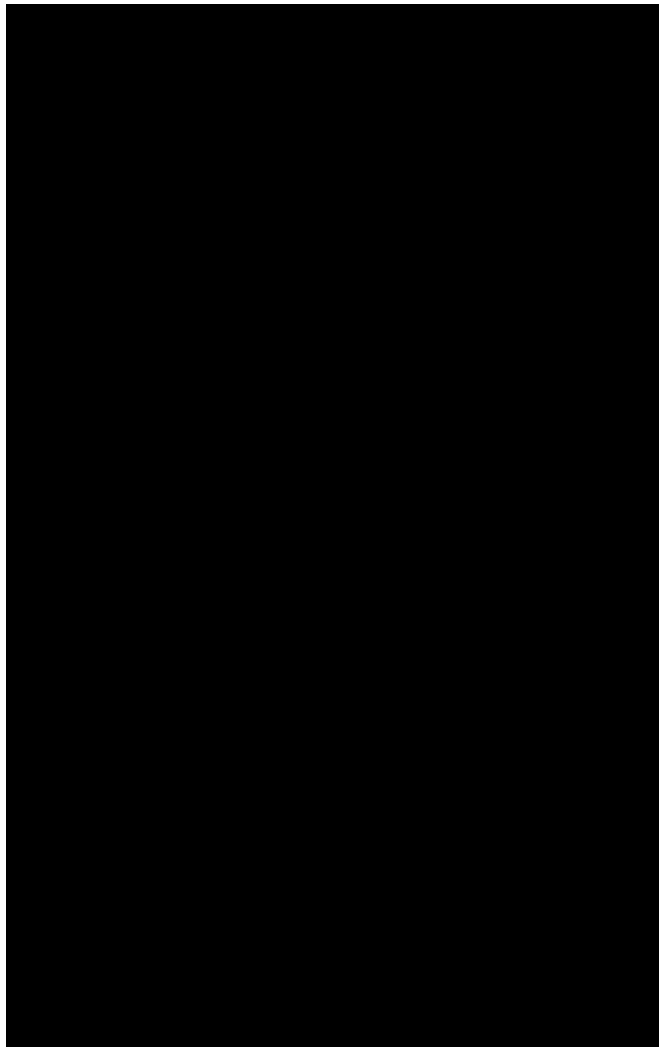

## GENETICS

### New Method of Isolating the Tetrads of Agarics

HITHERTO the usual technique for isolating tetrads from Agarics has been to detach a piece of gill tissue from a mature sporophore and then, with the aid of a micromanipulator, to pick off whole tetrads directly from the fresh gill. There are three main difficulties inherent in this method: (*a*) because of the high numerical density of tetrads on the gill, it is very difficult to be sure that no extraneous spores have been picked up by the micromanipulator needle until the spores are parked on the germination medium; (*b*) it is impossible to distinguish unripe from ripe tetrads, and since the former are removed from their basidia with great difficulty, many abortive attempts to pick up tetrads are frequently made before a tetrad is successfully removed; (*c*) in the case of *Coprinus*, autolysis of the gill can greatly limit the time available for isolation.

In the course of analysing tetrads from *Coprinus lagopus* (for the purpose of finding new centromere markers to be used for the extension of mapping) a new method has been developed. If a piece of gill tissue is taken from a mature sporophore, placed on a microscope slide and allowed to dry, the spores can be scattered on to the surrounding glass by sweeping the micromanipulator needle across the dried gill. Among the spores on the glass there is nearly always a high proportion of whole tetrads (Fig. 1), and these can very easily be removed from the

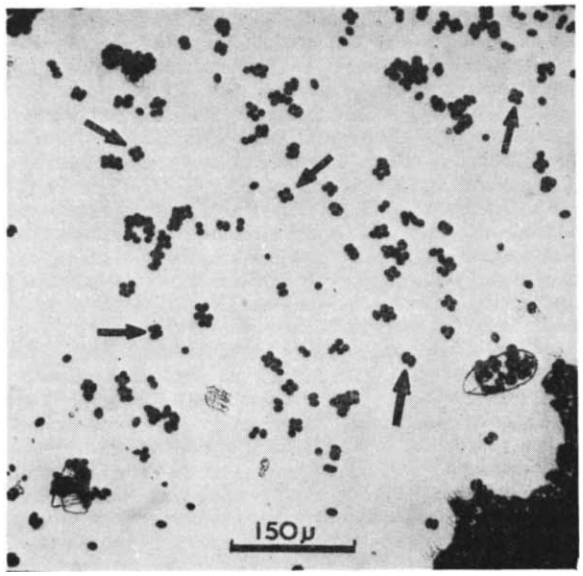


Fig. 1. Photomicrograph of spores scattered from dried gill. There are more than twenty tetrads in this field which are available for picking-off; five of them are arrowed as examples

glass. Drying may be hastened by placing the slide carrying the gill into a Petri dish containing a layer of dry silica gel.

During drying the four spores of each tetrad become aggregated into a single mass so that, when drying is complete, each tetrad tends to behave as a single unit. This presumably accounts for their easy removal as whole tetrads by this somewhat drastic method.

The great advantage of this 'on-glass' technique is that whole tetrads can be stored as such for long periods: in one case tetrads isolated four months after scattering still gave 85 per cent germination. Thus many tetrads can be isolated over a comparatively long period of time as and when it is convenient to do so, with the advantage that all originate from the same fruiting body. Slides carrying scattered spores are best stored over silica gel at room temperature.

That the on-glass technique still provides a random sample of tetrads was demonstrated by a cross in which 104 tetrads were isolated from fresh gill using the old method, and a further 49 isolated from glass. Both sets of data were completely homogeneous.

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David Moore*

Department of Botany,
University of Hull.

* Present address: The John Innes Institute, Bayfordbury, Hertford.

## AGRICULTURE

### Carbon- and Nitrogen-levels of a Medium in Relation to Growth and Nematophagous Activity of *Arthrobotrys oligospora* Fresenius

During the past few years, attempts have been made to enhance the nematode-trapping activity of nematophagous fungi in the soil by adding various carbohydrates[1,2]. As a preliminary investigation to similar experiments, we cultured *Arthrobotrys oligospora* and measured its growth and nematophagous activity on semi-synthetic media having different levels of carbon and nitrogen.

The basic medium had the following ingredients: dipotassium phosphate, 1·0 g; magnesium sulphate, 0·5 g; potassium chloride, 0·5 g; ferrous sulphate, 0·01 g; yeast extract, 3·0 g; agar, 15·0 g; and distilled water to make 1 l. of medium. Dextrose and ammonium nitrate were added to this medium at the rates shown in Table 1. Eight replicate Petri dishes of each medium were inoculated with a culture of *A. oligospora*, which had been growing on Lima bean agar.

After 2 days of incubation at room temperature, the fungal colonies were about 40 mm in diameter. A standard number of free-living nematodes (*Rhabditis* (*Cephaloboides*) *oxycerca* de Man. 1895), which had been surface sterilized in a 1 : 10,000 solution of mercuric chloride and washed thoroughly with sterile water, was then added to each of four of the original eight replicate cultures on each medium. The nematodes were allowed to be trapped for 1 week. The remaining four replicate cultures on each medium were grown for an additional 3 days, after which the diameters of the fungal colonies were measured.

The effect of the levels of dextrose and of ammonium nitrate of the medium on the nematode-trapping activity of *A. oligospora* was determined by transferring the contents of the plates to Baermann funnels and counting the extracted nematodes the next day. The number of nematodes recovered is assumed to be inversely proportional to the number trapped by the fungus.

Table 1. Effect of Carbon- and Nitrogen-levels on Growth and Nematophagous Activity of *Arthrobotrys oligospora*

| Carbon- and nitrogen-levels (mg/l.) | | Diameter of colony (mm) | | Nematodes recovered | |
|---|---|---|---|---|---|
| Dextrose | Ammonium nitrate | Mean* | *S.D.* | Mean† | *S.D.* |
| 50 | 5 | 80 | 2·6 | 630 | 41·3 |
| 100 | 10 | 78 | 1·8 | 711 | 88·7 |
| 200 | 20 | 81 | 0·3 | 197 | 64·6 |
| 400 | 40 | 72 | 0·2 | 177 | 18·9 |

* Mean of four colony diameters.
† Mean of six counts.

Media low in dextrose and ammonium nitrate supported less nematophagous activity of *A. oligospora* than media containing higher concentrations of these compounds (Table 1). As there was little difference in the amount of radial growth made by the fungus on these media, the effect of the carbon- and nitrogen-levels is assumed to have been on its nematode-trapping activity. The sharp decline in nematophagous activity at a level of dextrose and of ammonium nitrate between 100–200 and 10–20 mg/l., respectively, suggests the existence of a critical point or range in nutritional levels which determines a saprophytic or parasitic mode of existence for the fungus.

These observations cast some doubt on the validity of Duddington's[3] suggestion that nematophagous fungi may be most actively parasitic when grown on media of low nutritional value. Rather, these findings support Cooke's[2] view that nematode-trapping fungi need an organic energy source, other than nematodes, in order to remain in an active nematophagous state. The results of unpublished greenhouse investigations[4] indicate that small quantities of dextrose and ammonium nitrate added to soil enhance the nematode-trapping activity of *A. oligospora*.

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Th. H. A. Olthof*
R. H. Estey

Department of Plant Pathology,
Macdonald College of McGill University,
Montreal, P.Q., Canada.

* Present address: Research Station, Canada Department of Agriculture, Vineland Station, Ontario, Canada.

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