

## THREE NEW SPECIES OF BOTRYOTINIA ON RANUNCULACEAE<sup>1</sup>

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### Abstract

Three new species of *Botryotinia* on *Caltha palustris* L., *Ranunculus septentrionalis* Poir., and *Ficaria verna* Huds. (Ranunculaceae) are described as *B. calthae* Hennebert and Elliott, *B. ranunculi* Hennebert and Groves, and *B. ficariarum* Hennebert. Each of the three species has a *Botrytis* state of the *B. cinerea* complex, and they thus constitute additions to the species already segregated from that complex, i.e. *Botryotinia fückeliana*, *B. convoluta*, *B. draytoni*, and *B. pelargonii*. The *Botrytis* state of *B. ficariarum* can be distinguished morphologically.

While *B. ranunculi* is a North American species and *B. ficariarum* an European one, *B. calthae* is reported from both continents.

### Introduction

*Botryotinia*, a genus of the Sclerotiniaceae, was erected by Whetzel (1945) for four species formerly assigned to *Sclerotinia* Fuckel, but which differ from the true *Sclerotinia* species mainly in their erumpent, planoconvexoid sclerotia which are firmly attached to the substrate, and in the possession of a conidial state belonging to the form-genus *Botrytis* Pers. The type species is *Botryotinia convoluta* (Drayton) Whetzel in which the conidial state is a *Botrytis* of the *cinerea* type or *B. cinerea sensu lato*, and the other species included were *Botryotinia fückeliana* (De Bary) Whetzel, of which the conidial state is *Botrytis cinerea* Pers. or *B. cinerea sensu stricto*, and *Botryotinia ricini* (Godfrey) Whetz. and *B. porri* (v. Beyma) Whetz. In the latter two species the conidial states would not be considered *Botrytis* species of the *cinerea* type.

Buchwald (1949) added three species to *Botryotinia*, *B. narcissicola* (Gregory) Buchw., *B. polyblastis* (Gregory) Buchw., and *B. sphaerosperma* (Gregory) Buchw. The *Botrytis* states of these species are not of the *cinerea* type.

Røed (1949) described another species, *Botryotinia pelargonii*, based on apothecia which he obtained from cultures of a *Botrytis* of the *cinerea* type isolated from *Pelargonium*. Although the imperfect state of this fungus greatly resembles that of *Botryotinia fückeliana*, the apothecia can be distinguished by the ascospore measurements.

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Seaver (1951) published two *Botryotinia* combinations, *B. porri* (v. Beyma) Seaver, which is a later homonym and synonym of *B. porri* (v. Beyma) Whetzel, and *B. draytoni* (Buddin and Wakefield) Seaver, another species with a *Botrytis* state of the *cinerea* type. Dennis (1956) suggested that it was close to *Botryotinia convoluta* (Drayt.) Whetz.

Buchwald (1953) described *Botryotinia globosa* on *Allium ursinum* with the conidial state *Botrytis globosa* Raabe. Viennot-Bourgin (1953) described *Botryotinia squamosa* on *Allium cepa* with the conidial state *Botrytis squamosa* Walker. Yamamoto (1956) added *Botryotinia allii* (Sawada) Yamamoto with the conidial state *Botrytis byssoidea* Walker, and *Botryotinia arachidis* (Sawada) Yamamoto.

Hainsworth (1948) described a *Botrytis* blight of tea flowers in India that he believed was caused by another *Botryotinia* of which he had collected apothecia under the tea plants. However, he did not name it or provide a formal description. Sarmah (1956) referred to this fungus as *Botryotinia theae* Hainsworth along with another species that he also attributed to Hainsworth, *Ciborinia theae*. However, both these names are *nomina nuda*. Dr. V. Agnihotrudu, Tocklai Experiment Station of the Indian Tea Association, Cinnamara, India, has informed the senior author that no type material of either of these fungi has been preserved.

The genus *Botryotinia* Whetz. at the present time consists of 13 species, of which four might be considered to have conidial states belonging in the *Botrytis cinerea* complex, *Botryotinia fuckeliana*, *B. convoluta*, *B. draytoni*, and *B. pelargonii*.

*Botryotinia fuckeliana* is widely distributed and recorded from many different hosts, particularly as the conidial form *Botrytis cinerea* Pers. as typified by the Persoon collection L 910.262.845 in Herb. L (Groves and Loveland 1953). However "it does not necessarily follow that all other isolates of *Botrytis* species of the *cinerea* type belong to *Botryotinia fuckeliana*", as stated by Groves and Loveland in 1953. Already Whetzel and Drayton, in 1932, had distinguished *Botrytis convoluta* from the *B. cinerea* complex on the basis of its characteristic sclerotia, and Drayton, in 1937, showed it to be the imperfect of the distinctive species *Sclerotinia convoluta* Drayt. Later, *Sclerotinia draytonii* Buddin & Wakefield (Dennis and Wakefield 1946) and *Botryotinia pelargonii* Røed (1949) were segregated. In 1953, Groves and Loveland foresaw that further work would "make it possible to recognize other specific entities in this great *B[otrytis]*. complex".

It is now proposed to describe three more species each of which has a *Botrytis* state of the *cinerea* type. Two of these new species particularly were known to Whetzel. One of them occurs on *Caltha palustris* L., the other on *Ranunculus septentrionalis* Poir. For many years Whetzel observed them in the field near Ithaca, N.Y., throughout their entire life history. In the spring of 1930 in Europe, he collected apothecia and an associated *Botrytis* on *Ficaria verna* Huds. and considered that this was still another species of *Botryotinia* on Ranunculaceae. Although he had notes on all three of these species he did not publish a description of any of them.

Dr. F. L. Drayton was also quite familiar with the species on *Ranunculus* and *Caltha* and he initiated experiments at Ottawa to study their sexual

behavior. This work was carried on by Groves, especially with the species on *Ranunculus*, and with the species on *Caltha* by Mrs. C. A. Bowerman and Miss Mary E. Elliott, who also carried out morphological studies.

For all three of these species, names have already been published twice by Buchwald (1939, 1949), but in each case, they constituted 'nomina nuda' because of the absence of description. Buchwald, who knew of the existence of the species from his association with Whetzel and recognized the species on *Caltha* in Denmark, mentioned them as '*Botrytis calthae*', '*Botrytis ficariacearum*', and '*Botrytis ranunculi*' in 1939. In 1949 he assigned them all to '*Sclerotinia (Botrytis)*' as new species and under the authorship of Whetzel. Since in the meantime Whetzel published the genus *Botryotinia*, which was accepted by Buchwald, it appears, therefore, that Buchwald did not intend publication of the Whetzel names and that his use of them was merely casual mention.

The three specific epithets we are using were originally chosen by Whetzel and used by him in his notes or on his herbarium specimens. Since we will indicate later the reasons why Whetzel did not use the epithet *ficariae* but *ficariarum*, we only state here our preference for the genitive plural *ficariarum* rather than *ficariacearum*.

Terminology used in the description of the apothecial anatomy is that defined by Korf (1958).

***Botryotinia calthae*** Hennebert and Elliott n. sp. Figs. 1, 2, 7, 8, 13, and 17.

*Apothecia* solitaria vel plura e sclerotiis orientia; *discus* stipitatus discoideus carnosus umbilicatus vel planus, hymenio pallide brunneo, margine integro fuscior, exteriore leve pallidior, 1-4(5) × 0.5-0.8 mm; *stipes* cylindraceus, in majore parte colore simili disco, in basali parte atrobrunneus cum fuligineo fasciculato tomento, 2-20 × 0.5-1.5 mm; apothecii secti *hymenium* 160-190 μ, subhyalinum; *asci* inoperculati octospori cylindracei ad basim attentuati (103) 120-160 (190) × (6)8-11(14) μ jodo positivi; *ascosporae* uniseriatae obliquae continuae hyalinae naviculiformes subacuminatae non guttiferae (8) 11.5-16.5 (18) × 5-8.8 μ, plerumque 13.8 × 7.5 μ; *paraphyses* paucae filiformes tenues septatae subhyalinae 2.5-3 μ lat., ad basas ramosae, ad apicales 4-4.5 μ clavatae et rotundatae; *subhymenium* brunneum 50 μ, e textura intricata, hyphis 3-8 μ crass.; *excipulum* intus e textura intricata ad porrectam, hyphis levibus flexuosis furcatis septatis 4-9.5 μ crass., formans palum marginalem prominentem compactum 20 μ crass. brunneum; *excipulum* extus 65-90 μ, e textura globulosa ad angularem, cellulis tenuitunicatis subhyalinis 19-27 × 8-9 μ, exterioribus projicientibus pilos 13-30 × 3-6.5 μ hyalinos flexuosos leves; *stipes* e textura porrecta, hyphis interioribus 3-12 μ crass. bifurcatis et anastomosis, cellulis 70-110 μ long., hyphis exterioribus 11-16 μ crass., cellulis 34-40 μ long., ad summas stipitis hyalinis et projicientibus pilos 2-3 μ crass. tenuos ramosos, ad basas fuligineis et projicientibus pilos longos fuligineos septatos 75-125 × 3.5-4.5 μ. *Stromata*, sclerotia elongata acuminata nigra plano- vel concavo-convexa 3-18 × 0.5-1.5 μ, in petiolo hospitis formata; in vitro rotundata saepe immersa parva 1-3 mm diam. irregulariter distributa sed saepe aggregata et radialiter posita, matrice brunnea tincta; *cortex* e cellulis plerumque 14 × 10.5 μ; *medulla* e hyphis compactis 3.5-6 μ crass.

*Spermatia* in natura non visa, in vitro in spermodochiis, 2.5–3  $\mu$  ennata. *Conidiophorus status Botrytis* similis vere *B. cinereae* Pers.; conidia (7) 8–16.5 (18.5)  $\times$  5.8–9 (10)  $\mu$ . *Habitat*: in paludibus parasitica in *Calthae palustris* L. vivis foliis et petiolis, apotheciis conidiisque e sclerotiiis post hiemem ennatis. Europa et America boreali. *Typus*: in R. P. Korf, *Discomycetae Exsiccatae*, Fasc. IV et in herb. G.L.H. 3094.

*Apothecia* arising one to three on the sclerotium on overwintered petioles, usually not very long stipitate, disc at first dark, globoid, opening by a central pore, cupulate inside, then becoming shallow cup-shaped to flattened, central part slightly umbilicate to plane, or sometimes umbonate, pale brown but lightened towards the edges, margin slightly evident, entire, thick, dark brown, underside apparently smooth, whitened, 1–4 (5) mm in diameter, 0.5–0.8 mm thick. Stipe cylindrical, of variable length, depending upon the depth the sclerotium is buried, 2–20  $\times$  0.5–1.5 mm, in the upper portion concolorous with the outer surface of the disc, sometimes hairy around the apex, the lower portion 1–4 mm in length, dark brown to black and provided with agglutinated rhizoidal tufts among which organic and soil particles are enmeshed.<sup>4</sup>

In axial section: *hymenium* 160–190  $\mu$  thick, subhyaline; *asci* inoperculate, cylindrical, attenuate towards the base, rounded at the apex, which is thickened and provided with an iodine positive pore, eight-spored, (103) 120–160 (190)  $\times$  (6) 8–11 (14)  $\mu$ ; *ascospores* one-celled, hyaline, navicular, subacuminate at both ends, usually uniseriate and oblique, sometimes biseriate, (8) 11.5–16.5 (18)  $\times$  5–8.8  $\mu$ , averaging 13.8  $\times$  7.5  $\mu$ ; *paraphyses* slender, 2.5–3  $\mu$  wide, as long as or a little longer than the asci, once to three times branched in the lower 35  $\mu$  from the base, septate, cells 10–25  $\mu$  long, slightly clavate towards the tip, up to 4–4.5  $\mu$  wide with a rounded end. *Subhymenium* about 50  $\mu$  thick, light brown, of a textura intricata, composed of filamentous and globose cells 3–8  $\mu$  wide. *Medullary excipulum* 65–90  $\mu$  thick, hyaline, of textura intricata to porrecta, compact, hyphae smooth, undulate, more or less parallel to the outer surface of the disc, bifurcate or laterally branched, 4–9.5  $\mu$  wide, mostly 6.5–7  $\mu$ , septate, cells 30–150  $\mu$  long, the portion under the center of the disc of a loose textura intricata, developing at the margin of the disc a palisade 20  $\mu$  thick of paraphysis-like hyphae, compactly parallel 4–5  $\mu$  wide, subhyaline. *Ectal excipulum* 65–90  $\mu$  thick, subhyaline, of textura globulosa, cells thin-walled, 19–27  $\times$  8–19  $\mu$ , mostly 16  $\times$  14  $\mu$ , the outermost more globose or pyriform, projecting to form hyaline, flexuous, smooth, hair-like processes, sometimes septate or bifurcate, rounded at the end, 13–30  $\times$  3–6.5  $\mu$ , cells at the margin of the disc ovoid, pyriform, or irregularly inflated at the base. *Stipe* of a textura porrecta, in *medullary* portion hyphae 8–12  $\mu$  diam. cells 70–110  $\mu$  long, variably constricted at the septa, often bifurcate, with abundant anastomoses between hyphae through a prominent pore 1.5–2.5  $\mu$  in diameter, in *ectal* portion also of a textura porrecta but compact, with hyphae 11–16  $\mu$  wide and cells 34–40  $\mu$  long, throughout most of the length of the stipe hyaline to subhyaline and thin-walled, at the base becoming fuliginous, with

<sup>4</sup>Such rhizoidal tufts at the base of the stipe here described in three species of *Botryotinia* are for the first time, as far as we know, mentioned in this genus. They have been previously observed in some species of *Monilinia* Honey (Honey 1928, 1940) and in some species of *Ciborinia* (Groves and Bowerman 1955; Batra and Korf 1959; Batra 1960).

the outermost wall dark brown to black and thickened, towards the apex of the stipe the walls of the hyphae often projecting to form vesicles which elongate into hyaline, septate, irregularly branched hairs 2–3  $\mu$  wide, of variable length depending on the environmental conditions, often 20–40  $\mu$  long, towards the base of the stipe forming fuliginous, flexuous, fasciculate hair-like hyphae of the rhizoidal tufts, 75–125  $\mu$  long, 3.5–4.5  $\mu$  wide.

*Stroma*, a definite sclerotium of the plano-convexoid type, concave below, slender and elongate, acuminate at both ends, striate above, grooved below along the entire length, formed in petioles and along the larger veins of the leaves, under the cuticle, firmly attached to the veins, black, 3–18  $\times$  0.5–1.5 mm; in section, typical of the genus, *ring cells* in two to five layers, 9–14  $\times$  5–12  $\mu$ , mostly 14  $\times$  10.5  $\mu$ , *medullary hyphae* hyaline, 3.5–6  $\mu$  wide, septate in cells 20–40  $\mu$  long, compactly arranged.

*Spermidium* not observed in the field, a spermodochium in culture, white to ochraceous, globose, 50–100  $\mu$  broad; spermatophores repeatedly branched, metulae subglobose or shortly elongate, divergent, 5–10  $\times$  4–8  $\mu$  bearing three (five) phialides inflated, curved, 4–8  $\times$  2.5–3.5  $\mu$  having a narrow neck and very slight collarette; spermatia phialospores, globose, 2.5–3  $\mu$  in diameter.

Conidial state *Botrytis* of the *cinerea* type. Conidiophores arising in tufts from sclerotia on overwintered petioles of the host at the same time or before the apothecia, and from the mycelium in necrotic lesions on leaves and petioles. Conidia ellipsoid, ovoid, or subglobose, (6.9) 8.1–16.5 (18.4)  $\times$  5.8–9.1 (9.9)  $\mu$ , mostly 12  $\times$  7  $\mu$ , quotient length/width 1.75 in average.

#### *Cultural Characters*

On potato-dextrose (2%) agar, at 20–22° C, aerial mycelium abundant, felty and tufted, grey to dirty ochraceous, with sporulation occurring in spots, and abundant, very large, greenish brown to black appressoria on the side and bottom of the petri dish; sclerotia present but difficult to observe. At a lower temperature (14° or 5° C) in darkness, medium becoming irregularly or spotted chestnut brown to dark brown, aerial mycelium scanty, arachnoid, producing abundant, minute, white or dirty white spermodochia as well as the hyaline intramatrical mycelium; conidiophores and appressoria few or none; sclerotia typically well developed, originating under the surface of the agar, those closest to the surface becoming later erumpent, black, shiny because of the agar film, many others deeply imbedded in the agar and adherent to the bottom of the petri dish, varying in size, usually small, 1–3 mm in diameter, sometimes larger, ordinarily showing a strong tendency to aggregate in very large irregular groups, crust-like, often radially oriented, plano-convexoid to globose, surrounded by a diffuse, brown-stained zone in the agar. On potato-dextrose agar in tube, conidiophores and appressoria more abundant. On sterilized wheat, at 14° and 5° C, very numerous, small, regularly shaped sclerotia 1–2 mm in diameter, dull black, plano-convexoid or hemispherical, slightly rugose, ordinarily aggregated into large discontinuous crusts.

*Habitat*.—Parasitic on living leaves and petioles of *Caltha palustris* L. in swampy places, developing apothecia from overwintered sclerotia in decayed petioles.

*Distribution*.—Europe (Belgium, Denmark) and North America (United States and Canada).

*Type*.—In R. P. Korf, Discomyceteae Exsiccatae, Fasc. IV (in Herb. CUP, BPI, DAOM, K, LAH, MICH, NY, PC, PR, UC, UPS) and in Herb. G.L.H. 3094, collected at Michigan Hollow, Tompkins Co., N.Y. by R. P. Korf, G. L. Hennebert, and others, May 13, 1962.

*Specimens Examined*

(1) Apothecia and conidia from sclerotia on overwintered petioles of *Caltha palustris*: UNITED STATES: McLean, Lloyd-Cornell Preserve, N.Y., CUP 17479; CUP 19171; CUP 23535; CUP 25274, DAOM 4447; CUP 27322; Labrador Lake, near Apulia, N.Y., CUP 25930; G.L.H. 2065; G.L.H. 3123; Michigan Hollow, Tompkins Co., N.Y., G.L.H. 2057; G.L.H. 3094 and R. P. Korf, Discomyceteae Exsiccatae, Fasc. IV (in preparation) (Type); Malloryville bogs, Tompkins Co., N.Y., G.L.H. 3086, CUP 45676; CANADA: Tenaga, Que., CUP 26450; Mud Lake, Gatineau Co., Que., DAOM 45948; DAOM 67083; Hoare's Creek, Gatineau Park, Gat. Co., Que., DAOM 67082-a; DAOM 67082-b; DAOM 56310; DAOM 56326; DAOM 56325; G.L.H. 2106; Fortune Lake, Gatineau Park, Gat. Co., Que., DAOM 84068. *Europe*: Lovenjoel, Brabant, Belgium, G.L.H. 1091; G.L.H. 1092, G.L.H. 1115.

(2) Conidia only on overwintered petioles: UNITED STATES: McLean, Lloyd-Cornell Preserve, N.Y. CUP 31443; G.L.H. 2013. EUROPE: Lovenjoel, Brabant, Belgium, G.L.H. 1093; G.L.H. 1090.

(3) Conidia on living leaves and petioles of *Caltha palustris*: UNITED STATES: Lichtfield, Herkimer Co., N.Y., CUP 17763; Labrador Lake, near Apulia, N.Y., CUP 1312; G.L.H. 2065; CANADA: South March, Carleton Co., Ont., DAOM 45988; Camp Fortune, Gatineau Co., Que., DAOM 43144; Fortune Lake, Gatineau Park, Gat. Co., Que., G.L.H. 2040. EUROPE: near Perreux, Switzerland, H. H. Whetzel E 73 in CUP; Lyngby, near Copenhagen, Denmark, H. H. Whetzel E 77 in CUP; Bollennozen, Sealand, Denmark, C. Ferdinandsen Herb. in CP; Nyteboda, Sweden, N. F. Buchwald Herb. in CP; Hacquegnies, Hainaut, Belgium, G.L.H. 414; Lovenjoel, Brabant, Belgium, G.L.H. 821; G.L.H. 1089.

Single-ascospore and mass-ascospore strains of this species are maintained in the DAOM (Ottawa) culture collection.

The connection between the apothecial, conidial, and sclerotial states has been established partly on circumstantial and partly on cultural evidence. In the spring, apothecia and conidia may be found arising from the same sclerotium, and cultures from ascospores, conidia, sclerotial tissue, and infected leaf tissue are identical.

In attempts to confirm the connection by passage from conidia to ascospores, we carried out several series of experiments using mass-conidial, mass-ascospore, and single-ascospore isolates. The technique used followed the experimental schedule from Drayton (1937) and as outlined by Groves and Loveland (1953) and in ordinary use at the Plant Research Institute, Ottawa. This technique has been successful for the production of apothecia in other species of *Botryotinia*, such as *B. convoluta* (Drayton 1937), *B. fuckeliana* (Groves and Drayton 1939; Groves and Loveland 1953), and many other Sclerotiniaceae.

As early as 1938 some limited preliminary attempts were made to produce apothecia in this species. However, these first attempts were unsuccessful and no further experiments were tried with it until 1954. Since then four different experimental series have been attempted by Mrs. C. A. Bowerman and Miss M. E. Elliott using fresh isolates from both ascospores and conidia, and growing the cultures at 5° and 14° C. A fifth series was established recently by Miss M. E. Elliott and the senior author using both Canadian and European isolates of the species. All these experiments have also been unsuccessful, and so far we have not obtained any apothecia of this species in culture, and have, therefore, no information concerning its sexuality.

Whetzel first saw this species in 1917 when he received from H. D. House, N.Y. State Museum, Albany, a collection of largely necrotic leaves of *Caltha palustris* covered with *Botrytis* conidiophores (CUP 17763). In 1919, at Labrador Lake, N.Y., Whetzel himself observed a severe *Botrytis* epidemic in *Caltha palustris*, when a large number of plants were entirely killed. His notes of 1927 indicate his first observation of the sclerotia on overwintered petioles, and his appreciation that in culture, the fungus is "rather typical of the *Botrytis cinerea* type". Two years later, in 1929, the first apothecia were found in the field at McLean, N.Y., by Whetzel, who noted: "this appears to be an undescribed species of *Botryotinia*." (Whetzel's notes S 672, CUP 17479). The generic name *Botryotinia* was not yet published, but since 1927, Whetzel had been using it in his own notes as a tentative new generic taxon which had to be segregated from *Sclerotinia* Fuckel; however, in the CUP herbarium, the fungus was provisionally labelled *Sclerotinia calthae* n. sp.

During his trip through Europe in 1930, Whetzel only collected the *Botrytis* state on *Caltha palustris*. However, Professor N. F. Buchwald, who had seen the fungus in New York State in 1931, recognized it later in Denmark and included the species in his *Fungi Imperfecti* (1939) as a 'nomen nudum', stating that "the author has personally observed, on decaying stems of *Caltha palustris*, apothecia of *Sclerotinia* as well as colonies of *Botrytis*, which no doubt belong together." (Buchwald 1939, translated from Danish). Buchwald (1949) repeated his statement, assigning the species to *Sclerotinia* under the name "*Sclerotinia (Botrytis) calthae* Whetzel n. sp. (*Caltha palustris*)", [nomen nudum]. Buchwald added in a footnote that this species had been made the type of the new genus *Verpatinia* Whetz., under the name *B. calthicola* Whetz., but *Botryotinia calthae* and *Verpatinia calthicola* are entirely different.

The latest record of *Botryotinia calthae* in Europe is that reported here from Belgium. The collections were made by the senior author on March 20 and 23, 1960 (G.L.H. 1089 to 1093, 1115).

In Canada, many collections of the fungus have been made since 1937, by Dr. F. L. Drayton, J. W. Groves, and their collaborators. The species appears common in swampy places in the area around Ottawa.

From the field observations, the life history of the fungus may be worked out. At the end of spring, in June, sclerotia are developing imbedded in the rotting tissues of infected petioles of *Caltha palustris* which have been killed and are lying in the swamp at the base of the plants. These sclerotia overwinter still attached to the exposed tissues of the petioles, the superficial

tissues having disappeared. Alternating variation of the water level in the swamp in the spring seems to favor an early production of conidia from the sclerotia. It is about 1 or 2 weeks later that the first apothecia will develop from the same sclerotia. The date of the first appearance of the conidial and the apothecial states depends on the local climate. The first conidium production from sclerotia occurs at about the time when the *Caltha* shoots are just coming out; it is during the last half of April in the area around Ithaca, N. Y., and 2 weeks later in the Gatineau Park, Que., near Ottawa, but has been observed in the first week of March in Belgium. The earliest apothecial initials which have been recorded were observed on April 19, at McLean, N. Y., but on March 20 at Lovenjoel, Belgium. The host plants are then partly developed but not yet flowering. They come into full bloom during the period of production of apothecia. This period is quite long, extending from April 19 to May 25 in New York State, and from May 9 to May 31 at Ottawa. No data on the end of the period of fructification are available for Belgium at present. As soon as the first conidiophores have developed from the overwintered sclerotia, it is usual to notice the first infection of the plants at ground level. There the young shoots or the petioles of the young leaves are killed. The brown water-soaked lesions extend along the young blade and the under surface is soon covered with a short, dense, light grey layer of *Botrytis* conidiophores. Leaf blades may also show large necrotic lesions, which are circular or indefinite, brown with a light brown center, sometimes zonate, from water-soaked to dry. The more recent lesions often extend to the margin, and are V-shaped towards the petioles. In very humid weather, they may eventually invade the entire blade and run down through the petioles. Later, in June, it is common to observe on still living leaves such necrotic areas which have dried out and stopped their development. Parts of them placed in a moist chamber for a while soon become covered with *Botrytis* conidiophores. Whether the lesions on the leaf blades have originated from ascospore or conidial infections we do not know. The few inoculation experiments the senior author made have only been positively conclusive for direct infection of the shoots by conidia. However, since the development of conidia and early infection of the shoots precede the development of apothecia, it might indicate that the ascospores play a minor role in the spread of grey mould of *Caltha*.

***Botryotinia ranunculi*** Hennebert and Groves n. sp. Figs. 3, 5, 9, 10, 14, and 18.

*Apothecia* solitaria vel plura e sclerotiis orientia; *discus* stipitatus discoideus carnosus umbilicatus vel planus, hymenio brunneo, margine integro crasso fuscio, exteriore leve pallidior, 1.5–5 × 0.6–1.2 mm; *stipes* cylindraceus, in majore parte simili colore disco, in basali parte brunneus cum ochraceo tomento, 2–12 × 0.5–1.5 mm; apothecii secti *hymenium* 145–160 μ, pallide brunneum; *asci* inoperculati cylindracei octospori ad basim attenuati (100) 110–125 (176) × (7) 8–9 μ, jodo positivo; *ascosporae* uniseriatae obliquae continuae hyalinae ellipsoideae obtusae non guttiferae 10.2–16.2 (18) × 3.6–6.6 (7.8) μ, plerumque 14.4 × 5.6 μ; *paraphyses* numerosae filiformes tenues ramosae septatae pallide brunneae 2–3 μ lat., ad apicem 3–3.5 μ clavatae et rotundatae; *subhymenium* 25–50 μ, e textura intricata, hyphis 3–5 μ crass.; *excipulum* intus e textura intricata, hyphis levibus flexuosis furcatis septatis 6.5–13 μ crass.,



formans palum marginalem prominentem compactum brunneum 20–25  $\mu$  crass. e hyphis 2–3.5  $\mu$  lat.; *excipulum* extus 65–90  $\mu$ , e textura globulosa ad angularem, cellulis tenuitunicatis subhyalinis 20–50  $\times$  20–25  $\mu$ , exterioribus globosis moniliformibus 10–20  $\times$  9–14  $\mu$  projicientibus pilos hyalinos flexuosos breves leves; *stipes* e textura porrecta, hyphis interioribus bifurcatis anastomosis 6.5–12  $\mu$  crass., cellulis 80–140  $\mu$  long., hyphis exterioribus hyalinis 10–17  $\mu$  crass., cellulis 40–55  $\mu$ , ad basas stipitis fuligineis projicientibus pilos longos subhyalinos streptiformes septatos non ramosos. *Stromata*, sclerotia elongata acuminata nigra plano-vel concavo-convexa, 3–15  $\times$  1–1.5  $\mu$  formata in petiolo aut nervo hospitis; in vitro rotundata vel applanata, superficialia, media, 4–7  $\times$  3–5  $\mu$ , regulariter concentrice distributa, matrice non tincta; cortex e cellulis plerumque 5–6  $\mu$  diam., medulla e hyphis 2.5–4  $\mu$  crass. laxe intertextis. *Spermatia* in natura non visa, in vitro in spermadochiis, globosa 2.5–4  $\mu$  diam. e phialibus hyalinis 4–10  $\times$  2–3.5  $\mu$  ennata. *Conidiophorus status Botrytis* similis vere *B. cinereae* Pers., conidia (7) 11–15 (17)  $\times$  (5.5) 6–10 (11.5)  $\mu$ . *Habitat*: in sylvis paludosis parasitica in *Ranunculi septentrionalis* Poir. vivis foliis, petiolis caulibusque, apothecis conidiisque e sclerotiis post hiemem enatis. America boreali. *Typus*: in CUP 25278 et in DAOM 4457 et 7690, e natura vitroque.

*Apothecia* arising singly on the overwintered sclerotia in the field, but very numerous, up to 30, on sclerotia in vitro, usually not very long stipitate, *disc* at first globose, pinhead-shaped, dark, opening by a central pore, paler inside, becoming funnel-shaped, then expanding to plane, often with a central depression, fleshy, stout, 0.6–1.2 mm thick, 1.5–5 mm in diameter, hymenium brown, margin entire, thick, dark brown, underside apparently smooth and glabrous, paler, *stipe* 2–12 mm high, 0.5–1.5 mm in diameter cylindrical to irregularly cylindrical, tapering towards the base, smooth, glabrous, mostly concolorous with the outer surface of the disc, sometimes darkening downwards, with basal tuft of radiating rhizoidal hyphae, up to 500  $\mu$  long, subhyaline to ochraceous, undulate or twisted for most of their length, sometimes very shortly branched, appressed to the sclerotium.

In axial section: *hymenium* 145–160  $\mu$  thick, subhyaline to light brown, *asci* inoperculate, cylindrical, slightly attenuate towards the base, rounded at the apex, which is thickened and provided with an iodine-positive pore, eight-spored, (100) 110–125 (176)  $\times$  (7) 8–9  $\mu$ , ascospores hyaline, long ellipsoid, sometimes flattened on the sides, obtuse, one-celled, very variable in size, 10.2–16.2 (18)  $\times$  3.6–6.6 (7.8)  $\mu$ , averaging 14.4  $\times$  5.6  $\mu$ ; in culture asci similar sometimes containing more than 8 spores, up to 28 not infrequent, spores being then from very small, globose, or oblong to larger than normal and elliptical; ascospores germinating readily, also in the ascus itself, producing spermatia on one or two short phialides; *paraphyses* abundant, light brown, slender, 2–3  $\mu$  wide, as long or usually a little longer than the asci, septate with cells 15–20  $\mu$  long, laterally branched at any level, not or slightly clavate at the tip up to 3.5  $\mu$ , and obtuse. *Subhymenium* about 25–50  $\mu$  thick, brownish, of a textura intricata, hyphae 3–5  $\mu$  wide. *Medullary excipulum* 40–50  $\mu$  thick, thickening progressively from the margin towards the stipe, hyaline, of a textura intricata to porrecta, hyphae smooth, flexuous, more or less parallel to the under surface of the disc and going down into the stipe, bifurcate or laterally branched,

constricted at the septa but often swollen near the septa, 6.5–13  $\mu$  wide, mostly 8–8.5  $\mu$  where not swollen; at the margin of the hymenium paraphysis-like hyphae, densely fasciculate, septate, with cells 20–25  $\mu$  long, branching and anastomosing, forming a palisade 25–40  $\mu$  thick and slightly raised, the tips of these hyphae being slightly clavate and recurved outwards; at the central part of the disc, medullary excipulum of loose textura intricata, hyphae 8.1–9.4  $\mu$  wide, regular, frequently anastomosing. *Ectal excipulum* 65–90  $\mu$  thick subhyaline, of textura globulosa to angularis, cells thin-walled, the innermost more or less elongate, forming a palisade, 20–50  $\times$  20–25  $\mu$ , the outermost more globose, sometimes in monilioid chains, 10–20  $\times$  9–14  $\mu$ , often 12–13  $\mu$  in diameter, projecting to form hyaline, flexuous, smooth, hair-like processes, usually continuous and simple, rounded at the end, up to 30  $\mu$  long, 3–6  $\mu$  wide, at the margin of the hymenium, cells ovoid or pyriform projecting into short, hair-like processes. *Stipe* of textura porrecta, in *medullary* portion hyphae hyaline, 6.5–12  $\mu$  wide, mostly 9.5  $\mu$ , frequently anastomosing by juxtaposition through pores 2–2.5  $\mu$  wide, septate, cells 80–140  $\mu$  long, often bifurcately branched; in *ectal* portion hyphae 10–17  $\mu$  wide with shorter cells, 40–55  $\mu$  long, along the entire length of the stipe, hyaline to subhyaline, thin-walled with few projections, at the base external cells brownish, projecting into long twisted rhizoidal hyphae up to 1000  $\mu$  long, 2.5–3.5  $\mu$  width, septate, each twist varying from 6.5 to 12  $\mu$  in length.

*Stroma*, a definite sclerotium of the plano-convexoid type, concave below, slender, fusiform, black, formed within or upon the runners, petioles and largest veins of the leaves of the suscept, firmly attached to the tissues, varying greatly from 3 to 15 mm in length, usually 1–1.5 mm in width; in section, typical of the genus, rind cells polygonal, in two to three layers, 4–8  $\times$  4–6.5  $\mu$ , mostly 5–6  $\mu$  diameter, outer and lateral walls of the exterior layer of cells rather thick and almost black, *medullary hyphae* hyaline, 2.5–4  $\mu$  wide, straight or flexuous, loosely interwoven and imbedded in an abundant gelatinous matrix,<sup>5</sup> much branched, septate, sometimes constricted at septa, cells 13–40  $\mu$  long. In culture, sclerotia typically loaf-shaped, superficial, irregular but well defined, dull black, but covered by a webby white mycelium, firmly attached to the substratum, forming a continuous ring at the edge of the culture along the glass, varying in size from 4 to 7 mm in length, 3 to 5 mm in width; in cross section as on the suscept, showing a characteristic loose interweaving of hyphae.

*Spermidium* not observed in the field, a spermodochium in culture, appearing as minute, pearly, globose, slimy, whitish masses, up to 200  $\mu$  broad, spermatophores hyaline, repeatedly branched, primary and secondary metulae globose, up to 17  $\mu$  diameter, last metula shorter and more slender, 3–8  $\times$  3–5  $\mu$  bearing (two) to three to (five) phialides inflated, curved, 4–10  $\times$  2–3.5  $\mu$  having a narrow neck and a minute collarette; spermatia phialospores, globose, hyaline, 2.5–3.5 (4)  $\mu$  in diameter.

<sup>5</sup>This is the appearance of the medulla in section; but actually we do not know whether we observe *hyphae* with thin double walls leaving intrahyphal spaces filled by a 'gelatinous' substance of probable secretional origin, or whether we observe *lumina* and *internal walls* of hyphae of which the external walls extremely 'gelatinized' constitute the 'gelatinous matrix' excluding interhyphal spaces. Recent investigations by Novello and Korf [1962] showed that the 'gelatinous matrix' is also found *in vitro* on other substrates than agar, proving that agar does not enter into its composition.

Conidial state *Botrytis* of the *cinerea* type. *Conidiophores* arising in tufts from the overwintered sclerotia and from the undersurface of the leaves of the suspect, brown, readily proliferating into long branches. *Conidia* ellipsoid, ovoid, or subglobose, (7) 11–15 (17)  $\times$  (5.5) 6–10 (11.5)  $\mu$ , average 12.1  $\times$  7.2  $\mu$ , quotient length/width 1.69.

#### *Cultural Characters*

On potato-dextrose agar, at room temperature, the aerial mycelium forms minute tufts or flocci of a peculiar whitish grey color. These flocci are usually crowded forming a dense, thin felt usually covering the entire surface of the agar. Small olivaceous appressoria are formed in the tube, usually sparingly, on the glass along the lower edge of the slant. Minute, brownish, speck-like microconidial masses often develop in great abundance over the surface or submerged in the agar. The development of sclerotia is rather slow; mature sclerotia usually appearing only after some weeks, not very numerous, scattered over the surface and regularly formed along the edge of the petri dish. They are hemispherical, loaf-shaped, deeply irregularly lobed, dull black, covered with whitish webby mycelium, larger than those of *B. calthae*. Conidiophores are sparingly produced in petri dish cultures after the substratum begins to dry out. They are typical *Botrytis* conidiophores of the *cinerea* type. On sterilized wheat at 14° C and 5° C numerous sclerotia are uniformly developed, 3–5  $\times$  3–10 mm in size.

*Habitat*.—Parasitic on living leaves, petioles, and stems of *Ranunculus septentrionalis* Poir., in clear grassy swampy woods, developing apothecia from overwintered sclerotia in decayed stems and petioles.

*Distribution*.—North America (New York State).

*Type*.—from Labrador Lake, Onondaga Co., N.Y. Coll. H. H. Whetzel, F. L. Drayton, and others, May 5, 1936, in Herb. CUP 25278, DAOM 4457 (from the field) and DAOM 57690 (in culture from DAOM 4457).

#### *Specimens Examined*

(a) Apothecia and conidia at the type locality: DAOM 4457, CUP 25278 (type collection), CUP 16197; CUP 15618; apothecia and conidia obtained in culture at the Plant Research Institute, Ottawa: DAOM 57690; DAOM 26222, 26262, 12036, 12393, 19487, 12033 (all successively derived from the type collection).

(b) Conidia on leaves at the type locality: CUP 17284.

Single ascospore and mass ascospores strains of this species are maintained in the DAOM culture collection.

There is no doubt about the connection of the perfect and imperfect states of *Botryotinia ranunculi*. The circumstantial evidence, many times observed in the laboratory as well as in the field, has been experimentally confirmed by the work on the sexuality of the fungus.

Whetzel recorded in his notes the successful production of apothecia *in vitro* in 1927–28. From his second collection of the species on May 12, 1927 (CUP 15618), he obtained ascospore cultures (presumably mass ascospores) on potato-dextrose agar. On August 2, 1927, from 2-month-old cultures he transferred the sclerotia to moist cotton in dishes and held these in the dark

at 9° C until January 10, 1928, when initials of apothecia were observed to be developing. The dishes were then placed in the light in a cool greenhouse. Two weeks later, some apothecia had matured and more of them were just beginning to expand the cup. The apothecia were very abundant; for example one large fused sclerotial mass produced over 30 cups (Fig. 18D). Single ascospore isolates were obtained which produced typical mycelium, sclerotia, conidiophores, and conidia of *Botryotinia ranunculi*.

Preliminary work on the sexuality of this fungus was begun in 1936. One mass and four single ascospore cultures from DAOM 4457 were grown on wheat at 7° C for about 3 months and then put on moist sterilized sand in culture dishes. The transfers to sand were made in two ways, first by cutting out pieces of the wheat culture about 2 cm square and placing them on the sand, and second by removing individual sclerotia from the wheat culture to the sand. A set of the five isolates was placed in each of two dishes, one of which was spermatized with a mixture of spermata, and the other left unspermatized.

The sand cultures were put at 0° C for 1 month, then moved to 5° C for 6 weeks, then to 10° for 3 weeks, and finally transferred to the greenhouse. A month later apothecia were mature in all the isolates in the spermatized dish except one, but none appeared in the unspermatized dish. However, 3 weeks to a month later apothecia appeared in all the unspermatized cultures also. Apothecia of this series have been preserved in DAOM 57690.

Examination of these dishes revealed that spermata were being produced, in some instances actually on the fundaments themselves. This suggested that the fungus was self-fertile and the unspermatized cultures had been fertilized by their own spermata.

Apothecia were more abundant and sturdier from sclerotia which had been picked off individually than from the pieces of the wheat plates which were cut out and placed on sand. Possibly metabolic by-products in the wheat culture were responsible for inhibiting apothecium production to some extent.

The next experiment was designed to establish whether or not the fungus was self-fertile and to determine the most favorable temperature for its development. Seven single-ascospore cultures were grown on wheat at 5°, 10°, and 14° C for 2 months and then sclerotia were removed and placed on sand. Only one isolate was placed in each dish. Two sets were spermatized using spermata from single ascospore cultures and one set was left unspermatized.

On October 23, 1937, these dishes were placed in the greenhouse, and apothecia were mature on November 22, 1937 (DAOM 26222 and 26262) in the following crosses.

♂ \ ♀	SS6	SS10	SS2	SS13	SS11	SS12	SS14
SS2	-	-	-	-	+	+	+
SS10	-	-	+	+	-	-	-
Unspermatized	-	-	-	-	-	-	-

The two cultures that did not produce apothecia, also did not produce good sclerotia during the period of vegetative growth; hence these results were taken as clear evidence of self-sterility with bisexual reaction. It was considered probable that in the earlier series spermatia had been washed from one isolate to the other since the isolates had not been kept in separate dishes.

In every instance where apothecia were produced they were much more abundant in the cultures grown at 5° although some appeared in the other cultures. This temperature was used exclusively in all later experiments.

Then on December 13, 1937, a mature apothecium developed in the cross of SS<sub>12</sub> × SS<sub>10</sub>, thus duplicating the results of the previous experiment in which apothecia appeared in the unspermatized dish considerably later than those in the spermatized one.

Another experiment was then carried out using six single ascospore cultures and crossing these in all combinations, but the results were inconclusive since some of the isolates appeared to have staled and did not produce good sclerotia. However, where apothecia were produced there was again clear evidence of self-sterility and bisexual reaction, but again, as in the earlier series, the results were confused by sporadic appearance of a few apothecia some weeks later in supposedly incompatible crosses.

A fresh set of single ascospores was isolated from the apothecia in this last series and another experiment set up in which eight single ascospore cultures were used as females and five were used as males. The results are shown in the following table:

♂ \ ♀	SS2	SS15	SS17	SS21	SS23	SS25	SS16	SS26
SS2	-	-	-	-	-	-	+	+
SS18	-	-	-	-	-	-	+	+
SS21	-	-	-	-	-	-	+	+
SS12	-	+	+	+	+	+	-	-
SS26	+	+	+	+	+	+	-	-
Unspem- atized	-	-	-	-	-	-	-	-

This was clear evidence of self-sterility and bisexual reaction, but in this series also the phenomenon was encountered of a few apothecia developing some weeks later in some unspermatized isolates and theoretically incompatible crosses.

In searching for an explanation for this behavior the possibility was considered of the species being facultatively homothallic, or of self-sterility not being complete. It was thought possible that, failing fertilization by spermatia of opposite reaction, a delayed fertilization might occur with spermatia of the same reaction. The species might represent an intermediate stage in the evolution of bisexuality.

If this were so we would expect, on genetic grounds, that all the single-ascospore cultures derived from a selfed isolate or incompatible cross would be of the same sexual group.

A series was set up using six single-ascospore cultures all of which we left unspermatized with the intention of getting single-spore cultures from an unspermatized isolate, but no apothecia whatever were produced in this series.

However, in another series, single ascospores were obtained from apothecia from single-spore cultures that had apparently been selfed. Two isolates,  $SS_{16}$  and  $SS_{18}$ , were known to be of opposite sexual reaction and 10 single ascospores were isolated from each of these. The sclerotia from which these apothecia arose were spermatized only with spermatia from the same isolate.

If these apothecia had developed as a result of self-fertilization we would expect that all crosses of  $SS_{16}$  single spores  $\times$   $SS_{18}$  and  $SS_{18}$  single spores  $\times$   $SS_{16}$  would be fertile whereas crosses of  $SS_{16}$  single spores  $\times$   $SS_{16}$  and  $SS_{18}$  single spores  $\times$   $SS_{18}$  would be sterile or at least exhibit the delayed development of apothecia.

These crosses were made but the results were not as expected. Instead, the single ascospore cultures of both isolates were found to contain two groups of which one reacted positively to  $SS_{16}$  and negatively to  $SS_{18}$ , and the other positively to  $SS_{18}$  and negatively to  $SS_{16}$ . It therefore appeared that the apothecia from which these spores had been obtained had arisen as the result of cross-fertilization and not of self-fertilization.

The experimental technique was checked to see if cross-fertilization could have occurred at any point. The time factor seemed significant, for apothecia usually appeared in fertile crosses about 3 weeks to a month after the dishes were placed in the greenhouse, whereas about the same period elapsed again before the apothecia appeared in theoretically incompatible crosses or unspermatized cultures. This suggested that fertilization had occurred at some time after the dishes were placed in the greenhouse.

At this stage it was the usual practice to keep them moistened by adding a little sterile water from time to time. When apothecia were present in a dish, the lifting of the lid to add water usually provided sufficient stimulus to induce spore discharges which were often so heavy as to produce visible clouds of spores above the apothecia. Since it had already been shown by Drayton (1934) that ascospores of *Stromatinia gladioli* may produce spermatia directly on germination it seemed probable that fertilization had been effected in these unexplained instances by contamination with air-borne ascospores from neighboring dishes. The delay in the appearance of the apothecia was satisfactorily explained on this hypothesis and it was further supported by the complete absence of any apothecia whatever in the series in which no compatible crosses had been made.

A further series was run in which a number of crosses of isolates of known reaction were made. Care was exercised to avoid opening any dish after apothecia appeared in it and the results of this experiment agreed with theoretical expectations. Apothecia were produced in compatible crosses but none appeared in incompatible crosses or unspermatized cultures.

This species is, therefore, hermaphroditic and self-sterile, with the cultures falling into two sexual groups that are intragroup sterile and intergroup fertile.

*Ranunculus septentrionalis* is a North American species widely distributed in eastern Canada and United States. Throughout that area, the only records of a '*Botrytis cinerea* Pers.' on this species are from Wisconsin and New York state according to the U.S.D.A. Index of Plant Diseases in the United States, 1960, but no apothecial state is mentioned. Whetzel discovered the fungus at Labrador Lake, and subsequently collected it only from that place. On September 3, 1924, he first collected the conidial state on leaves, and on May 12, 1925, collected the apothecia on sclerotia on the ground.

*Botryotinia ranunculi* appears to be pathogenic to *Ranunculus septentrionalis*. No attempts to establish the pathogenicity and the host-specificity of the species have been carried out. However, the constant association of the fungus with the rotting debris of the host, and the general occurrence of necrotic lesions caused by the same fungus on living leaves, leave little doubt of its pathogenic character.

The first sign of attack on the suspect appears on half-expanded leaves where a water-soaked area is delimited in the leaf blade. The fungus progresses into large irregular not marginate, but sometimes zonate, light to dark brown, necrotic lesions, reaching the petioles as well as the margin of the leaf. Later in the season petioles and stems may become entirely blighted. But the infection may become arrested at any point where the leaves are partially or entirely killed. When the decayed organs of the plants are lying on the ground of the swamp, fusiform sclerotia develop in the tissues, firmly attached to the veins. They become exposed by the disintegration of the softer superficial tissues during the winter but remain attached to the remnants of the vascular tissue. In the spring, during the month of May, the sclerotia give rise to apothecia and conidia. The earliest and the latest dates of collection have been May 12 and May 21. According to Whetzel's observations in 1927, it appears that apothecia first develop and mature from sclerotia still lying in the water. Only some time later do tufts of conidiophores appear on the sclerotia. At that time primary infections can already be observed on the plants, which suggests that the ascospores play a primary role in the appearance of the earliest symptoms of the disease.

***Botryotinia ficariarum*** Hennebert n. sp. Figs. 4, 6, 11, 12, 15, 16, and 19.

*Apothecia* solitaria vel plura e sclerotiis orientia; discus stipitatus discoideus carnosus, umbilicatus vel planus, hymenio pallide fulvo, margine integro tenue, superiore translucido, inferiore fuscio, excipulo leve fuscio, 2-5 × 0.5-0.8 mm; *stipes* cylindraceus persimilis colore disco, cum subhyalino tomento in basali parte, 1-25 × 0.3-1 mm; apothecii secti *hymenium* 150-190 μ, subhyalinum; *asci* inoperculati cylindracei octospori ad basim attenuati, 126-174 × 7-11 μ, jodo positivo; *ascosporae* continuae hyalinae ellispoideae obtusae uniseriatae obliquae biguttiferae, 10.6-17.5 × 4.4-7.5 μ, plerumque 14.8 × 5.8 μ; *paraphyses* tenues ramosae septatae anastomosae subhyalinae 1.5-2.5 μ lat., ad apicem 2.5-3.5 μ clavatae et rotundatae; *subhymenium* 25-40 μ, e textura porrecta ad intricatam, hyphis (4) 5.4-8.1 (13.5) μ crass. hyalinis

levibus saepe anastomosis, ad extremas cellularum inflatis, formans e hyphis angustis palum marginalem non prominentem at compactum  $10-20 \mu$  crass. et brunneum in tertiis seu mediis inferioribus partibus; *excipulum*, extus  $30-60 \mu$ , e textura globulosa, cellulis tenuitunicatis subhyalinis  $13-30 \times 12-20 \mu$ , exterioribus globosis moniliformibus  $7-13 \times 6-10 \mu$  projicientibus pilos breves hyalinos flexuosos levesque; *stipes* e textura porrecta, hyphis interioribus  $7.5-13.5 \mu$  crass. bifurcatis anastomosis, cellulis  $65-95 \mu$  long. hyphis exterioribus idem crassitudine, cellulis  $15-30 \mu$ , hyalinis, ad basalem partem stipitis fuscioire projicientibus pilos parce streptiformes subhyalinos septatos breviter ramosos. *Stromata*, sclerotia elongata acuminata nigra plano- vel concavo-convexa  $3-12 \times 0.5-1.5 \mu$  formata in petiolo aut nervo hospitis; in vitro rotundata vel applanata, superficialia, magna,  $3-15 \times 2-8 \mu$  regulariter concentricè distributa, matrice non tincta; cortex e cellulis plerumque  $6-7 \mu$  diam., medulla e hyphis  $2-4 \mu$  crass., superioribus compactis, inferioribus laxis et diverticulatis. *Spermatia* in natura non visa, in vitro in sporodochiis, globosa,  $2.5-3.5 \mu$  diam., e phialibus hyalinis inflatis  $4-10 \times 2-3 \mu$  ennata. *Conidiophorus status Botrytis* similis *B. cinereae* Pers, conidiophori breves, crassi, apicaliter parce ramosi, conidia in densis capitulis ellipsoidea vel saepius oblonga, rarius piriformia,  $(8.8) 10-15 (18) \times (4.5) 5-7 (9.5) \mu$ , proliferationes conidiophorum breve saepiusque axiales. *Habitat*: in sylvis, parasitica in *Ficariae verna* vivis foliis, apotheciis e sclerotiis post hiemem ennatis. Europa. *Typus*: in Herb. BR, DAOM 84719 et G.L.H. 1066 (apothecia sclerotiaque) & 1114 (conidia).

*Apothecia* arising singly, or two to three from the overwintered sclerotium, very delicately but often long stipitate, *disc* at first pinhead-shaped, externally dark brown, then funnel-shaped, expanding to plane or slightly recurved at maturity, upper surface light fawn, margin delicate, entire, light colored towards the upper edge of the hymenium, dark brown and thicker in the lower part, under surface brown, pruinose, 2-5 mm diameter 0.5-0.8 mm thick. *Stipe* cylindrical very variable in length depending on the depth the sclerotium is buried under the surface of the forest litter, very slender, cylindrical, short on bare ground, 1-25 mm long, 0.3-1 mm in diameter, glabrous or pruinose, light fawn, slightly darkening at the base, with a basal tuft of radial, fasciculate, subhyaline, rhizoidal hyphae.

In axial section: *hymenium*  $150-190 \mu$  thick, subhyaline, *asci* inoperculate, cylindrical, slightly attenuate towards the base, rounded at the apex, which is thickened and iodine-positive, eight-spored,  $126-174 \times 7-11 \mu$ , *ascospores* hyaline, long ellipsoid to oblong, often asymmetric with a flattened side, obtuse, biguttulate, narrower and more elliptical than in *B. calthae*,  $10.6-17.5 \times 4.4-7.5 \mu$ , averaging  $14.8 \times 5.8 \mu$ , readily producing spermatia in the asci, producing one to three germ tubes and becoming septate on germination; *paraphyses* subhyaline, filiform,  $1.5-2.5 \mu$  wide, branched at any level, septate, anastomosing, clavate at the tip up to  $3.5 \mu$  on the uppermost  $20 \mu$  of their length. *Subhymenium*  $25-40 \mu$  thick, brownish, of a textura intricata, hyphae  $2-4 \mu$  wide. *Medullary excipulum*  $75-150 \mu$  thick, hyaline, of a textura porrecta in lateral and lower parts, hyphae varying from 4 to  $13.5 \mu$  in width, mostly  $5.4-8.1 \mu$  wide, smooth, flexuous, more or less parallel to the under surface, bifurcate, often swollen at the ends of the cells, often anastomosing,



the anastomoses occurring either between contiguous or non-contiguous hyphae, the latter forming long conical side branches, septate and narrowest at the point of union; at the margin of the hymenium hyphae brownish, narrower, 2–3  $\mu$  wide, paraphysis-like, densely fasciculate, forming a palisade 10–20  $\mu$  thick, reaching one-third to one-half of the height of the hymenium, slightly clavate at the tip, subhyaline and bending outwards; in the central part of the disc, of a textura intricata, hyphae regularly 6–8  $\mu$  wide. *Ectal excipulum* 30–60  $\mu$  thick, subhyaline, of a textura globulosa, cells thin-walled, the innermost large, globose, 13–20  $\mu$  in diameter or ellipsoid, 13–30  $\times$  12–20  $\mu$ , the outermost, more or less globose, sometimes in moniloid chains, 7–13  $\times$  6–10  $\mu$ , projecting to form diverticulate, flexuous, hair-like processes, 4  $\mu$  wide and up to 20  $\mu$  in length. *Stipe* of textura porrecta, in *medullary* portion hyphae hyaline, 7.5–13.5  $\mu$  wide, often bifurcate and anastomosing, cells 65–95  $\mu$  long; in *ectal* portion hyphae compactly arranged, 9.5–13.5  $\mu$  wide, constricted at the septa to 5.5–8  $\mu$  wide, cells inflated, 16–30  $\mu$  long, externally covered by a very loose web of narrower hyphae, 5–8  $\mu$  wide, septate, composed of cells which are short, panduriform, or in moniloid chains, 13.5–27  $\mu$  long; towards the base of the stipe, groups of small cells can be observed amongst the ectal cells, projecting in tufts of four to nine rhizoidal hyphae, which are 3–4  $\mu$  wide, subhyaline, septate, discontinuously twisted, very short branched, 50–80  $\mu$  long.

*Stroma*, a definite sclerotium of the plano-convexoid type, slender, fusiform, obtuse, or acuminate, usually concave and grooved below, sometimes completely enclosing the susceptible tissues of which debris remains imbedded in the medulla, firmly attached to the petioles of the susceptible but overwintered sclerotia appearing free by reason of the disintegration of the host tissue, dull black, striate, slightly rough, 3–12  $\times$  0.5–1.5 mm. In culture on petioles, as in nature but sometimes larger 2–15  $\times$  1–1.5 mm; in culture on leaf blades as in plain agar, large, plane to loaf-shaped, lobulate, superficial, strongly attached to the medium which is depressed, dull black, slightly rugose, 3–15  $\times$  2–8 mm. In section typical of the genus, *ring cells* of the upper surface in two to three layers, 4–10  $\times$  4–8  $\mu$ , mostly 6–7  $\mu$  in diameter, polygonal, more or less isodiametric, the external layer black, thick-walled, those of the under surface in three to four layers, larger, 6–15  $\times$  3–9  $\mu$ , mostly 10  $\times$  7  $\mu$ ; *medullary hyphae* hyaline in the upper part compactly interwoven, flexuous, branched, 2–4  $\mu$  wide, septate; in the lower part, less compactly interwoven, some long, flexuous, regular, few branched, 2.5–3.5  $\mu$  wide, with cells 20–35  $\mu$  long, others short, convolute, abundantly short branched, 2–3  $\mu$  wide.

*Spermidium* not observed in the field, a spermodochium in culture, white to yellowish, up to 800  $\mu$  in diameter; spermatophores repeatedly branched, mostly bi- or tri-furcate, metulae subglobose and short to slender, 5–8  $\times$  2.5–6  $\mu$ , each terminal metula bearing three phialides very slightly inflated, usually straight, 4–10  $\times$  2–3  $\mu$  having a narrow neck and a 1–3  $\mu$  long, funnel-shaped collarette; spermatia, phialospores globose, very abundant, 2.5–3.5  $\mu$  in diameter.

Conidial state *Botrytis* of the *B. cinerea* complex. *Conidiophores* arising singly or in groups of two or three from the under surface of the leaf of the susceptible, very regular in shape, 350–500  $\mu$  high, subhyaline to olivaceous

brown, paler to hyaline towards the tip, short, 11–17  $\mu$  wide, basal cell globose up to 23  $\mu$  in diameter, towards the tip alternately branched, the three or four branches and the extremity of the axis branching in the same way 1–2 times, all branches becoming much septate with prominent septa, and their terminal cell inflating into an ampulla producing simultaneously 15–22 (25) conidia on cylindrical denticles 1–3  $\mu$  long and 1–2  $\mu$  in diameter, forming 15–20 heads of conidia in a dense cluster up to 125  $\mu$  broad. *Conidia* subhyaline, globose at first until 2.5–3  $\mu$  in diameter, then ellipsoid, oblong, or pyriform, sometimes ovate, 8.8–18  $\times$  4.5–9.5  $\mu$ , mostly 10–15.5  $\times$  5–7  $\mu$ , averaging 13.2  $\times$  6.4  $\mu$ , attenuate at the base to a quite conspicuous scar bearing a frill 1–2  $\mu$  long; after dispersal of the conidia, sporogenous cells and entire branches collapsing in an accordion-like manner and falling off leaving the axis of the conidiophores as an unbranched stump with one terminal and one to two lateral, abrupt, prominent, circular scars; successive axial proliferations developing sometimes from the terminal scar, short, similar to the previous stage of development, increasing progressively the height of the conidiophores up to 1800  $\mu$ .

#### *Cultural Characters*

On potato-dextrose agar, the mycelium develops in a radial way like *Botryotinia porri*, but it is pure white, less abundant, and does not stain the substratum, which remains translucent. Sclerotia form soon, uniformly distributed in concentric rings, but take a long time to become black and fully mature. They are all superficial, firmly attached to and depressing the surface of the substratum, loaf-shaped then flattened, typically concave below, the lower rind being formed much later than the upper rind, very variable in size. Conidiophores and conidia not observed in culture. Spermatia are fairly abundant, but in very small spermodochia on this medium. A few appressoria develop along the glass. On sterilized wheat, aerial mycelium may be fairly abundant, often in arachnoid, nest-like tufts, dirty white to a light greenish grey; sclerotia very numerous, typically loaf-shaped, concavo-convexoid, becoming black in a few weeks, but remaining for a long time white on the surface of attachment along the glass. A few conidiophores and conidia have been observed in the spring on an overwintered sclerotium, placed on sand in a petri dish (G.L.H. 1887, dish XXVI) conidia measuring 9.2–18.4  $\times$  5.8–8.1  $\mu$ , average 13.36  $\times$  6.58  $\mu$  (quotient L/W = 2), but reisolations were not obtained. Spermatia are very abundant after some months on that medium at 5° and 14° C; the spermodochia are 1–2 mm diameter, white to yellowish. Only a few appressoria develop.

*Habitat*.—Parasitic on living leaf blades of *Ficaria verna* Huds. (syn. *Ranunculus ficaria* L, *Ficaria ranunculoides* Roth.) in clear woods and bushwoods, developing apothecia from overwintered sclerotia early in the spring.

*Distribution*.—Europe (Belgium, Holland, Denmark, Switzerland, Germany).

*Type*.—Apothecia and sclerotia (G.L.H. 1066), and conidia (G.L.H. 1114) on *Ficaria verna* Huds., in woods, Castle of Comte de Beaufort, Linden, Brabant, Belgium, Coll. by G.L. and Lidwina Hennebert, March 6 and 28, 1960, in Herb. BR, DAOM 84719 and G.L.H. 1066.

*Specimens Examined*

(a) Apothecia and sclerotia under *Ficaria verna*: BELGIUM: Linden, Brabant, Herb. BR, G.L.H. 1066, DAOM 84719 (Type collection); Heverlée, Brabant, G.L.H. 1067, DAOM 84769; Durace, Limburg, G.L.H. 1073; Lovenjoel, Brabant, G.L.H. 1087; HOLLAND: near Lisse, H. H. Whetzel's European collection E 18 in CUP; Sassenheim, H. H. Whetzel E 38 in CUP.

(b) Conidial state on leaves of *Ficaria verna*: BELGIUM: Linden, Brabant, BR, G.L.H. 1114, DAOM 84719 (Type collection); G.L.H. 647, DAOM 84770; Heverlée, Brabant, G.L.H. 376; G.L.H. 427; G.L.H. 1112, DAOM 84767; Lovenjoel, Brabant, G.L.H. 1113, DAOM 84768; Durace, Limburg, G.L.H. 385; Beloeil, Hainaut, G.L.H. 384; HOLLAND: Sassenheim, H. H. Whetzel E 52 in CUP; SWITZERLAND: along the Lake of Neuchatel, near Cortaillod, H. H. Whetzel E 74 in CUP; DENMARK: Ermelunden, Sealand, N.F. Buchwald Herb. in CP; GERMANY: (as *Botrytis ficariae* m.) Rolfshagen bei Odesloe, 3.5.1903, O. Jaap (Fl. v. Schleswig-Holstein) in B; Forbach im Loter, Herapel, 12.4.1912, A. Ludwig, in A. Ludwig Herb. n. 1738, in B; Dillkreiss, an der Ohell bei Burg, 1.5.1940, A. Ludwig, in A. Ludwig Herb. (Fl. v. Hessen-Nassau), in B; mit *Uromyces ficariae*, Kr. Siegen, Erlenniederwald im Weierbachtal bei OberdresseIndorf, 2.5.1936, A. Ludwig, in A. Ludwig Herb. (Fl. v. Westfalen), in B.

Single-ascospore and mass-ascospore strains of this species are maintained in the DAOM culture collection.

*Botryotinia ficariarum* is actually genetically connected with a *Botrytis* state which, in nature, has only been observed on the under surface of the lesions in *Ficaria* leaves. A sclerotium of the fungus bearing tufts of conidiophores has never been found in nature as is commonly observed in *Botryotinia calthae* or *B. ranunculi*. Even when sclerotia have overwintered on the surface of the soil or buried in the soil or covered by leaves, under wet or under drier conditions, they produce only apothecia in spring. A number of sclerotia appearing similar to those of *B. ficariarum* have been collected bearing only conidiophores, but isolations from conidia demonstrated that they belonged to other species of the *Botrytis cinerea* complex. (G.L.H. 1068, 1069, 1070, 1098). It could then be concluded that there is no circumstantial field evidence for the connection between the *apothecia* and *conidia*, and the apothecial fungus might be classified in the genus *Ciborinia* as characterized by Whetzel (1945).

However, apothecia are always observed under *Ficaria verna*, and the season of apothecial development, which is the earliest as far as we know for species of *Botryotinia*, entirely precedes the occurrence of *Botrytis* lesions on the leaves. It is only 1 to 2 weeks after the first apothecia have matured that the first lesions have been observed, and the first conidiophores appear on these lesions. This suggests that primary infections on leaves are caused by ascospores and this has been demonstrated experimentally. Artificial inoculation of *Ficaria verna* under aseptic conditions by ascospores from apothecia collected in the field have been successful, the lesions on the leaves later becoming covered with conidiophores and conidia. Whetzel's unpublished notes (E 38) indicate that he had carried out the same experiment with the same result. Furthermore, ascospores discharged into a fresh sterile extract of *Ficaria* leaves in Van Tieghem cells produced spermatia, appressoria, conidiophores, and conidia.

after a few days. In addition isolations from ascospores, sclerotia, and conidia, and from the margin of leaf lesions all show the same cultural characteristics.

By keeping necrotic leaves bearing *Botrytis* conidiophores on the under surface of the blade in a moist chamber at about 14°–16° C for 1 month, it was possible to observe the progression of the infection along the petioles, followed by the later development of fusiform black sclerotia which were identical with those collected in the spring bearing apothecia. Cultures of the *Botryotinia* on fresh sterile leaves imbedded in plain agar medium also produce these typically fusiform sclerotia on the veins and the petioles, together with the lobulate sclerotia characteristic of cultures on potato-dextrose agar.

These observations provide convincing evidence of the connection between the perfect and imperfect states of the fungus, but it would be desirable to confirm it by production of apothecia from conidial isolates and vice versa.

In experiments carried out by Miss M. E. Elliott and the senior author in 1960–61, abundant apothecial fundaments were produced in crosses of poly-ascospore isolates. Unfortunately the culture dishes were subjected to excessive drying at one period and the apothecia did not mature.

Whetzel's first apothecial collection E 38, made in Holland in 1930, is labelled '*Sclerotinia*', but along with it and bearing the same number is a packet labelled '*Botrytis* stage, on leaves of *Ficaria verna* inoculated by ascospores'. This *Botrytis* agrees with the specimen in Whetzel's collection E 52, made in the same locality and labelled '*Botrytis ficariarum* n. sp.' Whetzel chose that specific epithet instead of '*ficariae*', because he expected that the apothecial state would be a *Sclerotinia* species and wanted to avoid possible confusion with *Sclerotinia ficariae* Rehm.

Rehm (1896) described *Sclerotinia ficariae* from a Magnus collection of apothecia on sclerotia among plants of *Ficaria verna* Huds. at Berlin, and Rehm assumed that it was parasitic on the roots of this plant. A part of the type collection is preserved in Rehm's Herb. in Herb. S. and labelled "*Sclerotinia Ficariae* Rehm. nov. spec. 8.ii.75 Unter *Ranunculus Ficariae* in bot. Garten zu Berlin 4.67 [April 1867] leg. Prof. Magnus [ascospores] 6–9 × 3–3.5–5 hyalin, 1-zellig, 8, 1-reihig [ . . . , asci . . . ] c. 90 × 6–7 [ . . . ; scr. Rehm]". Another part of the type collection is preserved in Magnus Herb. in Herb. HBG and labelled "*Peziza* mit Sclerotien unter *Ranunculus Ficaria* wachsend Berlin. Hort. Bot. Berolin. 14.4.67 P. Magnus [scr. P. Magnus] *Sclerotinia Ficariae* Rehm nov. sp. 8.11.75 [ascospores] 6 × 3–3.6 hyalin, 1-zellig, 8, 1-reihig [ . . . , asci . . . ] c. 90 × 6–7 [ . . . ; scr. Rehm]". Rehm considered his species to be distinct from *Sclerotinia tuberosa* (Hedw.) Rehm on account of its smaller size. *Sclerotinia ficariae* is described as having sclerotia 2–10 × 3–5 mm, cup 2–4 mm in diameter, asci 90–100 × 6–8 μ, and ascospores 6–8 × 3–3.5 μ while *Sclerotinia tuberosa* is described by Rehm (1896) as having sclerotia up to 30 × 15 mm, cups of 10–30 mm in diameter, asci 120–150 × 8–10 μ and ascospores 15–18 × 6–8 μ.

Six other collections of *S. ficariae* have also been examined: (1) Rehm Ascomyceten. No. 1204, "In Wurzeln von *Ranunculus ficaria*. Feldkirch, in Voralberg [Prov. in Tyrol, Austria] 4.1898. Rick. S.J. [authentic]" in DAOM, NY, S; (2) Rabenhorst-Pazschke, Fungi europaei et extra-europaei. 4272.

"Ad terram inter *Ranunculos ficarias*, Tyrone prope Feldkirch [in Voralberg Prov., Austria] mai 1898. leg. J. Rick." in DAOM, S; (3) Auf Knollen von *Ranunculus ficaria*, Hassellake Grossbehnitz, Westhavelland. 17.4.[19]04 W. Kirschstein in W. Kirschstein Herb. in Herb. B; (4) idem as (3), 26.4.1905, W. Kirschstein in W. Kirschstein Herb. in Herb. B; (5) Auf sclerotierten Knollen von *Ficaria vulgaris*, Hassellake Grossbehnitz, Westhavelland, April 1921, W. Kirschstein in W. Kirschstein Herb. in Herb. B; (6) on soil among *Ficaria verna*, Sassenheim, Holland, April 7 1930 H.H. Whetzel E 39 in Herb. CUP. All these collections seem to be of the same species in agreement with the type collection. It is evident from these collections that *Sclerotinia ficariae* Rehm is definitely distinct from *Botryotinia ficariarum*; Whetzel, who examined several of these collections, reached a similar conclusion (Whetzel notes in CUP). *S. ficariae* has sclerotia of the *S. sclerotiorum* type rather than of the *Botryotinia fuckeliana* type, and the apothecia differ in habit, shorter ascospores, and wider paraphyses and in the marginal palisade of excipular hyphae reaching the surface of the hymenium.

The life history of *Botryotinia ficariarum* is quite simple. Ascospores from apothecia appearing in the early spring, at the beginning of March, in Belgium, from overwintered sclerotia, are responsible for the primary lesions on the leaf blades. These lesions are dry, uniformly brown, circular, slowly extending to the margin and invading the blade towards the petioles; before the end of March they are covered with a very short, light grey down of *Botrytis* conidiophores. The entire blade is finally invaded, collapsing and hanging at the tip of a still healthy, green petiole. The fungus continues to progress down through the petioles, which collapse on to the ground. The fungus does not invade the other leaves through the crown and the crown and rhizomes of the plant remain healthy. In May and June, sclerotia develop on the decayed petioles, which disintegrate during the winter.

Because of lack of experimental data, we cannot state whether or not the conidia are able to cause secondary infections of the leaf blades. However, it has been observed that the disease does not develop any further 1 or 2 weeks after the end of the apothecial season, i.e. about the beginning of April. The leaf spots may enlarge but their number does not increase.

It is interesting to notice that another species of *Botrytis* occurs on *Ficaria verna*, occasionally killing entire plants. In contrast to *B. ficariarum*, the crown of the plant is attacked at the soil level and the fungus grows up through the petioles into the leaf blades of the collapsed plant. Conidiophores and conidia typical of the *B. cinerea* complex are very abundant. Isolations have demonstrated that this is undoubtedly a species distinct from *B. ficariarum*, and somewhat similar to *B. calthae*. This *Botrytis* was observed on *Ficaria verna* at Lovenjoel, Belgium, in a station of mixed *Ficaria verna* and *Caltha palustris*. At that time the conidial state of *B. ficariarum* was not observed, whereas *B. calthae* was producing abundant conidia. Thus it is possible that *Ficaria verna* is susceptible to *B. calthae*.

Whetzel made a similar observation in 1930 when he cultured his *Botrytis* collection E 32 and E 35 (in CUP) from *Ficaria verna* near Velzen, North Holland, and noted that the cultures resembled those isolated from *Caltha* in New York, and could be of *Botryotinia calthae*.

The simultaneous incidence of certain other fungi on *Ficaria verna* along with *B. ficariarum* should be noted. The senior author observed *Peronospora ficariae* Tul., aecidial states of *Uromyces rumicis* (Schum.) Winter and *Uromyces poae* Rabenh., and the teleutospores of *Uromyces ficariae* (Schum.) Lev. in frequent association with *Botryotinia ficariarum*. The *Botryotinia* appears overgrowing the teleutospores or the aecia on the leaves of the suscepr, and seems responsible for the necrosis of the adjacent tissues of the leaf. The necrotic lesions are not limited to the margin of the rust spot but may extend over the entire leaf and become covered with *Botrytis* conidiophores. We adopt here the conclusion Greene (1944) deduced from a similar relationship between a species of *Botrytis* of the streptiform type and *Puccinia rubigo-vera* (DC.) Winter on *Thalictrum dasycarpum* Fisch. and Lall. "Probably", Greene stated, "the *Botrytis* gained a foothold on the tissues killed by the rust and then functioned as a weak parasite." A similar relationship is reported on *Allium ursinum* L. between *Botryotinia globosa* Buchwald (conidial state *Botrytis globosa* Raabe) and the aecia of a *Melampsora* species, probably *M. allii-populina* Kleb., then identified as *Caecoma allii-ursini* Winter (Hennebert 1958).

Brenchley and Johnstone (1955) reported that *Peronospora ficariae* could be overgrown by a *Botrytis* on *Anemone* sp. and this fungus may also be overgrown by *Botryotinia ficariarum* when it occurs on *Ficaria verna*.

*B. ficariarum* can produce infections on leaves of *Ficaria verna* by itself but it may be aided by the presence of these other parasites.

### Discussion

Obviously there are many similarities between these three species of *Botryotinia* occurring on Ranunculaceae but similarity does not necessarily mean identity. Field observations and cultural studies on them have been made over a period of many years by several mycologists including Whetzel, Drayton, Buchwald, Groves, and Hennebert and all have agreed that they are distinct taxa.

*B. ficariarum* is known only from Europe, *B. ranunculi* only from North America, but *B. calthae* occurs in both Europe and North America.

Probably *B. ficariarum* is the most distinct of the three species. The conidial state can be recognized in the field by the short conidiophores and dense clusters of conidia. The septa of the fertile branchlets are rather conspicuous as in other species such as *B. globosa* Buchw. or *B. squamosa* Viennot-Bourgin. The proliferations of the conidiophore are usually axial and short.

The conidia of *B. ficariarum* are a little longer in proportion to their width than those of either *B. calthae* or *B. ranunculi*; the quotient length/width is usually close to 2 but may vary from 1.6 to 2.3.

In a broad sense the conidial state of *B. ficariarum* can be considered a member of the *Botrytis cinerea* complex, but when found growing in the field along with a typical *B. cinerea* it can be distinguished.

The sclerotia of *Botryotinia ficariarum* are quite similar in shape to those of *B. calthae*, but the medullary hyphae are much narrower, and in the lower part of the sclerotium the medulla is less compact and composed of variously branched hyphae.

The apothecia of these three species are quite difficult to distinguish. The ascus and spore measurements in all three are very similar although in *B. ranunculi* both the asci and spores tend to be slightly narrower. From the sections examined it would appear that *B. ficariarum* can be distinguished by the structure of the excipulum around the hymenium.

This marginal excipulum consists of two parts, an inner part consisting of a palisade of paraphysis-like hyphae arising from the medullary excipulum at the point where it converges with the hypothecium, and an outer part consisting of inflated, projecting hyphae tips. In both *B. ranunculi* and *B. calthae* the palisade layer extends up to the level of the hymenium, but in *B. ficariarum* is only well developed in the lower third or half of the height of the hymenium. In gross appearance this condition can be observed as a light, delicate, hymenial margin exceeding the darker, stouter excipular margin.

*B. calthae* and *B. ranunculi* are more difficult to distinguish on morphological characters. The conidiophores and conidia are very similar in both species and are typical of a *Botrytis* of the *cinerea* type. In culture the sclerotia of *B. calthae* are smaller, often more or less embedded in the substrate and tending to form crust-like masses whereas in *B. ranunculi* they are larger, more elongated, and more superficial, although firmly attached to the substrate. In section the sclerotium of *B. calthae* is much more compact in structure than that of *B. ranunculi*. *B. calthae* tends to produce a dark brown stain in potato-dextrose agar which is not evident in *B. ranunculi*.

The ascus and spore size provide perhaps the most clear-cut criteria for distinguishing these two species. The asci of *B. ranunculi* rarely exceed 125  $\mu$  in length or 9  $\mu$  in diameter and the ascospores rarely exceed 5  $\mu$  in diameter. In *B. calthae* the asci are mostly over 125  $\mu$  in length and frequently up to 11  $\mu$  in diameter and the ascospores are mostly 5.5–7.0  $\mu$  in diameter.

In section the marginal excipulum appears thinner and less pronounced in *B. calthae* and the medullary hyphae are narrower, 6.5–8  $\mu$  in *B. calthae* and 9.4–10.8  $\mu$  in *B. ranunculi*. The base of the stipe is usually black, covered with straight, fuliginous, rhizoidal hyphae, whereas in *B. ranunculi* the rhizoidal hyphae are subhyaline and twisted.

As noted in the introduction, four species of *Botryotinia* have already been distinguished in the *Botrytis cinerea* complex: *Botryotinia fuckeliana*, *B. convoluta*, *B. draytoni*, and *B. pelargonii*. All of these species may be distinguished from the three species described here by the size of their asci and ascospores.

In other species of *Botryotinia* such as *B. porri* (v. Beyma) Whetz., *B. squamosa* Viennot-Bourgin, *B. globosa* Buchwald, *B. narcissicola* (Gregory) Buchw., the conidial state is quite distinct from *Botrytis cinerea* Pers. and the members of the *B. cinerea* complex and provides sufficient distinction from the three species described here.

Since all three of these species are pathogenic to species of the family Ranunculaceae it is of some interest to consider the host range. There is some evidence that *Botryotinia calthae* may occur on *Ficaria verna* but otherwise they seem rather specific to host.

Most records of *Botrytis* infection on Ranunculaceae have been attributed to "*B. cinerea* Pers." Known susceptibles in this family include the following:

*Aquilegia vulgaris* L., *Anemone cronaria* L., *Anemone* spp., *Clematis viticella* L., *Delphinium ajacis* L., *Delphinium cultorum* Voss., *Helleborus niger* L., *Nigella damascena* L., *Paeonia lactiflora* Pall., *P. officinalis* L., *P. suffruticosa* Andr., *P. moutan* Ait., *Ranunculus asiaticus* L.

*Botrytis paeoniae* Oud. is well known as a pathogen of *Paeonia* spp. but it is also known that another *Botrytis* species of the *cinerea* type may also attack this host. Whetzel (1915) wrote, "my own studies indicate that there are at least two distinct species of *Botrytis* attacking and causing identical symptoms in the peonies, both in this country and in Europe. The species while quite distinct in structure are very similar in their life habits and effects on the peonies. One forms large sclerotia while the other forms very minute ones." A large number of isolations made from peonies in Belgium confirm that statement. The *Botrytis* with large sclerotia is typically one of the *B. cinerea* complex, but does not seem to be identical with any of the *Botryotinia* species here described on Ranunculaceae.

This would suggest that more intensive morphological studies would reveal still more species of *Botryotinia* occurring on species of this family.

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NOTE: Figs. 1-19 follow.

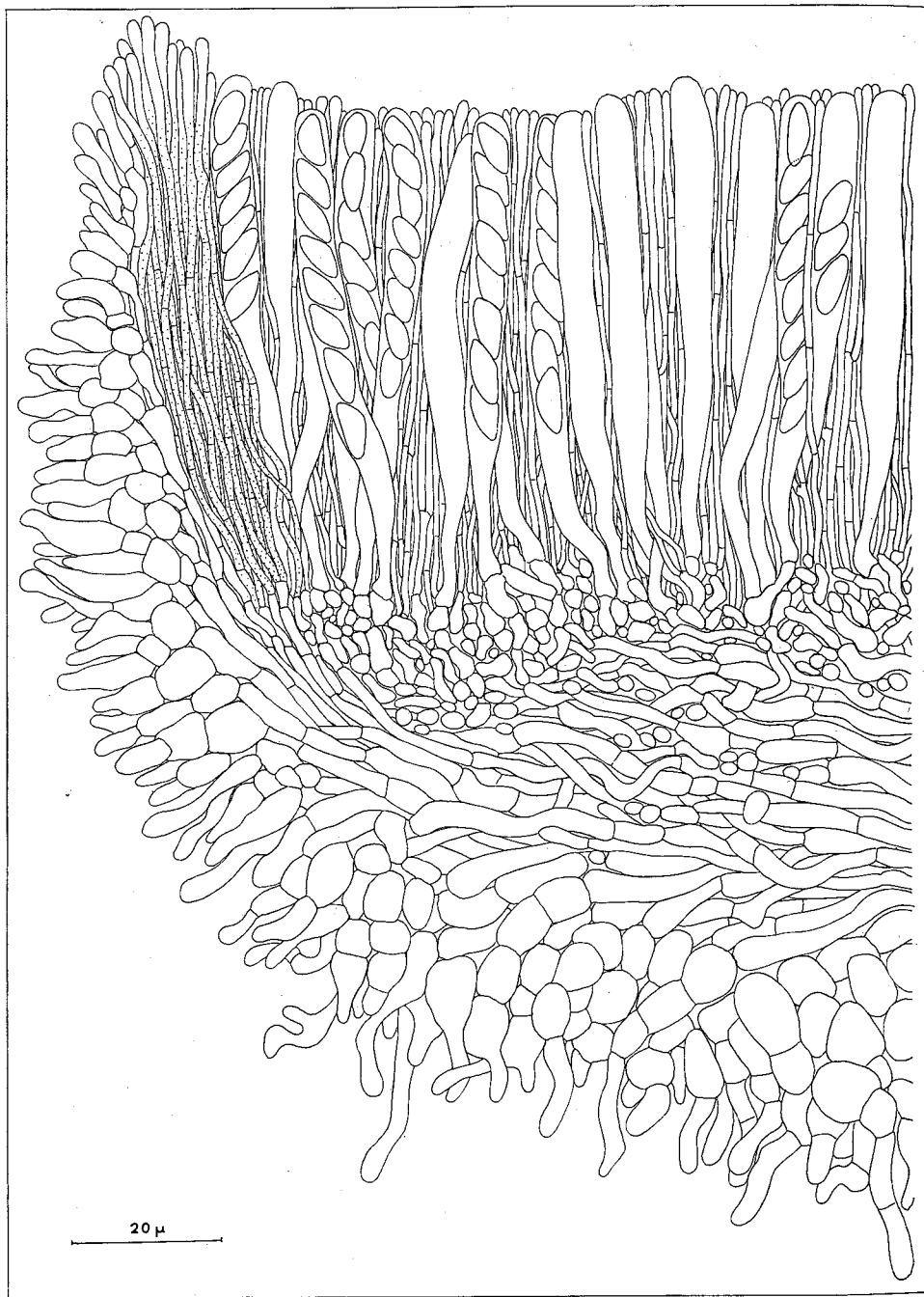
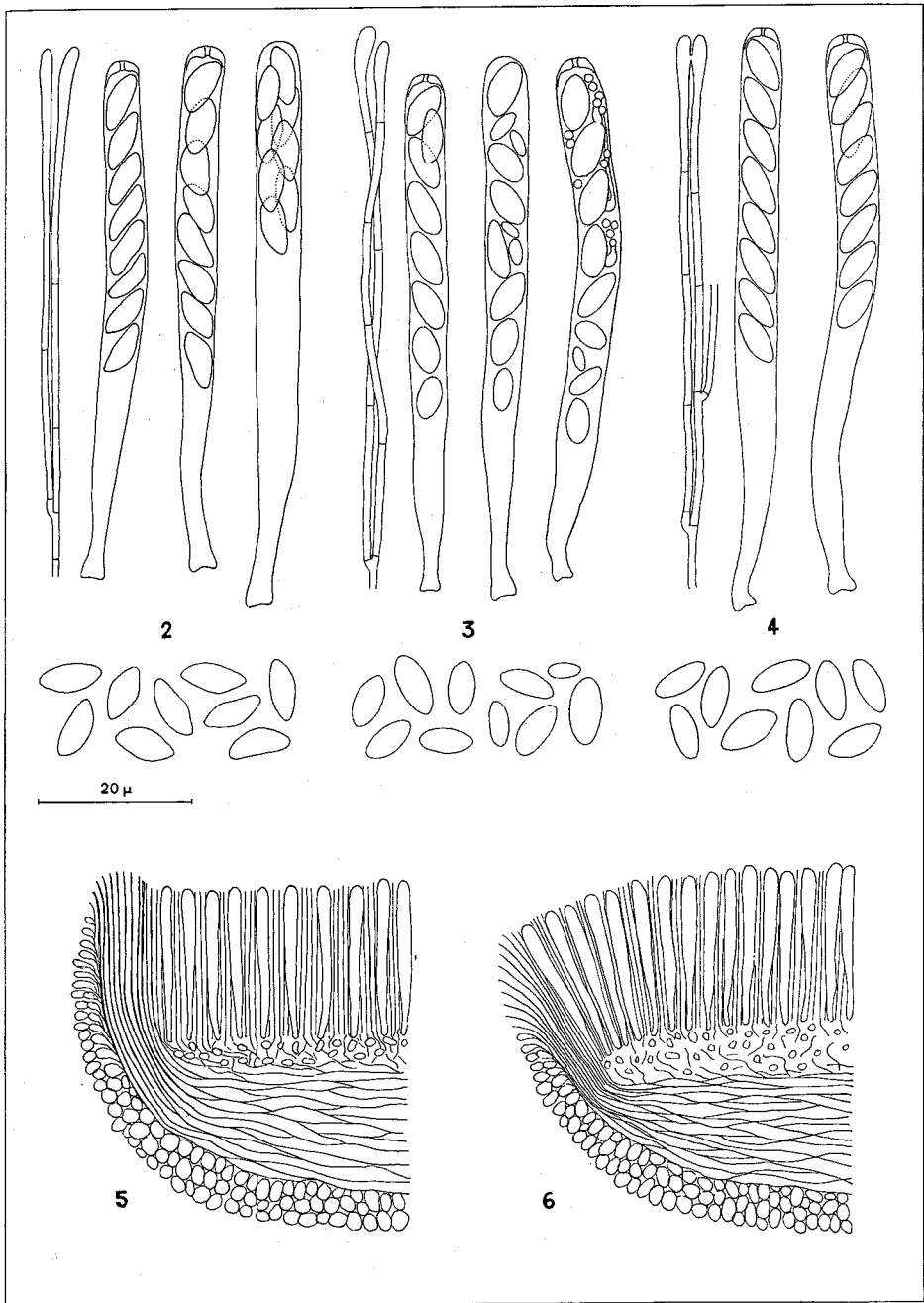
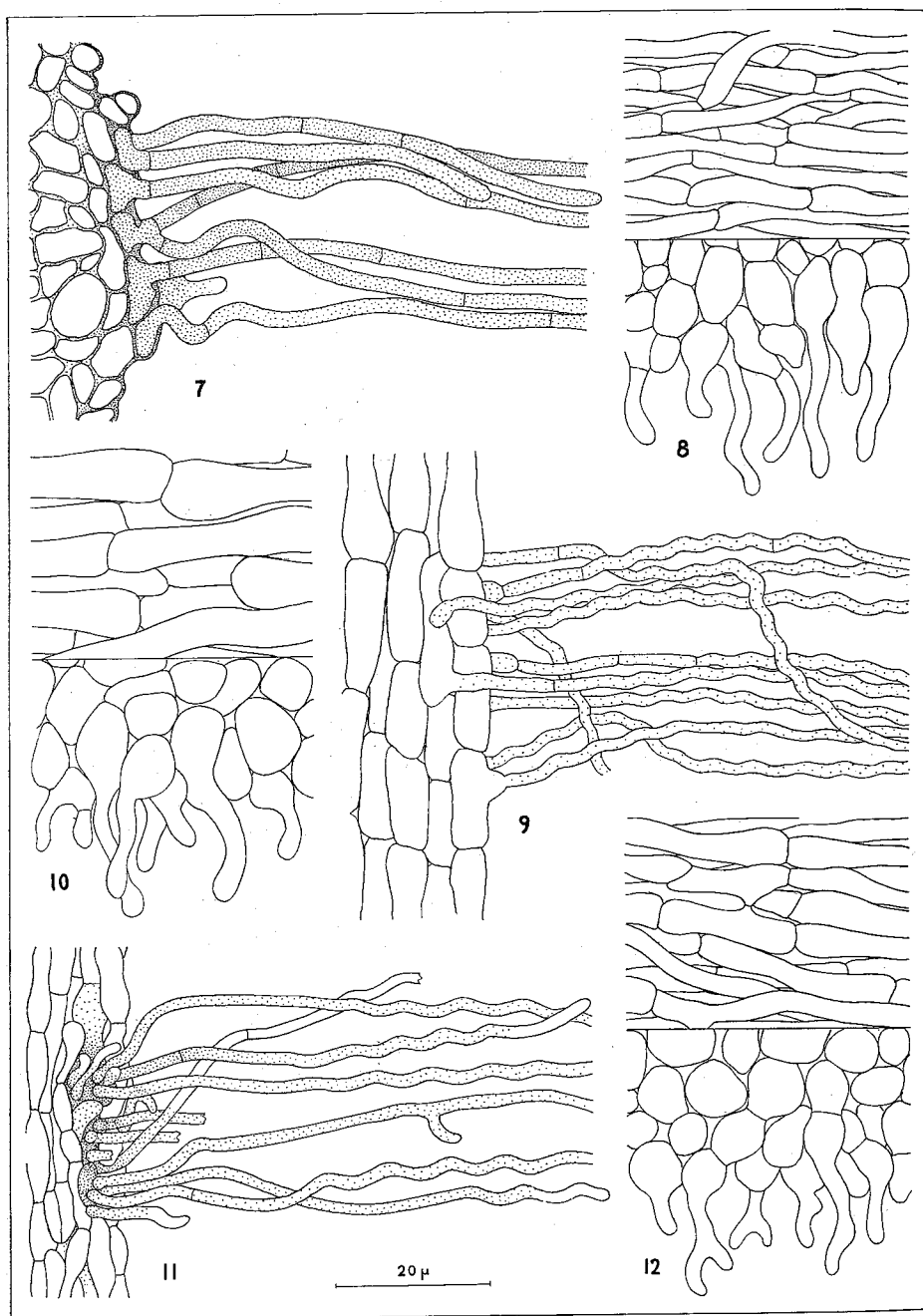


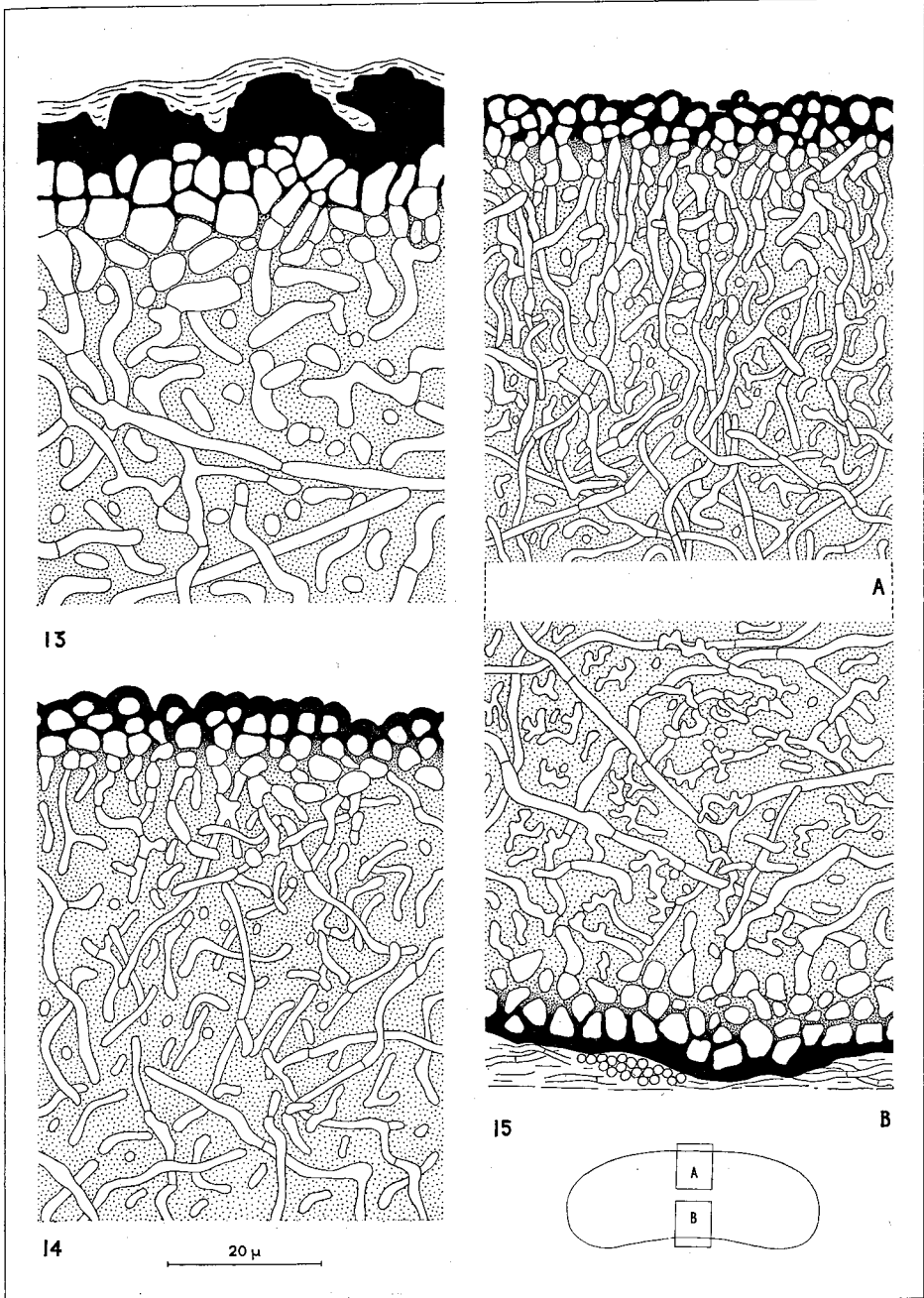
FIG. 1. *Botryotinia calthae*. Edge of the apothecium in axial section, G.L.H. 2059, camera lucida drawing,  $\times 500$ .



FIGS. 2, 3, 4. Ascospores, asci, and paraphyses: 2, *Botryotinia calthae*, G.L.H. 2059; 3, *B. ranunculi*, CUP 25278; 4, *B. ficariarum*, G.L.H. 1066;  $\times 500$ . FIGS. 5, 6. Schematic view of the edge of the apothecium in axial section: 5, *B. ranunculi*, and 6, *B. ficariarum*.



FIGS. 7, 9, 11. Rhizoidal hair-like hyphae near the base of the stipe (7, in transverse section, 9, 11, in longitudinal view). FIGS. 8, 10, 12. Portion of the ectal and medullary excipulum of the apothecia (in axial section). FIGS. 7, 8, *Botryotinia calthae*, DAOM 56310; FIGS. 9, 10, *B. ranunculi*, CUP 25278; FIGS. 11, 12, *B. ficariarum*, G.L.H. 1066;  $\times 500$ .



FIGS. 13, 14, 15. Sclerotia from culture on potato-dextrose in vertical section. Fig. 13, *Botryotinia calthae*, G.L.H. 2059; Fig. 14, *B. ranunculi*, from DAOM 15618; Fig. 15, *B. ficariarum* G.L.H. 1066, A, upper part, B, under part,  $\times 500$ .

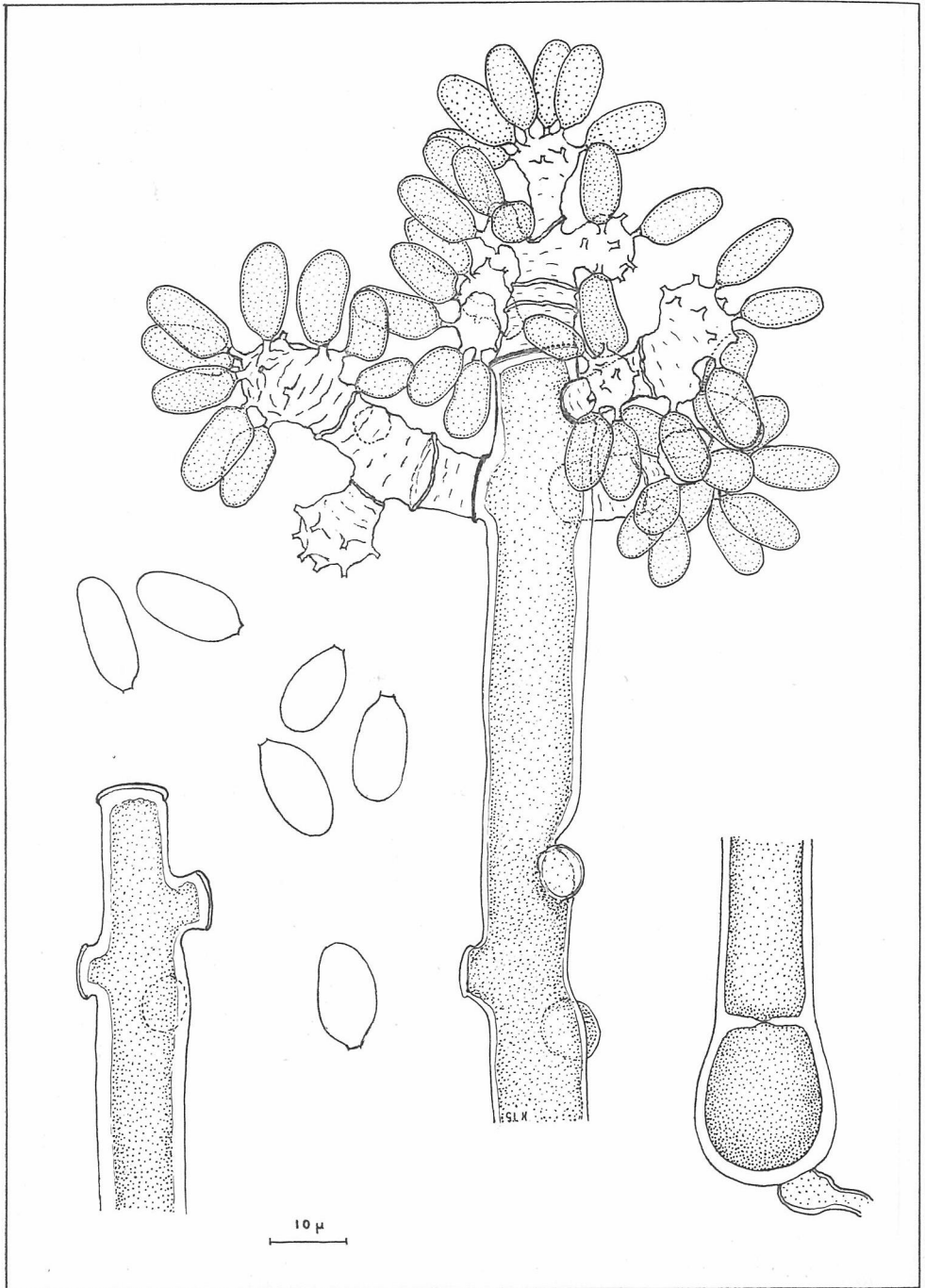


FIG. 16. *Botryotinia ficariarum*, conidiophores and conidia, G.L.H. 647,  $\times 1000$ .

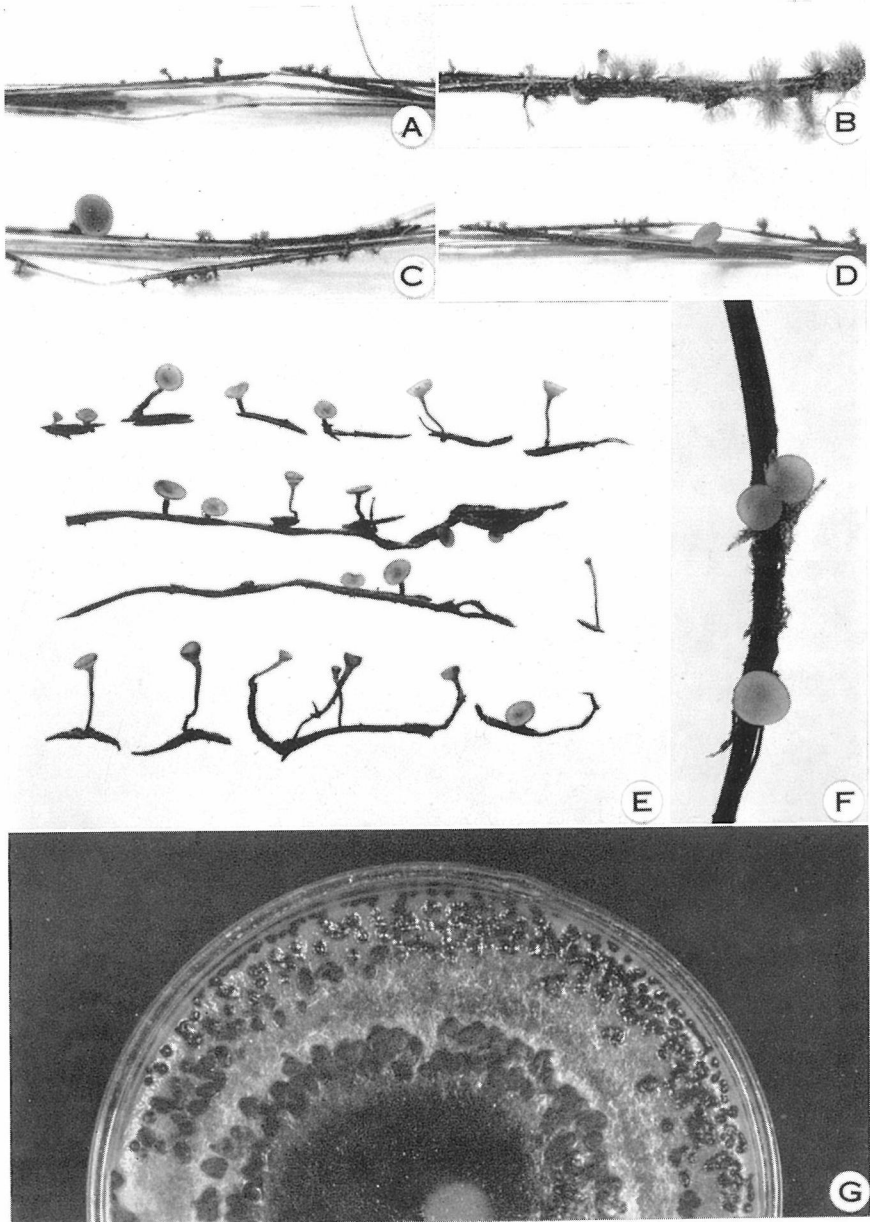


FIG. 17. *Botryotinia calthae*. A, B, C, D, simultaneous apothecial and conidial development from sclerotia on petioles in nature,  $\times 1.5$ ; A, C, D, from G.L.H. 2059; B, from CUP 19171; E, F, apothecia on petioles, E, natural size, CUP 16197, F,  $\times 2$ , CUP 23535; G, 2-month-old culture on potato-dextrose agar, natural size, G.L.H. 2059. (Photographs B, E, F, from Herb. CUP, Cornell University, Ithaca, N.Y.)

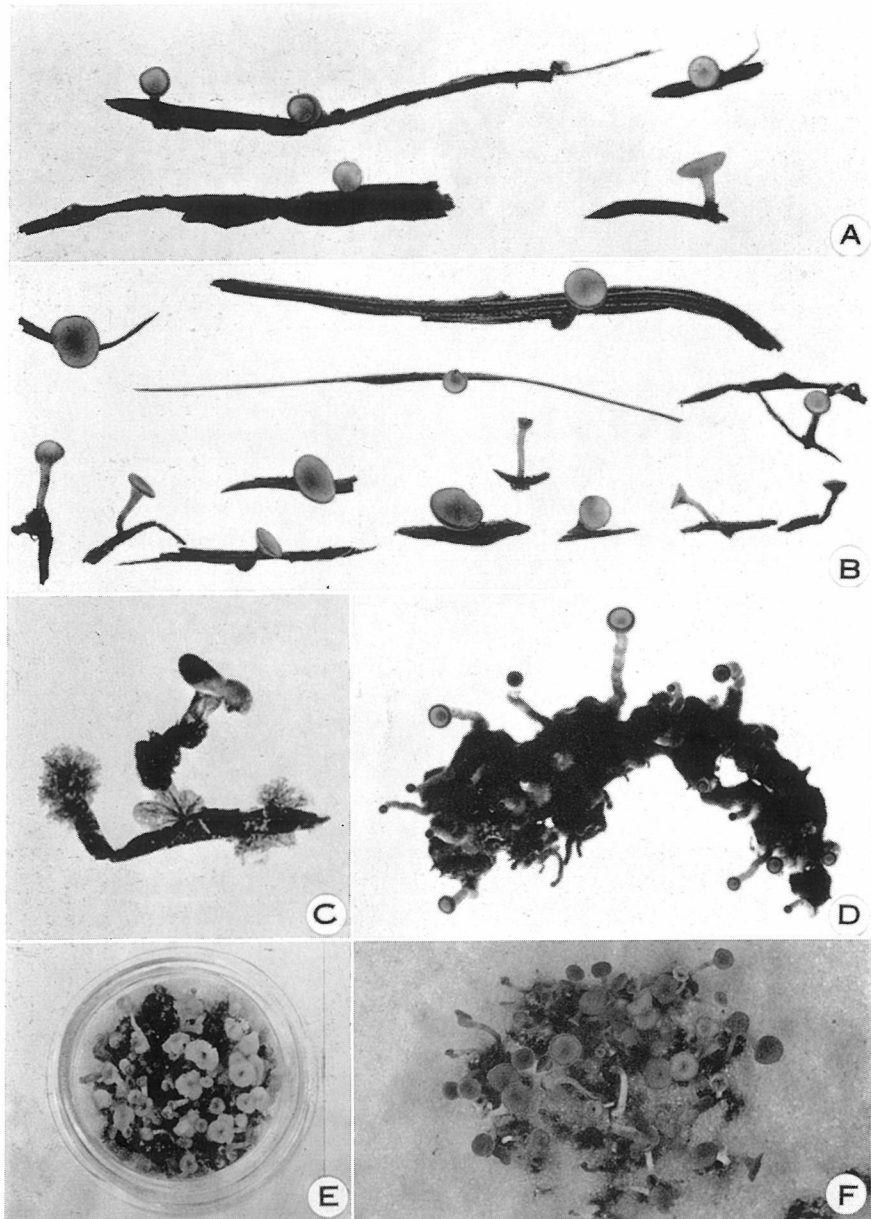


FIG. 18. *Botryotinia ranunculi*. A, B, apothecia on overwintered petioles from nature,  $\times 2$ ; A, type collection CUP 25278; B, CUP 15618; C, conidial production on a fertile sclerotium kept in moist chamber,  $\times 2.5$ , CUP 15618; D, E, F, apothecial development from cultivated sclerotia in laboratory, D,  $\times 2$ , CUP 15618, E,  $\times 0.9$ , DAOM 12393 ( $\text{♀}$  SS 16  $\times$   $\text{♂}$  SS 2), F, natural size, DAOM ( $\text{♀}$  SS 10  $\times$   $\text{♂}$  mass 2 + SS 10). (Photographs A, B, C, D, from Herb. CUP, Cornell University, Ithaca, N.Y.)



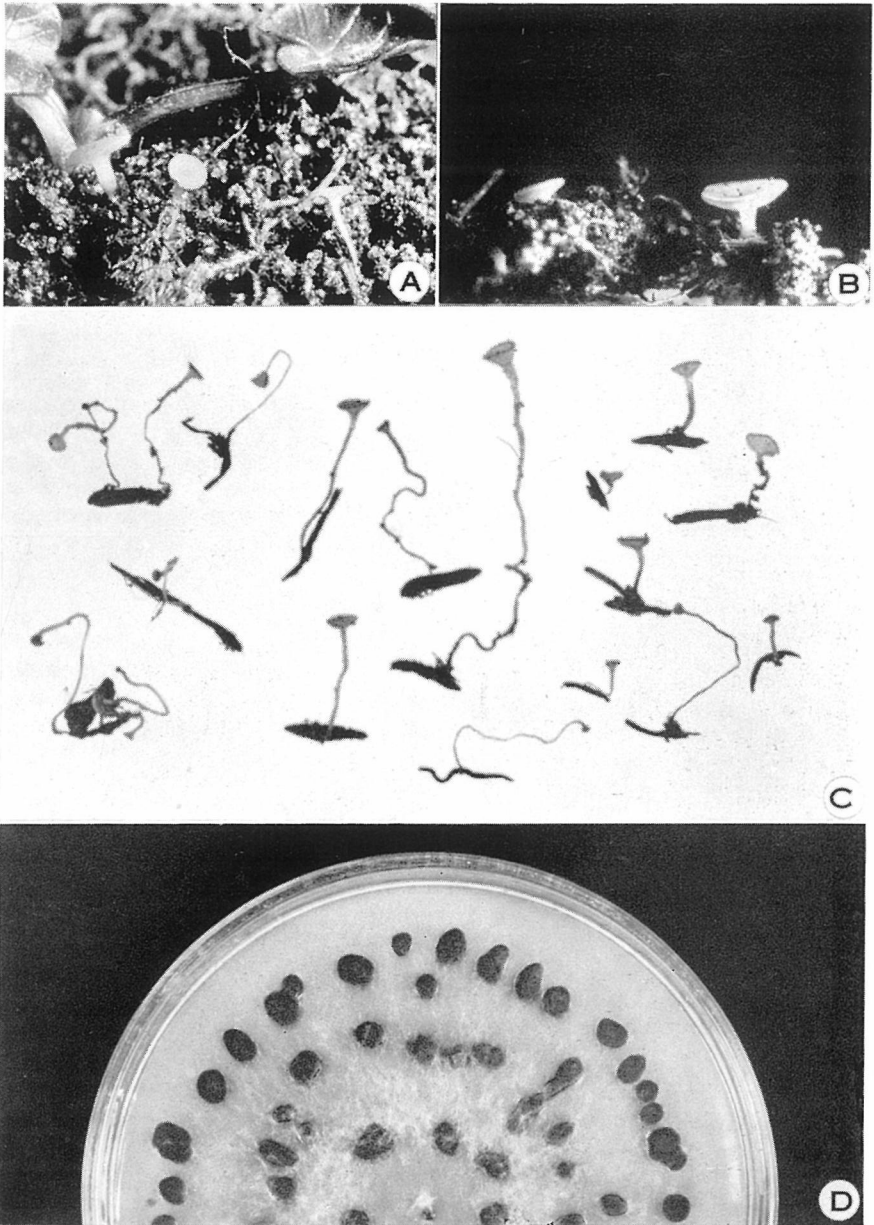


FIG. 19. *Botryotinia ficariarum*. A, B, apothecia in nature,  $\times 2$ ; A, under *Ficaria verna*, type collection G.L.H. 1066; B, G.L.H. 1067; C, type collection,  $\times 2$ , G.L.H. 1066; D, 2-month-old culture on potato-dextrose agar, G.L.H. 1073.