Microsphaera diffusa, the Perfect Stage of the Soybean Powdery Mildew Pathogen

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ABSTRACT

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Profuse development of cleistothecia of a powdery mildew pathogen was observed on soybeans grown in the greenhouse. The pathogen was identified as *Microsphaera diffusa*. Cleistothecia were white at first, then during maturation changed sequentially to yellow, tan, brown, and finally black. Mature cleistothecia were 92 to 125 μm in diameter and possessed about twenty appendages 124 to 212-μm long that were dichotomously branched four to five times at the tips. Multiple pyriform asci contained up to six light-yellow ovoid ascospores which measured 9 × 18 μm. A relatively simple and rapid procedure is described for handling leaf material with powdery mildew for scanning electron microscopy (SEM). The cleistothecia of *M. diffusa* were hemispherical with deeply convoluted outer walls and appendages originating in a ring from the base. A comparative SEM study of *Erysiphe polygoni* is presented.

Powdery mildew, commonly considered a problem of greenhouse-grown soybeans, *Glycine max* (L.) Merr., is being found in Illinois and in other regions with increasing frequency in field-grown soybeans (3, 4, 5, 7, 8, 10). Dunleavy (4) reported significant yield losses in naturally-infected, field-grown soybeans. Despite the common occurrence of this disease, relatively little is known about the pathogen.

Paxton and Rogers (10) first conclusively identified the cause of soybean powdery mildew as *Microsphaera diffusa* Cke. and Pk. in 1974, which confirmed the identifications of *Microsphaera* sp. by Lehman in 1947 (8) and Demski and Phillips in 1974 (3). Earlier reports of soybean powdery mildew identified the pathogen either as *Erysiphe polygoni* DC. ex Mérat (7, 14) or *diffusa* (6), but in these reports the perfect stage was not described to support either identification. These conflicting reports have caused some confusion in the literature (3, 5, 11). In 1976, Roane and Roane (11) reported the occurrence of *E. polygoni* and *M. diffusa* together, as the dual cause of soybean powdery mildew. They found "...islands of *Microsphaera*-type surrounded by *Erysiphe*", and upon examination of ascospores from dark cleistothecia with unbranced appendages concluded that both fungal species were present in their material.

Results presented here, a portion of which have been previously reported (9), agree with the work of Paxton and Rogers (10) and identify *M. diffusa* as the cause of soybean powdery mildew. Results of an examination of the perfect stage of *M. diffusa* are presented. Scanning electron microscopy (SEM) provided rapid critical examination of the cleistothecia of *M. diffusa* and *E. polygoni*. Some comparisons of these two fungi are presented.

In early September 1976, after the completion of this study, cleistothecia also were observed (for the first time in Illinois) on soybeans with powdery mildew (natural occurrence) in fields in Ogle and Bureau counties (Plant Clinic, University of Illinois, *unpublished*). Examination of these cleistothecia showed the powdery mildews to be *M. diffusa*.

MATERIALS, METHODS, AND RESULTS

In December 1975 we observed profuse development of cleistothecia on soybeans with powdery mildew (natural occurrence) in the greenhouse. Soybean cultivars Amsoy and Wells, which had been mechanically inoculated at the primary leaf stage with bean pod mottle virus, subsequently showed powdery mildew which eventually covered all but the most recently emerged leaves. Cleistothecia first were noted 52 days after planting when plants were flowering, beginning to set pods, and had four trifoliolate leaves each. The significance of prior systemic virus infection on development of powdery mildew was not investigated; however, we have noted that powdery mildew often develops more readily on virus-infected plants than on other plants in the greenhouse. The role of host plant physiology in the development of powdery mildew infections has been previously reviewed by Yarwood (15) and Schnathorst (13).

Observations and photomicrographs of isolated cleistothecia were made from material mounted in water on glass slides. Infected tissues also were photographed. Measurements were made using a light microscope

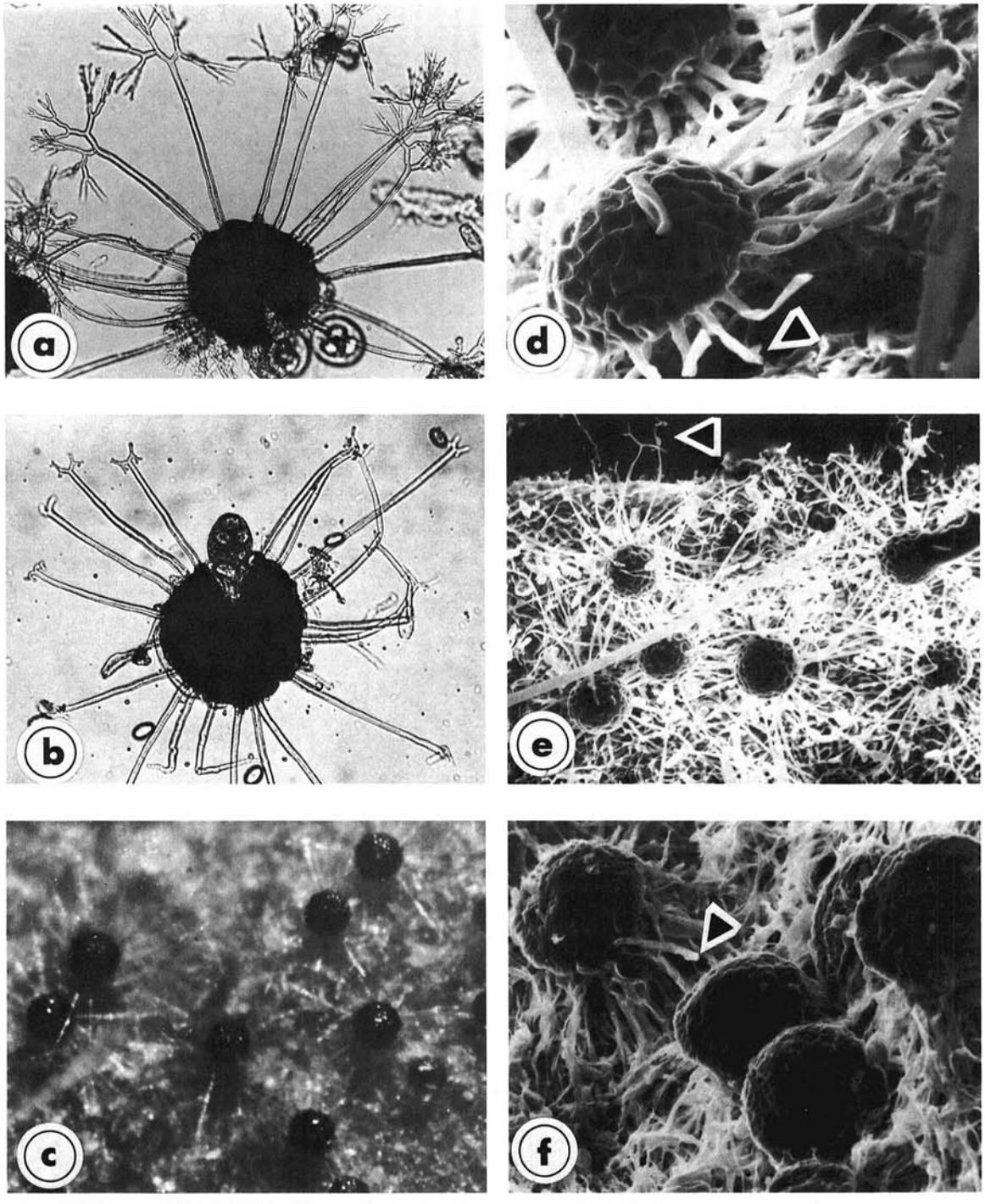

Fig. 1-(a-f). Cleistothecia of *Microsphaera diffusa* (a-e) and *Erysiphe polygoni* (f), including: **a)** mature cleistothecium with dichotomously branched appendages and multiple asci (× 170); **b)** immature cleistothecium with appendages in the early stage of branching and immature ascus showing granular appearance (× 170); **c)** cleistothecia on a leaf surface of *Glycine max* showing the pattern of refracted light which indicates a rough outer wall (× 70); **d and e)** scanning electron micrographs of cleistothecia on *G. max* leaf surfaces showing the convoluted outer wall, hemispherical shape, and basal origin of appendages. Note the beginning of branching (arrows) of appendages (× 370 and ×90, respectively); and **f)** cleistothecia of *E. polygoni* on a leaf surface of *Polygonum aviculare*, showing the slightly rough surface of outer walls, ellipsoidal shape, and basal myceloid appendages (arrow) radiating into the dense mycelium (× 220).

equipped with an ocular micrometer. The range of 100 or more measurements of each of the characters examined is reported.

On older leaves, which had been infected longer, formation and maturation of cleistothecia over older mycelial colonies occurred almost simultaneously, and resulted in a nearly uniform maturation of cleistothecia over entire leaves. On younger, more recently infected leaves, formation and maturation of cleistothecia began near the centers of powdery mildew colonies on older mycelium and progressed outward. This developmental sequence characteristically resulted in groups of cleistothecia with the more mature stages in the centers of the groups surrounded by increasingly more immature stages toward their peripheries.

Immature cleistothecia were white when first formed and then matured, becoming yellow, tan, brown, and finally black. Mature cleistothecia were 92 to 125 μm in diameter and usually appeared spherical in the light microscope. As they matured from dark brown to black, appendages began to form. Mature cleistothecia had about 20 appendages, which measured 124 to 212-μm long and were dichotomously branched four to five times at the tips (Fig. 1-a). Branches were narrowly divergent, related like the arms of a Y. Branching was not observed on appendages of less than about 100 μm long and developed only as appendages lengthened during the final stages of maturation. Early after the onset of branching the appendages sometimes appeared to have recurved branches (Fig. 1-b). Fully branched forms did not appear recurved (Fig. 1-a).

Multiple, pyriform asci with short attachment stalks were released through broken cleistothecial walls. Each ascus contained up to six light yellow ovoid ascospores measuring 9 $\times$ 18 μm. Immature asci with granular contents and poorly delineated ascospores were characteristically associated with cleistothecia having unbranched appendages or appendages in the early stages of branching (Fig. 1-b). The organism was identified as *M. diffusa* based on these observations.

Cleistothecium walls refracted light in such a way as to indicate a rough or pitted surface (Fig. 1-c). To confirm this observation, infected leaf material bearing abundant cleistothecia was air-dried 48 hr at room temperature, cut into 3 to 5-mm^2 pieces, mounted with an epoxy cement on scanning electron microscope stubs, coated with gold-palladium in a Denton DU-503FP vacuum evaporator, and examined in a JSM U-3 scanning electron microscope. Results were recorded photographically using Polaroid PN-55 film.

The SEM confirmed the convoluted reticulated texture of the cleistothecial walls and showed the cleistothecia to be hemispherical with a ring of appendages borne basally and extending radially, parallel to the leaf surface (Fig. 1-d). The SEM photomicrographs also detailed the intertwined condition of appendages with other appendages and surface mycelium (Fig. 1-e). This characteristic made it difficult to distinguish branching except in areas where appendages extended beyond the edges of the leaf surface (Fig. 1-e).

The SEM technique also was used in a comparative study of the cleistothecia of *E. polygoni* to examine the surface of the cleistothecium wall as a potentially useful character for distinguishing it from *M. diffusa*. Fresh leaf material of *Polygonum aviculare* L. and an herbarium specimen of *Oenothera biennis* L. bearing mature cleistothecia of *E. polygoni* were prepared and examined as outlined for *M. diffusa*. *Polygonum aviculare* material was from two collections: one made by D. P. Rogers, University of Illinois, and the other by the senior author. *Oenothera biennis* also was provided by D. P. Rogers from material originally collected and identified by F. S. Earle, 18 September 1885, and maintained as entry no. 6616 of the Mycological Herbarium, University of Illinois.

Similarities between cleistothecia of *E. polygoni* and *M. diffusa* were clearly shown by SEM. Cleistothecia of both fungi appeared to be flat-bottomed, were about the same diameter, and had appendages borne basally (Fig. 1-d, e, f). The appendages of *E. polygoni* usually were entwined with surface mycelium (Fig. 1-f), similar to the situation observed with *M. diffusa*; however, careful tracing of *E. polygoni* appendages showed them to be myceloid. Slight differences were observed in the shape and surface texture; cleistothecia of *E. polygoni* appeared somewhat less hemispherical than those of *M. diffusa* and were compressed vertically, resumbling half of an ellipsoid (Fig. 1-f). Although the cleistothecium walls of *E. polygoni* generally appeared to be less convoluted than those of *M. diffusa*, variability in this character precluded its use as a differentiating criterion.

DISCUSSION

Our results confirm the identification of *M. diffusa* as the causal organism of soybean powdery mildew (10) and agree with other morphological descriptions of this fungus (1, 2, 3, 8, 10, 12). In addition to confirming the observations of Lehman (8), who described the cleistothecia of *Microsphaera* as 'sub-spherical', our SEM studies of *M. diffusa* revealed a pitted cleistothecium surface and a basal origin of appendages.

Although small differences between the cleistothecia of *M. diffusa* and *E. polygoni* were observed, the major similarities dictate that at present the only reliable distinguishing characteristic between these two fungi is the presence or absence of branched appendages. Salmon (12), noted specimens earlier identified as *Erysiphe* ". . . which clearly belonged to *Microsphaera*", and he characterized them as having "a very slow development of apical branching . . .". In discussing *M. symphoricarpos*, which he considered synonymous with *M. diffusa*, Salmon pointed out that ". . . the appendages are very slow in reaching their full development . . .". We, therefore, reemphasize the point made by Salmon and others (3, 10) that correct identification of *M. diffusa* is dependent upon observation of fully mature cleistothecia, including appendages, and that misidentification as *E. polygoni* easily can occur if investigators are not cognizant of the morphological changes that occur during development and maturation of the cleistothecia of *M. diffusa*.

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