30 × 38–50 μ; oogonium 36–43 × 31–50 μ; oospore 30–41 × 30–37 μ; androsporangium 10–11 × 12–14 μ; dwarf male stipe 10–13 × 23–43 μ; antheridium 6 × 6 μ.

Var. omnibus partibus gracilioribus; oogoniis singulis vel 2–3 continuis, operculo apertis, circumscissione superiore, angusta; oosporis subglobosis vel rarius globosis, oogonia non complentibus, velfere complentibus, membrana laevi, nannandribus subrectis, plerumque in cellulis suffultoriis vel oogoniis, rarius in cellulis vegetativis ceteris prope oogonia sedentibus; cellulis vegetativis 9–18 μ latis, 30–50 μ longis; cellulis suffultoriis 23–30 μ latis, 38–50 μ longis; oogoniis 36–43 μ latis, 31–50 μ longis; oosporis 30–41 μ latis, 30–37 μ longis; cellulis androsporangiis 10–11 μ latis, 12–14 μ longis; nannandribus 10–13 μ latis, 23–43 μ longis; cellulis antheridiis 6 μ latis, 6 μ longis.

Occurred in small numbers in fruiting condition in collection of November 27 and more abundantly in that of December 1. Habitat: Epiphytic on grass leaves in shallow water. Type specimens: Author's Pacific collection #178; Chicago Natural History Museum; Farlow Herbarium; Herbarium University of Michigan; Philippine National Herbarium. Fig. 15–16.

SUMMARY

Two collections of fresh-water algae from a small swamp near the village of Palo, Leyte Province, the Philippine Islands, contained the following newly described species of the genus *Oedogonium: O. visayense, O. philippinense, O. paloense, O. circumlineatum,* and *O. hians* Nordst. & Hirn var. *leytense.*

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SEPARATION AND MOUNTING OF LEAF VEIN SKELETONS AND EPIDERMIS [1]

R. T. Whittenberger and J. Naghski

During studies on the recovery of rubber from cryptostegia, it was desirable to isolate and measure the full extent of the leaf veins (Whittenberger *et al.,* 1945). Although previous workers (Evans, 1928; Loomis and Shull, 1937; Sharman, 1942) described methods for separating vein tissue (xylem) from other plant tissues, the method developed by Naghski and associates (1945) proved particularly adapted to cryptostegia leaf. The method, an anaerobic fermentation process, has been used also for the isolation of protoplasts from vegetable leaves (White *et al.,* 1948). The present paper describes the method as applied to the preparation of vein skeletons and epidermis from leaves of a number of species. In addition, a technique for mounting fragile leaf vein skeletons is presented. Specimens thus prepared may be of use to morphologists and physiologists, both in classroom studies and in research.

Separation of veins and epidermis.—Preferably sound, undamaged leaves are chosen, since insect-damaged areas and gummy or resinous spots do not ferment properly, as indicated in fig. 2 and 5. The leaves usually are given a few pinpricks to insure penetration by the bacteria. They are then boiled in water for 5–15 min. to eliminate oxygen and inhibitory substances that may be present. The extractives are discarded. Then the leaves are introduced into a flask, submerged in freshly boiled tap water or mineral salt solution,[2] cooled to about 40°C., and inoculated with a 10 per cent by volume of a 12–18-hr. broth culture of *Clostridium roseum* McCoy and McLung. Inocula more than 24 hr. old usually are sluggish and show a prolonged lag period. This anaerobic bacterium vigorously digests cellulose cell walls but leaves lignified, suberized and cutinized walls essentially unaltered. Anaerobiosis is established in the flasks by use of a water trap and by flushing with carbon dioxide or nitrogen for 2–5 min. Fermentation proceeds rapidly at 35–40°C. Sterility and aseptic conditions need be observed only in the preparation of inoculum, for *C. roseum* maintains an essentially pure culture when incubated anaerobically, even with unsterilized leaves.

The time required for completion of fermentation varies with the type of leaf, although a period of 2–3 days usually is sufficient. Thick, xeromorphic leaves require a longer period than loosely compacted mesomorphic ones. Frequently the completion of fermentation is indicated by the inflation of leaves with gas and the simultaneous separation of the epidermis from the mesophyll.

The fermented leaves are placed in water and separated manually into their component parts under a dissecting microscope. Often both the

[1] Received for publication June 25, 1948.

[2] The solution contained 0.3 g. of KH_2PO_4, 0.7 g. of K_2HPO_4, 0.2 g. of NaCl, 0.2 g. of $MgSO_4 \cdot 7H_2O$, and 0.1 g. of $CaSO_4 \cdot 2H_2O$ per liter.

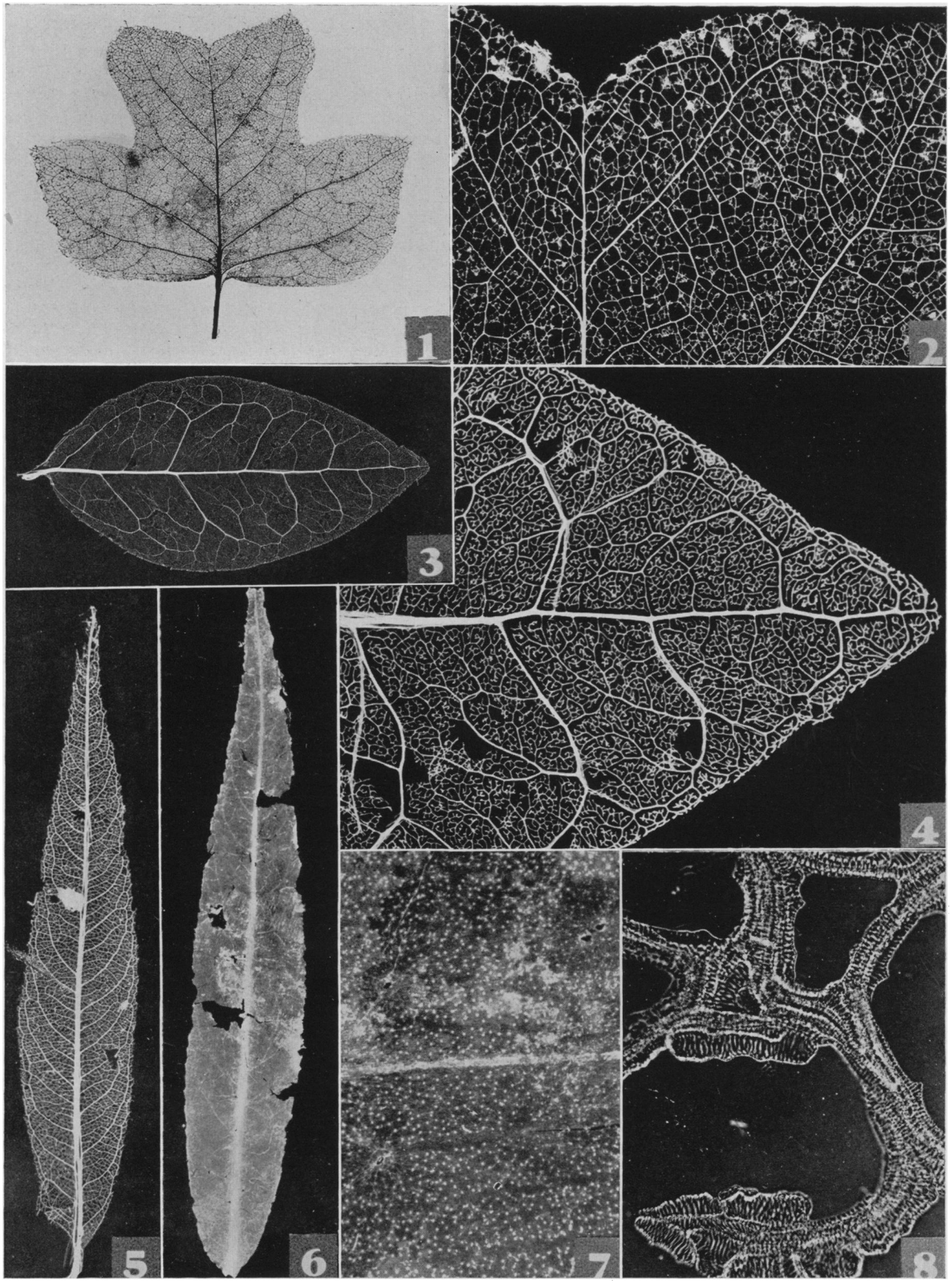

Fig. 1–8. Specimens shown in fig. 2–7 were mounted on cleared photographic plates and used as negatives for the prints.—Fig. 1. Leaf vein skeleton of *Liriodendron tulipifera* mounted on photographic printing paper. ×0.8.—Fig. 2. Photographically enlarged portion of *Liriodendron tulipifera* leaf vein skeleton. ×4.—Fig. 3. Leaf vein skeleton

lower and upper epidermis in their entirety may be floated free of the veins and mesophyll, after their severance from each other at the leaf margin with a scalpel or scissors. Vein skeletons are separated from the mesophyll parenchyma protoplasts by a jet of water from a bulb syringe. If separation is unduly difficult, the mesophyll may be tapped gently against the bottom of the container with a finger or the tissues may be immersed briefly (1–15 min.) in Javelle water. The separated tissues are washed by immersion in clear water. They may then be stained in a dilute aqueous solution of an appropriate dye (eosin Y, safranin O, *etc.*) or mounted unstained directly from water.

MOUNTING OF SPECIMENS.—Permanent mounts are obtained by floating the vein skeleton (or epidermis) on to the emulsion surface of a photographic plate. The plate is passed under the specimen, and then, slightly tilted, is drawn upward through the water. When the upper end of the plate is lifted above the surface of the water, the specimen is anchored to it. Complete lifting of the plate usually results in the uniform spreading of the specimen. If the specimen is curled or distorted, however, it should be reimmersed and remounted until satisfactory spreading is obtained. When dry, the specimen is firmly fixed to the emulsion, with a minimum of distortion.

The background for the specimen may be varied by pretreatment of the plate. A light, opaque background, which gradually darkens on exposure to light, is obtained with untreated plates, whereas a black background results if the exposed plate is treated successively with developing and fixing solutions. Fogged, overaged, or otherwise discarded plates may be used in the latter case. A white background may be produced by using double-thickness printing paper, previously treated in a fixing bath and washed. Specimens mounted thus are dried on a ferrotype tin. Finally, a clear background is obtained if the plate is put directly in a fixing bath and washed. Specimens mounted on such plates may be used satisfactorily as negatives in making photographic prints (fig. 2–7).

MAINTENANCE OF CULTURES.—A stock culture of *C. roseum* is easily maintained as a spore suspension in dry sterile soil, in which it remains viable for a long period and, above all, retains its cellulose-digesting ability. Cultures kept in the actively growing state by frequent transfers soon lose their cellulose-digesting activity.

Although the organisms are obligate anaerobes, they grow freely in open tubes or flasks if the medium is deep and freshly steamed (to drive off dissolved oxygen), and if the inoculum is introduced into the bottom of the medium. The inoculum can be produced readily by any medium suitable for growth of anaerobic organisms. A medium consisting of 16 ml. of blackstrap molasses, 1 g. of glucose, 5 ml. of corn steep liquor,[3] and 1 g. of $(NH_4)_2SO_4$ per liter of mineral salt solution (Allison and Hoover, 1934) and adjusted to *p*H 6.8–7.2 is satisfactory. Addition of 0.1 per cent sodium thioglycolate, 0.25 per cent agar, and 0.2 per cent of a 1–1000 solution of methylene blue produces a semi-solid medium useful in initiating growth from the stock spore suspension.

DISCUSSION.—The use of microorganisms to separate plant vascular skeletons is not new. Evans (1928) retted corn stems by placing them in a warm place for a few days. Loomis and Shull (1937) suggested the immersion of leaves in an algae tank until the mesophyll was eaten away by microorganisms, leaving an intact vascular skeleton. Sharman (1942) fermented corn stems and roots in a corn grain or pea seed infusion in preparing specimens for classroom work. Loomis and Shull (1937) also described a chemical method in which soda lime was used for separating vascular tissue.

All the retting methods just referred to are dependent on the chance that suitable microorganisms will find their way into the cultures. Under these conditions frequent failures might occur. Although cellulose-fermenting microorganisms are ubiquitous, their environmental requirements are so exacting that considerable time may be required for proper enrichment. These methods were ineffective with cryptostegia leaf in a reasonable amount of time, and soda lime, acting on the parenchyma cell walls less specifically than the enzymes produced by the microorganisms, also gave poor results.

The majority of leaves were easily separable into their component parts after *C. roseum* fermentation. Leaves fermented included *Liriodendron tulipifera* L. (fig. 1, 2), *Ligustrum japonicum* Thunb. (fig. 3, 4), *Salix vitellina* L. (fig. 5, 6), *Philodendron verrucosum* Mathieu (fig. 7), *Cryptostegia grandiflora* R. Br. (fig. 8), *Coleus blumei* Benth, *Poa pratensis* L., and *Saintpaulia ionantha* Wendl. Leaf skeletons of some of the monocotyledons used were difficult to obtain intact, owing principally to the lack of a sufficiently strong vein network between the larger parellel veins. The organism did not ferment satisfactorily the leaves of *Rhododendron maximum* L., *Kalmia latifolia* L., and *Robinia pseudacacia* L. As a precaution against the possibility that one plant species might contain toxic principles which would prevent *C. roseum* from acting on other species, leaves of each species were retted in separate cultures. The cause of the poor retting of certain spe-

[3] This liquor is available commercially from concerns that manufacture corn starch.

of *Ligustrum japonicum*. ×1.—Fig. 4. Photographically enlarged tip of skeleton of fig. 3. ×4.—Fig. 5. Leaf vein skeleton of *Salix vitellina*. ×1.—Fig. 6. Upper epidermis of *Salix vitellina* leaf, stained with safranin. Contact print, ×1.—Fig. 7. Lower epidermis of *Philodendron verrucosum* leaf, stained with safranin. Distribution of stomata is indicated by the white dots. Photographically enlarged, ×8.—Fig. 8. Leaf veinlets of *Cryptostegia grandiflora*, mounted in glycerin jelly. Photomicrograph, ×380.

cies remains unknown, and the effects of age, composition of leaf, *etc.*, were not investigated.

The preservation of fragile leaf skeletons was attended with difficulties. Evans (1928) and Sharman (1942) obtained specimens of stems or roots which were mechanically strong and needed no mounting. However, the spiral character of the unsupported leaf vein tracheids and vessels made them susceptible to severe distortion during drying. Unlike most algae, they had no gelatinous coating which would make them adhere to herbarium mounting paper. Skeletons floated on to a glass plate and pressed with another plate shrank and fragmented as they dried. Coating them with glycerin or glycerin-jelly likewise gave poor results, although small pieces of the skeletons could be mounted satisfactorily in this medium (fig. 8). Methods of mounting which involved solvent dehydration and subsequent coating of the skeletons with various polymeric substances were inconvenient and only partially successful because the delicate veinlets curled and broke. However, they could be quickly and effectively mounted on a moistened gelatin surface (photographic plate or printing paper).

SUMMARY

A brief fermentation of leaves with *Clostridium roseum* digests the mesophyll parenchyma cell walls sufficiently to permit the ready separation of intact epidermis and veins from the parenchyma protoplasts. The epidermis and even the most fragile vein skeletons are mounted easily on the emulsion surface of a photographic plate or printing paper. This gives permanent, durable, and undistorted specimens suitable for research and classroom study.

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GENETICS OF GLOMERELLA. V. CROZIER AND ASCUS DEVELOPMENT [1]

H. E. Wheeler, L. S. Olive, C. T. Ernest, and C. W. Edgerton

PREVIOUS STUDIES of certain members of the genus *Glomerella* have demonstrated the existence of strains which differ sexually as well as morphologically. Some of these strains produce fertile perithecia, some produce nearly sterile perithecia, while others are non-perithecial but produce large numbers of conidia. When grown in close proximity on suitable culture media, cross fertilization occurs and ridges of perithecia form on the lines of contact between certain strains. In earlier investigations certain results were obtained which, on a genetic basis, have not been explained satisfactorily. For example, perithecia formed by crossing perithecial and non-perithecial strains are frequently found to contain asci from which all eight spores produce cultures of the non-perithecial, parental type. With the hope that more precise information concerning the nuclear cycle of this fungus might provide an explanation for some of its problems of inheritance and variability, a cytological study was undertaken. In a previous paper Lucas (1946) described the three nuclear divisions in the ascus. The present paper deals with the origin and development of croziers and asci.

[1] Received for publication June 28, 1948.

MATERIALS AND METHODS.—For the present investigations, non-permanent, iron-propiono-carmine smear preparations, made as recommended by Sass (1940), were used almost exclusively. Young perithecia were transferred directly from cultures on oatmeal agar to a drop of the combined stain and fixative on a glass slide. A coverslip was added and enough pressure applied to force the contents of the perithecia out into the surrounding fluid. The preparation was then examined microscopically and, if necessary, heated gently for a few minutes before the coverslip was sealed in place with glycerine jelly. Preparations usually improved markedly after aging for a few days at room temperature. They also seemed to last much longer if stored in a refrigerator. The propiono-carmine method has a tendency to cause a swelling of the material, which often renders some of the finer cytological details easier to observe. It causes a noticeable swelling of chromosomes and nucleoli.

A modification of the above method was devised for staining whole young perithecia. Perithecia for staining were grown on a drop of oatmeal agar placed on a cover slip. A thin medium containing about 0.5 per cent agar gave the best results. The