THE NITROGEN NUTRITION OF THE PEACH TREE

I. SEASONAL CHANGES IN NITROGENOUS CONSTITUENTS IN MATURE TREES

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Summary

Seasonal changes in the concentration of total nitrogen, free amino acid nitrogen, arginine, and glutamine plus asparagine in flower buds, flowers, fruit, leaves, roots, new shoots, and 2–3-year-old shoots of 25-year-old peach trees have been studied over an annual cycle.

Peak concentrations of each constituent were found in tree tissues before growth commenced and after growth ceased (woody tissues only), while a minimum was found at the end of the growing season. In most tissues this pattern of change was not influenced by the amount of nitrogen supplied or by the time of application of the nitrogenous fertilizer, but the concentration of the constituents was usually proportional to nitrogen supply. From the evidence available, it is suggested that nitrogen accumulates in proportion to supply in woody tissues of mature peach trees after the cessation of shoot growth in late summer, and that this stored nitrogen is mobilized for new growth during the next growing season.

Since dormant storage tissues usually contained high concentrations of arginine but only low concentrations of glutamine plus asparagine, it is suggested that arginine is a more important constituent of the storage nitrogen of mature peach trees than are the amides.

I. INTRODUCTION

Few studies have been carried out to determine to what extent the nutrition of perennial plants, such as fruit trees, is influenced in the current season by the carry-over of nutrients from the preceding season or seasons. This paper is the first of a series of three dealing with the nitrogen nutrition of the peach tree from this point of view.

The concentration of total nitrogen and free amino acid nitrogen in tissues of peach trees undergoes a pronounced annual rhythm with a peak concentration in winter and a minimum concentration in midsummer (Schneider 1958; Radu 1961). This rhythm has been attributed to storage of nitrogen in tree tissues during autumn and winter and mobilization of stored nitrogen for new growth during the next growing season. According to Oland (1954, 1959) and Schneider (1958), arginine and the amides play an important role in the storage and mobilization of nitrogen in apple and peach trees.

The present investigation was carried out to study seasonal changes in the concentration of total nitrogen, free amino acid nitrogen, arginine, and the amides (glutamine plus asparagine) in mature peach trees which were given two treatments differing in both time and rate of application of nitrogenous fertilizer.

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II. Methods

(a) *Source of Tree Material*

The trees used (cv. Golden Queen on Elberta rootstock) were part of a peach fertilizer trial (PHF block) at the Horticultural Research Station, Tatura, Vic., and were 25 years old at the beginning of the experiment. The soil type was Shepparton fine sandy loam. Trees were given 3 lb ammonium sulphate per tree per year in August (N_1 trees), or 6 lb ammonium sulphate per tree per year as a split application in August and January (N_2 trees). Trees also received an annual dressing of 2 cwt superphosphate per acre and the soil was periodically limed. These fertilizer treatments had been applied to the trees for the previous 18 years. Fertilizers were broadcast on the surface of the soil and the orchard was usually irrigated afterwards. All trees were irrigated as required during the growing season. Under these conditions, the annual canning yield, increase in butt circumference, and weight of prunings per tree per year were significantly higher for N_2 than for N_1 trees. In addition, the foliage colour of N_2 trees was always darker green than that of N_1 trees during the latter half of each growing season.

(b) *Sampling Procedure*

Samples of flower buds, flowers, whole fruit or fruit flesh, new shoot leaves (including petioles), roots, new shoots, and 2–3-year-old shoots were taken from the trees at monthly intervals or less over a period of 1 year, commencing in June 1961. On each occasion, tissues were harvested from a group of 10 trees in each treatment at approximately the same time of day (8–11 a.m.). In the laboratory, tissues from each treatment were quickly subdivided into the abovementioned fractions. Dirt and spray materials were removed from the tissues by washing in tap water and then in distilled water. Roots less than $\frac{1}{4}$ in. in thickness and all leaves, except the mid-shoot leaves on the new shoots, were discarded. Shoot and root tissues were subdivided into bark (tissues external to the xylem) and wood parts. Bulky tissues were chopped finely with a pair of secateurs. Each tissue was then mixed well and subsamples were taken by grab-sampling for subsequent dry matter, total nitrogen, and free amino acid nitrogen analyses.

(c) *Methods of Analysis*

(i) *Dry Matter Content.*—Tissue samples were oven dried to constant weight at 80°C and the percentage dry matter content of the tissues was calculated.

(ii) *Total Nitrogen Analyses.*—Tissues were oven dried to constant weight at 80°C, ground in a Casella grain mill to pass a sieve with pores 2·5 mm in diameter, and stored at room temperature in screw-lid glass jars. Tissues were analysed in duplicate by the macroKjeldahl procedure, using copper sulphate as catalyst. Results were expressed as a percentage of the dry matter content of the analysed tissue.

(iii) *Free Amino Acid Nitrogen Analyses.*—Free amino acid nitrogen was extracted from the fresh tissues as soon as possible after sampling. Tissue samples were temporarily stored in polythene bags in a refrigerator if the extractions could not be carried out immediately after sampling. The extraction method used was as

follows: 2·0 g tissue was ground for a total of 15 min with 3 × 10 ml volumes of 75% aqueous ethanol in a mortar. The extracts were filtered through glass wool, bulked, and centrifuged. Each supernatant was poured into a beaker, a few drops of n-octyl alcohol were added to prevent "bumping", and the extracts were evaporated to dryness *in vacuo* at less than 35°C in a vacuum desiccator connected to a vacuum pump. Each residue was taken up in 3 ml 10% aqueous isopropanol and the concentrated extract was transferred to a small polythene vial. The vials were stored at −16°C.

The free amino acid content of the extracts was examined by two-dimensional paper chromatography. 20 μl of each extract were spotted onto 8-in. square sheets of Whatman No. 1 paper under a stream of warm air. Duplicate chromatograms were developed in darkness at 20±3°C in n-butanol–acetic acid–water (12 : 3 : 5 v/v) and then in phenol–water (4 : 1 w/v), according to the procedures described by Smith (1960). After development one chromatogram of each pair was dipped through a solution of 2% ethanolic ninhydrin and 0·1% pyridine (Possingham 1956) and was then heated at 80°C for 5 min. The colour and size of each spot which appeared was noted and the pattern of spots on the chromatogram was made permanent by dipping the chromatogram in a solution of 1% ethanolic copper nitrate. The other chromatogram of each pair was treated with isatin and Sakaguchi reagents which are specific for proline and arginine respectively (Smith 1960). The identity of the ninhydrin-positive compounds was established from their R_F characteristics, by co-chromatography with standard amino acids and amides, and by the use of specific chromogenic tests. In this way, the following amino acids and amides were detected in tissue extracts: cystine, aspartic acid, glutamic acid, lysine, serine, glycine, threonine, asparagine, arginine, glutamine, α-alanine, β-alanine, proline, γ-aminobutyric acid, pipecolic acid, tyrosine, phenylalanine, and leucine.

A semiquantitative estimate of the concentration of free amino acid nitrogen in the extracts was made using spot size and colour intensity as the criteria of concentration. Thus the concentration of each of the nine major amino acids and amides in each extract, viz. aspartic acid, glutamic acid, serine, asparagine, arginine, glutamine, α-alanine, γ-aminobutyric acid, and proline, was judged to fall within an arbitrary scale ranging from 0 to 5, where 0 indicated that the compound was not detected and 5 indicated that the compound was present as a very large, deeply coloured spot. The concentration of amino acid nitrogen in each extract was then estimated by adding together the individual values for the nine amino acids and amides. This method only gave a rough estimate of the concentration of free amino acid nitrogen in tissue extracts, and differences between treatments were not considered meaningful unless they were very large.

III. Results

(*a*) *Seasonal Changes in the Concentration of Total Nitrogen*

The pattern of seasonal change in concentration of total nitrogen in tree tissues was closely similar in both treatments (Figs. 1 and 2), and there was no sharp increase in concentration immediately following fertilizer application. However, the concentration of total nitrogen was usually higher in N_2 tissues than in the corresponding N_1 tissues (N_2/N_1 ratio > 1).

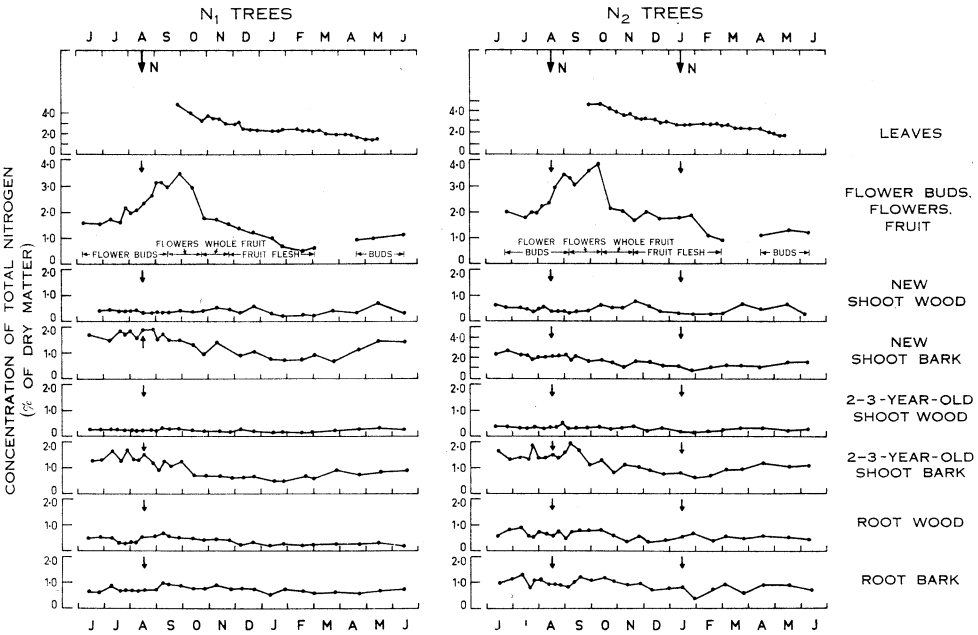

Fig. 1.—Influence of nitrogen supply on seasonal changes in the concentration of total nitrogen in tissues of 25-year-old peach trees. ↓N, application of 3 lb ammonium sulphate per tree.

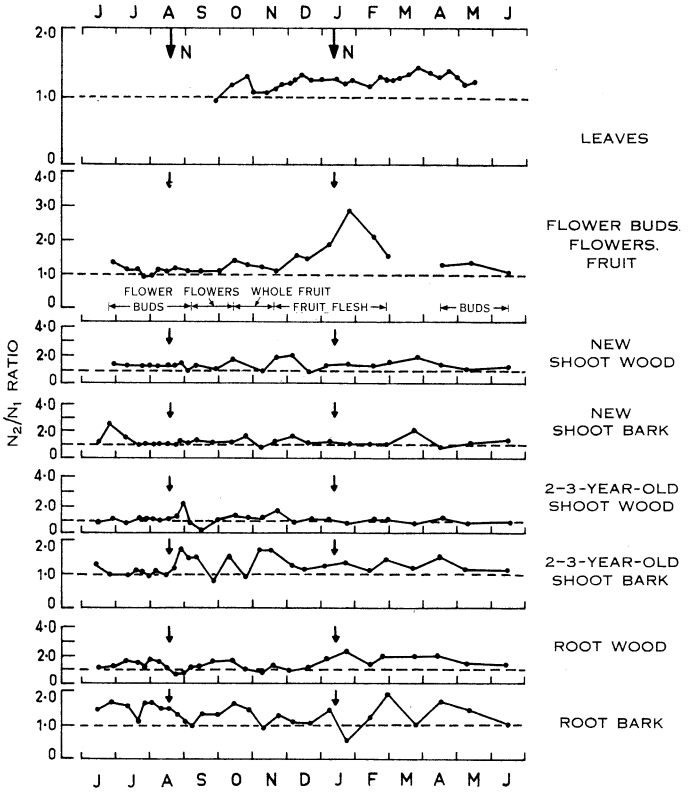

Fig. 2.—Seasonal changes in the ratio of the concentration of total nitrogen in N_2 *v.* corresponding N_1 tissues of 25-year-old peach trees. ↓N, application of 3 lb ammonium sulphate per tree.

In early spring the concentration of total nitrogen increased very rapidly in bud tissues and a peak concentration was reached at flowering. At the same time the

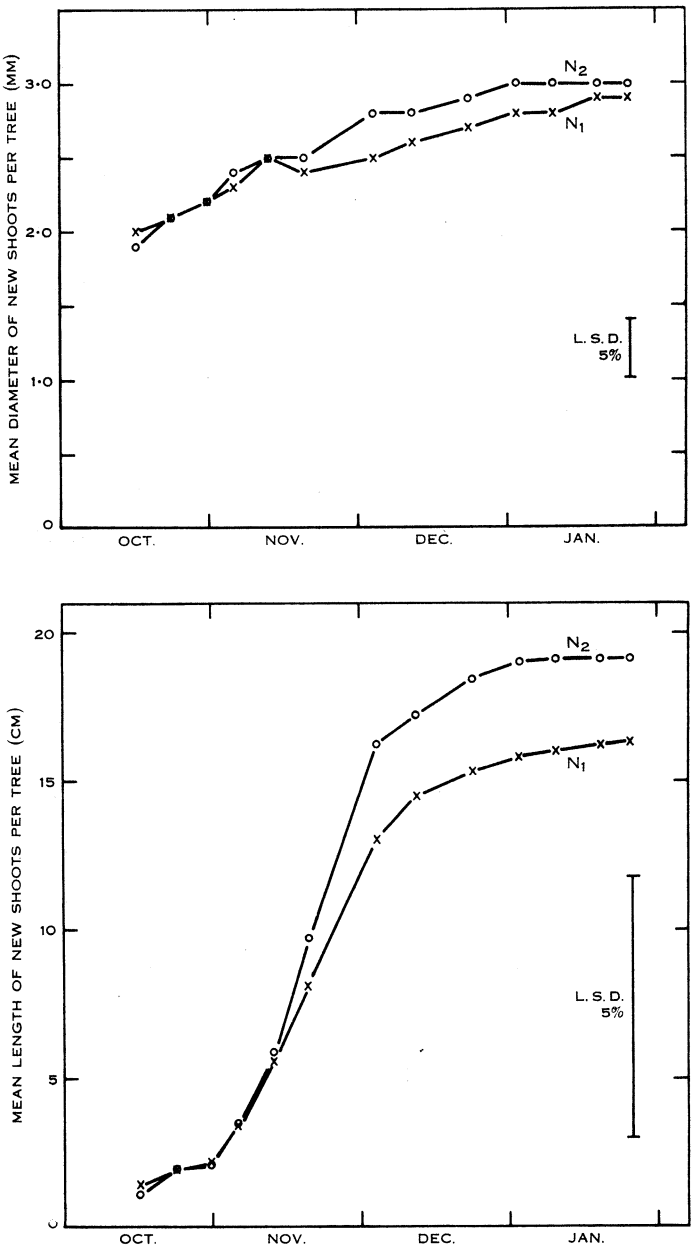

Fig. 3.—Influence of nitrogen supply on the mean extension and diameter growth of new shoots per tree during 1961–62.

concentration of total nitrogen in the shoot bark tissues fell rapidly, and since this was prior to commencement of shoot growth in October (Fig. 3), it is suggested that

the nitrogen which was stored in these tissues was translocated upwards to the developing buds. As the growing season progressed, the concentration of total nitrogen in the leaves, flowers and fruit, and shoot bark tissues declined slowly indicating that growth dilution was taking place or that nitrogen was translocated to other tissues. However, after shoot growth ceased in late summer (Fig. 3), the concentration of total nitrogen in shoot bark tissues rose again presumably due to storage of nitrogen.

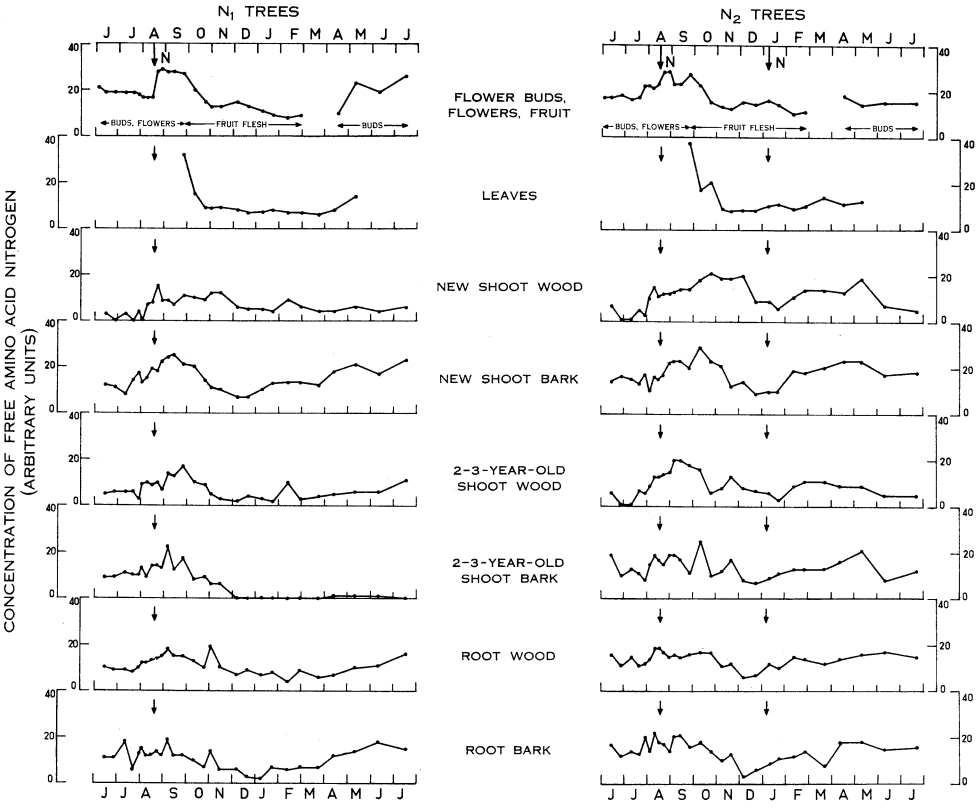

Fig. 4.—Influence of nitrogen supply on seasonal changes in the concentration of free amino acid nitrogen in tissues of 25-year-old peach trees. ↓N, application of 3 lb ammonium sulphate per tree.

(*b*) *Seasonal Changes in the Concentration of Free Amino Acid Nitrogen*

The pattern of seasonal change in concentration of free amino acid nitrogen in tree tissues was closely similar in both treatments except in 2–3-year-old shoot bark (Fig. 4). In this tissue the concentration of free amino acid nitrogen rose to a peak in autumn in the N_2 trees but did not similarly increase in the N_1 trees. This suggests that the concentration of free amino acid nitrogen in 2–3-year-old shoot bark was increased by the summer application of nitrogenous fertilizer. Levels were usually higher in N_2 tissues than in the corresponding N_1 tissues, especially in autumn when the concentration increased more rapidly in the N_2 tissues than in the corresponding N_1 tissues.

Peak concentrations were observed in woody storage tissues in spring (at flowering) and autumn, and this is consistent with the hypothesis that storage nitrogen is mobilized in spring and that storage nitrogen re-accumulates when growth ceases. The decline in concentration in many of the storage tissues in winter probably indicates that other nitrogenous compounds are synthesized at this time at the expense of the amino acids and amides.

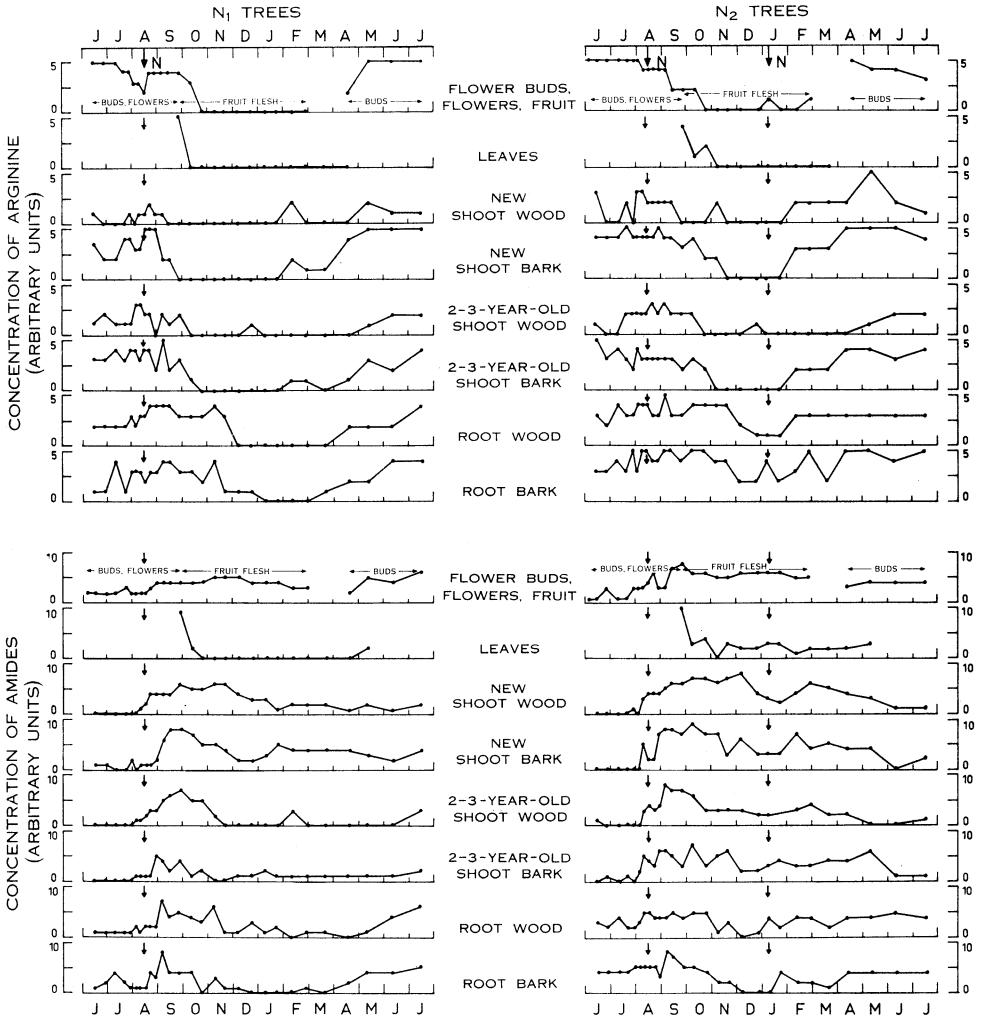

Fig. 5.—Influence of nitrogen supply on seasonal changes in the concentration of arginine and the amides in tissues of 25-year-old peach trees. ↓N, application of 3 lb ammonium sulphate per tree.

(c) *Seasonal Changes in the Concentration of Arginine and the Amides*

Seasonal changes in the concentration of arginine conformed to the same pattern in corresponding tissues from both treatments (Fig. 5). This pattern was not influenced by the time of application of the nitrogenous fertilizer or by the amount

of nitrogenous fertilizer applied, but the concentrations found were dependent upon the nitrogen supply. Arginine was present in very high concentration in woody storage tissues and buds in late autumn, winter, and spring, but the concentration in tree tissues fell to a minimum during the growing season. It is evident from Figure 5 that the decline to a minimum occurred earlier, and re-accumulation took place more slowly, in storage tissues of N_1 trees than in storage tissues of N_2 trees.

Seasonal trends in the concentration of the amides (glutamine plus asparagine) were different from those recorded for arginine but were similar in corresponding tissues of both N_1 and N_2 trees (Fig. 5). The concentration of the amides in tree tissues was very low in winter, exept in root and bud tissues, but was high in spring and autumn. As in the case of the other nitrogenous constituents, the concentration of the amides in tree tissues was proportional to the amount of nitrogen supplied.

IV. Discussion

The results obtained have confirmed and extended previously reported observations on seasonal trends in the concentration of total nitrogen and free amino acid nitrogen in tissues of mature peach trees (Schneider 1958; Radu 1961). Peak concentrations of each constituent were found in tree tissues before growth commenced and after growth ceased (woody tissues only), while a minimal concentration was found at the end of the growing season. In most tissues this overall pattern of change was not influenced by the amount of nitrogen supplied or by the time of application of the nitrogenous fertilizer, but the concentrations observed were usually proportional to nitrogen supply. It is concluded that nitrogen accumulates in woody tissues of the peach trees after shoot growth ceases in late summer, and that this stored nitrogen is mobilized for new growth during the next season. However, this conclusion must be regarded as tentative since results have been expressed on a concentration basis. A different conclusion might have been reached if results could have been expressed on an absolute, i.e. per tree, basis. For example, it is known that nitrogen accumulates on an absolute basis in storage tissues of young peach trees before growth ceases, but a corresponding increase in the concentration of nitrogen does not take place at this time because of growth dilution (Taylor 1966). This clearly illustrates the limitations inherent in the use of large orchard trees for this type of study.

After shoot growth ceased, the accumulation of nitrogen in N_2 tissues was much more rapid than in the corresponding N_1 tissues. This was probably partly a response to the summer fertilizer application and partly a response to the higher soil nitrogen content which had been built up in the N_2 plots compared with the N_1 plots over the years that these fertilizer treatments had been applied.

Since dormant storage tissues usually contained high concentrations of arginine but only low concentrations of the amides, it is suggested that arginine is a more important constituent of the storage nitrogen of mature peach trees than are the amides. Results of a quantitative study have shown that this is indeed the case for young peach trees (Taylor and May 1967).

It is possible that the metabolism of arginine and the amides is closely linked in the peach tree. For example, the fall in concentration of arginine in storage

tissues in spring may be related to the increase in concentration of the amides in the same tissues in spring. Thus arginine may be hydrolysed to release ammonia and this could be temporarily stored as amide nitrogen. In winter, however, arginine may be synthesized at the expense of the amides present in the woody shoots.

V. Acknowledgments

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