Summary of parameters influencing anesthesia and recovery

Medium (500 ml)	Initial pH	Final pH	Δ pH	Δ Time (h)	Time to crawling (h)	Time to feeding (h)	Time to egg laying (h)
a) Experimental (Nembutal present at a final concentration of 0.8%)							
DCW	7.00	8.49	1.49	15.45	12	21	36
0.01 M Trizma	7.00	7.63	0.63	15.25	3	5	23
0.02 M Trizma	7.01	7.69	0.68	14.00	3	4	24
0.05 M Trizma	7.00	7.52	0.52	16.00	3	8	30
b) Controls (Nembutal absent)							
DCW	7.00	8.49	1.49	13.45			
0.01 M Trizma	7.05	8.69	1.64	18.25			
0.02 M Trizma	7.00	7.54	0.54	16.50			
0.05 M Trizma	7.00	7.40	0.40	14.00			

creases the percentage of undissociated pentobarbital would increase favoring membrane penetration. Aerated DCW, at an initial pH 7.0 stabilizes within 2 h at pH 8.4–8.5. This phenomenon is also observed without aeration over a period of 15 h. At pH 8.5 approximately 70% of the soporific would be in the less effective ionized form. In order to favor membrane diffusion by pentobarbital a Trizma (Sigma Chemical Co.) buffer system was employed. Three molarities of buffer were investigated and evaluated with respect to ΔpH, anesthetization time, recovery time to crawling, feeding and oviposition (table). Animals maintained in Trizma buffered DCW were found to continue normal activities for several days, showing signs of discomfort only in the 0.05 M solution. Using this system, ΔpH over the course of anesthetization could be kept to one third of that using DCW alone. Recovery time leading to crawling and the onset of feeding was reduced to $\frac{1}{3}$ and $\frac{1}{4}$ respectively. Little difference was seen between using Trizma buffer at 0.01 M, 0.02 M or 0.05 M so the least concentration was adopted. At the initial pH 7.0 approximately 92% of the pentobarbital is present in the unionized form, while at the final pH 7.6 about 76% remains unionized, favoring efficient anesthetic activity.

The reduction in recovery time, when using a Trizma buffered anesthesia medium is difficult to interpret. The limitation of ΔpH may reduce osmotic stress. Alternatively, prolonged exposure to ionized pentobarbital may be more toxic to the animal than is the unionized form. Although recovery time is enhanced we were unable to reduce the anesthetization time.

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Identification of rooting depth and measurements of plant root activity in situ and in toto under field conditions using a gamma probe technique

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Summary. A technique for measuring root activity of agricultural crops in totality in situ under field conditions has been developed for the first time. The method essentially consists of measuring the activity emanating from roots after plant injection of a gamma radiating isotope using a probe. This also helps continuous monitoring of root growth changes over time.

Breeding of crop varieties with deep and voluminous root system ensures efficient utilization of scarce agricultural inputs. Research on the identification of such varieties has been greatly handicapped by the lack of suitable techniques for studying the root distribution. In recent years, radio-isotope techniques have become available for measuring root activity. Extensive investigations on the root activity distribution have been carried out using ^{32}P[2-7] and ^{86}Rb[8-12] by an injection tech-

Table 1. Percent radioactivity measured in roots (transformed to $\sin^{-1}\sqrt{P}$ in pearl millet using 5 gamma isotope

Soil depth (cm)	Percent activity measured in roots using					SEM	CD at 0.05	CV (%)
	^{86}Rb	^{134}Cs	^{65}Zn	^{59}Fe	^{54}Mn			
0–20	33.89[a]	43.62[b]	37.04[ab]	40.50[ab]	42.21[b]	1.37	7.44	6.0
20–40	32.22[c]	26.96[ab]	29.39[bc]	30.02[c]	26.40[a]	0.53	2.87	3.2
40–60	27.85	24.41	27.31	24.25	23.19	1.20	–	8.2
60–80	25.54	22.56	24.77	23.32	21.84	1.38	–	10.1

Superscript letters indicate order of statistical significance.

nique. The activity measurements do not directly indicate the amount of roots, owing to difficulties in the quantitative injection of the radioactive isotope. However, when the results are calculated on the basis of relative distribution of counts within the sample volume, they provide an accurate picture of the active root profile in the soil[13]. The biological concentration of the activity in the few millimeters from the apex in the root tissue produces only a negligible error, as the sample layer thickness usually ranges from about 5 to 20 cm[14].

These methods rely on laboratory measurements made on soil samples drawn from several regions within the rhizosphere after injecting the plant with a radioactive isotope. The main difficulties encountered in using these methods have been the low count rate and sampling errors owing to the uneven distribution of the root system in the soil profile. The use of gamma emitters such as ^{86}Rb and a larger sample size have considerably improved the counting efficiency[8]. But sampling for non-uniform root proliferation in the soil still creates large variations between replicates. The ideal methods for quantifying the root distribution would therefore require the measurement of root activity in totality and in situ. This paper describes the development and use of gamma probe to meet these requirements and also to permit a continuous monitoring of the root growth.

Material and method. The equipment was, in principle, a portable battery operated single channel solid scintillator. It consisted of 3 components – a gamma probe for activity detection, an analyzer and a scaler. The probe has a sodium iodide crystal of diameter 6.99 cm and thickness 0.64 cm mounted on a photomultiplier. The crystal was enclosed on the top and sides with a 3 cm thick lead shield. The contents of the probe were held tightly together in a cylindrical aluminium casing. The crystal was exposed for activity detection through the lead shield and aluminium casing by an aperture, the 'eye'. The photomultiplier was then connected to the scaler through an analyzer with a high voltage unit and suitable amplifier-descriminator system.

The main purpose of using this probe was to obtain information on the root activity in totality within specific soil zones. Therefore the elimination of the interference from zones above and below necessitated collimation for the eye. The present system was designed for a horizontal collimation of 38 cm and vertical collimation of 13 cm for half activity measurement by the eye at a center-to-center distance of 30 cm.

Standardization for root activity measurements. Into the test plant, pearl millet var HB-4, carrier-free ^{86}Rb with a total activity of about 50 µCi was injected into the second internode of all the tillers with a 50-µl microsyringe, by simultaneously sucking the sap from the first internode using a hypodermic syringe. The observations on the root activity were made, 5 days after injection, in 4 vertical holes drilled at specified distances from the plant by lowering the probe to different depths. These 4 holes were drilled at the corners of a square with the plant at its center. The depth of the hole was such as to include most of the root system. The actual distance of the hole from the plant depended on the inter-row distance and the minimum distance possible from the plant, consistent with the root spread. In the present experiment, holes drilled at a distance of 30 cm from the plant and to a maximum depth of 90 cm were found to be suitable. The diameter of the hole was sufficient to facilitate free movement of the probe. It was noted that the counts obtained in these 4 holes did not differ significantly. Therefore, the procedure adopted for routine estimation was to use only 1 hole for each plant.

It was necessary to ensure that the radioactive count rates measured at different depths were not affected by the activity in the injected stem. This was done by measuring the count rates in different soil zones, first in the presence of the injected shoot, then by shielding the plant with a thick lead brick, and finally by completely excising the above ground parts. The count rates obtained from these 3 sets of readings, analyzed as randomized block design[15] were not statistically different. This non-interference of activity from the shoot was due to the collimation of the crystal. Therefore, it was possible to monitor root activity changes without destroying or shielding the plant. The measured count rate was corrected for attenuation by soil, contribution from adjacent layers, and radioactive decay, to arrive at actual counts.

Comparative merits of gamma emitters. 5 gamma emitters, namely ^{59}Fe, ^{65}Zn, ^{86}Rb, ^{54}Mn and ^{134}Cs were injected in doses of about 40 µCi as described earlier, into separate plants in the field. The activity was measured in situ using the gamma probe. The average percentage activity, after equilibriation for 5 days, was obtained using each of these isotopes for a period of 40 days, from the flowering to harvesting stages of the plant. The results are presented in table 1.

The differences between isotopes in the soil layers were marginal. ^{59}Fe and ^{65}Zn can be seen to represent fairly the average picture. This is also found to be true after detailed statistical processing, over time and soil layers[16]. Since both these isotopes have a high efficiency for detection, among these 2 isotopes ^{59}Fe may be more suitable owing to its comparatively shorter half-life and consequent lower contamination in the

Table 2. Comparison of the ^{59}Fe activity measured by the probe with other methods

Method	Percent (transformed to $\sin^{-1}\sqrt{P}$) activity in the soil depth cm			
	0–20	20–40	40–60	60–80
Root weight distribution in cores	63.75[b]	15.38[a]	13.29[a]	14.85[a]
Activity pattern in separated roots from cores	40.11[a]	32.65[b]	24.06[b]	20.78[b]
Moisture extraction pattern	34.32[a]	29.81[b]	25.33[b]	27.50[c]
Root activity as measured by the probe	39.19[a]	29.18[b]	28.09[b]	23.83[bc]
SEM	1.71	1.58	1.53	1.47
CD at 0.05	5.91	5.45	5.31	5.10
CV %	6.7	10.2	11.6	11.8

Superscript letters indicate order of statistical significance.

Table 3. Distribution of the root activity in the 4 layers (%) as monitored by using ^{59}Fe

Soil depth cm	Percent activity in roots				
	5th day	10th day	20th day	30th day	40th day
0–20	31.81	37.81	42.35	42.01	42.00
20–40	25.02	26.91	25.00	24.80	24.51
40–60	23.15	20.15	19.80	20.05	20.01
60–80	20.02	15.13	12.85	13.14	13.48

field. Percentage activity distribution, as measured by using ^{86}Rb, is also close to the average values but suffers from the drawback that its detection efficiency is much lower, owing to the fact that only 8% of gamma rays are emitted with energies above 1 MeV. Further, its short half-life makes long term monitoring for root activity difficult.

Identification of the root activity by probe using ^{59}Fe was compared with other field methods of study, namely; percent root weight distribution in the 4 layers as obtained by total separation[17] by washing from the soil cores of 19 cm diameter and 20 cm deep, their ^{59}Fe activity, and the moisture extraction pattern[18] in the 4 soil layers of depth 0–80 cm. The results are presented in table 2. The activity measured by the probe and in separated roots was similar to the root activity depicted by the moisture extraction pattern, but differed significantly from that of the root weight distribution. This could be due to the contribution of non-active and dead roots to total weight. However, there was a linear relation between the relative activity represented by ^{59}Fe and the root weight in the 20–80 cm soil which largely comprises the active roots and root hairs. These might not contribute much to the total root weight, but very much to absorption, owing to the large soil-root interface contact. Since the activity was measured after flowering, when root growth of cereals ceases[19], the ^{59}Fe distribution possibly represents the mobile[20] phase of iron in the plant.

The root activity monitored over a 40-day period from flowering, using ^{59}Fe for pearl millet in different soil layers, is presented in table 3. There is no significant change in the root growth from flowering to harvest 10 days after the injection. The principal objective, i.e. reducing the variability due to sampling has been achieved by the in situ measurement of root activity using the gamma probe. Changes in root growth can be continuously monitored without any disturbance of the root profile in the soil. The vertical extension of the root system into different layers can be thoroughly established. This method completely eliminates laboratory processing, which enhances its feasibility for scanning a variety of rooting habits effectively in a shorter time with less personnel.

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Announcements

USA

23rd Hanford life sciences symposium 'Interaction of biological systems with static and ELF electric and magnetic fields'

Richland, Washington, 2–4 October 1984

Symposium organizers: Larry E. Anderson, Bruce J. Kelman, Richard J. Weigel. Sponsored by US Department of Energy and Battelle Memorial Institute, Pacific Northwest Laboratories.

The symposium will provide a forum for discussions of all aspects of research on the interaction of static and extremely-low-frequency (ELF) electric and magnetic fields with biological systems. These systems include simple biophysical models, cell and organ preparations, whole animals, and man. Dosimetry, exposure system design, and the role of artifacts in ELF bioeffects research will be discussed. The symposium will include research which is directly related to contemporary problems associated with electric power transmission and use (ac and dc). It will also include research on the fundamental mechanisms of interaction between static of ELF fields and biological processes.

For further information write, before 1 August 1984 to the symposium secretary: Patricia M. Bresina, Biology and Chemistry Department, Batelle, Pacific Northwest Laboratories, P.O. Box 999, Richland WA 99352, USA.

Austria

6th international symposium on 'Prevention and detection of cancer'

Vienna, 26–29 November 1984

The program of the symposium includes overview lectures, panels, poster sessions, scientific exhibits and special workshops designed for critical appraisal of current data. For information, please write to 'Prevention and detection of Cancer, AMEX P.O.B. 790459, DALLAS, Texas 75379/USA.