Tracer studies in the coffee plant
(*Coffea arabica* L.) (*)

E. MALAVOLTA
L. NEPTUNE MENARD
J. D. P. ARZOLLA
O. J. CROCOMO
H. P. HAAG
W. L. LOTT

E. S. A. "Luiz de Queiroz", University of S. Paulo
Piracicaba, S. Paulo, Brazil

(*) Supported by the Rockefeller Foundation and by Brazilian National Research Council.

1. INTRODUÇÃO

Coffee is by far the most important source of strong currency to Brazil. On the average, Brazil obtain every year circa one billion dollars by selling coffee beans abroad.

Nevertheless, the average yields are very low. Since most of the varieties are capable of outstanding perfomances when properly grown, the is not doubt that the main reason for the small productions obtained in most coffee plantations is the lack of adequate management. It is well known among research workers and farmers as well, that proper fertilization is in most cases the key for higher yields.

Despite the main problem in this matter being that os finding the most suitable levels of nitrogen, phosphorus, and potash, there are also other important questions which attracted our attention, namely: the control of zinc deficiency in the field; the methods of application of phosphatic fertilizers, since phosphorus in many cases the limiting factor; the possibility of supplying nitrogen to the coffee trees as sprays to the leaves. These problems have been investigated in our laboratory and experimental fields with the aid of the tracer technique. In this paper an account of the results so far obtained is to be given.

2. RADIOACTIVE ZINC (Zn^{65}).

2.1. *Aim of the experiments.* Under Brazilian conditions, the typical symptoms of zinc deficiency in coffee plants were observed for the first time in 1953 (FRANCO and MENDES, 1953). The deficiency appeared in several regions of the state of S. Paulo, mainly in coffee plantations located in "terra roxa", a red soil derived from basalt. The abnormality is characterized by the presence of small, distorted leaves, frequently showing chlorotic spots. The branches when affected show a reduced number of leaves, except on the extremities which present a rosette made out of leaves with the symptomatology described.

Several experiments were undertaken to study: (1) the root absorption of tagged zinc chloride as compared to the leaf absorption; (2) translocation of the radiozinc as affected by the method of leaf application; (3) the absorption of the radiozinc as a function of the concentration of heavy metals micronutrients, namely iron, manganese, copper and molybdenum supplied to the nutrient solution; (4) the uptake of zinc when supplied to the soil.

2.2. *Foliar and root application.* Experiments (1), (2) and (3) listed above were varried out with 8 month old plants grown in nutrient solution. In order to find out the relative efficiencies of leaf and root applications in the uptake of zinc, weekly doses of $Zn^{65}Cl_2$ were supplied totalizing 0.15 mc After 8 weeks of growth plants were harvested and assayed for Zn^{65} by standard procedures. The results appear in Table 2-1.

Type of application	Total c.p.m. found	As percentage of activity supplied
Root	18,348	5.0
Leaf		
upper surface	33,958	12.0
lower surface	118,530	42.6
both surfaces	57,455	20.5

TABLE 2-1

The effect of method of application on the uptake of zinc by coffee plants

It is clear that the absorption markedly varied as a function of the type of application. Brushing the radiozinc solution in the upper surface of the leaves caused little absorption which is easy to understand since therein the number of stomatal openings is very small; however, when the tagged zinc was applied to the lower surface, an intense absorption resulted. When both leaf surfaces, were given radiozinc, the highest absorption was to be expected; actually this was the case as showed by the increase in number of counts per unit of dry weight; however, since the leaves of the plants receiving double brushing (lower and upper surface) were small, the reduction in leaf area limited the total uptake. It is important to compare root and leaf absorption; by examining Table 1 it follows that rott uptake was remarkably less intense; this suggests that in field conditions, wherein the absorption would be further decreased by soil fixation, the control of zinc deficiency in coffee cannot be accomplished by supplying this micronutrient in furrows or broacasting it; leaf sprays are probably the only way to apply zinc in coffee plantations showing symptoms of deficiency. The soil experiment to be dealt with later on proved this assumption to be true.

Translocation was measured only in the leaf treated plants. Maximum rate of translocation was found when the tagged zinc was brushed in the lower surface of the leaves, being of the order of 12 per cent of the activity supplied. However, a considerable amount of Zn^{65} was found in the treated leaves; this probably indicates the retention of the radiozinc by a metabolic system located in the mesophyll; the export via phloen, beyond any doubt, was meant to satisfy the need of zinc presented by the meristems of the growing regions. It is interesting to mention that coffee plants showed a translocation of the same order as tomato plants (MALAVOLTA and PELLEGRINO, 1954).

2.3. *Effecf of micronutrients.* In the experiment designed to verify the absorption of zinc as influenced by the concentration of heavy metals micronutrients, the nutrient solution contained various concentrations of iron, manganese, copper, and molybdenum as indicated in Table 2-2. Inert zinc was supplied at a uniform rate of 0.05 p.p.m.

Treatment	Countings per minute	Activity absorbed as % of the activity supplied
Control (*)	18,348	5.0
minus Fe	20,061	5.5
10 p.p.m. Fe	18,579	5.1
minus Mn	18,606	5.5
5 p.p.m. Mn	12,066	3.3
minus Cu	24,923	6.9
0.2 p.p.m. Cu	7,723	2.1
minus Mo	17,992	5.0
0.1 p.p.m. Mo	10,935	3.0

(*) 1.0 p.p.m. Fe, 0.50 p.p.m. Mn, 0.02 p.p.m. Cu, 0.01 p.p.m. Mo

TABLE 2-2

Absorption of Zn^{65} from the nutrient solution as influence by the levels of Fe, Mn, Cu, and Mo

Data in Table 2 show that by raising tenfold the level of Mn, Cu, and Mo, the absorption of Zn^{65} decreased to nearly fifty per cent. Iron, under the experimental condition, apparently did not affect the uptake of the radiozinc; it is well known that when high concentrations of phosphorus are present, insoluble iron phosphates are formed rather than zinc phosphates which are less insoluble; this experiment provided suitable conditions for such reactions to take place: pH near 6.5, and a P concentration as high as 0.001 M. It follows then, that instead of depressing zinc absorption the high iron concentration made the zinc penetration into the cells some what easier. Hence the present data seem to confirm BIDDULPH's (1953) findings.

With respect to the decrease in the zinc uptake noted when high levels of Mn, Cu, and Mo were present, one is led to assume some kind of interionic competition. It is very likely that such ions, before entering the cell, are fixed to the same points of attachment or reactive centers (see the papers by OVERSTREET and JACOBSON, 1952; EPSTEIN and HAGEN, 1952).

2.4. *Soil application.* The two types of soils selected for this particular experiment support most of the coffee plantations in the State of S. Paulo "arenito de Bauru", a light sandy soil which came from a sandstone, and "terra roxa legitima", a red soil derived from basalt. Besides liberal amounts of macronutrients, young coffee plants received two doses of inert zinc, namely 65 and 130 mg $ZnCl_2$, thoroughly mixed with five kilos of soil contained in metal enamelled pots; radiozinc was supplied to give a total activity of 10^6 c.p.m. per plant.

It was found that after one month, in the plants grown in "arenito de Bauru" the activity absorbed as per cent of the total supplied was not affected by the dosis of inert zinc. The highest value observed was around 0.1 per cent. For the "terra roxa" plants, the situation was almost the same; there was, however, a slight increase in the absorption of the Zn^{65} where the higher dosis of carrier zinc had been applied: a little above 0.2 per cent of the activity supplied was absorbed.

These results clearly show that the young coffee plants practically did not absorb the zinc supplied; two reasons at least could be pointed out to explain such a finding: (1) zinc fixation by an exchange with magnesium ions or by filling up "holes" in the octahedral layer of clay aluminosilicates, probably montmorillonite in the case of the "arenito de

Bauru" and kaolinite in the "terra roxa" soil; (2) no need for fertilizer zinc in the particular stage of the life cycle under which the experiment was set up — since the data from chemical analysis are roughly paralel to the above mentioned.

2.5. *General conclusion.* When one compares zinc uptake from nutrient solution, leaf brushing or from fertilizer placed in the soil, a practical conclusion can be drawn, supported by the results reported in this paper: the control of zinc deficiency in coffee plants under field conditions should not be attempted by adding zinc salts to the soils: the soil applications used so extensively in other countries seem not to be suitable for our conditions; hence, zinc sprays should be used whenever necessary.

3. RADIOACTIVE PHOSPHORUS (P^{32}).

3.1. *Aim of the experiment.* In Brazil and in other coffee growing areas phosphatic fertilizers are generally placed in furrows arround the trees or in holes dug at various distances from the plant. These methods of rather deep placement are employed in order to obviate the phosphorus fixation. Recently, however, BONET and RIERA (1954) in Puerto Rico, by using tagged superphosphate showed that adult coffee plants absorbed circa 9-14 per cent of fertilizer phosphorus when the material was topdressed in the canopy area; less than 3 per cent was absorbed, on the other hand, when the superphosphate was placed either in circular furrows of various depths or in holes.

The present experiments were then undertaken in order to compare different methods of soil application of superphosphate against foliar sprays; the relative uptake by plants receiving grass mulch was compared to that shown by coffee trees not receiving such a treatment; finally the distribution of the P^{32} in the soil profile was studied.

3.2. *Absorption of the radiophosphorus.* The experiment designed to study the best way to supply superphosphate to coffee plants was installed in "terra roxa" soil with 2 year old trees. The fertilizer was supplied in the following ways: (1) topdressed in a circular area around the plant; (2) placed in the bottom of a circular furrow 15 cm deep; (3) placed in the bottom of a semicircular furrow also 15 cm deep; (4) sprayed directly to the leaves. In each case 150 gm of ordinary superphosphate tagged with 5×10^9 c.p.m. as $H_3P^{32}O_4$ were supplied per plant, four replicates being used. In the case of the fourth method of distribution, the total dosis was divided up in 5 applications with 30 gm of material in 3 liters of water each. Plants were allowed to absorb the superphosphate for a month; then the first and second pairs of leaves were collected from the second branch from top. These leaves were formed after the application of P^{32} was made. Chemical analysis and radioactive assay were carried out by standard procedures. Radioautography of the leaves from the sprayed plants was also made. A parallel experiment was run in the greenhouse in order to check for the absorption and translocation of the leaf applied superphosphate; in this experiment the tagged material was brushed in a given set of leaves.

The mulch experiment was carried in "arenito de Bauru" with 8 year old plants. In the treated plants, the heavy blanket of dried grass was taken apart and then the superphosphate was topdressed in the bare soil in a circular strip 0.7 m wide. Afterwards the mulch was returned to ist original position around the trees.

The results of the experiment on the methods of applications are summarized in Table 3-1. It is seen that topdres-

Methode of application	c.p.m./mgm. P	Per cent of P from fertilizer	Relative
Topdressed	483	10.2	100.0
Circular furrows	116	2.4	23.5
Half circular furro w	83	1.7	16.6
Spray	1.563	38.8	372.4

TABLE 3-1

sing the superphosphate was the most efficient way to supply phosphorus to the coffee trees when soil applications were concerned; instead of leading to a heavy loss due to fixation this method proved to be better than the others which, as pointed out, were used till recently by most of the coffee farmers. It is important to mention the excellent agreement between these data and those obtained by BONET and RIERA

(1954). The best uptake, however, was obtained by spraying the fertilizer to the leaves; this method seems to be then, a very promising way to supply phosphorus to the coffee trees, particularly in the small plantation where care of the orchard in easier. The greenhouse experiment and the radioautographs proved that the high activity found in the sprayed leaves was due to actual absorption and not to surface contamination.

In the mulch experiment there was no statistical difference between treated and untreated plants. This was not expected since the plants under mulch had many more roots near the surface of the soil. The result can be explained, however, on basis of the heavy rains which occurred during the experimental period and on the sandy nature of the soil: these two factors must have contributed to a substancial movement of the phosphorus to deeper layers of the soil.

3.3. *Soil studies*. In order to have an idea about how deep the phosphorus went in the two types of soil described above, extractions were made of HCl 0.2 N soluble phosphate, by taking samples at various depths. Only countings were then made in these extracts. The results are summarized in Table 3-2. It can be seen that most of the radiophosphorus applied

Depth (cm)	Soil	
	"Arenito de Baurú"	"Terra roxa"
0 — 2,5	4,266	1,378
2,5 — 7,5	683	50
7,5 — 15,0	73	25

TABLE 3-2

Activity (c.p.m.) in aliquots of HCl 0.2 N soil extract

was fixed in the upper layer of the soil; since, however, the root system of the coffee plant in the soil types used in these experiments is rather shallow, it is likely to assume that this was the reason for the good uptake found when the superphosphate was topdressed.

3.4. *General conclusions.* The experiments this section dealt with proved conclusively that labour and money can be saved in the coffee plantations when superphosphate is topdressed instead of being placed in furrows or holes which are costly to make and lead to an inefficient use of the material supplied. On the oher hand the spray application offers a good way to apply phosphorus in the small, intensively cultivated plantations: it can be used also to quickly overcome phosphorus deficiency in trees growing in soils which fix phosphorus strongly.

4. RADIOACTIVE CARBON (C^{14}).

4.1. *Aim of the experiments.* The possibility of correcting nitrogen deficiency in coffee under field conditions was demonstrated by MALAVOLTA et al. (1958-a, b). Two year old coffee plants showing acute symptoms of nitrogen starvation received three sprays of a 2.5 per cent solution of urea. Two weeks later, a remarkable recovery was plainly visible, despite faint signals of biuret toxicity. The effect of the urea spray lasted for almost one year. It was felt desirable to study the metabolism of that fertilizer in the coffee leaves. A nice piece of work along the same lines had already been published by CAIN (1956) who dealt only with the variations in the nitrogenous fractions. By using C^{14} labelled urea it would then be possible to follow the fate of the carbonic moiety of the urea.

4.2. *Absorption and metabolism of urea-C^{14}.* Young coffee plants 6 month old were grown in nutrient solution until the group from which nitrogen had been omitted showed symptoms of deficiency of this element. Newly matured leaves were then brushed with a 3 per cent solution of tagged urea to supply 9.5×16^{6} c.p.m. per plant. After 9 hours the plants were harvested, a couple of them being left in the greenhouse for a longer period in order to let translocation to take place. The fresh leaves were first extracted with 80 per cent cold ethanol in a blendor; a chloroform extraction was then made to separate lipids from the water soluble material. In the water extract the usual nitrogenous fractions were determined by standard procedures, only slight modifications being introduced; radioactivity assay was also made. Suitable aliquots, on the oher hand, were submitted to paper chromatography, free aminoacids, sugars and organic acids being detected and counted for C^{14}.

The main results were as follows: (1) As far as absorption is concerned, there was a very good agreement between the chemical assay for nitrogen and the C^{14} countings, as shown in Table 4-1. The recovery of the N absorbed by the leaves was higher than 95 per cent; of the C^{14}, however, only 60 per cent was accounted for in the leaves; this is easy to understand by assuming some diffusion of the $C^{14}O^2$ took place.

	Normal		Deficient	
	Nitrogen (mg)	Radiocarbon (c.p.m.)	Nitrogen (mg)	Radiocarbon (c.p.m.)
Applied	56.00	9.5×10^6	56.00	9.5×10^6
Not absorbed (*)	3.36	5.8×10^5	2.69	4.2×10^5
Absorbed	52.64	8.9×10^6	53.31	9.1×10^6
Absorption, %	94.0	93.9	95.2	95.2

(*) found in the washings.

TABLE 4-1

Uptake of the labelled urea by coffee plants

(2) In the healthy plants the following aminoacids became tagged: glutamic, serine, threonine, lysine, alanine, citrullyine, histidine, valine, arginine, glycine and proline (see Table 4-2); glutamine and asparagine also were radioactive; alanine, glutamine and proline were the metabolites which showed higher incorporations of C^{14}. In the nitrogen-deficient plants, after treatment with labelled area, only three aminoacids not found in the control plants showed up, the three of them being radiactive, namely citrulline, arginine and proline. Since two of these aminoacids belong to the urea cycle and since, on the other hand, prolin emay arise from ornithine, that is, from the third component of that cycle, it seems possible that a mechanism rather similar to that found in animals exists in coffee leaves; recent data from other laboratories support this view (DELWICHE and KASTINGS, 1958) with evidence obtained in other higher plants.

(3) With respect to water soluble carbohydrates, two radioactive sugars were detected in the normal plants, glucose

and fructose, and only one, fructose, in the nitrogen deficient ones (see Table 4-3).

Aminoacid	Normal	Deficient
Glutamic	850	650
Clycine	700	600
Serine	600	600
Asparagine	1.225	765
Threonine	—	—
Lysine	—	—
Alanine	500	500
Glutamine	2.430	1.275
Citrulline	775	750
Hystidine	—	—
Arginine	725	700
Proline	800	1.000

TABLE 4-2

Activity in c.p.m. found in the free aminoacids

(4) Citric, cis aconitic, isocitric and succinic were the organic acids which showed incorporation of the carbon derived from the urea supplied to the leaves; their activity was much higher than that found in the aminoacids, as shown in Table 4-3. No activity was found in alpha ketoglutaric acid, what can be explained assuming its rapid conversion into glutamic and into glutamine which had appreciable activity.

	Normal	Deficient
Glucose	3,030	—
Fructose	2,424	1,770
Citric	3,330	3,150
Isocitric	2,060	1,170
Cis aconitic	2,360	1,358
Succinic	2,060	1,150

TABLE 4-3

Activity (c.p.m.) found in sugar and organic acids

4.3. *Translocation of the C^{14}.* The translocation of the C^{14} as detected by radioautography was very poor, only in the N deficient plants being more ative. Even so, only the new organs (leaves and small branches) showed some radioactivity.

4.4. *General conclusions.* Confirming results obtained in the field, the experiments herein described showed that coffee plant is able to absorb a great deal of urea through the leaves; this type of application seems then quite satisfactory to correct nitrogen deficiency under field conditions.

The radioactivity assay of free aminoacids showed that upon the addition of urea-C-14 to the deficient leaves, only three aminoacids showed up, all of them being radioactive; since these aminoacids are either derived from or part of the urea cycle, it is suggested that this mechanism, well known in animals, exists also in higher plants, a view supported by evidence obtained independently in other laboratories.

Tricarboxylic acids showed more activity than the aminoacids what was due to the fact of them being formed frist. As far as activity is concerned, glucose and fructose had an intermediate position between aminoacids and organic acids.

5. SUMMARY.

Due to the great importance of coffee to the Brazilian economy, a good deal of the work carried out in the "Laboratório de Isótopos", E. E. A. "Luiz de Queiroz", Piracicaba, S. Paulo, Brazil, was dedicated to the study of some problems involving that plant.

The first one was designed to verify a few aspects of the control of zinc deficiency which is common in many types of soils in Brazil. An experiment conducted in nutrient solution showed that the leaf absorption of the radiozinc was eight times as high as the root uptake; the lower surface of the leaves is particularly suited for this kind of absorption. Among the heavy metal micronutrients, only iron did not affect the absorption of the radiozinc; manganese, copper, and molybdenum brought about a decrease of fifty per cent in total uptake. In another pot experiment in which two soils typical of the coffee growing regions were used, namely, a sandy soil called "arenito de Bauru" and a heavy one, "terra roxa", only O.1 and 0.2 per cent of the activity supplied to the roots was recovered, respectively. This indicates that

under field conditions the farmer should not attempt to correct zinc deficiency by applying zinc salts to the soil: leaf sprays should be used wherever necessary.

In order to find out the most suitable way to supply phosphatic fertilizers to the coffee plant, under normal farm conditions, an experiment with tagged superphosphate was carried out with the following methods of distribution of this material: (1) topdressed in a circular area around the trees; (2) placed in the bottom of a 15 cm deep furrow made around the plant; (3) placed in a semicircular furrow, as in the previous treatment; (4) sprayed directly to the leaves. It was verified that in the first case, circa 10 per cent of the phosphorus in the leaves came from the superphosphate; for the other treatments, the results ware, respectively: 2.4, 1.7, and 38.0 per cent. It is interesting to mention that the first and the last methods of distribution were those less used by the farmers; now they are being introduced in many coffee plantations.

In a previous trial it was demonstrated that urea sprays were an adequate way to correct nitrogen deficiency under field conditions. An experiment was then set up in which urea-C^{14} was used to study the metabolism of this fertilizer in coffee leaves. In was verified that in a 9 hours period circa 95 per cent of the urea supplied to the leaves had been absorbed. The distribution of the nitrogen of the urea was followed by standard chemical procedures. On the other hand the fate of the carbonic moiety was studied with the aid of the radiochromatographic technique. Thus, the incorporation of C^{14} in aminoacids, sugars and organic acids was ascertained. Data obtained in this work gave a definite support to the idea that in coffee leaves, as in a few other higher plants, a mechanism similar to the urea cycle of animals does exist.

6. LITERATURE CITED.

BIDDULPH, O. 1953 — Translocation of radioactive mineral nutrients in plants. A conference on the use of isotopes in plant and animal research, *TID-5098*: 48-58.

BONNET, J. A. and A. R. RIERA. 1954 — Radioactive studies with p32 in tropical soils and crops of Puerto Rico. Trans. Vth Internatl. Congr. Soil Sci. (Léopoldville) *2*: 369-375.

CAIN, J. C. -956 — Absorption and metabolism of urea by leaves of coffee, cacao and banana. Proc Am. Soc. Hort. Sci. *67*: 279-286.

EPSTEIN, E. and C. E. HAGEN. 1952 — A kinetic study of the absorption of alkali cations by barley rocts. Plant Physiol. *27*: 457-474.

FRANCO, C. M. and H. C. MENDES. 1953 — Deficiência de microelementos em cafeeiro (Nota preliminar). Bol. Super. Serv. Café *28*: 19-22.

KASTING, R. and C. C. DELWICHE. 1958 — Ornithine, citrulline, and arginine metabolism in watermelon seedlings. Plant Physiol. *33*: 350-354.

MALAVOLTA, E. and D. PELLEGRINO. 1954 — Estudos sôbre a distribuição do radiozinco no tomateiro (*Lycopersicum esculentum*). An. E. S. A. "Luiz de Queiroz" *11*: 78-84.

MALAVOLTA, E., J. D. P. ARZOLLA and H. P. HAAG, 1957 — Absorption of urea sprays by coffee leaves under field conditions. Plant Physiol. *32* (Suppl.): XIV.

MALAVOLTA, E., J. D. P. ARZOLLA, H. P. HAAG, T. COURY e O. J. CROCOMO. 1958 — Nota sôbre a aplicação da ureia em pulverização no cafeeiro. Rev. Agric. *32*: 223-226.

OVERSTREET, R. and L. JACOBSON. 1952 — Mechanism of ion absorption by roots. Ann. Rev. Plant Physiol. *3*: 189-206.