

# MYCOTAXON

Vol. XIV, No. 1, pp. 247-260

January-March 1982

## THE BUBBLE-TRAP PROPAGULES OF BEVERWYKELLA, HELICOON AND OTHER AERO-AQUATIC FUNGI

JOHN MICHAELIDES AND BRYCE KENDRICK

Department of Biology, University of Waterloo  
Waterloo, Ontario, Canada N2L 3G1

### ABSTRACT

The development and ecological role of the floating propagule of Beverwykella pulmonaria (Beverwijk) Tubaki and Helicoon elegans (corda) Arnaud are illustrated and compared with those of other aero-aquatic fungi.

Although delineated by van Beverwijk as long ago as 1951, the so-called aero-aquatic fungi have only recently been recognized and studied as an ecological grouping comparable to the amphibious (also known as 'aquatic' or 'Ingoldian') hyphomycetes (Webster & Descals 1981). The amphibious forms are now well known for: (1) their ability to sporulate underwater, a process that is (2) accelerated by turbulence, (3) requires high oxygen tension, and (4) gives rise to tetra- or multi-radiate (or in some forms, sigmoid) conidia -- all features that adapt these fungi to life in flowing water (lotic systems).

The aero-aquatic fungi, on the other hand: (1) develop conidia only in air and hence (2) need no turbulent stimulus; (3) their vegetative mycelia can tolerate low oxygen tensions; and (4) they have elaborate floating propagules (the word conidium hardly seems adequate for many of these structures). These features fit the aero-aquatic fungi for life in ponds (lentic systems).

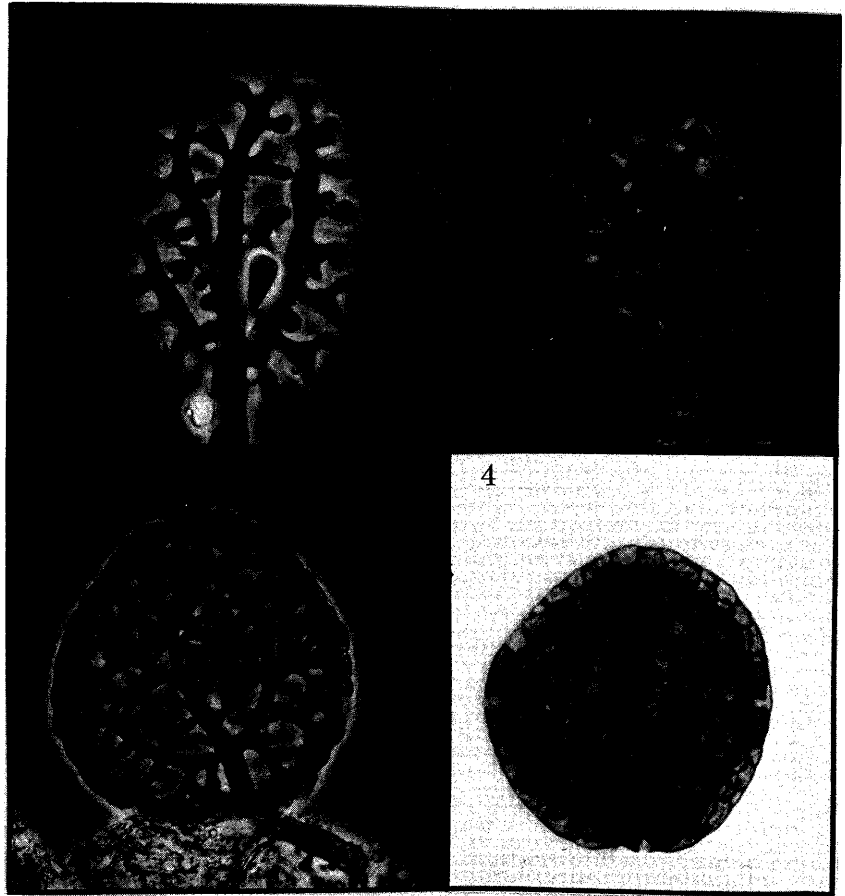
Work in this laboratory has established that the amphibious group plays an important role in colonizing and conditioning the allochthonous organic matter (mostly tree leaves) that constitutes the major energy input to many temperate streams. This role makes the fungi and the leaves they "condition" vital components in the diet of detritivorous benthos such as Gammarus (Amphipoda, Crustacea), and thus in energy flow through the stream community (Bärlocher & Kendrick 1974). With their ability to grow at low temperatures (and hence under ice-cover) some of these fungi even help the invertebrate community to survive the winter months in north temperate and boreal streams (Bärlocher & Kendrick 1973).

We are currently investigating what we believe is the parallel ecological role of aero-aquatic fungi in woodland ponds. During this work we have been greatly impressed by the variety of developmental pathways that lead to the bubble-trap propagule: the precise three-dimensional coiling of Helicoon and Helicodendron; the delicate dichotomously branched frameworks of Clathrosphaerina and Candelabrum; the porous hyphal masses of Aegerita; but none of these displays a sophistication to match that of Beverwykella.

This hyphomycetous anamorph has been encountered frequently during our studies of ponds in the Laurel Creek Conservation Area, Waterloo, Ontario. As far as we can ascertain, this is the first record of the fungus for North America. Previous records are from Europe and Japan (Van Beverwijk 1954, Tubaki 1975). Its large, unique propagules (Fig. 4) arise regularly on various kinds of leaf and twig litter collected from the pond and incubated in darkness at 15°C.

This fungus was originally described as Papulaspora pulmonaria v. Bev. (Van Beverwijk 1954). Tubaki (1975) recognized that it was not congeneric with the type species, Papulaspora sepedonioides Preuss (see Weresub & LeClair 1971 for a discussion of Papulaspora and some similar genera), and erected a new genus, Beverwykella Tubaki, to accommodate it.

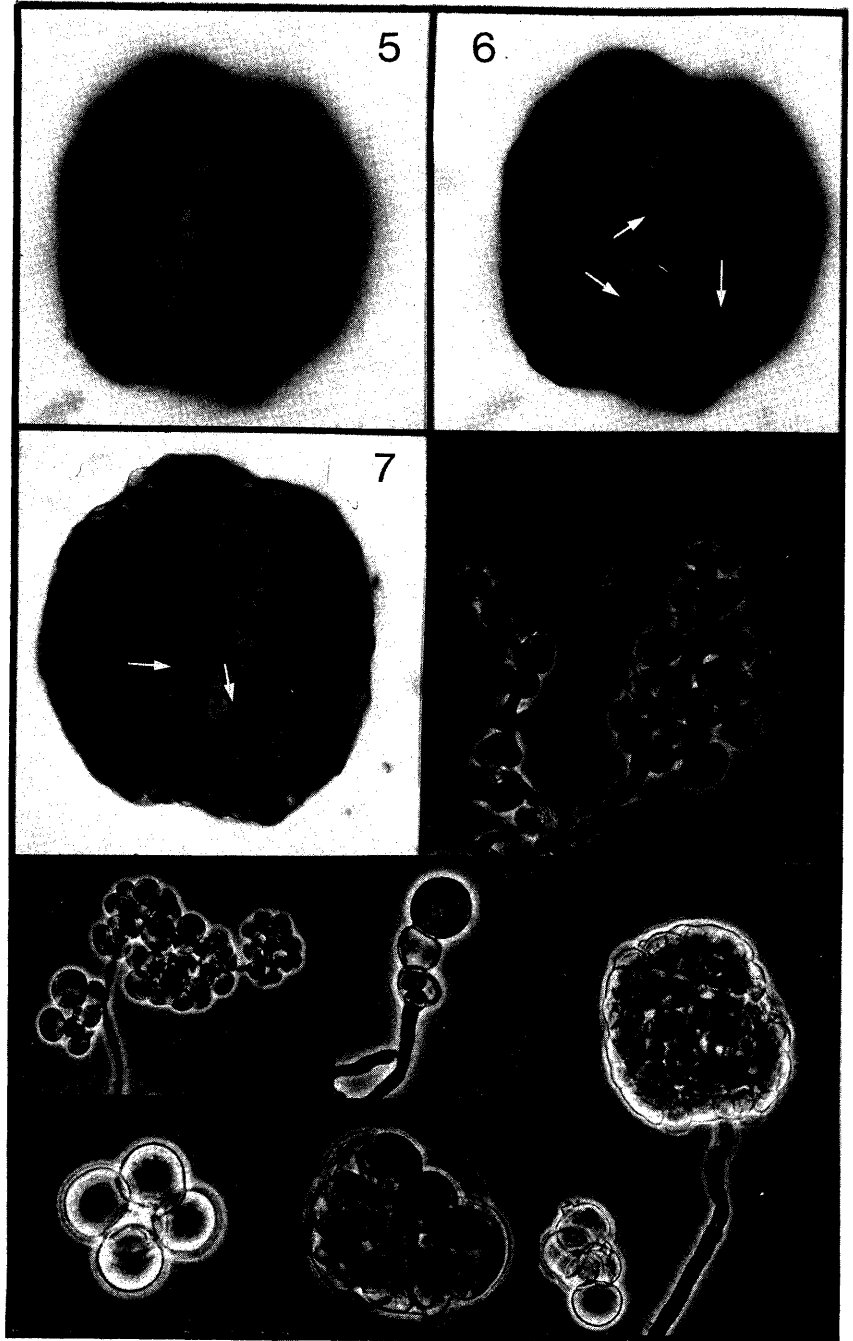
The mycelium of B. pulmonaria grows within natural leaf and twig substrates which are usually submerged in the pond. When the pond dries up, or leaves are kept moist (but not submerged) in dishes in the laboratory,



Figs. 1-4. Beverwykella pulmonaria: Conidium development. 1. Branching fertile hypha 1040X. 2. Swelling outer cells of the branches 1100X. 3. Outer cells in contact with each other forming a complete integument 700X. 4. Mature detached conidium 400X.

septate, hyaline conidiophores emerge from the substrate, pierce the surface film of water, and project into the air. Soon several branches arise from the conidiophore, often in opposite or almost opposite pairs just below each septum. These branches are longer in one plane than in the other. They curve gently upward until they are growing more or less parallel to the main axis; sometimes the curvature continues and the branch grows back toward the apex of the central axis. Then at several points along each branch, but always on the side away from the main axis (abaxial), many small lateral branches develop. These in turn quickly branch at their tips, becoming dichotomous or trichotomous, and the tips of the new branches become swollen (Fig. 1). At this point the whole structure bears some resemblance to young conidiophores of Botrytis or Chromelosporium, in which many synchronous holoblastic conidia are formed around the inflated tips of conidiophore branches. However, this aspect soon changes, as the pear-shaped tips of the branches continue to swell without differentiating into, or producing, conidia (Fig. 2). Soon an emerging symmetry becomes apparent, as each of these independent vesicles, although not growing in perfect synchrony with the others, touches its neighbours and fuses with them, establishing a complete integument around what is clearly a biological balloon (Fig. 3). At maturity all the swollen tip cells have met, have become polygonal as a result of mutual pressure, and have rendered the whole structure airtight (Figs. 4,5). Fig. 3 shows an optical section through a mature conidium. Fig. 4, of its exterior, shows that the finished propagule is not spherical, but disc- or lozenge-shaped (note that all the cells on one surface of the propagule are in focus). Figs. 4 and 5 also show that the propagule, or at least the cells of its integument, become darkly pigmented at maturity. This pigment is widely believed to enhance the waterproofing of cell walls and hence their ability to withstand arid conditions. Although this may sound out-of-place in a pond, the fact is that these propagules are not formed until the gradual drying up of the pond in summer brings the leaves to the water surface, and since

Figs. 5-14. Beverwykella pulmonaria. 5. Surface of propagule 580X. 6-7. Internal structure of propagule. Arrows indicate internal branches and central skeleton 6:610X, 7:590X. 8-14. Abnormal propagules of the fungus developing on artificial substrates. 8:490X, 9:590X, 10:310X, 11:340X, 12:840X, 13:790X, 14:480X.



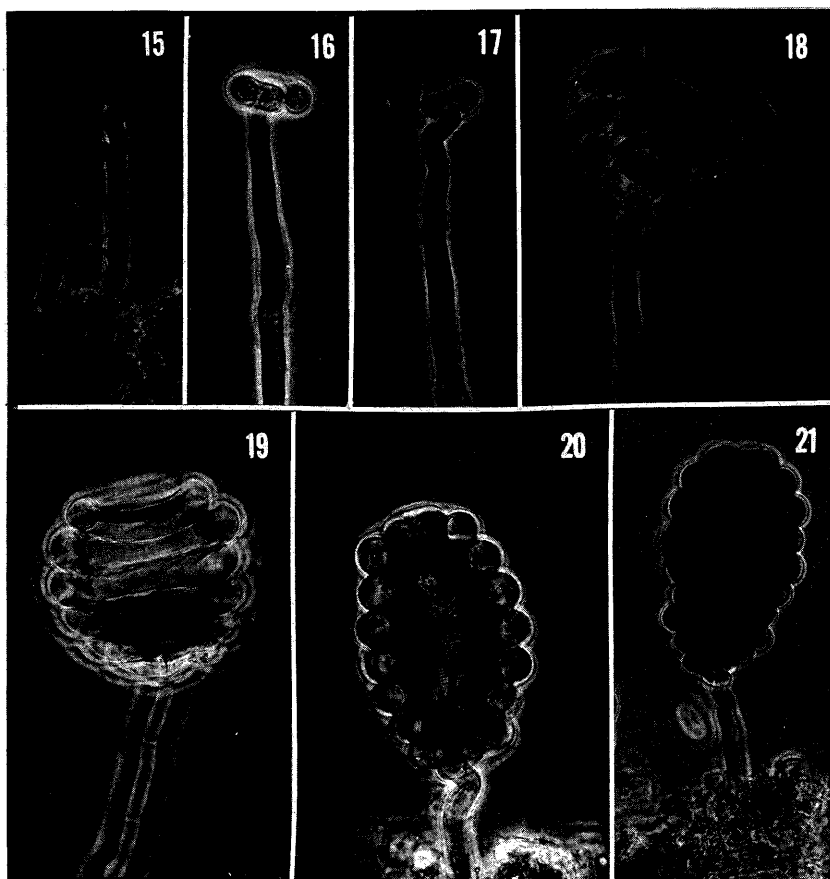
the water level may well recede further, the propagules may be left high and dry for weeks or months before autumn rains fill the pond again.

That is when the propagule fulfills its design potential; it is detached from the single point of attachment by the rising water and floats away. Although mature, pigmented propagules conceal their origins and internal structure fairly well, Figs. 5-7 show how, as one focuses down from the surface 'skin', one can gain some impression of what is so easily visible in the as-yet-unpigmented propagule seen in Fig. 3.

On artificial substrates such as Difco PDA and MA the development of propagules is abnormal. Propagules resembling those developing on natural substrates are uncommon, and when observed they are very small (Fig. 14). Most propagules consist of a main axis bearing lateral dichotomous and trichotomous branchlets with spherical cells at their apices. These branchlets do not, however, develop in the precise manner seen on the natural substrate, but occur irregularly along the main axis, producing individual cells (Fig. 11) or clusters of cells at various levels (Figs. 8-10). The cell aggregations can act as propagules (Figs. 12,13) but clearly lack the elegance and, presumably, the selective value of those formed under natural conditions.

Cancellidium Tubaki (which we have not recorded in our ponds), has propagules which at maturity are superficially similar to, and functionally identical with, those of Beverwykella, but develop in a very different way. As Webster & Descals (1981) show, a number of contiguous branches arising more or less simultaneously from the apex of the conidiophore grow up in a parallel cluster -- a palisade -- around a central space, ultimately converging at the top and producing a hermetically sealed propagule.

Perhaps even more elegant are the hollow helicosporous conidia of Helicoon Morgan, represented in our ponds by H. elegans (Corda) Arnaud and H. ellipticum (Peck) Morgan. In this genus the fertile hypha becomes sinuate, then begins to coil upon itself, always maintaining contact between successive gyres (Figs. 15-17). At first each coil is slightly wider than the one below (Fig. 18); later, successive coils are gradually narrower (Fig. 19), ultimately giving rise to a hollow, airtight,



Figs. 15-21. Helicoon elegans: the development of the propagule. 15. Sinuate fertile hypha 470X. 16-18. Fertile hypha coiling, producing gyres of increasing diameter 16:590X, 17:580X, 18:610X. 19. More advanced stage, upper half of conidium showing gyres of decreasing diameter 520X. 20. Completely enclosed conidium 560X. 21. Mature melanized conidium 460X.

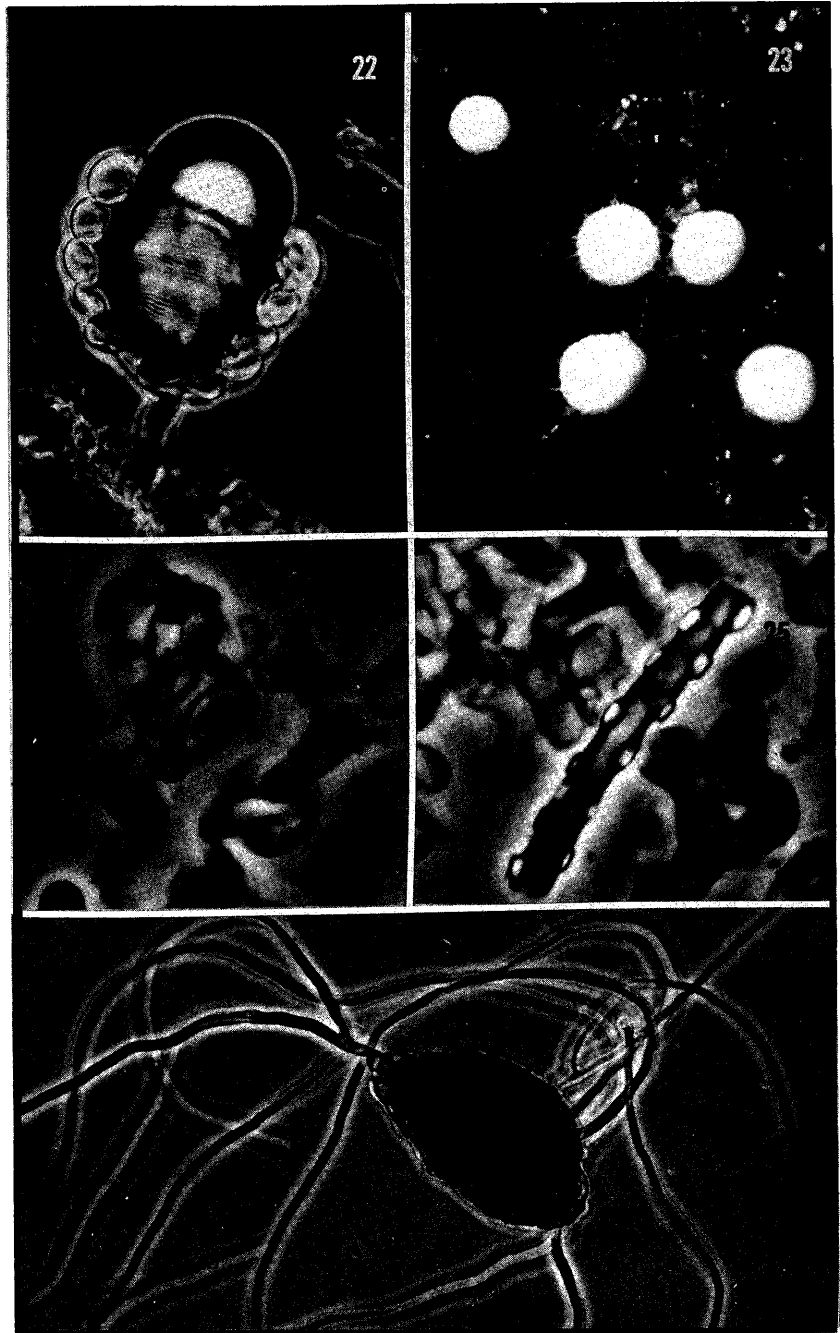
barrel-shaped structure (Figs. 20-21). It is obvious that the finished product will float. Less obvious are the bubble-trapping propensities of half-grown helicospores. Fig. 22 shows that even a partly-completed barrel -- a basket-shaped structure widely open at the upper end -- will trap a bubble as large as that in a mature spore. This may well be a bonus that gives the fungus some advantage if the water rises before the spore is fully developed.

The propagules of Beverwykella, Cancellidium and Helicoon seem to us to constitute a superb example of convergent evolution. Although we do not know the teleomorphs of these fungi (assuming that such exist), we have no reason to suppose that they are closely related. We are clearly looking at three independently evolved structures which are solutions to the same problem. This is a classical example which deserves to be more widely known among biologists.

Helicodendron Peyronel, represented in our ponds by H. trigitziense (Jaap) Linder, H. paradoxum Peyronel, H. luteo-album Glen-Bott, and H. tubulosum (Reiss) Linder, has smaller individual barrel-shaped helicosporous conidia which, however, characteristically give rise to further similar spores, and thus eventually produce branched chains of bubble-traps which presumably split up during the dispersal process. H. tubulosum is one aero-aquatic anamorphic fungus that has been connected to its teleomorph. This has been shown in culture to be Lambertella tubulosa Abdullah & Webster, a member of the Sclerotiniaceae (Abdullah & Webster 1981).

Clathrosphaerina van Beverwijk produces more or less spherical but skeletal propagules: the centre is hollow, and the periphery is sketched in by interconnected hyphae which form an open lattice. Although we have not seen this genus in our ponds, Bärlocher, McKay & Wiggins (1978) record C. zalewskii van Bev. from a pond in Southern Ontario. The teleomorph of this fungus is reported to be Hyaloscypha zalewskii Descals & Webster (Descals & Webster

Fig. 22. Helicoon elegans, a partly developed conidium with air bubble 620X. Figs. 23-25. Aegerita tortuosa. 23. Propagules 480X. 24. Propagule elements with clamps 2150X. 25. Encrusted setose projection 2500X. 26. Beverwykella pulmonaria propagule germinating 410X.



1976). It is clear that this configuration is more economical of resources than the completely sealed propagules of Beverwykella, Cancellidium and Helicoon. It is equally clear that Clathrosphaerina is not evolving toward the condition found in any of those three genera. It represents a fourth distinct pattern.

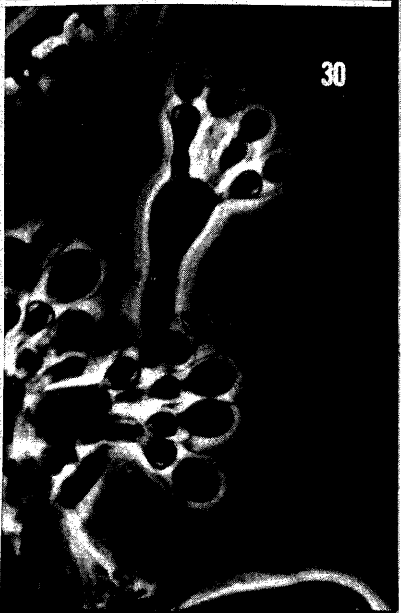
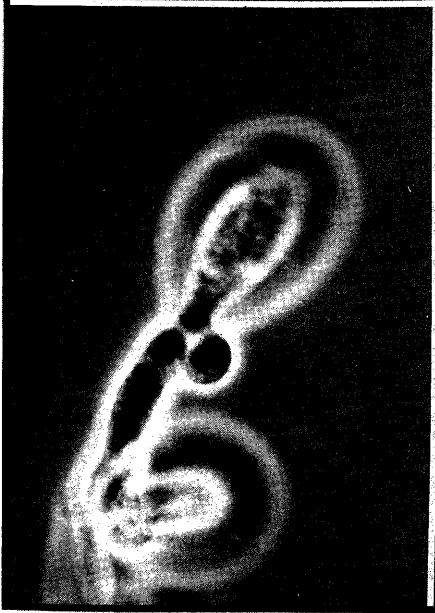
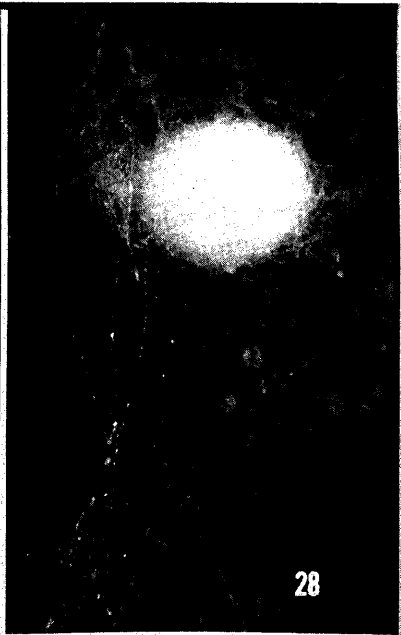
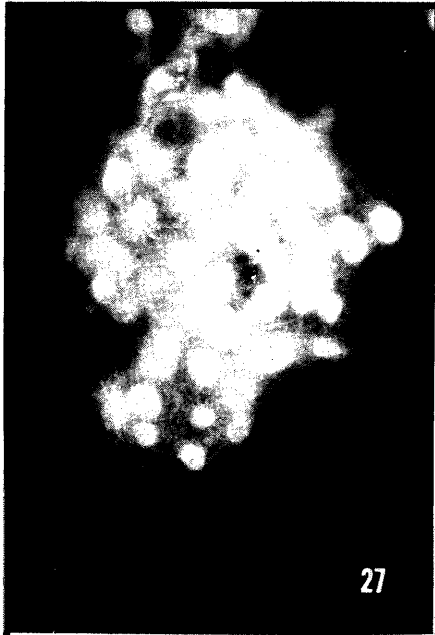
Spirosphaera van Beverwijk represents a lower level of coordination in development than the fungi mentioned so far. S. floriformis van Beverwijk occurs in our ponds, and produces a mass of coiled, interwoven hyphae which traps air incidentally, it would appear, rather than by design. This may represent an earlier stage in the evolution of a true 'bubble-trap': almost like an immature Beverwykella propagule that never seals itself.

Aegerita Persoon, represented in our ponds by A. tortuosa Bourd. & Galz., the anamorph of the basidiomycete, Subulicystidium longisporum (Pat.) Parm. (Jülich 1975) may also be a partially adapted form. The propagule is almost like a condensed miniature colony: hyphae branch repeatedly as they grow away from the centre. The result is a semi-solid, but still relatively buoyant structure (Figs. 23-24).

A. tortuosa also has setose projections with surface encrustations (Fig. 25). We observe that these appear to entrap some air, as do the incurved arms arising from spores of Peyronelina glomerulata Arnaud ex Fisher, Webster & Kane, which also occur in our ponds.

The fossil fungus Pesavis Elsik & Jansonius, described by Elsik & Jansonius (1974) from the upper palaeocene, is suggested to have aero-aquatic affinities since it produced propagules which resemble those of modern aero-aquatic hyphomycetes (Smith & Crane 1979). The propagules consisted of a central cell from which two incurving primary septate arms arose, their tips meeting or overlapping, and forming a complete circle. From the primary arms, inwardly directed secondary hyphae were produced, their tips meeting at the centre of the circle like spokes of a wheel. It is conceivable that air could have been trapped in the empty space between the secondary arms of the propagule.

Figs. 27-30. Limnoperdon incarnatum. 27. Cluster of fruit bodies 16X. 28. Individual basidioma with loose hyphae 60X. 29. Clamped hypha 1850X. 30. Basidia with basidiospores 1850X.



All our examples of aero-aquatic fungi so far have been hyphomycetous anamorphs; but we have also encountered one aero-aquatic teleomorph: the recently described Limnoperdon incarnatum Escobar, in which the basidioma itself is a small hollow sphere. This unique gasteromycete was originally discovered floating on water in a marsh near Lake Union, Seattle, Washington (Escobar, McCabe & Harpel 1976). We know of only one other report in the literature: Tubaki (1977) found L. incarnatum on wood blocks in brackish water in Japan. We have collected this fungus (the first record for Canada, and for Eastern North America), on wood of Acer saccharum Marsh, and Fagus grandifolia Ehrh., which we had experimentally immersed in one of our ponds for six months. Perhaps significantly, the fungus did not fruit on wood of Pinus strobus L. immersed for the same period. Fig. 27 shows a cluster of basidiomata. The loosely aggregated, rather hydrophobic hyphae of the outer wall can be seen in Fig. 28. Figs. 29 and 30 show characteristic internal elements -- clamped hyphae, and typical gasteromycetous basidia with blunt sterigmata and symmetrically attached basidiospores. It is intriguing to note that the lycoperdales have evolved two distinct ways of using water as an agent of passive spore dispersal.

We have shown how the propagules of several aero-aquatic fungi develop. All are passively dispersed by floating away from their point of origin. Successful dispersal in fungi may require movement only over very short distances -- just far enough, perhaps, to infect a nearby newly fallen leaf. Both our direct observations and our sampling indicate that these propagules, once released, are to be found almost exclusively at the surface of the pond: they do not sink. Yet one of the ecological assets of the aero-aquatic fungi is the ability to withstand virtually anaerobic conditions in substrates lying at the bottom of ponds. How do they get there? We know that some propagules are eaten by such animals as frog tadpoles and caddisfly larvae, but we do not yet know if they can survive passage through the gut. Barron (1961) provided information on the strategy adopted by one aero-aquatic fungus, Helicodencron tubulosum (Riess) Linder var. phialosporum Barron, to ensure delivery of inoculum to the benthic substrate. The helicosporeous propagule is essentially a long, coiled hypha. At some

point subsequent to liberation, this hypha disarticulates at the septa, converting a single floating propagule into a rain of smaller, less buoyant fragments, each presumably capable of initiating a new mycelium in appropriate surroundings.

What is the fate of the other large propagules we have described? We have not observed any comparable fragmentation in Beverwykella. We have seen Beverwykella propagules germinating from many cells simultaneously with germ tubes radiating in all directions (Fig. 26). Is this the norm, or is there some as yet undiscovered mechanism for fragmentation? Again it seems that the fungi may have evolved along two separate paths, one toward a multiplicity of small 'part-spores', one toward large propagules with correspondingly large food reserves and exploratory capacity.

The major influx of new substrate to our ponds takes the form of autumnally shed tree leaves. These, due to their large surface area to weight ratio, tend to float on the surface of the pond for some time before becoming waterlogged and sinking. During this period they may well encounter the floating propagules of the aero-aquatic fungi. Surface tension effects would tend to hold the two together while inoculation occurred. Preliminary experiments show that leaf extracts stimulate germination of Beverwykella propagules. The buoyancy of new substrate could be the major evolutionary stimulus leading to the evolution of the aero-aquatic propagule.

Since exploration for aero-aquatic fungi is still in its infancy, we expect further designs of bubble-trap propagule to be discovered. We commend the genre to mycologists as eye-catching examples of convergent evolution.

#### ACKNOWLEDGMENTS

We thank Dr. T. Matsushima and Dr. T. R. Nag Raj for reviewing this paper. Financial support in the form of a Natural Sciences & Engineering Research Council of Canada Operating Grant to Bryce Kendrick is gratefully acknowledged.

## LITERATURE CITED

- Abdullah, S.K. and Webster, J. 1981. Lambertella tubulosa sp.nov., Teleomorph of Helicodendron tubulosum. Trans. Brit. Mycol. Soc. 76: 261-263.
- Bärlocher, F. 1973. Hyphomycetes as intermediaries of energy flow in streams. Ph.D. thesis. University of Waterloo, 154 pp.
- Bärlocher, F. and Kendrick, B. 1974. Dynamics of the fungal population on leaves in a stream. J. Ecol. 62(3) 761-791.
- Bärlocher, F., Mackay, R.J. and Wiggins, G.B. 1978. Detritus processing in a temporary vernal pool in Southern Ontario. Arch. Hydrobiol. 81: 269-295.
- Barron, G.L. 1961. Studies on species of Oidiendron, Helicodendron and Stachybotrys from soil. Can. J. Bot. 39: 1563-1571.
- Elsik, C.W. and Jansonius, J. 1974. New genera of Paleogene fungal spores. Can. J. Bot. 52: 953-959.
- Escobar, G.A., McCabe, D.E. and Harpel, C.W. 1976. Limnoperdon, a floating Gasteromycete isolated from marshes. Mycologia 68: 874-880.
- Jülich, W. 1975. Studien an Cystidien. 1. Subulicystidium Parm. Persoonia 8: 187-190.
- Smith, P.H. and Crane, P.R. 1979. Fungal spores of the genus Pesavis from the Lower Tertiary of Britain. Bot. J. Linn. Soc. 79: 243-248.
- Tubaki, K. 1975. Notes on the Japanese Hyphomycetes VI. Candelabrum and Beverwykella gen. nov. Trans. Mycol. Soc. Japan. 16: 132-140.
- Tubaki, K. 1977. Brackish water fungi and their relationships to marine fungi. IMC2 Abstracts: 681.
- Van Beverwijk, A. 1954. Three new fungi: Helicoon pluriseptatum n.sp., Papulaspora pulmonaria n.sp. and Tricellula inaequalis n.gen., n.sp. Anton. van Leeuwenh. 20: 1-16.
- Webster, J. & Descals, H. 1981. Morphology, Distribution and Ecology of Conidial Fungi in Freshwater Habitats. pp. 295-355. In "Biology of Conidial Fungi". G.T. Cole & B. Kendrick eds. Academic Press, New York.
- Weresub, L.K. and LeClair, P.M. 1971. On Papulaspora and the bulbiferous basidiomycetes Burgoa and Minimedusa. Can. J. Bot. 49: 2203-2213.