Rhizomorphs and perithecial stromata of *Podosordaria tulasnei* (Xylariaceae)

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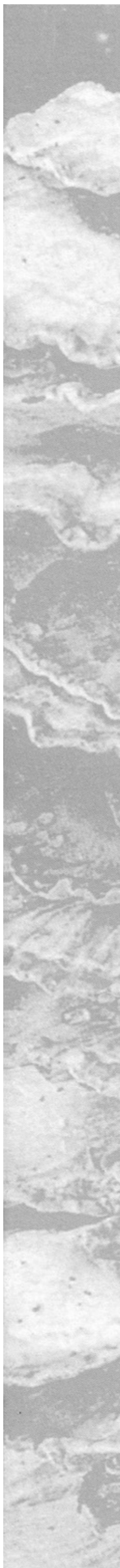


After incubation on moist filter paper, *Podosordaria tulasnei* frequently formed sterile stromata on rabbit pellets collected from the field. These developed extensive submerged rhizomorph systems when pellets were incubated on sand, but further differentiation and sexual reproduction occurred only when pellets bearing such structures were buried in soil. The present report describes the development of subterranean rhizomorphs, which are rare among ascomycetes, and the production of mature perithecial stromata. *Podosordaria tulasnei* is a true coprophilous fungus but, like some other members of the genus, has a high capacity to explore the surrounding soil. No anamorphic state was found in our material or in pure culture.

Keywords: coprophilous fungi, perithecial stroma, *Podosordaria tulasnei,* rhizomorph, Xylariaceae.

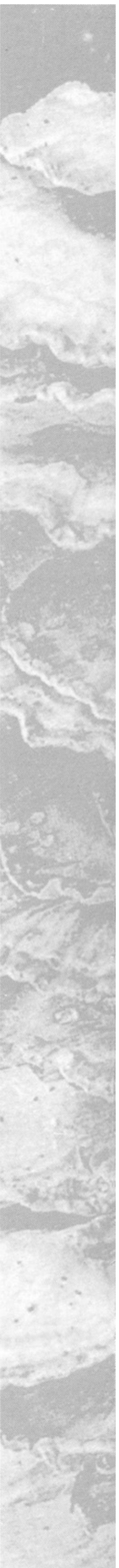
A well-defined succession of fruiting bodies of coprophilous fungi can be observed when fresh herbivore dung is incubated on moist filter paper in the light (see Dix & Webster, 1995). Rabbit pellets are particularly suitable for such studies (Kuthubutheen & Webster, 1986*a,b*). In the course of our investigations of the coprophilous mycota on rabbit pellets, along with many familiar species we observed black, pointed structures resembling sterile stromata of the lignicolous *Xylaria hypoxylon* (L.) Grev. However, under the conditions employed, these structures remained sterile even after many weeks of incubation. The fruiting of certain coprophilous fungi such as the bird's nest fungus, *Cyathus stercoreus* (Schwein) De Toni, is initiated when the colonized substratum is buried in soil (Webster & Weber, 1997), and we decided to incubate rabbit pellets with immature stromata under similar conditions. This stimulated the development and maturation of perithecial stromata, allowing the fungus to be identified as *Podosordaria tulasnei* (Nitschke) Dennis (Dennis, 1968; Krug & Jeng, 1995).

Development of rhizomorphs

Rabbit pellets from a wide range of habitats and locations were placed in glass crystallising dishes on a layer of beach sand (2 - 3 cm deep) to which a minimal volume of water was added. The dishes were covered with transparent lids and incubated in the light at room temperature (about 20°C) or in a garden shed (10 - 15°C) for several weeks. The material was slightly moistened approximately once every week with tap water from a hand sprayer.

Within 10 - 14 days, several pellets developed mycelial cords which extended horizontally to other pellets or burrowed into the sand, often growing along the inside surface of the glass dish (Fig 1). The tip of a cord was pointed and consisted of appressed, forward-pointing, white blunt hairs (3 - 4 µm diam). Within 5 mm of the tip these hairs gave way to a greyish-brown or black layer of *textura prismatica* consisting of dark-walled, often hexagonal cells (20 - 30 x 8 - 15 µm) which were elongated at right angles to the long axis of the cord. These cells made up a melanized surface rind which was only one cell layer deep. Beneath this was a medulla consisting of rope-like aggregates of intertwined, thick-walled hyaline cells (5 - 7 µm diam) with very narrow lumina (Fig 2). These medullary hyphae were of uniform width throughout the rhizomorph.

Below the sand surface, rhizomorphs appressed to the glass walls of the dish were wider (1 - 2 mm), flattened, branched, anastomosing, and occasionally running in parallel



strands. At the base of the dish, such dark flattened strands were often 4 - 5 mm wide. Close to the glass surface, the dark rind was sometimes incomplete so that the white medulla was exposed. Submerged rhizomorphs extended beneath the sand for several cm. At certain points they grew to the surface where their tips either appeared as white-tipped mycelial cords as described above, or differentiated into perithecial stromata under suitable conditions.

Development of perithecial stromata

The formation of mature perithecial stromata was stimulated when pellets with rhizomorphs were removed from the sand-filled glass dishes and immersed just beneath the surface of garden soil in shallow trays. Within several weeks, the rhizomorph tips had grown towards the soil surface where they formed knobbly swellings which contained perithecia with depressed circular ostioles, visible with a hand lens. These perithecial stromata were of the same colour as the rhizomorph rind but they were very variable in form, sometimes branched, often cylindrical or bulbous and tapering to a pointed tip (Fig 3). Occasionally, a stroma formed a flattened black rosette about 3 - 10 mm diam, bearing clusters of swollen perithecia. From the level of soil surface down to a depth of approximately 2 cm, these stromata were clothed with dark hairs which extended into the soil, binding particles of soil, humus and sand (Fig 3).

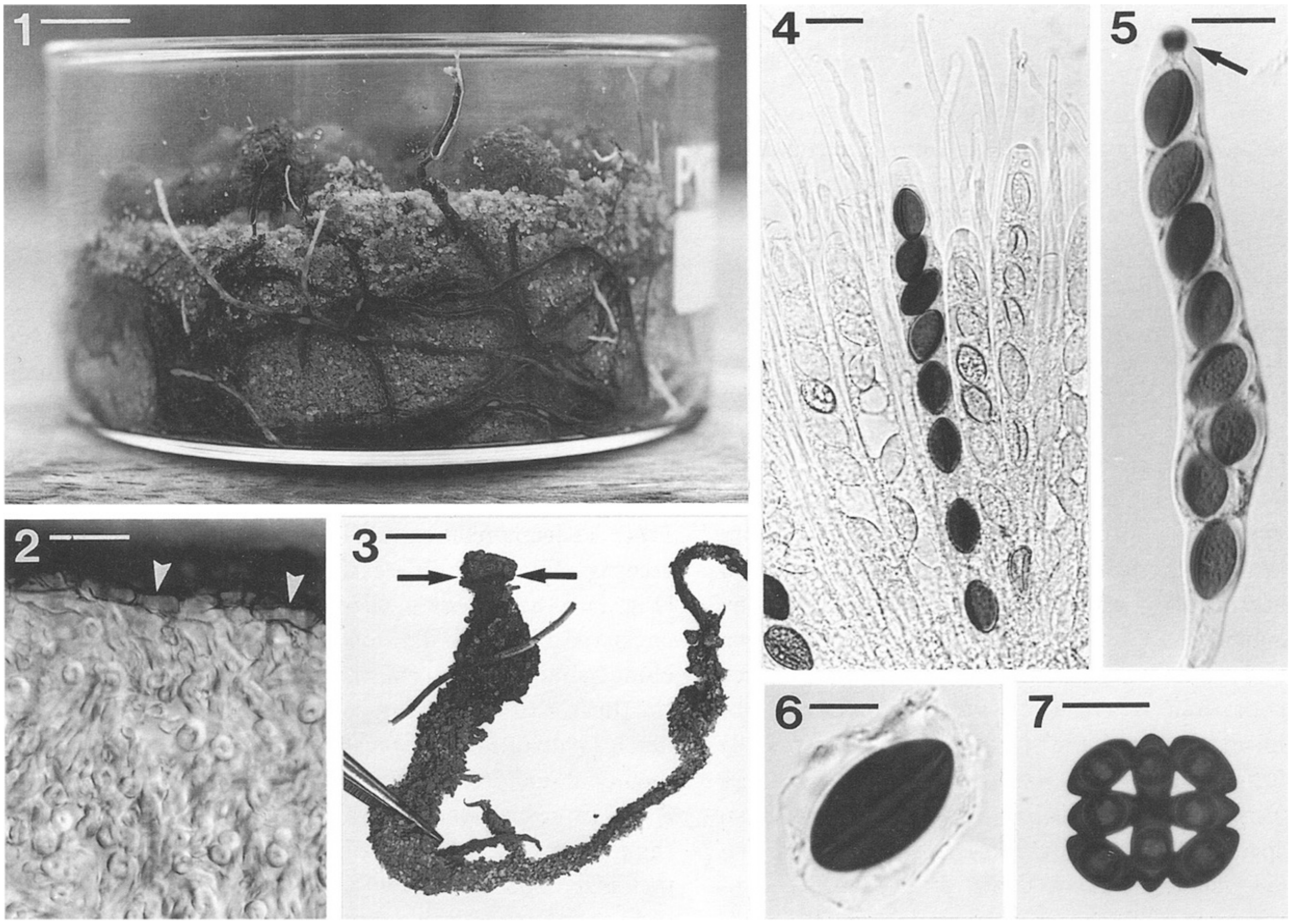
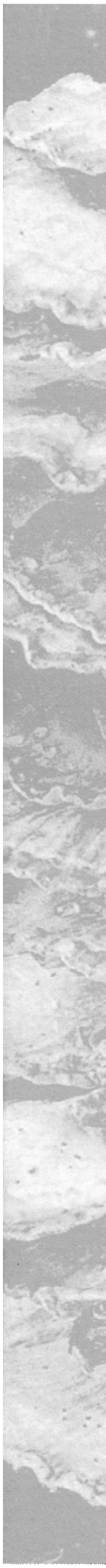


Fig 1 Formation of submerged rhizomorphs of *P. tulasnei* following incubation of rabbit pellets on the surface of moist sand. Bar = 1 cm. Fig 2 Transverse section through a rhizomorph. The cortex consisting of a single layer of melanized cells (arrowheads) encloses a medulla of hyaline but thick-walled hyphae which are tightly packed. Bar = 25 μm. Fig 3 Rhizomorph which has developed a perithecial stroma. The soil level is indicated by the arrows. Immediately below the soil level, a dense hyphal meshwork has bound soil particles and plant debris. Bar = 5 mm. Fig 4 Squash preparation of maturing and immature asci. Note the long, pointed paraphyses which extend beyond the ascus tips. Bar = 25 μm. Fig 5 Ascus dissected from a perithecium and mounted in dilute iodine. Note the stained apical apparatus (arrow). Each ascospore is surrounded by a gelatinous epispore. The germ slits are visible in some spores. Bar = 25 μm. Fig 6 Single ascospore removed from a slightly immature ascus. The epispore and longitudinal germ slit are clearly visible. Bar = 10 μm. Fig 7 A cluster of 8 ascospores discharged from a perithecial stroma and mounted dry. Note the occurrence of a de Bary bubble in the centre of each spore. Bar = 25 μm.



The perithecia of *P. tulasnei* required a further four weeks to form mature asci (Fig 4). In fresh material, the asci measured 165 - 250 x 20 - 22 µm, appreciably longer than the values of 160 x 20 µm given by Dennis (1968) who might have based his measurements on dry material. The ascus tip contained a massive apical apparatus which stained deep blue with iodine (Fig 5). The black ascospores were elliptic-fusoid, resembling a rugby football in shape. They had a longitudinal hyaline germ slit running along the whole length of the spore (Fig 6). Each ascospore was surrounded by a mucilaginous epispore (Fig 6) which often held several spores together during discharge; 8-spored projectiles were most frequently obtained (Fig 7).

Upon drying, the cytoplasm within each ascospore cavitated and a single spherical air bubble became visible (Fig 7). These are called de Bary bubbles because they were first described by him (de Bary, 1887). Despite their presence, the spores remained viable. On re-wetting, the de Bary bubbles immediately disappeared. Following incubation in saturated pancreatin at pH 9.0 for 5 h at 37°C (Harper & Webster, 1964), the ascospores germinated readily on dung extract agar or yeast extract agar. The body of the spore swelled, causing the germ-slit to widen, and two germ tubes developed, one from each end of the spore. Although Krug & Jeng (1995) state, without giving a reference, that an anamorph has been reported, we failed to observe conidia either on stromata developing from rhizomorphs or on mycelium developing in pure cultures derived from germinating ascospores. The absence of an anamorph in *P. tulasnei* raises the question as to whether this taxon is correctly classified in the genus *Podosordaria*. According to Rogers *et al.* (1998), the type species of the genus, *P. mexicana* Ellis & Holw. *apud* Holw., has an anamorph 'more or less assignable to *Geniculosporium*'. In contrast, species of the genus *Poronia* have disarticulating conidial elements assigned to *Lindquistia*, and Rogers *et al.* (1998) state that they firmly accept in *Podosordaria* and *Poronia* only those taxa with anamorphs known by culturing. They do not offer an opinion on the classification of similar fungi lacking an anamorphic state.

Ecology of *Podosordaria tulasnei*

Rhizomorphs and mycelial cords are well-known in basidiomycetes and have been described in great detail (Townsend, 1954; Butler, 1966; Thompson, 1984). However, it is less widely appreciated that they also occur among ascomycetes, even though they were noticed as early as 1863 by the Tulasne brothers (see Tulasne & Tulasne, 1931) whose description of the subterranean cords of *Xylaria pedunculata* Fr. (= *P. pedunculata* (S. F. Gray) Dennis) closely matches that given here for *P. tulasnei*. In fact, all three *Podosordaria* spp. recorded from Britain, *i.e. P. tulasnei, P. pedunculata* and *P. leporina* (Ellis & Everhart) Dennis (Richardson & Watling, 1997), produce rhizomorphs (Dennis, 1968; Ellis & Ellis, 1998). It has been suggested that these rhizomorphs serve to produce stalked perithecial stromata in order to enhance ascospore dispersal (Rogers, 1979). However, this is certainly not the situation in *P. tulasnei* in which perithecia are produced directly at the soil surface (Fig 3). At least in this species, therefore, the rhizomorphs may represent an adaptation for reaching the surface from dung which has been buried *e.g.* by blown sand or the burrowing activities of rabbits. Further, *Podosordaria* spp. are thought to be adapted to dry conditions (Whalley, 1996), and the strong cell walls in the hyphae making up the rhizomorph of *P. tulasnei* may play a role in water conservation.

We were intrigued by the density of hyphae ramifying into the soil especially in regions directly underneath perithecial stromata, a characteristic feature of *P. tulasnei* (Krug & Cain, 1974). These hyphae may assist in anchoring the stroma. They were seen to penetrate humus particles and fragments of woody debris, and we believe that nutrients and/or water may be derived from the soil in this way. Similarly, Dennis (1968) noted the occurrence of *P. pedunculata* 'deeply rooting in dung or richly manured soil'. Whilst clearly being coprophilous, these *Podosordaria* spp. also possess a high capacity for colonization of soil in the vicinity, which is unusual for coprophilous fungi. More commonly the reverse situation holds, *i.e.* dung being colonized by soil-borne fungi.

The frequency with which *P. tulasnei* rhizomorphs have appeared in collections of rabbit dung incubated under appropriate conditions indicates that this fungus must be rather more common than currently recorded. Close search around established rabbit latrines in the field should reveal perithecial stromata arising at ground level from subterranean rhizomorphs.

References

Butler, G. M. (1966) Vegetative structures. In *The Fungi – An Advanced Treatise* **2** (edited by Ainsworth, G. C. & Sussman, A. S.), pp. 83-112. Academic Press: New York, London.

de Bary, A. (1887) *Comparative Morphology and Biology of the Fungi* (English translation). Clarendon Press: Oxford.

Dennis, R. W. G. (1968) *British Ascomycetes.* Cramer Verlag: Lehre, Germany.

Dix, N. J. & Webster, J. (1995) *Fungal Ecology.* Chapman & Hall: London.

Ellis, M. B. & Ellis, J. P. (1998) *Microfungi on Miscellaneous Substrates* (enlarged edition). Richmond Publishing Co.: Slough.

Harper, J. & Webster, J. (1964) An experimental analysis of the coprophilous fungus succession. *Transactions of the British Mycological Society* **47**: 511-530.

Krug, J. C. & Cain, R. (1974) A preliminary treatment of the genus *Podosordaria. Canadian Journal of Botany* **52**: 589-605.

Krug, J. C. & Jeng, R. S. (1995) A new coprophilous species of *Podosordaria* from Venezuela. *Canadian Journal of Botany* **73**: 65-69.

Kuthubutheen, A. J. & Webster, J. (1986*a*) Water availability and the coprophilous fungus succession. *Transactions of the British Mycological Society* **86**: 63-76.

Kuthubutheen, A. J. & Webster, J. (1986*b*) Effects of water availability on germination, growth and sporulation of coprophilous fungi. *Transactions of the British Mycological Society* **86**: 77-91.

Richardson, M. J. & Watling, R. (1997) Keys to fungi on dung. British Mycological Society: Stourbridge.

Rogers, J. D. (1979) The Xylariaceae: systematic, biological and evolutionary aspects. *Mycologia* **71**: 1-42.

Rogers, J. D., Ju, Y.-M. & San Martin, F. (1998) *Podosordaria*: a redefinition based on cultural studies of the type species, *P. mexicana*, and two new species. *Mycotaxon* **67**: 61-72.

Thompson, W. (1984) Distribution, development and functioning of mycelial cord systems of decomposer basidiomycetes of the deciduous woodland floor. In *The Ecology and Physiology of the Fungal Mycelium. British Mycological Society Symposium* **8** (edited by Jennings, D. H. & Rayner, A. D. M.), pp. 185-214. Cambridge University Press: Cambridge.

Townsend, B. B. (1954) Morphology and development of fungal rhizomorphs. *Transactions of the British Mycological Society* **37**: 222-233.

Tulasne, L. R. & Tulasne, C. (1931) *Selecta Fungorum Carpologia* **II**. (English translation by Grove, W. B.). Clarendon Press: Oxford.

Webster, J. & Weber, R. W. S. (1997) Teaching techniques for mycology: 1. The bird's nest fungus, *Cyathus stercoreus. Mycologist* **11**: 103-105.

Whalley, A. J. S. (1996) The xylariaceous way of life. *Mycological Research* **100**: 897-922.

From the Editors – at the hyphal apex

- We welcome the first issue of *Field Mycology* which appeared in January. The addition of this new publication will mean some adjustments in distribution of material between it, *Mycologist* and *Mycological Research*. Book reviews, for example, will appear in all three but will be allocated according to their appropriateness. The *Mycologist* will continue as the Society's mainstream journal for general and scientific articles, new British Records, archival foray reports, book reviews and other established features.

- For reasons of ill health, Susan Isaac, for the first time after no less than 27 consecutive issues, has been unable to provide her knowledgeable 'Mycology Answers' column. We wish her a speedy recovery and look forward to publishing her next contribution in the near future.

- We have had an urgent request from Roland Fox for a good photograph of *Clematis* wilt caused by *Ascochyta clematidina,* to illustrate one of his 'Fungal Foes' items. The BMS slide collection also has a great need for high quality transparencies of plant diseases which, in turn, could be variously reproduced in the *Mycologist.* If you have slides you would like to contribute, please send them either to us or to Gill Butterfill at Kew. We can also have a copy made if you wish to retain the original.

- This issue sees the start (p. 6) of a new series on 'Useful Fungi of the World' by David Pegler. Future issues will include reports on Mu-Erh, Manna, Ling-zhi, and Stone fungus and fungus stones.

GH/RTM