

A HISTOCHEMICAL STUDY OF THE COMPOSITION OF SPORE ORNAMENTATIONS IN OPERCULATE DISCOMYCETES

DONALD H. PFISTER

Department of Plant Pathology, Cornell University, Ithaca, N. Y. 14850

SUMMARY

Spore marking material of selected operculate discomycetes has been examined histochemically using aniline blue and hydroxylamine-ferric chloride, tests specific for callose and pectins respectively. Contrary to earlier reports based on nonspecific stains, the marking substance is apparently neither callose nor pectin. Solubility tests also indicate that callose is not present.

INTRODUCTION

Since the publication of Mme. Le Gal's "Recherches sur les ornements sporales des discomycètes operculés" (1947), the spore markings of operculate discomycetes have generally been considered to be composed of callose-pectic substances ("calloso-pectiques"). Mangin (1890) appears to have been the first to distinguish callose-pectic substances in fungi on the basis of staining reactions. The first report of callose-pectic substances in spore markings of operculate discomycetes was that of Le Gal (1942). Her final identification of these compounds (Le Gal, 1947) was also based on typical and supposedly specific staining reactions: callose compounds were stained with cotton blue C4B in lactic acid, while pectic substances were distinguished by their reaction with methylene blue, naphthalene blue, and ruthenium red. Because today the above staining procedures are not considered specific for callose and pectic substances, solubility tests and staining procedures known to be specific were employed, in this study, in an attempt to verify Le Gal's conclusions. Of the three discomycetes used in this preliminary study, *Peziza ostracoderma* Korf was not used by Le Gal.

MATERIALS AND METHODS

Tests for callose.—1) The aniline blue visible light method (Jensen, 1962) was employed. Dried material of *Peziza succosa* Berk. and *Lamprospora crec'hqueraultii* (Cr. & Cr.) Boud. was rehydrated in aerosol

(Fisher Scientific Co.), sectioned on a freezing microtome, and placed in a 0.005% solution of aniline blue. Also fresh material of *Peziza ostracoderma* was sectioned and stained as above. Controls were onion root tips which had been killed, fixed, embedded in paraffin, and sectioned; as well as freehand sections of fresh grape, rose, and snapdragon stems. With the above process callose, when present, should be stained a clear, bright blue.

2) Saturated aqueous solutions of calcium chloride and stannous chloride were used to test the solubility of the marking material. Sections of *P. ostracoderma* and *P. succosa* were mounted in the solutions and observed over a 24 hr period.

Test for pectin.—Hydroxylamine-ferric chloride (Reeve, 1959) following procedures given by Jensen (1962) was employed. Freehand sections of fresh *P. ostracoderma* were tested. Controls were freehand sections of apple fruit treated as above. Pectic substances should stain pink-red.

RESULTS

Tests for callose.—The ornamentations of neither the fresh nor dried spores were stained blue by aniline blue, indicating the absence of callose. Material of *P. ostracoderma* which was left in the staining solution for 7 days showed no coloration of the ornamentations. In the controls, the typical blue was noted in the sieve plates of the sieve tubes after 2 hr.

The spore ornamentations of *P. ostracoderma* and of *P. succosa* were insoluble in saturated aqueous solutions of calcium chloride and stannous chloride.

Test for pectin.—Sections of *P. ostracoderma* treated with hydroxylamine-ferric chloride did not show the characteristic red-pink color, in or associated with the markings. In the controls the middle lamellae of the apple fruit tissue were stained a bright pink.

DISCUSSION

The "callose" component.—Callose has been reported in fungi by various authors (Currier, 1957; Le Gal, 1942, 1947; Locquin, 1943; and Mangin 1890, 1910) based on staining reactions. Only Currier's (1957) study with bakers yeast is based on the accepted aniline blue reaction.

Since callose has generally been characterized by its reaction with aniline blue (Currier, 1957; Jensen, 1962), Le Gal's (1947) use of cotton blue C4B is questionable; it was, however, recommended by Locquin (1943), in a different formulation, as a specific stain for callose in fungi. No confirmation of the specificity of cotton blue C4B (also

known as Poirrier's blue) for callose was found in the literature. The lack of the typical callose staining reaction indicates that the marking substance is not callose.

Physical qualities of callose can be used as a means of characterizing callose. Mangin (1890) and Currier (1957) gave similar characteristics for callose. Callose is described as colorless, amorphous, insoluble in solutions of alkali carbonates, water, alcohol, Schweizer's reagent (cuprammonia); soluble in cold KOH and NaOH, sulfuric acid, calcium chloride, and stannous chloride but reported as only swelling in ammonia. In this study only stannous chloride and calcium chloride have been used in solubility tests. The negative results obtained indicate that the marking substance is not callose.

Inconsistencies in the literature concerning solubility and reactions of the marking material in KOH are worth noting. The destruction of the ornamentations of *Cheilymenia* spores with 7% KOH was reported by Denison (1964). McKnight (1968) reported alteration in the size and shape, as well as obliteration of the surface pattern of the spores in the Discineae in 3% KOH. Kanouse (1958) found that the spores of some of the species of *Trichophaea* became swollen and smooth when mounted in 2½% KOH. She did not indicate that the substance had been dissolved, but rather implied that it was spread out over the expanded spore surface. Malençon (1929) found that markings in a species of *Scutellinia* ("*Ciliaria*") were swollen and finally disappeared when mounted in KOH (concentration not given). Le Gal used dilute solutions of KOH routinely to swell the ornaments, but she stated this never caused a complete destruction of spore markings. Thus, the action of KOH on the marking substance seems to vary greatly from genus to genus.

The "pectic" component.—Ruthenium red, as employed by Mangin (1893), the common stain used for testing the presence of pectin, has been questioned as to its specificity by Ritter (1925), Harlow (1928, 1930), and Reeve (1959). Thus the specificity of ruthenium red, Le Gal's method for testing the presence of pectin, may justifiably be questioned. For this reason only the hydroxylamine-ferric chloride test was used in this study. This test has been shown by Albersheim et al. (1960) to be specific for pectins to the ultrastructural level. Neither methylene blue nor naphthalene blue, both used by Le Gal (1947), are considered specific for pectins if not accompanied by extraction procedures. The negative results obtained in this preliminary study indicate that the substances stained red in ruthenium red by Le Gal were probably not pectic in nature.

ACKNOWLEDGMENTS

The author wishes to thank his colleagues in the Department of Plant Pathology for their valuable suggestions and criticisms. In particular, sincere thanks go to Dr. R. P. Korf for his valuable suggestions in the preparation of this manuscript. The research was supported in part by National Science Grant GB-8548.

LITERATURE CITED

- Albersheim, P., D. Mühlethaler, and A. Frey-Wyssling.** 1960. Stained pectin as seen in the electron microscope. *J. Biophys. Biochem. Cytol.* **8**: 501-516.
- Currier, H. B.** 1957. Callose substances in plant cells. *Amer. J. Bot.* **44**: 478-488.
- Denison, W. C.** 1964. The genus *Cheilymenia* in North America. *Mycologia* **56**: 718-737.
- Harlow, W. H.** 1928. Contributions to the chemistry of the plant cell wall. III. The reliability of staining reagents in microchemical studies of plant cell walls. *Bull. N. Y. State Coll. Forest.* **1**: 3-16.
- . 1930. The chemical nature of the middle lamella. *Bull. N. Y. State Coll. Forest.* **3**: 5-11.
- Jensen, W. A.** 1962. Botanical histochemistry. W. H. Freeman and Co., San Francisco. 408 p.
- Kanouse, Bessie B.** 1958. Some species of the genus *Trichophaea*. *Mycologia* **50**: 121-139.
- Le Gal, Mme. Marcelle.** 1942. Mode de formation des ornements sporales chez quelques discomycètes operculés. *C. R. Acad. Sci., Paris* **214**: 125-128.
- . 1947. Recherches sur les ornements sporales des discomycètes operculés. *Ann. Sci. Nat. Bot.* **XI** **8**: 73-297.
- Locquin, M.** 1943. Une nouvelle technique d'étude des périspores amyloïdes; application au développement des spores de *Fayodia bisphaerigera* (Lange) Kühner. *Bull. Mens. Soc. Linn. Lyon* **12**: 110-112.
- McKnight, K. H.** 1968. Artifacts on spores of Discineae induced by common reagents. *Mycologia* **60**: 723-727.
- Malcençon, G.** 1929. Observations sur les ornements des spores chez les champignons. *Arch. Bot. Bull. Mens.* **3**: 121-129.
- Mangin, M. L.** 1890. Sur la callose, nouvelle substance fondamentale existant dans la membrane. *C. R. Acad. Sci., Paris* **110**: 644-647.
- . 1893. Sur l'emploi de rouge de ruthénium en anatomie végétale. *C. R. Acad. Sci., Paris* **116**: 653-656.
- . 1910. Nouvelles observations sur la callose. *C. R. Acad. Sci., Paris* **151**: 279-283.
- Reeve, R. M.** 1959. A specific hydroxylamine-ferric chloride reaction for histochemical localization of pectin. *Stain Technol.* **34**: 209-211.
- Ritter, G. J.** 1925. Distribution of lignin in wood. *J. Industr. Engin. Chem.* **17**: 1194-1197.

Accepted for publication November 15, 1969.