis, growth responses are difficult to measure, and tree nutrition cannot be controlled precisely. It is necessary to take each of these factors into account when critically studying the relationship between stored nutrients and plant growth and in the experiment reported in this paper the problem was overcome by growing young trees in sand culture. The present investigation was carried out to determine how important storage nitrogen is for the growth of young peach trees and to determine the chemical composition and distribution of storage nitrogen in dormant trees. However, because of their small size and presumably small storage volume, the growth of young trees might not be as dependent upon the level of storage nitrogen as would the growth of large mature trees.

II. Methods

(*a*) *Experimental Design*

The experiment was carried out from June 1963 to March 1965. During the first year three levels of nitrogen were supplied to the trees. Further nitrogen treatments were superimposed on these during the second growing season, viz:

First year	N_1	N_3	N_9
Second year	N_1N_0	N_3N_0	N_9N_0
	N_1N_3	N_3N_3	N_9N_3
	N_1N_9	N_3N_9	N_9N_9

where N_0 indicates that no nitrate nitrogen was supplied, and N_1, N_3, and N_9 indicate that nitrate nitrogen was supplied at 20, 60, and 180 p.p.m. respectively. Overall, there were 108 trees in the experiment, i.e. 9 treatments $\times$ 4 replicates $\times$ 3 harvests. The experiment was laid out according to a randomized block design, where each block was a replicate and consisted of a factorial of treatments $\times$ harvests.

(*b*) *Preparation of Trees prior to Treatment*

(i) *Source of Trees.*—In June 1963, 135 one-year-old peach trees (Golden Queen scion on Elberta rootstock) were selected at a commercial nursery for uniformity of size, for the presence of leaf buds on tree stems, and for freedom from disease and damage.

(ii) *Planting Procedure.*—At planting, tree roots were washed clean, dried between sheets of blotting paper, and the trees were weighed. Tree roots were severely pruned to remove as much of the storage tissue of each root system as possible. The roots of the pruned trees were then serially immersed in solutions of 5·25% sodium hypochlorite (2 min) and 200 p.p.m. terramycin hydrochloride (30 min) in an attempt to prevent subsequent infection of trees with *Agrobacterium tumefaciens* (crown gall). However, galls were detected subsequently on about 20% of the trees. Pruned trees were planted in 4-gal metal pots (lined with epoxy resin) which contained 26 kg sieved, steam-sterilized river sand. Potted trees were placed in a glasshouse and tree tops were cut back to within 6 in. of the graft union. Both root and top prunings were weighed. The sand was thoroughly leached with distilled water and a

mulch of 3 kg sterilized gravel was placed on the surface of each pot. Distilled water was supplied as required to each pot until treatments were commenced.

(*c*) *Application of Nitrogen to Pruned Trees*

Trees were stratified according to their fresh weight after pruning. The stratified trees were divided into four equal groups, each of which constituted a range of tree weights and was designated a "replicate". Within each replicate the nine nitrogen treatments were allocated to trees at random. Tree positions within each replicate were changed in a random manner at monthly intervals during the growing season but the positions of the replicates in the glasshouse were not changed.

In both years of the experiment, 2 litres of complete nutrient solution with or without nitrogen (as nitrate) 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 excess liquid drained into a half-gallon polythene container 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.

In the first year, nutrient applications were commenced on July 27, 1963, and were continued until the end of June 1964. They were continued after the cessation of shoot growth in March 1964 to allow for possible further uptake of nitrogen during autumn and early winter. In July and August 1964, distilled water only was applied to the trees at a rate of 2 litres per tree per week.

One-third of the trees were harvested in July 1964, and, in the first week of August 1964, the remainder were repotted into fresh sand which had been sieved, acid-washed (see Hewitt 1952), and steam-sterilized. The trees were repotted so that growth during the second year would not be influenced by the level of nutrients which had accumulated in the sand during the first year. It was assumed that the acid-washing treatment would remove all available nitrogen from the sand. The pH of the acid-washed sand was $6{\cdot}8 \pm 0{\cdot}03$.

Second-year treatments were commenced on September 5, 1964, when the trees were at the "pink-tip" stage of bud-burst, and were continued until all trees had been harvested in March 1965.

(*d*) *General Tree Management*

After bud-burst in the first year, all shoots were removed except one which became the new stem of the tree. Root suckers were pinched off and leaves which abscissed during the first year were discarded. During the second year, flower buds were removed at bud-burst and root suckers were pinched off, but this tissue was added back to the pots in an attempt to maintain the nitrogen balance. In addition, the leaves which abscissed during the season were collected and placed in paper bags attached to each pot. In both years of the experiment, trees were sprayed with Bordeaux mixture at bud-burst to control "curl leaf" fungus. Insecticides were also applied when necessary.

(*e*) *Seasonal Tree Growth Measurements*

Measurements of tree growth were made at intervals of 3–4 weeks during the first and second growing seasons. During the first year, the total length of tops per tree and the stem diameter per tree at a fixed point (two readings at right angles to one another) were measured, while in the second year, the total length of new shoots, the total number of new shoots, and the stem diameter at the same fixed point were recorded for each tree.

(*f*) *Harvesting Procedures*

Trees were harvested at the times set out in the following tabulation:

Harvest No.	Harvest Date	No. of Trees Harvested	Remarks
1	July 13–19, 1964	36 (3 treatments × 12 replicates)	End of first year; dormant leafless trees
2	November 23–26, 1964	36 (9 treatments × 4 replicates)	Midway through second growing season
3	March 1–5, 1965	36 (9 treatments × 4 replicates)	End of second growing season

At each harvest, trees were thoroughly washed, blotted dry, weighed, and quickly subdivided into the following parts:

(1) Roots;
(2) Stock+stem+1-year-old shoots;
(3) Leaf+flower buds (replicates bulked), or new shoots.

The stock consisted of the above-ground portion of the rootstock. The number of buds, flowers, leaves, and shoots per tree was also recorded. Subdivided tissues were weighed, quick-frozen in dry ice, chopped finely, placed in labelled muslin bags, and stored at −20°C. If root galls were present on a tree they were cut off and oven-dried at 103°C for 16 hr. Abscissed leaf tissue was similarly treated.

(*g*) *Preparation of Tissue Samples for Analysis*

Frozen tissues were freeze-dried, weighed, and ground to pass a sieve with pores 1 mm in diameter. After mixing well, duplicate subsamples of each tissue were taken for dry matter analysis (oven-dried at 103°C for 16 hr) and the dry weight of each tree and its parts was calculated. The remaining tissue was placed in screw-lid glass jars and stored at −20°C until required. Oven-dried tissues were ground in the same way but were stored at room temperature.

(*h*) *Nitrogen Analyses*

Tree tissues were analysed in duplicate for their content of the following nitrogenous constituents—total nitrogen, nitrate nitrogen, soluble nitrogen, arginine nitrogen, total α-amino nitrogen, ammonium nitrogen, and amide nitrogen. Abscissed leaves and root galls were only analysed for total nitrogen. Apart from total nitrogen,

root galls were assumed to contain the same concentration of other nitrogenous constituents as healthy roots. Even if this was not correct, 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.

(i) *Total Nitrogen.*—The microKjeldahl method of McKenzie and Wallace (1954) was used except that the quantity of concentrated sulphuric acid used in the digestion step was increased to 2 ml, and the distillations were carried out in an apparatus described by Jennings (1962).

(ii) *Nitrate Nitrogen.*—Analyses were carried out according to the phenol-disulphonic acid procedure of Humphries (1955).

(iii) *Soluble Nitrogen.*—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 nitrogen from plant tissues (freeze-dried peach shoots) than did 80% aqueous ethanol. Citrate buffer (0·05M, pH 5·0) was used for the routine extraction of soluble nitrogen. The procedure was as follows: Freeze-dried ground tissue (0·5–1 g) was extracted for 24 hr at 2°C with 54 ml buffer in a stoppered 100-ml centrifuge tube (the extract was occasionally shaken during this time). After centrifuging, the supernatant was poured through a glass wool filter into a 250-ml volumetric flask. This process was repeated three times except that the extraction period was reduced to 1 hr. Successive extracts were bulked and made to volume with citrate buffer. After mixing, 75–100-ml aliquots of each extract were taken to dryness *in vacuo* in a rotary evaporator (water-bath temp. 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 nitrogen in 2-ml aliquots was determined by microKjeldahl analysis using the same method and equipment as described for total nitrogen analyses. However, a preliminary heating strip was necessary before digestion (extract acidified first) to reduce the volume of liquid in the flask, otherwise extracts frothed during digestion and nitrogen was lost.

(iv) *Insoluble Nitrogen.*—The concentration of insoluble nitrogen in the tissues was found by calculating the difference between the concentration of total and soluble nitrogen in each tissue.

(v) *Arginine Nitrogen.*—The concentration of arginine nitrogen in the citrate buffer extracts was estimated by the method of Gilboe and Williams (1956). In some cases it was necessary to dilute the extracts with distilled water before analysis otherwise pigments present in the extracts interfered with the test.

(vi) *Total α-Amino Nitrogen.*—The concentration of total α-amino nitrogen in the citrate buffer extracts was determined by the method of Rosen (1957). The acetate–cyanide reagent was freshly prepared before use (Grant 1963).

(vii) *Ammonium and Amide Nitrogen.*—Cold 80% ethanol was used to extract ammonium and amide nitrogen from freeze-dried tissues. Citrate buffer was not used as extractant because the method of assay for these nitrogenous fractions was pH-dependent and the use of buffered extracts would have complicated the assay procedure. Extractions were carried out in the same way as described earlier for soluble nitrogen except that the initial extraction period was for 4 hr and a total of 200 ml 80% ethanol was used per extraction. Each extract was acidified with

0·1N HCl, taken to dryness *in vacuo* in a rotary evaporator, and the residue was dissolved in distilled water and made to volume in a 25-ml volumetric flask. The concentration of these fractions in each extract was determined by the method of Pucher, Vickery, and Leavenworth (1935), except that the Nessler's reagent was prepared and used as described by Jennings (1962).

III. Results

Data recorded for trees infected with root galls were not separated out prior to statistical analysis because values for these trees fell within the range recorded for healthy trees.

(*a*) *Tree Growth during the First Year*

The fresh and dry weights of the trees and their parts, the number of shoots, leaves, leaf and flower buds per tree, the total length of tops, and the stem diameter per tree were all significantly increased with increased nitrogen supply (e.g. Fig. 1).

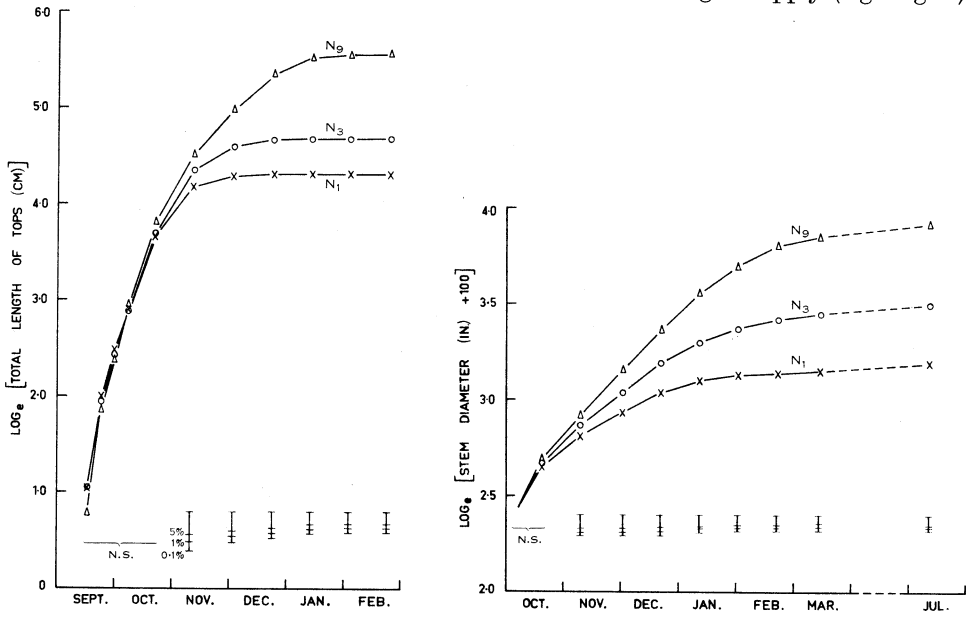

Fig. 1.—Influence of nitrogen treatment on the total length of tops per tree and the stem diameter per tree during the first growing season.

Increased nitrogen supply also increased the top/root ratio on a dry weight basis, indicating that the size of tree tops increased more so than the size of the roots. Nitrogen deficiency symptoms were first observed in November on trees in N_1 and N_3 treatments.

(*b*) *Tree Growth during the Second Growing Season*

(i) *At Harvests 2 and 3*

Tree growth, expressed as fresh weight or dry weight per tree (e.g. Fig. 2), was dependent on, and in proportion to, the nitrogen treatments applied in both years of the experiment, and in most cases differences between treatments were significant.

Similarly, the growth of each tree part usually showed a response to both sets of treatments. As noted for the harvest 1 data, the top/root ratio on a dry weight basis increased with increasing nitrogen supply.

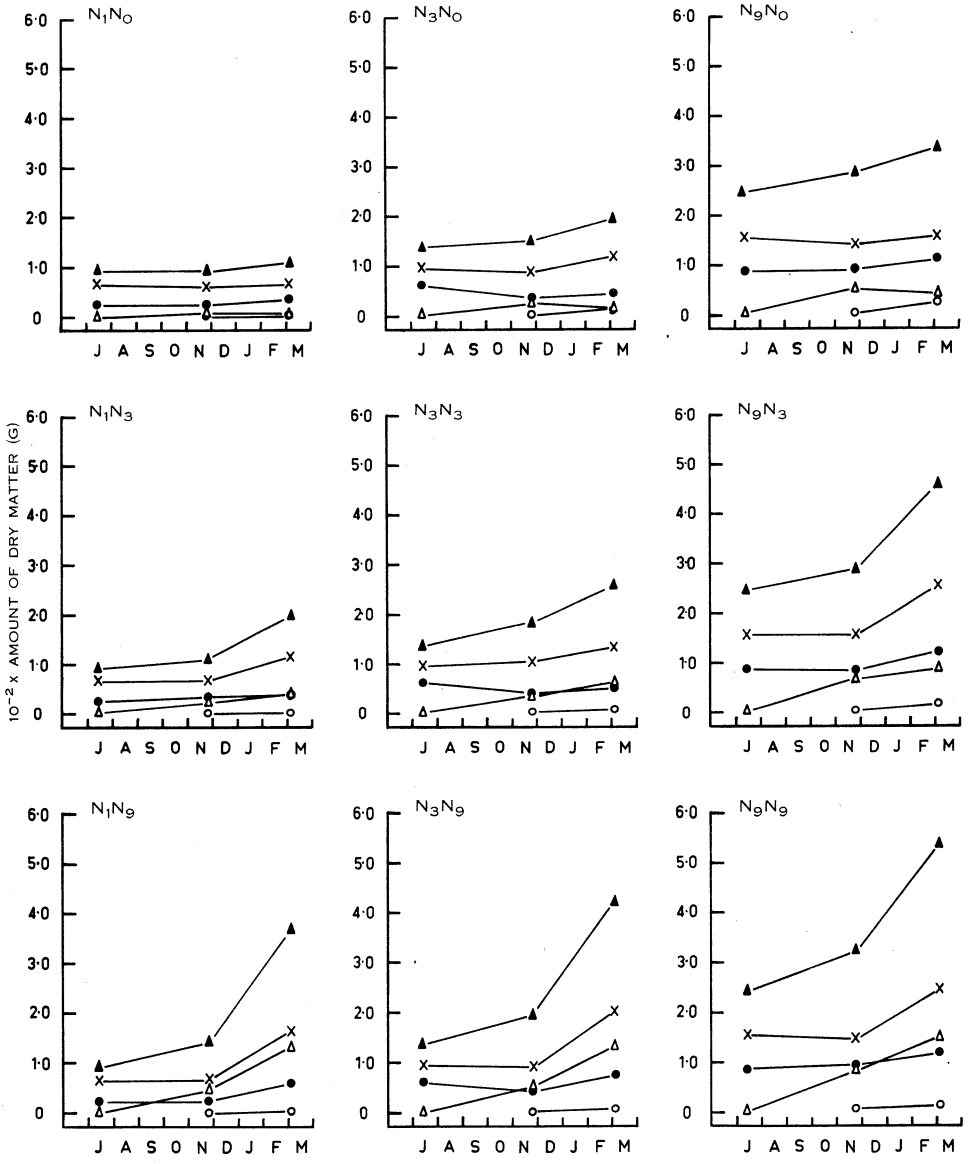

Fig. 2.—Influence of nitrogen treatment on the dry matter content of tree tissues during the second growing season. × Roots. ● Stock+stem+1-year-old shoots. △ Buds, new shoots. ○ Abscissed leaves. ▲ Whole tree.

Foliage colour ranged from dark green for the N_9 treatment to yellowish green for the N_0 treatment. Percentage leaf abscission per tree was inversely proportional

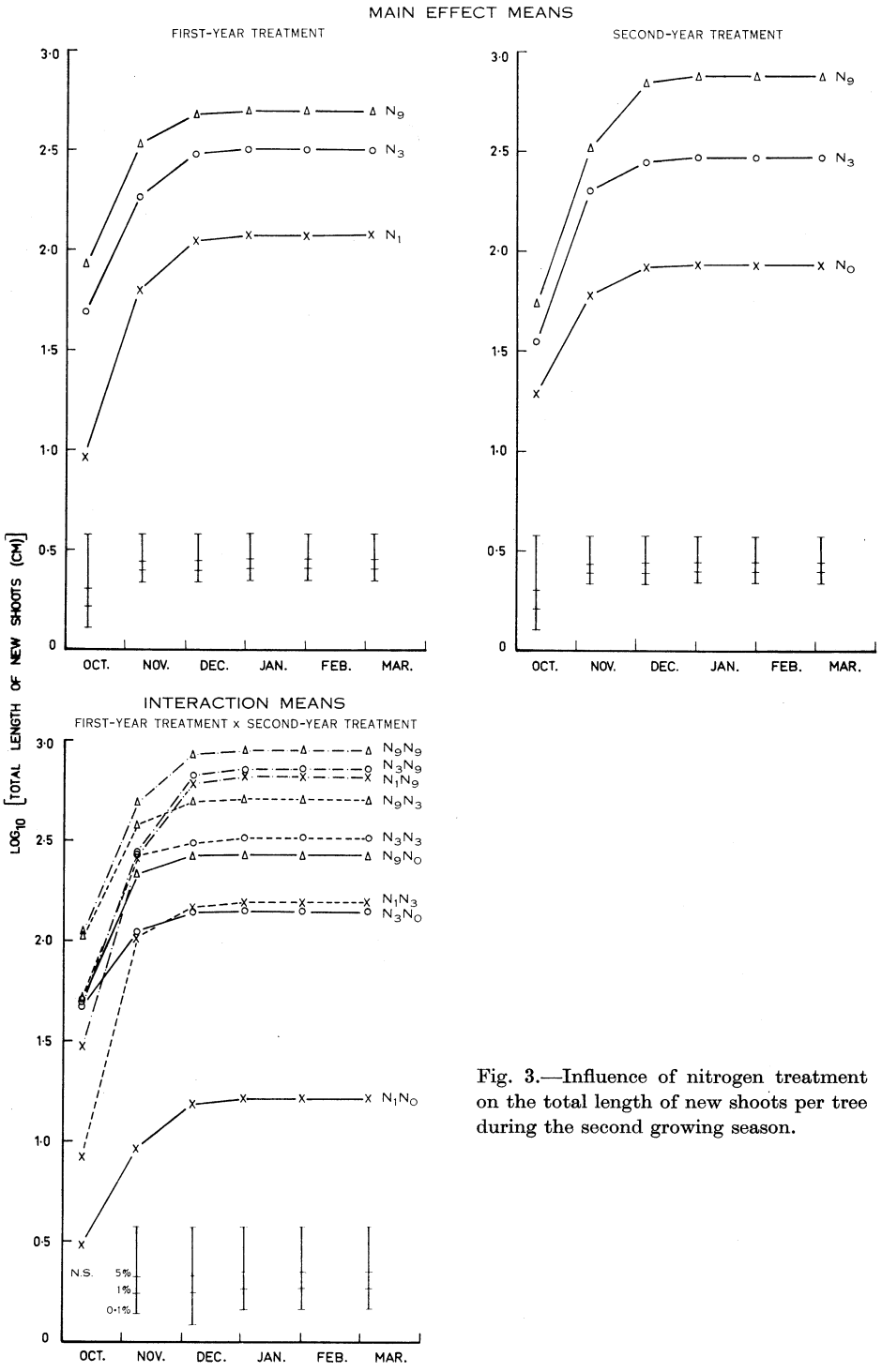

Fig. 3.—Influence of nitrogen treatment on the total length of new shoots per tree during the second growing season.

to nitrogen supply during the second year and was indicative of the severity of nitrogen stress in the trees.

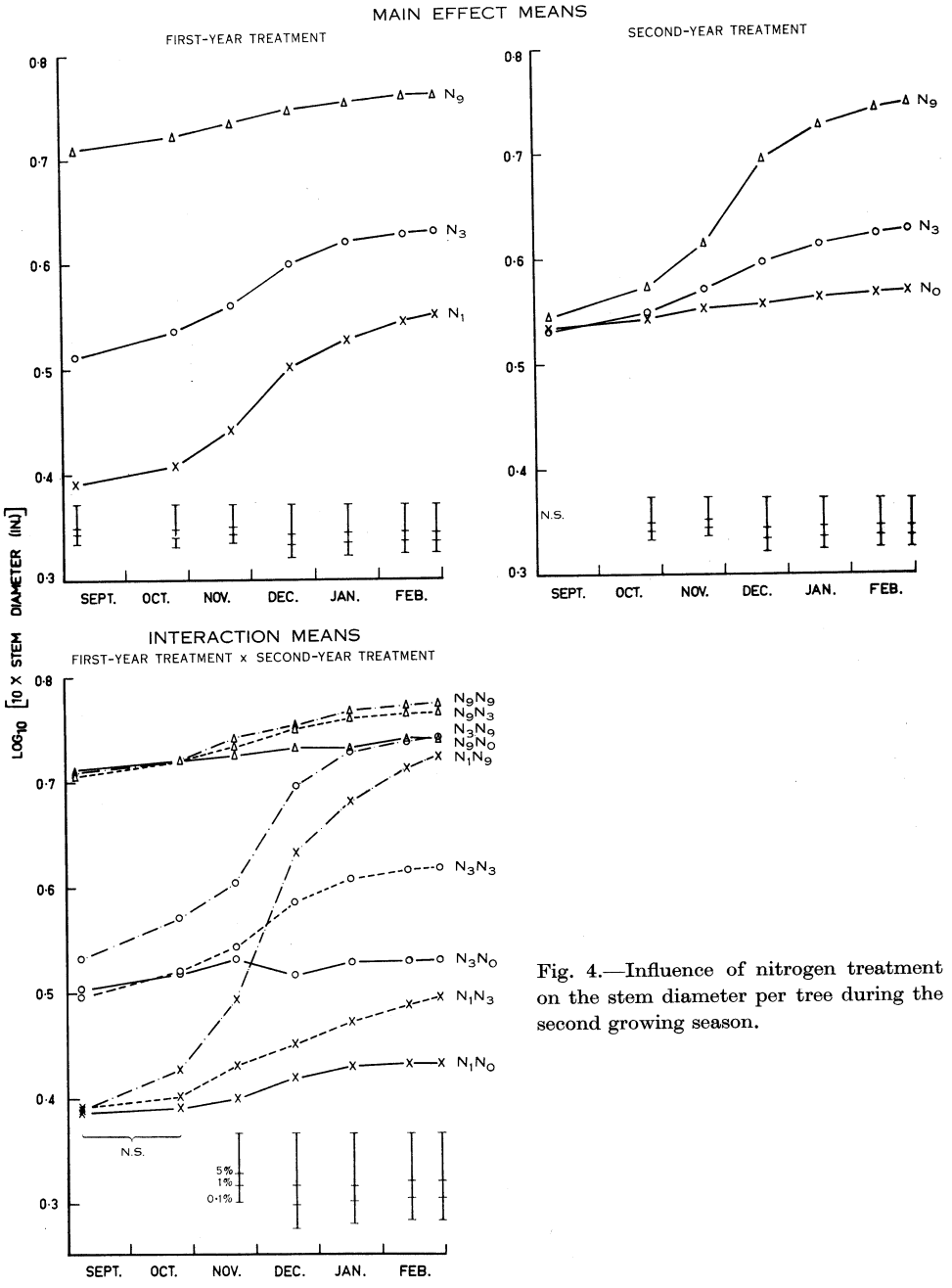

Fig. 4.—Influence of nitrogen treatment on the stem diameter per tree during the second growing season.

Significant negative interactions were found between first and second year treatments for all data recorded at harvest 3; i.e. the greater the nitrogen supply in

the first year, the smaller the effect of nitrogen application in the second year on tree growth, and vice versa.

(ii) *Seasonal Changes in Growth*

Seasonal changes in the total length of new shoots, in stem diameter, and in the total number of new shoots per tree are shown in Figures 3, 4, and 5.

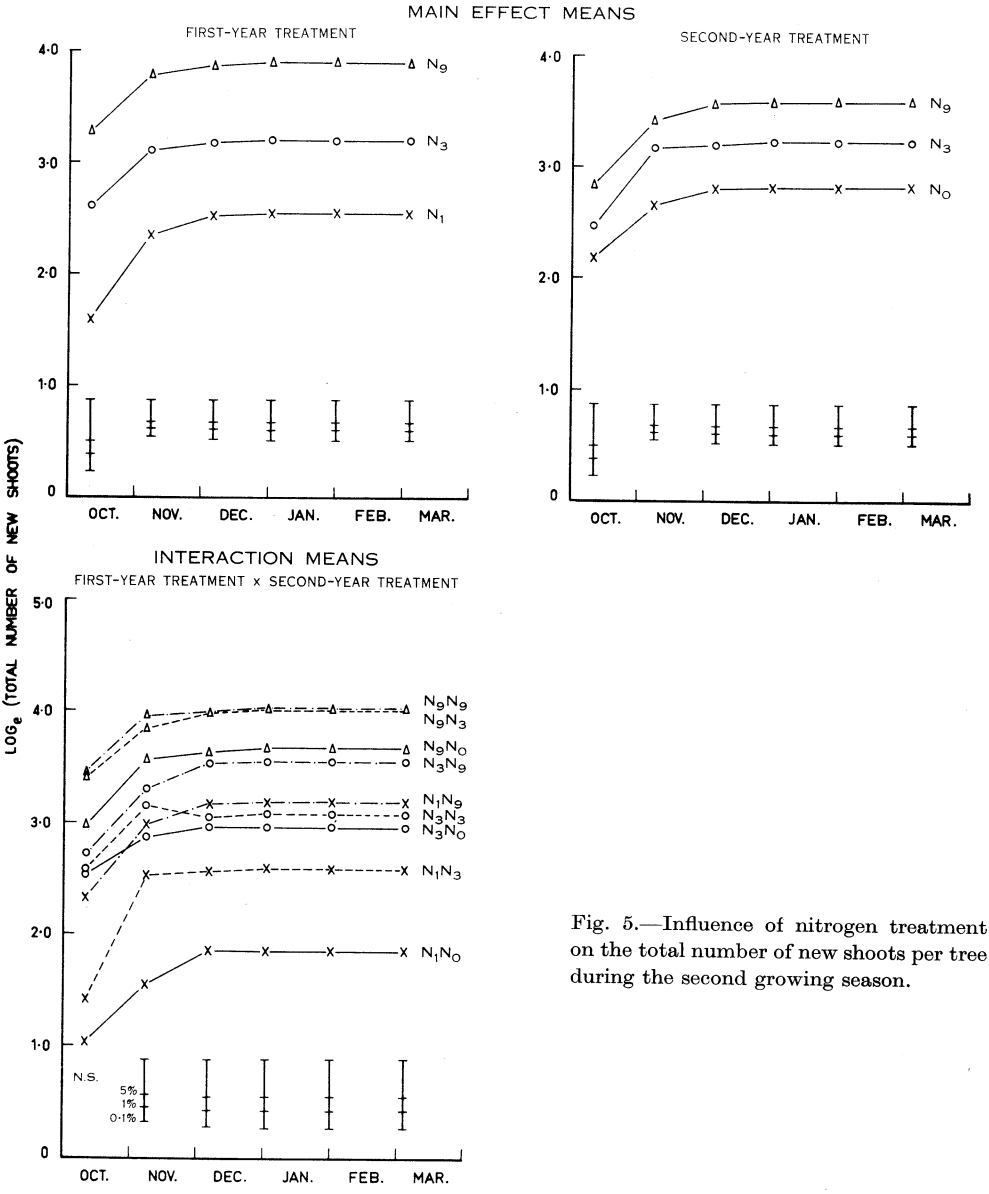

Fig. 5.—Influence of nitrogen treatment on the total number of new shoots per tree during the second growing season.

It is evident from Figure 3 that the total length of new shoots per tree was dependent on, and in proportion to, the level of nitrogen supplied in both years of the experiment. This result supports the hypothesis that the trees accumulated

nitrogen in proportion to supply during the first year and that the stored nitrogen was used for new growth during the second growing season. At the beginning of the growing season the influence of the first-year treatments on tree growth was more pronounced than that of the second-year treatments (cf. main effect means). However, after November, the current nitrogen supply had a pronounced effect on tree growth [cf. N_3N_9 and N_9N_3 treatments (Fig. 3) for example], and, since nitrogen deficiency symptoms were first seen in November on trees which did not receive any fertilizer nitrogen, it is suggested that the supply of storage nitrogen in these trees was exhausted by this time.

Similarly, seasonal changes in the stem diameter per tree (Fig. 4) and the total number of new shoots per tree (Fig. 5) were dependent on, and in proportion to, the nitrogen supply in both years of the experiment.

TABLE 1

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	Degrees of Freedom
Dry weight of new shoots at harvest 2	N_1N_0, N_3N_0, N_9N_0	0·966***	10
v. nitrogen per tree at harvest 1	N_1N_3, N_3N_3, N_9N_3	0·970***	10
	N_1N_9, N_3N_9, N_9N_9	0·828***	10
Length of new shoots at harvest 2	N_1N_0, N_3N_0, N_9N_0	0·843***	10
v. nitrogen per tree at harvest 1	N_1N_3, N_3N_3, N_9N_3	0·946***	10
	N_1N_9, N_3N_9, N_9N_9	0·784**	10
Dry weight of new shoots at harvest 3	N_1N_0, N_3N_0, N_9N_0	0·979***	10
v. nitrogen per tree at harvest 1	N_1N_3, N_3N_3, N_9N_3	0·987***	10
	N_1N_9, N_3N_9, N_9N_9	0·834***	10
Length of new shoots at harvest 3	N_1N_0, N_3N_0, N_9N_0	0·828***	10
v. nitrogen per tree at harvest 1	N_1N_3, N_3N_3, N_9N_3	0·714***	10
	N_1N_9, N_3N_9, N_9N_9	0·328†	10

** $P<0·01$. *** $P<0·001$. † Not significant.

(iii) *Correlation between Tree Growth and the Nitrogen Content per Tree*

As expected, a highly significant positive correlation was found between the amount of new shoot growth per tree at harvests 2 and 3 and the nitrogen content per tree at harvest 1 if the external nitrogen supply in the second year was low (Table 1). However, if a high level of nitrogen was supplied during the second year then the level of correlation was reduced and in one case was not significant.

(c) *Nitrogen Content of Tree Tissues*

(i) *Composition of Storage Nitrogen in Dormant Trees*

The influence of nitrogen supply on the amount of each nitrogenous constituent in dormant tissues, relative to values in the N_1 treatment, is shown in Table 2.

Nitrate was not detected in any of the tissues. It is evident that the amount of each constituent usually increased with increasing nitrogen supply and that levels of soluble nitrogen increased more rapidly than levels of insoluble nitrogen. From this latter result it is concluded that the storage nitrogen which accumulated in the young peach trees consisted mainly of soluble nitrogenous compounds.

Since the amount of arginine nitrogen in most tree tissues showed the greatest relative increase of any of the nitrogenous constituents with increasing nitrogen supply, it is concluded that estimation of levels of arginine nitrogen in the peach trees gave the most sensitive indication of the nitrogen status of the trees. The

TABLE 2

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

Values are percentages relative to the N_1 treatment

Tree Tissue	Nitrogen Treatment	Total Nitrogen	Insoluble Nitrogen	Soluble Nitrogen	Ammonium Nitrogen	Arginine Nitrogen	Amide Nitrogen	Total α-Amino Nitrogen
Roots	N_1	100·0	100·0	100·0	100·0	100·0	100·0	100·0
	N_3	197·1	162·5	280·2	190·9	293·9	204·7	291·8
	N_9	403·1	246·0	780·9	277·3	937·1	379·4	741·2
Stock + stem + 1-yr-old shoots	N_1	100·0	100·0	100·0	100·0	100·0	100·0	100·0
	N_3	236·0	182·2	410·5	153·3	464·6	330·3	447·3
	N_9	646·5	442·9	1307·2	253·3	1796·3	827·3	1394·5
Leaf + flower buds	N_1	100·0	100·0	100·0	100·0	100·0	100·0	100·0
	N_3	203·5	166·7	400·0	100·0	500·0	882·4	366·7
	N_9	503·5	389·6	1111·1	200·0	1383·3	1764·7	1000·0
Whole tree	N_1	100·0	100·0	100·0	100·0	100·0	100·0	100·0
	N_3	206·0	167·3	305·8	178·4	300·1	235·7	317·5
	N_9	459·8	295·8	882·8	273·0	1006·3	487·1	847·6

greatest relative increase in arginine nitrogen was found in the stem plus stock plus 1-year-old shoot fraction and therefore this is the most suitable type of tissue to use in any assessment of the nitrogen status of dormant trees. Similar results were obtained if the data were expressed on a concentration basis.

Ratios of amounts of nitrogenous constituents in tree tissues at harvest 1 are shown in Table 3. As expected from Table 2, the level of soluble nitrogen as a proportion of the total nitrogen content increased with increasing nitrogen supply. Almost all of the soluble nitrogen in the tissues was organic in nature and consisted mainly of arginine nitrogen. The arginine nitrogen content of the soluble nitrogen fraction increased with increasing nitrogen supply, indicating that the composition of this fraction was dependent upon the nitrogen supply. If this supply was low, then a greater proportion of the soluble nitrogen which accumulated consisted of amide

nitrogen. Generally, however, the levels of amide nitrogen constituted a very small part of the soluble nitrogen fraction. The total α-amino nitrogen content of the

TABLE 3

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

Expressed as percentages

Ratio	Nitrogen Treatment			Least Significant Difference		
	N_1	N_3	N_9	5% Level	1% Level	0·1% Level
Soluble nitrogen/total nitrogen						
Roots	30·4	42·6	57·4	3·6	4·9	6·6
Stock+stem+1-year-old shoots	24·1	41·2	47·8	3·3	4·4	6·0
Leaf+flower buds	16·3	30·8	34·8	4·0	7·3	16·2
Whole tree	28·3	41·5	53·7	3·1	4·2	5·6
Insoluble nitrogen/total nitrogen						
Roots	69·6	57·4	42·6	3·6	4·9	6·6
Stock+stem+1-year-old shoots	75·9	58·8	52·2	3·3	4·4	6·0
Leaf+flower buds	83·7	69·2	65·2	—	—	—
Whole tree	71·7	58·5	46·4	3·0	4·1	5·5
Arginine nitrogen/soluble nitrogen						
Roots	78·1	81·6	94·6	6·6	9·0	12·1
Stock+stem+1-year-old shoots	66·0	76·2	93·0	9·1	12·4	16·6
Leaf+flower buds	60·8	83·0	83·0	6·7	12·4	27·4
Whole tree	75·7	80·3	94·1	6·0	8·2	10·9
Total α-Amino nitrogen/soluble nitrogen						
Roots	31·4	33·0	30·0	1·5	2·1	2·8
Stock+stem+1-year-old shoots	25·1	27·3	26·8	1·7	2·3	3·1
Leaf+flower buds	33·1	31·0	30·1	2·2	4·0	8·8
Whole tree	30·2	31·4	29·0	1·4	1·9	2·5
Ammonium nitrogen/soluble nitrogen						
Roots	1·4	1·0	0·5	0·3	0·5	0·6
Stock+stem+1-year-old shoots	4·3	1·6	0·8	1·2	1·6	2·1
Leaf+flower buds	8·6	3·3	2·1	0·8	1·5	3·3
Whole tree	2·0	1·2	0·6	0·4	0·5	0·7
Amide nitrogen/soluble nitrogen						
Roots	7·1	5·3	3·6	1·1	1·5	2·0
Stock+stem+1-year-old shoots	9·7	7·4	5·9	2·7	3·7	5·0
Leaf+flower buds	4·2	4·2	3·2	2·7	5·0	11·0
Whole tree	7·5	5·8	4·2	1·1	1·5	2·0
Arginine+ammonium+amide nitrogen/soluble nitrogen						
Roots	86·7	87·9	98·7	6·1	8·2	11·0
Stock+stem+1-year-old shoots	80·0	85·2	99·7	7·5	10·2	13·6
Leaf+flower buds	73·6	90·5	88·3	5·0	9·3	20·5
Whole tree	85·2	87·3	98·9	5·4	7·4	9·8

soluble nitrogen fraction was not influenced by the nitrogen treatment. Irrespective of the treatment applied, almost all of the soluble nitrogen in the tissues was accounted for as arginine, the amides, and ammonia.

TABLE 5

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

Nitrogen Treatment	At Harvest 2 (harvest 2 − harvest 1 values)					At Harvest 3 (harvest 3 − harvest 2 values)				
	Roots	Stock + Stem + 1-year-old Shoots	New Shoots	Abscissed Leaves	Whole Tree	Roots	Stock + Stem + 1-year-old Shoots	New Shoots	Abscissed Leaves	Whole Tree
N_1N_0	−132·9	− 67·7	+ 142·5	+ 1·3	− 56·8	+ 5·1	+ 30·9	− 78·5	+ 34·3	− 8·2
N_1N_3	− 32·2	− 49·1	+ 449·4	+ 0·6	+ 368·7	+335·0	+ 69·1	+135·9	+ 27·9	+ 567·9
N_1N_9	+ 13·1	− 46·1	+1158·3	+ 2·1	+1127·4	+805·0	+135·9	+900·6	+ 73·7	+1915·2
N_3N_0	−361·0	−226·0	+ 425·6	+ 6·6	− 154·8	+ 95·3	+ 22·0	−239·7	+169·3	+ 46·9
N_3N_3	−171·8	−209·3	+ 757·6	+ 4·7	+ 381·2	+156·3	+ 58·7	+111·5	+119·0	+ 445·5
N_3N_9	−139·0	−171·3	+1402·8	+ 6·8	+1099·3	+870·3	+121·7	+738·4	+113·0	+1843·4
N_9N_0	−722·2	−560·5	+1145·1	+26·7	− 110·9	− 92·5	+ 65·3	−565·8	+428·3	− 164·7
N_9N_3	−711·1	−581·2	+1624·3	+30·6	+ 362·6	+470·8	+ 56·4	−371·5	+257·2	+ 412·9
N_9N_9	−523·9	−556·3	+2206·5	+44·2	+1170·5	+728·3	+129·2	+331·2	+278·6	+1467·3

from small differences in tree size, and therefore nitrogen content, at each harvest. It is also evident from Table 5 that the uptake of total nitrogen per tree at harvests 2 and 3 was approximately directly proportional to the external nitrogen supply.

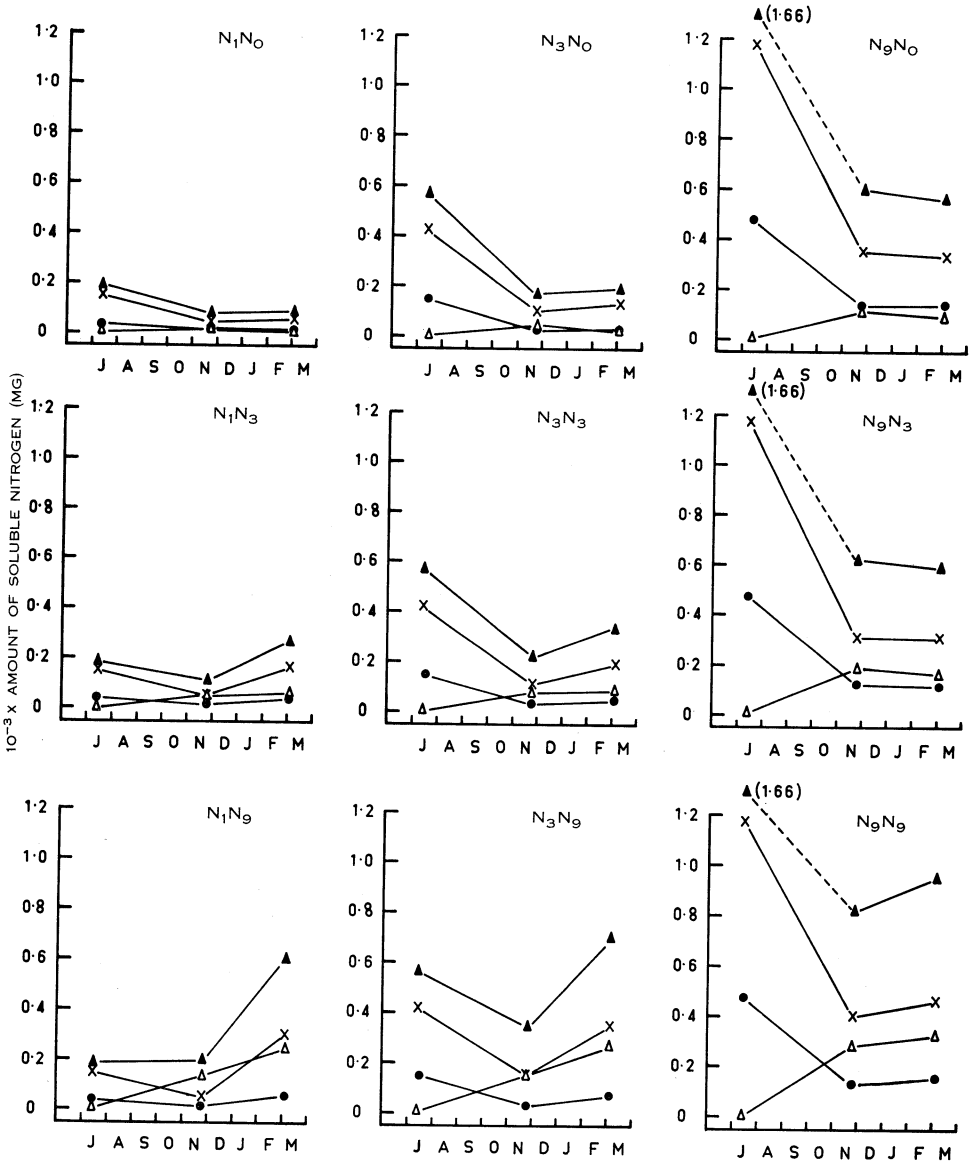

Fig. 7.—Influence of nitrogen treatment on the amount of soluble nitrogen in tree tissues during the second growing season. × Roots. ● Stock+stem+1-year-old shoots. △ Buds, new shoots. ▲ Whole tree.

During the second half of the growing season, the nitrogen content of the roots and old tree tops increased in all treatments except in roots of trees in the N_9N_0

nitrogen did not accumulate in tree tissues at this time, presumably because the demand for nitrogen exceeded the supply.

(3) *Insoluble Nitrogen* (*Fig. 8*).—The amount of insoluble nitrogen in tree tissues during the second growing season was proportional to the level of nitrogen supplied

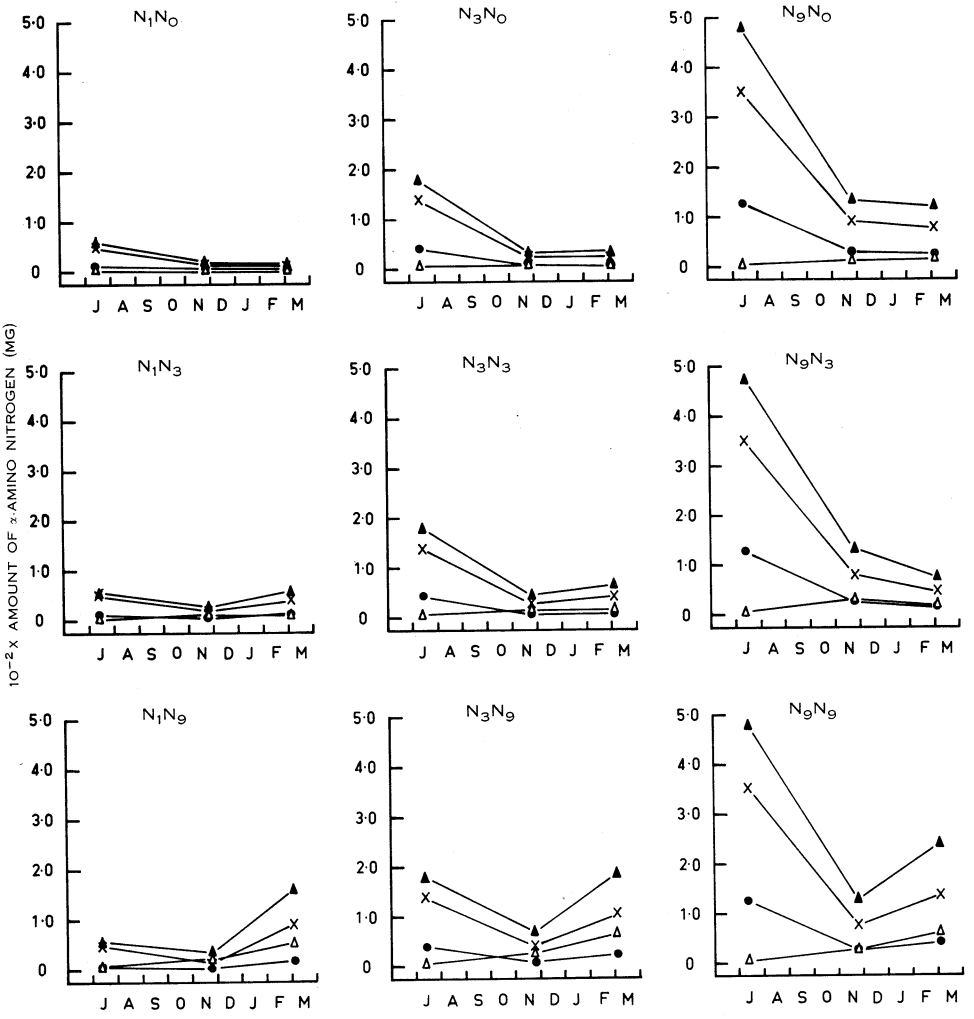

Fig. 10.—Influence of nitrogen treatment on the amount of total α-amino nitrogen in tree tissues during the second growing season. × Roots. ● Stock+stem+1-year-old shoots. △ Buds, new shoots. ▲ Whole tree.

in both years of the experiment. Since the amount of insoluble nitrogen increased throughout the growing season in most tissues it was not utilized in growth processes. However, there is evidence that small amounts of insoluble nitrogen in the stock plus stem plus 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 nitrogen in the new shoots of trees in the N_1N_0, N_3N_0, N_9N_0, and N_9N_3 treatments fell, presumably due to loss of insoluble nitrogen in the abscissed leaves (the extent of this loss was not measured).

(4) *Arginine Nitrogen* (*Fig. 9*).—The amount of arginine nitrogen in the trees fell sharply to a very low level during the first half of the growing season and remained low during the remainder of the season.

(5) *Total α-Amino Nitrogen* (*Fig. 10*).—The amount of total α-amino nitrogen in tree tissues was proportional to the nitrogen supply in the first and second years of the experiment. During the first half of the growing season, the amount of total α-amino nitrogen in tree tissues of all treatments declined, except in the new shoots where small increases occurred, but during the second half of the growing season the amount of total α-amino nitrogen increased in tree tissues in proportion to the external nitrogen supply (except in the N_9N_3 treatment). These seasonal changes were similar to those found for the amount of soluble nitrogen in tree tissues, and, since arginine did not accumulate in tree tissues during the latter half of the growing season, it is suggested that the soluble nitrogen which accumulated in the storage tissues at this time consisted largely of other free amino acids and amides.

IV. Discussion

The results strongly suggest that tree growth during the first half of the second growing season was made largely at the expense of nitrogen which had accumulated in woody tissues of the trees during the first year. However, apart from the influence of storage nitrogen on tree growth, tree size at the beginning of the second growing season could also have influenced subsequent growth, since tree size and the nitrogen content per tree at that time were both in proportion to the nitrogen treatment applied in the first year. Nevertheless, the results of the nitrogen analyses clearly show that stored nitrogen in tree roots and old tops was exported to the new shoots during the first half of the growing season, thus confirming the importance of storage nitrogen for tree growth.

Analysis of growth data obtained at the end of the second growing season indicated that there was a significant negative interaction between first and second year treatments, irrespective of the way in which tree growth was measured. For example, trees in the N_1N_9 treatment responded more markedly to the external nitrogen supply than did those in the N_9N_9 treatment, but this was not due to a greater uptake of nitrogen per tree in the former treatment. Apparently, factors other than nitrogen supply limited the growth of nitrogen-rich trees during the second year.

The finding that the storage nitrogen of dormant peach trees consists mainly of soluble organic nitrogen and that free arginine is the principal constituent of this fraction agrees well with the results of Oland (1959) for apple. Thus, as might be expected, the nature and chemical composition of storage nitrogen in different woody species of the family Rosaceae appear to be similar.

About two-thirds of the storage nitrogen in dormant, 2-year-old trees was held in root tissues irrespective of the previous nitrogen treatment. This uneven