

The Genus *Pithomyces* in South Africa

by

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ABSTRACT

Descriptions are given of South African isolates of *Pithomyces sacchari* (Speg.) M. B. Ellis, *Pithomyces chartarum* (Berk. & Curt.) M. B. Ellis and *Pithomyces karoo* Marasas & Schumann, sp. nov. *P. sacchari* and *P. chartarum* were isolated from *Medicago sativa* L. seed. *P. chartarum* was also isolated from dead leaves of *Lolium perenne* L. and *Sporobolus capensis* (Willd.) Kunth. plants from artificial pastures in the eastern Cape Province. *P. karoo* was isolated from stems of *Gnidia polycephala* (C.A. Mey.) Gilg and *Rhigozum trichotomum* Burch. from the Karoo, Cape Province and from *Avena sativa* L. stubble collected in the Orange Free State.

During the course of an investigation of the seedborne mycoflora of South African lucerne seed, *Pithomyces sacchari* (Speg.) M. B. Ellis and *P. chartarum* (Berk. & Curt.) M. B. Ellis were isolated from *Medicago sativa* L. seed produced in the Cape Province. *P. chartarum* and a new species, *P. karoo* Marasas & Schumann sp. nov., were isolated during the course of a mycological examination of several photosensitization syndromes in sheep in the Cape Province and Orange Free State.

Isolations were made by placing seeds or small pieces of plant tissue without surface sterilization on 1½% malt extract agar containing 100 mg/l of sodium novobiocin and incubating at 25° C. Morphological descriptions were prepared of cultures incubated on 1½% malt extract agar (malt extract: 15 g; agar: 17 g; distilled water: 1 l) and potato-carrot agar (potatoes: 20 g; carrots: 20 g; agar: 17 g; distilled water: 1 l). All spore measurements are based on spores mounted in lactophenol and were made with the aid of an oil-immersion lens at a magnification of ×1250.

A key to and morphological descriptions of the South African species of *Pithomyces* are given below.

- A. Conidia produced on potato-carrot agar and 1½% malt extract agar at 25° C predominantly smooth-walled or almost so, transverse septa 0-2-5, longitudinal septa 0-0-2, 9-15-28 × 4-6-10µ.....*P. sacchari*
- Conidia produced on potato-carrot agar and 1½% malt extract agar at 25° C definitely rough-walled.....B
- B. Conidia verruculose, transverse septa 0-3-5, longitudinal septa 0-2-3, 14-20-36 × 8-12-21µ..... *P. chartarum*
- Conidia coarsely verrucose, transverse septa 0-2-5, longitudinal septa 0-1-3, 10-22-41 × 9-15-20µ..... *P. karoo*

1. *Pithomyces sacchari* (Speg.) M. B. Ellis in Mycol. Papers 76: 17 (1960); Hughes, Mycol. Papers 50: 70 (1953); Lakshminarasimhan & Rama Rao, Curr. Sci. 38: 74 (1969).

Figures: 1, 2.

Colonies on potato-carrot agar at 25° C are flatly appressed with little aerial mycelium, white, beginning to sporulate after 5 days and becoming tinged black with spores, particularly in the centre. On 1½% malt extract agar at 25° C colonies are woolly, white to olive-grey with the reverse smoky-grey to black, beginning to sporulate after 4 weeks. *Vegetative mycelium* composed of hyaline, smooth or coarsely verrucose, branching, septate hyphae, 1-3µ diam., and pale brown, smooth or verrucose hyphae, 4-7µ diam., often forming strands. *Conidiophores* arise laterally on the aerial mycelium at right angles to the parent hyphae, peg-like or cylindrical, hyaline to subhyaline, thin-walled, straight or curved, non-septate, 2-6 × 1,5-2,0µ,

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frequently densely clustered and bearing conidia in sporodochium-like masses, following spore dispersal the remains of the conidiophores appear as denticles along the hyphae (Fig. 2). *Conidia* arise singly as blown-out ends of each conidiophore, obovoid or clavate, brown, smooth-walled when immature and at maturity, rarely slightly roughened, with 1–5 transverse and 0–2 longitudinal septa, mostly 2 transverse and 0 longitudinal septa (Fig. 1; Table 1), slightly constricted at the septa $9\text{--}28 \times 4\text{--}10\mu$ (Table 2). Each conidium bears a short, hyaline basal frill which is the upper part of the conidiophore (Fig. 1).

Specimens examined: Cultures on 1½% malt extract agar and potato-carrot agar: PRE 44584, PRE 44585, PRE 44586 (Mycological Herbarium), isolated from *Medicago sativa* L. seed, Oudtshoorn, Cape Province, 1970.

Considerable difficulty was experienced in assigning these three South African isolates to a species. The spores are very similar to those of *P. sacchari* (Speg.) M. B. Ellis as described by Ellis (1960), and to those of *Sporidesmium bakeri* Syd. var. 1 as illustrated by Hughes (1953). The latter variety was considered a synonym of *P. sacchari* by Ellis (1960). The immature and mature conidia of the South African isolates are, however, predominantly smooth-walled on the agar media used and in this respect most closely resemble the conidia of *P. graminicola* Roy & Rai as described by Roy & Rai (1968). An examination of the type collection of *P. graminicola* (IMI 126508, on *Saccharum minja*, Banaras Hindu University Campus, India) revealed that the conidia of this fungus are smooth-walled, pyriform to clavate with 0–2 transverse and no longitudinal septa, $7\text{--}12 \times 4\text{--}6\mu$ (mostly $9 \times 5\mu$) (Fig. 3). These spore characteristics are quite different from those of the three South African isolates (Fig. 1; Table 1, 2).

Dr. M. B. Ellis of the Commonwealth Mycological Institute, Kew, England, examined isolate PRE 44585 (= IMI 153452) and found that it produces: "short, obovoid, clavate and pyriform conidia with rough walls and longitudinal as well as transverse septa after 2 weeks on oat agar and I take this to be *Pithomyces sacchari*" (M. B. Ellis, personal communication).

P. sacchari has previously been recorded as a saprophyte on various plants from a number of countries (Hughes, 1953; Ellis, 1960) and has also been reported on leaves of *Saccharum officinarum* in Jamaica (Hudson, 1962) and in soil in India (Lakshminarasimhan & Rama Rao, 1969).

This is the first record of the occurrence of *P. sacchari* in the Republic of South Africa.

2. *Pithomyces chartarum* (Berk. & Curt.) M. B. Ellis in Mycol. Papers 76: 13 (1960); Hughes, Mycol. Papers 50: 66 (1953); Dingley, N.Z.J. Agr. Res. 5: 49 (1962).

Figure: 4

Colonies on potato-carrot agar at 25° C are flatly appressed with little aerial mycelium, white, beginning to sporulate after one to two weeks and becoming tinged black with spores. On 1½% malt extract agar at 25° C colonies are woolly or floccose, olive-grey in the centre with a white margin and a smoky-grey to black reverse, beginning to sporulate after one to four weeks, becoming black with a crust of spores, often giving rise to sectors which are more densely floccose than the rest of the colony and sporulate more heavily. *Vegetative mycelium* composed of thin-walled, hyaline, septate, smooth or verrucose, branching hyphae, 2–5µ diam., and dark-brown, smooth or verrucose, septate hyphae, 4–7µ diam., which may give rise to chains of verrucose, one-celled, dark-brown, intercalary chlamydospores, $10\text{--}20 \times 8\text{--}18\mu$. *Conidiophores* arise laterally on the aerial mycelium at right angles to the parent hyphae, hyaline, thin-walled, straight or curved, non-septate or one-septate, $3\text{--}15 \times 2\text{--}3\mu$, frequently clustered and bearing conidia in sporodochium-like masses. *Conidia* arise singly as blown-out ends of each conidiophore, at first fusoid, coarsely echinulate, hyaline

to pale brown, one-celled, becoming muriform and dark-brown. Mature conidia broadly ellipsoidal, rarely obovoid or clavate, dark-brown, verrucose, slightly constricted at the septa, with 2–5 transverse and 0–3 longitudinal septa, usually 3 transverse and 1–2 longitudinal septa in the central cells (Fig. 4; Table 1), $14\text{--}36 \times 8\text{--}21\mu$ (Table 2). Conidia become detached through fracture of the conidiophore wall and consequently each conidium characteristically bears a short, hyaline basal frill which is the upper part of the conidiophore (Fig. 4).

Specimens examined: Cultures on 1½% malt extract agar and potato-carrot agar: PRE 44581 (Mycological Herbarium), isolated from dead leaves of *Sporobolus capensis* (Willd.) Kunth., Robbehoek, Humansdorp Distr., Cape Province, September 1970, *Marasas* OP-9; PRE 44599, isolated from dead leaves of *Lolium perenne* L., Keokama, Humansdorp Distr., September 1970, *Marasas* OP-10; PRE 44582, isolated from *Medicago sativa* L. seed, Ladismith, Cape Province, June 1970, *Marasas* 306; PRE 44584, isolated from *M. sativa* seed, Oudtshoorn, Cape Province, July 1970, *Marasas* 366.

P. chartarum has previously been recorded as a saprophyte on paper and dead leaves and stems of many different plants from a number of temperate, sub-tropical and tropical countries, including Malawi, Rhodesia and Zambia (Hughes, 1953; Morris, 1956; Ellis, 1960; John, 1963; Sahni, 1966). This fungus has also been reported from Australia (Hore, 1960) and New Zealand (Dingley, 1962) where it is known to cause facial eczema in sheep; from the air on spore traps in Britain (Lacey & Gregory, 1962; Gregory & Lacey, 1964; Pawsey, 1964); from soil in Honduras (Goos, 1964) and Ontario (Barron, 1968); from wheat grains in Australia (Shipton & Chambers, 1966); from groundnuts and grasses in Texas (Taber, Pettit, Taber & Dollahite, 1968) and from human foodstuffs in Japan (Udagawa, Ichinoe & Kurata, 1970).

This is the first record of the occurrence of *P. chartarum* in the Republic of South Africa.

3. *Pithomyces karoo* Marasas & Schumann sp. nov.

Figures: 5, 6, 7, 8, 9, 10.

Hyphae ramosae, septatae, hyalinae vel brunneae, leves vel verrucosae, $2\text{--}6\mu$ crassae. *Chlamydosporae* intercalares vel terminales, solitariae vel catenulatae, uni- vel bi-cellulares, leves vel verrucosae, fuscae, crassitunicatae, $5\text{--}15 \times 5\text{--}10\mu$. *Conidiophora* singula ex apice lateribusque hypharum oriunda, simplicia, continua vel septata, cylindrica, recta vel flexuosa, hyalina, $3\text{--}25 \times 2\text{--}4\mu$. *Conidia* singula in apice conidiophori oriunda, ellipsoidea, obovoidea, obpyriformia, clavata vel sarcinaeformia, brunnea vel fusca, verrucosa, septis constricta, septis 0–5 transversalibus et 0–3 longitudinalibus praedita, $10\text{--}41 \times 9\text{--}20\mu$.

Colonies on potato-carrot agar at 25° C flatly appressed with little or no aerial mycelium, white, beginning to sporulate within 3 days and becoming tinged black with spores, particularly in the centre; at the edge of the petri dish old colonies sometimes develop a ring of downy white aerial mycelium or a brown ring of immersed spores that differ morphologically from the spores produced on the aerial mycelium. On 1½% malt extract agar at 25° C colonies are slow-growing, woolly or velvety, aerial mycelium in the centre Greyish Olive (Ridgway, Plate XLVI), beginning to sporulate within 3 days and becoming black in the centre with masses of spores that obscure the aerial mycelium; margin appressed with little or no aerial mycelium, white to Light Greyish Olive (Ridgway, Plate XLVI); colony reverse Cinnamon-Buff (Ridgway, Plate XXIX) to Vinaceous-Russet (Ridgway, Plate XXVIII) in the centre surrounded by a Light Yellowish Olive (Ridgway, Plate XXX) zone that becomes almost black in age and a white margin; young colonies of some single spore isolates produce an exudate that stains the surrounding agar Vinaceous-Russet (Ridgway, Plate XXVIII), but this exudate is not evident in old colonies. *Vegetative mycelium*

composed of several types of hyphae: hyaline, thin-walled, septate, branching, smooth or verruculose hyphae that are occasionally coarsely warted, $2-6\mu$ diam., and brown, multiseptate, smooth or verruculose hyphae that are often aggregated into strands, composed of cylindrical or ellipsoidal cells, $5-15 \times 3-6\mu$; the hyaline as well as brown hyphae occasionally give rise to intercalary or terminal, one- or two-celled, smooth or verruculose, brown, thick-walled, ellipsoidal or ovoid to subglobose chlamydospores that are solitary or produced in short chains, $5-15 \times 5-10\mu$ (Fig. 10). *Conidiophores* arise laterally on the aerial mycelium at right angles to the parent hyphae, hyaline, thin-walled, straight or curved, usually non-septate but occasionally with one or two septa, short and cylindrical, $3-25 \times 2-4\mu$ (Fig. 6); occasionally long, flexuous, septate, hyaline, thin-walled hyphae indistinguishable from vegetative hyphae, terminate in conidia (Fig. 7); following spore dispersal the remains of the conidiophores appear as denticles along the hyphae. *Conidia* aleuriospores that arise singly as blown-out ends of each conidiophore, at first fusoid, hyaline to straw-coloured, coarsely verruculose to warted, one-celled, becoming muriform and dark brown (Fig. 6). Mature conidia mostly ellipsoidal with broadly rounded ends occasionally obovoid, obpyriform, clavate, cruciform, sarciniform or curved, dark brown, thick-walled, coarsely verruculose to warted with obtusely rounded warts up to 2μ long and 2μ wide at the base, conspicuously constricted at the septa, with 0-5 transverse and 0-3 longitudinal septa, occasionally sarciniform with numerous transverse, longitudinal and oblique septa, mostly with 2 transverse and 1 or 2 longitudinal septa in the central and/or terminal cells (Figs. 5, 8; Table 1), $10-41 \times 9-20\mu$, mostly $20-24 \times 15-16\mu$, including warts, width measured in the broadest part (Table 2); conidia become detached through fracture of the conidiophore wall and consequently each conidium bears a short, hyaline basal frill which is the upper part of the conidiophore (Fig. 5). *Immersed conidia* are produced in the agar on slender, thin-walled, hyaline conidiophores that are poorly differentiated from the thin-walled, hyaline, immersed hyphae, $5-15 \times 1.5-2.5\mu$; hyaline, subhyaline to light brown, thick-walled, smooth or verruculose, ellipsoidal with broadly rounded ends or obovoid, obpyriform or cruciform, one- to four-celled, occasionally sarciniform and multi-celled, mostly two- or three-celled, $10-20 \times 10-15\mu$ (Fig. 9).

Specimens examined: Cultures on $1\frac{1}{2}\%$ malt extract agar and potato-carrot agar: PRE 44605 (Holotype), isolated from dead stems of *Gnidia polycephala* (C.A. Mey.) Gilg, Riekersfontein, Colesberg Distr., Cape Province, February 1970, *Marasas* OP-11; PRE 44606, isolated from *Avena sativa* L. stubble, Rusthof, Heilbron Distr., Orange Free State, March 1971, *Marasas* OP-12; PRE 44607, isolated from living stems of *Rhigozum trichotomum* Burch., heavily infested by scale insects and a sooty mould, Welverdiend, Hopetown Distr., Cape Province, March 1971, *Marasas* OP-13.

Type specimens in the form of dried-down cultures on agar have been deposited in the National Herbarium, PRE (Mycological Herbarium), Department of Agricultural Technical Services, P.O. Box 994, Pretoria, South Africa. A culture of the type strain (PRE 44605) has also been deposited in the Commonwealth Mycological Institute, Kew, England, under accession number IMI 155881.

P. karoo differs from *P. chartarum* in having coarsely verruculose conidia that predominantly have two transverse septa and are much more irregular in shape. The morphologically dissimilar immersed conidia found in cultures of *P. karoo* on PCA were not found in cultures of *P. chartarum*. The significance of these immersed conidia can not be interpreted at present. Marked differences between the spores of *Trichocladium opacum* (Corda) Hughes produced on aerial hyphae and those on immersed mycelium were also noted by Kendrick & Bhatt (1966).

A study is in progress to determine the effects of temperature on growth, sporulation and spore morphology of the South African *Pithomyces* isolates.

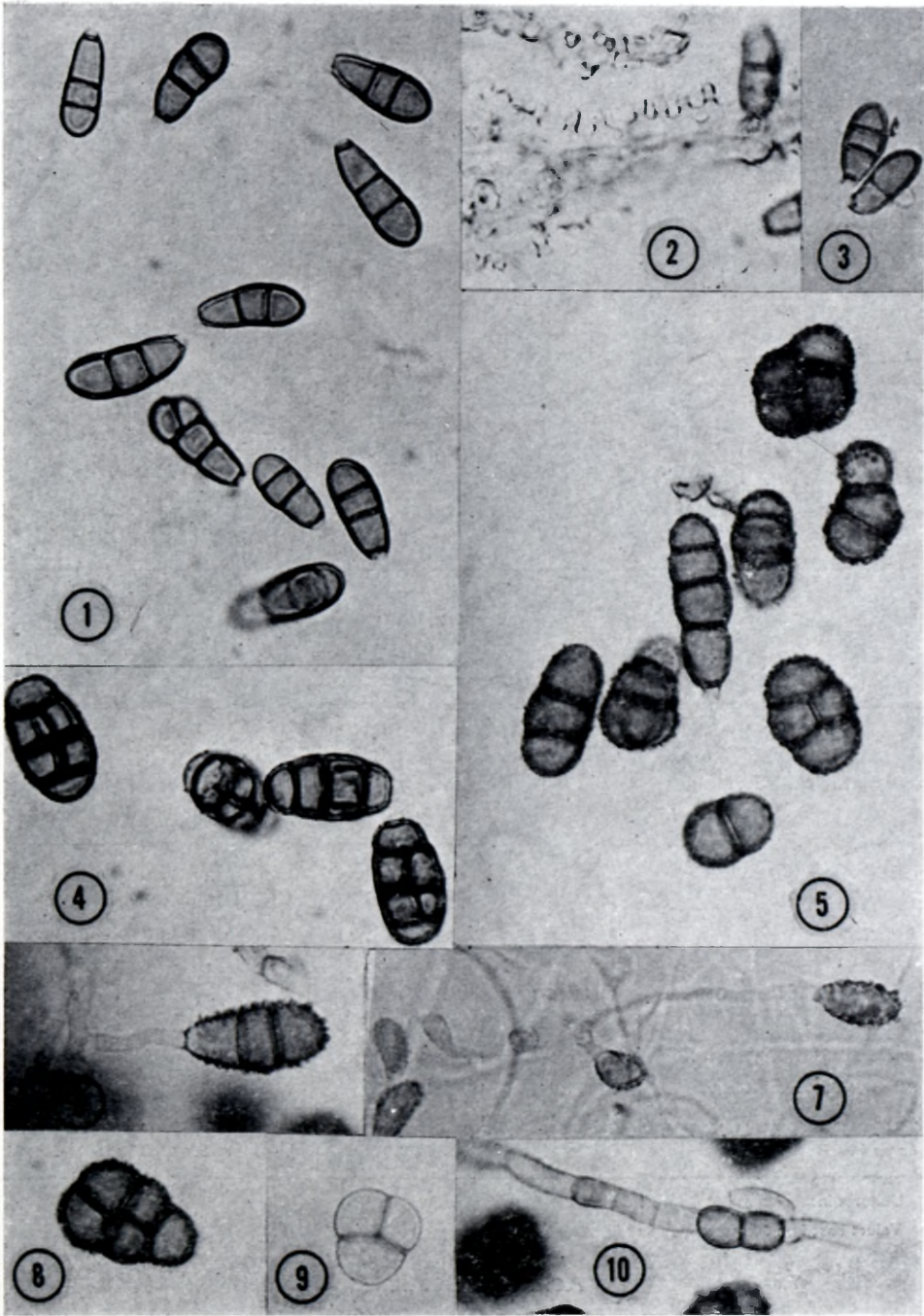


FIG. 1-2.—*Pithomyces sacchari*. Fig. 1, mature conidia. Fig. 2, denticulate hypha ($\times 1\ 000$).
 FIG. 3.—*Pithomyces graminicola* (Type), mature conidia ($\times 1\ 000$).
 FIG. 4.—*Pithomyces chartarum*, mature conidia ($\times 1\ 000$).
 FIG. 5-10.—*Pithomyces karoo* (Type). Fig. 5, mature conidia. Fig. 6, immature conidium on hyaline conidiophore. Fig. 7, aerial hypha terminating in a conidium. Fig. 8, mature conidium with a longitudinal septum in the terminal cell. Fig. 9, immersed conidium. Fig. 10, intercalary chlamydospore ($\times 1\ 000$).

TABLE 1.—Spore septation of *Pithomyces* species^a.

Species & isolate (PRE)	Septation (% of spores ^b)																	
	Potato-carrot agar						1½% malt extract agar											
	Transverse			Longitudinal			Transverse			Longitudinal								
	1	2	3	4	5	0	1	2	3	1	2	3	4	5	0	1	2	3
<i>P. chartarum</i> 44581.....	0	0	96	4	0	10	36	54	0	0	0	98	2	0	8	36	56	0
<i>P. chartarum</i> 44599.....	0	4	96	0	0	14	30	56	0	0	0	100	0	0	10	37	56	0
<i>P. chartarum</i> 44582.....	0	0	96	4	0	18	32	50	0	No spores			No spores					
<i>P. chartarum</i> 44583.....	0	0	70	24	6	8	30	62	0	0	20	70	8	2	6	44	48	2
<i>P. sacchari</i> 44584.....	8	60	26	4	2	74	20	6	0	No spores			No spores					
<i>P. sacchari</i> 44585.....	20	44	30	6	0	92	8	0	0	No spores			No spores					
<i>P. sacchari</i> 44586.....	26	48	24	2	0	90	10	0	0	No spores			No spores					
<i>P. karoo</i> 44605.....	34	58	8	0	0	42	38	20	0	22	68	10	0	0	30	44	26	0
<i>P. karoo</i> 44606.....	40	50	10	0	0	14	52	34	0	26	56	12	4	2	20	52	26	2
<i>P. karoo</i> 44607.....	36	48	12	4	0	6	46	46	2	32	60	6	0	2	18	40	38	4

^a Cultures incubated at 25° C for 21 days.^b Values based on 50 spores mounted in lactophenol.TABLE 2.—Spore measurements of *Pithomyces* species^a.

Species & isolate (PRE)	Spore size (μ) ^b					
	Potato-carrot agar			1½% malt extract agar		
	Range	Average	Mean	Range	Average	Mean
<i>P. chartarum</i> 44581.....	16–25 × 8–14	20,1 × 11,1	20 × 11	18–26 × 10–13	20,3 × 11,2	20 × 12
<i>P. chartarum</i> 44599.....	14–25 × 8–15	20,3 × 11,7	21 × 12	15–24 × 9–14	19,9 × 11,7	19 × 12
<i>P. chartarum</i> 44582.....	17–26 × 9–15	20,3 × 12,5	20 × 12	No spores		
<i>P. chartarum</i> 44583.....	19–30 × 9–16	22,6 × 12,8	21 × 12	18–36 × 12–21	25,9 × 16,8	25 × 16
<i>P. sacchari</i> 44584.....	10–22 × 5–10	15,7 × 6,5	15 × 6	No spores		
<i>P. sacchari</i> 44585.....	9–28 × 4–8	16,8 × 5,8	14 × 6	No spores		
<i>P. sacchari</i> 44586.....	9–25 × 5–8	15,1 × 6,1	16 × 6	No spores		
<i>P. karoo</i> 44605.....	10–27 × 9–17	19,8 × 12,9	20 × 15	16–31 × 12–19	22,3 × 14,3	23 × 16
<i>P. karoo</i> 44606.....	13–31 × 11–17	19,9 × 14,8	22 × 15	13–36 × 11–19	23,5 × 15,1	24 × 15
<i>P. karoo</i> 44607.....	16–41 × 11–17	22,0 × 14,8	21 × 15	13–41 × 10–20	21,1 × 15,0	21 × 15

^a Cultures incubated at 25° C for 21 days.^b Values based on 50 spores mounted in lactophenol.

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