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A SIMPLE METHOD FOR INDUCING SPORULATION IN SEED-BORNE FUNGI

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(With Plates 2–3)

A simple technique for inducing mass sporulation and for the study of morphological characters of fruiting structures in a number of seed-borne fungi has been worked out. Young cultures are grown on autoclaved leaves of *Pennisetum glaucum* (pearl millet) planted on the surface of water agar plates. Sporulation of a wide range of fungi was observed within a short period of incubation. The extent of sporulation was enhanced by incubating the cultures under near u.v. radiation but different fungi showed diverse requirements for initiation of sporulation. The advantage of the technique for mass sporulation of different fungi and its application are discussed.

Many seed-borne fungi fail to sporulate or do so weakly when isolated in pure culture and grown on conventional media. Often sporulation capacity declines or is lost after a few serial transfers on these media and the colonies may become completely mycelial or with very few sporophores and conidia. In *Drechslera* species, where it is desirable to observe the morphology of the developing conidiophore for purposes of taxonomy and identification, the agar slant cultures are often unsuitable due to heavy mycelial growth and feeble sporulation. For infection experiments, mass sporulation of the fungus is desirable and here again agar slant cultures on the usual media often do not satisfactorily serve the purpose.

Considering these points, an attempt was made to develop a suitable method for mass sporulation of seed-borne fungi in pure culture. Emphasis was laid on developing a simple and inexpensive technique which could be used easily in a laboratory or seed testing station in which sophisticated equipment is not readily available. Among several methods tried, the one described here appeared to be the simplest and cheapest and the results indicated that it was applicable with ease to obtain mass sporulation in a wide range of fungi.

MATERIALS AND METHODS

Water agar (Bacto Agar Difco, 1·5 %) was poured aseptically in sterile Petri dishes and allowed to set. Young leaves of growing seedlings of *Pennisetum glaucum* (L.) R.Br. were cut into small pieces, suspended in distilled water and autoclaved at 120 °C for 15 min. The leaf pieces were transferred to the surface of the agar substrate, one or two per plate, after excess moisture had been absorbed on a sterile blotter. A disk of mycelium

from the growing margin of a 48- to 72-h-old colony was aseptically transferred to the surface of the leaf piece and the plates incubated at the temperature optimum for growth of the fungus. In various experiments different seed-borne fungi were incubated under 12 h of near u.v., 12 h of daylight fluorescent tubes, and in complete darkness. Observations were made periodically from 48 h onwards to study the growth and sporulation.

EXPERIMENTAL RESULTS

Fungus growth took place on the leaf substrate in the form of thin creeping mycelia growing into the substrate. The sporulating structures began to form within 48–72 h and subsequently large masses of spores developed over the entire surface of the leaf pieces, extending in some cases to the agar substratum. The sporulating structures could be studied conveniently at different stages under the stereo-binocular microscope even at low magnifications without interference from the vegetative mycelium. In our studies a large number of seed-borne fungi were included and a full list of those tested is presented in Table 1.

Table 1. *Fungi tested for sporulation on leaf medium*

(Host names in parentheses)

1. *Alternaria porri* (Ell.) Ciferri (Coriander)
2. *A. sesami* (Kawamura) Mohanty & Behera (sesamum)
3. *A. zinniae* Pape (Zinnia)
4. *Colletotrichum graminicola* (Ces.) Wils. (sorghum)
5. *C. lindemuthianum* (Sacc. & Magn.) Bri. & Cav. (cowpea)
6. *C. truncatum* (Schw.) Andrus & Moore (soybean)
7. *Curvularia cymbopogonis* (C. W. Dodge) Groves & Skolko (rice)
8. *C. eragrostidis* (P. Henn) J. A. Meyer (rice)
9. *C. geniculata* (Tr. & Earle) Boedijn (rice and sorghum)
10. *C. intermedia* Boedijn (rice)
11. *C. oryzae* Bugnicourt (rice)
12. *C. pallescens* Boedijn (rice)
13. *C. trifolii* (Kauffman) Boedijn (rice)
14. *C. tuberculata* Jain (mungbean)
15. *C. uncinata* Bugnicourt (rice)
16. *Drechslera avenae* (Eidam) Scharif (oats)
17. *D. bicolor* (Mitra) Subramanian & Jain (pearl millet)
18. *D. catenaria* (Drechs.) Ito (English ryegrass)
19. *D. hawaiiensis* (Bugn.) Subramanian & Jain (fox tailed millet)
20. *D. longirostrata* (Subram.) Subram (rice, sorghum and finger millet)
21. *D. oryzae* (van Breda de Haan) Subramanian & Jain (rice)
22. *D. rostrata* (Drechs.) Richardson & Fraser (finger millet)
23. *D. tetramera* (McKinney) Subramanian & Jain (finger millet and fox tailed millet)
24. *D. tritici-repentis* (Died.) Shoemaker (ryegrass)
25. *Fusarium avenaceum* (Fr.) Sacc. (barley)
26. *F. graminearum* Schwabe (rice)
27. *F. equiseti* (Corda) Sacc. (pearl millet)
28. *F. poae* (Peck) Wollenw. (barley)
29. *Helminthosporium papaveris* Saw. (opium poppy)
30. *Nigrospora oryzae* (Berk. & Br.) Petch (rice)
31. *Pestalotia guepinii* Desm. (egg. plant)
32. *Phaeotrichoconis crotalariae* (Salam & Rao) Subramanian (rice)
33. *Plenodomus lingam* (Tode ex. Fr.) Höhnel (*Brassica oleracea*)
34. *Pyricularia oryzae* Cav. (rice — India, Korea and Egypt)
35. *Trichoconis padwickii* Ganguly (rice and pearl millet)

In several cases it was noticed that in their morphology the fruiting structures formed on this medium closely conformed with those observed on seeds in the 'blotter test'. For example, conidiophore morphology, attachment of conidia and their shape in different species of *Curvularia* as observed on this leaf medium conformed with earlier descriptions of *Curvularia* spp. on seeds (Benoit & Mathur, 1970). In *Drechslera* species the typical morphology of the developing conidiophores, shape of conidia and their attachment could also be followed with ease. *Colletotrichum lindemuthianum* (Sacc. & Magn.) Bri. & Cav., *C. truncatum* (Schw.) Andrus & Moore and *C. graminicola* (Ces.) Wils. produced typical acervuli with setae, and conidial masses were observed within 4 days, the young acervuli being differentiated almost simultaneously with the extension of mycelial growth within the leaf tissues. Pycnidia of *Plenodomus lingam* (Tode ex. Fr.) Höhnel were differentiated within 3 days and the purple spore exudate formed within 5 days after transfer. *Pyricularia oryzae* Cav. formed abundant conidia, covering the entire leaf surface with greyish conidiophores. In *Trichoconis padwickii* Ganguly, which normally does not sporulate on usual agar media, heavy sporulation was observed within 72 h in the rice as well as pearl millet isolate. Similar observations were recorded in many other fungi (Pls. 2 and 3).

HERBARIUM MOUNTS

A modification has been successfully employed for obtaining herbarium mounts of the sporulating structures of seed-borne fungi. It consisted in placing the leaf piece on a length of plain transparent autoclaved cellophane of the type PT 300 laid on the agar and then inoculating it with the fungus. After incubation, the cellophane was lifted off the agar plate and mounted in a plastic dish. This method was adapted from Carmichael (1963) who published a procedure for preserving dried mold colonies on cellophane. Unlike the procedure described by Carmichael, where the colony is pressed in a special type of plastic frame, in the present studies the material was not pressed and the sporophores were allowed to air dry. The herbarium material obtained thus maintained the fungus in an untouched in situ condition.

DISCUSSION

The simplicity of this procedure indicates its potential usefulness in the study of seed-borne fungi and fungi in general. The advantages include large-scale spore formation in diverse fungi isolated from different seed samples within a short period of incubation, without interference from mycelial growth. The autoclaved leaf pieces being transparent facilitate direct observation of the sporulating structures under the stereo-binocular as well as the compound microscope. The non-nutritive nature of the plain agar substrate permits examination of the cultures without fear of serious contamination even by opening the lid.

It was found that incubation under near-u.v. definitely increased sporulation but in most cases absolute requirement for induction of sporulation was not evident. In *Pyricularia oryzae* prolific sporulation was evident even in darkness, while in *Plenodomus lingam* the formation of pycnidia was

considerably delayed in darkness as compared with cultures incubated under near-u.v. or daylight. The effect of different environmental conditions on the extent of sporulation of various fungi on this leaf medium is being investigated and will be reported in due course.

Application of this technique for obtaining sporulation of other groups of fungi such as soil fungi needs investigation. It is also of interest to establish whether seed-borne fungi from different geographic locations representing different isolates will show differences in response to sporulation on this medium. The use of leaves from other hosts of the Gramineae including the cereals from temperate regions such as wheat, barley, and oats is being investigated and the indications are that besides pearl millet, leaves of other cereals may also prove to be favourable substrata for sporulation.

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EXPLANATION OF PLATES 2 AND 3

Figures 1–12 × 90

Fig. 1. *Alternaria sesami* on PDA. Heavy sporulation. The characteristics of conidiophores and conidia are partly visible only at the edge (3 days old).

Fig. 2. *A. sesami* on pearl millet leaf. Heavy sporulation. Characteristics of conidia and conidiophores clearly visible without interference from aerial mycelium. There is a reduction in the size of conidia as compared with conidia developed on PDA (3 days old).

Fig. 3. *Colletotrichum lindemuthianum* on PDA. A big shining mass due to coalescence of many acervuli; setae not observed in young cultures; sparse and submerged in old cultures (4 days old).

Fig. 4. *C. lindemuthianum* on leaf. Typical acervuli like those observed on seeds (4 days old).

Fig. 5. *Curvularia oryzae* on PDA. Heavy sporulation with profuse mycelial growth (3 days old).

Fig. 6. *C. oryzae* on leaf. Heavy sporulation with only creeping mycelium. Arrangement of conidia and conidiophores similar to those seen on seed (3 days old).

Fig. 7. *C. tuberculata* on PDA. Heavy sporulation with profuse aerial mycelium impairing the view of sporulation structures (3 days old).

Fig. 8. *C. tuberculata* on leaf. Heavy sporulation with little aerial mycelium (3 days old).

Fig. 9. *Drechslera bicolor* on PDA. Profuse greyish black mycelial growth with many conidia. Aerial mycelium obstructs the view of conidia and conidiophores (2 days old).

Fig. 10. *D. bicolor* on leaf. Heavy sporulation with only creeping mycelium (2 days old).

Fig. 11. *D. longirostrata* on PDA. Profuse mycelial growth with few small conidia not characteristic of the species (2 days old).

Fig. 12. *D. longirostrata* on leaf. Heavy sporulation with characteristic conidia and only creeping mycelium (2 days old).

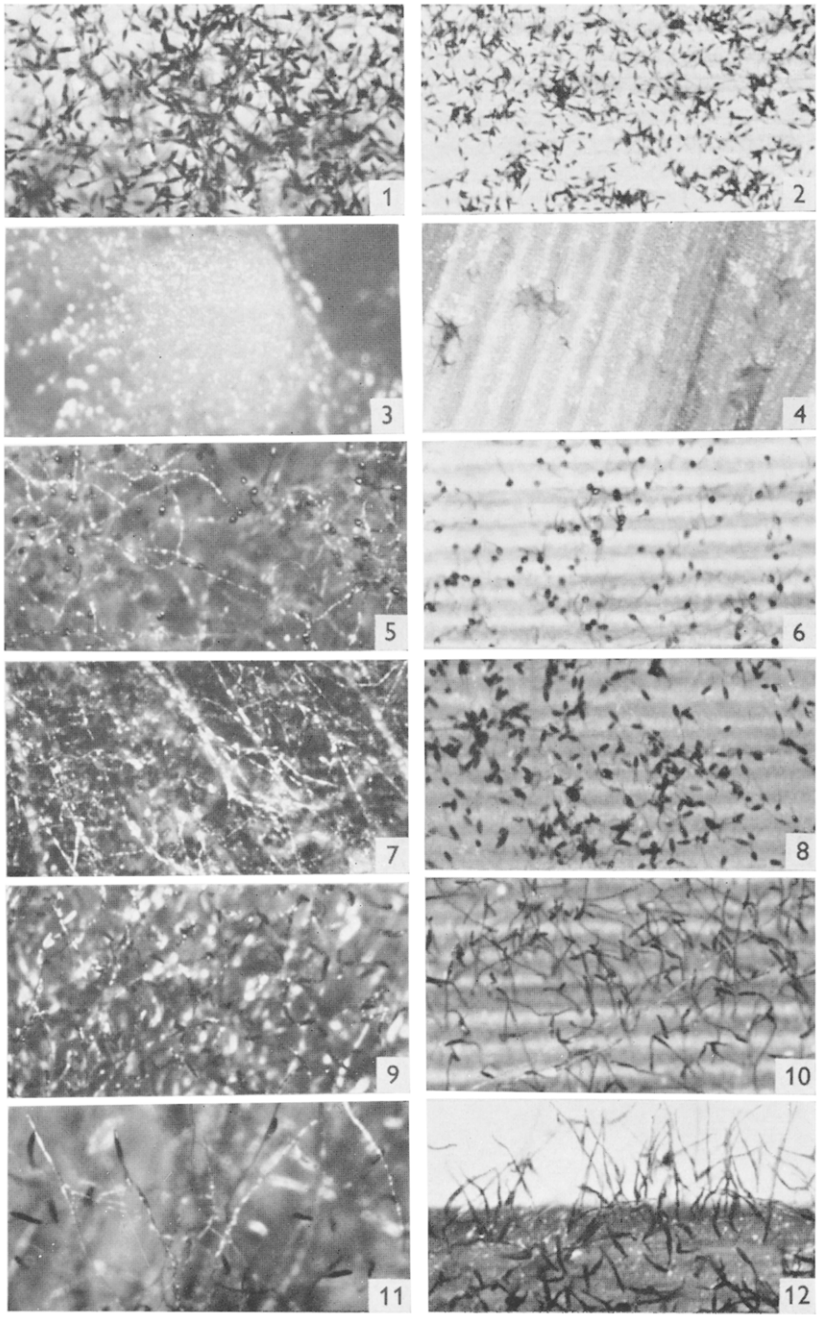
Figures 13–22 × 90

Fig. 13. *Fusarium graminearum* on PDA. Heavy mycelial growth with only few masses of conidia (5 days old).

Fig. 14. *F. graminearum* on leaf. Less mycelial growth with numerous masses of conidia (5 days old).

Fig. 15. *Pestalotia guepinii* on PDA. Profuse white mycelial growth covering masses of conidia (3 days old).

Fig. 16. *P. guepinii* on leaf. Distinct, well formed, small to big masses of conidia with only creeping mycelium (3 days old).



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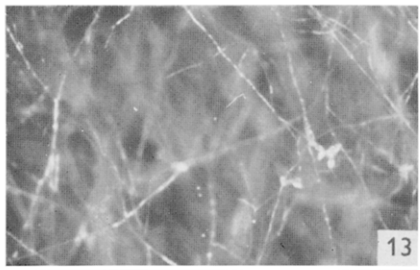
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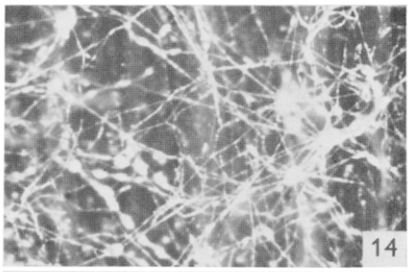
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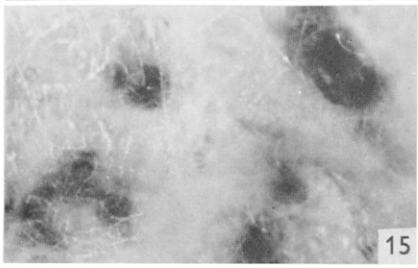
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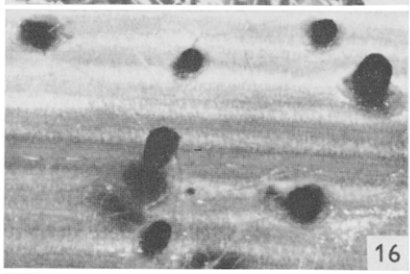
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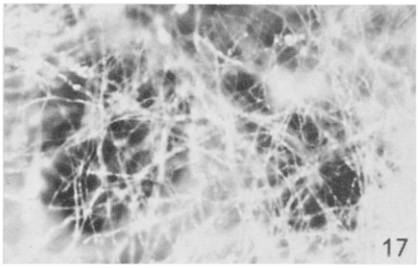
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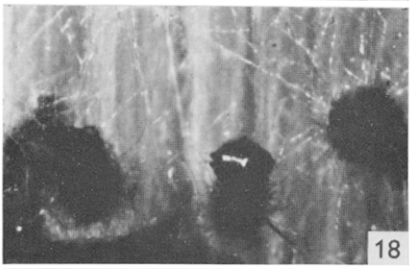
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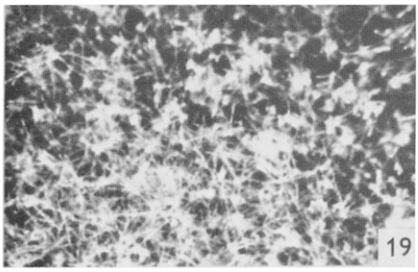
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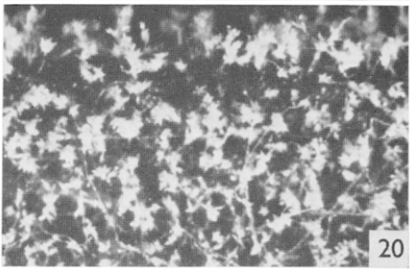
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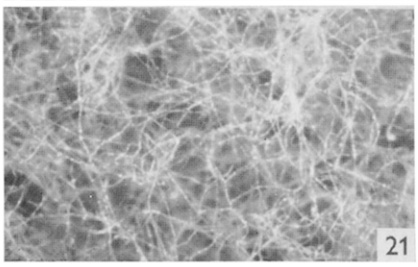
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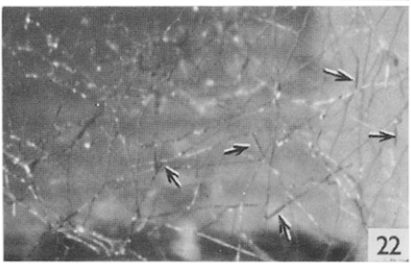
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Fig. 17. *Plenodomus lingam* on PDA. Colony slow growing, restricted with profuse silvery white mycelium covering few pycnidia. Purple ooze of conidia not distinct. Colony restricted even when old (5 days old).

Fig. 18. *P. lingam* on leaf. Fast-growing, with negligible amount of silvery white aerial mycelium; pycnidia distinct scattered all over the leaf surface. Purple ooze clearly visible (5 days old).

Fig. 19. *Pyricularia oryzae* on PDA. Heavy sporulation. Profuse mycelial growth presents slight obstruction in viewing clusters of conidia (5 days old).

Fig. 20. *P. oryzae* on leaf. Heavy sporulation with less mycelial growth. Clusters of conidia clearly visible (5 days old).

Fig. 21. *Trichoconis padwickii* on PDA. Only mycelial growth with no sporulation (4 days old).

Fig. 22. *T. padwickii* on leaf. Little aerial mycelium with many conidia; sporulation greatly increases with the incubation period (4 days old).

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