

*UPTAKE AND UTILIZATION OF AMMONIUM AND NITRATE IONS BY TEA PLANTS AS DETERMINED BY THE USE OF N¹⁵ LABELLED COMPOUNDS

T. Kularatne & V. P. Bhavanandan

The question whether tea prefers ammonium or nitrate as the form of nitrogen has been investigated previously by a number of workers at the Tea Research Institute of Ceylon, but the results have been variable. In order to obtain more information on this question, isotopically-labelled ammonium and nitrate compounds were used in further experiments. Actively-growing young plants of clone DT 1 established in sand culture were divided into two groups. One group of plants was given a balanced nutrient solution containing ammonium sulphate labelled with N¹⁵ as the only source of nitrogen. The other group of plants was also given the same balanced nutrient solution but containing potassium nitrate labelled with N¹⁵ as its only source of nitrogen. At the end of the treatment period, the nitrogenous components of the plants were fractionated into ammonium, amide, nitrate, caffeine, amino acid and protein nitrogen. The total nitrogen (N¹⁴ and N¹⁵) and labelled nitrogen (N¹⁵) content of each of these fractions were determined by the Kjeldahl and mass spectrometric methods respectively. The results obtained indicated that total nitrogen as well as the labelled nitrogen assimilated by the plants treated with ammonium nitrogen was twice as much as that assimilated by the nitrate-treated plants. In addition, the leaves, stems and roots from the plants were analysed for their phosphorus, potassium, calcium, magnesium, manganese and aluminium contents. The results of these determinations are discussed.

Organic and inorganic nitrogen fertilizer applied to soil are absorbed and assimilated by plants mainly in the form of nitrate and/or ammonium ions. Many plants are known to absorb and assimilate nitrate nitrogen, not just because it is the preferred form, but possibly because the nitrifying system in most soils is efficient, and more nitrogen is, therefore, available in this form. Some plants are reported to be able to absorb and assimilate ammonium nitrogen better than nitrate nitrogen. Although a plant may show a preference for one form or the other, it is seldom that a plant exclusively needs only one form of nitrogen. It is known that at least in culture experiments, ammonium nitrogen is useless or even damaging to certain plants. It has also been reported that sugar beets lack the ability to utilize nitrate. In order to work out an efficient policy for the application of nitrogen for a crop it is important to know whether the plant has a preference for nitrate or ammonium nitrogen.

In recent years, various forms of nitrogen fertilizers such as ammonium sulphate, calcium ammonium nitrate and urea have been used for tea. The earliest attempts at comparing the ammonium and nitrate nitrogen for tea were made by Eden (1942; 1943). The efficiency of ammonium sulphate, sodium nitrate and a mixture of these at three levels of nitrogen (33.6, 50.4, 67.2 kg/hectare) was investigated for mature tea in a field experiment. The responses for five years indicate that ammonium sulphate gave the best yield, followed by the mixture of ammonium sulphate with sodium nitrate. It is not stated whether these difference were statistically significant. The results might be assumed to be indication that ammonium fertilizer was better than nitrate fertilizer for mature tea under field conditions. It was possibly on these findings that ammonium sulphate was widely used as the source of nitrogen for tea.

* This paper was presented at the 26th Annual Sessions of the Ceylon Association for the Advancement of Science in December 1970.

Lamb (1954) carried out lysimeter studies in order to obtain more information. The drainage water, from two lysimeters with bare soil and two with tea plants, was collected and analyzed for total nitrogen, ammonium, nitrate and nitrite. His results indicated that tea plants absorbed considerable quantities of nitrates. Tolhurst (1968) tested ammonium sulphate, a mixture of ammonium sulphate with N-Serve, and calcium nitrate for young tea under field conditions. He found that at lower levels, ammonium nitrogen was superior to nitrate nitrogen and that the presence of N-Serve, a nitrification inhibitor, increased the efficiency of response to ammonium sulphate. At the higher levels of nitrogen, however, the differences were not marked.

The superior growth of young tea plants in soils fumigated with methyl bromide and the build up of ammonium nitrogen in these soils (Shanmuganathan and Fernando 1967) indicate the possible preference of young tea plants for ammonium rather than nitrate nitrogen. Nitrate reductase has been found in tea feeder roots but has not been detected in tea leaves (Bhavanandan 1971).

EXPERIMENTAL

Ten plants of clone DT1, propagated in the nursery from cuttings, were transplanted into sand pots. The plants were about one year old and of uniform growth. They were divided into two groups of five each. One group was supplied with a balanced nutrient solution containing ammonium sulphate as the only source of nitrogen. The other group was supplied with the same balanced nutrient solution but containing potassium nitrate as the only source of nitrogen. Each plant was given 100 ml of the appropriate solution once every two days for a period of seven weeks. At the end of this period two plants from each set were given a nutrient solution containing the N^{15} -labelled compounds and the remaining plants in both groups continued to receive N^{14} -nutrient solutions. Treatment with N^{15} -solution was continued for about one month. At the end of this period the sand in the pots was rinsed with demineralized water to leach out unabsorbed N^{15} compounds and the rinse water collected for analysis. After careful rinsing of the roots to wash off unabsorbed nutrients, the plants were cut into small pieces, dried, ground and analyzed. The nitrogen component of the plant was fractionated according to the schedule shown in Fig. 1. Subsequent studies showed that in this procedure, the caffeine was not completely removed by chloroform extraction. The unextracted caffeine slowly decomposed during distillation for the determination of ammonium, amide and nitrate and, therefore, gave slightly higher values for these fractions. The error in the values for ammonium and amide was not significant because the determination involved distillation for only 30 min. The error in the nitrate figure was, however, significant because the distillation continued for four hours.

The total-nitrogen content of each fraction was determined by the Kjeldahl procedure using sulphuric acid and hydrogen peroxide. After titration, the solutions were redigested with sulphuric acid/methanol (to remove boric acid) and the digests sent to the laboratories of the International Atomic Energy Agency in Vienna for analysis of % N^{15} excess. The three plants treated with unlabelled nutrient solutions were also removed, the roots washed lightly, separated into leaves, stems and roots and each of these components separately analysed for nitrogen, phosphorus, potassium, calcium, magnesium, manganese and aluminium.

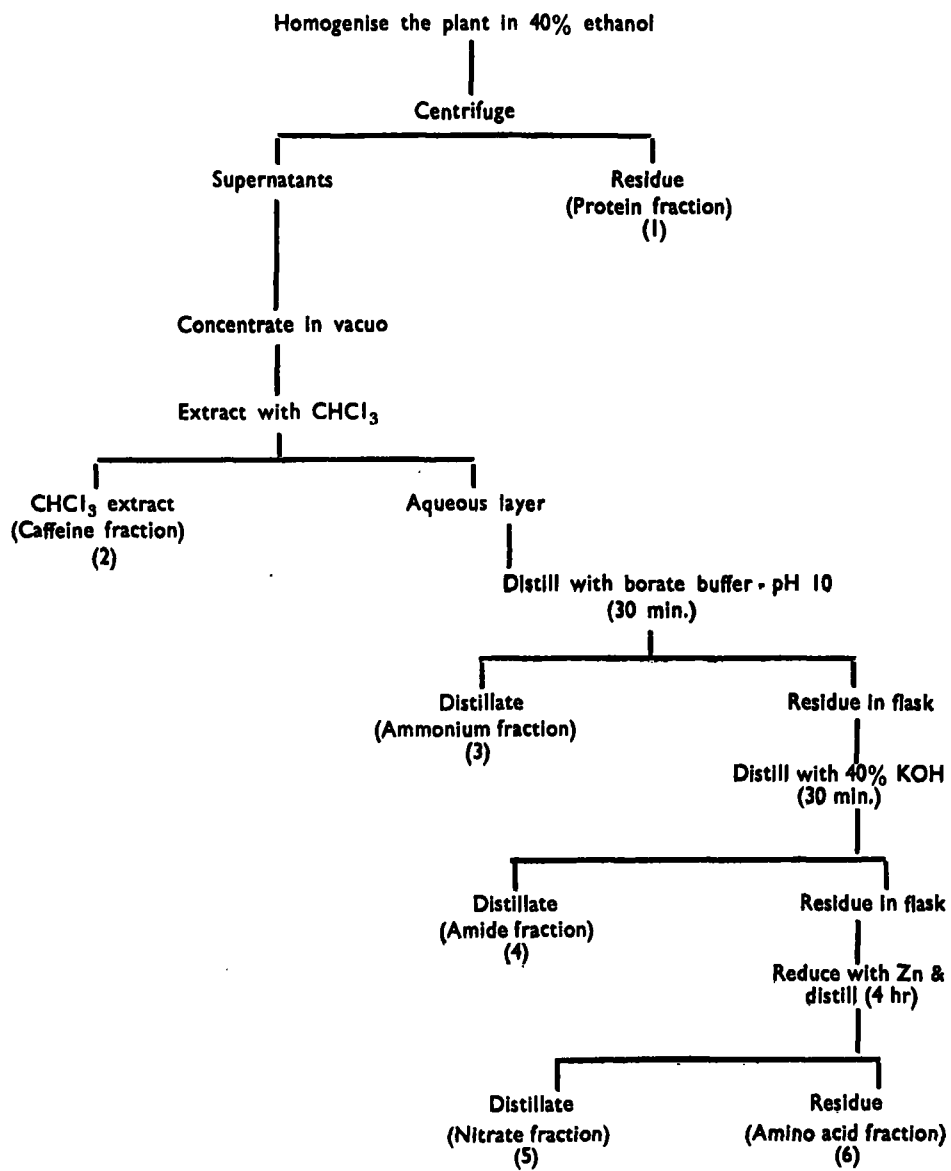


FIG. 1—*Fractionation procedure*

RESULTS AND DISCUSSION

From the results quoted by the earlier workers no precise conclusions can be drawn. The results of some of these investigations are even contradictory. The main reasons for inconsistency are probably the experimental techniques used. These are not capable of giving precise information on the problem under investigation. Although the field experiments of both Eden and Tolhurst have indicated that as an overall effect, ammonium sulphate was better than sodium nitrate or calcium nitrate, it is not possible to conclude from this that ammonium is preferentially absorbed and assimilated by the tea plant because in field experiments, the following factors come into action :

- 1 — The natural organic and inorganic nitrogen of the soil
- 2 — Additions and losses of nitrogen during the experiment due to natural phenomena; such as rain, biological action, leaf drop, pruning, *etc.*
- 3 — Uncontrolled transformations in the soil such as the ammonification of organic nitrogen, the nitrification of ammonium and the conversion of nitrate to ammonium, either by chemical reduction or by the activity of micro-organisms, with subsequent ammonifications of protein,
- 4 — Unbalanced nutrient supply, such as, the addition of sulphate with ammonium and the addition of sodium or calcium with nitrate.

Lysimeter experiments are also affected by these factors. For example, although the results of Lamb's lysimeter experiment at first glance indicated that tea bushes have taken up considerable quantities of nitrate, this is questionable. It is not clear as to whether tea plants in the lysimeter, used up ammonium nitrogen and whether only the balance was nitrified and leached out, or whether the ammonium nitrogen was firstly nitrified before some of it was absorbed by the plant and the balance leached out.

Our experiment was carried in a glass house using the tea plants grown in sand culture by the technique developed by (Pethiyagoda, Nagarajah & Krishnapillai 1969). In this method, the influence of the first three factors discussed above are absent. The influence of the fourth factor was overcome by using a balanced nutrient solution. Finally the use of N^{15} -labelled compounds enabled us to distinguish between the nitrogen already present in the plant and that taken up and assimilated during the experimental period. Another important advantage of the N^{15} -labelled compounds enabled us to distinguish between the nitrogen already present in the plant and that taken up and assimilated during the experimental period. Another important advantage of the N^{15} -labelled compounds is the ability to follow the metabolic pathway of the nitrogen absorbed by the plant. We were, therefore, able to determine not only the relative absorption, but also the efficiency of assimilation of the two forms of nitrogen by the tea plant.

The growth measurements, total nitrogen and N^{15} nitrogen absorbed by the plants in our experiment are presented in Tables 1 & 2.

It can be seen from the tables, that there is no obvious difference in the growth of the plants, as assessed by the total plant and root weights. The total nitrogen absorbed by the plant treated with ammonium is, however, twice that absorbed by the treated plant with nitrate. This is shown by the total nitrogen as well as the

TABLE 1 — *Growth and nitrogen absorption by young tea plants given in ammonium and nitrate nitrogen—Each figure represents the mean of 2 determinations*

| Medium | Fresh weight of plant (g) | Fresh weight of root (g) | Total nitrogen in plant (mg) |
|----------|---------------------------|--------------------------|------------------------------|
| Ammonium | 19.3 | 7.2 | 111.9 |
| Nitrate | 18.9 | 6.7 | 58.9 |

TABLE 2 — *N¹⁵ absorbed and percentage of N¹⁵ recovered by young tea plants given ammonium and nitrate nitrogen*

| Medium | Total N ¹⁵ —N in plant (mg) | % N ¹⁵ recovered by plant | N ¹⁵ /N ¹⁴ |
|----------|----------------------------------------|--------------------------------------|----------------------------------|
| Ammonium | 11.9 | 29.4 | 0.106 |
| Nitrate | 6.7 | 19.4 | 0.114 |

labelled nitrogen (N¹⁵) determinations in the two plants. The N¹⁵-nitrogen and N¹⁵/N¹⁴ ratio in the individual nitrogen fractions of the plants are given in Table 3.

It is seen that N¹⁵ in the nitrogen fractions is higher in the ammonium-treated plant, in all fractions except caffeine for which N¹⁵ is the same in both plants. It is also evident that there is a much larger accumulation of amino acids in the plants grown on ammonium nitrogen. The efficiency of assimilation of the two forms of nitrogen after uptake appears to be about the same as shown by the almost uniform incorporation of the labelled nitrogen into all fractions of the two sets.

The results of the elemental analysis of leaf, stem and roots from the two sets of N¹⁴ treated plants are given in Table 4 & 5.

TABLE 4 — *Elemental analysis of leaf, stem and root of plants grown in ammonium and nitrate medium*

| Medium | %N | | | %K | | | %Ca | | | %Mg | | |
|----------|------|------|------|------|------|------|------|------|------|------|------|------|
| | Leaf | Stem | Root | Leaf | Stem | Root | Leaf | Stem | Root | Leaf | Stem | Root |
| Ammonium | 3.77 | 1.47 | 2.31 | 1.95 | 0.73 | 1.73 | 0.28 | 0.11 | 0.10 | 0.21 | 0.01 | 0.06 |
| Nitrate | 2.79 | 1.20 | 1.68 | 2.35 | 2.75 | 2.05 | 1.38 | 0.50 | 0.50 | 0.26 | 0.31 | 0.23 |

TABLE 5 — *Elemental analysis of leaf, stem and root of plants grown in ammonium and nitrate medium*

| Medium | %P | | | Mn ppm | | | Al ppm | | |
|----------|------|------|------|--------|------|------|--------|------|------|
| | Leaf | Stem | Root | Leaf | Stem | Root | Leaf | Stem | Root |
| Ammonium | 0.43 | 0.26 | 0.34 | 350 | 86 | 111 | 460 | 540 | 690 |
| Nitrate | 0.42 | 0.53 | 0.46 | 288 | 111 | 68 | 410 | 230 | 410 |

TABLE 3 — N^{15} nitrogen (mg) and N^{15}/N^{14} ratio in the nitrogen fractions of the tea plants grown in ammonium and nitrate medium

| Medium | N^{15} nitrogen in nitrogen fractions (mg) | | | | | | N^{15}/N^{14} ratio | | | | | |
|------------|----------------------------------------------|-------|---------|----------|------------|---------|-----------------------|-------|---------|----------|------------|---------|
| | Ammonia | Amide | Nitrate | Caffeine | Amino Acid | Protein | Ammonia | Amide | Nitrate | Caffeine | Amino Acid | Protein |
| Ammonium N | 0.11 | 0.42 | 0.78 | 0.89 | 2.62 | 7.08 | 0.144 | 0.155 | 0.149 | 0.084 | 0.113 | 0.089 |
| Nitrate N | 0.04 | 0.11 | 0.37 | 0.90 | 0.30 | 5.01 | 0.129 | 0.186 | 0.224 | 0.092 | 0.169 | 0.112 |

The percentage of nitrogen in all components of the ammonium-treated plants are higher than those of the corresponding components of the nitrate-treated plants. This can be expected from the results given above, which indicate that more nitrogen was taken up by the ammonium treated plants but there was no corresponding increase in weight. Potassium, calcium and magnesium are consistently higher in the components of the nitrate-treated plants. The uptake of anion nitrate would, however, not interfere with the absorption of cations. There are no marked trends in manganese and aluminium contents. In the case of phosphorus, the quantity in the stem of the nitrate-treated plant is twice that in the ammonium-treated plants.

It can, therefore, be stated that our experiments indicate that there is preferential absorption of ammonium ions by young tea plants compared with nitrate ions. This is illustrated by the greater recovery of N^{15} nitrogen by the ammonium-treated plants as shown in Table 2. After absorption, however, the tea plants appear to be able to assimilate both forms equally efficiently for synthesizing amino acids, proteins, caffeine *etc.*

The effect of ammonium on the uptake of cations (potassium, calcium, magnesium) is evident from our results, but there was no influence on the uptake of the phosphate anion which is difficult to explain. Although, we start with a balanced nutrient solution, after absorption begins, this balance would be upset. A second connected problem is that although the pH of the solutions are initially the same, ammonium cultures become acid and nitrate culture become alkaline because of the charge on these ions being opposite. Such changes were also recorded in our experiment. In this connexion, it is interesting to note that the uptake of manganese and aluminium were not affected. These ions are more readily available in acid medium and if the pH changes were drastic the ammonium-treated plants should have taken up more of these ions. As the nutrient solutions were freshly supplied every other day, it is possible that the pH changes did not have a lasting influence.

It is assumed that in our experiment the sand was sterile and that the effect of nitrifying and other micro-organisms were absent or minimal. Again, it was hoped that the fresh supply of solution once every two days helped ensure sterile conditions and prevented nitrifications in the pots supplied with ammonium solution. We, however, detected greater moss growth on the sand in the nitrate-treated pots. This is a possible reason for the low recovery of N^{15} compounds from the drainage and leachate of the sand from the nitrate treatment.

This experiment was done with young tea plants. It has been shown in the case of other plants that preferences change as the plant goes through various stages of growth. The possibility remains that the behaviour of mature tea plant is different.

REFERENCES

- BHAVANANDAN, V. P. (1971). Report of the Agricultural Chemistry Division. *Rep. Tea Res. Inst. Ceylon* 1969, 2, 20-41.
- EDEN, T. (1942). Report of the Agricultural Chemist for 1941. *Bull. Tea Res. Inst. Ceylon*, 23, 52-57.
- EDEN, T. (1943). Report of the Agricultural Chemist for 1942. *Bull. Tea Res. Inst. Ceylon*, 24, 37-41.
- LAMB, J. (1954). Report of the Chemical Division for the year 1953. *Bull. Tea Res. Inst. Ceylon*, 35, 27-36.
- PETHIYAGODA, U., KRISHNAPILLAI, S. & NAGARAJAH, S. (1969). Studies on the mineral nutrition of tea—1. Techniques for growing tea plants in sand culture. *Tea Q.* 40, 88-102.
- SHANMUGANATHAN, N. & FERNANDO, S. R. A. (1967). Some observations on *Poria* control with methyl bromide. *Tea Q.* 38, 311-319.
- TOLHURST, J. A. H. (1968). Report of the Agricultural Chemistry Division. *Rep. Tea Res. Inst. Ceylon* 1967, 2, 25-41.

Accepted for publication : 15th January 1971.