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†CHEMICAL CHANGES IN YOUNG TEA PLANT (*CAMELLIA SINENSIS L.*) TISSUES FOLLOWING APPLICATION OF FERTILIZER NITROGEN

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The uptake of ammonium sulphate by 14-month-old potted tea plants grown in a glasshouse was studied over the 11-week period following application. Concurrent changes in the starch of root-wood, amino acids of xylem sap, and total nitrogen of leaves, stems, root-wood, and feeder roots were determined. Depletion of nitrogen from the soil at different depths and transformation of NH_4^+ to NO_3^- was also followed.

The results show that the uptake of nitrogen commences within 2 days of application as indicated by a marked increase in the amino-acid content of the xylem sap. Glutamine and, to a lesser extent, theanine were quantitatively the most important amino acids in the sap. The amino-acid content of the sap was a maximum at about the time rapid depletion of the ammonium of the soil took place. An interesting feature of the work is the reciprocal relationship between the changes in the starch of root-wood and amino acids in the sap a few days after fertilizer application. Studies on the ammonium and nitrate levels of the soil at different depths showed that transformation of NH_4^+ to NO_3^- occurred in the soil.

The response of the various tissues to applied fertilizer nitrogen and increase in the fresh weight of the shoot system showed similar trends and may be correlated with the depletion of ammonium-nitrogen from the soil.

The influence of nitrogen fertilizers on tea yield is well known (Fernando, Bhavanandan, Wettasinghe, and Manipura 1969). Ammonium sulphate is the most widely used fertilizer for tea. Recently urea and calcium ammonium nitrate have been tried as alternative forms of nitrogen (Bhavanandan and Manipura 1969). Field experiments with mature tea have indicated that ammonium sulphate gives better yields under certain conditions (Bhavanandan and Sunderalingam 1971). However, glasshouse experiments designed to evaluate the effects of the form of nitrogen nutrition (varying combinations of ammonium and nitrate) on growth of tea plants in sand culture, have indicated that the best performance is obtained when 60-80 per cent of the nitrogen is available in the form of nitrate (Pethiyagoda and Krishnapillai 1971). Ammonium sulphate is also used generously to fertilize young tea, some of the fertilizer mixtures containing as much as 50 per cent of this compound (Tolhurst 1961). Although nitrogen fertilizers are applied to tea at regular intervals, the mode of uptake of nitrogen from the soil by the tea plant is little understood.

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The primary objective of this work was to study the uptake of nitrogen by potted plants in the greenhouse following application of ammonium sulphate. Studies on the loss of soil nitrogen and changes in the quantities of nitrogenous compounds in the xylem sap and in the various tissues of the plant were carried out and the results are reported here. This is apparently the first attempt to present such comprehensive data on the uptake of fertilizer nitrogen by the tea plant as a whole.

MATERIALS AND METHODS

Plants used in the investigation

Plants of Clone TRI 2024 were grown in polythene bags under standard nursery conditions. They were transplanted after 6 months into cement pots (30 cm long, 25 cm (top), and 18 cm (bottom) diam.) filled with 1.5 kg of fumigated soil (pH 5.0) and placed in the glasshouse near the laboratory (1500 m elevation). During the first 3 weeks following transplanting, the plants received only tap water. They were then regularly fertilized with fertilizer mixture, T65 (Tolhurst and Visser 1961) for 8 weeks, and thereafter with fertilizer mixture, T200 (Tolhurst 1961), up to 10 weeks from the commencement of the experiment. The present study was made when the plants were 14 months old. One hundred uniform plants, each with a single stem, were selected for the experiment. The control of pests and diseases was carried out as a routine measure.

Fertilizer treatment

The plants were divided into two groups of 50 each for the experiment. One group was not given any additional nitrogen and served as control plants, whereas the other group was given 2.24 g nitrogen per plant in the form of ammonium sulphate (i.e. 10.6 g $(NH_4)_2 SO_4$. The $(NH_4)_2 SO_4$ was dissolved in distilled water (100 ml) and given in a single application on 1 October 1971. After the solution had soaked in, a further 100 ml of tap water was applied per pot. The control plants received 200 ml of tap water. All the plants were watered daily and it was ensured that no solution was leached from the pot throughout the course of the investigation.

Sampling

When required, two plants from each group were cut at a height of 10 cm above soil level and the xylem sap collected as described before (Selvendran and Sabaratnam, 1971). After sap collection the decapitated plants were removed from the pots and their roots washed carefully free of soil. The plants were then divided into morphologically different parts which were weighed and dried separately. The parts were (*a*) fully expanded young leaves (fifth and sixth); (*b*) stem (including bark and wood); (*c*) root-wood (woody roots without bark); and (*d*) feeder roots (those not woody).

Representative samples from each of these tissues from all the plants were dried in the oven at 100°C, milled, and subsampled for analysis. Because the composition of the xylem sap and nitrogen content of the various tissues of the control plants showed negligible variation during the course of the experiment, they were sampled for analysis less frequently.

Methods of analysis

The total nitrogen of the various tissues was determined by the micro-Kjeldahl procedure.

The amino acids were estimated by the method of Yemm and Cocking (1955) using alanine as the standard. The total amino-acid content of the sap was determined before purification. The two main amino acids, namely glutamine and theanine, of the sap samples were determined quantitatively after their separation by one-directional paper chromatography using phenol-water as the solvent.

The ammonium content of the sap was determined by the method of Hofmann (1963) and the starch content of the roots by the method of Hassid and Neufeld (1964).

Ammonia and nitrate content of soil

The ammonia and nitrate contents of 20-g soil samples were determined after extracting them with 200 ml of M/100 $CaCl_2$ solution. The mixture was mechanically shaken in stoppered glass bottles for 1 h, centrifuged and filtered. The ammonia was first distilled from the extract by heating with 5 g of MgO and trapped in 20 ml of 2 per cent boric acid and the nitrate was subsequently distilled by heating the remaining mixture with 5 g of Devarda's alloy and 5 ml of 40 per cent NaOH.

During the course of the investigation it was realized that amides interfered with the nitrogen determinations. Tests with glutamine and asparagine indicated that while treatment with MgO decomposed a negligible quantity of the amide nitrogen the subsequent treatment with Devarda's alloy and NaOH liberated about 50 per cent of the amide nitrogen. The soil samples would contain some amide amino acids (rhizosphere effect) especially after fertilization, hence the figures given for soil nitrate nitrogen would include some nitrogen liberated from the amide fraction.

Growth assessments

Fresh weights of the entire shoot system of the treated and control plants were determined at regular intervals.

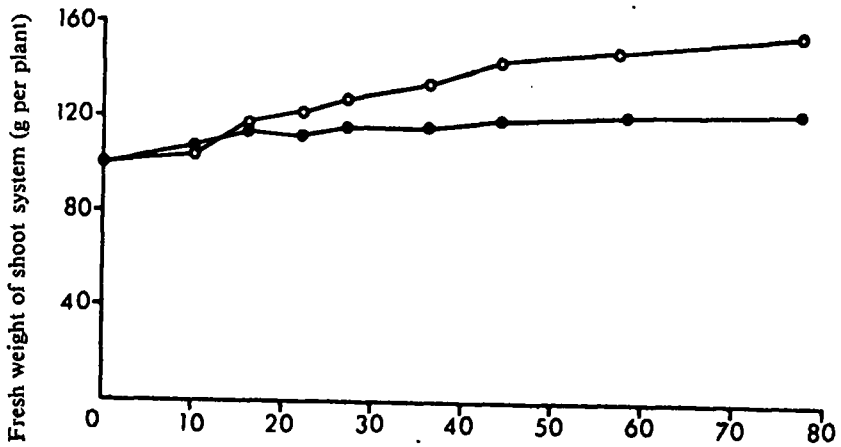

FIG.1 — *Shoot growth of tea plants fertilized with ammonium sulphate* (O) *and control* (●)

RESULTS

General observations

Visual differences between treated plants and controls were evident within 5 weeks of fertilizer application. The leaves of treated plants were darker green compared with controls and they had a much higher percentage of active terminal buds.

The treated plants also showed significantly better growth compared with controls (Fig. 1). Values in Figs. 1-7 are the means of determinations on two separate plants.

Nitrogen status of the soil

The nitrogen content (ammonium and nitrate) of the soil at different depths (top, middle, and bottom) over the 11-week period is shown in Fig. 2. The control pots had about 0.5 - 1 mg ammonium nitrogen and 0.2 - 0.5 mg nitrate nitrogen per 100 g soil at different depths. It seems that the NH^+_4 is fairly readily leached into the lower layers and its uptake is nearly complete after 5 weeks. More than 85 per cent of the applied nitrogen is taken up by the plant during this period. The increase in the nitrate in the lower layers with time is probably due to nitrification at the surface of the soil, followed by the relatively faster rate of movement of the nitrate into the lower layers. To test this hypothesis, 200 mg $(NH_4)_2\ SO_4$ and 226 mg KNO_3

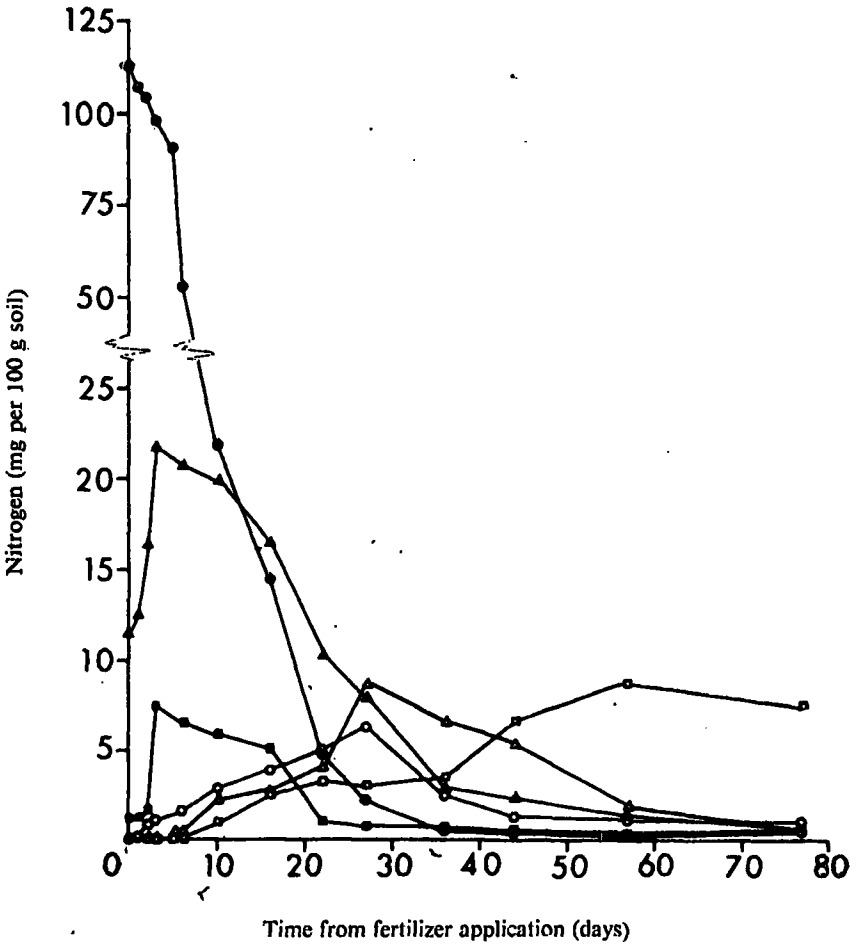

FIG. 2 — *Changes in the levels of ammonium and nitrate nitrogen in the soil in pots at different depths following ammonium sulphate application.*

Ammonium nitrogen: top soil (●), middle soil (▲), bottom soil (■).
Nitrate nitrogen: top soil (○), middle soil (△), bottom soil (□).

containing 41.2 mg and 34 mg nitrogen respectively, were applied to the top of a soil column (60 cm x 3.5 cm diam.) and eluted with distilled water. The eluate was collected in 200-ml fractions and the elution of NH_4^+ and NO_3^- was followed by chemical analysis. Sixteen fractions were collected and analysed. The elution sequence shown in Fig. 3 appears to support the hypothesis. The recovery of NH_4^+ and NO_3^- from the soil column was 70 and 98 per cent respectively.

Effect of fertilizer nitrogen on the composition of the xylem sap

Ammonia. The ammonium content of the sap increased considerably 3 days after application of $(NH_4)_2SO_4$ and declined after reaching a maximum after 4 to 5 days. It decreased to the level of controls after 8 weeks (Fig. 4) Considerable amounts of sulphur as sulphate, were also present during the first 3 weeks following fertilizer application.

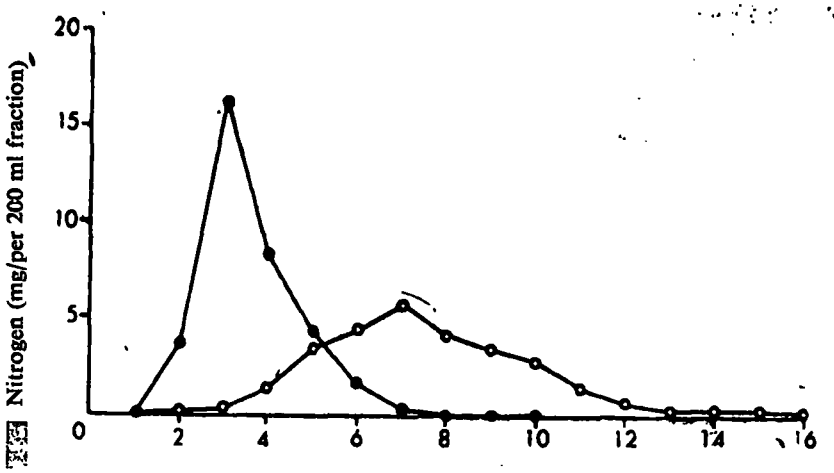

FIG. 3—*Elution sequence of nitrate (●) and ammonium (○) from a soil column (60 cm x 3 cm diam.) with distilled water as the eluting agent.*

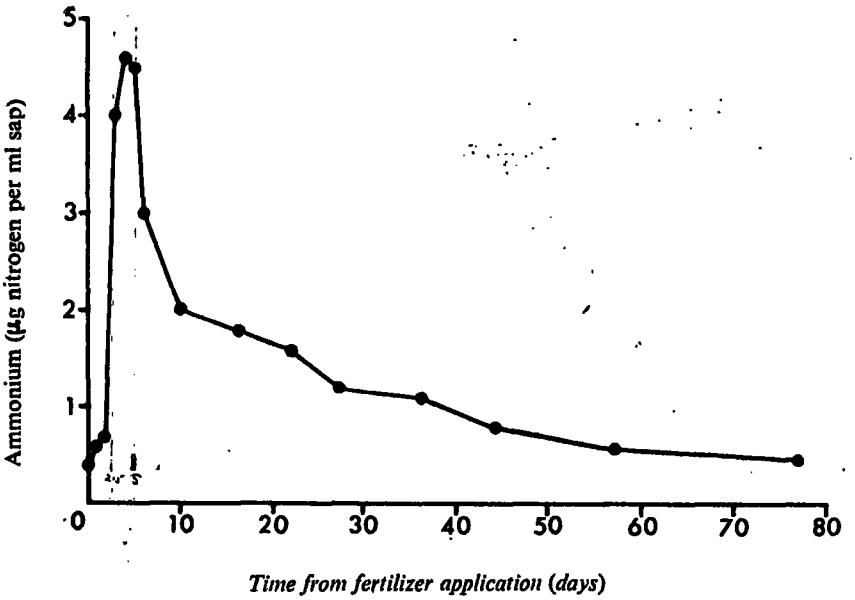

FIG. 4—*Changes in the level of ammonium in the sap of tea plants following ammonium sulphate application.*

Amino acids. The results of analyses are shown in Fig. 5. The total amino-acid content of the sap increased considerably 2 to 3 days after fertilizer application, reaching a maximum after 10 days and decreased thereafter. It dropped to the value of controls after 8 weeks. Glutamine and theanine were the most impotant amino-acids quantitatively, and accounted for up to 85-90 per cent of all amino nitrogen present. The increase in glutamine was considerably greater than that of theanine, Glutamic and aspartic acids increased slightly.

Effect of fertilizer nitrogen on the leaves, stems, and roots

The total nitrogen content of the various tissues are shown in Fig. 6 as a percentage of the dry weight. It is seen that application of fertilizer nitrogen increased the total nitrogen of the various tissues in a similar manner. After 6 weeks all the plants

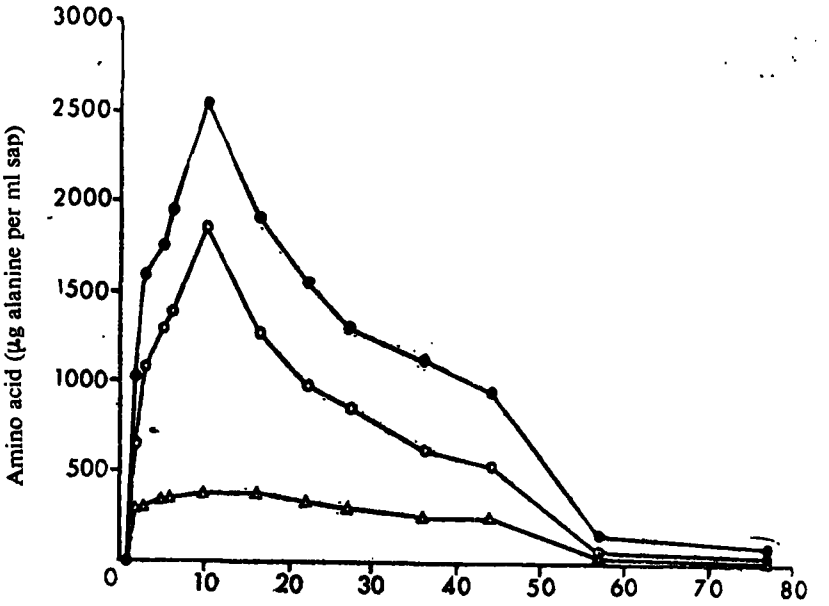

FIG. 5—*Changes in the levels of amino acids in the sap of tea plants following ammonium sulphate application.* (●) *total amino acids;* (O) *glutamine;* (Δ) *theanine.*

that received nitrogen had leaf-nitrogen values of approximately 4.2 per cent, while those from control plants were only about 3.0 per cent. The nitrogen content of the various tissues of the controls showed slight decrease during the 11-week period.

Effect of fertilizer nitrogen on the starch content of root-wood

The changes in the starch content of root-wood following fertilizer application are shown in Fig. 7. The starch content of the root-wood decreased appreciably 2 to 3 days after fertilizer application and remained at a low level for about 3 weeks. After 5 weeks the starch content of the roots increased to a value higher than the controls.

The feeder roots contained about 1 per cent starch on a dry-weight basis.

DISCUSSION

The growth of tea plants fertilized with ammonium sulphate was studied over the 11-week period following application. It was found that the new growth as measured by the fresh-weight increase of the shoot system was primarily due to

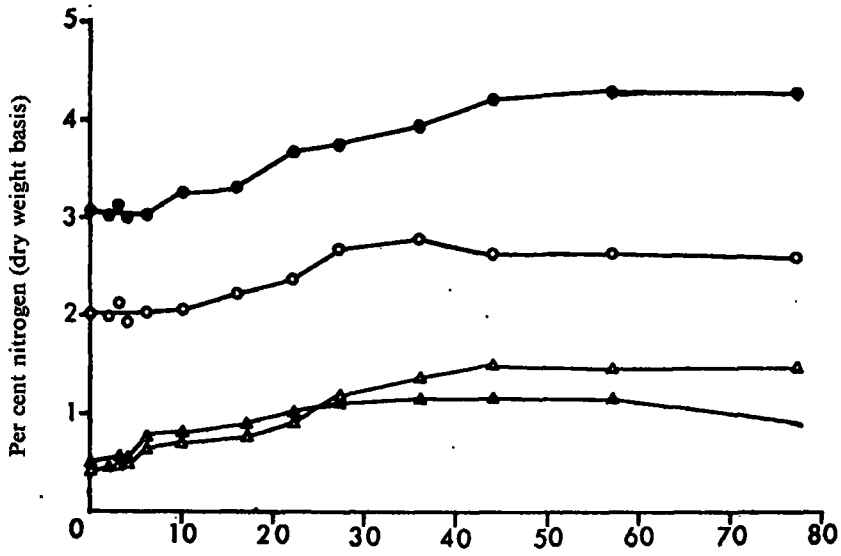

FIG. 6—*Changes in the nitrogen content of the various tissues of tea plants following ammonium sulphate application.*
(●) leaves (▲) stems (△) root-wood (○) feeder roots.

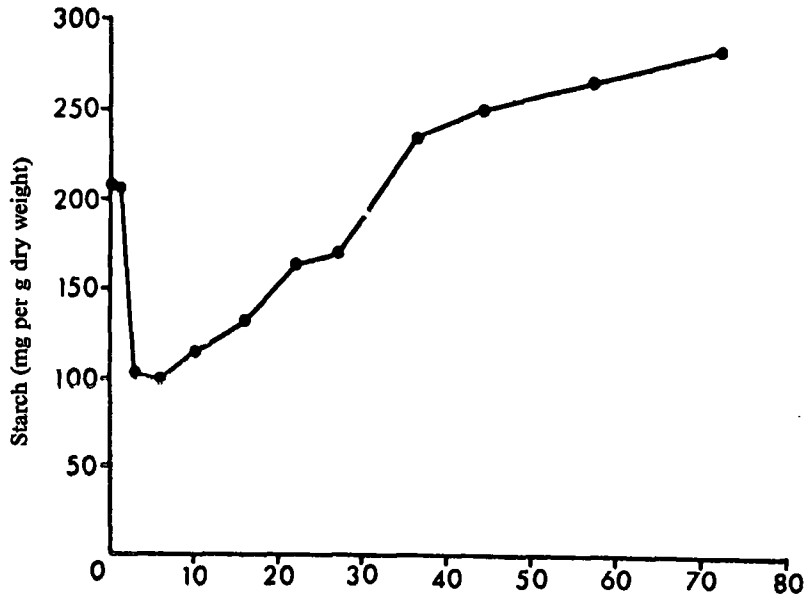

FIG. 7—*Changes in the level of starch in the root-wood of tea plants following ammonium sulphate application. Values are mg starch (as glucose) per g dry weight.*

breaking of dormancy, i.e. production of active terminal buds and the subsequent growth of these buds. It has been shown that the production of active terminal buds is stimulated by exogenosly applying certain growth regulators to dormant buds (Kulasegaram, 1969) and by application of fertilizer nitrogen (Kulasegaram and Kathiravetpillai, 1971).

The initial rapid rise of amino acids (and NH^{+}_{4}) in the sap following nitrogen application suggests that the absorbed nitrogen may be translocated as these compounds. The amino-acid content of the sap was a maximum at about the time rapid depletion of the ammonium of the soil took place. It is thus of interest that the analysis of xylem sap provides a useful index of ammonium-nitrogen uptake by the plant. Similar studies with somewhat comparable results have been made with apple trees by Hill-Cottingham and Bollard (1965), Tromp and Ovaa (1969), and Hill-Cottingham and Cooper (1970).

An interesting feature of the present work is the reciprocal relationship between the changes in the starch of root-wood and amino acids in the sap. As seen in Figs. 5 and 7, 2 to 3 days after fertilizer application, the starch content of the root-wood decreased, whereas the amino acids of the sap increased sharply. These results suggest that the additional α keto acids required for nitrogen fixation are probably provided by degradation of starch. A similar phenomenon takes place during post-prune growth (Selvendran, 1970). Depletion of carbohydrate reserves during nitrogen assimilation has been shown with both higher (Yemm and Willis, 1956) and lower plants (Syrett, 1953; Yemm and Folkes, 1954).

By 5 weeks after fertilizer application, due to increased chorophyll content of leaves and 'other factors', sufficient carbohydrates, are manufactured through photosynthesis and translocated from the aerial parts to roots, that the excess carbohydrates are accumulated as starch in the root-wood. Hence the starch content of the root-wood increased. The starch reserves of roots therefore represent an accumulation of material in excess of the immediate needs of the plant for growth and maintenance. These reserves are utilized when the demands for growth exceed the supply from photosynthesis (e.g. recovery from pruning, fertilizer up-take and high soil temperatures). If the starch reserves are depleted to low levels in response to increased nitrogen levels and higher soil temperatures, it appears that the plants are placed in a more vulnerable position to disease susceptibility and delayed bud-break following dormancy and recovery from pruning.

Fig. 6 represents the response of the various tissues of the plants to applied fertilizer nitrogen as well as the subsequent redistribution of nitrogen within these plants, in response to growth. The fresh-weight curve of the shoot system and increase in nitrogen of the various tissues showed similar trends and may be correlated with the depletion of ammonium-nitrogen in the soil. A fair proportion of the currently absorbed nitrogen would be utilized for new growth (Grasmanis and Nicholas, 1971) and some of it appears to be stored as metabolic reserves in the roots, stems, and leaves. The tissue most frequently sampled in order to assess fertilizer uptake by tea plants is the mature leaf and the results of this experiment justify this choice. Although these complete analyses of soil and plant tissues following fertilizer application are only practicable with small potted plants, the results may prove useful in advisory work on tea where problems relating to fertilizer applications and post-prune growth are not uncommon.

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