

JIM WELLS: Case, I believe Zineb or Parzate sprayed on the plants as a preventative spray in May or June will correct this but you must get at it early.

H. HOITINK: Dithane works equally as good as Zineb for blackspot, it might be better to go to Dithane because we think it helps control die-back also.

MODERATOR FLEMING: Our next speaker is Dr. R. K. Horst of Cornell University. He spent 5 years doing tissue culture work for Yoders Bros. and though he's not doing much of this now at Cornell, his subject is "Modern Propagation with Tissue Culture."

MODERN PROPAGATION WITH TISSUE CULTURES

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It is fashionable today in modern day research (both animal and plant investigations) to relate research projects in some manner to tissue culture. Most of the interest in tissue culture has centered around the increased concern of mankind for the answers to the complex question of the cause and cure of cancer. Cancer or malignant tumors is one of the most feared diseases of man. Tissue culture has served as a means of studying the ways in which tumor-type growths develop and ways in which these types of growths can be retarded or inhibited. Tumor cells, on dividing, produce tumor cells regardless of whether one is dealing with plant or animal cells; however, the conversion to normal tissue growth from callus-type tissue growth has been done with certain plant tissues (4, 10, 11). It is the implication and importance of this type of technique which we want to consider in relation to the propagation of plant materials.

We want first to define what we mean when we talk about plant tissue culture. The organization and differentiation of cells into a specialized complex may be termed a tissue or more simply, a tissue is a group of organized cells. Meristematic tissue is often used in tissue culture work. This is a region of the plant where active cell division is occurring, such as the terminal bud of a plant. It is not always essential to use meristematic tissues. Pith tissues have been used with some success and this tissue is found in the center portion of the stem. Theoretically, one could use any tissue of the plant because each cell contains the genetic code for that particular plant. In order to simplify our discussion we will discuss only the use of meristematic tissues for tissue culture.

The terminal meristem is deeply imbedded within a terminal bud. Leaves grow around the very small meristem and tightly enclose it in a sterile-type growth environment. When

one carefully removes the leaves found in a terminal bud, the leaves are found to be smaller and smaller until an area can be observed where the leaves appear to be horn-like appendages on either side of a mound of cells. This can only be observed with the aid of a dissecting microscope. The horn-like appendages are the first leaves and the mound of cells are cells which have not yet differentiated into plant parts. A piece of meristematic tissue which is approximately 0.5 to 1.0 mm in size is obtained by cutting just below the first leaves. When this meristematic tissue is placed on a very specific synthetic medium, the cells will continue to actively divide and if the proper medium is utilized the cells will differentiate into plant parts such as leaves, stems, and roots. Several types of media have been utilized (3, 8, 13). In general, plant tumor cells will generate tumor cells and normal cells will generate normal cells.

The crown gall disease of plants is caused by a certain bacterium which can induce normal plant cells to transform to tumor cells within a matter of hours. Once this transformation has taken place, the tumor cells will proliferate of their own accord. Cells from a crown gall tumor will grow profusely and indefinitely on a simple culture medium without the influence of the bacterium or the plant. Normal plant cells will not grow on this medium; however, the conversion of callus tissue growth to normal tissue growth can be induced by manipulating the ingredients of the medium (1). The difference between tumor-type tissue and callus-type tissue is not fully understood. Tumor tissues are induced to form by an external stimulant such as a bacterium or a virus and the resultant cellular growth is uninhibited proliferation of cells which is completely undifferentiated and to the present time irreversible. Callus tissue type growth is also a proliferation of undifferentiated cells but no external stimulant is required and callus tissues can be induced to convert to normal tissue growth. An example of callus tissue growth is that cell growth which occurs over a plant wound. Callus tissue growth versus normal cell growth has been found to be due, in general, to the influence of hormones. There are two types of hormones which regulate cell growth and cell division. Auxins regulate the cell's growth in size, and the cytokinins act together with auxins to trigger cell division. The regulation of these hormones in a synthetic medium may well have a profound effect on the propagation of plant material.

The most important purpose of meristem tissue culture to the plant industry in the past has been the elimination of virus from infected plant tissues. Thermotherapy is used along with tissue culture to accomplish this purpose (5, 6, 7, 12). Virus infected plant material is exposed to continuous high temperatures such as 37°C (98°F). After a period of exposure, meristem tissue is taken from the heat exposed plant. The heat does not kill the virus but only inhibits the move-

ment and multiplication of the virus particles within the plant tissues so that terminal meristems are frequently virus-free.

Recently a great deal of interest has been induced in the orchid industry with the use of meristem tissue culture as a means of propagating orchid tissues. (9) This is seemingly going to revolutionize the orchid industry. A once long term procedure will be greatly accelerated. In addition, the possibility of obtaining virus-free orchid tissues along with the advantages to propagation gave added incentive to this effort.

The build-up of quantities of plant material in a test tube culture has also been an exciting possibility. Callus-like tissue growth can be induced to proceed in test tube culture at will. If this can be followed by breaking these callus-like growths apart and then obtaining a transformation of the various cell pieces to normal cell growth with resultant leaf, stem, and root formation, a rapid plant build-up can be obtained. The advantages are quite obvious. The space required to build up a large quantity of plant material in test tube culture as compared with greenhouse culture is held to a minimum. Greenhouse space costs would be greatly reduced in addition to the cost saving in watering, spraying, and fertilizing the plant material. The plant could be maintained disease-free indefinitely in the test tube. This type of culture also serves to eliminate wilt pathogens. Bacterial and fungal wilt pathogens readily grow on these synthetic meristem tissue culture media so that cultures exhibiting these types of growth can be eliminated. Some success has been obtained in performing the transformation from callus cell growth to normal cell growth with carnations (2) and work is presently proceeding to test chrysanthemum, geranium, and rose tissues.

Meristem tissue cultures may also be used to ship plant tissues across state lines and from country to country in a disease-free condition. Plant quarantines may be greatly simplified with the use of such a procedure in the future.

In conclusion, meristem tissue culture is a laboratory tool which in the near future may be used in the plant industry for propagation of plant materials. A tissue piece may be removed from plants aseptically and placed in a test tube on a specific nutrient medium. After approximately 2 weeks one may have an abundant callus cell growth and by placing the culture on a rotating wheel in order to keep the medium and cells agitated, the callus cells can be broken apart into small cellular pieces. These cellular pieces can then be transferred aseptically to another culture nutrient medium which would transform the callus cell growth to normal cell growth with resultant leaves, stem, and roots on each cellular piece. This may take another 2 weeks. These miniature plants may then be planted into pots in the greenhouse to grow on to mature plants.

LITERATURE CITED

- 1 Braun, A. C. 1965. The reversal of tumor growth. *Scientific American* 213:75-83.
- 2 Hackett, W. P. and J. M. Anderson 1967. Aseptic multiplication and maintenance of differentiated carnation shoot tissue derived from shoot apices. *Proc Amer. Hort. Soc.* 90 365-369
- 3 Halperin, W. and D. J. Philips 1964. Obtaining pathogen-free stock by shoot tip culture. *Phytopathology* 52 1242-1244.
- 4 Heller, R. 1953. Recherches sur la nutrition minerale de tissus vegetaux cultives in vitro. *Ann. Sci. Nat Bot Biol. Veg* 14 1-223
- 5 Kassanis, B. 1954. Heat therapy of virus-infected plants. *Ann Appl. Biol* 41:470-474
- 6 Morel, G. M. 1960. Producing virus-free Cymbidiums. *Am. Orchid Soc. Bull.* 29:494-497
- 7 Morel, G. M. 1964. Tissue culture — a new means of clonal propagation of orchids. *Am Orchid Soc. Bul.* 33 473-478.
- 8 Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant* 15 473-497
- 9 Sagawa, Y., T. Shoji and T. Shoji. 1966. Clonal propagation of Cymbidium through shoot meristem culture. *Am. Orchid Soc. Bul* 35 118-122
- 10 Skoog, F. and C. O. Miller 1957. Chemical regulation of growth and organ formation in plant tissues cultivated in vitro. *Soc Exper Biol Symp* 11:118-131
11. Steward, F. C. et al 1958. Growth and organized development of cultured cells. I. *Amer. J. Bot.* 45 693-703. II. *Am J. Bot.* 45 705-708 III *Am J. Bot* 45:709-713.
- 12 Stone, O. M. 1963. Factors affecting the growth of carnation plants from shoot apices. *Ann Appl Biol.* 52 199-209.
13. White, P. R. 1963. *The Cultivation of Animal and Plant Cells*, 2nd ed. Ronald Press, New York

MODERATOR FLEMING: Thank you very much Dr. Horst. Any questions?

GUS MEHLQUIST: Do you know of anyone who has successfully used this method to propagate typical woody plants? I could not find anything in the literature.

KEN HORST: No I do not but we are trying to do some work with roses at the present time. I don't know if we will be successful but I think if we had the proper medium it could be done.

MODERATOR FLEMING: Our next speaker is Dr. L. V. Edgington from the University of Guelph where he has been working for the past 3 years with systemic type fungicides and his subject this morning concerns systemic fungicides: Dr. Edgington.

CONTROL OF PLANT DISEASES WITH SYSTEMIC CHEMICALS

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A chemical which is taken up by a plant and transported within the plant may be considered as systemic. If the chemical controls diseases of the plant caused by fungi, bacteria,