POLARIZED LIGHT IN VEGETABLE HISTOLOGY.*

BY C. W. BALLARD, A.M.

Many of our standard references on vegetable histology, while making mention of the polarizing apparatus, do not give much space to the effects of polarized light upon the different plant tissues. Illustrations of starch grains and crystals viewed under this form of illumination are to be found in many of these works, but further details are lacking. As the effects of polarized light are so minutely described in texts upon the microscopy of minerals and microchemical analysis, it has occurred to the writer to consider very briefly the appearances of some plant tissues and their contents when observed through the polarizing microscope.

There are several types of polarizing apparatus supplied by microscope manufacturers. The apparatus is generally separable from the microscope unless the instrument is designed for petrographic work exclusively, in which case the polarizer is built in the body tube. Of separable polarizers, the type which has a fixed analyzing prism and a revolving polarizer adjusted above the ocular is preferred to that in which the polarizer is inserted above the objective. The latter form can only be used on instruments having a revolving stage and, unless used with special objective clamps, hinders free changing from one power to another. While a graduated revolving stage is necessary in critical operations, it is not indispensable in the average work of the vegetable histologist. The most satisfactory objective for use in this work is the 8 mm. (No. 3), although the 5 mm. (No. 4) may be used. Higher powers than the 5 mm. do not give satisfactory results as the field, with crossed prisms, is too dark to note details. It is better to use a comparatively low objective with a high ocular. The combination used in this work was 5 mm. (No. 4) objective with 10 x (No. 5) ocular. Polarization work may be performed by artificial light if a ground glass screen is placed between the source of light and the mirror of the microscope. Best results are obtained with a 100-watt concentrated tungsten filament lamp, hooded so as to shield the stage and the eyes of the observer.

From the standpoint of polarization, vegetable tissues and cell contents are divided into (*a*) those which have no effect upon the light rays reflected through the apparatus, and (*b*) those which modify the direction or speed of vibration of these rays. Materials in subdivision (*a*) are termed isotropic; those in subdivision (*b*), anisotropic. Among the points of interest or of direct value in the examination of vegetable substances by polarized light, the three here stated are perhaps the most essential:

1. Partial or total extinction, or disappearance of the object when prisms are rotated.
2. Markings observed upon rotation of the prisms.
3. Coloration (pleochroism) upon rotation, in some cases running through the shades of the spectrum.

Partial or total extinction may be due to the thickness of the object, as it has been observed that fiber-masses, which ordinarily would not extinguish, are invisible under crossed prisms if the mass is thick or the light weak. Among the tissues which undergo partial or total extinction are cork, epidermis, aleurone, resins and volatile oils. The markings observed under crossed prisms in differ-

* Read before Scientific Section, A. Ph. A., Atlantic City meeting, 1916.

ent cellular elements or cell contents are in some instances very characteristic and may well be considered diagnostic. Starch grains, mucilage deposits and crystals are among the elements which exhibit characteristic markings upon rotation of the prisms. Many substances and cellular elements show wonderful color changes during rotation. We may classify fibers by their response to this test. The true isotropic crystals, or those classified as isometric, should not show coloration. Crystals of all classes, with the exception of the isometric, will exhibit a color change during rotation of the prisms.

PLANT TISSUES UNDER POLARIZED LIGHT.

Cork.—The masses of cork tissue in the powders examined were in most instances too thick for positive determination. In sections with corky layer attached, the only change under crossed prisms was a clearer differentiation of the individual cell walls.

Specimens examined: Powdered Cascara, Xanthoxylum and Quebracho; sections of Cascara, Xanthoxylum and Oak.

Root Epidermis.—No change could be observed in either powdered specimens or sections. Tissue totally extinguishes under crossed prisms.

Specimens examined: Powdered Sarsaparilla, Belladonna and Calumba; sections of Sarsaparilla, Belladonna and Calumba.

Collenchyma.—This element is usually difficult of differentiation because of the small intercellular spaces. Under polarized light the cell-walls and limits are clearly defined.

Specimens examined: Sections of Peppermint Stem.

Conducting Tissues (Vessels, Tracheids and Medullary Rays).—The only result observed in the conducting tissues when viewed by polarized light is a general clearing of the structural details. Such tissues show no signs of extinguishing. The crossed prisms, by diminishing the light ordinarily appearing through the porous parts of the vessel, serve to clearly define the pores, both border and central, with any markings present.

Specimens examined: Powdered Sarsaparilla, Belladonna, Hydrastis, Squill, Spigelia and Quassia; sections of Sarsaparilla, Quassia and Pumpkin Stem.

Trichomes (Plant Hairs).—Details of structure are cleared, as are junction points in multicellular and multiseriate types. Extinction as well as coloration characterizes some trichomes.

Specimens examined: Powdered Digitalis, Lobelia, Peppermint, Cannabis, Senna, Mullein, Eupatorium and Salvia.

Fibrous Tissues.—Aside from clearing details of structure, the most practical use of polarized light in dealing with fibers, depends upon the phenomenon of pleochroism. We may separate the fibrous tissues into pleochroic and non-pleochroic classes. Cinchona fibers exhibit this property to the greatest extent, giving many beautiful shades of color. Cinnamon fibers exhibit but slight color change under crossed prisms, while fibers of cotton root bark show no trace of coloration under this manipulation. None of the fibers examined showed complete extinction. The crystal-bearing fibers are seldom pleochroic, although the crystals contained therein may exhibit coloration. Polarization serves admirably to clear questions of presence or absence of crystal-sacs and defines accurately the outlines of the crystals.

Specimens examined: Powdered Cinchona, Cinnamon, Cotton Bark, Quassia, Sandalwood, Scoparius, Licorice, Cascara, Frangula, Quebracho; sections of Cinchona, Cinnamon, Quassia, Licorice, Cascara, Frangula.

Stone Cells.—Some types exhibit a slight but definite coloration, but the majority of specimens examined were lacking in this quality. Accurate definition of pores and striations may be secured. Thick masses, as those occurring in cubeb, are apt to extinguish.

Specimens examined: Powdered Ruellia, Cinnamon, Cascara, Aconite, Cubeb, Physostigma, Pimenta, Chionanthus, and Oak.

CELL CONTENTS UNDER POLARIZED LIGHT.

Cystoliths.—These amorphous calcium carbonate deposits present the appearance of white granular aggregates in the dark field of crossed prisms. In water mounts of powders containing cystoliths there is sometimes a purplish tinge to the mass; this coloration is more pronounced when they are viewed with an instrument in which the analyzer has been substituted for the usual condenser. Cystoliths if very thick will extinguish under crossed prisms.

Specimens examined: Powdered Cannabis Indica and Ruellia; sections of Cannabis Indica Leaf, Ficus Leaf and Ruellia.

Inulin.—Fragments of this substance appear as white masses in the dark field. Neither coloration nor characteristic markings were observed.

Specimens examined: Powdered Inula, Taraxacum and Lappa; sections of Taraxacum and Lappa.

Aleurone.—Cells containing this substance become entirely dark or completely extinguish under crossed prisms.

Specimens examined: Powdered Fenugreek and Mustard; sections of Mustard.

Oil Globules.—The globules of volatile oil in the specimens examined totally extinguish in the dark field.

Specimens examined: Powdered Cubeb, Pepper and Mustard.

Crystals.—It is difficult to apply principles of optical or chemical crystallography to the crystalline deposits of calcium oxalate occurring in plants, because of the many transition forms and fragments present. The location of a perfect crystal in vegetable powders is rather the exception. The crystals of senna and cascara, which the vegetable histologist would without hesitation classify as cubical, in a majority of instances do not react to polarized light as isometric or cubic crystals should. Isometric crystals are classed with the isotropic bodies and should not undergo change during rotation of the prisms. Most so-called cubic crystals in plant tissues would be classified by the crystallographic worker as anisotropic bodies by reason of the changes occurring during rotation of the prisms. This apparent lack of agreement with laws of crystal formation, in the case of these cubic crystals, may be due to limitations of crystal growth by the cell membranes in the living plant. In powdered drugs it might be occasioned by partial fracture of the crystals during grinding. All plant crystals undergo more or less coloration during rotation of the prisms. Coloration is most pronounced in the case of the acicular and the larger prismatic crystals. In the dark field prismatic crystals usually exhibit several black bands running parallel with the visible outer boundaries of the crystal. Cubic crystals usually show one black band parallel with the visible boundaries. The acicular, rosette and microcrystalline types did not show these bands. Rosette and microcrystals may or may not show coloration in the dark field.

Specimens examined: Powdered Senna, Licorice, Frangula, Cascara, Quillaja, Sarsaparilla, Belladonna, Squill, Rhubarb, Castanea, Polygonatum and Quebracho; sections of Sarsaparilla, Licorice, Cascara, Belladonna, Phytolacca and Rhubarb.

Starch.—The main characters to be observed in the examination of starches by polarized light are striations, positions of the crossed lines in the light and dark fields, and comparative width of the arms of the crossed lines. These cross lines are characteristic of all starches. The intersection point of the lines is always through the hilum or anatomical center of the grain. The outline of the cross depends to considerable extent upon the outline of the grain. The width of the angle formed by the cross lines and the relative widths of the arms appear to be fairly constant for each starch. As starches of botanically related drugs show similarities in the shape and formation of the hilum, so do their polarization figures agree. When the prisms are slowly rotated, the cross lines follow the rotation and sections of the grain which were white under crossed prisms will assume a dark color; the cross lines under these conditions will be outlined in white. Striations appear to be accentuated when viewed by polarized light. In granules having an oval outline, the polarization figure always extends in the direction of the long axis. If the grain is spherical the arms of the figure are usually equidistant from each other and their point of intersection is in the center, corresponding with the hilum. Water mounts will show to best advantage, although permanent mounts may be used.

Specimens examined: Wheat, Rye, Corn, Barley, Maranta and Potato Starches, Aconite, Hydrastis, Cimicifuga, Sarsaparilla, Colchicum, Galangal and Iris Starches.

Mucilage Cells.—Materials to be examined for mucilage are best mounted in glycerin, as an aqueous medium may affect the mucilage deposits. This substance when viewed by polarized light gives effects similar to those observed in the examination of spherical starch grains. The arms of the cross lines are equidistant from each other and always at right angles. The figure is that of a maltese cross. The cross lines change from light to dark upon rotation. Under polarized light in dark field, the mucilage sacs of powdered mustard are readily visible even in very thick mounts.

Specimens examined: Powdered Mustard and Ulmus.

Resins and Gum Resins.—None of the resins or gum resins examined exhibited characters of note when viewed by polarized light. The resin masses in the powdered drugs examined extinguish under crossed prisms.

Specimens examined: Powdered Jalap, Sanguinaria, Serpentaria, Gamboge, Myrrh.

In summarizing we may conclude that the polarizing apparatus is of value in determining minute structural details which in many instances would otherwise escape observation. In sections where the cells are in such close proximity that intercellular space is apparently lacking and cell-walls appear to be continuous, the actual details may be easily and rapidly determined by the apparatus. The writer has found polarized light a readily applied and certain aid in micro-analyses involving determinations of starches, crystals, fibers and mucilage-sacs.

DEPARTMENT OF PHARMACOGNOSY,
Columbia University School of Pharmacy.