

# THE ESTIMATION OF CANE ROOT DEVELOPMENT AND DISTRIBUTION USING RADIOPHOSPHORUS

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## Introduction

Soil sampling for the purpose of fertility assessment is normally confined to the plough layer with little or no attention to the rooting pattern of the crop in question or the availability of subsoil nutrients. However, there is little doubt that a thorough understanding of the development and activity of plant roots in their soil environment is essential to a balanced soil fertility research programme.

Most methods of root examination in the field are time consuming and destructive, and do not necessarily indicate the activity of roots at a particular location in the soil. More recent methods involve placement of a substance which can be absorbed by roots and readily detected in the plant, at various positions in the soil. These are relatively non-destructive and are, therefore, suitable for the continuous study of root development. Various soluble substances, such as dyes, elements not normally encountered to any extent in plants, or radio-isotopes of nutrient elements, have been used as tracers, but the latter are most promising. Accordingly, radio-isotope placement techniques have been applied to root development studies on a number of crops. Hall *et al* (1953) used radiophosphorus (P-32) to study the root systems of maize, peanuts, cotton and tobacco, while Boggie and Knight (1962) examined the root development of grasses and other plant species growing on deep peat with the same radio-isotope.

The basic requirements of the radio-isotope technique are as follows:

- (a) Injection or placement of the radio-isotope, at a particular depth and distance from the plant, in such a way that there is a reasonable probability of roots reaching the zone encountering it.
- (b) No appreciable movement of the radio-isotope from the point of placement during the study.
- (c) When taken up, the radio-isotope should be easily detectable and readily distributed throughout the plant.

Requirements (b) and (c) are most closely met by P-32.

Two procedures frequently used to apply P-32 at various depths in the soil are:

- (i) *the boring method*—a small diameter hole is augered and P-32 solution or P-32-tagged fertilizer poured down a tube (Lawton *et al*, 1954; Lipps *et al*, 1957), and
- (ii) *the injection method*—P-32 solution is injected by means of a needle and syringe (Hall *et al*, 1953; Murdock and Engelbert, 1958).

In the present study a combination of the two methods was employed.

Methods involving placement of fertilizer in solid form are undesirable as it is impossible to estimate the extent to which the large amount of readily available nutrient has affected the normal behaviour of the roots. Injection of carrier-free P-32 solution leaves the phosphate status at the injection site unaltered and is therefore preferable.

Interpretation of results using the P-32 injection method is complicated by a number of important factors; (i) variation from point to point in the soil of the amount of soil P with which the added P-32 exchanges; (ii) continuous exchange between ions in roots and soil; (iii) radiation damage to plant tissue. In addition, P-32 uptake is not necessarily indicative of the activity of roots with respect to the absorption of other ions or water (Newbould and Taylor, 1965).

To eliminate the effect of fluctuations in the total phosphate content of the plant, P-32 uptake is best expressed as a fraction of this quantity, or in other words, as specific activity (Wood, 1964). Both Hall *et al* (1953) and Waugh (1963) contend that this is the most meaningful measure of uptake. Moreover, since the P-32 distribution in the plant tends with time to become the same as that of the natural phosphorus (Wood, 1964), variation in the position on the plant from which a sample is taken should be less critical. The specific activity in a plant arising from a particular depth-distance treatment has little meaning, unless considered in relation to the total possible uptake from all treatments. Results from each sampling should therefore be expressed on a relative basis. Any comparison of specific activities between sampling dates to obtain a measure of the rate of P-32 uptake is invalidated by factors such as changes in specific activity of the soil phosphate resulting from soil reactions.

This paper concerns a study of the rate of development and relative activity of different parts of the root system of the sugarcane variety N:Co.382 on deep coastal sands, employing a P-32 injection technique. An attempt has been made to relate the root development and activity to changes in available soil moisture as measured by cylindrical gypsum blocks.

## Experimental Method

The experiment was located on a Clansthal sand with a  $\pm$  6-inch layer of soft weathered sandy ferricrete occurring at about 5 ft. and overlying a sandy clay loam. Setts were planted in October 1964 in 4ft. 6in. rows. The cane received superphosphate

in the furrow at planting and subsequent topdressings of sulphate of ammonia, muriate of potash and a 4-1-6 NPK mixture.

The treatments involved injecting P-32 solution at six depths (3 in., 9 in., 1 ft., 3 ft., 5 ft. and 7 ft.) and four distances (3 in., 9 in., 18 in. and 36 in.) relative to the cane row. A split plot design and three replicates was employed, with depth as whole plot treatments and distance as subplot treatments. Each whole plot consisted of a 50 ft. row of cane with a guard row on either side. Subplots were formed by dividing whole plots into four equal lengths of 12½ ft. The nett subplots consisted of a 3 ft. length of row receiving five equally spaced 20 cc. injections on each side of the row at the required distance and depth. Plate 1 illustrates the field experimental layout and injection procedure.

the black polythene pipes, which had been placed in the soil to within two inches of the desired injection depth, to facilitate insertion of the needles to depths of 1 ft. or greater. These were left in position for the duration of the experiment, to permit reinjection at intervals of ten or more weeks to compensate for radio-active decay. Accordingly, seven applications of P-32 were made during the life of the plant crop, with an additional one about three months after harvesting to study the initial feeding pattern of the first ratoon.

Each injection contained 0.05 millicuries P-32, making a total of 0.5 millicuries per treatment. Autoradiographs of cross-sections of injections at different depths in the soil showed that the P-32 spread approximately half an inch from the point of injection. There was little downward movement of P-32

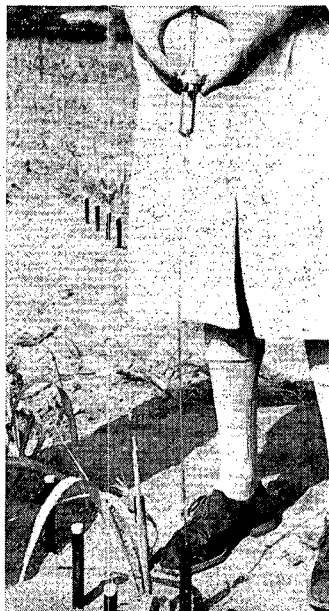


**PLATE 1:** Field experimental layout and injection procedure.

A. Filling syringe and inserting needle.

B. Injecting P-32.

C. Transferring needle from one polythene pipe to another.



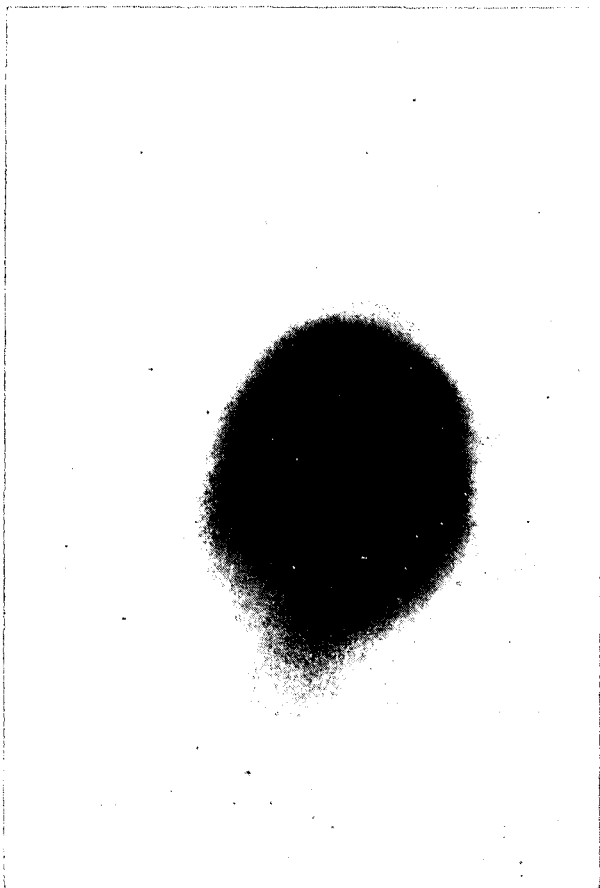
B



C

Injections were made with a 20 cc. veterinary syringe and gauge 11 stainless steel needles of various lengths, similar to those used by Murdock and Engelbert (1958). Plate 1 shows clearly the tops of

injected six inches below the soil surface, even after being leached with five inches of rain over a period of 50 days and a final ten inches of water within a few hours (Plate 2).



**PLATE 2:** Autoradiograph of P-32 injection leached with 5 inches of rain and 10 inches of water.

P-32 uptake was determined by taking third-leaf samples from the cane within each nett subplot, using a punch sampler, at one-, two- or three-weekly intervals depending on the growth rate. After wet-ashing, the P-32 content of the samples was measured with a Geiger-Muller pour-in counter and scaler and the total P by a molybdophosphoric blue colour method.

In February 1965 sets of cylindrical gypsum soil moisture blocks, manufactured according to the method of Pereira *et al* (1958), were installed adjacent to each treatment at the corresponding injection depth and distance, omitting the 3 inch depth. Readings of percentage available moisture were taken at weekly intervals for the remainder of the experiment, using a Bouyoucos moisture meter. To check the moisture block results, the Viatic Ra/Be neutron probe was used to make periodic measurements of absolute moisture at various depths down the profile. For this purpose a ten foot aluminium access tube was installed in each of the three replications, midway between a row of cane and the centre of the interrow.

The experiment was harvested at the beginning of June 1966. As an indication of the degree of uniformity of growth, nett subplot weights, stalk heights and population were recorded.

The variability of phosphate, exchangeable cations, and other soil properties down the profile was estimated from analyses of composite soil samples from each whole plot, taken adjacent to the treatments at the corresponding depth and distance. For resin-extractable P and exchangeable P (E-value) measurements, subsamples ground to 60 mesh were equilibrated with P-32 solution for periods of one or two weeks, and the labelled soil phosphate extracted by shaking the suspension overnight with Dowex 1-X8 anion exchange resin (Cooke and Hislop, 1963).

### Results and Discussion

The variability of the soil chemical and physical properties down the profile is shown in Table I, and the effects of repeated heavy dressings of lime-rich filter-cake can be clearly seen. Most striking were the high Ca and P levels in the plough layer, and the very high pH values which persisted down the profile. Initially some of the ratoon cane in this experiment showed the characteristic symptoms of lime-induced iron-deficiency chlorosis.

The phosphate fertility patterns estimated by sulphuric acid extraction, resin extraction and P-32 equilibration for one week were all essentially similar. However, the variation in the measured E-value with depth was drastically reduced by increasing the equilibration period to two weeks. This uncertainty in the E-value, coupled with the lack of a reliable estimate of this parameter at 7 feet, made it difficult to apply corrections to the P-32 uptake so that variations in exchangeable P could be accounted for, as recommended by Newbould and Taylor (1966). However, Hall *et al* (1953) suggest that lower specific activities at sites in the soil with high levels of labile P may be equalized by greater absorption of P by the roots at these sites. The advisability of applying corrections therefore remains uncertain.

In calculating the results, the mean specific activity due to each individual treatment was taken as a percentage of the sum due to all treatments. This was used as an estimate of the relative root activity at the various position in the soil. As an indication of the natural variability of the results, and the significance of treatment effects, analyses of variance of the log-transformed specific activity data from two samplings selected for large apparent depth and distance effects, are presented in Table II, together with the coefficients of variation of the stalk height and weight. The high coefficients of variation, and non-significance of treatment effects in the one instance indicate that the method is only semiquantitative and emphasize the need for caution in drawing conclusions regarding differences in root activity.

**TABLE I**  
Variability with depth of soil chemical and physical properties

Soil Properties	Depth (Ft.)					
	$\frac{1}{4}$	$\frac{1}{2}$	1	3	5	7
<i>Phosphate (ppm)</i>						
(i) O.02N-H <sub>2</sub> SO <sub>4</sub> Extractable ..	93	97	10	4	17	3
(ii) Resin Extractable after—						
1-week pre-equilibration ..	22.4	21.4	4.2	1.4	5.1	0
2-week ..	25.0	23.7	5.8	3.3	5.4	0
(iii) Exchangeable (E-Value)						
1-week equilibration .. ..	41.9	45.9	10.9	6.2	22.9	—
2-week .. ..	53.0	54.9	21.4	30.4	38.4	—
<i>Other Chemical Properties</i>						
Ca (ppm) .. .. .	2250	2720	380	140	330	460
Mg (ppm) .. .. .	48	55	19	11	23	210
K (ppm) .. .. .	41	34	23	20	36	29
Na (ppm) .. .. .	23	25	7	5	8	28
pH (1:2.5 soil:water) .. ..	8.83	8.91	8.73	8.38	8.22	6.75
Organic Matter % .. .. .	1.30	1.46	0.77	0.64	0.87	0.87
<i>Mechanical Analysis</i>						
Clay % .. .. .	10	11	8	7	13	34
Silt % .. .. .	4	4	3	2	2	5
Fine sand % .. .. .	49	52	51	53	50	30
Coarse sand % .. .. .	37	33	38	38	35	31
Textural Group .. .. .	Loamy Sand	Loamy Sand	Sand	Sand	Loamy Sand	Sandy Clay Loam

**TABLE II**  
Analyses of variance of log-transformed specific activity data and variability of stalk height and weight

	Sampling Date	
	5 Jul 1965	28 Mar 1966
C.V. % .. .. .	48	35
<i>F-Values:</i>		
Depth effects .. .. .	0.79	1.73
Distance effects .. .. .	30.27**	8.90**
Depth x distance effects ..	6.10**	1.12
<i>C.V. % at harvest:</i>		
Average cane height in nett subplots .. ..		12
Average stalk wt. in nett subplots .. ..		18

\*\*Significant at 1% level.  
\* " " 5% "

The estimated root activity patterns at different stages of growth are illustrated in Figure 1, which shows the mode and rate of root development. When considering this figure, it must be remembered that sampling on a given date provided an estimate of the integrated P-32 uptake over the whole period from the previous application of P-32 to that date. This explains the change in the measured root distribution pattern which sometimes occurred after a fresh injection of P-32, the new pattern being more indicative than the old of the root distribution prevailing at the time of sampling. Although a given P-32 injection sometimes had a small residual effect on the results from the next, radio-active decay prevented this effect being carried over to results from subsequent injections.

Figure 2 presents graphically changes in moisture status that occurred at various depths and distances from the time the cane was 20 weeks old. The shaded areas represent the amount of available water present between the 1 and 15 bar tension limits and are clearly related to the pattern of rainfall distribution occurring during the course of the experiment. Each curve represents the mean of three gypsum block readings.

The effects of the prolonged drought at the beginning of 1965 on available moisture are clearly seen. By the beginning of April the surface foot of soil was almost completely dry, moisture reserves at 3 ft. were dwindling, and even the soil at 5 ft. was showing signs of drying. Figure 1 shows that, at the end of April, P-32 uptake from 5 to 7 ft. accounted for a significant proportion of the total, whereas two weeks earlier there had been little indication of significant root activity at these depths. It is most likely that this sudden stimulation of root activity at depth was largely due to the relatively large supply of available moisture still present there. The neutron probe results for the period January to June 1965, recorded in Table III, reveal a pattern of moisture

removal similar to that in Figure 2. Both methods indicated a sudden large decrease in available moisture at 7 ft. at the beginning of May, confirming the arrival of significant quantities of active roots. The decline in the relative contribution of roots near the surface during the latter half of April was probably due mainly to the depletion of moisture.

Good rain from early June replenished profile moisture and caused a renewed flush of surface roots, as shown by the large increase in the relative P-32 uptake from the surface at the beginning of July, and confirmed by root washing. The general pattern at this stage suggests that roots growing six inches to three feet from the surface had not yet recovered from the setback due to the drought, while those at depth continued to make a significant contribution. However, by the end of September, root activity in the surface nine inches again predominated. These results indicate that good rainfall and adequate subsoil moisture favour root proliferation near the surface.

The general root distribution remained fairly constant until late February 1966, when a dry period at the height of the growing season again caused severe moisture depletion in the surface foot of soil. The effects were noticeable at the end of March, an apparent decrease in the relative surface root activity coinciding with an enhanced contribution from five to seven feet. The large removal of moisture at the seven foot depth and 36 inch distance during this period (see Figure 2) implies considerable root activity in this region, confirming the P-32 results. However, since the dry spell was much shorter and less severe than the 1965 drought, the general relationship between soil moisture depletion and P-32 uptake was less clearly defined.

Although the results for the ratoon crop have been included in Figure 1, they can only be regarded as qualitative due to extremely poor tillering in many

TABLE III

Absolute soil moisture measurements made with the neutron probe during the period January—June 1965 (in inches water per 6 inches soil)

Date	Age of Cane (Wks)	Depth (Ft.)						Rainfall (In.)	
		$\frac{1}{4}$	$\frac{1}{2}$	1	3	5	7	Previous Week	During Interval
12 Jan .. .. .	12	0.67	0.65	0.59	0.39	0.97	2.02	1.12	2.18*
28 Jan .. .. .	14 $\frac{1}{2}$	0.57	0.52	0.44	0.42	1.03	1.97	0.52	1.40
18 Feb .. .. .	17 $\frac{1}{2}$	0.45	0.37	0.29	0.30	1.11	2.18	0.31	0.46
18 Mar .. .. .	21 $\frac{1}{2}$	0.39	0.33	0.26	0.21	0.77	2.00	Nil	0.93
25 Mar .. .. .	22 $\frac{1}{2}$	0.38	0.31	0.25	0.20	0.72	1.98	Nil	Nil
2 Apr .. .. .	23 $\frac{1}{2}$	0.39	0.32	0.26	0.20	0.72	1.95	0.08	0.08
8 Apr .. .. .	24 $\frac{1}{2}$	0.43	0.36	0.29	0.20	0.69	1.92	Nil	Nil
14 Apr .. .. .	25 $\frac{1}{2}$	0.40	0.33	0.24	0.17	0.66	1.89	0.16	0.16
23 Apr .. .. .	26 $\frac{1}{2}$	0.46	0.38	0.29	0.22	0.68	1.88	0.26	0.28
30 Apr .. .. .	27 $\frac{1}{2}$	0.46	0.38	0.29	0.22	0.69	1.85	0.26	0.26
6 May .. .. .	28 $\frac{1}{2}$	0.34	0.28	0.20	0.11	0.59	1.69	Nil	Nil
20 May .. .. .	30 $\frac{1}{2}$	0.36	0.29	0.21	0.12	0.58	1.75	0.01	0.11
3 June .. .. .	32 $\frac{1}{2}$	0.66	0.64	0.62	0.40	0.65	1.74	3.38	3.38

\* Rainfall during previous three weeks.

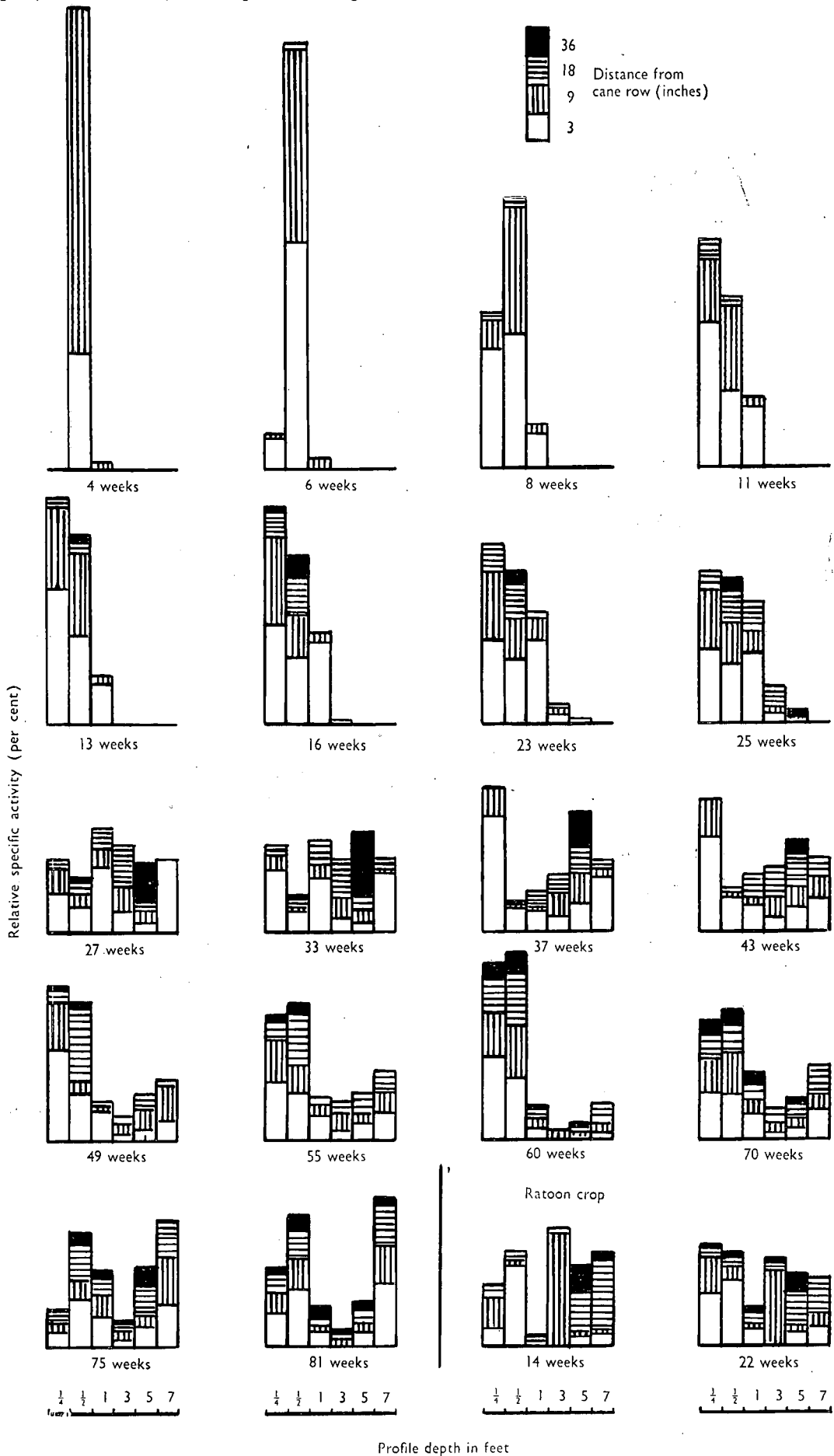


FIGURE 1: Sugarcane root activity patterns (estimated), at different stages of growth

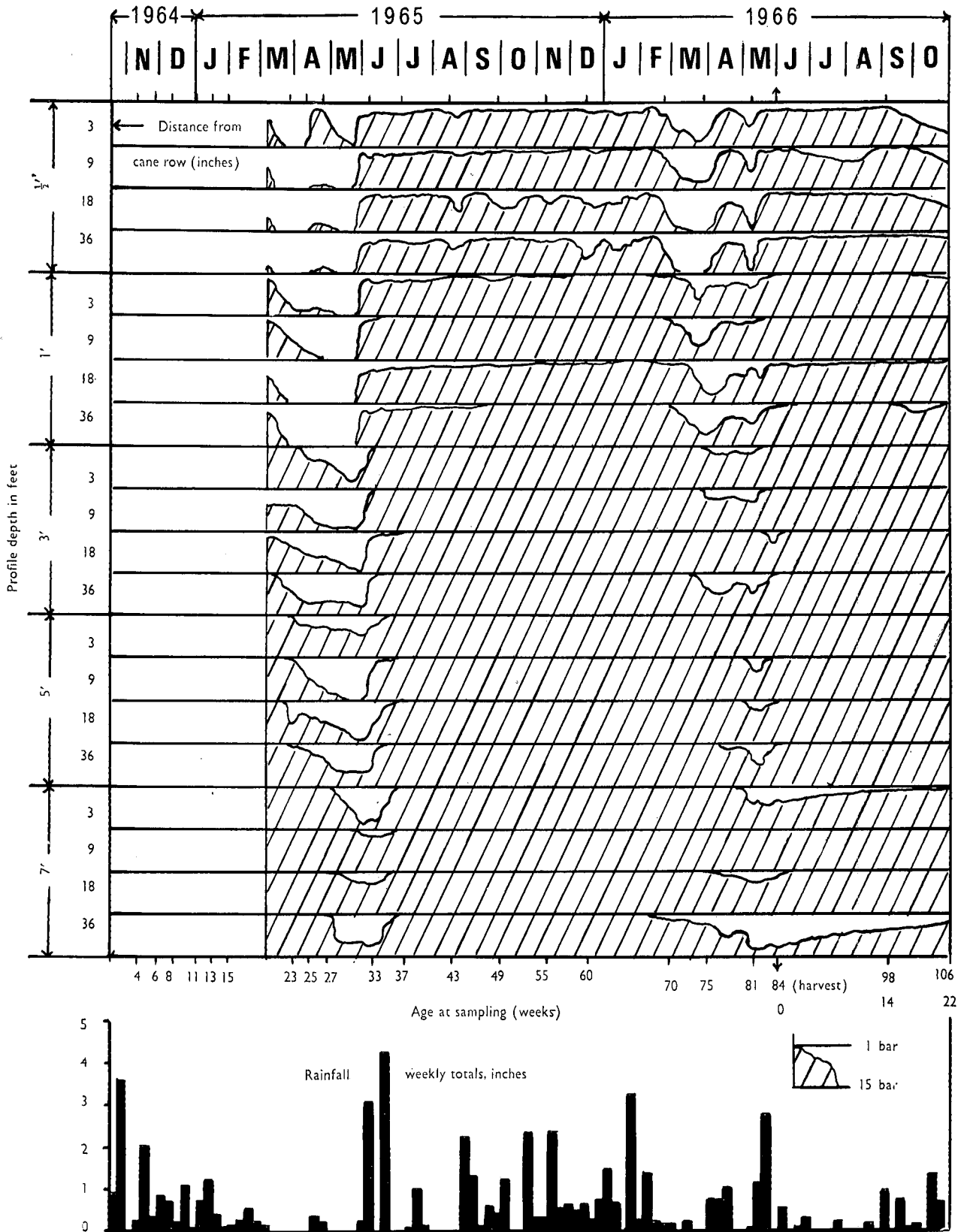


FIGURE 2: Changes in available soil moisture under sugarcane to a depth of 7 feet

subplots and large growth variations. Despite this, they indicate significant P-32 uptake from seven feet only 14 weeks after harvest. Considering that the cane at this stage averaged less than two feet in height due to retarded growth over the winter months, it is considered unlikely that new shoot roots would have reached a depth of seven feet in this time. These results therefore suggest that the plant crop root system, which was still active at this depth shortly before harvest, continued to sustain the ratoon crop to some extent at least four months after cutting. Since the crop was still small, absence of visible moisture extraction at five and seven feet was not unexpected.

### Conclusions

The radio-isotope injection method as described is subject to considerable natural variability. For this reason, the results are at best semiquantitative, but nevertheless useful in estimating the development of the sugarcane root system and giving some idea of the comparative activity of roots at different depths and distances relative to the cane row. Despite its drawbacks, it is one of the most promising methods yet devised for studying root patterns in situ in the field.

The results indicate that root activity predominates close to the surface under conditions of normal rainfall. However, the frequently observed drought resistance of sugarcane grown on Natal coastal sands can be attributed to the fact that active roots are able to penetrate to considerable depths in these soils and proliferate where moisture is still available.

The evidence obtained for continued functioning of the plant cane root system for at least four months after harvesting is not surprising, as the stool remains a living entity, and there is therefore no reason to presuppose that all roots attached to it should cease to function immediately. It is quite feasible that some could become dormant, when relieved of much of the task of sustaining growth, and may start functioning again if or when required. In the Root Observation Laboratory at the Sugar Experiment Station, Glover (1967) has noted persistence of turgid root hairs on old suberized roots of living plants for periods of up to four months, and branching of these roots 160 days after growth has apparently ceased.

It must be emphasized that the experiment was not designed as a study of phosphate fertilizer placement and the authors have therefore not attempted to draw any conclusions in this regard. Its value lies mainly in increasing our understanding of the rate of development and distribution of the sugarcane root system on a light textured soil with a deep profile, and root behaviour as influenced by soil moisture conditions.

### Summary

A description is given of a field experiment, using a P-32 injection method, to study in situ the development and distribution of the sugarcane root system on Natal coastal sands throughout the life of the plant crop, and during the initial stages of growth of the first ratoon. The behaviour of the root system

was studied in relation to soil moisture conditions as measured by gypsum blocks, while using the Viatic Ra/Be neutron probe as a check on absolute moisture variations down the profile.

Although initial root development was found to be mainly confined to the surface foot of soil, there were signs that the roots had reached a depth of five feet by 20 weeks. A prolonged drought early in 1965 caused gradual depletion of soil moisture from the surface downwards, giving rise to sudden appearance of significant amounts of active roots at a depth of seven feet.

In general the results indicate that root activity predominates close to the surface under normal rainfall conditions. Drought resistance of cane grown on coastal sands is explained by the ability of active roots to penetrate to considerable depths where moisture is still available. Evidence was obtained that plant cane roots apparently continue to function for some time after harvest.

The advantages and limitations of the P-32 injection technique are discussed.

### Acknowledgements

The authors would like to record their thanks to the Agronomy Section for supplying the neutron probe data, and to the Chemical Services Laboratory for analyses of soil samples.

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### Discussion

**Mr. Armstrong:** In work with radioisotopes has any evidence come to light to show that uptake from one root is distributed to all leaves in the plant?

**Mr. G. H. Wood:** We intend to investigate this in our new root laboratory. Some work has been done on the distribution of P-32 in a plant but not yet in great detail.

**Mr. von der Meden:** In Figure 2, did the soil moisture in the top six inches ever reach below two bars tension throughout the two years?

**Mr. G. H. Wood:** In none of the three replications did we ever obtain a hundred percent moisture reading in the top six inches.

**Mr. Moberley:** The fact that where chlorosis occurs it is more evident in ratoon plants seems to indicate that ratoon roots are not feeding from depths below the high calcium strata.

**Mr. G. H. Wood:** Results for the ratoon crop are qualitative. The uptake from seven feet may be relatively small. It is now thought that chlorosis is not due to pH but to an excess of phosphate which the plant may take up from new roots it puts out near the surface, thus upsetting the iron phosphate ratio.

**Mr. Coignet:** In coffee, also, when there is an excess of phosphate new shoots show the effects of iron chlorosis although iron is available in the soil.

**Mr. G. H. Wood:** A recent paper states that there is the same amount of iron in chlorotic and non-chlorotic plants but the chlorotic plants contain a greater amount of phosphate.

**Dr. Roth:** The higher the amount of carbohydrate that has to be decomposed after the first crop by micro-organisms the more acids will be produced. They may influence the uptake of phosphates in the roots as phosphates are acid soluble.