Pacific Southwest
Forest & Range
Experiment Station

P. O. BOX 245
BERKELEY, CALIFORNIA 94701

1968

U. S. Forest Service
Research Note
PSW-170

ABSTRACT: Fresh and chemically fixed sectioned plant material can be quickly stained by applying a Brook Cyte-Chrome I polychrome stain. Staining time averaged only about 10 minutes. And exact timing of staining and de-staining is not as critical as with most of the commonly used stains. The over-all quality is comparable to that of the traditional stains.

RETRIEVAL TERMS: dyes; microscopy; photomicrography; staining.

OXFORD: U598.65:U778.31.

Forest Service
U.S. Department of Agriculture

A Useful Single-Solution Polychrome Stain for Plant Material . . . Brook Cyte-Chrome I[1]

STANLEY L. KRUGMAN JULIA F. LITTLEFIELD

Several of our current studies in forest genetics require the staining of large numbers of slides of chemically fixed plant material. Many excellent staining procedures for plant material are available, but most of them involve long and elaborate techniques.[2,3] To make staining easier and faster, we tried using several rapid staining methods in which a single solution containing a mixture of dyes was used. We found the rapid fast green-safranin counter staining method of Gram and Jorgensen[4] to be a useful technique for staining fresh or frozen material, but quality proved to be inconsistent in the staining of chemically fixed plant material. We then tried Brook Cyte-Chrome I stain (BCC),[5] a commercial multiple-stain developed for exfoliative cytology. It has proved to be rapid and efficient in staining many types of plant material.

MATERIAL AND METHODS

We routinely use BCC for sectioned material of developing and mature pine (*Pinus* sp.) seeds and needles of various pines and firs (*Abies*). To determine how well it stains other plant material, including fungi, we have also prepared and stained sections of woody branches of red fir (*Abies magnifica* A. Murr.) infected with dwarf-mistletoe (*Arceuthobium campylopodum* Engelm.), the roots and hypocotyls of Monterey pine (*Pinus radiata* D. Don) seedlings infected with *Verticicladiella wagenerii*

---

[1] Trade names and commercial enterprises and products are mentioned solely for necessary information. No endorsement by the U.S. Department of Agriculture is implied.

Kendrick, and needles of sugar pine (*Pinus lambertiana* Dougl.) infected with *Hypodermella arcuata* Derker. In each case, the plant material was first chemically fixed with either FAA (90 ml. of 50 percent ethyl alcohol, 5 ml. of glacial acetic acid, 5 ml. of formalin) or Belling's modified Navashin's fluid (solution A: 5 g. of chromic acid, 50 ml. of glacial acetic acid, 320 ml. of water; solution B: 200 ml. formalin, 175 ml. of water; equal volumes of solutions A and B mixed just before fixing).

After fixation, the plant material was embedded in Tissuemat and cut into serial sections 10 to 12 μ thick. A portion of the sections stained with fast green and safranin[3] served as a check of the staining quality of the BCC stain.

A set of similar sections was then stained with BCC using the following procedure for paraffin-embedded material:

1. Deparaffinize sections in xylene for 2 or 3 minutes.
2. Rinse the deparaffinized sections in 50 percent xylene, 50 percent ethanol (V/V) for at least 2 minutes. Agitate the slides to remove any remaining paraffin.
3. Rinse sections in a solution of 20 percent water, 80 percent isopropanol (V/V). Only two to five dips were necessary here.
4. Rinse in 75 percent water, 25 percent isopropanol (V/V). Again, only a few dips were needed.
5. Take care to see that the stain is thoroughly mixed when poured from stock solution and at the time the slides are placed in the solution; otherwise, the plant material tends to stain predominantly blue. We found that 2 minutes in undiluted stock BCC were enough to stain adequately most material. We have found it useful to place the dish containing the stain on a magnetic stirrer both before and during staining. For some material, such as the female gametophyte of the pine seed, longer periods (3 to 4 minutes) in the stain are needed, so various staining periods should be tried first.
6. After adequate staining, rinse the slides for 30 seconds in 3 percent HCl in absolute isopropanol. This rinse can serve as a destaining procedure, if needed, but it also sharpens the staining of the nucleus. Care must be taken because after 30 seconds the cytoplasm will destain quickly.
7. Rinse the stained slides for 1 minute in absolute isopropanol, and then dip them in xylene.

Staining time was about 10 minutes when the above procedures were followed.

The slides are now ready for coverslip mounting. The manufacturer of this stain recommends that heat not be used with smears in the mounting procedure, since heat catalyses the development of blue color. It recommends the use of "Apochromount,"[6] a relatively rapid-drying mounting medium. We found that Apochromount was useful in that the slides could be handled within 20 to 30 minutes after mounting. No loss in staining quality of plant material was found, however, when the slides were heated to less than 40°C. after mounting in "Apochromount" or with either "Piccolyte" or "Permount."

BCC was also used to stain fresh fir and pine needles frozen and sectioned in a cryostat. With fresh material, we started at step 4 with rinsing in 75 percent water, 25 percent isopropanol. In step 5, the time in the stain varied from 1 to 3 minutes, depending on the desired intensity of cytoplasm staining. We then proceeded as before through steps 6, 7, and the mounting described above.

RESULTS

BCC stained to some degree all the types of plant material examined except the hyphae of the fungus *Verticicladiella wagenerii*. Cell walls generally stained blue to pale violet or pale yellow, and the nucleus stained red to orange. The color of the cytoplasm after staining was highly variable, ranging from pale blue in the pine female gametophyte to deep green for the cells of the transfusion tissue and mesophyll of needles.

In the fir branch material the cell walls of the tracheids stained a pale yellow while the sieve cells were a deep purple to a dark blue. However, the stain tended to understain the tracheids. The walls of the phloem ray parenchyma stained a blue green, which readily separated it from the surrounding cells. The cortical cytoplasm stained green in contrast to its purple cell walls.

The infected hypocotyls of Monterey pine had these same staining features. The tracheids stained a light yellow and the sieve cells of the phloem stained a deep blue. Unlike the woody branches material the cortex stained purple. The fungus *Verticicladiella wagenerii*, which infected these pine seedlings, was not stained by BCC. The hyphae could be distinguished, however, because of their natural brownish color and the distinctive staining of the host tissue.

DIFFERENTIAL STAINING

In both the pine and fir needle sections there was useful differential staining of cell parts. The outer walls of the characteristically lignified epidermal cells stained a light yellow, as did the cuticle. The outer layer of the wall of the fiber-like cells of the hypoderm was also stained yellow, while the inner layer stained a lavender color. In contrast, the mesophyll cell walls were stained blue green. The portion of the endodermis cell wall toward the outside of the needle stained a light blue while the portions toward the inside stained yellow. The cell walls of the transfusion tissue were mostly pale violet and the walls of the phloem sieve cells a light blue. This differential staining of the cell walls was also typical of the vascular tissues in the hypocotyl and woody pine branches. In general then, BCC stained lignified, cutinized, and suberized walls a yellow color.

In the above material the cytoplasm stained green, brown or even yellow. The cytoplasm of the epidermal cells stained green, that of mesophyll cells stained green, brown or yellow, and that of the phloem sieve cells a light blue.

GENERAL STAINING

The general staining of cell walls and cytoplasm by BCC proved helpful in distinguishing the extent of mistletoe penetration in the woody branches of red fir. The cytoplasm of the advanced cells of the mistletoe-penetrating structure (sinker) appeared purple in contrast to the light green cytoplasm of the host cortical cells. The cytoplasm of the cells of the central portion of the mistletoe penetrating-structure stained yellow to orange, which readily separated it from the host tissue.

Similarly, the developing hypterothecium of *Hypodermella arcuata* stained a deep purple; the color readily distinguished it from the light blue of the cells of the host tissue, *Pinus lambertiana* needles.

Staining quality of BCC was independent of the type of chemical fixation. There were no apparent differences between those sections fixed with FAA or Navashin's fluid.

BCC did not stain fresh material as well as chemically fixed material. In the limited number of fresh sections stained with BCC, there was a predominance of blue. Cell walls tended to be over-stained, but the nuclei were adequately stained. Cytoplasm staining

was erratic, and in some sections the cytoplasm failed to stain at all. Causes for the lower quality of staining of fresh material as compared to fixed material are not known. We are making further trials to determine if staining and acid rinsing time can be changed to improve staining quality.

BCC is well suited for both black-and-white and color photomicrography. Its staining quality permits softer and more intergrading shades of color than safranin-fast green. With color photomicrography BCC produced subtle differences between cells of the same tissue, as well as between tissues.

For most plant material studied, BCC was found to be a useful stain. Lipophylic cell walls tended to stain yellow, non-proteineous cell walls a blue. With an increasing protein concentration red tones predominated over blue. The over-all quality of staining was comparable to that of traditional stains. In addition, BCC has real advantages for certain types of routine staining. The single solution of different dyes is prepared at time of purchase, and diluting is optional. Exact timing of both staining and destaining was found not to be as critical as with most stains. Staining takes only about 10 minutes, an average staining time for most plant material. With a suitable cover slip mounting medium, slides can be ready for viewing in less than 1 hour from the time staining begins.

FOOTNOTES

[2]Johansen, D. A. *Plant microtechnique*. New York: McGraw-Hill Book Co., Inc. 523 pp., illus. 1940.

[3]Jensen, W. A. *Botanical histochemistry*. San Francisco: W. H. Freeman & Co. 408 pp., illus. 1962.

[4]Gram, K., and Jorgensen, Erik. *An easy, rapid and efficient method of counter-staining plant tissues and hyphae in wood-sections by means of fast green or light green and safranin*. Saertryk of Friesia (Copenhagen) 4(4-5):262-266. 1953.

[5]Obtained from Aloe Scientific Division, Brunswick Corporation, South San Francisco, Calif. The manufacturer has not released the exact formula of the stain because the patent is pending. In general terms, 'Brook Cyte-Chrome I' consists of numerous biological dyes (certified if obtainable), acids, and special ingredients in 75 percent isopropanol.

[6]Obtained from Aloe Scientific Division, Brunswick Corp.

The Authors

STANLEY L. KRUGMAN is responsible for the Station's studies of genetics of western conifers, with headquarters in Berkeley and Placerville, Calif. Native of St. Louis, Mo., he was graduated from the University of Missouri (B.S., in forestry, 1955), and earned M.S. (1956) and Ph.D. (1961) degrees at the University of California, Berkeley. JULIA F. LITTLEFIELD, a biological technician with the forest genetics research staff, is a 1967 graduate of Mills College.