A REVIEW OF TECHNIQUES FOR STUDYING ROOT SYSTEMS

by
J. S. Yorke

FOREST RESEARCH LABORATORY
ST. JOHN'S, NEWFOUNDLAND
INFORMATION REPORT N-X-20

CANADA
DEPARTMENT OF FORESTRY
AND RURAL DEVELOPMENT

FORESTRY BRANCH
APRIL, 1968

A REVIEW OF TECHNIQUES FOR STUDYING ROOT SYSTEMS

by

J.S. Yorke

Forest Research Laboratory

St. John's, Newfoundland

Information Report N-X-20

Forestry Branch

April, 1968

CONTENTS

A REVIEW OF TECHNIQUES FOR STUDYING ROOT SYSTEMS

by

J.S. Yorke

INTRODUCTION

About 375 B.C. Theophrastus discussed differences between the morphology of root systems of several species (Hort 1916). Nowadays, of course, it is widely known that species may be broadly classified into those with tap-root systems and those with fibrous root systems. Nevertheless additional knowledge of roots is fragmentary and depends largely on the method by which it was obtained. Hence methods of studying the root systems deserve some scrutiny. Considering the variety of techniques available, recent reviews are few and are not sufficiently comprehensive for many purposes. The present review was prepared to fill this omission in the literature and has been divided into 'general techniques' and 'techniques of measuring specific parameters of root growth'. Owing to the vast amount of pertinent literature, the author has been forced to select references which, in his opinion, are the most relevant. In doing so, it is hoped that there are no important omissions.

GENERAL TECHNIQUES

The most comprehensive surveys to date are those of Pavlychenko (1937), Rogers (1939), Troughton (1957) and McKell (1962). Recently Schuurman and Goedewaagen (1965) have reviewed methods used at the Institute for Soil Fertility, Groningen, Holland, and have presented a bibliography of 158 references. Methods of studying excised roots and intact roots in sterile

conditions have been reviewed by Butcher and Street (1964) and Hewitt (1966) respectively and are outside the scope of the present review.

Unfortunately a classification of techniques tends to be an unsystematic catalogue. This is unavoidable to some extent because some methods which are different in principle have certain similar features. Hence in this review general techniques for studying root systems are grouped under the following arbitrary headings.

1. Destructive measurements of root systems.
2. The use of tracers to measure root systems.
3. The observation of growing roots.
4. Methods of isolating part of a root system.
5. The estimation of a root system using the shoot to root ratio.

Destructive measurements of root systems

Direct but destructive measurements of root systems may be obtained by mapping the roots in situ or by removing the roots by excavation. Indirectly the size of root systems may be estimated from analyses of soil cores containing roots. (The use of tracers in the plant to measure root growth is somewhat special and therefore will be discussed separately.) It may also be required to separate roots and soil, to distinguish between living and dead roots and to preserve material for future study.

Mapping root systems in situ. The classical technique of mapping the form of a root system as elaborated by Weaver (1926) is well documented and has been used by numerous workers. A trench is excavated near the stem of a plant and the soil of one wall is removed using a handpick or a spray of

water (Robertson 1955) until a vertical bisect of the root system is exposed. The positions of the exposed roots are then plotted on squared paper. Haas and Rogler (1953) sprayed the roots with white paint before picking away the last traces of soil and then photographed the profile. Roots on the profile may be mapped according to their metabolic activity (Jacques and Schwass 1956) or their thickness (Schuurman and Goedewaagen 1965) or their degree of branching (Robertson 1955). Trenches dug tangentially to the root system are useful when studying plants with large root systems, such as trees. Many workers have also examined the distribution of roots in a horizontal plane (e.g. Lyford and Wilson 1964). Much work in which root systems are mapped is not adequately replicated, a weakness considering the variability of root systems. Schuurman and Goedewaagen (1965) noted that the reliability of observations on a single plant may be increased by mapping several radial profiles.

The removal of root systems from soil. The simplest method used by innumerable workers is to remove root systems from soil by digging. Rivers and Faubion (1963) used a 'Tree Ogger', a device with 6 blades which removes an inverted cone of soil. However, this device has limited application in research since only a portion of a root system can be removed. Instead of digging Stoeckler and Kuender (1938) used a jet of water and Van Breda (1937) used compressed air to excavate roots. The disposal of used water presents a major difficulty in hydraulic excavation.

A convenient alternative to exposing roots in situ is to transport a block of soil to a washing site, e.g. the 'soil block' method (Pavlychenko 1937), the 'monolith' method (Weaver and Voigt 1949) and the 'soil elution'

method (Upchurch 1951) which differ mainly in the size of the block that is removed.

Unfortunately these techniques do not permit measurements of the orientation of individual roots. Hays (1886) constructed a steel frame with layers of 'two-inch' wire netting at depth intervals of two or three inches. The frame was filled with sand and corn was planted in the centre. At certain stages of growth the sand was washed away and the roots were left hanging over the wire. Recently the technique has been revised using nylon stockings (Hironaka 1959) or glass fibre frames (Pittman 1962). King (1892) used a method which was a modification of that used by Hays. A block of soil one foot thick by several feet long and deep was isolated, reinforced with wire netting, and then riddled by a series of cross rods. The aerial parts of the plant were supported by a layer of plaster of paris at the top of the block. Soil was then washed away to expose the roots. Using the same principle, Blaser (1937) attached the cross-rods to a back board ('pin board' or 'nail board') and forced the whole unit into the side of a trench. The block of soil penetrated by the pins was then cut away using steel ropes and transported to a washing site. This method is at present used extensively in Holland (Schuurman and Goedewaagen, 1965). Hudig (1939) used two pin boards at right angles in order to retain the position of the roots in three dimensions.

The measurement of root systems by sample cores. Instead of exposing a whole root system, the distribution of the roots may be estimated by removing soil cores by augers or borers at selected positions relative to the plant stem. The auger is less useful than the borer because the soil is broken up and also

the volume of the sample cannot easily be determined. Although hand borers of many types have been described, their designs are subject to the same criteria. If the internal diameter is too small, the internal friction with the soil becomes excessive and the sample is unduly compressed. Schuurman and Goedewaagen (1965) used borers with an internal diameter of seven centimeters, having found that an internal diameter of four centimeters was too small. They overcame this problem by increasing the internal diameter of the borer just behind the cutting edge. Williams and Baker (1957) prevented the soil from compacting by using a borer split lengthwise and held together by a cap at the top and a ring at the bottom. Provided sampling is sufficiently replicated, the size and shape of the borer does not affect the accuracy of sampling (Simon and Eich 1955; Zaplatin 1963).

The separation of roots and soil. The above methods require the separation of roots from soil. Generally the block of soil containing roots is soaked and sprayed with water and manipulated (by hand or mechanical agitation) until the roots can be separated from the soil suspension with a sieve. Manual techniques can be both tedious and arduous and hence there has been some attempt at mechanization. Upchurch (1951) passed water through 33-gallon drums containing the samples to wash out the roots which were then removed by a 16-inch sieve. Gates (1951) placed soil containing roots into screen-bottomed cradles which were agitated in tanks of water until the roots were washed out and could be collected in a sieve. Fribourg (1953) soaked blocks of soil plus roots in screen-bottomed trays in 50-gallon drums of water and subsequently removed the trays and sprayed the blocks with a flared-nozzle garden hose. Fehrenbacher and Alexander (1955) put the samples into

suspension by shaking and then separated roots from soil with a 16-inch sieve. Ogden (personal communication) attempted to use an ultrasonic washer to separate roots from soil, but found that the roots tended to disintegrate.

The task of separating roots from soil varies greatly depending on whether the roots are short or long, thick or thin, branched or unbranched; or whether the soil is sand or clay, with a high or low organic matter content. Hence special additional washing techniques are sometimes employed for certain combinations of these factors. When the organic matter content is high, the separation may be achieved by floating the roots in a tray of water and picking out foreign material by hand (Schuurman and Goedewaagen 1965). Jacques (1945) attempted to speed up the separation by flowing a suspension of roots and foreign organic material over a surface of rubber conical projections, where the long pieces of roots were retained and the short pieces of organic matter were washed away. Some workers have added dispersing agents to the suspension to facilitate separation. Bates (1948) used calcium hydroxide solution. Schuurman and Goedewaagen (1955) dried the soil at 105°C and then soaked it in a sodium pyrophosphate solution (134 gm. per 50 liters of water) or a solution of detergent. Barley (1953) ground up a sample of roots and soil to pass through a 5 mm. sieve. The mixture was then placed in a sodium chloride solution (density 1.2 gm/cc) and the root debris and organic matter were separated from the mineral particles by centrifugation and by filtering the supernatant liquid, when more than 80% of the root material was recovered. Later Barley (1955) modified the technique by placing the ground mixture in a mineral solution ("Calgon", 200 gm/liter), shaking occasionally, and then decanting off the organic

fraction by hand. Pure sand can be used to avoid the difficulty of separating organic matter and root material. Subsequently, the total content of organic material attributed to the roots can then be determined accurately by ashing (Willard and McClure 1932) or by digestion (Smith and Mabbit 1953). However, when the soil contains a very high content of organic matter, the absolute separation of roots by any system is impossible. As Barley (1955) pointed out, terms such as 'underground yields', 'root and other plant residues', 'root fibre' and 'underground plant materials' are used to indicate that organic materials other than roots are present at the end of the separation process.

The separation of living and dead roots. For some purposes it is desirable to estimate the proportions of living and dead roots in a system or sample. The simplest means is by colour (Hason and Stoddart 1940). Visual separation may be aided when the cortex and lateral roots breaks away from dead roots (Schuurman and Goedewaagen 1965). Several workers have used vital stains such as 2, 3, 5 triphenyl tetrazolium bromide (Jacques and Schwass 1956) and tetrazolium chloride (Goedewaagen 1954). Studkey (1941) identified living and dead roots by the presence of dividing cells in the apices. Cepikova (1942) used catalase activity and the volume of colloids as parameters. The tensile strength of living roots is considerably less than dead roots (Shalyt 1963). Greenham and Cole (1949, 1950) determined the electrical conductivity of the tissues of large roots by inserting a probe; dead roots had a higher electrical resistance than living roots. Taper, King and Hutchinson (1963) found that the electrical conductivity of ethanol extracts of apple terminal shoots on 'dwarfing' rootstocks was high, and of shoots on

'vigorous' root stocks was low. Ueno, Yoshikaro and Okada (1967) distinguished between living and dead roots by their relative ability to accumulate ^{14}C which was supplied through the shoots.

The preservation of roots. It is often necessary to store washed roots before they are measured. The roots are usually dried: Schuurman and Goedewaagen (1965) noted that when dried roots are wetted they regain their original pliable nature, and that "even the root hairs do not appear to be greatly affected". Roots may also be stored by freezing or by placing them in an aqueous solution of formaldehyde. Schuurman and Goedewaagen (1955) removed roots using the 'nail board' method and then sprayed them with cellulose so that the relative orientations of the preserved roots could be examined at a later date.

An interesting technique has been described by Lund and Beals (1965) to preserve the orientation of soil particles at the root surface. A sample of soil containing roots was put through a dehydration series combined with a plastic embedding technique, before being sectioned. Coloured plastic facilitated the identification of pore space.

The use of tracers to measure root systems.

In recent years tracers have been used extensively to measure root systems. Tracers have included non-radioactive lithium (Sayre and Morris 1940) as well as various radioisotopes: ^{32}P, ^{86}Rb, ^{131}I and ^{42}K. In studies of atomic fall-out other radioisotopes including ^{90}Sr, ^{89}Sr and ^{137}Cs have been used (see Evans and Dekker 1965). The general procedure has been to 'place' the tracer at measured distances from the stem, and then test for its presence in the shoot, which is assumed to indicate that the root system

extends to the approximate position of the placement. Radioactive tracers have been placed in various ways. Robertson, Kang, Ramirez, Werkhoven and Ohlrogge (1966) directed individual roots into containers of soil mixed with tracers. Hall, Anderson, Chandler, Reid and Van Bavel (1953) drove holes in the ground into which the tracers were placed. This technique has been improved by Boggie and Knight (1962) who injected radioisotopes into deep peat through specially designed tubes. Burton, Vane and Carter (1954) removed a core of soil in order to place a tracer. The more elaborate system devised by Murty, Moser and Hobbs (1963) consisted of a series of tubes, and each possessing horizontal slits at intervals, which were positioned vertically in the soil and down which Geiger-Müller tubes were lowered. Emission of the radioactive tracer could then be measured at the positions of the slits. When a series of such tubes are used, the movement of the tracer in the soil as well as the time at which the tracer is first evident in the shoot can be determined. The relative rate of spread of the roots can be estimated from this information.

In a few studies a tracer has been incorporated in one plant and its presence has then been determined in neighbouring plants. Thus Rakhteenko (1958) demonstrated the transfer of ^{32}P between grass plants and Woods and Brock (1964) followed the movement of ^{32}P and ^{45}Ca in a mixed stand of trees.

The observation of growing roots

The observation of roots growing in solid media. The most widely used method of observing growing roots (as opposed to destructive measurement) is through observation windows, which may be the glass-lined wall of a

trench or the glass side of a soil-filled box. There are many modifications of these basic techniques. For example, Rogers and Head (1962) described a 'root-observation laboratory, 92 feet long, seven feet wide and seven feet high, which contained 48 glass windows. Glass-faced boxes may be held in slots in the ground (Lavin 1961). Bilan (1964) grew plants in large plastic tubes containing soil. Various media other than soil have been used, including vermiculite (Johanson and Muzik 1964), and glass and acrylic beads of which the latter are most suitable (Bloomberg 1963).

The use of observation windows is subject to certain limitations. Firstly, the observed roots constitute a small and unknown portion of the total root system. To increase this proportion Lavin (1961) used a window that sloped at 30° to the vertical and subsequently calculated that 38% of the roots were visible. Secondly, the environment of the roots adjacent to the glass may be sufficiently different from that of the non-visible roots to make observations misleading. Temperature differences may be important. Leonard and Head (1958) stated that they had some difficulty in observing the roots due to the growth of algae on the inner face of the glass. Light will affect root growth, although this factor may be minimized if the roots are observed using a dark green light source (Larsen 1962).

An interesting application of glass-faced boxes was that of Barber (1962) who grew corn plants against the glass in soil mixed evenly with ^{86}Rb. An autoradiograph made adjacent to the glass demonstrated the depletion of the isotope from the soil in the vicinity of the roots.

Sphagnum moss was placed around roots by Jacques and Edmond (1952) and could be removed to permit observations of root growth. However, to what extent root growth is affected by mechanical disturbance and by the

antibiotic substances produced by the moss is uncertain.

The observation of roots growing in liquid media. Many workers have suspended root systems in aerated nutrient solution in plexiglas- or glass-sided vessels; specialized applications of this technique used in studies of root tropisms have been described by Larsen (1962). Schuurman and Goedewaagen (1965) trained roots from soil-filled pots into glass cylinders containing nutrient solution. Leyton and Rousseau (1958) trained roots along square-sectioned tubing containing aerated nutrient solution. However, it should be noted that the rate of growth of the roots may be affected by the width of the tubing (Ohlrogge 1962). In all cases where roots are grown in nutrient solutions, results should be viewed with caution. The root will not be subjected to tactile stimuli and also the orientation of roots, which is normally constant when the growing media is solid, may vary considerably and thereby affect growth.

The observation of roots growing in humid chambers. Many studies of geotropism have been carried out in humid chambers (see Larson, 1962). Lyon and Yokoyama (1966) devised an interesting technique to study the orientation of the seminal roots of wheat seedlings. A plastic tube with 12 lateral tubes was filled with moist vermiculite and a cereal seedling was held in each lateral tube so that the roots projected into moist air. Photographic records were obtained from two directions (i.e. by turning the main tube) enabling the angles of the roots to be measured in three dimensions. However, almost without exception, geotropic studies have been carried out with very young seedlings and thus the techniques are not directly applicable to more adult plants.

The 'fox-box' technique which was used by Clayton and Lamberton (1964) - also see Went (1957) and Hewitt (1966) - allows roots to be grown in humid chambers for long periods. The roots are suspended in an enclosed chamber and are sprayed with nutrient solution from time to time. However, besides suffering the same criticisms as cited for liquid media, the fox-box technique cannot be related to natural conditions in which roots grow.

The observation of roots growing on damp surfaces. The objections mentioned above may be met by growing roots over damp surfaces, a technique well known in elementary classwork where bean roots are grown down the side of jam-jars. Seedlings have been grown down vertical sheets of moist filter paper on Petri dishes (see Larsen 1962), on filter paper in polythene bags (Eliasson 1961) or on filter paper between glass sheets (Yorke 1967). Davidson and Milthorpe (1966) grew seedlings on sheets of black glass (43 x 28 cm) supported by a metal frame at 12° to the horizontal with nutrient solution circulating over the roots. Roots have also been grown on sheets of agar (Jones, Metcalfe and Sexton 1954). Younis (1954) placed seedlings in a plexiglas box containing a thin sheet of wood covered by successive layers of cotton wool, flannel and black cloth. The seedlings were wedged into a holder by pieces of sponge and the roots grew down over the surface of the pad which was kept moist by constantly dripping water or nutrient solution. Pel'tsikh (1963) sowed seeds on glass plates covered by a white cloth. The plates, placed at 35-40° in clean wet sand, were removed after a week or two, dried, cleaned of sand and sprayed with a 0.5% solution of ninhydrin in butyl alcohol to develop the remaining traces of amino acids. In this way it was possible to obtain an 'autograph' of the root system.

Although ingenious, practical applications of this technique are few. Particular advantages of these techniques are that besides receiving nutrients via the substrate, the roots are well aerated, are anchored by root hairs, and can be readily observed.

Methods of isolating part of a root system

In many studies of plant nutrition 'split-root' techniques are used so that interplant variation may be reduced to a minimum. The reader is referred to Hewitt (1966, Section 3.6) for examples not cited below.

The characteristics of a root to grow according to the local environment of its individual roots has been exploited by many workers. In a study of geomagnetotropism, Woolley and Pittman (1960) inserted a wedge of soil containing a radioactive tracer (^{32}P) into a container. The side of the container with the wedge was orientated to different points of the compass and by determining the uptake of ^{32}P, it was possible to demonstrate that the greatest number of roots were orientated toward the north side of a plant. Most workers, however, have used barriers to separate portions of the system between which the roots are to be divided. For example, to train root systems to a particular depth or soil horizon, De Roo and Wiersum (1963) grew whole root systems down plastic tubes 2.5 or 5.0 cm. in diameter. However, some investigations require waterproof barriers which are ion-impermeable and through which roots may be grown from one local environment into another. Such barriers may be composed of paraffin wax (Weaver, Jean and Crist 1922), soft wax (Hunter and Kelley 1946), or a mixture of paraffin wax and rosin (Stone and Hulkey 1961). It has been reported that after

penetration the roots became sealed to each type of barrier. A cheap and useful split-root technique has been developed by Gis'kin and Kohnke (1965) who fixed wax-impregnated cheese-cloth over slits in the sides of ice-cream cartons.

Sometimes, however, individual rather than groups of roots are studied. To simulate the placement of fertilizers in field conditions, Wilkinson (1961) led an individual root into a 5-gallon can containing vermiculite and fertilizer. In another experiment, single roots were led from a can, via a plastic tube, into glass cylinders containing layers of fertilizer sandwiched between soil. Plastic and glass 'cells' for individual roots are described by Kang and Ohlrogge (1961) and Evans and Vaughan (1966) respectively. Such cells may have an important role in the study of the growth of individual roots.

The estimation of root systems using shoot to root ratios

In a given environment, the shoot to root ratio tends to remain constant (Troughton 1957). Smith (1964) found that the ratio of root spread to crown width averaged 1.1 for open-grown and 0.9 for forest-grown Douglas fir, compared with 2.4 for lodgepole pine in both situations. However, the method should be used with caution. McMinn (1963) noted that the apparent proportionality between crown width and root spread of Douglas fir will be altered by factors affecting root spread, e.g. slope, proximity of other trees and the presence of old roots. Other work indicates that the symmetry of a root system may be distorted by geomagnotropism (Woolley and Pittman 1960). Nevertheless, shoot to root ratios may be very useful where a particular species is distributed over large, uniform areas of land.

TECHNIQUES FOR MEASURING SPECIFIC PARAMETERS OF ROOT GROWTH

A root system grows in terms of extent and direction, although these two aspects have been integrated rarely. Extent of root growth can be divided into the processes of elongation and multiplication, whereas direction of growth includes growth movements (or nutations) and tropisms. These components of root growth and their measurements are presented schematically in Fig. 1. It is not the object of this review to discuss why a particular parameter should be chosen, although it is relevant to outline briefly methods of measuring root growth.

Wet and dry weights are probably the most usual way of measuring root growth, although neither measurement can be related directly to surface area, volume or orientation. Cahoon and Morton (1961) used a domestic spin dryer to remove surplus water from washed roots. This was quicker than the usual procedure of blotting roots and subsequent measurements of moisture content were more consistent.

The volume of a root system may be determined by the amount of liquid that the roots displace (Andrew 1966). It may also be calculated from photographs of a root system by assuming that the root is in a branched cylinder, and then substituting the average radius and total length in the formula $\pi r^2 l$, where r=radius and l=length (Hackett, personal communication).

Surface area may also be calculated approximately using the formula $2\pi rl$. Carley and Watson (1966) compared a titration method with a more rapid, although slightly less accurate, gravimetric procedure to measure surface area. In the titration method, washed air-dried roots were dipped in 3N HCl for 15 seconds, drained for 5 minutes and the remaining acid was

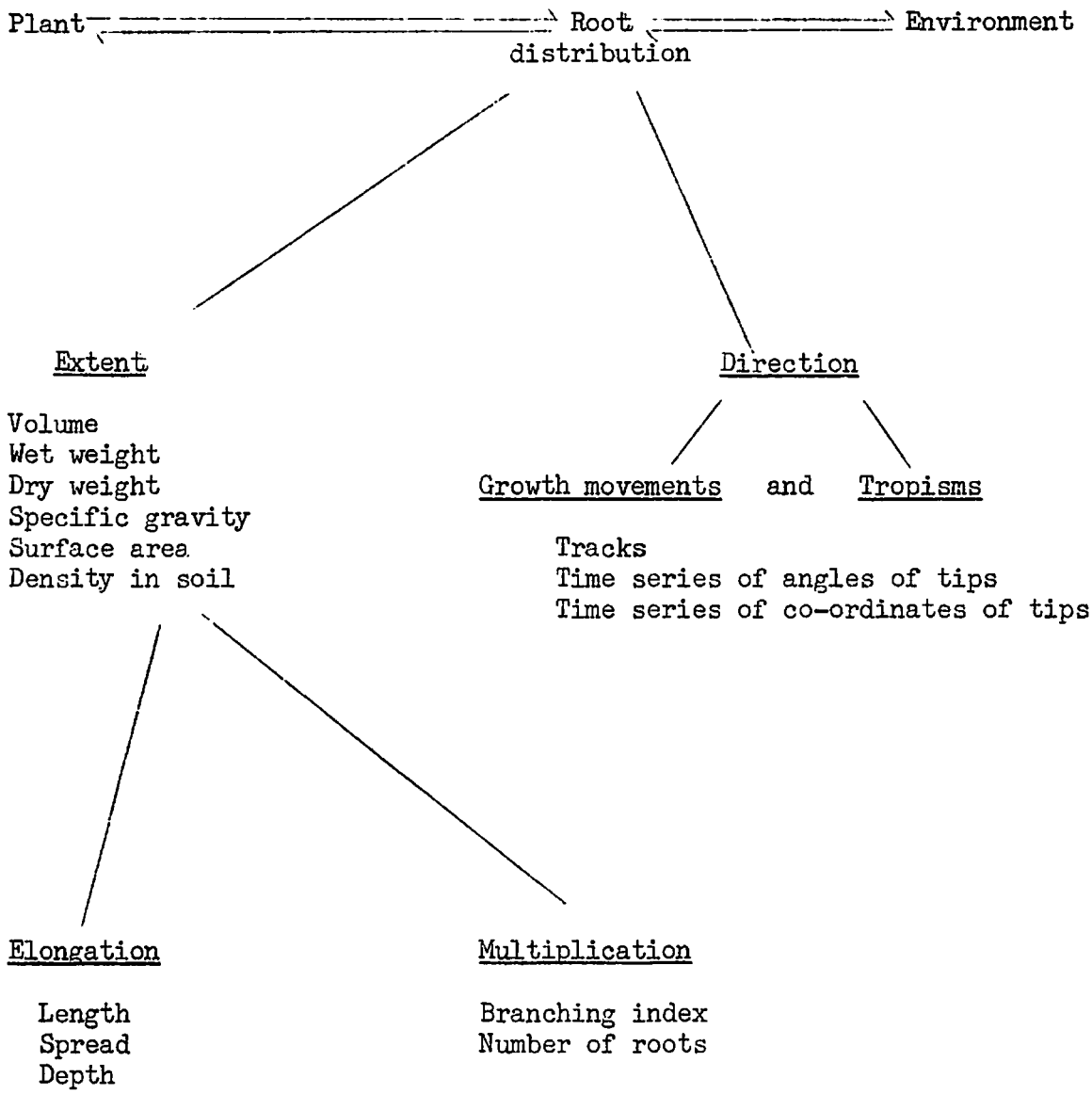

Fig. 1 Schematic analysis of the components of growth of a root system and the parameters used for their measurement.

eluted off in 250 ml. of distilled water. The elutant acid was titrated with 0.3 N NaOH and a phenolphthalein indicator. The relative area of root surface per plant was expressed as milliliters of 0.3 N NaOH. In the gravimetric method air-dried roots were dipped for 10 seconds in a weighed 600 ml. beaker of calcium nitrate solution (1 part water: 6 parts $Ca(NO_3)_2$), surplus liquid was allowed to drain into the beaker for 30 seconds and the beaker was reweighed. The loss of calcium nitrate solution was assumed to be proportional to the root surface area.

The density of exposed roots at the end of a soil sample core can be determined simply by counting (Fitspatrick and Rose 1963) or by comparison with a series of standard cards (Schuurman and Knot 1957). Blydenstein (1966) estimated density by counting the number of roots that passed through a 1 x 2 inch wire frame held within 3 inches of the base of a plant. An alternative method is to introduce ^{32}P into the root system (via the shoot) and then measure the radio-activity of sample cores previously ashed at 500°C (Racz, Rennie and Hutcheon 1964). Of course the density can also be accurately determined by washing roots out of a soil core, although this method is more time-consuming.

Elongation of individual visible roots can be measured directly, but the only practical way to measure a whole root system is indirectly from photographs (Yorke 1967). Quicker methods of estimating the total length of a sample of roots which depend on the number of intersections between roots and overlying straight lines have been described by Head (1966) and Newman (1966). Theoretically the straight lines should be of the same length and should be orientated at random; however, in practice the number

of intersections of roots with a non-random grid has been found to be highly correlated with root length.

Multiplication of roots can be equated with density, although this measurement could be misleading due to individual roots being recorded several times. Robertson (1955) classified roots according to a branching index, i.e. roots were "unbranched", "once branched" or "branched branches".

Growth movements and tropisms have rarely been recorded together and have even less frequently been related to the extent of root growth. Individual roots must be recorded separately. Classical methods used by Darwin (1880) were to observe the 'path' of a root growing against a smoked glass screen and also to follow the movement of a glass filament attached to a root tip. Both methods have obvious disadvantages. Movements of roots have usually been measured by recording a time series of the angle of the longitudinal axis of the root tip (Larsen 1962). Unfortunately the angle of the root tip besides being somewhat difficult to measure gives no direct indication of the growth rate or of the zone of maximum elongation. A method of determining the angles of root axes at set distances from the root tip has been described by the author (Yorke 1967). To avoid the difficulties inherent in measuring angles, a time series of the co-ordinates of the root tip may be determined from photographs (Spurný 1966). Special techniques for demonstrating chemo- and hydro-tropisms of roots are discussed by Ziegler (1962 a,b).

A final point worth mentioning is how different root systems of plants of the same species might be distinguished. A method used by Nielson (1964) was to wash a monolith of soil containing roots of plants

whose tops were treated with ^{14}C - urea. The washed roots were pressed and dried and the presence of the radio-active root system was detected by autoradiography. Unfortunately, besides causing considerable disturbance to the roots, the method cannot be applied to plants with natural root grafts. Litav and Harper (1967) also labelled plants and then picked out segments of roots from the surface of a vertical bisect through the soil containing the root systems. The frequency of four possible pairing classes (labelled +, unlabelled -: ++, +-, -+, --) was assembled as a 2 x 2 contingency table and 'undermixing' and 'overmixing' was detected by a χ^2 test for departure from random association.

CONCLUSION

As was pointed out earlier the results of studying the growth of a root system will be limited by the method employed. For instance, if distribution is determined by excavating a whole root system, the rate or direction of growth cannot be ascertained with certainity. Conversely, if the rate and direction of root growth behind observation windows is measured, the growth of non-visible roots must be largely ignored. Hence, from the outset of a root investigation it is necessary to have a clear idea of the particular facet of root growth that is to be measured and only subsequently should the technique of measuring the roots be chosen. This elementary principle has not always been adhered to.

It is very striking that considered against the background of mechanical aids and automation that marks recent scientific progress, very few of the methods described to study roots are even moderately

mechanized. Most of the procedures are both arduous and tedious to use and because of this, frequently limit the scale of experimentation. Furthermore, the greatest expense usually encountered in field experimentation is the cost of employing assistants. For this reason alone, it is surprising that more attention has not been given to mechanizing the techniques.

Because of the general lack of quick and effortless techniques, the attitude of many workers to the study of roots is somewhat evasive. As Wiersma (1959) pointed out, most the information regarding root growth in the field has been obtained only incidentally from experiments with other objectives. Indeed the fact that knowledge of root growth is exceedingly fragmentary may be attributed to the lack of a single method by which it would be possible to study the growth of a root system in the field in terms of extent and direction, from germination to senescence. The author has attempted to remedy this matter by growing pea root systems for periods up to one month from germination, in a single vertical plane on damp filter paper between glass sheets (Yorke 1967). This method, however, although proving instructive, is obviously still not ideal.

It appears that much more attention should be given to integrating the various topics embraced within the general subject of root systems into a concise description and understanding of how root systems grow. How does a root system develop to produce its 'mature' form? Where are the longer roots located in a system and how do their rates and direction of growth differ from the shorter roots? How do environmental factors affect both the whole root system and individual roots? Once the precise morphological

pattern of development has been determined, the next step would be to investigate the physiological control of the process. As yet most of the basic information pertaining to root physiology has been obtained from experimentation using excised culture, and other specialized and artificial techniques and therefore the results cannot always be readily applied to problems of roots growing in field conditions.

Finally, the growth of the root system in whole plants is usually dependent on either the cotyledons or the shoot. There is increasing evidence that the growth of all organs of a plant are very closely integrated and, in all root studies, care should be taken to remember this.

ACKNOWLEDGEMENTS

This review was largely prepared during the tenure of a Post-graduate Studentship of the Ministry of Agriculture, Fisheries and Food, U.K.

LITERATURE CITED

ANDREW, R.H. (1966). A technique for measuring root volume in vitro. Crop Sci., 6, 384-386.

BARBER, S.A. (1962). A diffusion and mass-flow concept of soil nutrient availability. Soil Sci., 93, 39-45.

BARLEY, K.P. (1953). The root growth of irrigated perennial grasses and its effect upon soil temperature. Aust. J. agric. Res., 4, 283-291.

_____ (1955). The determination of macro-organic matter in soils. Agron. J., 47, 145-147.

BATES, G.H. (1948). An investigation into the cause and prevention of deterioration of leys. J. Br. Grassld Soc., 3, 177-184.

BILAN, M.V. (1964). Acrylic resin tubes for studying root growth in tree seedlings. J. Forest Sci., 10, 461-462.

BLASER, R.A. (1937). A rapid quantitative method of studying roots growing under field conditions. J. Am. Soc. Agron., 29, 421-423.

BLOOMBERG, W.J. (1963). A translucent rooting medium for the observation of Douglas fir seedling roots. Pl. Dis. Reptr., 47, 455-458

BLYDENSTEIN, J. (1966). Root systems of four desert grassland species on grazed and protected sites. J. Range Mgmt., 16, 93-95.

BOGGIE, R. AND A.H. KNIGHT (1962). An improved method for the placement of radioactive isotopes in the study of root systems of plants growing in deep peat. J. Ecol., 50, 461-463.

BUTCHER, O.N. AND H.E. STREET (1964). Excised root culture. Bot. Rev., 30, 513-586.

BURTON, G.W., E.H. DE VANE AND R.L. CARTER (1954). Root penetration, distribution and activity in southern grasses measured by yields, drought symptoms and p^{32} uptake. Agron. J., 46, 229-233.

CAHOON, G.A. AND E.S. MORTON (1961). An apparatus for the quantitative separation of plant roots from soil. Proc. Am. Soc. hort. Sci., 78, 593-596.

CARLEY, H.E. AND R.D. WATSON (1966). A new gravimetric method for estimating root-surface areas. Soil Sci., 102, 289-291.

ČEPIKOVA, A. (1942). (Root systems of perennial herbage plants and when they die out. (A preliminary communication)). (Russian). Proc. Lenin Acad. Sci. (9-10), 28-9. Cited by Troughton (1957).

CLAYTON, M.F. AND J.A. LAMBERTON (1964). A study of root exudates by the fog-box technique. Aust. J. Biol. Sci., 17, 855-866.

DARWIN, C. (1880). The power of movement in plants. Publ. Appleton and Co., London

DAVIDSON, J.L. AND F.L. MILTHORPE (1966). The effect of defoliation on the carbon balance in Dactylis glomerata. Ann. Bot., N.S., 30, 185-198.

DE ROO, H.C. AND L.K. WIERSUM (1963). Root training by plastic tubes. Agron. J., 55, 402-405.

ELIASSON. L. (1961). Responses of pea roots to growth substances. Physiologia Pl., 14, 803-812.

EVANS, E.J. AND A.J. DEKKER (1965). ^{90}Sr concentration in deep - and - shallow rooting species as affected by placement of ^{90}Sr and fertilizer in soil. Can. J. Soil Sci., 45, 289-296.

EVANS III, E.C. AND B.E. VAUGHAN (1966). New methods for effecting water-tight seals on corn roots. Pl. Physiol., 41, 1077-1078.

FEHRENBACHER, J.B. AND J.D. ALEXANDER (1955). A method for studying corn root distribution using a soil-core sampling machine and shaker type washer. Agron. J., 47, 468-472.

FITSPATRICK, E.G. AND L.E. ROSE (1936). A study of root distribution in prairie claypan and associated friable soil. Am. Soil Survey Assoc. Bull., 17.

FRIBOURG, H.A. (1953). A rapid method for washing roots. Agron. J., 45, 334-335.

GATES, C.T. (1951). The quantitative recovery of root systems in pot experiments. J. Aust. Inst. Agric. Sci., 17, 152-154.

GISKIN, M.L. AND H. KOHNKE (1965). Measuring root responses to soil properties with a single plant. Agron. J., 57, 96-97.

GOEDEWAAGEN, M.A.J. (1954). Beoordeling van de vitaliteit van het wortelstelsel. Landbk. T., 66, 553-554. Cited by Schuurman and Goedewaagen (1965).

GREENHAM, C.G. AND D.J. COLE (1949). Diagnosis of death in roots. Nature, Lond., 164, 669.

_____ _____ (1950). Studies on the determination of dead or diseases tissues. I. Investigations on dead plant tissues. Aust. J. Agric. Res., 1, 103-115.

HAAS, H.J. AND G.A. ROGLER (1953). A technique for photographing grass roots in situ. Agron. J., 45, 173-174.

HALL, N.S., J.H. ANDERSON, W.V. CHANDLER, P.H. REID AND C.H.M. VAN BAVEL (1953). A tracer technique to measure growth and activity of plant root systems. N. Carolina Agri. Expt. Sta. Tech. Bull., 101.

HASON, W.R. AND L.A. STODDART (1940). Effect of grazing upon bunch wheatgrass. J. Am. Soc. Agron., 32, 278-289.

HAYS, W.M. (1886). Corn, its habit of root growth, methods of planting and cultivating, notes on ears and shoots or suckers. Minnesota Agri. Expt. Sta. Bull., 5.

HEAD, G.C. (1966). Estimating seasonal changes in the quantity of white unsuberized root in fruit trees. J. hort. Sci., 41, 197-206.

HEWITT, E.J. (1966). Sand and water culture methods used in the study of plant nutrition. Tech. Bull. 22, Commw. Bur. Agric., 2nd Edition, pp. 547

HIRONAKA, M. (1959). Personal communication of McKell (1962).

HORT, A. (1916). Translator of: Enquirey into plants and minor works on odours and weather signs, by Theophrastus. Publ. Harvard Univ. Press., Mass.

HUDIG, J. (1939). Klinisch grondonderzock in de tropen. Landbk. T., 51, 372-389.

HUNTER, A.S. AND O.J. KELLEY (1946). A new technique for studying the absorption of moisture and nutrients from soil by plant roots. Soil Sci., 62., 441-450.

JACQUES, W.A. (1945). Root development in some common New Zealand pasture plants. IV. A method of root separation. N.Z. Jl. Sci. Technol., A26, 367-371.

_____ AND D.B. EDMOND (1952). Root development in some common New Zealand pasture plants. V. The effect of defoliation and root pruning on cocksfoot (Dactylis glomerata) and perennial rye grass (Lolium perenne). N.Z. Jl. Sci. Technol., A34, 231-248

_____ AND R.H. SCHMASS (1956). Root development in some N.Z. Pasture plants. VII. Seasonal root replacement in perennial rye grass (Lolium perenne), Italian rye grass (L. multiflorum) and tall fescue (Festuca arundinaceae). N.Z. Jl. Sci. Technol., A37, 579-584.

JOHANSON, N.G. AND T.J. MUZIK (1964). Some effects of 2, 4-D on wheat yield and root growth. Bot. Gaz., 122, 188-194.

JONES, R.L., T.P. METCALFE AND W.A. SEXTON (1954). The relationship between the constitution and the effects of Chemical compounds on plant growth. IV. Derivatives and analogues of 2-benzoylbenzene acid. J. Sci. Fd. Agric., 5, 32-38.

KANG, B.T. AND A.J. OHLROGGE (1961). The response of corn roots to high nutrient concentration within a single root culture cell. Proc. Indiana Acad. Sci., 70, 254-259.

KING, F.H. (1892) Natural distribution of roots in field soils. Rep. Wis. agric. Exp. Sta., pp. 112. Cited by Weaver (1926).

LAIRD, A.S. (1930). A study of the root systems of some important sod forming grasses. Florida Agri. Expt. Sta. Bull., 211.

LARSEN, P. (1962). Geotropism an introduction. Handb. PflPhysiol., XVII/2, 34-73.

LAVIN, F. (1961). A glass-faced planter box for field observations on roots. Agron. J., 53, 265-268.

LEONARD, E.R. AND G.C. HEAD (1958). Technique and preliminary observations on growth of the roots of glasshouse tomatoes in relation to that of the tops. J. Plant. Sci., 33, 171-185.

LEYTON, L. AND L.Z. ROUSSEAU (1958). Root growth of tree seedlings in relation to aeration. In: Physiology of forest trees (Edt. K.V. Thimann). Norald Press, N.Y.

LITAV, M. AND J.L. HARPER (1967). A method for studying spatial relationships between the root systems of two neighbouring plants. Pl. Soil, 26, (2), 389-392.

LUND, Z.F. AND H.O. BEALS (1965). A technique for making thin sections of soil with roots in place. Proc. Soil Sci. Soc. Am., 29, 633-635.

LYFORD, W.H. AND B.F. WILSON (1964). Development of the root system of Acer rubrum L. Harvard Forest Paper No. 10, Publ: Harvard Univ., Mass.

LYON, C.J. AND K.Y. YOKOYAMA (1966). Orientation fo wheat seedling organs in relation to gravity. Pl. Physiol., 41, 1065-1073.

MCKELL, C.M. (1962). Root studies. In: Pasture and range research techniques (Edt. D.E. McCloud). Publ: Cornell Univ. Press, Ithaca, N.Y. 173-179

MCMINN, R.G. (1963). Characteristics of Douglas fir root systems. Can. J. Bot., 41, 105-122.

MURTY, G.S., H.C. MOSER AND J.A. HOBBS (1963). A tracer technique for rapid determination of nutrient movement in soil. Proc. Soil Sci. Soc. Am., 27, 277-478.

NEWMAN, E.I. (1966). A method of estimating the total length of root in a sample. Jnl. appl. Ecol., 3, 139-145.

NIELSON Jr., J.A. (1964). Autoradiography for studying individual root systems in mixed herbaceous stands. Ecology, 45, 643-646.

OHLROGGE, A.J. (1962). Some soil root plant relationships. Soil Sci., 93, 30-38.

PAVLYCHENKO, T.K. (1937). The soil block washing method in quantitative root study. Can. J. Res., C15, 33-57.

PEL'TSIKH, I.L. (1963). Novyi metod i izucheniya kornevoi sistemy v pervyi period razvitiya rastenii. Bot. Zhur., 48, 694-695. Abstract in Biol. Abstr., 45, 52545.

PITTMAN, U.J. (1962). Growth reaction and magnetism in roots of winter wheat (Kharkov 22 M.C.). Can. J. Pl. Sci., 42, 430-436.

RACZ, G.J., D.A. RENNIE AND W.L. HUTCHEON (1964). The ^{32}P injection method for studying the root system of wheat. Can. J. Pl. Sci., 44, 100-108.

RAKHTEENKO, I.N. (1958). O peremeshcheii mineral'nykh pitatel' nykh veshchesty iz odnogo rastenniya v drugoe pri vzaimodeistvii ikh kornevykh sistem. Bot. Zhur., 43, 695-701. Abstract in Biol. Abstr., 45, 83763.

RIVERS, G.W. AND J.L. FAUBION (1963). A uniform technique for comparing root systems of sesame (Sesamun indicum L.), Crop. Sci. 3, 182-183.

ROBERTSON, J.A., B.T. KANG, F.M. RAMIREZ, C.H.E. WERKHOVEN AND A.J. OHLROGGE (1966). Principles of nutrient uptake from fertilizer bands. VII. ^{32}P uptake by brace roots of maize and its distribution within the leaves. Agron. J., 58, 293-296.

ROBERTSON, J.H. (1955). Penetration of roots of tall wheat grass in wet saline alkali soil. Ecology, 36, 755-757.

ROGERS, W.S. (1939). Root studies. VII. A survey of the literature on root growth, with special reference to hardy fruit plants. J. Pomol., 17, 67-84.

_____ AND G.C. HEAD (1962). A new root-observation laboratory. Ann. Rep. E. Malling. Res. Sta., 54-55.

SALTER, P.J. AND D.H. DREW (1965). Root growth as a factor in the response of (Pisum sativum L.) to irrigation. Nature, Lond., 206, 1063-1064.

SAYRE, J.D. AND V.H. MORRIS (1940). The lithium method of measuring the extent of corn root systems. Pl. Physiol., Lancaster, 15, 761-764.

SCHUURMAN, J.J. AND M.A.J. GOEDEWAAGEN (1955). A new method for the simultaneous preservation of profiles and root systems. Pl. Soil, 6, 373-381

_____ AND M.A.J. GOEDEWAAGEN (1965). Methods for the examination of root systems and roots. Publ: Wageningen: Centre for Agric. Publ. and Documentations, pp. 36.

SHALYT, M.S. (1959). Apparat dlya opredeleniya rastyazhimosti i prochnosti kornei rastenii konstruktsii G.P. Kas'Kuma: V.I. Samkovskogo. Bot. Zhur., 44, 177-184. Abstract in Biol. Abstr., 45, 97114.

SIMON, W. AND D. EICH (1955). Probleme und Methoden der Wurzeluntersuchungen (unter besonderer Berucksichtgung leichterer Boden). Z. Acker- u. PflBau., 100, 179-198.

SMITH, A.M. AND L.A. MABBIT (1953). The contribution to soil organic matter and N from cereal residues and undersown crops. J. Soil Scie., 4, 98-106.

SMITH, J.H.G. (1964). Root spread can be estimated from crown width of Douglas fir, Lodgepole pine and other British Columbia tree species. For. Chron., 40, 456-473.

SPURNY, M. (1966). Spiral feedback oscillations of growing hypocotyl with radicle in Pisum sativum L. Biologia Pl. 8, 381-392.

STRYDOM, E. (1964). A root study of onions in an irrigation trial. S. Afr. J. agric. Sci., 7, 593-560.

STOECKELER, J.H. AND W.A. KUENDER (1938). The hydraulic method of excavating the root system of plants. Ecology, 19, 355-369.

STONE, J.F. AND J.R. MULKEY Jr. (1961) Pliable, root permeable layers for separation of portions of experimental plant root systems. Science, 133, 329-

STUCKEY, I.H. (1941). Seasonal growth of grass roots. Am. J. Bot., 28, 486-491.

TAPER, C.D., R.S. KING AND A. HUTCHINSON (1963). Note on the estimation of apple rootstock vigour by the electrical conductivity of water extract. Can. J. Pl. Sci., 43, 228-230.

TROUGHTON, A. (1957). The underground organs of herbage grasses. Bull. 44 Commonw. Bur. Past. Fld. Crops., pp. 163.

UENO, M., K. YOSHIHARA AND T. OKADA (1967). Living root systems distinguished by the use of carbon - 14. Nature, 213, 530-532.

UPCHURCH, R.P. (1951). The use of trench-wash and soil elution methods for studying alfalfa roots. Agron. J., 43, 552-555.

VAN BREDA, N.G. (1937). An improved method in the study of root bisects. S. Afr. J. Sci., 34, 260-264.

WEAVER, J.E. (1926). Root development of field crops. McGraw-Hill Book Co. Ltd., N.Y.

_____ F.C. JEAN AND J.W. CRIST (1922). Development and activities of roots of crop plants. Carnegie Inst. Wash. Pub., 316.

_____ AND J.W. VOIGT (1949). Monolith method of root sampling in studies on succession and degeneration. Bot. Gaz., 111, 286-299.

WENT, F.W. (1957). In: Experimental control of plant growth. Publ. Chronica Botanica Co., Waltham, Mass.

WIERSMA, D. (1959). The soil environment and root development. Adv. Agron., 11, 43-51.

WILKINSON, S.R. (1961). Influence of nitrogen and phosphorus fertilizers on the morphology and physiology of soybean roots. PhD. Thesis, Perdue Univ.

WILLARD, C.S. AND G.M. McCLURE (1932). The quantitative development of tops and roots in blue grass with an improved method of obtaining root yields. J. Am. Soc. Agron., 24, 509, 514.

WILLIAMS, T.E. AND H.K. BAKER (1957). Studies on the root development of herbage plants. I. Technique of herbage root investigations. J. Br. Grassld. Soc., 12, 49-55.

WOODS, F.W. AND K. BROCK (1964). Interspecific transfer of ^{45}Ca and ^{32}P by root systems. Ecology, 45, 886-889.

WOOLLEY, D.G. AND U.J. PITTMAN (1966). ^{32}P detection of geomagnetism in winter wheat roots. Agron. J., 58, 561-562.

WREN, M.J. AND J.W. HANNAY (1963). Ageing in roots in groundsel (Senecio vulgaris). I. The root system of seedlings cultured aseptically in darkness. New Phytol., 62, 249-256.

YORKE, J.S. (1967). Studies of root growth with particular reference to the root system of Pisum sativum L. PhD. Thesis, Univ. Wales.

YOUNIS, A.F. (1954). Experiments on the growth and geotropism of roots. J. exp. Bot., 5, 357-372.

ZAPLATIN, P.I. (1963). K metodike opredeleniya vesa kornei Bot. Zhur., 43, 867-869. Abstract in Biol. Abstr., 45, 56922.

ZIEGLER, H. (1962a). Chemotropismus. Handb. Pflphysiol., XVII/2, 396-431.

_____ (1962b). Hydrotropismus. Handb. PflPhysiol., XVII/2, 432-456.