

Pliable, Root-Permeable Layers for Separation of Portions of Experimental Plant Root Systems

Abstract. A description is given of simply made, sturdy, pliable layers which may be placed in rooting media for studies of divided root systems. Roots readily penetrate the layers but form a tight seal in doing so. The layer remains impermeable to water and ions.

In the study of plants under controlled conditions it is often necessary to separate portions of the root system of a growing plant by special layers in the root medium in order to confine ions, water, and microorganisms to the desired regions. In such instances it is frequently desirable to fabricate the layers from materials impermeable to ions, water, and microorganisms but readily penetrable by roots. Such materials have been reported (1). Some materials have been cumbersome and have had to be formed in place when needed. Thin layers are desirable but have not always been attained. This report describes a material which possesses required impermeability and which can be penetrated by roots; furthermore, it possesses strength and simplicity of design and forms layers which are reasonably thin.

The layers are composed of a 1:1 mixture (by weight) of paraffin wax and rosin. The substance is prepared by melting and mixing the two components in a common container. When the mixture has reached the boiling point, a quantity (75 to 80 ml, for example) of the liquid is poured on the surface of water which has been heated to 60°C. The water, ½ in. deep, is contained in a shallow pan (a pan 12 in. in diameter and 1½ in. deep has sufficed for this purpose). Water of this depth seems to exhibit a stability of surface desirable for preparation of the layers. The poured mixture is allowed to cool until firm.

The material, prepared according to these specifications of quantity of mixture, temperature, and size of pan, produces a layer about 11 in. in diameter and about 1 mm thick. The layer is of rather uniform thickness throughout, thinning out somewhat at the very edge. The thickness obtained is primarily a function of the initial temperature of the water: lower temperatures produce thicker layers. The best results were obtained by pouring the melt down a glass rod which was in contact with the water at one end. This eliminated the formation of bubbles in the layer. What temperature produces a layer of a given thickness depends upon the composition of the paraffin and rosin and is best determined by experimentation.

The cooled layer can be removed

from the pan by hand, molded, cut, handled, and pressed into shape to conform to a vessel, then sealed into place with a piece of heated metal or ceramic. A soldering iron has sufficed for this purpose. If the material is accidentally torn, repair by the same procedures is possible. When used in a pot experiment, the material showed no evidence of decomposition over a 3-month period. It appeared to have no toxic or inhibitory effect on the roots of either white clover (*Trifolium repens* L.) or common Bermuda grass (*Cynodon dactylon* L.).

The material appears to be impermeable to ions. In a study in which radioisotopes of strontium were used, no movement of the ions through layers of the wax material was observed. The material is impermeable to water. It appears, further, that a tight seal exists between the material and a root which has penetrated the layer. Penetration of roots has not produced measurable movement of water or ions past the layer.

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References

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Effect of Mitomycin C on Lateral Root-Tip Chromosomes of *Vicia faba*

Abstract. Lateral root-tip chromosomes of *Vicia faba* were treated with mitomycin C. Chromatid aberrations were observed from 24 through 96 hours after treatment. The effect of mitomycin C was not altered appreciably by changes in pH, temperature, oxygen tension, or lack of adenosine triphosphate. The relationship of mitomycin C to other radiomimetic compounds is discussed.

In two recent papers Sekiguchi and Takagi (1, 2) demonstrated two remarkable effects of mitomycin C. They showed an inhibition of deoxyribonucleic acid synthesis by phage-infected *Escherichia coli* in the presence of mitomycin C (1) and the production of what the authors termed "abnormal DNA" by bacteriophages infecting *E. coli* in the presence of a high level of mitomycin C (2). Sekiguchi and Takagi also observed that, although DNA synthesis was inhibited in *E. coli* during treatment with mitomycin C, there was

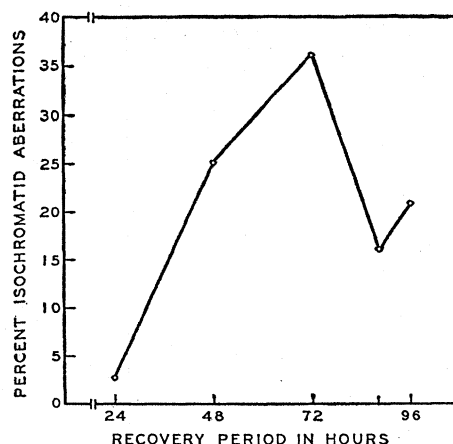


Fig. 1. Frequencies of isochromatid aberrations obtained at different fixation times after treatment.

no such inhibition of protein or ribonucleic acid synthesis (1). It seemed, then, that mitomycin C would be an excellent agent to use in determining whether the "rejoining system" would be inhibited in the absence of DNA synthesis, as it has been shown to be in the absence of protein synthesis (3). During tests to determine whether rejoining was in fact inhibited by mitomycin C, chromosomal aberrations were observed in root-tip cells treated with the agent alone. This is a report of the ensuing study of mitomycin C as a member of a rapidly increasing list of interesting radiomimetic compounds.

The material used in the experiments consisted of lateral root tips of the broad bean, *Vicia faba* (4). As has been previously described (5), the roots were grown in shell vials in the dark at 25°C for 7 days after an initial 24-hour period of soaking. The tap water was changed daily during growth and recovery (the latter being the time interval between treatment and fixation). Twenty-four hours before treatment the roots were placed in an incubator at 17°C. Treatments were carried out at 20°C, unless otherwise stated, and recovery took place at 17°C in the dark. The root tips were treated with 0.05-percent colchicine for 3 to 4 hours before fixation in cold alcohol and acetic acid (3:1), and slides were prepared as Feulgen squashes. The treatments were carried out in shell vials. The method for the introduction of gases has been described previously (6). Pretreatment with dinitrophenol (DNP) for 2 hours was carried out to test the effect of energy deprivation on the mitomycin C effect (7).

Various concentrations of mitomycin C were tried. It was found that concentrations of 0.1 percent, 0.01 percent for 1 hour, and 0.001 percent for 2 hours were lethal, death of cells occur-