6. Barksdale, T.H., J.M. Good, and L.L. Danielson. 1972. Tomato diseases and their control. U.S. Dept. of Agr. Handb. No. 203.
7. Barksdale, T.H. and E.J. Koch. 1969. Methods of testing tomatoes for anthracnose resistance. Phytopathology 59:1373–1376.
8. Batson, W.E. and K.W. Roy. 1982. Species of *Colletotrichum* and *Glomerella* pathogenic to tomato fruit. Plant Dis. 66:1153–1155.
9. Gourley, C.O. 1966. The pathogenicity of *Colletotrichum dematium* to table beets and other hosts. Can. J. Plant Sci. 56:531–536.
10. Griffing, B. 1956. Concept of general and specific combining ability in relation to diallel crossing systems. Austral. J. Biol. Sci. 9:463–493.
11. Hayman, B.I. 1954. The theory and analysis of diallel crosses. Genetics 39:789–809.
12. Miller, A.N. 1983. The inheritance of resistance to tomato anthracnose as caused by *Colletotrichum dematium*. MS Thesis, Univ. Maryland, College Park.
13. Robbins, M.L. and F.F. Angell. 1970. Tomato anthracnose: a hypodermic inoculation technique for determining genetic reaction. J. Amer. Soc. Hort. Sci. 95:118–119.
14. Robbins, M.L. and F.F. Angell. 1970. Tomato anthracnose: inheritance of reaction to *Colletotrichum coccodes* in *Lycopersicon* spp. J. Amer. Soc. Hort. Sci. 95:469–471.
15. Schaffer, H.E. and R.A. Usanis. 1969. General least squares analysis of diallel experiments: a computer program-DIALL. N.C. State Univ. Genetics Dept. Res. Rpt. 1.
16. Steel, R.G.D. and J.H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill, New York.
17. Stevenson, W.R. 1977. Use of captafol and chlorothalonil on reduced application method schedules for tomato disease control in Indiana. Plant Dis. Rptr. 61:803–805.
18. Stevenson, W.R., G.E. Evans, and T.H. Barksdale. 1978. Evaluation of tomato breeding lines for resistance to fruit anthracnose. Plant Dis. Rptr. 62:937–940.
19. Strickberger, M.W. 1976. Genetics. MacMillan, New York.

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A Method for Studying the Three-dimensional Distribution of Roots Grown in an Artificial Medium

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Abstract. **A method is described for studying the 3-dimensional distribution of roots grown in a medium consisting of small pieces of glass. After growing to a desired size, the plant is sacrificed by evaporating all water from the media with flowing air. To visualize the undisturbed root system, an immersion oil with the same refractive index as the glass is added to the glass container in which the plant was grown.**

There are numerous methods to study the distribution and morphology of root systems grown in soil and soilless media. These methods have been reviewed recently by Böhm (1). Excavating root systems is tedious, time-consuming, and, unless great care is exercised, only shows the gross structure of the root system (5). Other methods, such as the cage method (3), and the needleboard method (4) were developed for ease of use or to show more structure. However, all methods require a great deal of labor to remove physically the medium from the roots and often result in damage (6). Washing media from the roots damages smaller roots by either removing them with the medium or displacing them from their original orientation, causing them to cling to larger roots (1). There are currently no methods to visualize undisturbed 3-dimensional root systems.

Plants grown in soilless media consisting of coarse, inert material such as vermiculite, show a root distribution similar to that occurring in soil, while plants grown in other inert media such as sand or perlite, show root development similar to those grown in liquid cultures (2). Root growth and development often is studied in liquid culture to facilitate observation of the roots. However, any similarity to the 3-dimensional root distribution found in soil clearly would be coincidental.

An inert, transparent solid will seem to disappear when immersed in a liquid with the same refractive index. Flooding the root system of plants grown in a medium consisting of transparent glass, with a liquid having the same refractive index as the glass, will cause the medium to become transparent and allow the undisturbed roots to be viewed in their original 3-dimensional orientation. This paper describes such a technique for viewing roots.

Preparation of media. Glass used for the growing media must be transparent and free of inclusions and surface deposits. Safety plate glass (3.2 mm in thickness) was broken into small pieces and sieved. Pieces retained between the 3.2-mm and 1.5-mm screens yielded suitable-sized particles for growing plants. This procedure yielded about 1 liter of broken glass pieces per 22.5 cm^2 of sheet glass. Other types of glass proved unsuitable either because they contained small bubbles or surface deposits or because it was difficult to obtain enough glass pieces of the correct size.

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Immersion oil. A sample of broken safety glass was matched to an immersion oil of the same refractive index by immersing the glass in a series of oils with varying refractive indexes until the glass became invisible. Since the refractive index of both the glass and the immersion oil varies slightly with the wavelength of light, the immersion oil was matched against the glass using yellow light at 589.3 nm. R.P. Cargille Laboratories, Inc., (Cedar Grove, NJ 07009) matched an immersion oil to the refractive index of the glass, and furnished the immersion oil used in this study. The immersion oil that matched the safety glass had a refractive index of 1.516 at 25°C and was composed of a mixture of aliphatic and aromatic hydrocarbons.

Reflection, refraction, and dispersion of light makes it impossible to see an image clearly through a layer of broken glass pieces (Fig. 1). A rectangular grid can be seen through the glass container above the broken glass, but not through the top layer of broken glass. Immersion oil in the bottom of the container renders the broken glass in the bottom of the container transparent, so that the grid can be seen easily.

Cultural practices. Various horticultural crops were grown for up to 2 months in the broken glass medium in a rectangular glass container 10 × 10 × 20 cm deep (Fig. 1). Plants were subirrigated by flooding the container with solution for 3 min every 30 min through an offset hole in the bottom of the container. The container drained completely through the same hole; yet adequate solution adhered to the broken glass to permit growth without wilting of the plants between irrigations. Plants grown included apples, azaleas, boxwoods, carrots, chrysanthemums, cucumbers, lima beans, peas, potatoes, tomatoes, tulips, watermelon, and wheat. Microbial growth on the broken glass media and on the glass walls of the container was a problem when material was grown for over one month.

Adding the immersion oil. When a plant reached the desired developmental stage, the glass medium was rinsed 3 times with distilled water before all water was evaporated by forcing dry air through the offset hole in the bottom of the container. Evaporating all water was essential because water severely clouded the immersion oil. After drying overnight, enough immersion oil was added to the container to cover the glass. The plant whose root system is to be studied is killed by this technique because: 1) since the immersion oil is not water-soluble, all the moisture must be evaporated from the root system; and 2) the immersion oil is phytotoxic. A refinement of this technique would be the formulation of a nontoxic, water-soluble immersion liquid having the same refractive index as the clear solid support. This new immersion fluid would allow repeated observation of the same root system during development and growth, and would reduce the number of plants needed for growth studies using destructive sampling.

The viscosity of the immersion oil and the small pores in the media resulted in the trapping of many small air bubbles in the media. Application of a vacuum at ⅓ atm for a few minutes facilitated removal of these bubbles. The root system could be viewed or photographed in its undisturbed, 3-dimensional con-

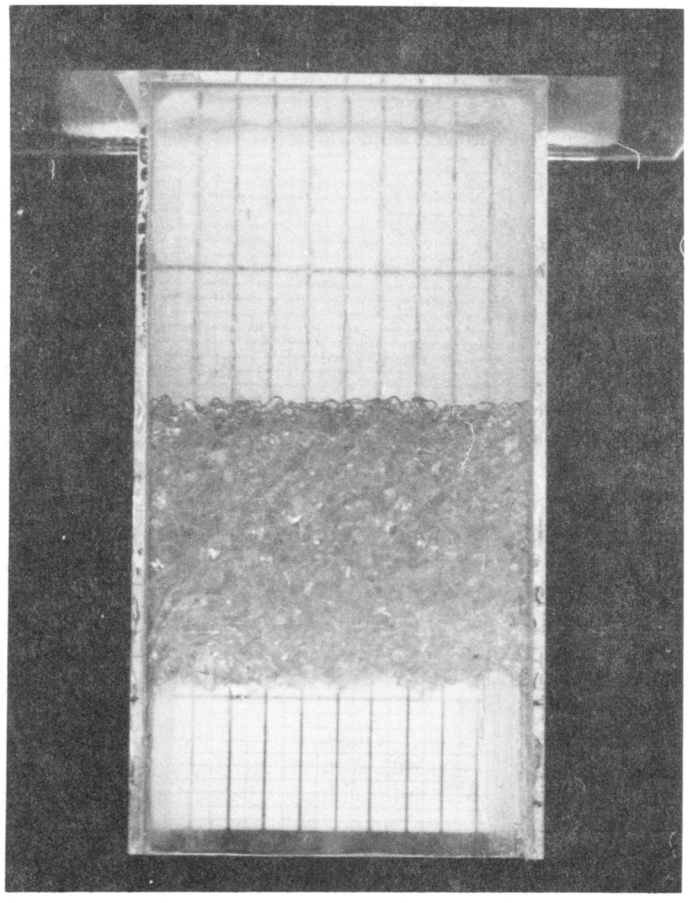

Fig. 1. Glass container (10 × 10 × 20 cm) filled with sized, broken glass pieces and half-filled with immersion oil having the same refractive index as the broken glass. Note transparency of bottom-half filled with immersion oil.

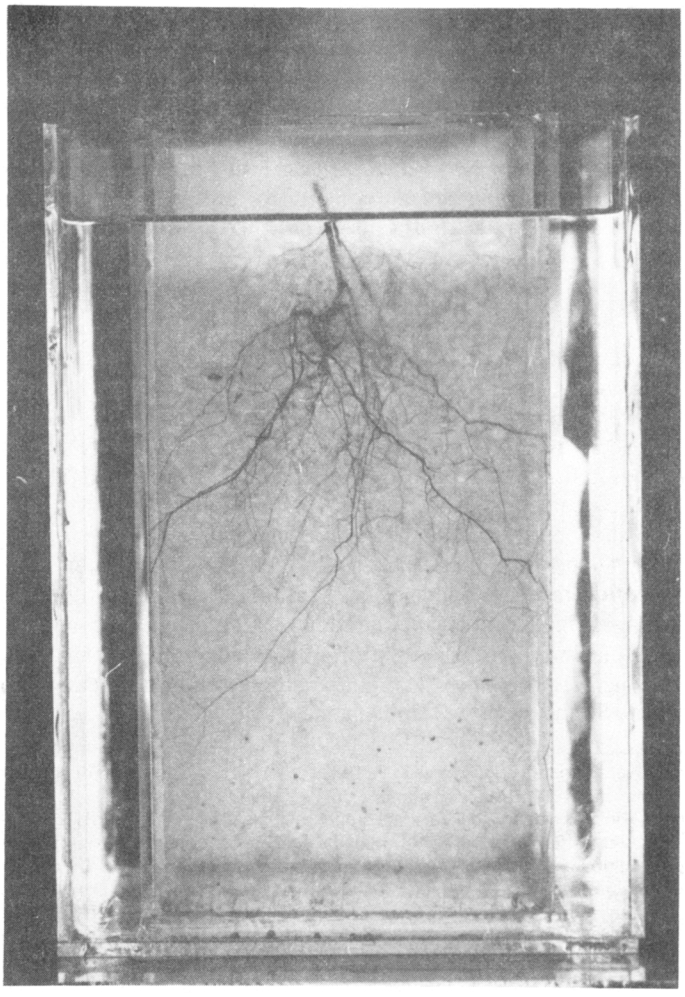

Fig. 2. Root system of an apple tree grown for 2 months in broken glass from a 3-week-old transplant. See materials and methods for procedure to render glass media transparent.

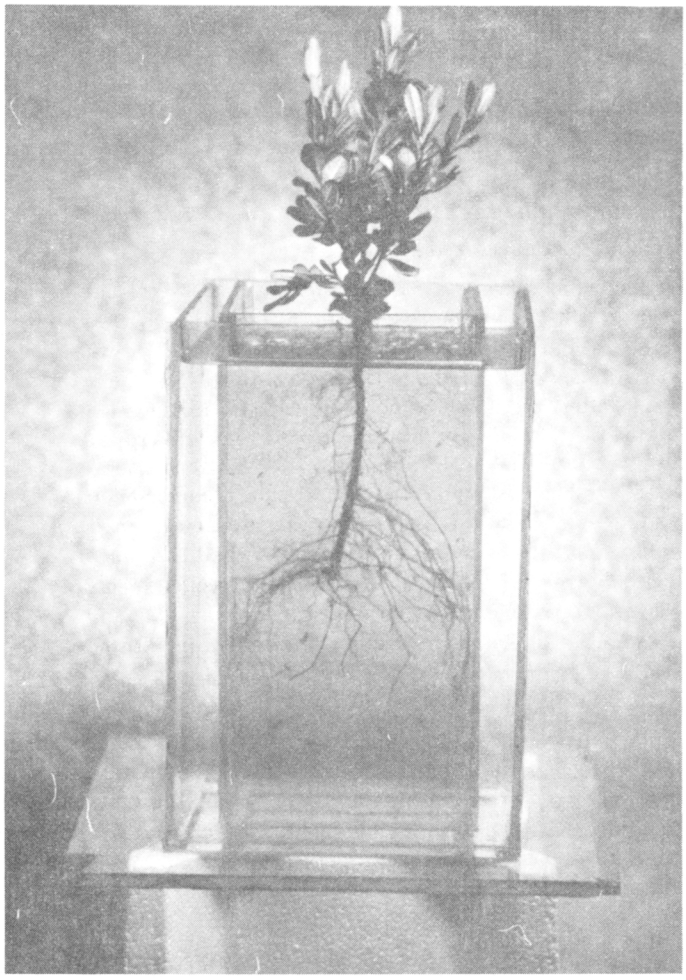

Fig. 3. Root system of a boxwood shrub grown for 1 month in broken glass from a 4-week-old transplant. See materials and methods for procedure to render glass media transparent.

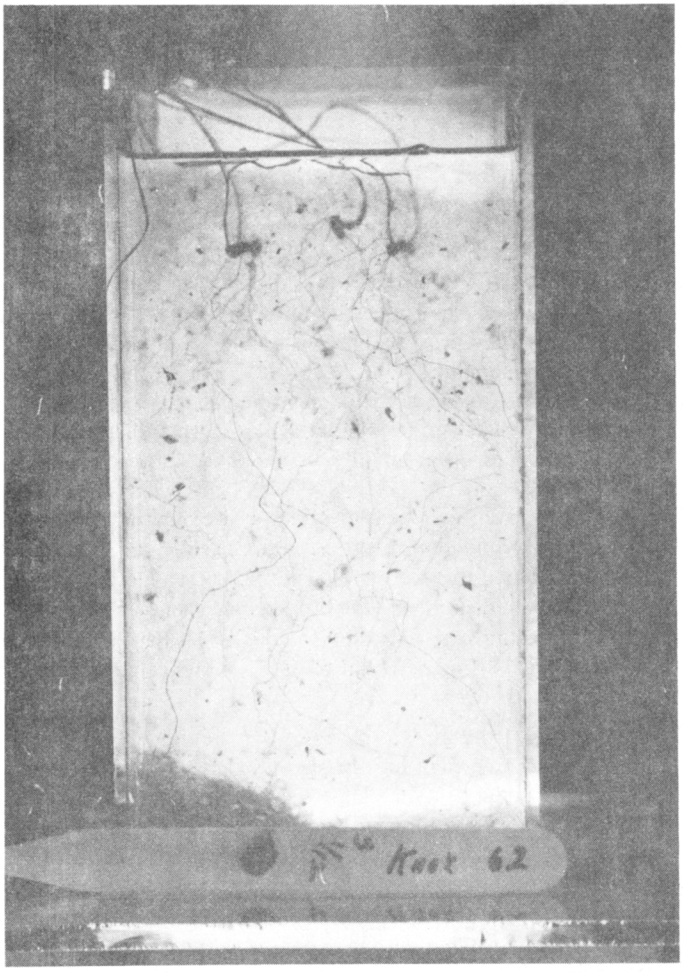

Fig. 4. Root system of wheat plants grown in tap water.

figuration a few minutes after the vacuum had been released. Application of a vacuum resulted in the evaporation of some of the more volatile components of the immersion oil, and a slow change in the refractive index of the immersion oil. If a few cycles of vacuum at ⅓ atm for around 5 minutes were used, then the change in refractive index was slight and the immersion oil could be used many times.

Viewing the root system. It was often necessary to experiment with lighting the chamber to get the best conditions for viewing and photographing the roots. Back lighting gave a distinct view of heavily pigmented roots such as apple (Fig. 2), azalea, and boxwood (Fig. 3). Other plants such as cucumber, tomato, pea, and wheat had very fine, colorless roots that were difficult to see and to photograph. Side plus back lighting made these fine, colorless roots more visible (Fig. 4), but lighting techniques alone cannot make small, translucent roots easy to see. A refinement of this technique would be the selection of a dye to stain roots and make them more easily seen.

Conclusion. The technique described in this paper for viewing the undisturbed 3-dimensional distribution of roots should facilitate the study of the effects of soil environment, chemicals, and microorganisms on root distribution. Differences in root distribution among cultivars and species should also be easy to study.

Literature Cited

1. Böhm, W. 1979. Methods of studying root systems, Springer-Verlag, New York.
2. Mac Key, J. 1973. The wheat root. Proc. 4th Intern. Wheat Genet. Symp., Missouri Agr. Expt. Sta., Columbia, Mo. p. 827–842.
3. Pittman, U.J. 1962. Growth reaction and magneotropism in roots of winter wheat (Kharkov 22 M.C.). Can. J. Plant Sci. 42:430–436.
4. Portas, C.A.M. 1973. Development of root systems during growth of some vegetable crops. Plant & Soil 39:507–518.
5. Rogers, W.S. and G.A. Booth. 1960. The roots of fruit trees. Scientific Hort. 14:27–34.
6. Welbank, P.V., M.J. Gibb, P.J. Taylor, and E.D. Williams. 1974. Root growth of cereal crops. In: Rpt. Rothamsted Exp. Sta. 1973, Part 2, p. 26–66.