NEW SPECIES OF SPIROSPHAERA

By G. L. HENNEBERT

Laboratory of Systematic and Applied Mycology, University of Louvain, Heverlee, Belgium and Centraalbureau voor Schimmelcultures, Baarn, the Netherlands

(With Plate 1 and 3 Text-figures)

Spirospheara beverwijkiana and S. minuta collected from decaying leaves under water in the Netherlands and Belgium are described as new species, in addition to the type species, S. floriformis van Beverwijk.

The mycological material left by the late Miss Agatha van Beverwijk, formerly director of the Centraalbureau voor Schimmelcultures, was composed mainly of microscopical slides, living or desiccated cultures and some plant material collected in the field and kept in moist chambers. The material, accompanied by some notes, was used by her at the time in her investigations on hyphomycetes, when she described Candelabrum spinulosum van Beverwijk (1951a), Clathrosphaerina zalewskii van Beverwijk (1951b), and Papulaspora pulmonaria van Beverwijk (1954), three of the fungi of that peculiar ecological group that she called 'aero-aquatic' on account of their way of growth and sporulation. She also described (van Beverwijk, 1953), besides seven other helicosporous fungi, the new genus Spirospheara with the single species S. floriformis, another of those remarkable fungi. Later, she observed species that she left unnamed.

In view of the various contacts I had with Miss van Beverwijk concerning the study of these fungi, I was asked by the Centraalbureau voor Schimmelcultures to continue her investigations and to have the use of her collections. Since then I have been able to study additional collections and isolates. The results of these investigations will be reported in various notes, of which this is the first.

METHOD OF ISOLATION AND CULTURE

Van Beverwijk's success in the study of these fungi was due to her method. Instead of collecting plant material, decaying pieces of bark and wood, in moist places or near stagnant waters, as Linder did, she preferred to collect from under the water surface, in bogs, ponds, ditches, fishponds and garden fountains. Most of her collections were composed of decaying leaves.

The aero-aquatic fungi are characterized by the development of a vegetative mycelium in plant material under water and in the water film covering this substratum, and by the production of immersed conidio- phores developing the conidium just above the water surface. In most
species, the conidia show a globose and complex structure allowing the retention of some air inside acting as a float. During the collection of plant material, the spores are shed and the fungus can hardly be detected in a fresh specimen. Back in the laboratory, the collections are put in covered Petri dishes and kept for a few days to several weeks with enough water to maintain a thin liquid film over them. The dishes with lids are deposited in large translucent containers to prevent the surface of the leaves from drying out. Soon, new spores develop, sometimes profusely, at the surface of the water film covering the leaves or on the still very wet portions of these; the spores are supported by fragile conidiophores, the length of which depends on the depth of the water film, and are readily shed by water motion. This method developed by van Beverwijk permits ready observation and isolation of these fungi under undisturbed conditions.

To isolate these fungi, spores are picked off under a x 45 magnification of a stereoscopic microscope with a fine needle and then transferred to Petri dishes containing a suitable agar medium. Another successful method is to remove with the aid of a loop, a portion of the water film bearing floating spores from the surface of the leaf and to spread this by streaking on the surface of the agar. The spores or spore fragments, when germinated, are transferred to another dish or tube to check the purity of the culture. The medium used by van Beverwijk for her isolation was cherry agar; I used yeast–dextrose–asparagine agar (YDAA) with antibiotics added.

A sufficient quantity or an excess of water on the agar surface simulates the natural conditions for growth and favourably influences the development of vegetative and fertile hyphae under the water and sporulation on the water surface. Cultures of old, no longer sporulating strains of Spiro–phaera beverwijkiana, after being macerated, taken into suspension in sterile water and poured on a suitable agar medium, recovered their ability to sporulate; subsequent transfers of spore suspensions progressively gave heavier sporulation. In view of the slow growth of species of Spiro–phaera the most satisfactory way used for culturing these fungi has been this spore suspension method.

The media used are the following:

(1) Cherry agar, prepared from a decoction of 200 g cherry fruit, 25 g agar/l, the cherry extract and the agar being mixed after separate sterilization.

(2) 1% malt agar (MA 1%), made from a diluted brewery malt extract made up to 1% sugar content with a Balling’s saccharimeter (equivalent to 1% Difco malt extract), and 20 g agar/l.

(3) Yeast–dextrose–asparagine agar (YDAA), containing 1 g yeast extract Difco, 0.75 g asparagine, 10 g glucose, 0.5 g K$_2$HPO$_4$, 0.25 g MgSO$_4$·7H$_2$O, 0.05 g FeCl$_3$ and 25 g agar/l.

(4) Yeast–dextrose–peptone agar (YDPA), composed of 5 g yeast extract Difco, 20 g glucose, 10 g peptone, 25 g agar/l.

(5) Oat agar, made of a decoction of 30 g oatmeal with 5 ml glycerol, 0.2 ml lactic acid and 15 g agar/l.

(6) Potato–dextrose agar (PDA), prepared from a decoction of 200 g potato tubers, with 20 g glucose and 20 g agar/l.

SPIROSPHAERA FLORIFORMIS van Beverwijk, Trans. Br. mycol. Soc. 36, 121, 1953 (as ‘floriforme’) (type species). (Pl. 1, fig. 1; Text-fig. 1)

Van Beverwijk (1953) gave a good description and illustrations of the species. It is here illustrated again and redescribed for comparison with

Text-fig. 1. Spirosphaera floriformis. Spore development, mature spores and appressoria-like chlamydospores. Type, x 1000.
the additional species. I did not collect the fungus myself but have examined the following collections:

NETHERLANDS: (1) the type collection from decaying *Betula* leaves under the water surface of a pond, Eerder Esh, Ommen, prov. Overijssel, Nov. 1947, A.v.B. 10.o.2T (CBS 402.52 = IMI 52.467 = GLH 6766); (2) from *Betula* leaves in a pond, same station, presumably same date, A.v.B. 14.o.2B, slides and dried cultures (GLH 6750); (3) from *Betula* leaves in a pond, Groeneveld, Baarn, prov. Utrecht, 10.6.1952, A.v.B. (GLH 6744); (4, 5) from *Quercus* leaves, Ommen, prov. Overijssel, May 1951, A.v.B. (GLH 6744-A, 6744-B); (6) from a *Rhododendron* leaf, same station, July 1949, A.v.B. (GLH 6748). ENGLAND: (7) from *Quercus* leaves in a pond, Haslemere, Surrey, England, Nov. 1948, J. I. Glenn-Bott (CBS 403.52 = GLH 6767).

Colonies on cherry agar at first hyaline, becoming maroon brown, warm sepia to dark purple brown, the reverse is sepia. *Hyphae* prostrate, often fasciculate, septate, branched, 3–5 μ diam, sparsely aerial, at first hyaline, then umbre brown. On the surface of the leaves, the hyphae bear large dark brown cells, 5–18 × 4–7 μ, thick-walled, appressoria-like. *Conidiophores* little-differentiated erect hyphae, arising from the immersed mycelium, single or fasciculate, simple or branched, septate, 3–5 μ wide, of variable length depending on the depth of the water, short under drier conditions, sometimes inflated at the base when developed on the leaf, producing at their end, just above the water surface, one or two spore bodies which are called conidia or spores.

*Conidia* globose balls of threads, pure white at first, then pale ochre-yellow, tawny to fuligineous at maturity, 50–150 μ diam., consisting of more or less densely interwoven spirals of rarely more than one coil, each developed as a single lateral branch on the previously formed spirals, growing towards the centre, rarely outwards, coiling and intertwining, then branching again in the same way. Filaments of the coils 4–8-septate, 3.5–5.5 μ wide, with cells 6–16 μ long, at first hyaline and cylindrical, finally maroon brown to fuscous, inflated outwards and constricted at the septa. When crushed, the mature conidium breaks apart into single cells or multicelled curved segments of coils which can readily germinate.

As in the other described species, a variation in colour as well as in the conidium formation exists between strains. These two characters seem to be correlated. Indeed, isolates (4) and (5) have a mycelium remaining whitish to creamy, which is unable to develop better-formed conidia than very loose coils, whereas the typical strains are much darker coloured and develop more compact spore balls. The width of the hyphae and of the coils is, however, typical for the species. There is no doubt, as I also found in *S. beverwijkiana*, that such strains fit the range of variability of the species.

Spirosphaera beverwijkiana sp. nov. (Pl. 1, fig. 2; Text-fig. 2)

Fungus imperfectus helicosporalis. Coloniae restrictae, pulvinatae, velutinae, ochraceae vel brunneae, interdum albae aut griseae, lente crescentes, hyphis repentibus, paucis aeriis, subhyalinis vel pallide brunneis, septatis, 2–3.5 μ crassis. Conidiophora ut laterales et erecti rami hypharum, unum conidium ferentia. Conidia glomerulosa, laxa vel compacta, orbicularia, elongata vel difformia, pallide brunnea, 30–85 μ in diametro, e cylindraceis 1–4 involutis 12–17 μ latis laxe intricatis helicibus compositae, ut singulis lateralibus rami primiarum helicum formatis. Fila cylindracea, regularis, 4–18-septata,
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tarde parce inflata et constricta, in fragmenta sub pressione labens. Chlamydosporae intercalares, brunnescentes, globosae, 7–8 μ diam.


Text-fig. 2. *Spirosphaera beverwijkiana.* Spore development, mature spores and intercalary chlamydospore. Type, × 1000.
Imperfect fungus, helicosporous hyphomycete.

Colonies small, very slowly growing, dense, tuberculate or pulvinate, first pale, later ochraceous to chocolate brown, sometimes greyish brown or even chalk white, dark brown on the reverse side. Hyphae mostly prostrate or immersed in the covering layer of water, sparsely aerial, branched, sometimes fasciculate, septate, hyaline to brownish, 2–2.5 μ diam., with well-marked walls and septa. Conidiophores little differentiated, arising as lateral branches on hyphae, erect, simple, septate, of variable length, bearing usually a single conidium. Conidia formed eccentrically by coiling and branching of the extremity of the conidiophore. They are loose balls of threads, subglobose, elongate or of an irregular shape, 18–85 μ broad, each spiral as a single lateral branch of one of the basal cells of the previous spiral. Filament regular, 2.5–4 μ wide, 4–18-septate, at first cylindrical and hyaline finally slightly inflated and brown, breaking apart when crushed into cells or segments of coils which are able to germinate. Chlamydospores intercalary, single, globose, pale brown, 7–8 μ diam.

Habitat: in aero-aquatic conditions on decaying leaves of deciduous trees (Quercus pedunculata Ehrh., Q. coccinea Vaughan, Fagus sylvatica L., Betula verrucosa Ehrh., Populus tremula L.) just under the surface of stagnant waters in the Netherlands and Belgium.

Type: GLH 8641-A, plant material and dried cultures, in author's herbarium at the University of Louvain, Belgium, in Herbarium BR, Jardin Botanique de l’Etat, Bruxelles, and in the Collection CBS 469.66, Centraalbureau voor Schimmelcultures, Baarn, Netherlands.

The specimens examined are all from decaying leaves of deciduous trees under water. Hosts and localities are as follows:


Living cultures of the type (1), of isolates (13), (15), (19), and of the white variant of (13) are maintained at the Centraalbureau voor Schimmelcultures. The same strains and seven others are preserved at the Laboratoire de Mycologie systématique et appliquée, University of Louvain.

The fungus was observed for the first time by van Beverwijk in the Netherlands in 1947. Since then it has been collected many times at all seasons. Recently I discovered it in Belgium in the same kind of habitat.
Quercus leaves are the most usual substratum. Examinations of decaying leaves of other species, Acer campestris, Carpinus betulus and Corylus avellana have not yet yielded any colony of the fungus. S. beeverwijkiana is easily isolated. The spores germinate readily on all media used, but its growth is very slow. On YDPA and on YDAA, a single-conidial culture reaches 8–10 mm only after 4 weeks. In subcultures originating from a spore suspension inoculum, the colonies are still smaller. On the other media, such as oat agar, the colonies are particularly small. Sporulation appears on YDPA and YDAA first and is abundant, it occurs much later on MA and oat agar and never on PDA.

The colour of the fungus is very variable, depending on the strain and the nature of the medium. Single conidial strains are differentiated from each other by the two related characters of sporulation and colour. A spore seeding originating from a single-conidial culture may give different strains. Dark brown strains produce small and compact conidia, those that are tawny brown or pale brown to ochre produce looser and larger conidium balls and the chalk white ones show only a very loose and imperfect spiral formation.

Spirosphaera minut a sp. nov. (Pl. i, fig. 3; Text-fig. 3)


Helicosporous hyphomycete.

Coloniess mall and restricted, velvety, dense, whitish to pale creamy yellow, growing very slowly, with a pale brown to red brown reverse. Hyphae immersed in the substratum or in water, also aerial but sparse, tiny, fragile, straight or undulate, knobby, septate and branched, sometimes fasciculate in ropes of 2 to 6, hyaline, to pale coloured, 1.5–2 μ wide, with thin walls and septa. Conidiophores develop as undifferentiated erect simple lateral branches of hyphae, of variable length depending on the depth of water, bearing a single conidium on its tapering and contorted end which is hidden in the middle of the first coil. Conidia borne centrically at the end of the conidiophore; they are loose balls, of a more or less globose shape, white, 15–35 μ diam, composed of 1–3 loosely interwoven spirals of 1–4 coils and 7–12 μ, diam. Filament of the coils regular, uneven or knobby outwards, 1.5–2 μ wide, 1–5-septate, hyaline, the basal cells each bearing a single similarly coiled and interwoven lateral branch. The conidium body retains air inside and floats; the conidia are deciduous and break apart, when forcibly crushed, in cells or segments of coil which are capable of germination. Chlamydospires are not developed.

Habitat: in aero-aquatic conditions, on decaying leaves of deciduous

Type: GLH 8640, plant material and dried cultures, in author's herbarium, University of Louvain, in Herbarium BR, Jardin Botanique de

Text-fig. 3. *Spirospheara minuta*. Spore development, mature spores and hyphae. Type, x 1000.
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l'Etat, Bruxelles, and at the Centraalbureau voor Schimmelcultures, Baarn, under CBS 475.66.

The specimens examined are all from decaying leaves. Hosts and localities are listed as follows:

(a) On Betula alba L. and B. verrucosa Ehrh. NETHERLANDS: (1) in a ditch Hooge Vuursche, Baarn, prov. Utrecht, 25. iii. 1966, G.L.H. (GLH 8640 = CBS 475.66, TYPE);


Living cultures of the type (1) and of collections (3) (9) and (10) are maintained in the CBS culture collection at Baarn; the same and also an isolate of (5) at the University of Louvain.

Spirosphaera minuta has been observed twice and grown once in a mixed culture with a species of Dasyseephyus by van Beverwijk. Only two slides and a dried slant were preserved. After seeing van Beverwijk's slides, I discovered the fungus again in the field and isolated it.

S. minuta differs from the two other species chiefly in its small size. On decaying leaves where it often grows in association with S. beverwijkiana, its spores are the smallest and the whitest ones, and might be easily mistaken for young spores of the other species. But under the microscope, the constant smallness of the spore filament is evident. Single spore isolations repeatedly demonstrated the difference between these species.

Like S. beverwijkiana, S. minuta in culture is of variable color. On YDPA, it is pale creamy white when young, cream or greyish cream when mature, ochre, tawny to dark brown on the reverse side. On YDAA, the colonies are similar when young to those developed on YDPA, but are, when mature, felt grey or testaceous with a chamois-coloured margin; their reverse varies on that medium from dark bistre to red brown or dark brown.

This fungus grows slowly, single colonies producing a flat velvet growth becoming more pulvinate when colonies are crowded together. It sporulates after 10 days from the centre at the surface of the agar, under cover of the aerial hyphae. The sporulation is sparse on most of the media, except on YDPA, the only medium where it is typical and abundant. Unlike S. beverwijkiana, the sporulation is less profuse on YDAA than on YDPA. On cherry agar and MA, the fungus does not develop well.

In culturing the fungus, some difficulties may be encountered because of its very slow growth. The colonies reach only 2–3 mm diam after 3 weeks from a single spore inoculation. Abundant growth can only be obtained by transferring a spore suspension in water to freshly prepared
YDPA. The quantity of suspension poured on the surface of the medium
must be sufficient to keep the agar wet during some days. Under these
conditions, the germination and growth of the sporelings occur quickly.

The most important feature for distinguishing *S. minuta* from the other
species of *Spirosphaera*, in culture as well as in natural substrates is its spore
morphology. The spore filament is conspicuously smaller, 1·2-1·6 µ as
against 2·5-4 µ in *S. beverwijkiana* and 3·5-5·5 µ in *S. floriformis*. The spore
diameter does not exceed 35 µ in *S. minuta*, while it reaches 85 µ in *S.
beverwijkiana*, although both species can have spores of the same size
between 18 and 35 µ. The spores of *S. floriformis* are considerably larger
(50-150 µ). Furthermore, the conidiophore of *S. minuta* tapers to a slender
and contorted end hidden in the middle of the first spore coil, while that
of *S. beverwijkiana* does not vary in width and bears the spore eccen­
trically. Finally, the spore filament of *S. minuta* is uneven or crooked out­
wards, whereas that of *S. beverwijkiana* is regular or, finally inflated and
constricted like that of *S. floriformis*.

**DISCUSSION**

In establishing the genus *Spirosphaera*, van Beverwijk (1953) gave the
following definition: ‘Fungus aeroaquaticus, mycelio repente, ramoso,
septato, hyalino vel fusco. Conidiophora non multum distincta. Conidium
constat e spiris ramosis septatis.’ Without altering her concept, I would
add ‘et intertextis’ since she stated further in her paper that the inter­
woven spirals make the distinction between *Spirosphaera* and *Helicodendron
Peyr.*

This original generic diagnosis seems to me still lacking in precision for a
clear segregation. Van Beverwijk (1953), describing the type species, had
already noticed the existence of structures similar to those seen in the
developing conidium of *Spirosphaera floriformis* in the figures of Hotson
(1912), illustrating the early stages of development of the bulbils of
*Papulaspora spinulosa* Hotson. The difference, as she remarked, should
appear in the mature spores. However, Hotson’s description and illustra­
tions are, in my opinion, more suggestive of the relationship of *P. spinulosa*
with the species of *Spirosphaera* than with *P. sepedonioides* Preuss, the type
species of *Papulaspora* Preuss.

The question may, nevertheless, arise whether the reproductive bodies
of *Spirosphaera* should be called bulbils. The definition of a bulbil, accord­
ing to Hotson (1912) is ‘a reproductive body of more or less definite form,
composed of a compact mass of homogenous or heterogenous cells which
may be few or many, but are always developed from primordia of more
than one cell.’ I would probably consider this latter characteristic as too
restrictive, if not somewhat inconsistent. This definition of a bulbil, how­
ever, does not seem to apply to the spores of *Spirosphaera*. As in helico­
sporous fungi, the spores of *Spirosphaera* are not compact masses of cells, but
contain small spaces filled with air unlike those of *Papulaspora*. Similarly,
*Spirosphaera* is adapted to an aero-aquatic habitat, *Papulaspora* seemingly
not.

*Malbranchea* Penz. & Sacc. is also an interesting genus to compare with
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Spirosphaera. It is characterized by an abundant and wide mycelium, bearing arborescent conidiophores composed of coiled or sinuous septate branches, the cells of which spontaneously break off as arthrospores at maturity. The genus shows evident affinities with Chrysosporium Corda or Coremiella Bub. & Krieg. The branching and coiling of the conidiophore may vary from species to species and may present a very similar picture to that of a young or mature Spirosphaera spore. I know of a still unnamed species of Malbranchea developing balls of thread, outwardly identical with the spores of Spirosphaera minuta in structure and size. However, the difference is evident. In this Malbranchea sp., the ball is the conidiophore, is hydrophilic and its coiled branches break apart readily as arthrospores becoming dispersed within the water. In Spirosphaera, the ball is the spore, is hydrophobic and spreads out as a unit, floating and drifting on the surface of water.

Another similar conidium development to that observed in Spirosphaera has been described and illustrated by Arnaud (1952) under the provisional name 'Ophioidendron laocoont Arnaud'. This fungus, the type species of a new genus, is said to have 'conidiophores incolores, d'abord simples et dressés, formant au sommet un petit nombre de rameaux enroulés en hélices lâches puis en hélices coniques serrées du type Helicoon'. Arnaud noticed that the spores observed were seemingly not mature. If the illustration did not suggest strongly an interweaving development of the coils and, therefore, the young state of a Spirosphaera spore (possibly S. beuwerwijkiana), the fungus could be easily taken for a species of Helicodendron. Because of the absence of original material, the question cannot be elucidated. I prefer then to propose the exclusion of the generic name and the specific epithet, for being nomenclaturally a nomen invalidum (being a nomen provisorium and lacking a Latin diagnose) and taxonomically a nomen dubium.

Still one more remarkable character of Spirosphaera is that the coiled branches in the spore are never opposite, but single. A study is being carried out on another helicosporous fungus known as Strumella olivatra (Sacc.) Sacc., the spore of which, like Spirosphaera, are balls formed as the result of repeated development of coiled branches from the end of the conidiophore. Unlike Spirosphaera, however, each cell of the coils may form two or more opposite orthogonal branches near the distal septum instead of a single one.

The three species of Spirosphaera here described thus constitute a clearly homogenous genus which van Beverwijk first recognized. For greater precision, its diagnosis may be given now as follows:

**SPIROSPHAERA** van Beverwijk


No perfect state is known for either of the three species. No primordia or similar body were observed in culture. Investigations have been made in an
attempt to connect one of the Spirosphaera with the ascomycetes frequently found in association in nature. Amongst others, two discomycetes, identified as Trichopeziza punctiformis Fr. sensu Sacco and Dasyscyphus fuscescens (Pers. ex Fr.) Rehm, were always found in mixed growth with S. beverwijkiana and S. minuta. Cultured from single ascospores they reproduced fruiting bodies in artificial conditions but never formed conidia. Their cultural characters were, in any case, quite different from those of Spirosphaera.

I am much indebted to the late Miss Agatha van Beverwijk for her enthusiastic collaboration in the study of these remarkable fungi.

I wish to accord my sincere thanks to the authorities of the Centraalbureau voor Schimmelcultures, Baarn, and particularly to Dr J. A. von Arx, Director and Dr M. B. Schol-Schwarz, previous Director ad interim, for having entrusted to me the continuation of the investigations of Miss van Beverwijk and for their generous assistance and criticism during that work.

REFERENCES


EXPLANATION OF PLATE I

Fig. 1. Spirosphaera floriformis. Young and mature spores from culture on cherry agar (type).
Fig. 2. Spirosphaera beverwijkiana. Young and mature spores from culture on YDAA (type).
Fig. 3. Spirosphaera minuta. Spores from culture on YDPA (type). All phase contrast, ×400.

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