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Abstract

The plant pathogenic fungi Fusarium solani f. sp. cucurbitae and Fusarium solani f. sp. pisi are among the few Fusaria with a known sexual stage.

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The plant pathogenic fungi Fusarium solani f. sp. cucurbitae and Fusarium solani f. sp. pisi are among the few Fusaria with a known sexual stage. They are therefore increasingly employed for genetic studies of plant pathological problems (Van Etten and Kistler 1988 Adv. Plant Pathol. 6:189-206).

Taxonomically these fungi are often referred to as mating populations, MP I and MP VI, respectively, of Nectria haematococca. They are typical heterothallic ascomycetes, producing perithecia and ascospores when the + and - mating types are brought together on a crossing medium (V-8, vegetable juice agar medium) and illuminated with daylight or "daylight lamps" during development.

In most asci the spores are not found in a linear array. Isolation of unordered spores in asci is possible but tedious due to the small size. Instead it is often preferred to let the ascospores germinate before isolation. Larger numbers can be processed when a growth inhibitor is added to the germination medium or by the use of genetically colonial strains. The methods described here are concerned with the procurement of large numbers of unordered ascospores.

Ascospores ooze from the ostioles of mature perithecia 11 to 14 days after fertilization. They can be collected as random spores using a fine needle or in a drop of water on a small loop under the dissecting binocular. With some experience this involves no serious risk of contamination with vegetative spores, conidia. However, it may be difficult to collect enough ascospores for counting in the hemocytometer or for selection of rare recombinants, etc.

During work with colonial and microcyclic mutants (Kolmark 1984 Mol. Gen. Genet. 198:12-18) it was noticed that ascospores could be collected in masses when a Petri plate with a mature cross had been turned upside down. Such ascospores could easily be suspended and transferred in water using a pipette. In crosses with good fertility more than 1 x 10^6 ascospores could be collected from one plate. Repeat batches could be obtained over a period by shifting the lids every day or two. Hardly any vegetative spores were mixed with the ascospores provided that hyphae had not been allowed to grow onto the lid.

An interesting question was whether the ascospores dropped down passively, or whether Fusarium, like Neurospora, possessed a mechanism for active discharge. Some testings of this were made using taller plastic boxes with inside dimensions of 6.5 x 10.0 x (height) 6.0 cm.

When the perithecia were ready to expel the spores, two microscope slides (2.5 x 7.0 cm) were placed across the box with an angle approximately 30° to the perithecial surface. The slides were covered on the bottom side with growth medium containing Triton X-171, 0.025% w/v, to retard spreading.

Distinct colonies (due to the Triton) were found on the exposed probes, testifying that ascospores are indeed discharged by an active mechanism.

The vertical height above the surface of any shot spore when it was stopped on the medium surface is: h = a (27/70) where a is the distance measured from the lower end of the microscope slide to the spore and the figures 27 and 70 are constants, for the given set, of the highest position of the slide, and the total length of the slide, respectively, all measured in mm.

Using this method, five different crosses were sampled every second day over a period of more than one week. The maximal shooting height was found to be rather constant, varying in the range 15 to 19 mm for all crosses over the sampling period. A sharper borderline with many microcolonies usually developed 2-3 mm below the few spores at the maximal height.

The shooting height (or range) for Fusarium is presumably considerably shorter than that for Neurospora. However, it is sufficient for ascospores to adhere to drops of moisture on the lid of a standard Petri dish in the upright position. We found that adhesion could be improved by means of a thin layer of glycerol applied on the inside of the lid.

Presumably, oozes of ascospores stuck together on top of ostioles are in time blocking the free ejection from many perithecia in the upright position. The researcher may take advantage of this if it is wanted to study a smaller number of offspring from singular perithecia, while large numbers for selection studies, etc. may preferentially be secured from the lids of overturned plates.

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