it eliminates many of the difficulties attendant upon experiments involving hanging drop cultures.

CHARLES E. HADLEY

ZOOLOGICAL LABORATORY,
HARVARD UNIVERSITY

A MICRO-TECHNIQUE FOR OBSERVING OIL PENETRATION IN CITRUS LEAVES AFTER SPRAYING

THE rapidly increasing use of white oil of high viscosity in spraying for the control of scale pests of citrus has been followed in many instances by injurious effects, such as fruit drop, leaf drop, dead-wood, reduction of bloom and reduction of crop, etc.; hence the need for a careful study, by means of the microscope, of the penetration and disposition of oil within the plant tissue.

The problem presented several difficulties. The exigencies of the case precluded the employment of the ordinary solvents used in histological technique. It was necessary to fix or clear the specimen without disturbing or dissolving the oil. Consequently, the use of alcohol, xylol or any other of the essential oils was prohibited, as was also the paraffin method of imbedding which necessitates infiltration with chloroform or ether.

It was desirable to examine both flat sections, cleared and stained, and also fixed cross-sections. This has been accomplished by the use of an aqueous solution of pyridin to dissolve chlorophyll, and Oil Red O[1] dissolved in aqueous-pyridin as a stain.

The technique is substantially as follows:

For Flat Sections:

Immerse specimen in a 60 per cent. aqueous solution of pyridin (60 parts pyridin to 40 parts distilled water).

Heat over water bath. When discolored pour off and refill. Repeat till solution remains clear and specimen becomes transparent. (This can be conveniently done with the use of a small shell vial inserted through the center of a flat cork and floated on water bath.)

Immerse in saturated solution of Oil Red O in 70 per cent. pyridin (70 parts pyridin to 30 parts distilled water) for 24 hours.

Differentiate in 50 per cent. pyridin, until stain ceases to stream. (This takes but a few minutes.)

Wash in running water one hour or more.

Pass through, first glycerine water (equal parts). Second glycerine.

Clear in carbol-glycerine (1 part carbolic acid to 2 parts glycerine). Heat gently in watch glass and observe carefully under dissecting microscope till clear. Specimen should be turned under side up, when oil drops can be distinctly seen.

Pass through glycerine again. (This is important and prevents clouding on the slide.)

Mount in glycerine jelly. Allow to harden and seal with clear Duco.

For Cross-Sections:

Fix in chrome-acetic acid 48 hours.

Wash in running water.

Immerse in 5 per cent. formalin 1 hour.

Wash in running water.

Immerse in 50 per cent. pyridin 10 minutes.

Stain in saturated solution of Oil Red O in 70 per cent. pyridin 24 hours.

Differentiate in 50 per cent. pyridin until color ceases to stream. (Watch carefully.)

Wash in running water.

Section in pith. (The freezing method might be used to advantage but has not been tried.)

Pass through, first glycerine water (equal parts). Second glycerine.

Mount in glycerine jelly and seal with clear Duco.

The oil stains a bright orange to deep red, depending upon the length of time allowed for the staining process. Heavy oils take longer to stain than light. Essential oils, lipoids and other fatty bodies, as well as cutin, also stain but are readily distinguished from the oil. Essential oils are confined to certain well-defined oil cells; they do not occupy the intercellular spaces. Lipoids and other fatty bodies stain deep scarlet, almost black. Cutin stains yellow and, if oil soaked, orange to red.

By employing this technique the writer has been able to observe the penetration of oil into the leaf, its translocation through the vascular system into the stem and across the medullary rays to its final deposition in the storage cells of the pith and old wood fibers. Oils of high viscosity choke the vascular system, to a greater or less degree depending upon the amount of oil, for an indefinite period of time.

HUGH KNIGHT

GLENDORA, CALIFORNIA

[1] F. Proescher, "Oil Red O a Rapid Fat Stain," *Stain Technology*, Vol. 2: 60.

SPECIAL ARTICLES

ON THE STRUCTURE OF THYMONUCLEIC ACID

THE plant nucleic acid is regarded as a tetranucleotide, each nucleotide being composed of phosphoric acid, a sugar (ribose) and a nitrogenous component. The evidence for this theory of structure is complete, inasmuch as it was possible to decompose the nucleic acid into the individual nucleotides, and each of the nucleotides into phosphoric acid and the complex consisting of the sugar and a base.

For the thymonucleic acid an analogous structure was suggested. The evidence, however, was incomplete, since it was impossible to decompose by chemical means the thymonucleic acid in such a manner