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Perfect States of *Stemphylium*. II.

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Abstract. – *Pleospora herbarum* (Fr.) Rabh. (anamorph = *Stemphylium herbarum* sp. nov.), *P. tarda* sp. nov. (anamorph = *S. botryosum* Wallr.), and *P. alfalfae* (anamorph = *S. alfalfae*) sp. nov. are described, discussed, and differentiated on the basis of type specimens and of isolates from *Medicago sativa*.

It has been traditional to cite ascosporic *Pleospora herbarum* (Pers.: Fr.) Rabenhorst and conidial *Stemphylium botryosum* Wallroth as components of the same holomorph. This tradition is defective in that it does not agree with the evidence available to us. The observations in my earlier work on *S. botryosum* and its ascomatous state remain experimentally valid (Simmons, 1969). However, the identification of the teleomorph as *P. herbarum* was ill-founded in 1969 (cfr. discussion, l. c., p. 5); a usage such as "*P. herbarum* complex" would have been more nearly supportable. On the basis of about 15 subsequent years of culture work with many isolates of *Stemphylium* and of *Pleospora*, I now am prepared to defend the statement that ascosporic *P. herbarum* and its anamorph constitute a species quite distinct from *S. botryosum* and its teleomorph.

The following discussion is based on comparison of several *Pleospora-Stemphylium* holomorphs that have developed in a moderate number of *Stemphylium* isolates from *Medicago sativa*. Conclusions are derived not only from culture comparisons but also from re-examination of the holotype specimens of *P. herbarum*, *S. botryosum*, and similar taxa.

1. *Pleospora herbarum* (Pers.: Fr.) Rabenhorst and its anamorph – Fig. 1.

The holotype of *P. herbarum* is the portion of E. Fries's *Scleromyceti Sueciae "38. Sph. herbarum Fries"* held in the herbarium (E) of the Royal Botanic Garden, Edinburgh, Scotland. (Other portions of this exsiccatum examined by me (FH, K, and UC) are almost totally pycnidial, as is reported to be the portion at UPS (Wehmeyer, 1953); none of these therefore is useful in typifying *P. herbarum*). The herbaceous stem fragments of the specimen at E bear abundant discrete ascocarps that are immersed/erumpent and that appear flattened-discoid or almost cupulate because of the

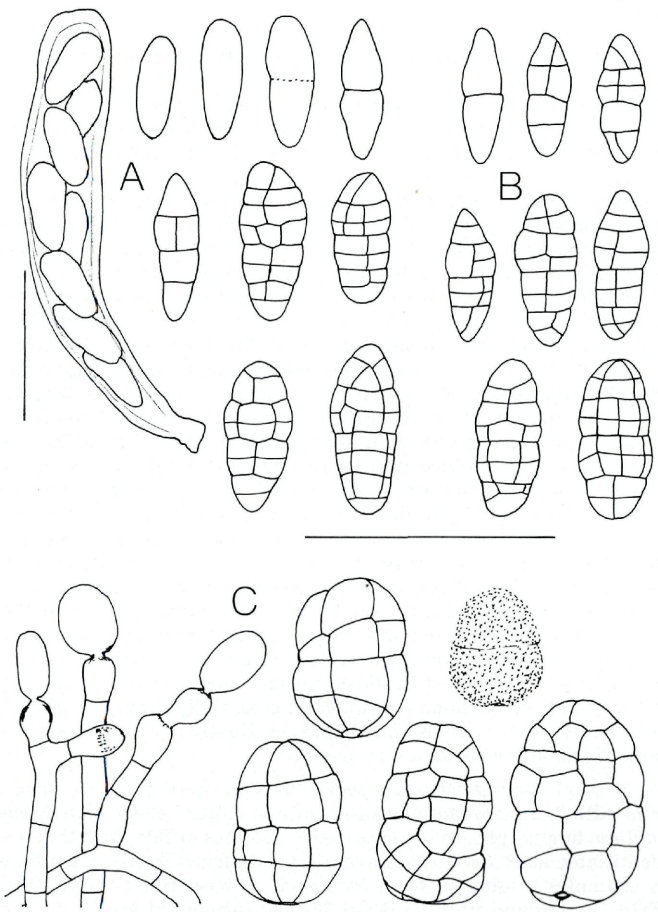

Fig. 1: *Pleospora herbarum/Stemphylium herbarum:* A. ascus and ascospores from holotype of teleomorph [E]. – B. ascospores, and C. conidia and conidiophores from culture of anamorph type [EGS 36-138]. – Ascus; bar = 50 µm; ascospore and conidium; bar = 50 µm.

collapse of their rather thin walls. Mature ascocarps about 250–300 µm diam. contain numerous asci and ascospores of all developmental stages.

The developmental pattern of typical *P. herbarum* ascospore shape and septation is illustrated in Fig. 1. Young aseptate ascospores are ellipsoid or just perceptibly obovoid (using the ascus base as point of reference); a median septum becomes visible as the ascospore elongates and widens slightly. By the time the initial septum is clearly defined, the developing ascospore is strongly constricted at the septum and each of its cells is almost conoid. A single transverse septum then is produced in each spore-half, and the upper half becomes distinctly larger than the lower. Further development of septa follows a general pattern, with minor variations: one more septum across the entire width of the spore may be produced in the top or bottom cell, after which a longitudinal septum is laid down in any or all of the transverse divisions; subsequent transverse septa are placed only across individual cells, as are a few secondary longitudinal septa, first in the largest, subapical quarter of the spore and eventually in any transverse sector. Young ascospores often are somewhat flat on one side; this inequilateral appearance carries through to many mature ascospores. In general, mature ascospores of typical *P. herbarum* are about 32–35 × 13–15 µm, dilute medium brown in color, obovoid, with 7 transverse septa (3–5 major and complete, others secondary) and 1 longitudinal septum in each of most or all of the initial four transverse divisions of the spore (plus secondary ones in any or all of the transverse subdivisions); septation in the apical quarter of the spore may be either of several elements at right angles to each other or of a simple Y-form construction; the mature ascospore is distinctly constricted at each of its three initial transverse septa. Asci at maturity are conspicuously bitunicate; essentially tubular with parallel walls and broadly rounded apex, gradually narrowed near the base and about 160 × 25 µm in size.

Several distinguishable species of *Pleospora* produce small, thin-walled, rapidly maturing ascomata in culture along with *Stemphylium* anamorphs. In my experience, isolates of this kind that are identifiable as *P. herbarum* have not been plentiful; two cited here as examples originated from *Medicago* in New Hampshire, USA. [EGS 12-138] and in India [EGS 36-138]. Abundant ascomata and mature asci develop within a month or less on 20% V-8 agar plates held at moderate temperatures. The conidial state is produced abundantly throughout this time; it is similar to *Stemphylium botryosum* but distinguishable from it on the basis of greater size range in culture and greater complexity of septation in mature conidia.

2. *Stemphylium botryosum* WALLROTH and its teleomorph – Fig. 2.

The holotype of *S. botryosum* (in herb. STR) has been described and illustrated in considerable detail by WILTSHIRE (1938) and by SIMMONS (1967, 1969). Diagnostic characteristics include conidium dimensions commonly near 33–35 × 24–26 µm, a length/width ratio near 1.0–1.5 (i. e., large numbers of conidia are almost as broad as they are long), a single conspicuous constriction at the median transverse septum, densely echinulate walls, pale wall and septum color, and a large basal scar-like zone (Fig. 2).

As is the case with typical *P. herbarum*, isolates of *Stemphylium* whose conidia retain essentially all *S. botryosum* characteristics in culture have not often come to my attention. Isolates that have produced mature ascocarps in culture for me originated in Ontario, Canada [EGS 04-118C], in New Hampshire, USA [EGS 08-069], and in New Zealand [EGS 30-124], all from *Medicago*. Ascomata produced in cultures of *S. botryosum* differ markedly from the thin-walled ones of *P. herbarum,* in that they develop slowly for several months as large (commonly 700 µm or more diam.), hard, internally undifferentiated, sclerotic bodies; ascus production occurs late in this period, with ascospores maturing after about 8 months. Asci are subcylindrical, about 200 × 40 µm; juvenile ascospores are oblong with obtusely rounded ends and are noticeably constricted at one or more of the initial three transverse septa; ascospores mature at a size of about 40 × 17 µm and with a broadly rounded apex, an almost flat base, three primary and four secondary transverse septa, and one complete series of longitudinal septa plus secondary ones initially in the broadest subapical transverse quarter of the spore and eventually in all of the primary quarters.

This *Pleospora* picture of large sclerotic ascomata commonly is ascribed to *P. herbarum,* but the concept is erroneous. On the contrary, the teleomorph of which *S. botryosum* is the conidial state is exactly the same as the one illustrated in SIMMONS (1969, p. 7); the discussion pertinent to it (l. c., pp. 6–8) is valid for the *Pleospora-S. botryosum* holomorph, with the following three corrections; the name of the "St. perf." is not *Pleospora herbarum;* no isolate I would now call *S. botryosum* matures its ascocarps rapidly; and the fungus that I then touted as "the best modern collection of this species which I have seen . . . [IMI 102, 649, on *Crambe*]" must be reconsidered, in that isolates of the same fungus from the same site and substrate produce conidia that follow a different developmental pattern and ascomata with ascospores quite different from those of the *S. botryosum* teleomorph.

Defining the *Pleospora herbarum-Stemphylium* holomorph and the *Pleospora-Stemphylium botryosum* holomorph in a type-re-

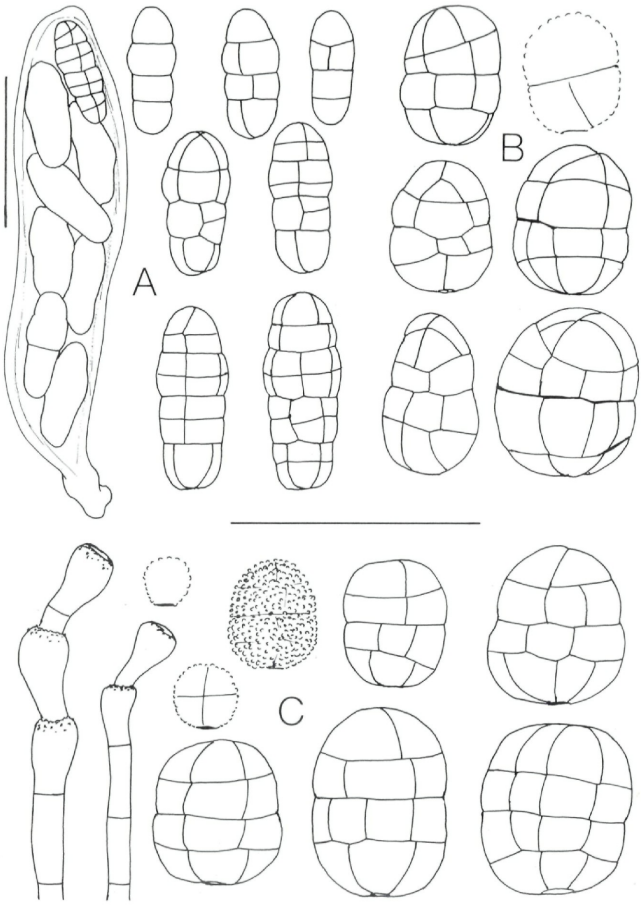

Fig. 2: *Pleospora tarda/Stemphylium botryosum:* A. ascus and ascospores, and B. conidia from culture of teleomorph type [EGS 04-118C]. – C. conidia and conidiophores from holotype [STR] of anamorph. – Ascus; bar = 50 µm; ascospore and conidium; bar = 50 µm.

stricted manner, with isolates of each to provide a progression of developmental stages, permits us to take a few more steps toward understanding the diversity and, I believe, the multiplicity of species that have been included in the *P. herbarum* species-complex (WEHMEYER, 1961). To the classic characters of ascocarp shape, size and texture; ascus shape and size; and ascospore shape, size, color and septation, we now are becoming accustomed to note differential factors such as ascocarp maturation time (fast, slow), juvenile spore shape and early development, and uniqueness of *Stemphylium* anamorphs.

3. Yet a third *Pleospora-Stemphylium* holomorph (Fig. 3) derived from *Medicago* invites incredulity, but it actually may help to explain some published observations related to leafspot diseases of alfalfa on a worldwide basis (most recently, COWLING, GILCHRIST & GRAHAM, 1981; IRWIN, 1984). Several isolates from *Medicago* in Western Australia are of a *Stemphylium* with conidia having somewhat the same morphology as those of *S. vesicarium* (WALLROTH) SIMMONS; thin-walled ascostromata mature rapidly in cultures of these isolates (within 25–30 days) in the manner of *P. herbarum,* but ascospores of this third species are markedly different from those of the first two discussed above.

Stromata are produced abundantly in V-8 and PCA plate cultures within 4–5 days and may contain asci with fully developed spores within 17 days. Ascostromata about 600 µm diam. are thin-walled, subspherical, with a short beak. Asci are subcylindrical, about 140 × 30 µm for many with either immature or mature ascospores but also as large as about 200 × 40 µm before rupture. Ascospores initially are narrowly ellipsoid, becoming narrowly obovoid when the first transverse septum is formed slightly below the middle of the spore; as many as five, occasionally six primary septa are produced across the total width of the developing spore, the remaining transverse septa being partial within cells delimited by longitudinal septa; longitudinal septa sometimes completely through a primary transverse division of the spore but just as frequently partial, vertical or oblique and without predictable pattern. The fully developed ascospore is medium yellow-brown, ellipsoid or narrowly obovoid, very often near 38 × 12 µm and reaching a maximum size of about 42 × 14 µm (l/w near 3.0), with 5–6 primary and additional secondary transverse septa (yielding what might be considered 7–9-septate spores) and 1–3 primary and secondary longitudinal septa in most of the transverse divisions of the spore; constricted at each of the initial three transverse septa.

Juvenile conidia are either rectangular-oblong or subspheroid to broadly ovoid, with walls subhyaline and minutely verruculose.

Oblong conidia quickly become 1–3 transversely septate, add secondary transverse and longitudinal septa in each of the 3–4 primary transverse divisions of the conidia, darken as septa and verruculae become brown, and enlarge to a size range of about

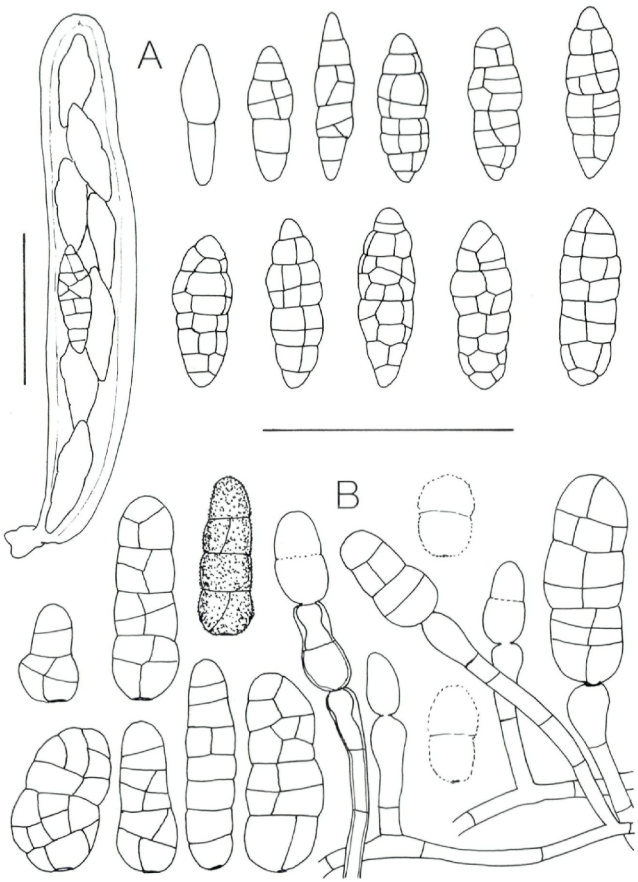

Fig. 3: *Pleospora alfalfae/Stemphylium alfalfae:* A. ascus and ascospores, and B. conidia and conidiophores from culture of holotype [EGS 36-088]. – Ascus; bar = 50 µm; ascospore and conidium; bar = 50 µm.

30–40 × 12–15 µm with a max. near 45 × 18 µm (l/w near 2.5). Juvenile spheroid and ovoid conidia develop 1 median transverse septum and become variously transversely and longitudinally septate in a somewhat unpredictable pattern of secondary walls, often with a spurious appearance of having 3 major transverse septa (the initial primary septum remains identifiable); large numbers of resultant broadly ovoid, often inequilateral conidia enlarge to a size range of about 32–35 × 16–19 µm (l/w near 2.0). Septa of most conidia thicken and darken in age; conidia of all shapes remain noticeably constricted at primary transverse septa.

Nomenclator and new species

1. *Pleospora herbarum* (Pers.: Fries) Rabenhorst ex Cesati & deNotaris, Comment. Soc. Crittog. Ital. 1: 217 (1863)
   = *Sphaeria herbarum* Persoon, Syn. meth. fung., p. 78 (1801)
   = *Sphaeria herbarum* Pers.: Fries, Syst. mycol. II(2): 511 (1823)
   Sanctioned name; *holotype* = E. Fries, Scleromyceti Sueciae no. 38, in herb. E.

Anamorphosis: ***Stemphylium herbarum*** Simmons, anamorph. nov.

Ex culturis in agaris PCA et V-8 descripta. – Sporulatio abundans. – Conidiophora ca. 3–5 µm diam., vulgo 15–20 µm long. vel. multo magis, in apice usque 8–10 µm inflata, simplicia vel prolificantia nunc crescentia apicali nunc persaepe crescentia subterminali. – Conidia initio oblonga vel late ovoidea vel subsphaerica, hyalina, minute verruculosa, postea late ovoidea vel late elliptica, aliquando inaequilateralia, distincte atque copiose verruculosa; septis transversalibus usque ad 6–7 seriatim et longitudinalibus 1–3 in segmentis transversalibus; ad 1–3 septa transversalia distincte constricta; dilute ochracea vel spadicea; in statu maturo usque ad ca. 35–45 × 20–27 µm. – *Typus:* partes ex EGS 36–138 (coll. H. K. Joshi in *Medicago sativa*, Jhansi, India, 1983 [IMI 276975]) desiccatae et in BPI (holotypus) et pro isotypis saltem in DAOM, IMI et NY conservandae.

2. ***Pleospora tarda*** Simmons, sp. nov.

Ex culturis in agaro PCA descripta. – Sporulatio anamorphosis abundans. – Primordia ascomatum aliquot sparsa, sclerotica, atra, crescentia tarde demum ad 700 µm diam. vel magis, post ca. 8 menses ascos et ascosporas maturas continentia. – Asci subcylindrici, ca. 200 × 40 µm, 8-spori. – Ascosporae juveniles hyalinae, oblongae utrinque obtusa, continuae dein 1–3 transverse septatae, valde constrictae; ascosporae maturae ochraceae, ca. 40 × 17 µm, apice late rotundo, basi late rotunda vel quasi plana, septis transversalibus primariis 3 et secundariis 4, initio serie una septorum longitudinalium primariorum demum septis secundariis in omnibus quartis transversalibus. – Typus: praeparatio in lamina vitrea ex EGS 04-118C (coll. W. G. Benedict in *Medicago sativa*, Ontario, Canada, 1953, in BPI (holotypus) conservanda [ex QM 1379 = ATCC 18522 = CBS 714.68 = IMI 135456].

Anamorphosis: *Stemphylium botryosum* Wallroth, Flora crypt. germ., pars post., p. 300 (1833). Holotype: "Ad Asparagam" in herb. Wallroth, STR.

3. ***Pleospora alfalfae*** SIMMONS, sp. nov.

Ex culturis in agaris PCA et V-8 descripta. – Sporulatio anamorphosis *(Stemphylium alfalfae)* copiosa. – Primordia ascomatum abundans in aliquot dies, atrantia, crescentia celeriter ad ca. 600 µm diam.; ascomata matura parietibus tenuibus, subsphaerica, rostellata, sporas plene evolutas in 17–25 dies ferentia. – Asci subcylindrici, 8-spori, plerumque ca. 140 × 30 µm, etiam ad ca. 200 × 40 µm in maturitatem. – Ascosporae juveniles anguste ellipsoideae, tum anguste obovoideae septum primum submedianum habentes; ascosporae maturae ochraceae, plerumque ca. 38 × 12 µm, ut maximum ca. 42 × 14 µm, ellipsoideae vel anguste obovoideae, septis transversalibus primariis 5–6 et secundariis 2–3, septis longitudinalibus primariis atque secundariis 1–3 in plurimas partes transversales sporae, ad 3 septa prima transversalia valde constrictas.

Anamorphosis: ***Stemphylium alfalfae*** SIMMONS, anamorph. nov.

Conidia juvenilia nunc oblonga transverse 1–3-septata nunc subsphaeroidea vel late ovoidea transverse 1-septata, subhyalina, minute verruculosa; conidia oblonga matura septis transversalibus usque ad 6–7 et longitudinalibus 1–2 in segmentis transversalibus, 30–40 × 12–15 µm, max. ca. 45 × 18 µm; conidia sphaeroidea vel ovoidea matura septis transversalibus 3–4 et longitudinalibus pluribus ordinatione variabili, plerumque ca. 32–35 × 16–19 µm; plurima conidia matura ad septa primaria transversalia constricta, septis incrassatis fuscatis. – TYPUS holomorphosis: partes ex EGS 36-088 (via IMI 269683, 1982, coll. *Medicago sativa*, WA, AUSTRALIA) desiccatae et in BPI (holotypus) et pro isotypis saltem in DAOM, IMI et NY conservandae.

Conclusion

It is with considerable hesitation that I have decided to establish names for distinguishable *Pleospora* teleomorphs with *Stemphylium* anamorphs that I have watched develop in axenic culture over the past 30 years. All examples thus far are considered to belong with the *P. herbarum* complex of species, as treated by WEHMEYER (1961) and by others (most recently CRIVELLI, 1983). WEHMEYER'S views about the complex were that within *Pleospora herbarum* "there is a wide range of variation in spore size, form, color, and secondary septation, and it will probably be possible to recognize several distinct taxa when this complex group is better known." He synonymized about sixty *Pleospora* names with *P. herbarum,* in most cases because type specimens and descriptions available to him did not yield usable data for making segregations.

The National Mycological Herbarium, Biosystematic Research Institute, Agriculture Canada, Ottawa [DAOM] generously has given me access to the Wehmeyer slide collection and herbarium, which are among their holdings. Numerous other institutions and individuals have been very helpful with specimens, cultures, and literature; these are cited in the text when appropriate. Even with such abundant support, pairing current isolates with already published *Pleospora* names and their types is a treacherous pursuit. Therefore,

when an old name can be taken up with any degree of certainty, I am doing so. When available type material is unconvincing, new names are being established.

The separation of typical *Pleospora herbarum/Stemphylium herbarum* from typical *Pleospora tarda/Stemphylium botryosum* will be disconcerting from many viewpoints, including bibliographic, for the traditional misinterpretation *(P. herbarum = S. botryosum)* is reinforced by multitudes of misidentified specimens. My view that it is both possible and taxonomically necessary to clarify the uniqueness of the two species follows closely our information from type specimens, developmental morphology, and phytopathology. And it seems only fair to add that the three species treated here are only the tip of an iceberg that now comprises certainly a dozen, perhaps even a score of distinguishable *Pleospora-Stemphylium* species.

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