Much more work is required to prepare a reagent specific for S. pannosa which might, for example, be used to identify conidia on spore trap slides. One special problem is that this fungus does not produce conidia abundantly like some powdery mildews so it is difficult to collect enough for injecting rabbits. Nothing is known about the chemical constitution of powdery mildew conidia but the present results with heat-treated, conidial preparations suggest that non-proteinaceous fractions may have been involved in the production of antibodies.

Dr B. E. J. Wheeler helped me with the manuscript and Dr Boreham and his staff gave valuable advice on the preparation of antisera. The work was carried out during the tenure of a University of London postgraduate studentship and the Henry George Plimmer Fellowship of Imperial College.

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# PRODUCTION OF FERTILE PERITHECIA OF PYRENOPHORA AVENAE IN CULTURE

## W. A. SHIPTON\* AND W. C. McDONALD

### Canada Department of Agriculture, Research Station, Winnipeg 19, Manitoba

Fertile perithecia of *Pyrenophora avenae* S. Ito & Kurib. have been found on the straw of species of *Avena* in nature (Dennis, 1935; Ito, 1930) but, except for one doubtful report, they have not been recorded in culture. Rathschlag (1930) claimed to have produced the perfect stage of *Drechslera avenacea* (Curtis ex Cooke) Shoem. on oatmeal agar under special conditions but his description and illustrations of ascospores do not agree with the original description of *P. avenae* (Ito, 1930). Dennis (1935) suggested that the discrepancy could be attributed to the cultural conditions under which the ascocarps were produced. Shoemaker (1962) placed Rathschlag's fungus in *Pleospora* and Luttrell (1958) believed that it was a contaminant, most probably *P. herbarum* (Fr.) Rab.

Attempts to produce fertile perithecia of *P. avenae* in culture have been

\* Present address: James Cook University of North Queensland, Australia.

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made in this laboratory for a number of years. Isolates of diverse origin were mated on various media but only in one instance were fertile perithecia produced and these contained only immature or aborted ascospores. Further studies with additional isolates and a wider variety of media were more successful.

A mixture of conidial suspensions from eighteen cultures of *P. avenae* from Canada, Scotland and Israel was transferred in duplicate to Petri dishes that contained Sach's agar and one of a number of other substrates (barley, oat, or wheat straw; barley, oat, wheat or sunflower seed; citrus, maize or oat leaves). Oatmeal was also tested.

The straw was treated for 24 h with propylene oxide as a sterilant and then aerated for 24 h. The grain was sterilized by boiling in water for 2-3 min and the leaves were autoclaved for 1 h at 1 bar gauge pressure. The straw or grain was half embedded in Sach's agar and the leaves were placed on the agar surface. The cultures were incubated at 16 °C with a 12 h photoperiod for 1 week and then half of them were transferred to a dark room at 1° for 1 week.

Single ascospore cultures were isolated from water agar in Petri dishes that had been inverted over mature perithecia from which mature ascospores were being ejected. The mating type of monoascospore progeny was determined by pairing cultures on oat seed or straw half embedded in Sach's agar. Measurements were made of specimens mounted in water and are based on twenty representative structures.

Fertile perithecia were produced on media containing oat seed or oat straw, but not on other substrates. They appeared 9 weeks after inoculation, irrespective of whether the cultures received a cold, dark period of I week. Less than half of the ascocarps examined were fertile and ascospores were not present in all asci.

The perithecia, asci, ascospores and conidia were typical of those described by Shoemaker (1962). Perithecia were large, dark brown to black, without a well defined beak, and were covered with numerous, long, dark brown setae. They were slightly ovate to broadly oval and bore numerous asci in a fascicle at the base of the perithecium. The asci were prominently bitunicate, cylindric to slightly clavate, with a broadly rounded apex and a short stipitate base. Asci measured  $180-315 \times 33-49 \ \mu m$  and contained from 1 to 8 ascospores; many asci were infertile.

Ascospores were biseriate in arrangement, light to medium yellow brown, cylindrical, with rounded ends. They were muriform with five transverse septa; ascospores with four to six septa were also observed. Up to four vertical septa were observed in the central cells but usually only two or three were present. The widest cell was usually the second from the apex, occasionally the third, and rarely the fourth. The ascospores measured  $33-77 \times 14-34 \ \mu\text{m}$ ; the average cell length was 10 \ \mu\text{m}.

Spermogonia were formed abundantly by some isolates. They were medium to light brown, globose or slightly ovate, not prominently beaked, and rarely bore setae. They measured  $28-88 \times 26-79 \ \mu m$  and exuded a mass of globose to ovoid spermatia in a droplet at the ostiole. Spermatia

were hyaline and  $1.5-2 \mu m$  diam. Development of spermogonia in culture was described by Smith & Putterhill (1932).

Conidia were usually cylindrical, light to medium yellow brown, and had a conspicuous scar. Pigmentation of the basal cell was somewhat less pronounced than in other cells. Conidia measured  $40-100 \times 8-19 \ \mu m$  and had two to six septa.

Monoascospore isolates grown on V-8 juice agar varied in their cultural characteristics. Some were uniformly dark grey to black, conidial; some were uniformly white or grey, cottony, with few conidia; and some were light or dark grey with a profusion of white or grey tufts on the surface.

Six ascospores were successfully isolated from a group of eight, presumably ejected from one ascus on to water agar, and the monoascospore cultures were divided into three distinct groups on the basis of cultural characteristics. These isolates were mated in all possible combinations and were also grown singly. Five matings produced perithecia that contained asci, and four plus and two minus mating types were identified. Culturally similar pairs of isolates were of the same mating type and cultures grown singly were sterile.

Both homo- and heterothallic species have been found in the Cylindro-Helminthosporium group of fungi. P. bromi (Died.) Drechs., P. lolii Dovaston and Pleospora trichostoma (Fr.) Ces. & de Not. are homothallic (Chamberlain & Allison, 1945; Dovaston, 1948; Simmons, 1952), while P. teres Drechs. is heterothallic (McDonald, 1963). The report by Paul & Parbery (1968) indicates that P. dictyoides Paul & Parb. may also be heterothallic. Evidence obtained from mating monoascospore cultures of P. avenae shows that this species is also heterothallic and bisexual although uniform production of fertile perithecia or of asci with full complements of eight ascospores was not obtained. Even under natural conditions many asci are without organized ascospores (Dennis, 1935) and it may be that the requirements for ascospore production are rather specific. Alternatively, hereditary factors may control the morphogenetic processes and prevent normal ascospore formation.

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# VIABILITY OF DISCHARGED AND RESIDUAL SPORES OF SOME COPROPHILOUS PYRENOMYCETES

#### I. J. HODGKISS

### Department of Botany, University of Hong Kong

During laboratory experiments to determine the influence of various physical and chemical factors on spore discharge from a number of coprophilous pyrenomycetes (Hodgkiss, 1968; Hodgkiss, 1969; Hodgkiss & Harvey, 1970) it was noted that the percentage viability of discharged spores varied both with age of perithecium and with prevailing conditions.

Table 1 illustrates the total numbers of spores discharged and their percentage viabilities in four species, namely, Sordaria macrospora, S. finicola, Pleurage anserina and P. taenioides, at four different temperatures. All four species appear to have different optimum temperatures for the production of viable spores, and also different optimum temperatures for maximum spore discharge and highest viability.

Table 1. Spore discharge and viability at different temperatures

| Temperature                          | S. macrospora |     | S. fimicola |                                  | P. anserina |    | P. taenioides |    |
|--------------------------------------|---------------|-----|-------------|----------------------------------|-------------|----|---------------|----|
| (°C)                                 | A*            | ́В† | A           | В                                | Α           | В  | Α             | В  |
| 5                                    | 354           | 0   | 76          | о                                | 92          | 26 | 86            | 71 |
| 15                                   | 212           | 27  | ġ1          | 43                               | <u>8</u> 9  | 32 | 124           | 69 |
| 23                                   | 129           | 21  | 80          | 64                               | 45          | 14 | 134           | 69 |
| 33                                   | 176           | 31  | 29          | 0                                | 22          | 13 | 26            | 58 |
| * A Spore discharge/perithecium/day. |               |     |             | † B Percentage of spores viable. |             |    |               |    |

Viability was assessed by growing perithecial isolates in high humidity chambers (85-90 % relative humidity) at each temperature under a 12 h light:12 h dark régime, and collecting the discharged spores on slides. These spores were then allowed to germinate at  $23 \pm 0.6$  °C under the same humidity and light conditions. After a 6-day period the spores were examined microscopically, and all those which had a visible germ-tube were counted as 'viable'.

In order to determine the effect of perithecial age on viability, one species, S. macrospora, was grown at a high humidity under a 12 h light: 12 h dark régime and at a temperature of  $23 \pm 0.6^{\circ}$ . Discharged spores were

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