Fluorescence Microscopy for the Observation of Nematophagous Fungi inside Soil

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Fluorochroming of soil samples makes it possible to study soil fungi in their natural habitat. This is demonstrated with some nematophagous (predacious) fungi.

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Fluorescence techniques have become an effective tool in microscopy. With the help of sophisticated equipment and an increasing number of fluorochromes, it is now possible to observe and differentiate a range of materials, microorganisms, electric, water or redox potentials, and even living and dead cells or tissues. Most of the fluorochromes also allow the unrestricted observation of living cells. Fluorescence microscopy has now been successfully adapted to observe soil-dwelling fungi in situ (Jensen, 1994; Jensen & Lysek, 1991; Saxena & Lysek, 1993). This has been made possible by the use of specific fluorochromes such as fluorescein-di-acetate (FDA), which stains actively metabolizing cells or hyphae, and calcofluor white, which marks the hyphal walls. Here, we present the results of the observation of soil-dwelling, predacious (mainly nematophagous) fungi. This ecological group can in part be well characterized by the morphology of their spores (Monacrosporium- or Arthrobotrys-type) and also by the presence of specific capture organs, which are often associated with the remains of captured nematodes. To give a better overview and to allow a comparison of fluorescence and conventional microscopical images, these structures are given as schemes in Fig 1. In addition Fig 2 shows a scanning electron microscopic view of the typical capture organ of Arthrobotrys oligospora Fresenius, the

![Fig 1 Schematics of the main structures of nematophagous fungi which are frequently found by fluorescence microscopy of soil samples: a) Sporophore and spores of Arthrobotrys; b) sporophore with terminal conidium of Monacrosporium; c), d), e) sticky branches and f) connected sticky branches of Monacrosporium (the glue is given by the dots); g) three-dimensional sticky network of Arthrobotrys (glue not given); h) and i) three-celled ring trap of Arthrobotrys in i) constricted. Bar represents 20 µm.](image1)

![Fig 2. SEM of a trap (three-dimensional sticky network) of Arthrobotrys oligospora. Bar represents 100 µm.](image2)
nematophagous hyphomycete on which most of our experiments were based and which is common in many types of soils (Nordbring-Hertz, 1988).

Materials and methods

Soils
We used sterilized (autoclaved) compost to avoid any effect of soil fungistasis and inoculated the fungi from pre-cultures on malt extract agar plates. Incubation was done in petri dishes at room temperature.

Fluorochroming
For this study, we used two stains, as follows. Fluorescein-di-acetate (FDA): this substance itself does not give any fluorescence; rather it is taken up by actively metabolizing cells or hyphae, where it is hydrolyzed enzymatically to yield free fluorescein, a true fluorochrome. Thus, only living and actively metabolizing microorganisms become visible when stained with FDA (Söderstrom & Erland, 1986; Corren et al., 1986; Jensen, 1994).

For the experiments a stock solution was prepared from 500 mg FDA dissolved in 100 ml acetone and stored at -18°C. A working solution was prepared by diluting 0.1 ml of the stock in 5 ml 60 mM phosphate buffer pH 6.88. Since it degraded very rapidly, the working solution was freshly prepared for every observation. It was mixed into the soil and microscopic observation started immediately; images were visible one minute after preparation. Due to the rapid photofading of fluorescein, staining lasted not longer than 20 minutes.

Calcofluor White: this stain (correct name Calcofluor white M2R new) is an optical brightener with a high affinity to β-1, 4-glucans such cellulose or chitin. It is thus specific for hyphal walls, and does not differentiate between living and dead tissue. Calcofluor stains many types of fungal structures, for example spores or fruiting body initials (Van Sengbusch, 1988; Cohen et al., 1987). A stock solution was prepared from 50 mg calcofluor dissolved

Fig 3 Typical sticky network of *A. oligospora* with vegetative hyphae and the digestive hyphae filling the corpse of a captured nematode. The soil particles are just visible. Staining: Calcofluor W. Bar represents 100 μm.

Fig 4 (left) Hyphae and sticky networks of *A. oligospora* in between soil particles. Calcofluor W staining – additional lateral white light is used to illuminate the soil particles. Bar represents 100 μm.
in 5 ml buffer (TRIS pH 9.0). The working solution consisted of 0.1 ml of stock solution in 9.9 ml buffer. Both solutions are stable and were stored in the dark at room temperature. For microscopic observations, the working solution was simply mixed with the soil to be investigated immediately prior to observation. The fluorescence was very stable; for example, mounts kept in a wet chamber could be studied for at least 24 h after staining.

**Treatment of soil samples**

In order to preserve the exact localization of hyphae, traps, or other specific structures and surrounding soil particles, the soil samples had to be treated very carefully. Soil samples were cut vertical from the surface to the bottom of the petri dish with cover slips to get a number of soil slides with a depth of 2.5 mm in average. Each soil slide was placed on one cover slip. Staining and manipulation were done with much care to avoid distortion of the native structure.
Observation was best through the cover slip in the hanging drop technique (Jensen, 1994; Jensen & Lysek, 1995).

**Microscopic and photographic equipment**

Observations were made using a Leitz Dialux 20 research microscope equipped with an (epi-) fluorescence illuminator PLOEMOPAK 2.4 supplied with a mercury lamp HBO 50 W (Osram). The filter blocks used were A, E3, 13 and N2 (Jensen, 1994). Photographs were taken with an MPS 11 microscope camera connected with an MPS 15 seminomat (Wild). Kodak negative films of 100, 200, 400 and 1000 ASA were used.

SEM-photos were taken using a Cambridge stereoscans 90 B 100/SE scanning electron microscope; for details see Neumeister (1994).

**Results and discussion**

Fig 3 shows calcofluor-stained fluorescence of a typical three-dimensional sticky network of *A. oligospora* hyphae between soil particles. The hyphae within the sticky network of the trap as well as the digestive hyphae filling the empty corpse of a captured nematode are clearly visible.

Due to the Calcofluor W staining, all hyphae give a similar fluorescence – a distinction of active or inactive hyphae is not possible. However, it may be assumed, at this state of degradation, that the digestive hyphae are only present as empty tubes of hyphal walls.

Fig 4 shows hyphae and capture organs of *A. oligospora* occupying a larger area; the clusters of traps found often in cultures are clearly visible, in addition to the soil particles which have been illuminated by additional lateral white light. Another type of capture organ, namely the short sticky branches of *Monacrosporium cionopagum* (Drechsler) is shown in Fig 5. These comparatively simple structures are typical for various *Monacrosporium*-species and can fuse to give larger structures (see Fig 5b). Such capture organs are typically intensively metabolizing and produce the glue to hold trapped nematodes as well as nematode-digesting enzymes.

Another group of capture organs, constricting rings, are shown in Fig 6. These act by very rapid inflation, triggered when a nematode touches the inside of the ring. The three cells enlarge their volume such that the ring is completely filled – the nematode is quickly ensnared and cannot escape. Fig 6 also shows a recently captured nematode, but due to the selectiveness of calcofluor W for glucans, it is stained poorly and hence is only visible as a shadow.

Fig 7 shows an example of double staining of *A. oligospora* with FDA and calcofluor. If the filters are chosen adequately, the stains highlight different cellular components: FDA (Fig 7a) stains actively metabolizing areas such as capture organs, while calcofluor W makes the entire complex visible.

Fluorescence microscopy can be used for the direct observation of a wide range of soil-dwelling fungi, not just the nematophagous fungi studied for this article. Staining of key taxonomic features (for example the developing conidiophores and spores of *A. oligospora*) make it an ideal tool for the rapid identification of species of fungi that grow actively in soil, and which would be extremely difficult to observe by conventional microscopy. The technique can also be used to study fungal growth in soils over time, which exhibited periodic effects (Jensen et al., 1997).

We expect fluorescence microscopy to play an increasing role in environmental mycology. Recently, for example, it has been used to study the colonisation of soils contaminated with urban pollutants (Neumeister et al., 1997) and to study the occurrence of fungi in the ventilation systems of buildings (Neumeister et al., 1996).

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**References**


Jensen, C. & Lysek, G. (1986) Fluorescence microscopy of fungi in native soil - improvements by additional sub-
Cookery Corner

SADDLED CHEESE

Until recently I had not eaten the 'Dryad's saddle' - *Polyporus aquosus*. Most books do not sing its praises but I have discovered that it is delicious. It has to be fresh and so young it's almost 'new born'! This recipe serves one and is simple and quick to prepare.

Ingredients

1 small round goats cheese
Olive oil for marinade
Fresh or dried herbs of your choice
A few rocket leaves
Salad dressing of your choice
Thin slices of Dryad's saddle
Oil to deep fry

Method

Marinate the goats cheese in olive oil and the herbs. I particularly like parsley, thyme and bay. You could also add crushed garlic, lemon zest or anything you fancy. This is better done overnight but a minimum of four hours should be sufficient.

Dress the rocket, or salad leaves could be substituted, and place onto a plate.

Bake the cheese in a moderate oven for 5-10 minutes until soft. Deep fry the slices of Dryad's saddle for a few minutes; do not cook for too long or they may become bitter. Place the cheese on the leaves and sprinkle the Dryad's saddle over the top.

Diana Bateman

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Volvariella succincta

Editor's note: Profiles of Fungi will continue in the November issue with No 97 Volvariella succincta, held over from the two other species of that genus which appear in this issue. Although it is a fungus which seems to be recorded rather infrequently, it has been illustrated in two short items in recent issues of the Mycologist. It is of particular interest as a mycoparasite.

Further correspondence is now in hand, together with a short summary paper by Sheila Wells, to be published in November, on the occurrence and distribution of this unusual species. Any other information will be welcome and could contribute to the debate, if received very soon!

G. Hadley