Trans. Br. mycol. Soc. **81** (3) 597–603 (1983) *Printed in Great Britain*

TOLYPOSPORIUM PENICILLARIAE, THE CAUSAL AGENT OF PEARL MILLET SMUT

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Sporeballs of *Tolyposporium penicillariae* varied from circular to near-polyhedral and measured 42–325 × 50–175 μm. The number of teliospores aggregated in balls varied from 200 to 1400. Teliospores were mostly circular and measured 7–12 μm diam. Maximum germination of teliospores aggregated in balls occurred at 30 °C and different patterns of germination were observed. Large variability was observed in cultural characters of the pathogen grown on different media. The fungus grew well within 3–5 days on potato or carrot agar at 35°. Growth of the fungus remained purely sporidial even after repeated subculturing. Sporidia were borne on promycelia, laterally and/or terminally, and these reproduced by budding in chains. Sporidia were spindle-shaped and varied in length from 8 to 25 μm. Individual separated teliospores seldom germinated. The use of sporidial inoculum in screening for smut resistance in pearl millet is discussed.

Smut, caused by *Tolyposporium penicillariae* Bref., is a common disease of pearl millet (*Pennisetum americanum* (L.) Leeke) in the semi-arid tropical regions of the world (Rachie & Majmudar, 1980), and has also been reported from the United States (Wells, Burton & Ourecky, 1963). Until 1963, smut of pearl millet was not considered of economic importance (Ramakrishnan, 1963) but more recently the disease has become a serious constraint in realizing the high yield potentials of F_1 hybrids in India and of improved varieties in Africa (Rachie & Majmudar, 1980). The disease is confined to the inflorescence (Ajrekar & Likhite, 1933; Bhatt, 1946). The infected ovaries are converted into oval or pear-shaped sori with large numbers of black or brown dusty spores (Mundkur, 1940).

At the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India, work on smut was initiated in 1977 with the major objective of identification of sources of resistance. In the process of developing an effective, large-scale field screening technique for smut resistance, studies were made on several aspects of the biology of the pathogen, some of which are reported in this paper.

MATERIALS AND METHODS

Smutted pearl millet inflorescence collected from the 1979–80 post-rainy season millet fields at the ICRISAT Centre were used as initial inoculum source in the various studies reported.

Seven media were tested for their relative efficacy to support growth of *T. penicillariae*: potato agar (PA) (200 g peeled potato extract, 15 g agar in 1 l distilled water); carrot agar (CA) (200 g peeled carrot extract, 15 g agar in 1 l distilled water); potato-dextrose agar (PDA) (200 g peeled potato extract, 20 g dextrose, 15 g agar in 1 l distilled water); potato extract (PE) (200 g peeled potato extract); carrot extract (CE) (200 g peeled carrot extract); potato pieces and carrot pieces. The media, after adjustment to pH 6, were autoclaved at 1·1 kg cm^{-2} for 20 min in conical flasks. PDA, PA and CA were plated in 9 cm diam plastic Petri dishes.

Unruptured mature smut sori were surface sterilized with 0·1 % mercuric chloride for 1 min, rinsed with sterile distilled water twice and crushed aseptically with sterile forceps to obtain the sporeballs to inoculate different media. Observations on topography, colour, margin, consistency and colony diameter on solid media and concentration of sporidia in liquid media were made after 10 days incubation at 35° under 40 W fluorescent lamps at an intensity of 3500 lux in a Percival I 35 CC incubator.

Potato agar plates inoculated with the fungus were incubated at five temperatures, 20, 25, 30, 35 and 40°, with five Petri dishes at each temperature. The growth was measured after 7 days incubation. Growth in shake-liquid culture was compared with that in still-liquid culture by measuring the concentration of sporidia in PE and CE.

Sporeballs were suspended in sterile water in cavity slides and incubated in moist chambers at 15, 20, 25, 30, 35 and 40°. Observations on germination were made after 12 h incubation. A sporeball was

considered to have germinated when at least one promycelium had emerged. In another set of similar experiments, observations were made at hourly intervals to determine the threshold time required for spore germination, sporidial production and sporidial liberation. Measurements of promycelia and sporidia were taken and camera lucida drawings made.

Observations were recorded on individual teliospores, separated by crushing the sporeballs underneath a coverslip on a glass slide. Teliospores were incubated in sterile distilled water in cavity slides at 30° and observations were made on germination after 12 h incubation and the camera lucida drawings were made.

RESULTS

Growth and cultural characteristics on different media

The maximum growth of *T. penicillariae* at 35° occurred on PA (mean size of 25 colonies in 5 replications $17{\cdot}0 \times 13{\cdot}4$ mm) and CA ($13{\cdot}8 \times 11{\cdot}8$ mm) and the minimum on PDA ($8{\cdot}9 \times 8{\cdot}2$ mm) (Fig. 1). Profuse growth was also obtained on the inoculated surface of carrot pieces and potato pieces covering more than 50% of the surface area (Fig. 2). In CE, growth occurred in the form of a thin layer covering the surface of the medium. In PE colonies were thin, scattered and floating on the surface. The detailed cultural characteristics are summarized in Table 1. In general, colonies on each medium grew for 10 days and later ceased to grow. Growth on all media was sporidial and no mycelial growth was seen (as confirmed by microscopic observations).

Effect of temperature on growth

The maximum growth of the fungus on PA occurred at 35° and the minimum at 40° (Table 2). At 20° and 25° the colonies initially remained dull, later turned white and raised. At 30°, the type of growth was similar to the growth at 35°. At 35° the shake-culture (in PE and CE) produced more sporidial growth ($3{\cdot}6 \times 10^7$ and $1{\cdot}5 \times 10^8$ sporidia/ml, respectively) than the still-culture ($2{\cdot}4 \times 10^7$ and $8{\cdot}3 \times 10^7$ sporidia/ml) (Figs 3, 4, respectively).

Effect of temperature on germination of teliospores

Germination of aggregated teliospores (sporeballs) was maximum at 30° (67·3%) and minimum at 15° (16%) (Fig. 5). In general there was a gradual increase in germination percentage with increase in temperature from 15 to 30° and a decline with further increase in temperature to 40° (23·5%). No germination occurred up to 9 h incubation; by 10 h, promycelia became visible with transverse septa; by 11 h sporidial formation had started on promycelia; by 16 h most sporidia were liberated from the promycelia.

Morphology

Sporeballs. The sporeballs varied from black to various hues of brown, from circular to near-polyhedral, and measured 42–325 μm × 50–175 μm (Fig. 6). The number of teliospores per sporeball varied from 200 to 1400 (based on observations of 50 sporeballs).

Teliospores. The teliospores were uniformly yellowish brown, globose to sub-globose with thickenings at 2 to 3 points in the exospure wall, and measured

Table 1. *Growth and culture characteristics of* Tolyposporium penicillariae *grown on different media at 35°*

Medium	Topography	Colour	Margin	Consistency
Potato extract	Thin colonies floating on the surface of the medium	Dull white	Wavy	Membranous
Potato pieces	Raised, finely ridged	Dull white–white	Lobate	Leathery
Potato agar	Thick, flat, broadly ridged, appearing like a frilled structure	Dull white	Wavy and lobate	Yeastoid
Potato-dextrose agar	Raised shiny colonies	Dull white	Entire	Yeastoid
Carrot extract	Thin layer covering the entire surface of the medium	Creamy	Entire	Membranous
Carrot pieces	Raised, finely ridged	White and Creamy	Wavy	Leathery
Carrot agar	Shiny colonies, raised in the centre, thin at periphery	Yellowish white	Entire	Yeastoid

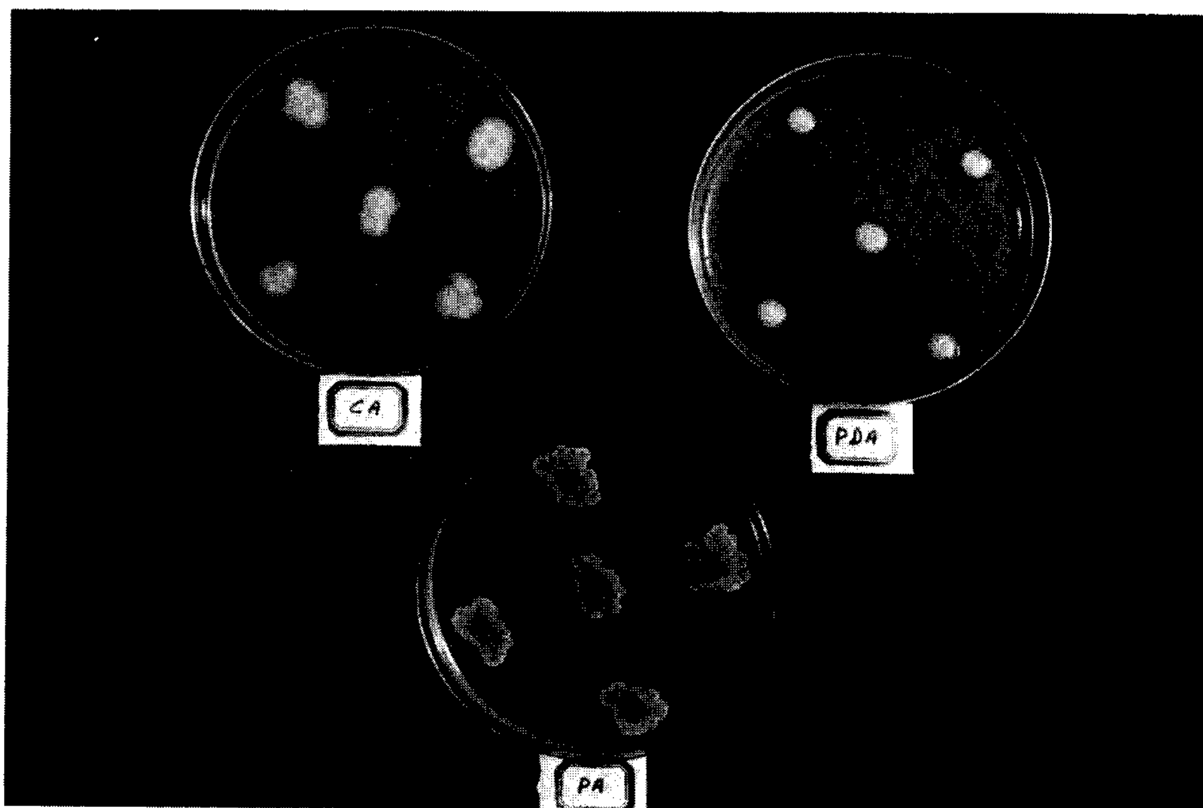


Fig. 1. Growth of *Tolyposporium penicillariae* on carrot agar (CA), potato dextrose agar (PDA) and potato agar (PA) after 5 days incubation at 35 °C.

Fig. 2. Growth of *Tolyposporium penicillariae* on carrot pieces (CP) and potato pieces (PP) after 5 days incubation at 35 °C.

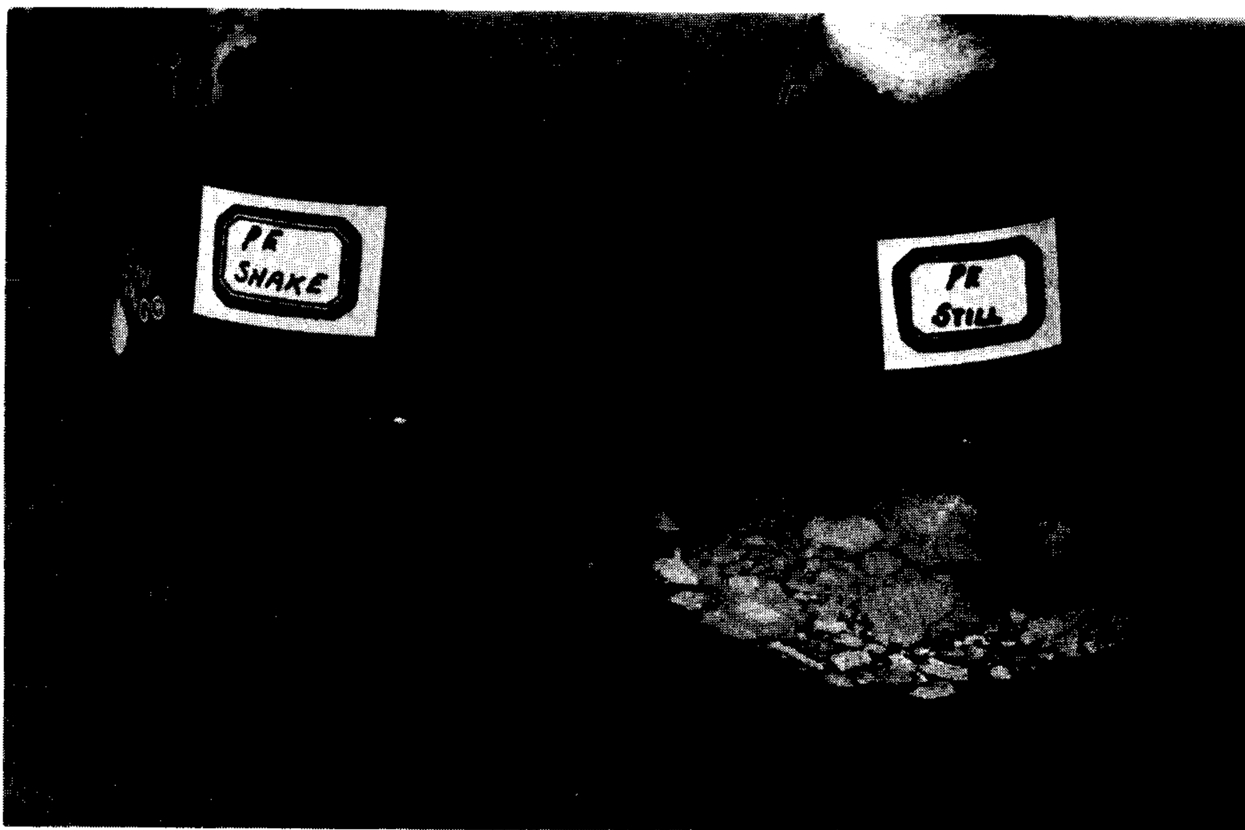


Fig. 3. Growth of *Tolyposporium penicillariae* in potato extract (PE) shake- and still-culture after 5 days incubation at 35 °C.

Fig. 4. Shake-culture of *Tolyposporium penicillariae* as compared to still-culture in carrot extract (CE) after 5 days incubation at 35 °C.

Table 2. *Culture characteristics of* Tolyposporium penicillariae *on potato agar at five temperatures of incubation*

Parameter	Temperature of incubation (°C) 20	25	30	35	40
Mean colony size* (mm)	9·7 × 6·8	10·0 × 7·5	11·5 × 8·6	12·6 × 9·4	1·9 × 1·1
Type of growth	Sporidial	Sporidial	Sporidial	Sporidial	Sporidial
Colour of colony	Dull white–white	Dull white–white	Dull white	Dull white	Dull white

* Mean of 12–88 colonies in 5 replications.

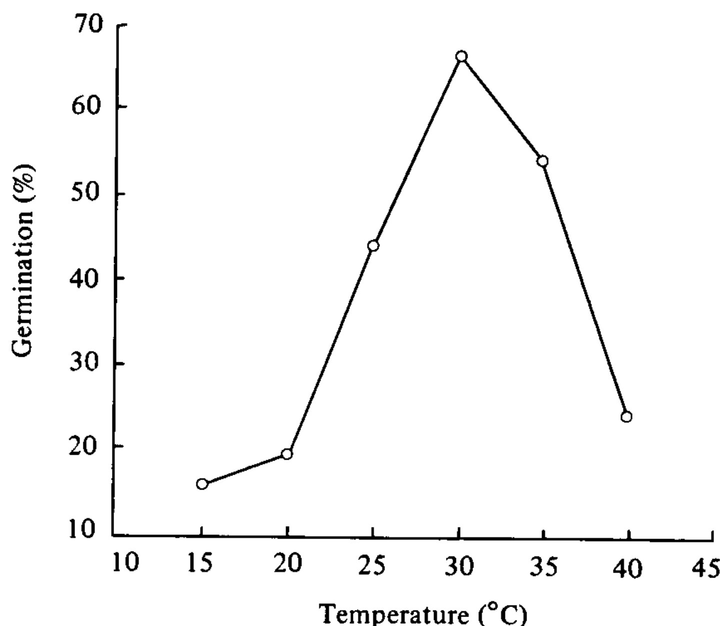


Fig. 5. Percentage germination of sporeballs of *Tolyposporium penicillariae* incubated for 13 h in water at six temperatures.

7·0–12·5 μm diam. Individual teliospores aggregated in sporeballs germinated in water by producing typical four-celled promycelia. However, some variations were noticed in the pattern of germination (Fig. 7). In some cases sporidia were borne either laterally or terminally on the four-celled promycelium while in others sporidia were produced on pointed branches, and by budding formed branched chains or clusters on 3- to 8-celled promycelia. Sporidia were also observed in branched chains without any promycelia. Individual, free teliospores invariably produced four-celled promycelia (Fig. 8). No teliospore was observed in the cultures.

Sporidia. Sporidia were hyaline, single-celled and spindle-shaped. They varied in length from 8 to 25 μm.

Chlamydospores. Cultures stored for 90 days at 10° produced chlamydospores which were intercalary and terminal and measured 4–8 μm diam (Fig. 9).

DISCUSSION

The growth of smut cultures may be mycelial, sporidial or a combination of both (Holton, Hoffmann & Duran, 1968). In these studies growth of *T. penicillariae* on all seven media was purely sporidial and this growth was maintained even after repeated subculturing. In contrast to these observations Pathak & Shekhawat (1980) quantified their results with *T. penicillariae* in terms of mycelial dry weight indicating mycelial growth.

Large variability exists in many species for spore wall ornamentation and spore germination which has contributed greatly to the dynamic taxonomic history of smut fungi (Holton *et al.*, 1968). Three different patterns of spore germination and formation of chlamydospores in the stored culture indicate the kind of variability that exists in *T. penicillariae*. Further studies are needed to understand the variability in this fungus by using monosporidial cultures and their interactions with the pearl millet host plant.

In screening for disease resistance, the availability of large quantities of effective inoculum is essential. In screening pearl millet for smut resistance, inoculation of individual plants at the boot leaf stage using *T. penicillariae* sporidial suspension provides good levels of infection (Bhatt, 1946; Thakur, Subba Rao & Williams, unpubl.). Growing the pathogen on a suitable medium which supports rapid sporidial growth of the pathogen is important for its use as an effective inoculum source. While several workers (Ajrekar & Likhite, 1933; Husain & Thakur, 1963; Pathak & Shekhawat, 1980; Tripathi & Bhaktavatsalam, 1977) reported different media to grow the pathogen, none of them aimed to produce large quantities of sporidial inoculum for large-scale field screening. Our results clearly

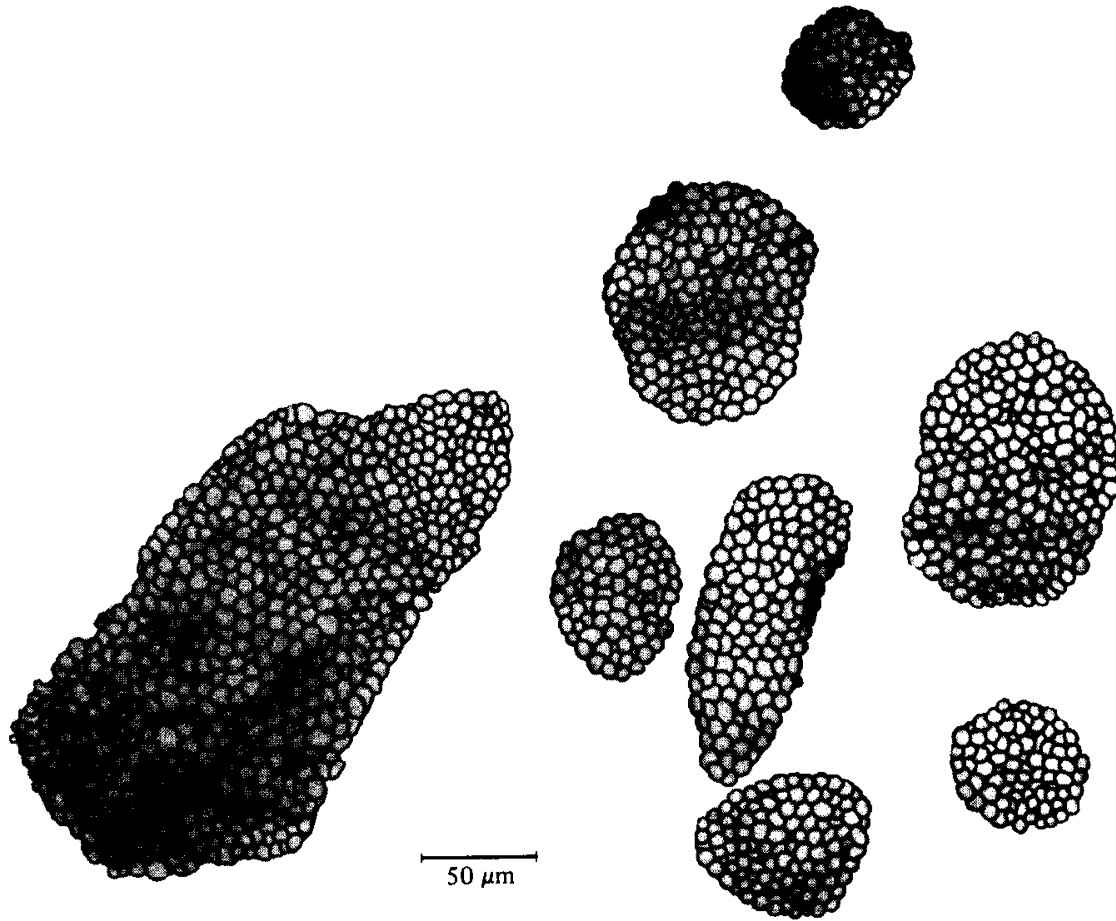


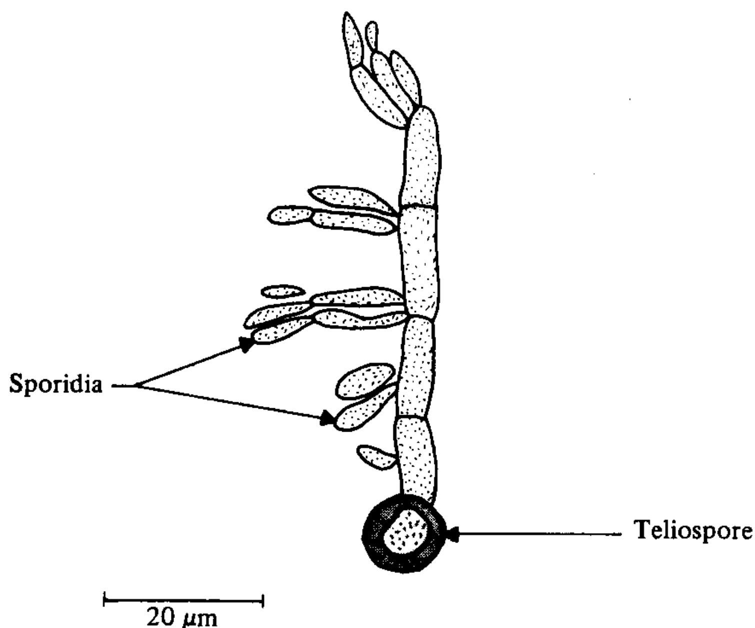
Fig. 6. Sporeballs of *Tolyposporium penicillariae*.

I
Terminal sporidia
Sporidial chain
Promycelium
Lateral sporidia

II

III
Sporidial cluster
Pointed branches
Promycelium
Sporidia in branched chains

Fig. 7. Patterns of teliospore germination in *Tolyposporium penicillariae*.



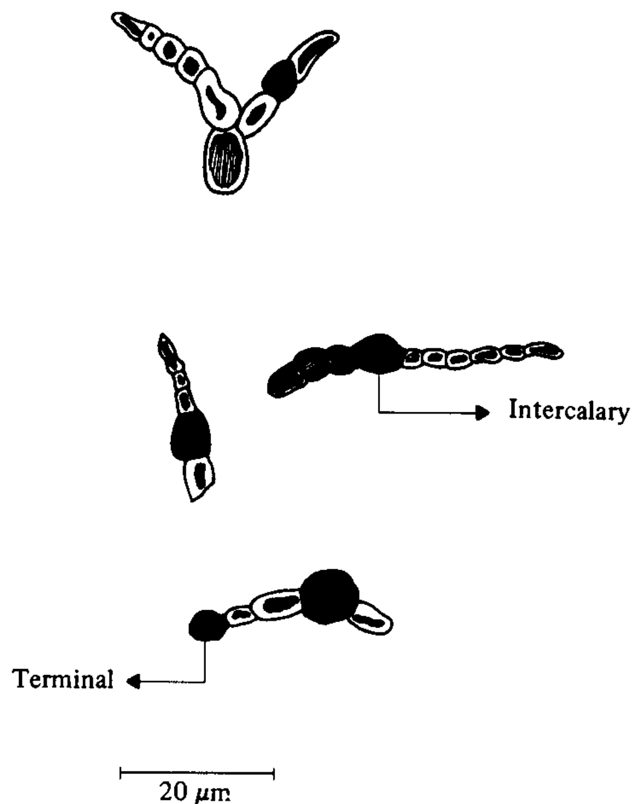


Fig. 8.

Fig. 9.

Fig. 8. A germinated teliospore of *Tolyposporium penicillariae*.
Fig. 9. Chlamydospores of *Tolyposporium penicillariae* in culture.

indicate that large quantities of sporidial inoculum can be produced in 3 days at 35° on a simple medium such as PA or CA and shake-culture in PE or CE. This inoculum has successfully been used for the past three crop seasons to screen hundreds of pearl millet lines for smut resistance at the ICRISAT Center.

The authors gratefully acknowledge Dr R. J. Williams, Principal Millet Pathologist, ICRISAT, for his continuing encouragement, inspiration and comments and suggestions on the manuscript.

Submitted as journal article no. JA 274 by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT).

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(*Received for publication 2 December 1982*)