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THE GENUS MASSARIOVALSA¹

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THE GENUS *Massariovalsa* was described by Saccardo (1882, p. 569) as a *Massariella* with perithecia arranged in a valsoid manner. The two-celled brown ascospores have a gelatinous envelope, and the asci are given as paraphysate. Apparently, only two species have been described in this genus, *M. sudans* (B. & C.) Sacc., the type species, and *M. caudata* E. & E. which the writer (1933) has shown appears under two descriptions, the first being a description of *Pseudovalsa* (*Prosthecium*) *bicornis* (Cke.) Sacc. and the second a description of a good species, *Pseudovalsa Ulmi* Wehm.

Berkeley's type collection (Kew Herb., ex Berkeley Herb. No. 3866) of *Sphaeria sudans* is in reality closely related to the genus *Melanconis*. It differs from most species of *Melanconis* in the possession of larger spores which show a gelatinous envelope at first, in the slight development of the ectostroma, and in the conidial stage. These are all differences of degree but seem sufficient for subgeneric separation at least.

Massariovalsa sudans occurs on a number of different hosts. Berkeley's type on *Acer* from South Carolina and a later collection on *Ulmus* from Pennsylvania are the same as all other collections which have been examined. Ellis reports this species on *Acer*, *Carya*, and *Quercus*. The writer has collected it on *Acer*, *Ulmus*, and *Nyssa* and has received mate-

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rial from J. H. Miller on *Acer*, *Nyssa*, and *Carya* from Georgia.

Material on all these hosts is very similar (fig. 1). The perithecia are formed in small circinate groups in the upper bark cortex, have thick parenchymatic walls and cause a slight pustulate swelling of the periderm. There is very little ectostroma formed, and this is practically obliterated by the convergent emergent ostioles. The resulting appearance on the surface is a very minute black disc, consisting of the more or less fused ostioles erumpent through the center of a flat swelling, often of loosened periderm. The ascospores (fig. 6) vary in size from 26-52 × 13-18.5 μ, but there is no definite correlation between spore size and host. The spores from *Acer* tend to run somewhat longer than those on *Ulmus* and *Carya*, but this may be due to the larger number of collections examined on *Acer*. On *Acer*, the perithecial stage is commonly accompanied by the conidial stage. No conidial pustules have been seen on the other hosts, except those developed in culture. On *Carya*, the pustules are firmer, more definite, irregularly scattered, and the ectostroma is more easily seen. These differences are probably due to the character of the hickory bark, but when first seen it was taken for a *Melanconis*. Cultural studies have proven it to be the same as *Massariovalsa sudans*. The placement of this genus in the Massariaceae is undoubtedly due to the slight ectostromatic development of the disc and the gelatinous envelope found about the spores. This latter character is found in some spe-

cies of *Melanconis* also, and cultural studies have shown that a definite ectostroma is formed. The paraphyses found in the young perithecium, furthermore, are broad, band-like, guttulate, and evanescent, as in *Melanconis*. The conidial stage is a modification of the *Melanconium* type with conidia formed within an enclosed chamber, instead of open cavities. The relationship is obviously with *Melanconis*.

In order to determine whether or not the occurrences of *M. sudans* on the various hosts represent separate species, single ascospore isolations were

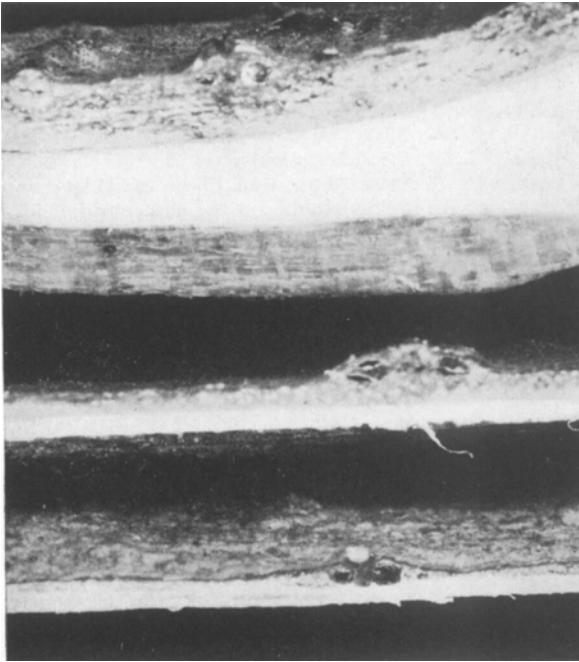


Fig. 1. Radial sections of stromata of *Massariovalsia sudans* (B. & C.) Sacc. on twigs of *Acer saccharinum* (above), *Carya glabra* (center) and *Nyssa sylvatica* (below).

made from three collections kindly sent the writer by J. H. Miller from Athens, Georgia. The hosts and dates of collection of these were as follows: *Carya glabra*, March 12, 1938; *Acer rubrum*, December 8, 1938; and *Nyssa sylvatica*, June 4, 1938. Certain cultural differences were observed between these host strains, but in general the results were similar. A general consideration of the strain from *Carya* will serve for all three strains. The varietal differences will be considered later.

Ascospores from *Carya glabra* were sprayed onto nutrient agar on March 26, 1938. Germinating spores were found within 24–48 hours. Only a small percentage of the spores germinated, and most of the germinating spores were cracked at the septum, with the two cells of the spore partially separated or entirely free from one another (fig. 7). Two to four, stout, gnarled, branching germ tubes, 2.5–5 μ in diameter, were put out from each spore, either from

the central ruptured septum or from the ends of the spore.

Growth was extremely slow on nutrient agar, colonies being only three to four centimeters in diameter after six months. Growth on oatmeal agar was somewhat more rapid. The surface of the agar is heavily blackened, and there is a superficial whitish to greenish-gray cottony mycelial growth. A few hemispheric stromata, of a similar greenish tint, were formed on oat agar after some six weeks. These split open, exposing a slimy inner surface. These stromata consisted of a mass of pale greenish to hyaline pseudo-parenchyma. In various internal areas, these cells put out short conidiophores which bore conidia at their apex. These conidia (fig. 8) were broadly ovoid, slightly flattened at the base where attached, hyaline at first but soon becoming olive brown and coarsely granular, finally with a thickened wall and homogeneous content and $26\text{--}33.5 \times 19.5\text{--}30 \mu$.

In the first twig inoculations the twigs dried out before fruiting occurred, because of the slow growth of the fungus. Autoclaved twigs of *Carya* sp. inoculated on November 2, 1938, showed numerous minute elevations of the periderm by December 29, and these later gave rise to erumpent, spheric, grayish stromata which finally showed the splitting off of a cap-like apical portion, similar to that found in agar cultures. The conidia were exuded in a black slimy mass and were identical with those found on agar.

Sections through these areas show that the pustules arose as rather widespread but interrupted areas of ectostroma on the bark surface. These ectostromata (fig. 4) are at first composed of loose, erect, hyaline, somewhat interwoven hyphae, 2–3 μ in diameter, causing a separation of the periderm from the bark cortex. These hyphae soon show a definite tendency to form a pseudo-parenchyma by the swelling and septation of the strands and the rounding up of the individual cells. This change occurs first at the base and in the central portions of these areas. As a result of subsequent increase in cell size, these central areas swell rapidly to an irregular spheric form and cause the pustulate ruptures of the periderm, and eventually the erumpent stromata.

The cells of this stroma are at first hyaline, filled with protoplasm and capable of continued growth. Upon exposure, the outer cells become thick-walled, olive-brown in color, and limit the expansion of the stroma. The inner cells remain hyaline and active, and at one or several points conidial formation is initiated. In these areas, the smaller more active cells may grow out directly as conidiophores, but the larger parenchymatic cells "sprout" by the rupture of the thin hyaline wall and the outgrowth of the naked protoplast as a conidiophore (fig. 9). The conidiophores are short, stout, $7\text{--}18 \times 2\text{--}4 \mu$, and often tapered to a point at the apex. The ruptured wall of the mother cell often appears as a sheath at the base. The conidiophores swell at the apex to form the ovoid conidium which is hyaline until it reaches nearly full size, when the wall becomes

thickened and a dark olive-brown. The conidia are $21.5-27 \times 17.5-22 \mu$. The formation of conidia continues centrifugally until one or a few large cavities result, limited by the outer wall of inert cells. The

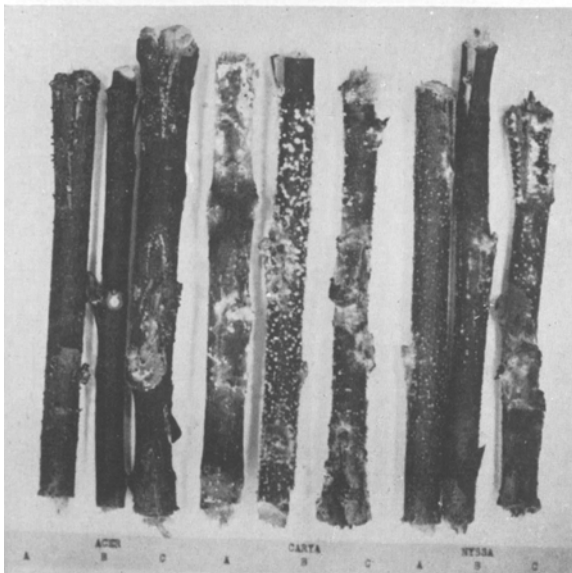
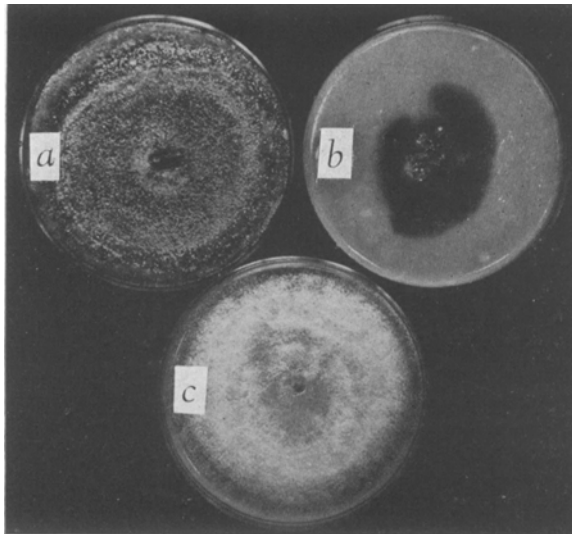


Fig. 2 (above). Colonies on oatmeal agar showing type of growth produced by strains of *Massariovalsa sudans* (B. & C.) Sacc. from (a) *Acer rubrum*, (b) *Carya glabra* and (c) *Nyssa sylvatica*.

Fig. 3 (below). Twig cultures of strains of *Massariovalsa sudans* (B. & C.) Sacc. from *Acer*, *Carya* and *Nyssa*, as indicated, on twigs of (A) *Acer saccharum*, (B) *Carya ovata*, and (C) *Ulmus americana*.

pressure resulting from spore production causes a rupture of the stroma, which usually takes place by the throwing back of a lid-like slice of the upper portion of the stroma.

Variations from the above life history are indicated in the following comparison of the three host

strains. Inoculations of the three strains were made in triplicate on oatmeal agar, and two series were carried for three weeks each. Certain definite differences appeared and were constant for each strain. These are described below and illustrated in figure 2.

On *Carya*: Colonies 2-4 cm. in diameter (after 3 weeks). Young colony with fine superficial tomentum which is grayish when viewed at a wide angle to the light. Soon forming a heavy dark green-black growth in the agar, giving the entire colony this color. Later a fine cottony growth of grayish mycelium may occur on the blackened stromatic areas. No pycnidia after three weeks.

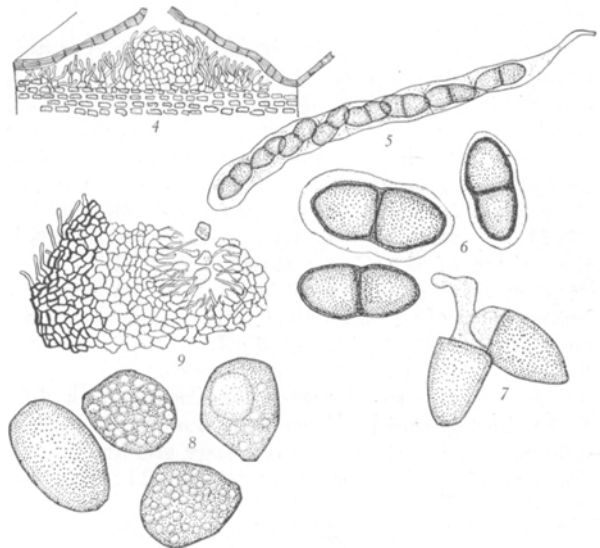


Fig. 4-9. *Massariovalsa sudans* (B. & C.) Sacc.—Fig. 4. Vertical section of young ectostroma, showing development of central plug of pseudoparenchyma.—Fig. 5. Ascus.—Fig. 6. Ascospores.—Fig. 7. Germinating ascospores.—Fig. 8. Conidia as formed in culture.—Fig. 9. Section of conidial locule and wall as formed in culture.

On *Acer*: Colonies 3-7 cm. in diameter. General color of the colony yellow-brown. Growth in concentric fan-like waves forming an outer tufted, superficial, yellow-gray mycelium behind which there is a more appressed superficial growth of darker yellow-brown hyphae arranged in a radiating system of very fine, loosely floccose, mycelial strands. Numerous grayish, superficial, spheric stromata, 0.5-1.0 mm. in diameter, with amber colored droplets present.

On *Nyssa*: Colonies 7-8.5 cm. in diameter. Surface growth of flocculent white mycelium, continuous or zonate in some colonies, 0.1-0.2 mm. thick. Agar with a characteristic tan to red-brown discoloration. Numerous minute, greenish-black stromata within the agar, which later proved to be perithecia.

On March 26, 1939, each of the strains from *Acer*, *Carya*, and *Nyssa* were inoculated onto three twigs each of *Acer*, *Carya*, and *Ulmus* (*Nyssa* twigs not being available). Figure 3 shows one set from this

series after eight weeks' growth. The growth of any one strain on the three hosts was very similar. There was somewhat greater superficial mycelial growth in the case of the *Carya* strain, but this may have varied with air moisture. The lack of growth of the *Acer* strain on *Carya*, for instance, was due to the drying out of the twig. The more important differences are cited below.

The *Nyssa* strain failed to produce any conidial stage in any of the many cultures carried, either on agar or on twigs. On the other hand, perithecia were produced in all these cultures but in none of the cultures from the other hosts. The perithecia formed were small and abnormally developed, but typical mature two-celled, brown ascospores were found in many of them.

The pycnidia formed by the strain from *Acer* did not open by the cap-like splitting of the apex, as is common in the *Carya* strain. The conidia of the *Acer* strain were exuded through a pore-like opening as fine coiled tendrils, rather than shapeless spore masses. The conidia of the *Acer* strain on twigs of *Acer* ($23-32 \times 19.5-23 \mu$) and *Ulmus* ($26.5-32 \times 18-21.5 \mu$) were slightly narrower and more ovoid than those of the *Carya* strain ($26.5-33.5 \times 21-25 \mu$ on *Acer* and $26.5-39 \times 23-30 \mu$ on *Ulmus*), but on twigs of *Carya*, both strains produced similar conidia ($26.5-33.5 \times 21.5-25 \mu$ for *Acer* strain and $25-33.5 \times 21-26.5 \mu$ for *Carya* strain).

These differences between the strains from different host genera are largely ones of physiological behavior, rather than of morphology and are, therefore, considered to be of varietal rather than of specific rank.

The conidial stage, obtained both in nature and in culture, is a *Melanconiopsis*. Ellis (1900) in describing this genus says "doubtless a stylosporous stage of a *Melanconis* or a *Massariovalsa*," and his type collection (N. Y. Bot. Gard., Ellis Coll., Kans., 1900, Bartholomew 2519) of *Melanconiopsis inqui-*

nans E. & E. on *Acer dasycarpum* is identical with the conidial stage obtained from the strain on *Acer*, showing the similar, somewhat narrower, conidia, $25-35 \times 14-18 \mu$.

This form genus can be considered as a modified *Melanconium* with enclosed locules. The differences between *Massariovalsa* and *Melanconis* might be considered of generic rank, but inasmuch as the genus *Melanconis*, as now circumscribed, has species with various affinities and variable conidial stages, the writer would prefer to place *Massariovalsa* as a sub-genus of *Melanconis*. These arrangements within the genus *Melanconis* will be considered in a forthcoming paper.

SUMMARY

Cultural studies of three strains of the one good species of *Massariovalsa*, *M. sudans* (B. & C.) Sacc., from *Acer*, *Carya*, and *Nyssa*, indicate certain differences considered to be of varietal rank.

The strains from *Acer* and *Carya* produced a conidial stage, identical with *Melanconiopsis inquinans* E. & E. The strain from *Nyssa* produced no conidia but numerous perithecia in all cultures.

The genus *Massariovalsa* is considered as a sub-genus of *Melanconis*.

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HYDROGEN-ION CONCENTRATION OF LEAF JUICE IN RELATION TO ENVIRONMENT AND PLANT SPECIES¹

Annie M. Hurd-Karrer

THE DATA reported in the present paper show the extent to which the acidity of the wheat plant is subject to change by the environment. Hundreds of pH determinations for plants in all stages of development, grown under different environmental conditions over a period of years, are summarized in the form of distribution curves that permit determination of the most typical value and the range of variation. Some of the data are tabulated to illustrate specific effects of growth factors.

Comparative acidities of other kinds of plants are shown by a compilation of pH values reported by other investigators.

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Numbers in parentheses refer to Literature Cited at end of this article.

The significance of acidity measurements on the expressed juice of plants is enhanced by Chibnall and Grover's conclusion (7) that since the buffering power of the vacuole fluid is greater than that of the cytoplasm the hydrogen-ion concentration of the expressed juice is a close approximation to that of the vacuole fluid in the living cell.

METHODS AND MATERIAL.—The plant material was macerated by grinding in a food grinder, or in a Nixtamal mill, and the juice expressed by squeezing it out by hand through muslin or cheesecloth. It was found that preliminary freezing of the plants had little effect on the hydrogen-ion concentration of the expressed juice. The use of high pressure has been advocated; but the writer found that values for