AN AUTORADIOGRAPHIC TECHNIQUE FOR DETAILED STUDIES WITH PLANT TISSUE

W. M. DUGGER, JR. AND D. E. MORELAND

DEPARTMENT OF BOTANY, UNIVERSITY OF MARYLAND, COLLEGE PARK, MARYLAND AND COLLEGE OF FORESTRY, STATE UNIVERSITY OF NEW YORK, SYRACUSE, NEW YORK

Received May 5, 1952

Within the past few years, reports in the literature indicate the increasing use of detailed autoradiographic techniques in problems concerned with the location of areas of deposition or concentration of radioactive isotopes in animal tissue. The usefulness and value of these techniques has increased with the availability of special nuclear track emulsions and the development of suitable laboratory procedures. These autoradiographic techniques have not been fully utilized in studies of plants although ARNON et al. (1), COLWELL (2), HARRISON et al. (8), and GROSS and SNIYDER (6) have used the gross or survey autoradiographic techniques. Several procedures have been used in conjunction with animal studies; and with minor modifications, these can be adapted to plant materials. A general review of these techniques as applied to animal studies has recently appeared in the literature (7). One of the techniques provides for a permanent mounting of the histological sections on the photographic emulsion throughout the exposure and processing periods, and it is this one that was used in the study. This method was first used with animal tissue by ENDICOTT and YAGODA (4) and EVANS (5). Microscopic examination with both high-dry and oil-immersion objectives is possible, and the deposited silver grains can be correlated with the tissues and cells containing the radioisotope.

Sections taken from Hydrangea leaves that had been exposed to an atmosphere containing radioactive carbon dioxide (C\textsuperscript{14}O\textsubscript{2}) for four hours were used in this study. Details of the chamber which was used and the procedure which was followed have been previously reported (3). At the end of the period of photosynthesis, the leaves were separated from the plants and rinsed with dilute hydrochloric acid to remove adsorbed C\textsuperscript{14}O\textsubscript{2}. Small strips were cut from the leaves and placed in an FAA fixing solution. The leaf tissue was then dehydrated and embedded in paraffin following usual procedures. Sections 15 microns in thickness were cut on a rotary microtome. Paraffin ribbons of the desired length were expanded in warm water at 42° C. Following expansion, the ribbons were transferred to water at 18 to 20° C. The ribbons hardened at these lower temperatures and could be handled more easily during subsequent operations.

In the dark room, Eastman nuclear track plates of the NTB or NTB2 type were soaked in distilled water at 20° C for five minutes. The ribbons were removed from the water with a section lifter or a small hair brush and placed on the swollen emulsion. This manipulation was conducted under a Wratten safelight, series 2. Soaking the plates prior to placing the ribbons in contact with the emulsion ensures good adhesion between sections and

143
emulsion and eliminates the use of an adhesive. With the sections in place, the plates were positioned on top of small corks in light-tight boxes. Calcium chloride was placed in the bottom of the boxes as a desiccant, and the plates were stored in a refrigerator for the period of exposure.

At the end of the exposure period the paraffin was removed by immersing the plates in xylene for 10 minutes. The xylene was then allowed to evaporate completely from the plates. Plates were developed without agitation in Kodak D-19 developer for 20 minutes at a temperature of 20° C. The plates were then rinsed in water, fixed in Kodak F-5 for twice the clearing period, and washed in running tap water for one hour. Immediately following washing, and without permitting the plates to dry, the sections were stained in Harris' haematoxylin for 20 minutes. Destaining in acid water can be used to reduce the intensity of the stain wherever necessary. Selection of the appropriate time interval will eliminate this step. Sections were then blued in alkaline water to the desired degree. The plates were passed through a dehydration series and were counterstained for approximately one minute with either eosin, yellowish-acid, or erythrosin, bluish-acid. The final xylene-alcohol wash of the dehydration series had been saturated with the desired stain. The use and selection of a counterstain will depend on the requirements of the worker. The plates were transferred to xylene and mounted immediately in balsam which produced a slide with a stained tissue section mounted permanently on the emulsion.

Figure 1 is a photomicrograph of a transverse section of a Hydrangea leaf. The leaf was exposed to 1% C\textsuperscript{14}O\textsubscript{2} for four hours at a light intensity of 260 foot-candles. The section was mounted on the emulsion and proc-
essed as outlined in the preceding section. Cellular detail can be observed in this focal plane. Shifting the focus to the underlying emulsion, as in figure 2, shows concentrations of silver grains corresponding to cellular fixation of the C\textsuperscript{14}. The stained cellular material of the leaf is easily distinguishable from the reduced silver grains of the autoradiograph. The leaf sections were 15 microns in thickness and were mounted on Eastman NTB2 plates having an emulsion thickness of 10 microns. The exposure interval was 26 days. The most dense concentration of deposited silver grains is correlated with cells of the palisade region. Untreated control sections showed only the normal background of grain formation. Green and red filters were used in preparing the photomicrographs to accentuate cellular details and the deposited silver grains.

The use of the isotope C\textsuperscript{14} as a source of radiation incorporated in plant material is ideal for autoradiographs. No special technique is required to prevent diffusion of C\textsuperscript{14} from the tissue during the preparations if it is in a fixed condition. The long half-life of C\textsuperscript{14} eliminates the necessity for rapid processing, and it is possible to use long exposure periods when the isotope is present only in minute quantities. In addition, the relative consistence in radiation activity permits accurate estimate of the time required to obtain an image of the desired density.

The authors wish to acknowledge the assistance of the Office of Naval Research and the Atomic Energy Commission in conjunction with this study.

LITERATURE CITED