ISSN 0308-3675

MERLEWOOD RESEARCH AND DEVELOPMENT PAPER

No 103

THE PHOSPHORUS DEFICIENCY BIOASSAY: SAMPLE AND DATA HANDLING PROCEDURES

by

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December 1984

Suggested citation:

HARRISON, A.F., DIGHTON, J. & SMITH, M.R. 1984.

The phosphorus deficiency bioassay: sample and data handling procedures. (Merlewood research and development paper no. 103) Grange-over-Sands: Institute of Terrestrial Ecology.

C O N T E N T S

SUMMARY

This research and development paper gives the practical details of the procedures used in the operation of the phosphorus deficiency bioassay, which may be used to determine the phosphorus nutritional status of trees and other plants.

The details cover aspects such as the laboratory working conditions, equipment required, root sampling and preparation procedures, the bioassay technique and data handling and computer procedures. The computer programmes, written in BASIC PLUS for a PDP 11/34 computer are presented in full.

Introduction

Five papers have now been published on the deficiency bioassay developed at Merlewood (Harrison & Helliwell, 1979; Dighton & Harrison, 1983; Brown & Harrison, 1983; Harrison, Dighton, Hatton & Smith, (in press) and Van Cleve & Harrison (in press)). The first paper outlines the first pot experiments with birch (Betula pendula) and sycamore (Acer pseudoplatanus) seedlings attempting to substantiate the method. The second, third papers and fourth papers describe the application of the method to forest stands of Sitka spruce, lodgepole pine, Norway spruce and upland grass swards, whilst the fifth paper uses the technique to detect rates of mineralization of P from a variety of forest floor litters.

The published research and recent unpublished studies suggest that the bioassay may be more sensitive than conventional needle analysis at detecting phosphorus deficiencies in trees. Work is now in hand to further validate the bioassay in the forest application, though it might have a wider application in agriculture as well. The fourth publication outlines some preliminary results with grasses and grassland swards.

It is in the first paper that the method is described as fully as the space in the paper would allow. This paper, thus, only gives the bare outlines of the method. Such short descriptions of the method in papers often grossly understate the amount of work and backup facilities involved in the application of methods.

We feel it would now be worthwhile describing in more detail the sample and handling procedures we adopt in the application of the method to either intact seedlings or to roots severed from trees in the field. It is particularly important when the bioassay is applied to 100-200 plants or roots per day, that smooth operating procedures are worked out.

This research and development paper contains, therefore: i) details of the field sampling of roots. ii) root washing procedures, iii) the full description of the bioassay procedures, including precautions necessary when handling ^{32}P (the latter are not intended to exempt researchers from further discussions with appropriate radiosafety officers of their institutions), iv) our present facilities for data handling, including computer programmes listed in full.

We hope all this information will be useful to prospective users of the bioassay, whether they be interested in the physiology and biochemistry of the plant response involved or the application of the technique to plant nutritional problems in the field.

Facilities, Equipment and Materials

Working conditions

The whole process of the bioassay is carried out in a registered grade 3 radioisotope laboratory. Registration of the laboratory with the Radiochemical Inspectorate, Department of the Environment for the use of ^{32}P is essential. When carrying out all activities laboratory coats are worn at all times. Appropriately calibrated contamination and radiation monitoring equipment is available at all times - we use a Mini-monitor model S, calibrated for us by the National Radiological Protection Board. Film badges are always worn to monitor personal radiation dose levels, but we have had no problems with radiation dose. Rubber surgical gloves are worn as a routine. Activities with radioactive solutions are carried out in a "fume cupboard" with a perspex screen containing arm ports to allow manipulation of the highly active solution (1 millicurie per ml) with the screen down i.e. between the solution and the operator. Few problems have been encountered with laboratory or pesonal contamination with ^{32}P when using this technique.

Main equipment

The central and essential piece of equipment for the bioassay is the Packard 2425 liquid scintillation counter. The machine takes 150 samples at once in three trays of 50 vials. It operates automatically and has a so-called "blackout" device, which means that it automatically continues its operation following a temporary power failure.

Data is printed out onto a teletype which simultaneously produces a punched paper tape (more modern machines have a micro computer attached for data capture). The machine is set to count ^{32}P by Cerenkov light and to count in two channels, so that the sample channels ratio, a function of counting efficiency, is automatically provided.

An electronic balance is used to measure root weights to a precision of 1 mg and water baths at 18°C are used to maintain bioassay solutions at constant temperature.

A digester is also used to digest random root samples for estimation of the effects of root quenching of the ^{32}P, reducing the counting efficiency to a variable extent depending on the root mass in the vial.

Solutions used

Potassium dihydrogen phosphate and calcium sulphate are included in the bioassay solutions. Whilst they are used at a concentration of $5*10^{-6}$ and $5*10^{-4}$ molar respectively, the solutions are made up at 10^{-5} and 10^{-3} M respectively. For the phosphorus- calcium sulphate-^{32}P solution, these solutions are then simply added to each other in equal proportions, mutually diluting the ions by half. In the case of the calcium sulphate solution used in the pretreatment of the roots, the 10^{-3} M solution is diluted with an equal volume of distilled water. Preparation of the solutions is carried out as in Fig.1.

The ^{32}P used is in the form of orthophosphate, purchased from Amersham International, under code PBS 11. 5 millicuries is usually purchased at a time, as to buy less is hardly less expensive, because of insurance, packaging and transport costs. The 5 millicuries can have a useful life of about 2 months, depending on the rate of usage. ^{32}P is also purchased as an absolute standard for calibrating the scintillation counter, with respect to its counting efficiency This source of ^{32}P is usually only 5 microcuries, at a concentration of 1 microcurie per ml and with a tolerance of 2.5%. Purchase of this ^{32}P varies as the need to recalibrate the counter depends on counter stability, modification or change of components which affects the instrument's counting efficiency.

Ordinary tap water is used to wash the roots free of physically adsorbed ^{32}P from the surface, as the water has a good level of purity. In any area where the water quality is questionable, perhaps an isotonic solution of KCl could be used, but this has not been tested.

Concentrated nitric-perchloric-sulphuric acid mixture is used to digest root samples, as no significant losses of ^{32}P occur when this acid mixture is used (for a background see Allen <u>et al.</u> 1974).

Fig. 1 **SOLUTIONS REQUIRED FOR BIOASSAY**

1. $CaSO_4 2H_2O$

   0.1722 g l^{-1} (Sol^n 1)

   ↓

   Dilute 1:1 with distilled water (D.W.)

   ↓

   5 x 10^{-4}M (Sol^n 2)

2. KH_2PO_4

   1.3069 g l^{-1}

   ↓

   1ml

   ↓

   make up to 1l (= 1 x 10^{-5}M)

   ↓

   Dilute 1:1 with Sol^n 1

   ↓

   5 x 10^{-6}M KH_2PO_4 in $CaSO_4$ (Sol^n 3)

Methods

Root sampling and preparation

When applied to roots from forest stands, roots are sampled from the surface soil organic material on the forest floor. Generally we try to obtain 50 10-20 cm lengths of root of about 1-3 mm diameter, which are usually mycorrhizal. These roots are taken randomly from ridges, flat areas and the walls of drainage ditches. Sample roots are left with soil or organic debris still attached and an appropriately coded label attached to one end. Roots are then laid between moist tissue paper in a plastic tray and kept cool (i.e. out of the sun) for transportation to the laboratory. When the bioassay is applied to grass swards, turfs of the grass are dug, moistened as necessary and transported to the laboratory intact. Individual plants are then teased out for analysis. Potted plants, provided they are not too big, are simply removed from the pot and treated as individual intact plants.

All roots are processed in a random fashion, not in the treatment blocks of the experiment being examined. A SORT programme is used to unscramble the data later in the procedure. Random treatment of roots is essential to prevent any inadvertant or subconscious bias in handling.

A diagramatic presentation of the sampling and washing sequence is given in Fig.2. The individual roots or whole root systems are washed with tap water to remove adhering soil particles, with as little abrasive action as possible.

Bioassay procedure

Before the bioassay procedure starts, enough glass counting vials, in cardboard trays, are filled with 15 ml of distilled water, to take the number of root samples required. Within an hour after washing, roots are immersed in $5*10^{-4}$ M calcium sulphate solution, usually in a plastic bucket, for thirty minutes. The immersion is considered to have two effects, firstly, to remove phosphorus from the free space of the root (unlabelled phosphorus diffusing into the ^{32}P labelled solution would alter the specific activity in an indeterminate way, thus reducing the precision of the bioassay) and, secondly, calcium ions may act to stimulate phosphorus uptake and help to maintain root cell membrane integrity.

Fig. 2 FLOW CHART OF ROOT SAMPLING AND HANDLING PRIOR TO THE BIOASSAY

SEEDLING PLANTS

Plants removed from containers avoiding root damage

↓

Rooting medium washed from roots using tap water by:
a) Root washing machine
b) Hose pipe
c) Water in buckets
with gentle rubbing with fingers where necessary

↓

Whole plants or roots from each tagged (aluminium pot label or freezer-bag tie tag)

FOREST TREES

SURFACE FEEDER ROOTS

Litter moved aside - root followed to terminal fine roots by gentle excavation

↓

10-15cm long root segment detached with scissors

↓

Tag roots

↓

Place in tray on damp tissue paper - cover with damp tissue - fill tray in layers

↓

In laboratory wash soil from roots in tap water

ROOTS FROM SOIL CORES

Divide soil core into horizons

↓

Break up each horizon carefully by hand and wash soil from roots in washing machine or by hand

↓

Root fragments aggregated to give suitable size for handling and tied together with tag

↓

TO BIOASSAY

N.B. Any intermediate storage between harvest and the bioassay, roots are kept moist under tissue paper in a cold room (2-5^{o}C). All samples to date have been processed within 72h of collection.

Whilst roots are being treated in calcium sulphate, ^{32}P labelled solutions are being prepared. Two litres of solution, containing calcium sulphate and potassium hydrogen phosphate at $5*10^{-4}$ and $5*10^{-6}$ M concentration respectively, are poured into each of two three litre beakers, referred to as A and B (for small numbers of samples, two beakers of solution are not necessary). The beakers are placed in the water bath at 18°C and allowed to reach the same temperature. To each beaker is added about 60 microcuries of ^{32}P taken from the multidose vial in which it is supplied, using a sterile hypodermic syringe (N.B. it only requires 0.1 ml of solution at an activity of 1 millicurie per ml to provide 100 microcuries). The volume required will increase as the ^{32}P decays at a rate of about 2% per day. The solutions in the beakers are then thoroughly mixed. Two 0.5 ml samples are taken from each of the two solutions to estimate the ^{32}P activity added, before the roots are immersed. The 0.5 ml samples of solution A, referred to as standards, are taken and transferred to the first two vials in the tray, using a fixed volume micro-pipette. Two similar samples are taken from solution B, but the vials containing these are not yet placed in the trays. Roots are immersed in the solutions, 15 to 30 at a time, depending on their size, using appropriate sized tongs. The time of immersion is noted on the control document, roots being immersed for exactly 15 minutes.

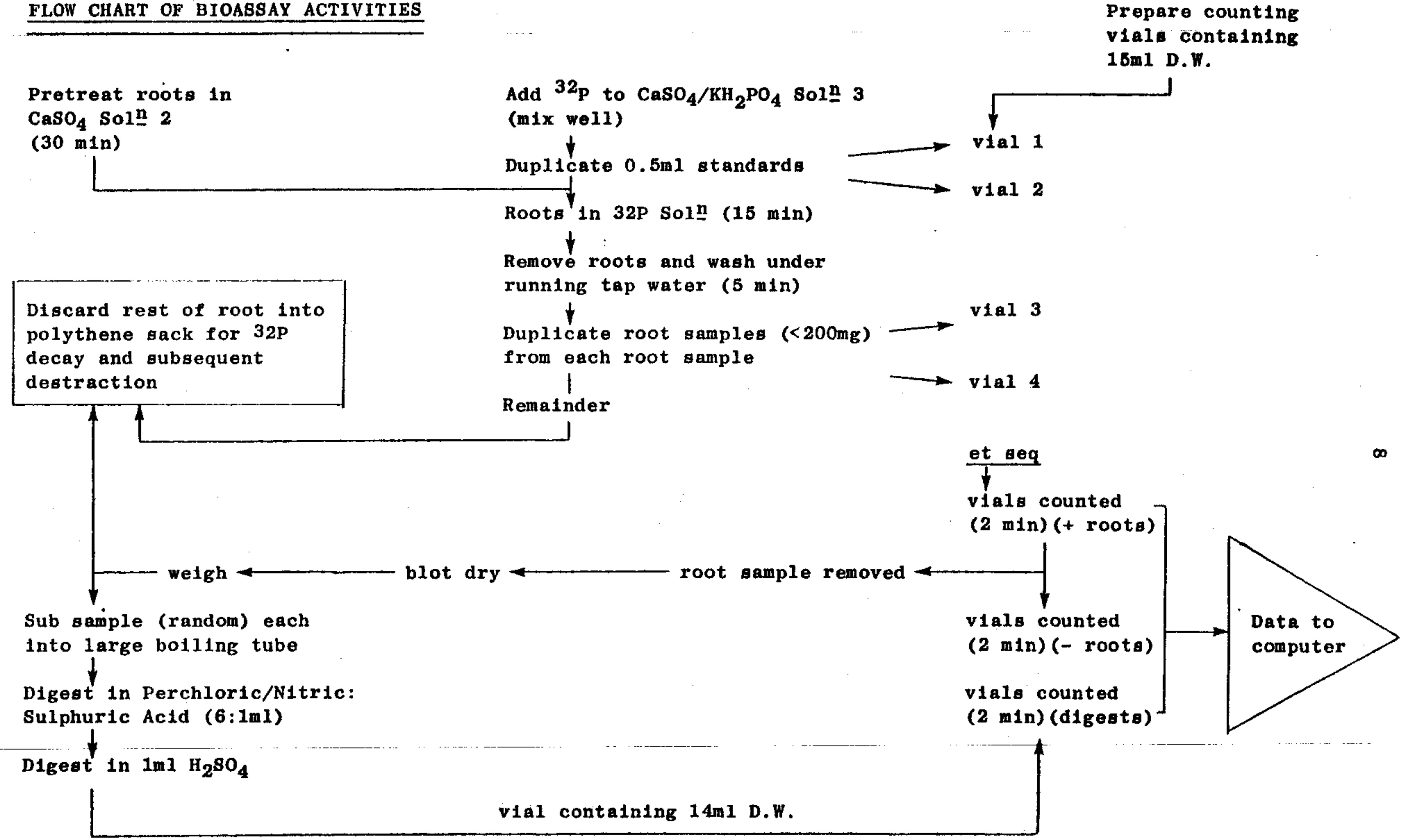
After 15 minutes in the solution, the roots are removed from the ^{32}P solution and immediately transferred to separate beakers into which tap water is directed via a polypropylene tube to the bottom of the beaker, and is allowed to flow over into a sink, designed for the disposal of ^{32}P. Roots are washed for 5 minutes and are then removed to trays covered with paper towels. The tray on which the roots are placed is kept at arms length from the person carrying out the bioassay, to reduce the risk of exposure to radiation. Roots in solution A are dealt with first. Two subsamples of lateral roots are taken from each root and placed in successive vials containing 15 ml of distilled water. A note of the root code, number or description is made on the control document against vials 3 and 4 (vials 1 and 2 containing the 0.5 ml standard from solution A).

The next root is subsampled likewise and the subsamples placed in vials 5 and 6 and the appropriate note made of the root number processed. When all the root samples from solution A have been processed in this manner, the standards of solution B are placed in the tray and root samples from solution B are processed similarly into successive vials.

When a tray of 50 vials is completed, the vials are transferred to a plastic counting tray and placed into the scintillation counter and counted for 2 minutes. The time at which the counting was started is recorded to enable the time elapsed between placing the roots in the solution and time counting was started to be calculated. Counting of three trays takes six hours and it takes 25 seconds for the results of each sample to be printed out and the vials changed in the counter, before counting is resumed.

After counting vials with the root samples in, roots are removed from the vials (very small pieces may be left in the vial), blotted dry with tissue paper and weighed to the nearest mg. The weight is noted against

Fig. 3 FLOW CHART OF BIOASSAY ACTIVITIES



the vial number on the control document. The vials are then replaced in the same order in the counting trays and returned to be counted as soon as practicable (counting vials containing roots should have precedence). The time at which the vials are recounted, without roots, is also noted on the control document, so that a further elapsed time can be calculated.

In order that an allowance may be made for the quenching effect of the root biomass in the vial, a stratified random sample of the roots is transferred to boiling tubes for digestion. Firstly, a series of roots is taken to cover the range of weights, from minimum to maximum, and then a series to cover the range of activity levels. A total of about 40 samples is necessary to permit a satisfactory regression equation to be developed. Whilst in the original paper (Harrison & Helliwell, 1979) it was suggested that there was no significant difference in equations for roots of the two different species studied, work with roots from trees in the field has shown it is necessary to run a digestion series for each batch of morphologically different root samples examined. The roots taken for digestion are noted on the control document. Samples are usually digested in a mixture of 3 ml of the nitric-pechloric acid mixture and 0.5 ml of concentrated sulphuric acid.

Digestion takes about 1.5 hours from start to finish. The digested sample is then washed out of the tube with 3 washes of 5 ml of distilled water, into a counting vial. The vials are then placed in a plastic counting tray and placed in the counter for counting under the same conditions as the root samples. The time of commencement of counting is noted for the calculation of elapsed time.

Whilst vials with roots, vials without roots and vials with digests are counted, the data are simultaneously printed out and punched onto paper by the teletype connected to the counter. The paper tape is required for transference of the data to the computer.

The solutions A and B can be re-used by immersing several batches of roots in them. However, further standard 0.5 ml samples of the solution must be taken to estimate the ^{32}P level in the solution before roots are immersed. Corrections for the reduction of ^{32}P by previous root samples can be allowed for in the computer programmes used to process the data. We usually continue to reuse the solutions until the level of activity drops below 50-60% of the origional, or roots of a different species of plant are to be processed. We do this in order to reduce the ammount of work preparing the ^{32}P solutions, to reduce the ammount of ^{32}P used, so reducing cost and we have to dispose of far less ^{32}P (we are allowed to dispose of a maximum of 1 millicurie per month). A diagramatic scheme of the bioassay activities is presented in Fig.3. An example of the control document on which the times, sample codes, root weights etc. are recorded is also given in Fig.4.

EXPERIMENT:

TRAY No.

Fig. 4

DATE	SOL^n	TIME OF STAND.	ROOT CODE	DIGEST TUBE NO.	ROW NO.	VIAL NO.	ROOT WEIGHT		
					0	0			
					0	1			
					0	2			
					0	3			
					0	4			
					0	5			
					0	6			
					0	7			
					0	8			
					0	9			
					1	0			
					1	1			
					1	2			
					1	3			
					1	4			
					1	5			
					1	6			
					1	7			
					1	8			
					1	9			
					2	0			
					2	1			
					2	2			
					2	3			
					2	4			
					2	5			
					2	6			
					2	7			
					2	8			
					2	9			
					3	0			
					3	1			
					3	2			
					3	3			
					3	4			
					3	5			
					3	6			
					3	7			
					3	8			
					3	9			
					4	0			
					4	1			
					4	2			
					4	3			
					4	4			
					4	5			
					4	6			
					4	7			
					4	8			
					4	9			

Computation

In the calculations, all carried out by computer, allowance is made for: i) background counts of the scintillation counter, when counting a vial of water, ii) counting efficiency (assuming the vials contain only homogenous solutions), iii) decay of the ^{32}P (hence the need to record the times of activities - the computer programme calculates the progressive elapsed times for each sample) and iv) the effects of root quench (a multiple regression equation is developed after i), ii) and iii) have been computed in the first stage of the programme). The counting efficiency equation is developed in the form of:

$$Y=aX + C$$

where X is the channels ratio value.

The channels ratio value is calculated by taking a series of vials containing 14 ml of distilled water into which 1 ml of a series of solutions containing varying ammounts of colour quenching substances (e.g. NaOH extracts of roots or soils from which roots have been extracted) and adding 10 microlitres of a 1 microcurie per ml calibrated ^{32}P solution. The vials are then counted under the same conditions as the root samples, i.e. in two channels, with the calculation of the channels ratio. The percentage counting efficiency is calculated from the CPM estimate of the channel with the wider spectrum (in the case of our instrument, the red channel) after allowing for the ^{32}P decay and background count in that channel and dividing that sum by the known amount of activity in DPM present in 10 microlitres of the standard, times 100.

The equation to allow for the effects of root quench takes the form of:

$$Y = aX + bR + cR^2 + d$$

where X is the estimated ^{32}P activity in DPM before allowance for root quench.
R is the root (moist) weight in mg.

The equation is derived from a multiple regression from the estimated ^{32}P content of the root (i.e. counts of the vials with roots minus the counts of the vials without roots, both after correction for background, counting efficiency and decay), root wieght and the estimated ^{32}P activity in the digest(after allowance for background counting efficiency and decay), for the roots which were selected for digestion.

If the ^{32}P solutions are re-used, allowance for the reduction in the rate of uptake of ^{32}P and phosphate due to a lower concentration of the ^{32}P labelled phosphate in the solution must be made (previous batches of roots have removed some of the phosphate). As the rate of ^{32}P uptake is linearly related to concentration over the low ranges of concentration in question, adjustment of the estimates of ^{32}P

uptake by the roots can be calculated by increasing the estimate of root uptake by the ratio of the ^{32}P activity in the solution before any roots were immersed to activity before the particular batch of roots in question. The computer programme CALPUP will automatically calculate the adjustment, if the appropriate inputs are given.

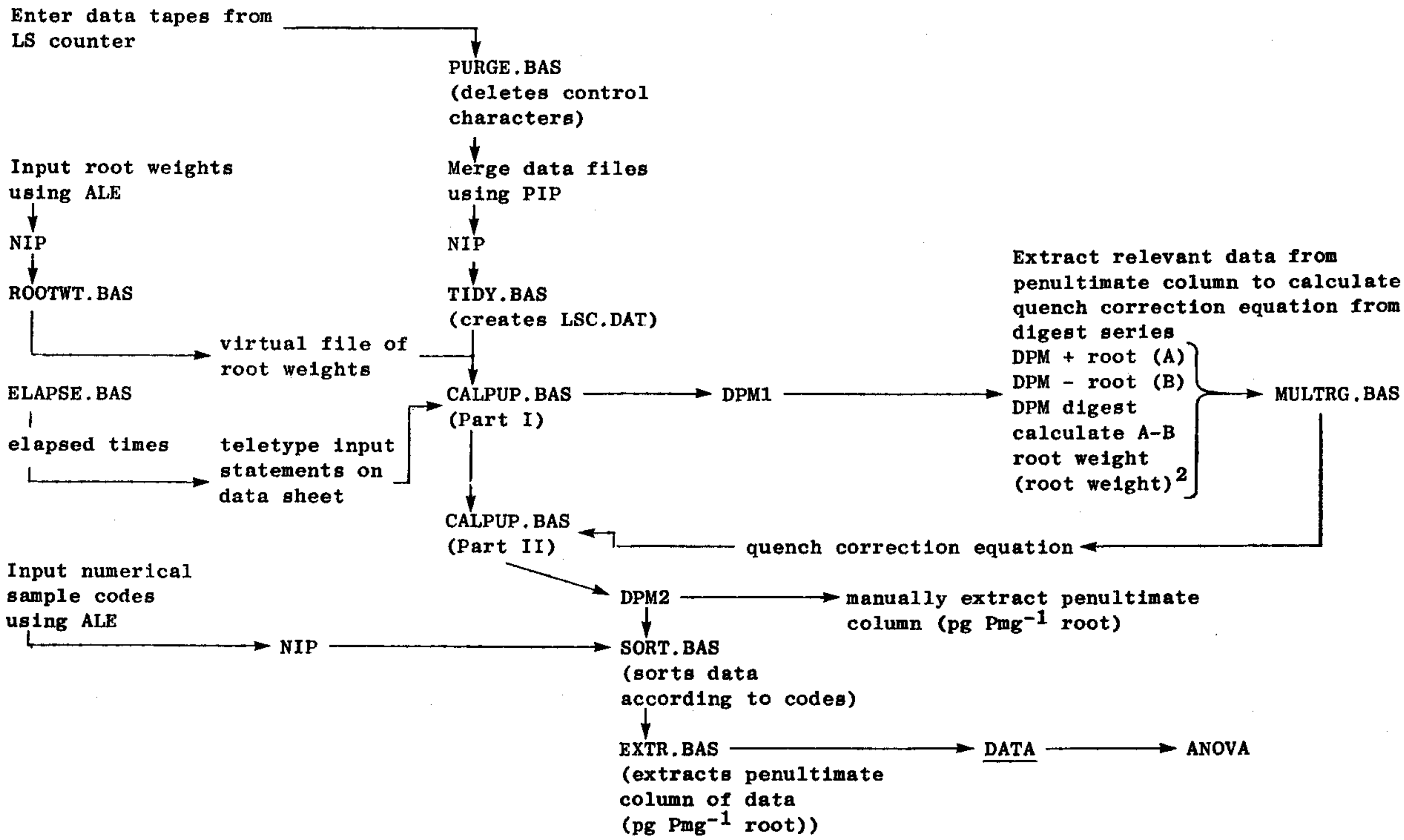
The data obtained from the liquid scintillation counter are transferred to a computer using the paper tape and the data are processed using a series of programmes. The sequence of events in the processing of the results are given in Fig.5.

The programmes in order of use are referred to as: PURGE.BAS, TIDYP.BAS, ELAPSE.BAS, ROOTWT.BAS, CALPUP.BAS (or BATPUP.BAS), SORT.BAS and EXTR.BAS, programme listings are given in Appendix II. PURGE.BAS is used to remove control and other odd unprinted characters generated by the counter, which cause problems in the execution of programmes. Data files are edited using the line-editor programme ALE at this stage. If data have been entered on several different tapes , the data files are merged together using the file merging facility of PIP (the peripheral input programme). After being processed using the Numerical Input Programme (NIP), a random access virtual file called LSCDAT.XXX (the XXX's being substituted by an appropriate extension) is created by TIDYP.BAS. This programme selects out the data, time of counting in minutes, total counts accumulated in the red channel in the counting time and the sample channels ratio, already calculated by the scintillation counter. Before the main programme CALPUP.BAS (or BATPUP.BAS) is used, rootweights are entered into a virtual file with ROOTWT.BAS and the appropriate elapsed times are calculated by using ELAPSE.BAS. CALPUP.BAS is effectively a programme with two major subroutines. Part 1 adjusts the counts per minute results for the background counts, decay of the ^{32}P (which has a half life of 14.28 days) and the efficiency with which each sample was counted, to give estimates of the disintigrations per minute in each sample. Teletype inputs for the first part of CALPUP are generated from the data on the control documents and the sequence of the tray counting (see worked example in appendix I).

Resulting intermediate calculations are stored in a file called DPM1.XXX. Data to generate the quench correction equation, required in subroutine 2, is extracted from the printout of DPM1.XXX. On the printout, the positions of standards is noted amongst the "with root" and "without root" data, and all lines of data are subsiquently numbered. The data corresponding to the digested samples in the "with" and "without root" data are extracted with reference to the corresponding control documents. With the inclusion of the root weight data, a file is created and a multiple regression equation is generated using MULTRG.BAS. Having generated a table of input data and entered the coefficients and constant for the quench correction equation, the second subroutine of CALPUP.BAS may be run.

Subroutine two of the programme subtracts the estimated ^{32}P of the vials without roots from the ^{32}P content of the vial with roots, adjusts for the effects of root quenching and calculates the estimated phosphorus uptake of the root samples in picogrammes per mg root. All the intermediate calculations are deposited in the file DPM2.XXX. Intermediate calculations are both printed out and stored on the disk.

Fig. 5 FLOW CHART OF USE OF COMPUTER PROGRAMS



Errors or oddities can then be easily edited out or corrected. After inputting the numerical codes of the root samples in the order in which the roots were processed, the SORT.BAS programme can then rearrange the data in DPM2.XXX into the desired order. EXTR.BAS can then extract the penultimate column of data in DPM2.XX (P uptake in pg mg^{-1} root) for analysis of variance or other statistical analysis.

Appendix I gives a fully documented run through a set of data derived from roots collected from Norway spruce processed through the bioassay.

ALLEN,S.E. *et al.* (1974). Chemical analysis of ecological materials. Blackwell, Oxford, U.K.

BROWN,A.H.F. & HARRISON,A.F. (1983). Effects of tree mixtures on earthworm populations and nitrogen and phosphorus status in Norway spruce (*Picea abies*) stands. In: New Trends in Soil Biology. Proc. VIII Int. Colloq. Soil Zoology. Louvain-la-Neuve. edited by Ph. Lebrun *et al.* Dieu-Brichart.

DIGHTON,J. & HARRISON,A.F. (1983). Phosphorus nutrition of lodgepole pine and Sitka spruce stands as indicated by a root bioassay. Forestry, 56, 33-43.

HARRISON,A.F. & HELLIWELL,D.R. (1979). A bioassay for comparing phosphorus availability in soils. J. appl. Ecol., 16, 497-505.

HARRISON,A.F., DIGHTON,J., HATTON,J.C. & SMITH,M.R. (in press). A phosphorus-deficiency bioassay for trees and grasses growing in low nutrient status soils. In: Proc. VI Colloq. Int. pour L' optimization de la Nutrition des Plantes, Montpellier.

VAN CLEVE,K. & HARRISON,A.F. (in press). Bioassay of forest floor phosphorus supply for plant growth. Can. J. For. Res. (in press).

Appendix I

A worked example of processing the data from the scintillation counter to calculate P uptake.

1. Record sheet (Table 1).

This sheet records the usage of the ^{32}P solution, the times of removal of standards and the sequence of root samples (root codes) passing through the bioassay solution and into the counting vials. Each beaker of bioassay ^{32}P solution (A and B) may be used more than once. Records of solution usage are made as this is required to estimate P removal from the solution by previous root batches.

Root samples taken for digestion are noted on the record sheet, together with the moist weight of the root determined after the first (with root) counting of the vials.

EXPERIMENT: GISBURN (ROOT POSITION ON PLOUGH LINE) TRAY NO. 1

DATE	SOL^n	TIME OF STAND.	ROOT CODE	DIGEST TUBE NO.	ROW NO.	VIAL NO.	ROOT WEIGHT (mg)
15/2/1984	A(1)	1132	Std		0	0	1st use of Sol^n A 0.5 ml standard
			Std.		0	1	"
			D2		0	2	129
					0	3	108
			R3		0	4	106
					0	5	133
			D3	21	0	6	102
					0	7	108
			F1		0	8	107
					0	9	89
			F1	25	1	0	237
					1	1	116
			D2		1	2	105
				13	1	3	55
			D2		1	4	125
					1	5	107
			R3		1	6	142
				3	1	7	78
			R1		1	8	40
					1	9	37
			D1		2	0	64
					2	1	84
	B(1)	1132	Std		2	2	1st use of Sol^n B 0.5 ml standard
			Std.		2	3	
			R1		2	4	63
					2	5	100
			F3		2	6	69
					2	7	75
			R2		2	8	76
					2	9	116
			R3	14	3	0	208
				18	3	1	136
			D1		3	2	65
					3	3	74
			D2		3	4	84
					3	5	81
			F3	20	3	6	173
					3	7	128
			F3	2	3	8	69
					3	9	67
			F1		4	0	140
					4	1	117
			R1		4	2	23
					4	3	86
	A(2)	1232	Std		4	4	2nd use of Sol^n A 0.5 ml standard
			Std		4	5	
			F2		4	6	71
					4	7	85
			D2		4	8	41
					4	9	53

2 Liquid Scintillation Counter Output (Tables 2 and 3).

In addition to the paper tape output a teletype printout of the LSC output is also obtained. Data sequence is according to tray and vial number following that on the record sheet. This output is used to number the lines of data to itemize the standards in order to make up the teletype inputs for the first part of the programme CALPUP.BAS.

This data is processed through PURGE.BAS to delete control characters and will be converted to a virtual file (LSCDAT.XXX) by TIDYP.BAS.

Table 2

15-2-'84
WITH-ROOT SAMPLES, COUNTING STARTED 12.16 (BACKGROUND COUNT = 34)

1ST50.TMP

1	100	2.00	35450	.7	21093	.7	17725.0	10546.5	.594	STANDARD A (1st use)
2	101	2.00	37528	.7	22767	.7	18764.0	11383.5	.606	
3	102	2.00	21183	.7	11135	1.0	10591.5	5567.5	.525	
	103	2.00	19492	1.0	10595	1.0	9746.0	5297.5	.543	
	104	2.00	23124	.7	13025	1.0	11562.0	6512.5	.563	
	105	2.00	28357	.7	15056	1.0	14178.5	7528.0	.530	
	106	2.00	2607	3.0	1452	4.0	1303.5	726.0	.557	
	107	2.00	3757	3.0	2112	3.0	1878.5	1056.0	.562	
	108	2.00	29729	.7	16418	1.0	14864.5	8209.0	.552	
	109	2.00	33444	.7	19738	1.0	16722.0	9869.0	.590	
	110	2.00	48685	.5	25173	.7	24342.5	12586.5	.517	
	111	2.00	21478	.7	11991	1.0	10739.0	5995.5	.558	
	112	2.00	2993	3.0	1674	4.0	1496.5	837.0	.559	
	113	2.00	6028	2.0	3533	3.0	3014.0	1766.5	.585	
	114	2.00	10356	1.0	5679	2.0	5178.0	2839.5	.548	
	115	2.00	28332	.7	16057	1.0	14166.0	8028.5	.566	
	116	2.00	38942	.7	21604	.7	19471.0	10802.0	.554	
	117	2.00	2547	3.0	1492	4.0	1273.5	746.0	.586	
	118	2.00	9152	2.0	5553	2.0	4576.0	2776.5	.606	
	119	2.00	10495	1.0	6378	2.0	5247.5	3189.0	.607	
	120	2.00	2547	3.0	1456	4.0	1273.5	728.0	.571	
22	121	2.00	2829	3.0	1542	4.0	1414.5	771.0	.545	
23	122	2.00	29911	.7	18274	1.0	14955.5	9137.0	.610	
24	123	2.00	30156	.7	18674	1.0	15078.0	9337.0	.619	STANDARD B (1st use)
25	124	2.00	23399	.7	13571	1.0	11699.5	6785.5	.579	
	125	2.00	22273	.7	11664	1.0	11136.5	5832.0	.523	
	126	2.00	5116	2.0	3020	3.0	2558.0	1510.0	.590	
	127	2.00	3089	3.0	1714	4.0	1544.5	857.0	.555	
	128	2.00	10881	1.0	5837	2.0	5440.5	2918.5	.536	
	129	2.00	7331	2.0	4187	2.0	3665.5	2093.5	.571	
	130	2.00	50420	.5	26594	.7	25210.0	13297.0	.527	
	131	2.00	49995	.5	27419	.7	24997.5	13709.5	.548	
	132	2.00	19991	1.0	11476	1.0	9995.5	5738.0	.574	
	133	2.00	8592	2.0	4601	2.0	4296.0	2300.5	.535	
	134	2.00	8493	2.0	4920	2.0	4246.5	2460.0	.579	
	135	2.00	10754	1.0	5866	2.0	5377.0	2933.0	.545	
	136	2.00	23363	.7	12516	1.0	11681.5	6258.0	.535	
	137	2.00	12765	1.0	6974	2.0	6382.5	3487.0	.546	
	138	2.00	1393	4.0	780	5.0	696.5	390.0	.560	
	139	2.00	3685	3.0	2070	3.0	1842.5	1035.0	.561	
	140	2.00	26385	.7	14410	1.0	13192.5	7205.0	.546	
	141	2.00	22371	.7	12379	1.0	11185.5	6189.5	.553	
	142	2.00	6217	2.0	3502	3.0	3108.5	1751.0	.563	
44	143	2.00	11371	1.0	6462	2.0	5685.5	3231.0	.568	
45	144	2.00	35280	.7	21931	.7	17640.0	10965.5	.621	
46	145	2.00	36707	.7	23143	.7	18353.5	11571.5	.630	STANDARD A (2nd use)
47	146	2.00	22436	.7	12968	1.0	11218.0	6484.0	.577	
	147	2.00	19093	1.0	10897	1.0	9546.5	5448.5	.570	
	148	2.00	5926	2.0	3423	3.0	2963.0	1711.5	.577	
50	149	2.00	9036	2.0	5175	2.0	4518.0	2587.5	.572	

Table 3

16-2-'84

1ST50.NRT   WITHOUT-ROOT SAMPLES, COUNTING STARTED 1005 (BACKGROUND COUNT = 34)

51	100	2.00	33227	.7	18958	1.0	16613.5	9479.0	.570	
52	101	2.00	35047	.7	20358	.7	17523.5	10179.0	.580	STANDARD A (1st use)
53	102	2.00	1429	4.0	774	5.0	714.5	387.0	.542	
	103	2.00	1112	4.0	629	5.0	556.0	314.5	.564	
	104	2.00	19026	1.0	11055	1.0	9513.0	5527.5	.580	
	105	2.00	22168	.7	12867	1.0	11084.0	6433.5	.580	
	106	2.00	1391	4.0	800	5.0	695.5	400.0	.575	
	107	2.00	1380	4.0	839	5.0	690.0	419.5	.607	
	108	2.00	3812	3.0	2175	3.0	1906.0	1087.5	.570	
	109	2.00	3681	3.0	2079	3.0	1840.5	1039.5	.564	
	110	2.00	3152	3.0	1618	4.0	1576.0	809.0	.513	
	111	2.00	1609	4.0	887	5.0	804.5	443.5	.550	
	112	2.00	1594	4.0	825	5.0	797.0	412.5	.516	
	113	2.00	580	5.0	363	7.0	290.0	181.5	.624	
	114	2.00	1173	4.0	692	5.0	586.5	346.0	.590	
	115	2.00	2292	3.0	1241	4.0	1146.0	620.5	.541	
	116	2.00	15871	1.0	9440	2.0	7935.5	4720.0	.594	
	117	2.00	1099	4.0	669	5.0	549.5	334.5	.608	
	118	2.00	4544	2.0	2679	3.0	2272.0	1339.5	.589	
	119	2.00	2493	3.0	1547	4.0	1246.5	773.5	.620	
	120	2.00	582	5.0	343	7.0	291.0	171.5	.587	
72	121	2.00	621	5.0	378	7.0	310.5	189.0	.609	
73	122	2.00	27721	.7	16019	1.0	13860.5	8009.5	.577	
74	123	2.00	27094	.7	15997	1.0	13547.0	7998.5	.590	STANDARD B (1st use)
75	124	2.00	6874	2.0	4050	2.0	3437.0	2025.0	.589	
	125	2.00	5860	2.0	3194	3.0	2930.0	1597.0	.545	
	126	2.00	1241	4.0	756	5.0	620.5	378.0	.609	
	127	2.00	578	5.0	326	7.0	289.0	163.0	.564	
	128	2.00	3965	3.0	2239	3.0	1982.5	1119.5	.564	
	129	2.00	1754	4.0	1043	4.0	877.0	521.5	.594	
	130	2.00	27888	.7	15864	1.0	13944.0	7932.0	.568	
	131	2.00	34511	.7	20196	.7	17255.5	10098.0	.585	
	132	2.00	6650	2.0	3959	3.0	3325.0	1979.5	.595	
	133	2.00	2167	3.0	1345	4.0	1083.5	672.5	.620	
	134	2.00	2942	3.0	1816	4.0	1471.0	908.0	.617	
	135	2.00	3551	3.0	2052	3.0	1775.5	1026.0	.578	
	136	2.00	4452	2.0	2285	3.0	2226.0	1142.5	.513	
	137	2.00	2370	3.0	1302	4.0	1185.0	651.0	.549	
	138	2.00	581	5.0	333	7.0	290.5	166.5	.572	
	139	2.00	1616	4.0	925	5.0	808.0	462.5	.571	
	140	2.00	2717	3.0	1554	4.0	1358.5	777.0	.572	
	141	2.00	3588	3.0	1988	4.0	1794.0	994.0	.554	
	142	2.00	1308	4.0	712	5.0	654.0	356.0	.544	
94	143	2.00	1183	4.0	670	5.0	591.5	335.0	.566	
95	144	2.00	32488	.7	19546	1.0	16244.0	9773.0	.601	
96	145	2.00	33276	.7	20319	.7	16638.0	10159.5	.610	STANDARD A (2nd use)
97	146	2.00	2453	3.0	1446	4.0	1226.5	723.0	.589	
	147	2.00	3988	3.0	2303	3.0	1994.0	1151.5	.577	
	148	2.00	1742	4.0	1032	4.0	871.0	516.0	.592	
100	149	2.00	2426	3.0	1370	4.0	1213.0	685.0	.564	

3 Teletype Input Instructions I (Table 4)

Each batch is associated with the number of trays of vials processed through the scintillation counter. The elapsed time between taking the standard 0.5 ml from the bioassay solution and the time of counting the related vials is calculated and entered here. Background counts are based on counts of vials containing distilled water only.

These inputs run the first part of CALPUP.BAS to correct crude DPM's for background and decay of the ^{32}P during the elapsed time.

Table 4

TELETYPE INPUTS FOR FIRST PART OF CALPUP.BAS.

ELAPSED TIME (min)	BACKGROUND	NO. BATCHES	1ST AND LAST SET NO.	WITH (1) OR WITHOUT (2) ROOTS	COUNT TIME	
44	34, 34	1	1, 50	1	2	
1340	34, 34	1	51, 100	2	2	
			417, 445	2	2	DIGESTS

4 DPM1 (Table 5)

This is the data output from the first part of CALPUP.BAS (Note: for all output files no extension name is given, an appropriate extension, related to the nature of the experiment, is usually appended).

The root weight file (a virtual file of root weights created through ROOTWT.BAS) is not required for the first part of CALPUP.BAS.

The first 50 vials only are shown here, counted firstly with roots, secondly, without roots and, thirdly, those root samples which were digested to correct for quenching. The appropriate vials from which roots were taken for digestion are marked on DPM1. The penultimate column (DPM corrected for background and decay) is manually extracted for the digested samples and compiled with the appropriate root weight data to form:

Table 5

```
RUNNH

ENTER THE EXTENSION FOR THE LSCDAT AND DPM FILES        ? VIR
Will you need root weights? NO
Have you allowed for Background, decay and C.E.         ? NO

** ROUTINE TO CORRECT FOR BACKGROUND, DECAY AND C.E. **

Minutes elapsed before counting started    ? 44
Backgrounds for with and without roots     ? 34,34
Total number of batches in matrix to be processed       ? 1
```

1. *Sample number*
2. *Counting time required when background is subtracted*
3. *CPM allowing for background subtraction*
4. *% counting efficiency (from channels ratio)*
5. *DPM = CPM x 100/CE*
6. *log DPM (after correction for decay)*
7. *DPM (after correcting for decay)*
8. *Elapsed time over which decay has occurred (min)*

```
BATCH 1

First and last set number in batch      ? 1,50
With (1) or without (2) roots           ? 1
Length of counting period   ? 2
```

1	2	3	4	5	6	7	8	
								WITH ROOT
1	2.00	17691.00	32.65	54184.00	10.90	54264.40	44.00	STANDARD A 1st USE
2	2.00	18730.00	33.50	55913.60	10.93	56001.10	46.42	
3	2.01	10557.50	27.77	38014.60	10.55	38077.20	48.84	
4	2.01	9712.00	29.04	33438.20	10.42	33495.90	51.26	
5	2.01	11528.00	30.46	37848.30	10.54	37916.80	53.68	
6	2.00	14144.50	28.13	50290.30	10.83	50385.50	56.10	
7	2.05	1269.50	30.03	4226.83	8.35	4235.18	58.52	DIGEST TUBE No 21
8	2.04	1844.50	30.39	6069.88	8.71	6082.36	60.94	
9	2.00	14830.50	29.68	49966.50	10.82	50073.30	63.36	
10	2.00	16688.00	32.37	51558.50	10.85	51673.00	65.78	
11	2.00	24308.50	27.21	89347.40	11.40	89553.00	68.20	DIGEST TUBE No. 25
12	2.01	10705.00	30.11	35559.90	10.48	35643.60	70.62	
13	2.05	1462.50	30.18	4846.61	8.49	4858.56	73.04	
14	2.02	2980.00	32.01	9308.53	9.14	9332.23	75.46	DIGEST TUBE No 13
15	2.01	5144.00	29.40	17497.70	9.77	17543.70	77.88	
16	2.00	14132.00	30.67	46076.80	10.74	46201.60	80.30	
17	2.00	19437.00	29.82	65176.20	11.09	65358.10	82.72	
18	2.05	1239.50	32.08	3863.26	8.26	3874.36	85.14	DIGEST TUBE No. 3
19	2.01	4542.00	33.50	13559.00	9.52	13599.00	87.56	
20	2.01	5213.50	33.57	15530.80	9.65	15577.90	89.98	DPM, ROOT (2)
21	2.05	1239.50	31.02	3995.30	8.30	4007.75	92.40	
22	2.05	1380.50	29.19	4730.00	8.46	4745.14	94.82	
23	2.00	14921.50	33.78	44171.40	10.70	44316.40	97.24	STANDARD B 1st USE
24	2.00	15044.00	34.42	43710.90	10.69	43852.90	99.66	
25	2.01	11665.50	31.59	36923.40	10.52	37055.60	102.08	
26	2.01	11102.50	27.63	40181.50	10.60	40323.20	104.50	
27	2.03	2524.00	32.37	7790.04	9.97	7824.70	106.92	
28	2.05	1510.50	29.89	5053.03	8.53	5071.69	109.34	
29	2.01	5406.50	28.55	18937.10	9.85	19008.50	111.76	
30	2.02	3631.50	31.02	11705.50	9.37	11750.60	114.18	
31	2.00	25176.00	27.91	90192.50	11.41	90547.60	116.60	DIGEST TUBE No 14
32	2.00	24963.50	29.40	84915.30	11.35	85258.60	119.02	DIGEST TUBE No. 12
33	2.01	9961.50	31.24	31891.00	10.37	32021.80	121.44	
34	2.02	4262.00	28.48	14965.30	9.62	15027.90	123.86	
35	2.02	4212.[illegible]	31.59	1333[illegible].10	9.50	13392.00	126.28	
36	2.01	5343.00	29.19	18306.70	9.82	18396.30	128.70	
37	2.01	11647.50	28.48	40898.30	10.62	41079.50	131.12	DIGEST TUBE No 20
38	2.01	6340.50	29.26	21699.30	9.99	21797.20	133.54	
39	2.10	662.50	30.25	2190.34	7.70	2200.40	135.96	DIGEST TUBE No 2
40	2.04	1809.50	30.32	5965.28	8.70	5993.17	138.38	
41	2.01	13150.50	29.26	44976.00	10.72	45189.90	140.80	
42	2.01	11151.50	29.75	37482.10	10.54	37663.40	143.22	
43	2.02	3074.50	30.46	10094.10	9.22	10143.70	145.64	
44	2.01	5651.50	30.81	18341.90	9.82	18433.70	148.06	
45	2.00	17606.00	34.56	50945.50	10.84	51204.60	150.48	STANDARD A 2nd USE
46	2.00	18319.50	35.19	52051.90	10.87	52320.70	152.90	
47	2.01	11184.00	31.15	35563.30	10.48	35750.00	155.32	
48	2.01	9512.50	30.95	30731.80	10.34	30895.60	157.74	
49	2.02	2929.00	31.45	9313.75	9.14	9364.15	160.16	
50	2.02	4484.00	31.09	14420.50	9.58	14499.70	162.58	

```
ELAPSED TIME T= 165 MINUTES.
```

Table 5 (continued)

Do you want to return to re-enter a new elapsed time ? YES

~~Minutes elapsed before counting started~~ ? 1340
Backgrounds for with and without roots ? 34,34
Total number of batches in matrix to be processed ? 1

BATCH 1

First and last set number in batch ? 51,100
With (1) or without (2) roots ? 2
Length of counting period ? 2

Without roots

51	2.00	16579.50	30.95	53563.00	10.93	56037.20	1340.00	Standard A 1st use
52	2.00	17489.50	31.66	55241.30	10.96	57797.90	1342.42	
53	2.10	680.50	28.97	2348.66	7.81	2457.56	1344.84	
54	2.13	522.00	30.53	1709.84	7.49	1789.27	1347.26	
55	2.01	9479.00	31.66	29939.80	10.35	31333.10	1349.68	
56	2.01	11050.00	31.66	34901.90	10.51	36529.00	1352.10	
57	2.10	641.50	31.31	2112.76	7.20	2211.65	1354.52	Digest tube No. 21
58	2.10	656.00	33.57	1954.17	7.62	2045.64	1356.94	
59	2.04	1872.00	30.95	6047.82	8.75	6331.33	1359.36	
60	2.04	1806.50	30.53	5917.29	8.73	6195.19	1361.78	
61	2.04	1542.00	26.92	5727.24	8.70	5996.70	1364.20	Digest tube No. 25
62	2.09	770.50	29.54	2608.37	7.91	2731.31	1366.62	
63	2.07	763.00	27.14	2811.76	7.99	2944.53	1369.04	
64	2.27	254.00	34.77	736.26	6.65	771.08	1371.46	Digest tube No. 13
65	2.12	552.50	32.37	1706.98	7.49	1797.88	1373.88	
66	2.06	1112.00	28.90	3847.31	8.30	4029.27	1376.30	
67	2.01	7901.50	32.65	24200.70	10.14	25351.00	1378.72	
68	2.13	515.50	33.64	1532.42	7.30	1605.44	1381.14	Digest tube No. 3
69	2.03	2238.00	32.30	6929.56	8.89	7260.33	1383.56	
70	2.06	1212.50	34.49	3515.74	8.21	3683.85	1385.98	
71	2.26	257.00	32.16	799.25	6.73	837.54	1388.40	
72	2.25	274.50	33.71	920.23	6.75	859.59	1390.82	
73	2.00	13824.50	31.45	43964.10	10.74	46079.70	1393.24	Standard B 1st use
74	2.01	13513.00	32.32	41749.20	10.69	43759.80	1395.66	
75	2.02	3403.00	32.30	10535.80	9.31	11045.10	1398.08	
76	2.02	2896.00	29.17	9722.55	9.25	10402.10	1400.50	
77	2.12	586.50	34.71	1739.83	7.51	1824.04	1402.92	
78	2.27	255.00	30.53	835.27	6.78	875.76	1405.34	
79	2.03	1948.50	30.53	6382.42	8.81	6692.53	1407.76	
80	2.09	843.00	32.65	2581.94	7.90	2707.41	1410.18	
81	2.00	13910.00	30.81	45144.90	10.77	47346.00	1412.60	Digest tube No. 16
82	2.00	17221.50	32.01	53794.20	10.94	56421.70	1415.02	Digest tube No. 17
83	2.02	3291.00	32.72	10057.90	9.26	10550.00	1417.44	
84	2.06	1049.50	34.49	3043.11	8.07	3192.26	1419.86	
85	2.05	1437.00	34.28	4192.47	8.39	4398.32	1422.28	
86	2.04	1741.50	31.52	5525.27	8.67	5797.04	1424.70	
87	2.03	2192.00	26.92	8141.45	9.05	8542.58	1427.12	Digest tube No. 20
88	2.06	1151.00	29.47	3905.82	8.32	4098.61	1429.54	
89	2.27	256.50	31.09	824.90	6.76	865.69	1431.96	Digest tube No. 2
90	2.09	774.00	31.02	2494.84	7.97	2618.41	1434.38	
91	2.05	1324.50	31.09	4259.57	8.41	4470.90	1436.80	
92	2.04	1760.00	27.92	5901.63	8.73	6194.94	1439.22	
93	2.11	620.00	29.12	2129.46	7.71	2235.48	1441.64	
94	2.12	557.50	30.67	1817.71	7.55	1908.36	1444.06	
95	2.00	16210.00	33.14	48706.80	10.95	51149.90	1446.48	Standard A 2nd use
96	2.00	16601.00	[illegible]	49152.00	[illegible]	51611.70	1448.90	
97	2.06	1192.50	32.30	3692.36	8.26	3877.45	1451.32	
98	2.03	1960.00	31.45	6232.49	8.79	6545.44	1453.74	
99	[illegible]	837.00	32.51	2574.71	7.90	2704.22	1456.16	
100	2.05	1179.00	30.53	3861.98	8.11	4056.46	1458.58	

DPM - 20CT (B)

ELAPSED TIME T= 1161 MINUTES.

Do you want to return to re-enter a new elapsed time ? NO
Do you want to close down at this stage ? YES
Program Terminated Correctly

Ready

Table 5 (continued)

BATCH 2

First and last set number in batch ? 417,445
With (1) or without (2) roots ? 2 ROOT DIGESTS
Length of counting period ? 2

DIGEST TUBE No.									
1	417	2.05	1327.50	35.76	3712.23	8.28	3952.02	1857.36
2	418	2.12	529.00	34.21	1692.73	7.50	1802.22	1859.78
3	419	2.06	1118.50	34.28	3263.24	8.15	3474.60	1862.20
4	420	2.01	6833.50	35.27	19377.40	9.93	20634.10	1864.62
5	421	2.02	3891.50	35.12	11079.30	9.38	11798.80	1867.04
6	422	2.00	18590.50	34.98	53142.10	10.94	56597.90	1869.46
7	423	2.04	1767.00	36.26	4873.80	8.55	5191.17	1871.88
8	424	2.16	422.50	35.90	1176.83	7.13	1253.56	1874.30
9	425	2.04	1575.00	34.28	4595.09	8.50	4895.11	1876.72
10	426	2.02	2936.50	34.28	8567.29	9.12	9127.39	1879.14
11	427	2.01	6816.00	34.21	19926.90	9.96	21231.40	1881.56
12	428	2.06	1070.00	35.62	3004.03	8.07	3200.95	1883.98
13	429	2.02	3465.50	34.42	10069.10	9.28	10730.00	1886.40
14	430	2.00	30114.00	34.06	88405.10	11.45	94215.60	1888.82
15	431	2.01	10917.50	34.21	31917.80	10.43	34018.40	1891.24
16	432	2.02	3156.50	35.34	8932.80	9.16	9521.46	1893.66
17	433	2.00	24853.00	34.84	71332.10	11.24	76039.10	1896.08
18	434	2.00	18024.00	33.57	53692.70	10.96	57240.30	1898.50
19	435	2.05	1247.50	35.55	3509.33	8.23	3741.51	1900.92
20	436	2.00	17047.00	34.98	48729.90	10.86	51958.10	1903.34
21	437	2.06	1059.00	34.13	3102.45	8.10	3308.25	1905.76
22	438	2.04	1864.50	34.42	5417.36	8.66	5777.20	1908.18
23	439	2.01	8307.00	34.06	24386.70	10.17	26008.60	1910.60
24	440	2.09	755.50	34.35	2199.65	7.76	2346.13	1913.02
25	441	2.00	46873.00	33.22	141118.00	11.92	150528.00	1915.44
26	442	2.01	6657.00	35.12	18952.80	9.91	20218.30	1917.86
27	443	2.03	2720.00	34.21	7952.04	9.05	8483.69	1920.28
28	444	2.02	3594.00	33.43	10751.60	9.35	11471.40	1922.70
29	445	2.00	26002.50	34.06	76335.00	11.31	81451.80	1925.12

ELAPSED TIME T= 1927.54 MINUTES.

Do you want to return to re-enter a new elapsed time ? NO
Do you want to close down at this stage ? YES
Program Terminated Correctly

5 Data Input for Quench Correction Calculation (Table 6)

Corrected DPM's for with, without root and digested samples are combined with root weight and root weight squared values. Data in columns Y,X1,X2 and X3 are fed into a multiple regression programme to derive the quench correction equation:

$$Y=X1(\text{DPM in root})+X2(\text{root wt})+X3(\text{root wt}^2)+K$$

In this example the root weight squared function does not contribute significantly to the regression, hence the quench correction equation is:

$$y=1.3(\text{DPM in root})+170.8(\text{root wt})-9065$$

This equation is substituted into a subroutine of the CALPUP.BAS programme.

Table 6

DATA EXTRACTION FROM DPM1 TO CALCULATE QUENCH CORRECTION

DIGEST TUBE NO.	DPM IN DIGEST (Y)	DPM + ROOT (A)	DPM - ROOT (B)	DPM IN ROOT A - B (X_1)	ROOT WT. (X_2)	ROOT WT^2 (X_3)
1	3952	5097	2773	2324	111	12321
2	1802	2200	866	1334	69	4761
3	3475	3874	1605	2269	78	6084
4	20634	21591	6602	14989	92	8464
5	11799	8174	2315	5859	104	10816
6	56598	45949	9603	36346	66	4356
7	5191	4497	197	4300	25	625
8	1254	1292	449	843	26	676
9	4895	6757	3343	3414	84	7056
10	9127	8658	1064	7594	61	3721
11	21231	24049	13156	10893	89	7921
12	3201	6584	4254	2330	39	1521
13	10730	9332	771	8561	55	3025
14	94216	90548	47346	43202	208	43264
15	34018	25643	1638	24005	114	12996
16	9521	15888	2067	13821	58	3364
17	76039	18163	17330	833	142	20164
18	57240	85257	56422	28835	136	18496
19	3741	8558	6168	2390	71	5041
20	51958	41079	8542	32537	173	29929
21	3308	4235	2212	2023	102	10404
22	5777	7144	1940	5204	116	13456
23	26009	25872	6366	19506	94	8836
24	2346	2818	994	1824	63	3969
25	150528	89553	5997	83556	237	56169
26	20218	18556	4525	14031	63	3969
27	8484	5120	499	4621	43	1849
28	11471	9638	1204	8434	69	4761
29	81452	71448	27150	44298	304	92416

```
OLD
Old file name--MULTRG.BAS

Ready

RUN
MULTRG  02:58 PM          14-Dec-84
STEPWISE OR NOT ?,ENTER S OR N? N
DEPENDANT VARIABLE? 1
ENTER INDEPENDANT VARIABLES TERMINATED -999
? 2
? 3
? 4
? -999
ENTER NUMBER OF TOTAL SETS AND VARIABLES? 29,4
INPUT NAME OF DATA FILE? DIG.NIF
DO YOU WANT TO TEST EACH VARIABLE FOR SIGNIFICANT CONTRIBUTION Y(ES) OR N(O)? N


MEAN AND VARIANCE OF DEPENDANT VARIABLE 27248.8  .126811E 10


MEANS AND VARIANCE OF INDEPENDANT VARIABLES
 2        14833.7   .345558E 9
 3        99.7242   4000.99
 4        13807.9   .388255E 9


MULTIPLE CORRELATION COEFFICIENT R 92.9081


COEFFICIENT OF DETERMINATION (R SQUARED) 86.3191 %

SUM SQUARES DUE TO REGRESSION = .306493E 11
 RESIDUAL MEAN SQUARE         = .194307E 9
 TOTAL SUM SQUARES            = .35507E 11
 RESIDUAL SUM SQUARES         = .485769E 10
 S.DEV FROM REGRESSION        = 13939.4

F RATIO IS  52.5787 WITH  3 AND  25 DEG. OF FREEDOM


 COEFF.        STANDARD ERROR        T             D.F.
 1.32261         .213394          6.19796          25
 300.306         155.231          1.93458          25
-.44875          .492654          .910882          25   Not significant

CONSTANT =-16121.7


CONFIDENCE LIMITS?,Y(ES) OR N(O)? N


ANOTHER RUN ?,Y(ES) OR N(O)? N
A.D.HORRILL       14-Dec-84

Ready
```

```
RUN MULTRG
STEPWISE OR NOT ?,ENTER S OR N? N
DEPENDANT VARIABLE? 1
ENTER INDEPENDANT VARIABLES TERMINATED -999
? 2
? 3
? -999
ENTER NUMBER OF TOTAL SETS AND VARIABLES? 29,4
INPUT NAME OF DATA FILE? DIG.NIP
DO YOU WANT TO TEST EACH VARIABLE FOR SIGNIFICANT CONTRIBUTION Y(ES) OR N(O)? N


MEAN AND VARIANCE OF DEPENDANT VARIABLE 27248.8  .126811E 10


MEANS AND VARIANCE OF INDEPENDANT VARIABLES
 2         14833.7   .345558E 9
 3         99.7242   4000.99


MULTIPLE CORRELATION COEFFICIENT R 92.6635


COEFFICIENT OF DETERMINATION (R SQUARED) 85.8651 %

SUM SQUARES DUE TO REGRESSION = .304881E 11
 RESIDUAL MEAN SQUARE         = .193033E 9
 TOTAL SUM SQUARES            = .35507E 11
 RESIDUAL SUM SQUARES         = .501886E 10
 S.DEV FROM REGRESSION        = 13893.6

F RATIO IS  78.9712 WITH  2 AND  26 DEG. OF FREEDOM


 COEFF.        STANDARD ERROR         T              D.F.
 1.29993          .21124           6.15379            26
 170.791         62.0803           2.75113            26

CONSTANT =-9065.87


CONFIDENCE LIMITS?,Y(ES) OR N(O)? N


ANOTHER RUN ?,Y(ES) OR N(O)? N
A.D.HORRILL        14-Dec-84

Ready
```

6 Teletype Input Instructions II (Table 7)

These input instructions are derived from the line numbers of the DPM1 file, the solution usage on the record sheets and the number of the appropriate root weights in the file ROOTS.VIR.

These instructions allow the second part of CALPUP.BAS to be run to correct the DPM1 file to produce DPM2.

Table 7

TELETYPE INPUTS FOR SECOND PART OF CALPUP.BAS.

FIRST USE OF SOLUTION?	YES
WHICH STANDARD (A or B)?	A
NO. OF STANDARDS COUNTED?	4
IDENTIFY STD. SET NOS.?	1, 2, 51, 52
NO. OF SAMPLES?	20
FIRST SET NO. FOR + ROOTS?	3
- ROOTS?	53
ROOT WT.?	1
VOL. USED FOR STD. (0.5 or 1ml)?	0.5

FIRST USE OF SOLUTION?	YES
WHICH STANDARD (A or B)?	B
NO. OF STANDARDS COUNTED?	4
IDENTIFY STD. SET NOS.?	23, 24, 73, 74
NO. OF SAMPLES?	20
FIRST SET NO. FOR + ROOTS?	25
- ROOTS?	75
ROOT WT.?	21
VOL. USED FOR STD. (0.5 or 1ml)?	0.5

FIRST USE OF SOLUTION?	NO
WHICH STANDARD (A or B)?	A
NO. OF STANDARDS COUNTED?	4
IDENTIFY STD. SET NOS.?	45, 46, 95, 96
NO. OF SAMPLES?	4
FIRST SET NO. FOR + ROOTS?	47
- ROOTS?	97
ROOT WT.?	41
VOL. USED FOR STD. (0.5 or 1ml)?	0.5

FIRST USE OF SOLUTION?	
WHICH STANDARD (A or B)?	
NO. OF STANDARDS COUNTED?	
IDENTIFY STD. SET NOS.?	
NO. OF SAMPLES?	
FIRST SET NO. FOR + ROOTS?	
- ROOTS?	
ROOT WT.?	
VOL. USED FOR STD. (0.5 or 1ml)?	

FIRST USE OF SOLUTION?	
WHICH STANDARD (A or B)?	
NO. OF STANDARDS COUNTED?	
IDENTIFY STD. SET NOS.?	
NO. OF SAMPLES?	
FIRST SET NO. FOR + ROOTS?	
- ROOTS?	
ROOT WT.?	
VOL. USED FOR STD. (0.5 or 1ml)?	

7 DPM2 (Table 8)

The second part of CALPUP.BAS corrects the DPM1 file for sample quenching and converts the ^{32}P uptake into actual measures of P-uptake (pg P mg^{-1} fresh weight of root). P-uptake is listed in the penultimate column and root weight in the final column of the computer printout.

This data may be sorted and extracted according to the sample codes using SORT.BAS and EXTRACT.BAS to arrange the data into a form suitable for analysis of variance.

Table 8

```
RUN
CALFUP  06:07 PM        17 Apr-84

ENTER THE EXTENSION FOR THE LSCDAT AND DPM FILES        ? VIR
Will you need root weights? YES
Name of file holding root weights            ? ROOTWT.VIR
Have you allowed for Background, decay and C.E.         ? YES

**  ROUTINE TO CALCULATE P UPTAKE Mg. ROOT-1  **

---    -- - -- ------- ----- --------------------

Enter the coefficients for the root quench equation
X1 : for DPM correction? 1.300
X2 : for Root Wt.     ? 170.0
X3 : for Root Wt. Sq.? 0
X4 : Constant? -9065


Is this a first usage of the assay solution             ? YES
Which standard are you dealing with  -  A or B          ? A
Number of standard counts available for batch           ? 4
Identify standard set numbers - as asked

? 1
? 2
? 51
? 52
Activity of first usage solution A = 56025.1

Number of samples in batch to be processed              ? 20
First  set  number of WITH    ROOT samples              ? 3
First  set  number of WITHOUT ROOT samples              ? 53
First  set  number of ROOT    WEIGHT value              ? 1
Is the standard 1ml or 0.5ml                  ? 0.5
```

1. Sample number
2. DPM in vial + root
3. DPM in root i.e.((bottle + root) - (bottle - root))
4. DPM in root corrected for P removal from bioassay solution in previous uses of solution
5. DPM in root corrected for quench effects
6. DPM in root (DPM mg root^{-1})
7. P uptake (pg P mg root^{-1})
8. Root weight (mg)

1	2	3	4	5	6	7	8
1	38077.2	35619.6	35619.6	62468.5	484.252	469.87	129
2	33495.9	31704.7	31704.7	52926.1	490.057	677.899	108
3	37916.8	6583.66	6583.66	58331.6	550.299	761.232	106
4	50385.5	13856.4	13856.4	79152.5	595.131	823.25	133
5	4235.18	2023.52	2023.52	13862.3	135.905	187.999	102
6	6087.36	4036.73	4036.73	17288.5	160.079	221.439	108
7	50073.5	43741.9	43741.9	74305.9	694.447	960.634	107
8	51673	45477.8	45477.8	73311.1	823.72	1139.46	89
9	89553	83556.3	83556.3	147833	623.77	862.866	237
10	35643.5	32912.3	32912.3	57084.5	492.108	680.736	116
11	4858.56	1914.03	1914.03	15185.1	144.62	200.054	105
12	9332.23	8561.14	8561.14	12460.9	226.562	313.405	55
13	17543.7	15755.8	15755.8	35091.9	280.735	388.342	125
14	46201.6	42171.7	42171.7	69272.7	647.409	895.566	107
15	65358.1	40006.4	40006.4	100154	705.311	975.662	142
16	3874.35	2268.92	2268.92	9294.07	119.155	164.828	78
17	13599	6338.69	6338.69	15445.7	386.143	534.155	40
18	15577.9	11894.1	11894.1	17505.9	473.133	654.489	37
19	4007.75	3170.21	3170.21	7076.28	110.567	152.948	64
20	4745.14	3885.56	3885.56	11450.9	136.32	188.573	84

Table 8 (continued)

```
Another batch on which to calculate root activity? YES

Is this a first usage of the assay solution                ? YES
Which standard are you dealing with  -  A or B             ? B
Number of standard counts available for batch              ? 4
Identify standard set numbers - as asked

? 23
? 24
? 73
? 74
Activity of first usage solution B - 44503.4

Number of samples in batch to be processed                 ? 20
First  set  number of WITH    ROOT samples                 ? 25
First  set  number of WITHOUT ROOT samples                 ? 75
First  set  number of ROOT    WEIGHT value                 ? 21
Is the standard 1ml or 0.5ml                ? 0.5
```

21	37055.6	26010.5	26010.5	49867.7	791.551	1378.44	63
22	40323.2	29921.1	29921.1	60435.2	604.352	1052.44	100
23	7826.2	6002.14	6002.14	12894.3	186.873	325.428	69
24	5071.69	4195.91	4195.91	10338.2	137.843	240.044	75
25	19008.5	12316	12316	28626.9	376.67	655.947	76
26	11750.6	9042.97	9042.97	26023.6	224.341	390.676	116
27	90547.6	43201.6	43201.6	144173	693.141	1207.06	208
28	85256.6	28834.9	28834.9	124997	919.099	1600.55	136
29	32021.8	21471.8	21471.8	43665.4	671.775	1169.86	65
30	15027.9	11835.7	11835.7	23110.5	312.304	543.859	74
31	13392	8993.67	8993.67	22691.8	270.14	470.433	84
32	18366.3	12589.2	12589.2	28672	353.975	616.425	81
33	41079.5	32536.9	32536.9	73886.7	427.091	743.752	173
34	21797.2	17698.6	17698.6	41133.7	321.357	559.624	128
35	2200.4	1334.72	1334.72	5580.72	80.88	140.848	69
36	5993.17	3374.76	3374.76	10169.7	151.787	264.328	67
37	45189.9	40719	40719	73593.8	525.67	915.423	140
38	37663.4	31468.5	31468.5	59001	511.804	891.275	117
39	10143.7	7908.26	7908.26	8050.26	350.011	609.523	73
40	18434.2	16525.3	16525.3	29587.6	344.042	599.128	86

```
Another batch on which to calculate root activity? YES

Is this a first usage of the assay solution                ? NO
Have first usage DPMs been entered          ? YES
Which standard are you dealing with      A or B            ? A
Number of standard counts available for batch              ? 4
Identify standard set numbers - as asked

? 45
? 46
? 95
? 95

Number of samples in batch to be processed                 ? 4
First  set  number of WITH    ROOT samples                 ? 47
First  set  number of WITHOUT ROOT samples                 ? 97
First  set  number of ROOT    WEIGHT value                 ? 41
Is the standard 1ml or 0.5ml                ? 0.5
```

41	35750	31872.5	34591.3	49536.8	697.701	965.135	71
42	30895.6	24356.2	26427.3	45617.3	536.674	742.386	85
43	9364.15	6559.94	7220.04	10111.2	246.615	341.144	41
44	14499.7	10443.2	11334.1	18032	355.415	491.649	53

```
Another batch on which to calculate root activity? NO
Program Terminated Correctly

Ready
```

Appendix II

Listing of the computer programmes used to calculate the P-uptake by root samples.

1 Programme "PURGE.BAS"

Programme to remove control and other odd unprinted characters generated on the paper tape by the scintillation counter.

```
1!      PROGRAM "PURGE.BAS"
        A program to get rid of "wierd" characters from L.S.C.tape.

2       Extend
\       PRINT
\       Input'Name of file to be "purged"',F$
\       Input'Clean file to be called     ',N$
\       OPEN F$ For input as file #1%
\       OPEN N$ For output as file #2%
\       On error go to 100
\       TABLE$=STRING$(32%,0%)
\       TABLE$=TABLE$+CHR$(J%) for J%=32% to 126%
\       TABLE$=TABLE$+STRING$(128%,0%)
10      INPUT Line #1,X$
\       D$=XLATE(CVT$$(X$,4%),TABLE$)
\       IF D$<>'' THEN PRINT #2,D$
20      GOTO 10
100     IF ERR=11 THEN RESUME 32000
200     KILL N$
\       PRINT RIGHT(SYS(CHR$(6%)+CHR$(9%)+CHR$(ERR)),3%)
\       RESUME 32100
32000   PRINT'PROGRAM ENDS OK'
32100   CLOSE #1,#2
32767   END
```

2 Programme "TIDYP.BAS"

Programme to set up a virtual file of purged data files produced by the scintillation counter.

```
1!      PROGRAM 'TIDYP'

        A program toset up a virtual file from the purged raw data
        files produced by the L.S. counter.

2       Extend
\       Dim #2%,L(2500,2)
\       Print
\       Input 'Purged raw - data file name',P$
\       Input 'LSCDAT matrix file to be called',L$
\       OPEN P$ for input as file#1%
\       OPEN L$ for output as file#2%
\       ON ERROR GO TO 100
\       R%=0%
10      INPUT #1, N(J%) For J%=0% to 8%
\       R%=R%+1%
\       L(R%,0)=N(1)
\       L(R%,1)=N(2)
\       L(R%,2)=N(8)
\       GO TO 10
100     CLOSE #1
\       IF ERR=11 AND J%=0% THEN PRINT'PROGRAM ENDS OK'\ GO TO 300
200     PRINT
\       PRINT'ERROR';err;'has occured'
\       PRINT'File';L$;'must NOT be used'
300     PRINT
\       PRINT'Parts of last lines entered into';L$;'reads'
\       PRINT'(Record number';R%;')'
\       PRINT L(R%,J%) For J%=0% to 2%
\       CLOSE #2
32767   END
```

3 Programme "CALPUP.BAS"

Programme to calculate DPMs corrected for background, decay of the radiotracer and quenching. Converts DPMs into actual P-uptake.

Programme "BATPUP.BAS"

is the equivalent to CALPUP designed to run the programme as a batch job to printout on the fast printer.

```
1!      PROGRAM "CALPUP"

        A program to calculate P uptake by rootsystems
        in the P-defficiency bioassay.

2 EXTEND
\       DIM #1%,L(2500,2)
\       DIM #2%,Y(2500,6)
\       DIM #3%,I(2500,6)
\        DIM #4%,V(2500)
\        PRINT
\        INPUT'ENTER THE EXTENSION FOR THE LSCDAT AND DPM FILES',EXT$
\        EXT$=LEFT(CVT$$(EXT$,38%),3%)
\        OPEN 'LSCDAT.'+EXT$ FOR INPUT AS FILE 1%
\        OPEN 'DPM1.'+EXT$   AS FILE 2%
\        OPEN 'DPM2.'+EXT$   AS FILE 3%
\        INPUT'Will you need root weights';ROOT$
\        IF LEFT(CVT$$(ROOT$,34%),1%)<>'Y' THEN 10
5        INPUT'Name of file holding root weights',root$
\        OPEN ROOT$ FOR INPUT AS FILE 4%
\!

10       input 'Have you allowed for Background, decay and C.E.',reply$
\        if left(cvt$$(reply$,34%),1%)='Y' then 50
20       print
\        print'**  ROUTINE TO CORRECT FOR BACKGROUND, DECAY AND C.E.  **'
\        print string$(57%,45%)
25       print
\        input'Minutes elapsed before counting started',,,t
\        input'Backgrounds for with and without roots',,,b1,b2
\        input'Total number of batches in matrix to be processed',n%
\                for m1%=1% to n%
\                print chr$(12%)
\                print'BATCH';m1%
\                print
\                input'First and last set number in batch',,n1%,n2%
\                input'With (1) or without (2) roots',,w
\                input'Length of counting period',,ct
\                print
\                if w=1 then x=b1 else x=b2

30                       for p%=n1% to n2%
\                        y(p%,0)=l(p%,0)*(l(p%,1)/(l(p%,1)-(l(p%,0)*x)))
\                        y(p%,1)=l(p%,1)/y(p%,0)
\                  y(p%,2)=(70.69*l(p%,2))-9.34
\                        y(p%,3)=y(p%,1)*(100/y(p%,2))
\                        y(p%,4)=log(y(p%,3))+(.693*(t/20563))
\                        y(p%,5)=exp(y(p%,4))
\                        y(p%,6)=t
\                        print USING'####',p%;
\                        print USING' ######.######',y(p%,x9%); for x9%=0% to 6%
\                        print
\                        t=t+ct+.42
\                        next p%
\                next m1%
\        print
\        print'ELAPSED TIME T=';T;'MINUTES.'
\        print chr$(12%)
\        input'Do you want to return to re-enter a new elapsed time',reply$
\        if left(cvt$$(reply$,34%),1%)='Y' then 25
40       input'Do you want to close down at this stage',reply$
\        if left(cvt$$(reply$,34%),1%)='Y' then 200
50       print
\        print'**  ROUTINE TO CALCULATE P UPTAKE Mg. ROOT-1  **'
\        print
\        print string$(48%,45%)
\        print
\        J%=1%
55       PRINT"Enter the coefficients for the root quench equation"
\        INPUT"X1 : for DPM correction";X1\INPUT"X2 : for Root Wt.     ";X2
\        INPUT"X3 : for Root Wt. Sq.";X3\INPUT"X4 : Constant";X4
60       print for l%=1% to 4%
\        input'Is this a first usage of the assay solution',reply$
\        if left(cvt$$(reply$,34%),1%)<>'Y' then 90
70       input'Which standard are you dealing with  -  A or B',d$
\        d$=cvt$$(d$,34%)
\        if d$='A' then d%=1%
else     if d$='B' then d%=4% else 70
80       s(d%)=0.0
\        goto 120
```

```
90      input'Have first usage DPMs been entered',reply$
\       if left(cvt$$(reply$,34%),1)='Y' then 110
100     input'Enter first usage DPMs  -  A then B',s(1),s(4)
110     input'Which standard are you dealing with  -  A or B',d$
\       d$=cvt$$(d$,34%)
\       if d$='A' then d%=1%
else    if d$='B' then d%=4% else 110
120     s=0.0
\       input'Number of standard counts available for batch',s2
\       print'Identify standard set numbers - as asked'
\       print
\               for l%=1% to s2
\               input s3%
\               s=s+y(s3%,5)
\               next l%
\       s=s/s2
\       if s(d%)>10 then 140
130     s(d%)=s
\       print'Activity of first usage solution ';d$;' =';s(d%)
140     print
\       input'Number of samples in batch to be processed',n3%
\       input'First  set  number of WITH    ROOT samples',h%
\       input'First  set  number of WITHOUT ROOT samples',l%
\       input'First  set  number of ROOT    WEIGHT value',j%
150     input'Is the standard 1ml or 0.5ml',e
\       if e<>1.0 AND e<>.5 then 150
160     print chr$(12%)
\               for k%=1% to n3%
\               i(j%,0)=y(h%,5)
\               i(j%,1)=i(j%,0)-y(l%,5)
\               i(j%,2)=i(j%,1)*(s(d%)/s)
\             v=v(j%)
\               K=V*V
167             I(J%,3%)=(X1*I(J%,0%))+(X2*V)+(X3*K)+X4
168             I(J%,4)=I(J%,3)/V
\               i(j%,5)=i(j%,4)*((155000*e)/s(d%))
\               i(j%,6)=v
\               print j%;
\               print tab(14*(n%+1%));i(j%,n%); for n%=0% to 5%
\               print tab(98);
\               if i(j%,5%)<=4000 then print i(j%,6) else print'*****',i(j%,6)

170             l%=l%+1%
\               h%=h%+1%
\               j%=j%+1%
\               next k%
\       print for k%=1% to 3%
\       input'Another batch on which to calculate root activity';reply$
\       if left(cvt$$(reply$,34),1)='Y' then 60
200     CLOSE 1%,2%,3%,4%
\       print'Program Terminated Correctly'
\ END
```

```
1!      PROGRAM 'BATPUP'

        This program will create and queue a batch job for
                the 'CALPUP' program.

        A.NELSON.        AUGUST 1983.

2 EXTEND
\       DIM M%(30%)
\       N$='NO'
\       Y$='YES'
10      PRINT
\       PRINT'"BATPUP" - Creates and queues batch job for CALPUP'
20      PRINT
\       PRINT'Enter the extension (max of 3 chrs) of the'
\       INPUT'LSCDAT, DPM1 and DPM2 files',EXT$
\       EXT$=LEFT(CVT$$(EXT$,38%),3%)
\       LSC$='LSCDAT.'+EXT$
\       DP1$='DPM1.'+EXT$
\       DP2$='DPM2.'+EXT$
\       IF NOT FNCHECK%(LSC$) OR NOT FNCHECK%(DP1$) OR NOT FNCHECK%(DP2$) THEN 20
40      ON ERROR GOTO 50
\       OPEN'CALPUP.BAS' FOR INPUT AS FILE #1%
\       CLOSE #1%
\       PRINT'Prog CALPUP.BAS found'
\       GOTO 60
50      PRINT CHR$(7%)
\       PRINT'The program CALPUP.BAS and data-file ';LSC$;' must be present'
\       PRINT'on the system disk for the job to run successfully.'
\       RESUME 32767
60      OPEN LSC$ FOR INPUT AS FILE #1%
\       CLOSE #1%
\       PRINT'File ';LSC$;' found'
\!

100     ON ERROR GOTO 110
\       PRINT
\       INPUT'JOB NAME',JOB$
\       JOB$=FNSCAN$(CVT$$(JOB$,38%),'.CTL')
\       OPEN JOB$ FOR INPUT AS FILE #1%
\       CLOSE #1%
\       PRINT CHR$(7%)
\       PRINT'File ';JOB$;' already exists - overwrite it';
\       INPUT X$
\       IF LEFT(CVT$$(X$,38%),1%)='Y' THEN 120 ELSE 100
110     IF ERR=5 THEN RESUME 120 ELSE GOSUB 1000 \ RESUME 100
```

```
120     ON ERROR GOTO
\       OPEN JOB$ FOR OUTPUT AS FILE #1%
\       PRINT #1%,'$JOB/CCL/NOLIMIT'
\       PRINT #1%,'$SET GAG;WIDTH 158'
\!

200     QUENCH%=0%
\       QUENCH$='165            I(J%,3%)=(A*I(J%,2%))+(B*V)+(C*K)+(D)'
\       PRINT
\       PRINT'Current QUENCH correction equation is...'
\       PRINT
\       PRINT QUENCH$
\       PRINT
\       INPUT'Will you need to enter one for this job',X$
\       IF LEFT(CVT$$(X$,38%),1%)<>'Y' THEN 250
210     QUENCH%=-1%
\       PRINT
\       PRINT'Enter the values for A, B, C and D as asked'
\               FOR J%=65% TO 68%
\               J$=CHR$(J%)
\               PRINT J$;
\               INPUT X
\               X$=NUM1$(X)
\               P%=INSTR(1%,QUENCH$,J$)
\               QUENCH$=LEFT(QUENCH$,P%-1%)+X$+RIGHT(QUENCH$,P%+1%)
\               NEXT J%
\       PRINT
\       PRINT'Revised QUENCH correction equation is...'
\       PRINT
\       PRINT QUENCH$
\       PRINT
\       INPUT'Is this correct',X$
\       IF LEFT(CVT$$(X$,38%),1%)<>'Y' THEN 200
220     PRINT
\       INPUT'Name of file holding ROOT weights',ROOT$
\       ON ERROR GOTO 230
\       OPEN ROOT$ FOR INPUT AS FILE #2%
\       CLOSE #2%
\       GOTO 240
230     GOSUB 1000
\       GOTO 220
240     ON ERROR GOTO
\       PRINT #1%,'$OLD CALRUP'
\       PRINT #1%,QUENCH$
\       PRINT #1%,'$RUN
\       PRINT #1%,'$DATA'
\       PRINT #1%,EXT$
\       PRINT #1%,Y$
\       PRINT #1%,ROOT$
\       GOTO 300
\!
```

```
250     PRINT #1%,'$RUN CALPUP'
\       PRINT #1%,'$DATA'
\       PRINT #1%,EXT$
\       PRINT #1%,N$
\!

300     PRINT
\       INPUT'Have you allowed for background, decay and C.E.';X$
\       IF LEFT(CVT$$(X$,38%),1%)='Y' THEN PRINT #1%,Y$ \ GOTO 400
310     PRINT #1%,N$
320     INPUT'Minutes elapsed before counting started',T
\       INPUT'Backgrounds for WITH and WITHOUT roots ',B1,B2
\       INPUT'Total no of batches in matrix to be processed',N%
\       PRINT #1%,T
\       PRINT #1%,B1;',';B2
\       PRINT #1%,N%
\               FOR J%=1% TO N%
\               PRINT
\               PRINT'BATCH';J%
\               INPUT'First and last set number in this batch',N1%,N2%
\               INPUT'WITH(1) or WITHOUT(2) roots',W
\               INPUT'Length of counting period',T
\               PRINT #1%,N1%;',';N2%
\               PRINT #1%,W
\               PRINT #1%,T
\               NEXT J%
\       PRINT
\       INPUT'Return to enter a new elapsed time',X$
\       IF LEFT(CVT$$(X$,38%),1%)='Y' THEN PRINT #1%,Y$ \ GOTO 320
330     PRINT #1%,N$
\       PRINT
\       INPUT'Do you want to close down at this stage',X$
\       IF LEFT(CVT$$(X$,38%),1%)<>'Y' THEN PRINT #1%,N$ \ GOTO 410
340     PRINT #1%,Y$
350     PRINT #1%,'$EOD'
\       PRINT #1%,'$EOJ'
\       CLOSE #1%
\       GOSUB 2000
\       GOTO 32767
\!

400     PRINT
        INPUT'Is this a first u[illegible] of the [illegible] solution',X$
\       IF LEFT(CVT$$(X$,38%),1%)<>'Y' THEN PRINT #1%,N$ \ GOTO 430
410     PRINT #1%,Y$
\       GOSUB 420
\       GOTO 500
\!
420     PRINT
\       INPUT'Which standard are you dealing with - A or B',X$
\       X$=LEFT(CVT$$(X$,38%),1%)
\       IF X$<>'A' AND X$<>'B' THEN 420 ELSE PRINT #1%,X$
430     RETURN
\!
```

```
430     INPUT'Have first usage DPMs been entered',X$
\       IF LEFT(CVT$$(X$,38%),1%)='Y' THEN PRINT #1%,Y$ \ GOSUB 420 \ GOTO 500
440     PRINT #1%,N$
\       INPUT'Enter first usage DPMs - A then B',A,B
\       PRINT #1%,A;',';B
\       GOSUB 420
\!

500     PRINT
\       INPUT'Number of std counts available for batch',N%
\       PRINT #1%,N%
\       PRINT
\       PRINT'Identify std set numbers as asked'
\       PRINT
\               FOR J%=1% TO N%
\               INPUT X%
\               PRINT #1%,X%
\               NEXT J%
\       PRINT
\       INPUT'Number of samples in batch to be processed',X%
\       PRINT #1%,X%
\       INPUT'First set number of WITH    ROOT samples',X%
\       PRINT #1%,X%
\       INPUT'First set number of WITHOUT ROOT samples',X%
\       PRINT #1%,X%
\       INPUT'First set number of ROOT  WEIGHT samples',X%
\       PRINT #1%,X%
510     INPUT'Is the standard 0.5 or 1ml',X
\       IF X<>1.0 AND X<>0.5 THEN 510 ELSE PRINT #1%,X
\       PRINT
\       INPUT'Another batch on which to calculate root activity',X$
\       IF LEFT(CVT$$(X$,38%),1%)='Y' THEN PRINT #1%,Y$ \ GOTO 400
520     PRINT #1%,N$
\       GOTO 350
\!

1000    PRINT CHR$(7%)
\       PRINT RIGHT(SYS(CHR$(6%)+CHR$(9%)+CHR$(ERR)),3%)
\       RESUME 1099
1099 RETURN
\!

2000    PRINT
\       PRINT'QUEUING JOB'
\       ON ERROR GOTO 2010
\       F$=MID(SYS(CHR$(6%)+CHR$(-10%)+JOB$),7%,6%)
\       Q$=SYS(CHR$(6%)+CHR$(-28%)+STRING$(4%,0%)+F$+'BA'+STRING$(16%,0%))
\       PRINT'JOB  QUEUED'
\       GOTO 2099

2010    PRINT'QUEUING FAILED'
\       PRINT
\       PRINT'Type QUE BA:=';JOB$;' to queue the job yourself.'
\       RESUME 2099
2099 RETURN
\!

3000 DEF FNSCAN$(Z$,E$)
\       Z$=CVT$$(Z$,-1%)
\       CHANGE SYS(CHR$(6%)+CHR$(-10%)+Z$) TO M%
\       M%=M%(29%)+SWAP%(M%(30%))
\       IF M% AND 8% THEN FNSCAN$=Z$ ELSE FNSCAN$=Z$+E$
3099 FNEND
\!

4000 DEF FNCHECK%(Z$)
\       ON ERROR GOTO 4010
\       FNCHECK%=0%
\       OPEN Z$ FOR INPUT AS FILE #1%
\       CLOSE #1%
\       GOTO 4090
4010    IF ERR=5 AND (Z$=DP1$ OR Z$=DP2$) THEN RESUME 4090
4020    GOSUB 1000
\       PRINT'FILE IS ';Z$.
\       GOTO 4099
4090    FNCHECK%=-1%
4099 FNEND
\!

32767 END
```

Programme "SORT.BAS"

Using a file of pairs of numerical codes for the root samples in the same sequence as the roots were processed, this programme sorts the DPM2 data file into a sequence according to a numerically ascending order of the codes. The programme also extracts the P-uptake data (penultimate column of DPM2 file) to create a file on which statistical analyses may be performed.

```
2 EXTEND
5 DIM N%(11000%)
10 DIM #3% , V(2500,6)
20 INPUT 'NAME OF VIRTUAL FILE (INPUT)', V$
30 OPEN V$ FOR INPUT AS FILE #3
40 INPUT 'NAME OF FILE HOLDING PAIRS OF CODES', P$
45 OPEN P$ FOR INPUT AS FILE #1
46 INPUT 'NAME OUTPUT FILE',Z$
47 OPEN Z$ FOR OUTPUT AS FILE#2
50 INPUT 'HOW MANY PAIRS IN IT', P%
55 X$=''
56 X$=X$+'######.## ' FOR J%=1% TO 7%
57 MAX%=0% \ MIN%=11000%
60 FOR J%=1% TO P%
70 INPUT #1,N%
80 INPUT #1,N1%
85 IF N%>MAX% THEN MAX%=N%
86 IF N%<MIN% THEN MIN%=N%
90 N%(N%)=J%
100 NEXT J%
200 FOR J%=MIN% TO MAX%
210 IF N%(J%)=0% THEN 250
220 N2%=N%(J%)*2
221 N1%=N2%-1
225      PRINT #2,USING'#####  ',J%;
226  PRINT#2,USING X$,V(N1%,K%);FOR K%=0% TO 6%
230  PRINT#2
235      PRINT #2,USING'#####  ',J%;
236  PRINT#2,USING X$,V(N2%,K%);FOR K%=0% TO 6%
240 PRINT#2
250 NEXT J%
300 CLOSE#1,#2 ,#3
310 PRINT'DONE'
32767 END
```