GAEUMANNOMYCES LEPTOSPORUS IN PAPUA NEW GUINEA

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ABSTRACT

Gaeumannomyces leptosporus *Iqbal developed on submerged decayed leaves held in water culture in the laboratory. The long perithecial necks, however, protruded into the air above the water surface. On removal of the substrate into a drier atmosphere, asci were extruded in a milky droplet at the tip of each neck. The ascospores were 3-septate, with one nucleus per cell. Simple, dark brown spherical to pear-shaped appressoria developed in hanging drops from ascospores germinating within asci and as released spores. No hyphopodia developed on sterile or asterile plant surfaces when inoculated with the isolate. Although this is only the second record of the fungus in the world, the first being in England, its occurrence in two such diverse locations probably indicates that it is of worldwide distribution.*

INTRODUCTION

Iqbal (1972) described *Gaeumannomyces leptosporus* as a new aquatic ascomycete occurring with immature perithecia on decaying submerged umbelliferous branches in the River Creedy, England. Mature ascospores developed after three weeks when the branches were incubated in plastic bags at 15° C. The present paper reports the occurrence of this fungus in Papua New Guinea.

Papua New Guinea Record

In March, 1976 this species (PNG 10147; IMI 202624) was found on brown, decaying unidentified leaves of a broad-leaved species, held for several weeks in water culture in Petri dishes in the laboratory, having been originally collected as brown submerged leaves from a stream at about 457 m altitude, about 24 km from Port Moresby.

Although the perithecia occurred on the submerged leaves in Petri dishes, the perithecial necks extended above the water surface. Perithecial necks of *Gnomonia papuana* reported recently by Sivanesan and Shaw (1976) did likewise in a similar situation. Also, as with *Gn. papuana*, when the leaf material was removed from the water culture into a dry Petri dish base, a white milky globule formed at the perithecial tip within about 15 minutes, the globule containing extruded asci. Later the globule became honey-coloured, adhering to the perithecial tip and resisting pressure with a needle, and at that stage contained ascospores (*Plate* I E).

Asci from the milky terminal globules when streaked on to potato dextrose agar (PDA) plates germinated slowly, mainly from both ends of the enclosed ascospores (*Plate* I D). Cultures established from single asci were at first white and began to produce long-necked perithecia after three weeks, as reported by Iqbal, mainly at the inoculum site; later perithecia were produced over the whole surface of the medium in cultures with a bacterial contaminant. Necks were up to 2.4 mm long, mainly at right angles to the medium surface, and appeared to show no geotrophic effect. Purified cultures later became grey, with an appressed growth which became slightly crust-like after about five weeks so that it could be lifted without much difficulty from the surface of the agar; perithecia did not form in the purified cultures on this medium.

As mentioned above, asci from the original substrate germinated very slowly. Asci from culture also germinated slowly in water in hanging drops, but the ascospores were even slower. No germination occurred in 24 hours, but by 69 hours, while only 7 out of 44 ascospores examined had germinated, 60 out of 62 asci examined had produced germ tubes. Whether the slow germination of asci and ascospores has some significance as a survival factor is not known.

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After 69 hours in the hanging drop, two of the asci had each produced a brown appressorium, while another had produced one hyaline and one brown appressoria. A few days later, in two traverses 3 out of 13 ascospores had germinated, one with an appressorium, while 11 out of 15 asci had produced appressoria, some with up to four, all dark brown.

The appressoria were simple and non-lobed, circular to pear shaped with a truncate base at the septum, 3.5 to 4.5 um wide and mainly 7.5 to 10.5 um long, at first hyaline, later dark brown, occasionally with a second intercalary appressorium immediately adjacent to the terminal one which formed first. In most cases the appressoria appeared at the tips of germ tubes which were 4.5 to 31.5 um long, mainly from the end cells, occasionally from the sides, of the ascus. In a few cases, the appressoria appeared sessile or nearly sessile on the ascus or ascospore (*Plate* II E, F).

No hyphopodia were noted when pieces of culture were inoculated on to live coleoptiles produced from surface sterilized germinating grains of wheat, oats, maize, rice and Triticale in Petri dishes lined with wet filter paper, or on leaves of *Auracaria hunsteinii* on detached shoots maintained alive in a humid atmosphere, or on sterile grass pieces on water agar in Petri dishes. In all these cases the hyphae radiated sparsely over the surface of the tissue around the inoculum piece without forming hyphopodia.

The fungus agrees well with Iqbal's description, with perithecial necks up to 2.4 mm long by up to 39 um wide (*Plate* I A), asci 75 to 90 x 4.5 to 5.8 um (*Plate* I B), and with a refractive body in the ascus tip 1.8 x 1.2 um in size, with median canal and slightly flattened base (*Plate* I C) and ascospores 66 to 81 x 1.0 to 1.5 um (*Plate* II A). The number of septa in the spores could not be distinguished in mounts of water, cotton blue lactophenol, lactic fuchsin or Lugol's solution. Ascospores mounted in a small drop of water, then allowed to air-dry on the slide, exhibited what appeared to be a slight thickening of the walls at three places along the spore (*Plate* II B), probably indicating the position of septa.

Asci and ascospores air-dried on slides and stained with Giemsa after acid hydrolysis in N HCI at 60° showed one nucleus per cell, that is, four nuclei per spore. The two centre groups of nuclei in the ascospores still retained in the asci were mainly stained deep purple, and each nucleus was more compact than the nuclei at each end of the spore. These latter were more reddish purple, more diffuse and more elongated than those in the two centre groups (*Plate* II C). In released ascospores the four nuclei were usually elongated (*Plate* II B). In most ascospores a small area at the tip of the spore was tinged red, but did not give a positive purple reaction. While many ascospores were lost during the staining and washing procedures, all the asci adhered firmly to the slides, perhaps indicating that some fluid from the extrusion globule had remained on the asci and was acting as a cementing agent.

No asexual state was found in the cultures on PDA, and none formed when pieces of these cultures were floated on water in Petri dishes, or when the isolate was subcultured on to sterile dead stems of *Barleria cristata* (a substrate often used in this laboratory) and no perithecia formed on the dead stems. Small very slightly coloured spherical knots of hyphae formed on some of the floating pieces of culture, but no further development took place.

DISCUSSION

Iqbal's collection consisted of immature perithecia on submerged decaying branches, and mature perithecia developed when the material was held in plastic bags, but whether this entailed a humid atmosphere only, or immersion in water in the bags, was not stated. In culture, Iqbal obtained perithecia without flooding, as did the present author.

Perithecia in the present collection formed on the submerged substrate, and were apparently mature, as asci were extruded within a short time when the material was removed from the water culture and allowed to air-dry.

It should be noted that these mature asci were not extruded until drying occurred, even though the tips of the perithecial necks protruded above the water surface into the air.

Whether the fungus colonized the leaves before immersion, or whether the submerged substrate was colonized by propagules from the stream, is not known. It is also not known whether asci and ascospores will mature if the substrate remains totally submerged, and

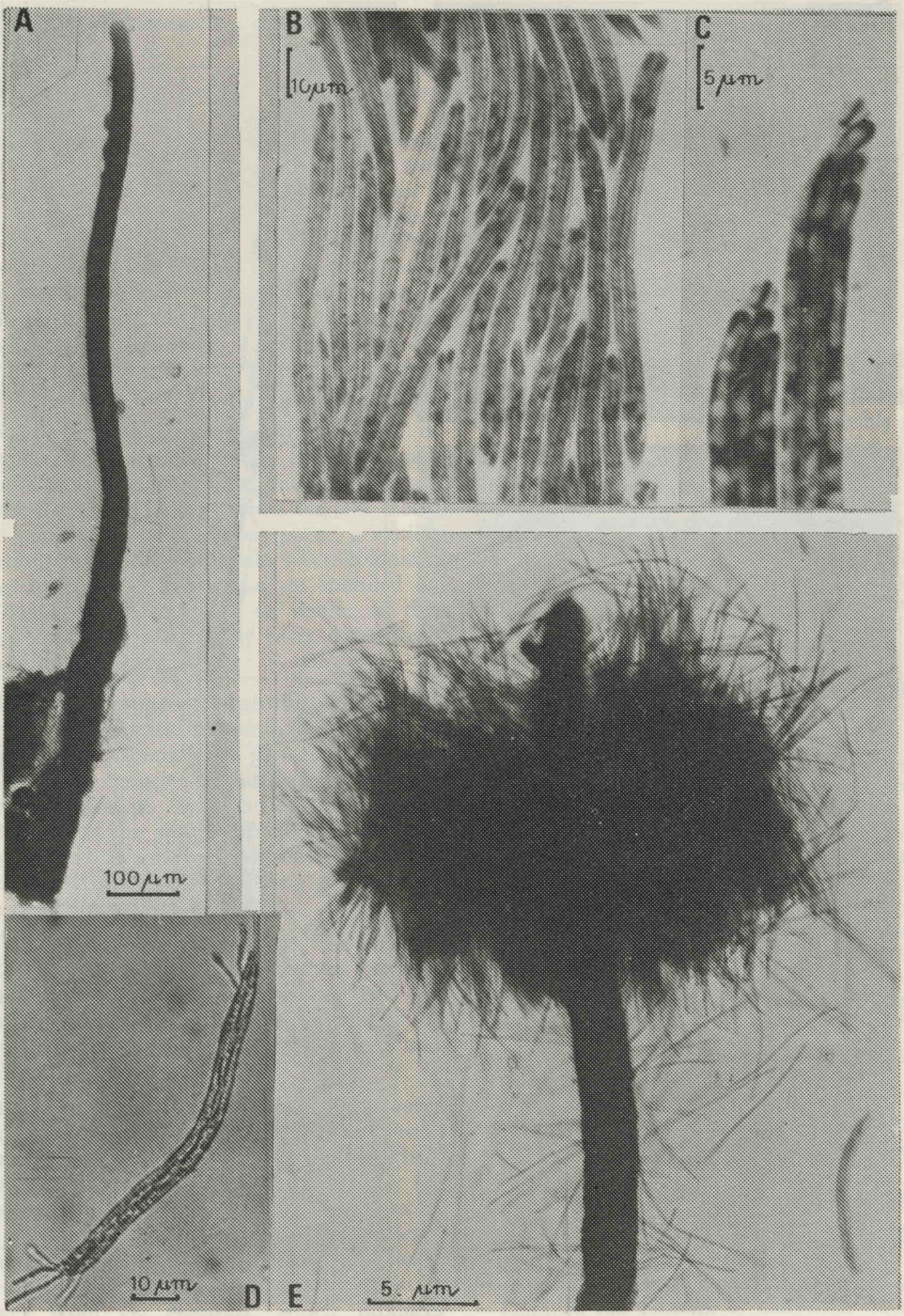

Plate I.— A. Perithecial neck from original substrate. B. Asci extruded in globule from neck tip in drier atmosphete; stained lactic fuchsin. C. Two ascus tips showing median canal in apical ring; stained cotton blue lactophenol. D. Extruded ascus with ascospores germinating at both ends; unstained. E. Ascospores in terminal globule some time after extrusion; only a few asci still remaining; stained lactic fuchsin

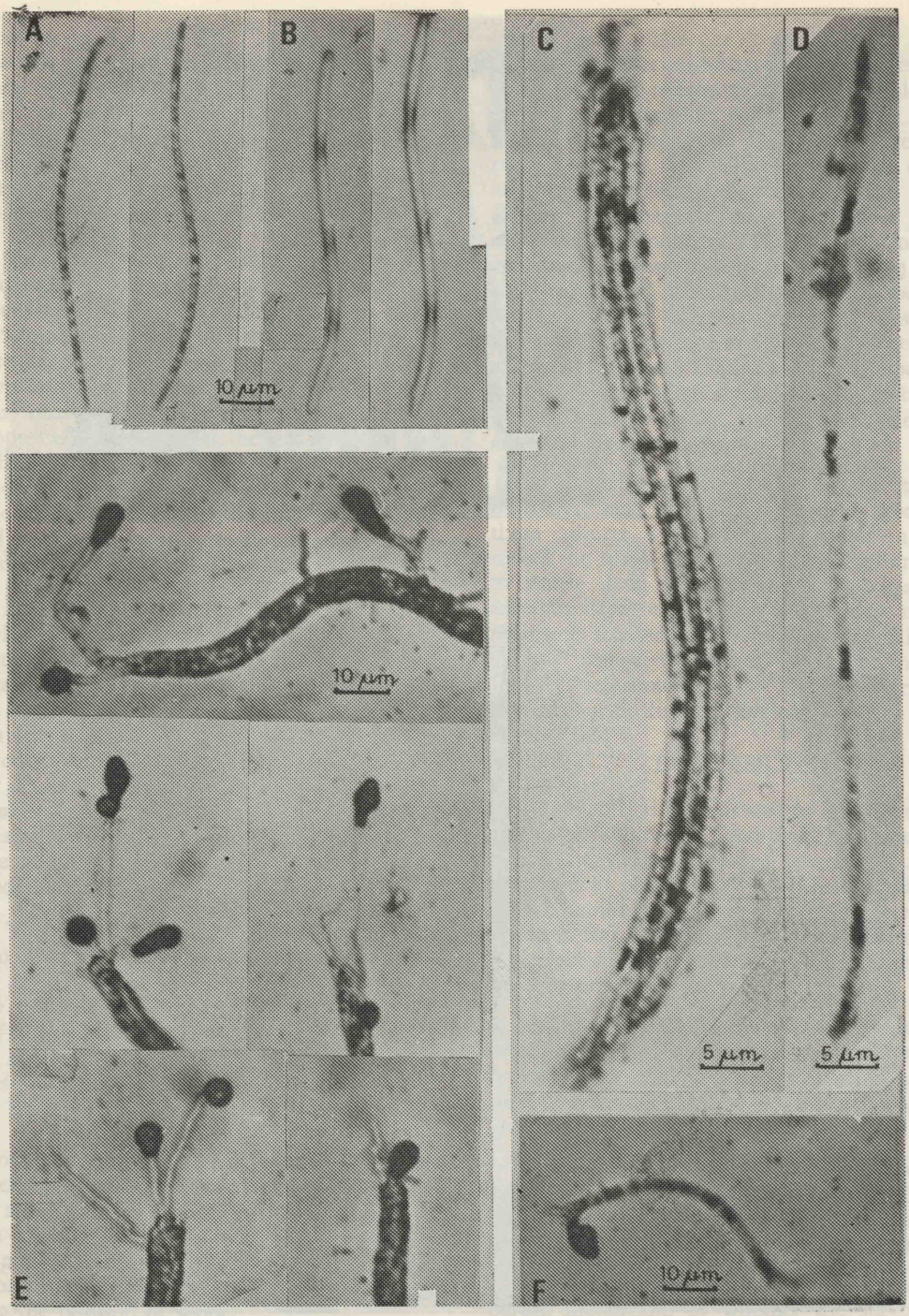

Plate II.—A. Two ascospores, stained cotton blue lactophenol. B. Two ascospores, air-dried, unstained and unmounted, showing position of septa. C. Ascus stained with Giemsa, showing four areas of nuclei in the enclosed ascospores; some nuclei out of focus. D. Ascospore stained with Giemsa showing four elongated nuclei per spore. E. Various appressoria from ascospores germinating within asci in hanging drops; unstained. F. Appressorium produced on germinating ascospore in hanging drop; unstained

Table 1.—Ascomycetes recorded as ejecting or extruding asci or ascospores when placed in a condition of lower humidity

Sordaria fimicola	Ingold & Mashall 1962*	ascospores	ejected
S. fimicola	Austin 1968*	"	"
Nectria galligena	Lortie & Kuntze 1963*	"	"
Wetted lichen fruit bodies	Ahmadjian 1967*	"	"
Anthostomella cylindrospora	Booth & Shaw 1967	"	extruded
A. cylindrospora	Shaw & Booth 1967	"	"
Claviceps purpurea	Hadley 1968*	"	ejected
Calonectria crotalariae	Rowe & Beute 1975	"	"
Leiosphaerella longispora	Sivanesan, Shaw & Brown 1976	& asci	extruded
Gnomonia papuana	Sivanesan & Shaw 1976	asci	"
Gaeumannomyces leptosporus	Shaw (herein)	"	"

* Cited by Ingold (1971)

whether, if maturation does occur, asci or ascospores are extruded below the water surface. Although extrusion in the present case occurred in the laboratory when the leaf pieces were removed to a drier atmosphere, a similar situation could occur in nature when colonized leaves or branches in streams are stranded on the banks, or on rocks, or on debris, as this would constitute a relatively drier atmosphere than the submerged state.

While it is apparent that this fungus can survive in an aquatic situation, it may not be fully aquatic, especially as perithecia are able to develop to maturity on nutrient agar without immersion, and as the perithecial necks protruded above the water surface in the water cultures, and as asci were extruded in the air when the substrate with perithecia was removed to a drier atmosphere.

The occurrence reported here adds another species to the number of ascomycetes (such as those listed in *Table* 1) whose asci or ascospores have been reported as ejected or extruded when the perithecia were placed in a condition of lower humidity.

It is not known as yet whether *G. leptosporus* produces hyphopodia on its substrates in nature. However, the fact that it did not produce them in several tests in the laboratory, although the germinating ascospores formed appressoria in hanging drops, may indicate that this species is somewhat different (apart from the smaller size of the asci and ascospores already noted by Iqbal) from *G. graminis*, which produces abundant hyphopodia.

The fact that this fungus has now been reported from two such diverse locations as about 51° N. latitude in England and about 9° S. latitude in Papua New Guinea probably indicates that it is of worldwide distribution.

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