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NEW SPECIES OF *SPIROSPHAERA*

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(With Plate 1 and 3 Text-figures)

Spirosphaera 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 (1951*a*), *Clathrosphaerina zalewskii* van Beverwijk (1951*b*), 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 *Spirosphaera* 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, fish-ponds 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 conidiophores 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 $\times 45$ 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 *Spirosphaera 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 *Spirosphaera* 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_2HPO_4, 0·25 g $MgSO_4 . 7H_2O$, 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.

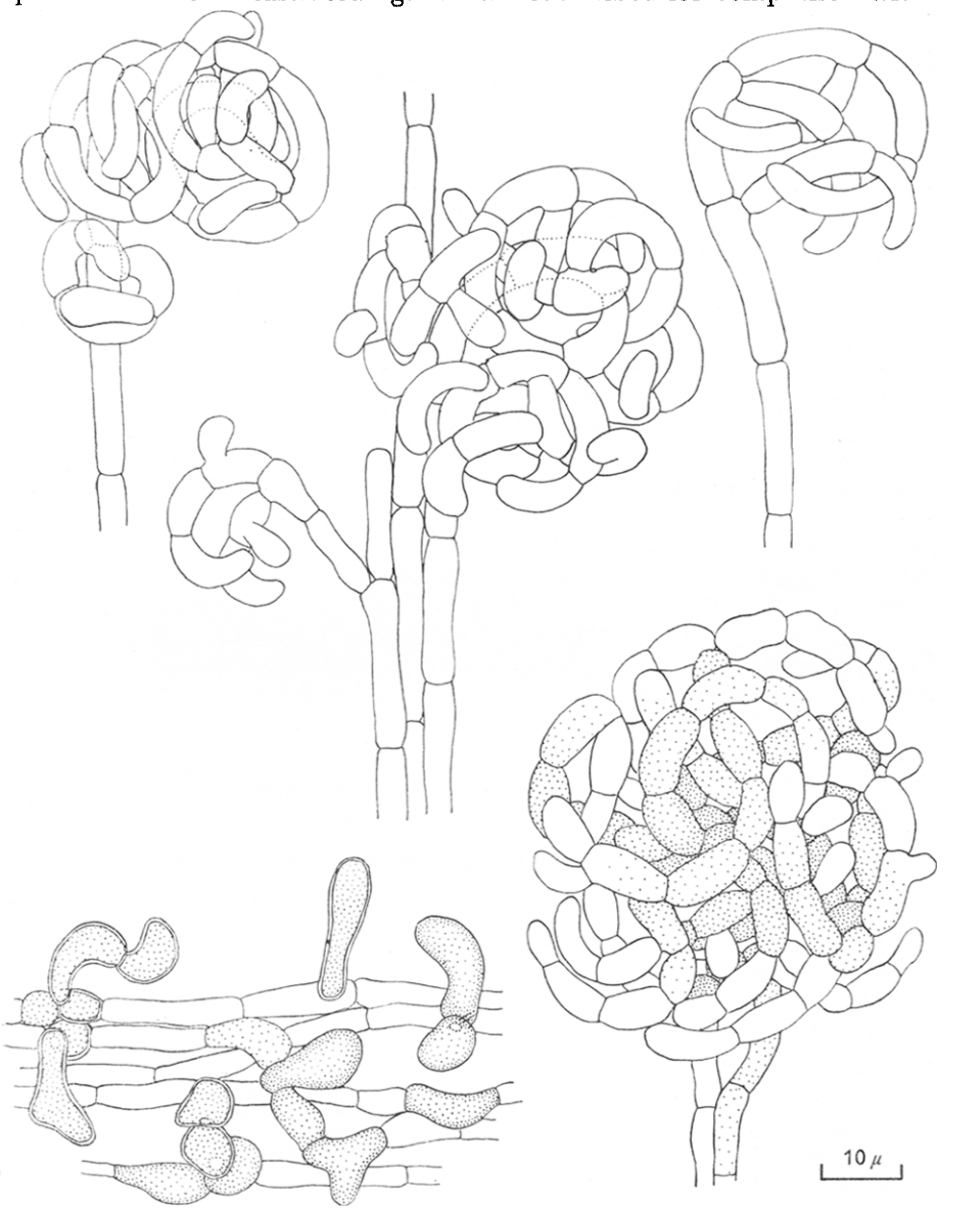
(5) Oat agar, made of a decoction of 30 g oatmeal with 5 ml glycerol, 0·2 ml lactic acid and 15 g agar/l.

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

SPIROSPHAERA van Beverwijk, *Trans. Br. mycol. Soc.* **36,** 120, 1953.

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, × 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. Overijsel, Nov. 1947, A.v.B. 10.0.2T (**CBS** 402.52 = **IMI** 52.467 = GLH 6766); (2) from *Betula* leaves in a pond, same station, presumably same date, A.v.B. 14.0.2B, 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. Overijsel, May 1951, A.v.B. (GLH 6764-A, 6764-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 umber 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.

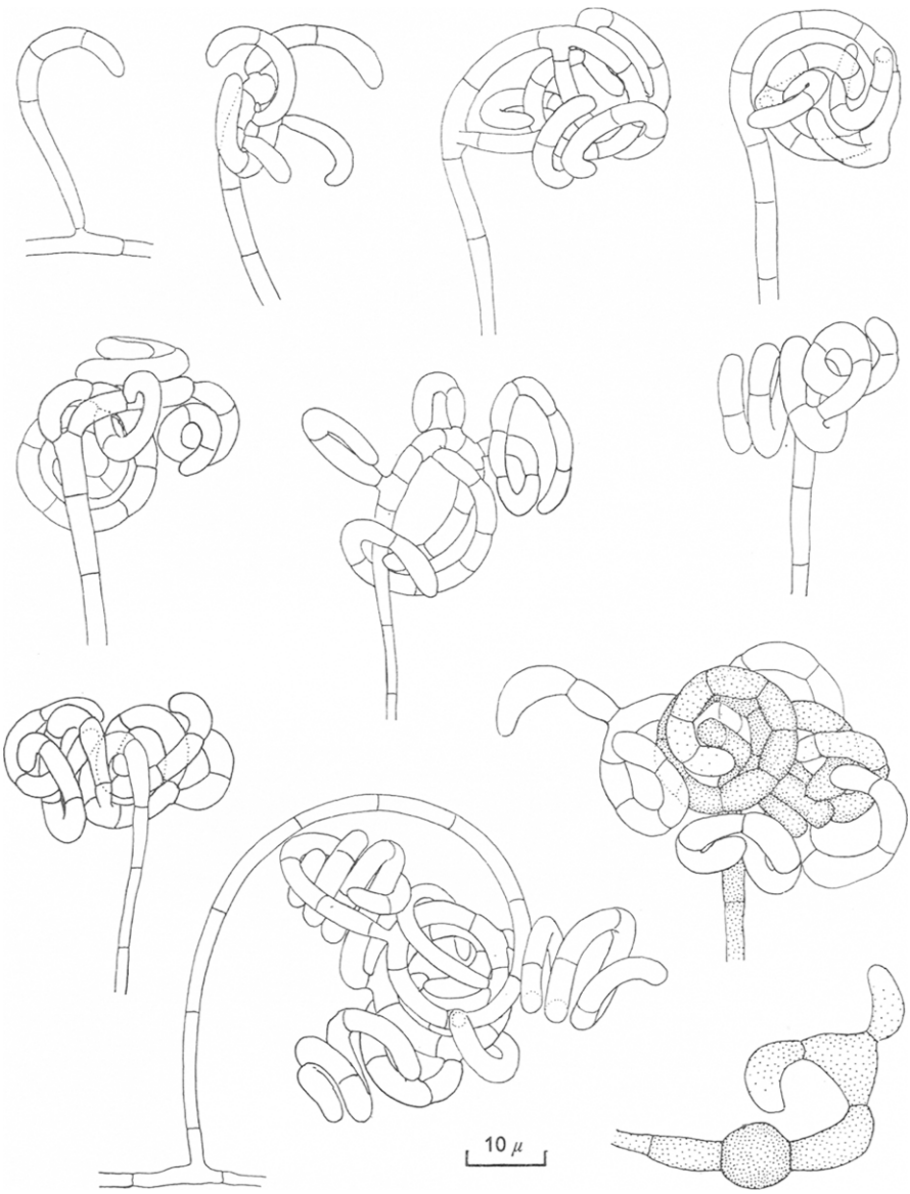
As in the other described species, a variation in colour as well as in the conidium formation exists between strains. These two characters seem to be correlated. Indeed, isolates (4) and (5) have a mycelium remaining whitish to creamy, which is unable to develop better-formed conidia than very loose 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 ramis primariarum helicum formatis. Fila cylindracea, regularis, 4–18-septata,

tarde parce inflata et constricta, in fragmenta sub pressione labens. Chlamydosporae intercalares, brunnescentes, globosae, 7–8 μ diam.

Habitat in foliis sub aqua putrescentibus *Quercus*, *Fagi*, *Betulae*, *Populi* specierum in Hollandia et Belgio. Typus: ut desiccata materia et cultura, GLH 8641-A = **CBS** 469·66, in Universitate Lovaniensi, et Horto Botanico Bruxellensi, Belgio, et in C.B.S., Baarn, Hollandia.



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* Vaugh. *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:

(*a*) On *Quercus pedunculata* Ehrh. NETHERLANDS: (1) Hooge Vuursche, Baarn, prov. Utrecht, 25. vi. 1966, G.L.H. (GLH 8641-A = **CBS** 469.66, TYPE); (2) ibid. (GLH 8641-B); (3, 4) Bilthoven, prov. Utrecht, 16. iv. 1966, G.L.H. (GLH 8718-B, 8719); (5–9) Middachten, prov. Gelderland, 2. xii. 1954, 22. iii. 1955, 24. vi. 1955, 31. vii. 1955, Nov. 1957, A.v.B. (GLH 6761, 6757, 6760, 6759, 6753); (10, 11) Groeneveld, Baarn, prov. Utrecht, 10. vi. 1952, Sept. 1953, A.v.B. (GLH 6752, 6755); (12) Baarnse Bos, Baarn, ibid. Jan. 1949, 1. x. 1949, one not dated, A.v.B. (GLH 6756, 6746, 6765); (13) Ommen, prov. Overijsel, 19. xii. 1957, A.v.B. (GLH 6754 = **CBS** 471.66, and a white variant GLH 6754-var. = **CBS** 472.66). BELGIUM: (14, 15) Meerdael Forest, Nethem, prov. Brabant, 16. v. 1966, G.L.H. (GLH 8874, 8875 = **CBS** 474.66).

(*b*) On *Quercus coccinea* Vaugh. NETHERLANDS: (16) Emst, prov. Gelderland, May 1952, A.v.B. (GLH 6758).

(*c*) On *Fagus sylvatica* L. NETHERLANDS: (17) Baarnse Bos, Baarn, ibid., 30. iii. 1960, A.v.B. (GLH 6751 = **CBS** 473.66).

(*d*) On *Betula verrucosa* Ehrh. NETHERLANDS: (18) Heerde, prov. Overijsel, 10. ix. 1961, A.v.B. (GLH 6762).

(*e*) On *Populus tremula* L. NETHERLANDS: (19) Hooge Vuursche, Baarn, prov. Utrecht, 25. vi. 1966, G.L.H. (GLH 8643-B = **CBS** 470.66).

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. beverwijkiana 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 minuta sp.nov. (Pl. 1, fig. 3; Text-fig. 3)

Fungus imperfectus helicosporalis. Mycelium album vel luteum, fragile, floccosum vel procubene, ex hyphis tenuibus, regularibus, septatis, ramosis et fasciculatis, hyalinis, 1·5–2 μ crassis. Conidiophora nulla vel brevia. Conidia glomerulosa, laxa, 15–35 μ in diametro, ex una vel pluribus laxe intricatis primariis spiris, 1–2 vicibus, planis vel helicoideis, hyalinis, 7–12 μ in diametro, 1–4-septatis, basalibus articulis lateraliter secundarios spirales ramos ferentibus, qui similiter tertios ferunt. Fila cylindracea, 1·5–2 μ, non constricta sed nodulosa, in fragmenta sub pressione labens. Chlamydosporae nullae. Habitat in foliis sub aqua putrescentibus *Quercus*, *Betulae*, *Fagi*, *Populi* et *Coryli* specierum, in Hollandia et Belgio. Typus: desiccata materia et cultura, GLH 8640 = **CBS** 475.66, in Universitate Lovaniensi et Horto Botanico Bruxellensi, Belgio, et in C.B.S. Baarn, Hollandia.

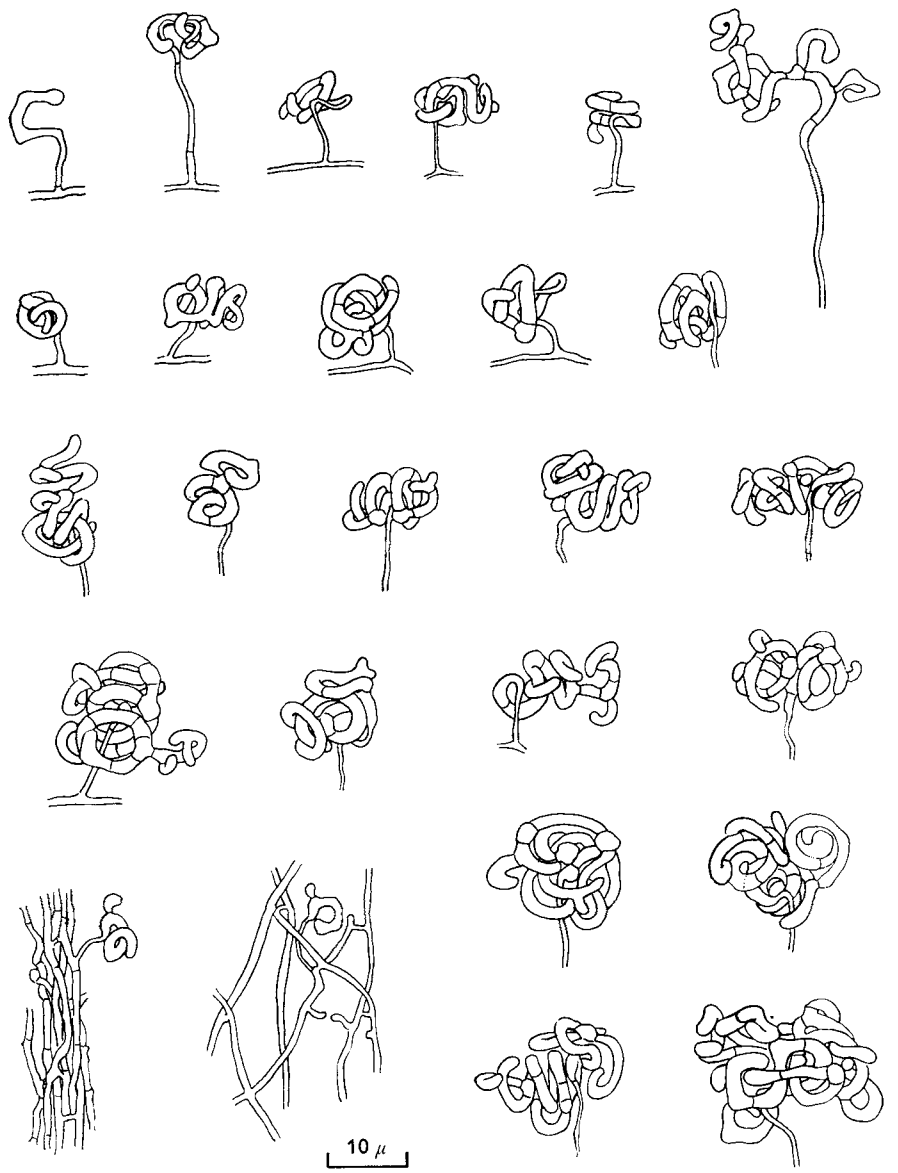
Helicosporous hyphomycete.

*Colonies*s 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. *Chlamydospores* are not developed.

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

trees (*Quercus pedunculata* Ehrh. and *Q.* species, *Betula alba* L., *B. verrucosa* Ehrh., *Fagus sylvatica* L., *Populus tremula* L., *Corylus avellana* L.) lying just under the surface of stagnant water in ditches or ponds in the Netherlands and Belgium.

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



Text-fig. 3. *Spirosphaera minuta.* Spore development, mature spores and hyphae. Type, × 1000.

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);

(*b*) On *Quercus pedunculata* Ehrh. and unidentified species. NETHERLANDS: (2) in a ditch, ibid. 25. iii. 1966, G.L.H. (GLH 8641-C) in association with *S. beverwijkiana*; (3, 4) in a ditch, Bilthoven, prov. Utrecht, 16. iv. 1966, G.L.H. (GLH 8718-A = **CBS** 476.66, GLH 8721); (5) in a ditch, Baarnse Bos, Baarn, 15. v. 1961, A.v.B., (GLH 6745); (6) Middachten, prov. Gelderland, 22. iii. 1955, A.v.B. (GLH 6763).

(*c*) On *Fagus sylvatica* L. NETHERLANDS: (7) in a pond, Hooge Vuursche, ibid. 25. iii. 1966, G.L.H. (GLH 8642); (8) in a ditch, Bilthoven, ibid., 16. iv. 1966, G.L.H. (GLH 8720).

(*d*) On *Populus tremula* L. NETHERLANDS: (9) Hooge Vuursche, ibid. 25. iii. 1966, G.L.H. (GLH 8643-A = **CBS** 498.66).

(*e*) On *Corylus avellana* L. BELGIUM: (10) in a pond, Meerdael Forest, Nethem, prov. Brabant, 16. v. 1966, G.L.H. (GLH 8904 = **CBS** 477.66).

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 *Dasyscyphus* 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 eccentrically. Finally, the spore filament of *S. minuta* is uneven or crooked outwards, 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 interwoven 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 illustrations 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, according 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, however, does not seem to apply to the spores of *Spirosphaera*. As in helicosporous 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

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 '*Ophiodendron laocooni* 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. beverwijkiana*), 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

Fungi imperfecti, hyphomycetes, helicosporei, aeroaquatici. Hyphae aqua submersae, septatae, hyalinae vel fuscae. Conidiophora non vero distincta, erecta, terminalia conidia ferentia. Conidia glomerulosa, e spiris intermixtis, ramosis et septatis, secundariis et singulis lateralibus ramis primariorum, composita.

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 Sacc. 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.

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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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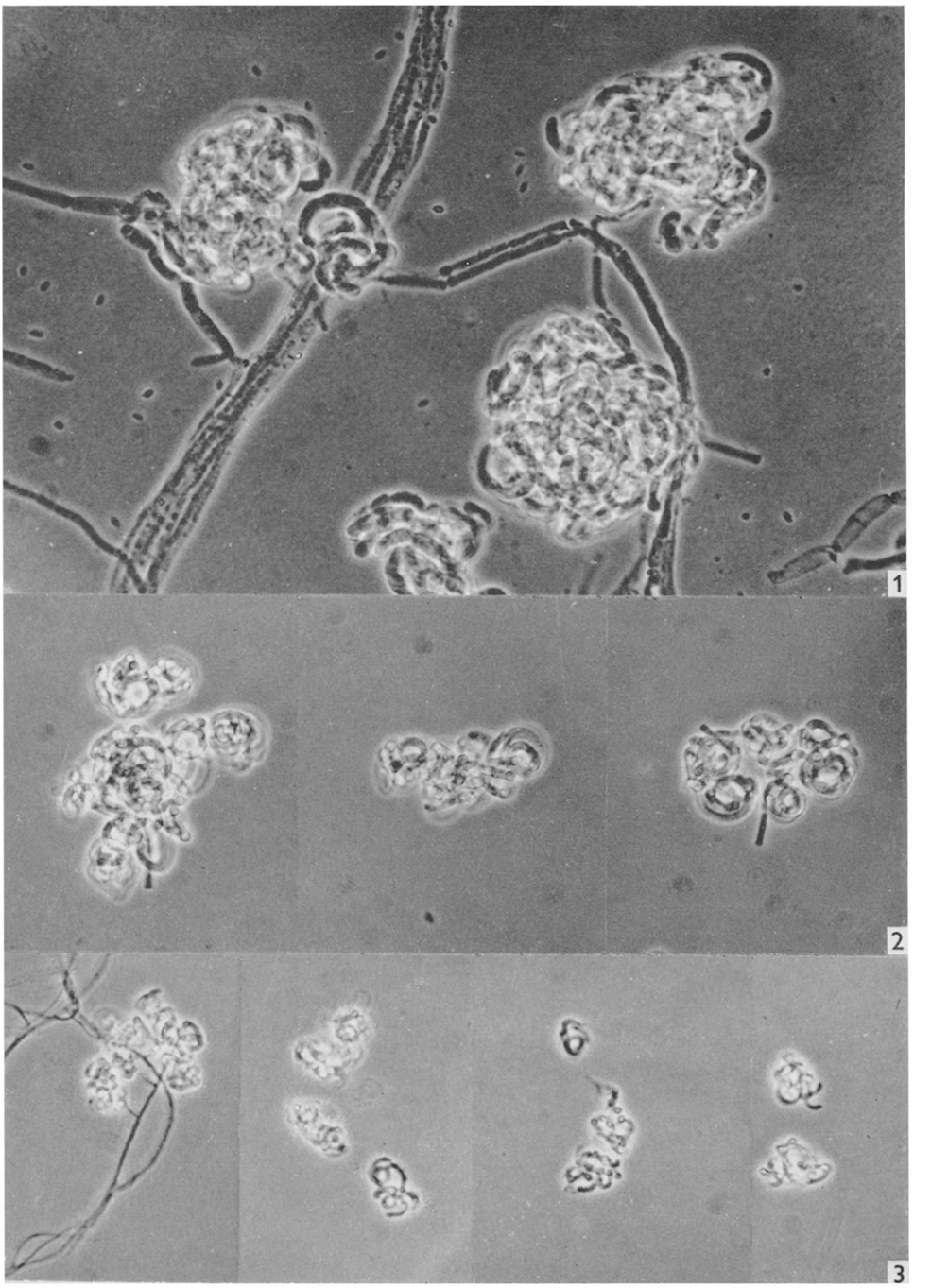

(*Facing p.* 24)