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LEPTOSPHAERIA NARMARI AND *L. KORRAE* SPP.NOV., TWO LONG-SPORED PATHOGENS OF GRASSES IN AUSTRALIA

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(With Plates 43 and 44 and 2 Text-figures)

Leptosphaeria narmari sp.nov. and *L. korrae* sp.nov., root and stolon parasites of various grasses in New South Wales, are described. Both grow readily in agar culture and can attack cereals and grasses in artificial inoculation tests. The generic position of the two species is discussed.

Smith (1965) described a patch disease of several turf grasses in New South Wales. First recognized in 1961, it was called spring dead spot because of its similarity to a disease of the same name occurring in America (Wadsworth & Young, 1960). Smith (1965) isolated a long-spored Pleosporaceous fungus from the N.S.W. specimens and showed that it was capable of reproducing the disease. He identified this fungus as *Ophiobolus herpotrichus* (Fr.) Sacc.

Subsequent work (Smith, 1967) showed that a second closely related species with shorter spores was a much more common cause of spring dead spot in N.S.W. It was determined as an undescribed species of *Leptosphaeria*. Re-examination of the collections originally cited by Smith (1965) as *O. herpotrichus* showed that they did not belong to this species but would be better placed as another undescribed species of *Leptosphaeria*.

These two species are described below, with comments on their taxonomic position. The herbarium abbreviations used are those of Lanjouw & Stafleu (1964).

Leptosphaeria narmari sp.nov. (etym. *narmar*, gramen, in lingua una aboriginum Australiae Centralis)

Pseudothecia erumpentia, aggregata vel remota, magna, usque ad 800 μm alta (cum collo) et 650 μm lata, nigra, ampulliformia vel late obpyriformia, cum corpore plus minusve globoso et cum collo obtusato 100–300 μm longo et 300–450 μm lato saepe cum 1 vel 2 annularis incrassatis; paries pseudothecii in corpore 60–75 μm crassus, usque ad 100 μm in collo, ex stratis pluribus cellularum fuscatarum complanatarum 7–12 × 4–7 μm constatus. In collo, canalis usque ad 150 μm latis, plerumque haematiticus et cum periphysibus hyalinis ascendentibus 45–70 × 2 μm vestitus. Asci clavati, basin versus attenuati et unguliformes, (100) 110–145 (155) μm longi, 11–13 μm lati, bitunicati, octospori; ascosporae biseriatae, pallide brunneae, anguste ellipticae vel fusiformes, saepe leviter arcuatae, (35) 45–62 (72) × 4–5 (6) μm, (3)–5–(7) septatae, ad septum medium parum constrictae. Pseudoparaphyses hyalinae, septatae, numerosae, 1·5–3 μm latae. Hyphae hospiti insidentes brunneae septatae ramosae 2·5–5 μm latae, saepe in filis 3–4 hypharum dispositae et sclerotia complanata fusca 40–400 μm diametro fascientes.

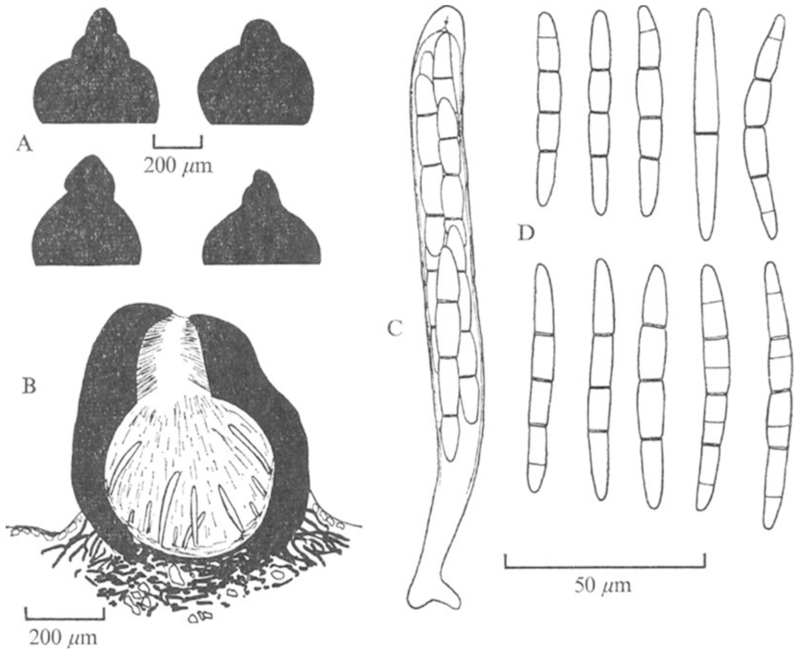

Fig. 1. *Leptosphaeria narmari.* A, Shapes of upper half of pseudothecia; B, cross-section of pseudothecium; C, ascus; D, ascospores.

In radicibus stolonibus et vaginis foliorum plurium Graminearum. Holotypus in stolonibus *Stenotaphri secundati* (Walt.) Kuntze, Leeton, New South Wales, Australia, Nov. 1966, P. Kable, **DAR** 16020.

Pseudothecia erumpent, occurring in clusters or singly, up to 800 µm high (including the neck) and 650 µm wide, black, flask-shaped to widely obpyriform (Pl. 43, fig. 2; Fig. 1 A), with a more or less globose body and a thick neck 100–300 µm long and 300–450 µm wide (Pl. 44, fig. 3), often with one or two thickened ridges around it. Pseudothecial wall 60–75 µm thick in body, to 100 µm thick in neck, composed of several layers of flattened brown cells 7–12 × 4–7 µm (Fig. 1 B). Neck canal up to 150 µm wide, lined with hyaline upwardly pointing periphyses 45–70 × 2 µm, often coloured by a reddish-brown material between the periphyses (Fig. 1 B). *Asci* clavate, narrowed towards the foot-like base, (100) 110–145 (155) × 11–13 µm, bitunicate, eight-spored (Pl. 44, fig. 4; Fig. 1 C). *Ascospores* biseriate, pale brown, narrowly elliptical to fusiform, often slightly bent, (35) 45–62 (72) × 4–5 (6) µm, (3)–5–(7) septate, slightly constricted at the central septum and sometimes very slightly at other septa (Pl. 44, fig. 5; Fig. 1 D). *Pseudoparaphyses* numerous, hyaline, septate, 1·5–3·0 µm wide. *Hyphae* on the host brown, septate, branched, 2·5–5·0 µm wide, often in strands of three or four and forming flattened dark sclerotia 40–400 µm diam.

Specimens examined. Only specimens with pseudothecia, asci and ascospores are listed. On *Cynodon dactylon* (L.) Pers., Hunters Hill, N.S.W., 23. vi. 1965, D. Reilly, **DAR** 14492; Turramurra, N.S.W., 27. x. 1965, F. Bagshaw, **DAR** 15500; Turramurra, N.S.W., 15. xi. 1965, A. M. Smith, **DAR** 15505; Yeoval, Molong district, N.S.W.,

6. x. 1966, F. O'Halloran, **DAR** 15949; artificial inoculation ex *Cynodon*, glasshouse, Rydalmere, N.S.W., 10. vi. 1966, A. M. Smith, **DAR** 15420. On *Hordeum vulgare* L., artificial inoculation ex *Cynodon*, glasshouse, Rydalmere, N.S.W., 20. x. 1966, A. M. Smith, **DAR** 15951*b*. On *Oryza sativa* L., artificial inoculation ex *Cynodon*, glasshouse, Rydalmere, N.S.W., 13. xii. 1965, A. M. Smith, **DAR** 14493*b*. On *Pennisetum clandestinum* Hochst. ex Chiov., Cobar, N.S.W., 19. xi. 1970, Mr Campbell, **DAR** 20806; Leeton, N.S.W., x. 1968, P. Kable, **DAR** 20657. On *Stenotaphrum secundatum* (Walt.) Kuntze, Yenda, N.S.W., 29. x. 1957, J. Walker, **DAR** 5374: Wellington, N.S.W., 8. vii. 1964, Mrs Paterson, **DAR** 13460; Yenda, N.S.W., 22. x. 1964, D. Reilly, **DAR** 14491; Leeton, N.S.W., November 1966, P. Kable, **DAR** 16020 (holotype); Jerilderie, N.S.W., 12. x. 1967, J. Entwistle, **DAR** 16577; Wagga Wagga, N.S.W., 23. vii. 1969, J. Kuiper, **DAR** 17497*a*; same locality, 11. viii. 1969, J. Kuiper, **DAR** 17504; Dubbo, N.S.W., 1. xii. 1970, L. Meakins, **DAR** 20740. On *Triticum aestivum* L., artificial inoculation from *Cynodon*, glasshouse, Rydalmere, N.S.W., 13. xii. 1965, A. M. Smith, **DAR** 14493*a*. Several collections on these hosts identified on mycelial and cultural characters have also been examined. Two specimens on *Cynodon transvaalensis* Davy from Kerang, Victoria (**DAR** 13727*a* and **DAR** 15419) are also thought to be *L. narmari* on the basis of cultural characters.

Leptosphaeria korrae sp.nov. (etym. *korra*, gramen, in lingua una aboriginum Australiae Meridionalis)

Pseudothecia magna, nigra, 400–600 μm alta (cum collo) et 300–500 μm lata, ampulliformia, cum corpore plus minusve globoso et cum collo 50–150 (200) μm longo, 200 (250) μm lato, saepe cum annulo incrassato; paries pseudothecii in corpore basi 80–120 μm, latere 40–80 μm, et in junctura cum collo 60–80 μm crassus, ex stratis pluribus cellularum fuscatarum complanatarum 10–18 × 4–7 μm constatus. In collo, canalis usque ad 80–100 μm latis, cum periphysibus hyalinis ascendentibus vestitus, et in pseudotheciis juvenilibus plerumque haematiticus. Asci cylindrici vel clavati, basin versus attenuati et unguliformes (145)150–185(200) × 10–13 (15) μm, bitunicati, octospori; ascosporae filiformes, in fasciculis leviter tortae, sibi parallelae, pallide brunneae, (1–3–6)–7–(15) septatae, (120) 140–170(180) × 4–5(5·5) μm, spora longissima rara ad 210 μm longa, ad extrema rotundatae. Pseudoparaphyses hyalinae, septatae, numerosae, 1·5–2·0 μm latae. Hyphae hospiti insidentes brunneae septatae ramosae 2·5–5 μm latae, saepe in filis 3–4 hypharum dispositae et sclerotia complanata fusca 50–400 μm diam. fascientes.

In radicibus, stolonibus et vaginis foliorum plurium Graminearum. Holotypus in stolonibus et vaginis foliorum *Cynodontis dactylonis*, bowling green, Blacktown, New South Wales, Australia, Nov., 1964, A. M. Smith, **DAR** 13723.

Pseudothecia erumpent, usually closely packed, 400–600 μm high (including the neck), 300–500 μm wide, flask-shaped, with a globose body and a thick neck 50–150 (200) μm long and 200 (250) μm wide (Pl. 44, fig. 6), often with a thickened ridge around it (Fig. 2A). Pseudothecial wall 80–120 μm thick at the base, 40–80 μm at the sides, and 60–80 μm at the junction with the neck, composed of several layers of flattened brown cells 10–18 × 4–7 μm (Fig. 2B). Neck canal up to 80–100 μm wide, lined with hyaline upwardly pointing periphyses and, in young pseudothecia, often coloured reddish-brown by some material between the periphyses. *Asci* cylindrical to clavate, narrowed towards the foot-like base, (145)150–185(200) × 10–13(15) μm bitunicate, eight-spored (Pl. 44, fig. 7; Fig. 2C). *Ascospores* filiform, slightly twisted in a bundle and parallel to one another, pale brown, (1–3–6)–7–(15) septate, (120)140–170(180) × 4–5(5·5) μm, an occasional very long spore to 210 μm, widest in the middle and tapering more towards the base than the apex, rounded at the ends (Pl. 44, fig. 8; Fig. 2D). *Pseudoparaphyses* hyaline, septate, numerous, 1·5–2·0 μm wide. *Hyphae* on the host brown, septate, branched,

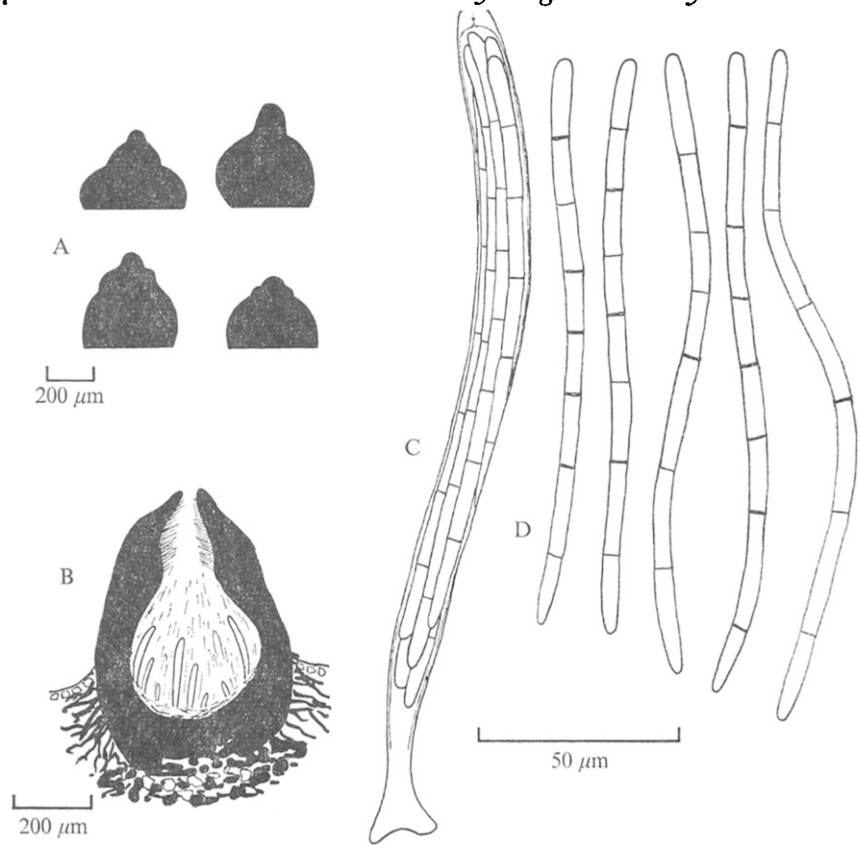

Fig. 2. *Leptosphaeria korrae*. A, Shapes of upper half of pseudothecia; B, cross-section of pseudothecium; C, ascus; D, ascospores.

2·5–5·0 μm wide, often in strands of three or four and forming flattened dark sclerotia 50–400 μm diam.

Specimens examined. Only specimens with pseudothecia, asci and ascospores are listed. On *Avena sativa* L., artificial inoculation ex *Cynodon* (**DAR** 13723), in glasshouse, Rydalmere, N.S.W., 31. v. 1965, A. M. Smith, **DAR** 14055. On *Axonopus compressus* (Swartz) Beauv., Pennant Hills, N.S.W., 24. vii. 1964, L. R. Fraser, **DAR** 13726. On *Cynodon dactylon* (L.) Pers., bowling green, Blacktown, N.S.W., November, 1964, A. M. Smith, **DAR** 13723 (holotype); same locality, 2. xii. 1964, A. M. Smith, **DAR** 13724; same locality, pseudothecia developed on plants kept in glasshouse, 8. iv. 1965, A. M. Smith, **DAR** 14002. On *Eremochloa ophiuroides* (Munro) Hack., in grass plots, Ryde School of Horticulture, Ryde, N.S.W., 14. x. 1965, A. M. Smith, **DAR** 14422. On *Oryza sativa* L., artificial inoculation, ex *Cynodon* (**DAR** 13723), in glasshouse, Rydalmere, N.S.W., 25. iii. 1965, A. M. Smith, **DAR** 14004; artificial inoculation, same locality, 8. vi. 1965, A. M. Smith, **DAR** 14053. On *Triticum aestivum* L., artificial inoculation ex *Cynodon* (**DAR** 13723), in glasshouse, Rydalmere, N.S.W., 20. v. 1965, A. M. Smith, **DAR** 14054; same locality, 2. ii. 1966, A. M. Smith, **DAR** 21036.

On their hosts, both *Leptosphaeria narmari* and *L. korrae* produce pseudothecia within leaf sheaths. In older specimens, where the host tissue has rotted away, the pseudothecia often appear superficial on the stolons. The thickened ridges often seen on the pseudothecia seem to be pressure ridges associated with the growth of the embedded fruiting bodies through layers of leaf sheaths.

Infected stolons are blackened by the growth of dark-brown hyphae through the tissues (Pl. 43, fig. 1). Both species also form an abundant superficial mycelium, with strands of brown runner hyphae and dark-brown flattened sclerotic bodies 40–400 μm diam, often fusing into larger crusts. These are composed of angular cells 5–10 μm diam, and beneath them abundant minute penetration holes into the host are present. Penetration also occurs through minute penetration pegs from single lateral and intercalary cells on the mycelium. They closely resemble the individual penetration cells and groups of cells described and figured by Mangin (1899) for *Leptosphaeria herpotrichoides* de Not. on wheat, and they seem to serve a similar penetration function to the infection cushions and hyphopodia of *Gaeumannomyces* spp. (Walker, 1972). The cushions could also serve as sclerotia, as the fungi can be isolated from them long after isolation from diseased tissue and mycelium is no longer possible.

Both species grow readily in agar culture. On potato dextrose agar, growth is white at first, and in *L. korrae* gradually darkens in colour from the centre of the colony. Eventually the aerial mycelium is dark grey and the mycelium on the agar surface dark grey to black in colour. In *L. narmari* the aerial mycelium changes from white to buff and the buff colour persists for several days, gradually darkening until the colonies resemble those of *L. korrae*. On PDA, the optimum temperature for radial growth of both species is 25 °C (4–5 mm per day). In the aerial mycelium both fungi form dark mycelial strands composed of only a few or up to 50 or more hyphae. No pseudothecia have developed in culture, and no conidial stages have been found for either species, in nature or in culture.

The reddish-brown colour of the neck interior is quite noticeable in young pseudothecia of both species, and occurs in pseudothecia produced on artificially inoculated plants as well as in those from natural infections. It was thought at first that the pigment may be a product of bacteria in the neck canal, but attempts to isolate pigment-producing bacteria failed (Smith, 1967). In mature and over-mature pseudothecia the pigment is often absent.

DISCUSSION

The limits of families and genera within the Pleosporales are still poorly defined and it is difficult to place with any certainty the two fungi described above. Traditionally, long-spored species such as *Leptosphaeria korrae* have been placed in *Ophiobolus* and shorter-spored species such as *L. narmari* in *Leptosphaeria*. However, *L. korrae* and *L. narmari* seem to be so closely related that to separate them in different genera only on the basis of ascospore length would obscure this relationship and not help in clarifying generic concepts in this group.

The possibility of placing *L. korrae* and *L. narmari* in *Ophiobolus* was rejected as they do not agree with the original concept of this genus proposed by Riess (1854) and re-affirmed more recently by Holm (1957). As Holm (1957) states, the raison d'être of the genus is the nature of the ascospores, which are scolecospores constricted in the middle and having a swollen cell in each half on either side of the constriction. The ascospores break easily at the central constriction. Since Saccardo (1883) transferred many names to *Ophiobolus*, the generic name has been used in a much wider sense for many different scolecospored perithecial and pseudothecial fungi. Until the limits of genera in the Pleosporaceae are more clearly defined, it is thought that the original concept of *Ophiobolus* is the only suitable one to follow. *Ophiobolus* and some related genera will be considered in further detail in a later publication (Walker, in preparation).

Of the fungi formerly placed in *Ophiobolus*, *L. korrae* resembles *O. herpotrichus* (Fr.) Sacc. (≡ *Phaeosphaeria herpotricha* (Fr.) L. Holm, 1957) and was originally recorded under this name by Smith (1965). *L. korrae* was examined by Professor L. Holm, who also sent us a specimen of *P. herpotricha*. *L. korrae* has pseudothecia with much thicker and stronger walls and wider ascospores than *P. herpotricha*. A pycnidial stage has been recorded for *P. herpotricha* (as *Ophiobolus herpotrichus*) by Webster & Hudson (1957), but no conidial stage has been detected for *L. korrae*. A survey of the descriptions in the literature of *Ophiobolus* spp. and other scolecospored ascomycetes did not show any with which *L. korrae* could be identified.

The genus *Leptosphaeria* is also a heterogeneous collection of species. Holm (1957) has separated many of these into related genera such as *Nodulosphaeria* Rabenh. and *Phaeosphaeria* Miyake, and his treatment has been followed wholly or in part by some later workers (Eriksson, 1967; Hedjaroude, 1969; Arx, 1970). Generally the species he leaves in *Leptosphaeria* have thick-walled ascocarps composed of thick-walled cells. He refers to this tissue as a scleroplectenchyma. Lucas & Webster (1967) use the name *Leptosphaeria* in a wide sense and define the genus to include species placed by Holm (1957) in various segregate genera.

Whilst *L. narmari* and *L. korrae* have thick-walled ascocarps, the cells of which also show wall thickening in some cases, they do not have a scleroplectenchyma as defined by Holm (1957) and would not fit exactly into his concept of *Leptosphaeria*. Moreover, both species have abundant periphyses in their ostiolar canal and this would exclude them from *Leptosphaeria* as treated by Hedjaroude (1969).

In view of the uncertainty of the generic limits of *Leptosphaeria*, the wide concept adopted by Lucas & Webster (1967) is followed for the present. In this sense, *L. narmari* can be placed satisfactorily in *Leptosphaeria*, and *L. korrae*, with scolecospores, is also included because of its evident relationship with *L. narmari*. Other authors also have placed scolecospored species in this genus. Holm (1957) included *L. cesatiana* (Mont. ex Ces. & de Not.) Holm and *L. tanaceti* (Fckl) Holm, which have ascospores 90–120 × 3·5–5·0 μm, and Barr (1967) included *L. hesperia* Barr, with ascospores 93–135 × 3–5 μm.

A survey of the descriptions of *Leptosphaeria* spp. on Gramineae did not show any with which the present fungi could be identified. *L. narmari* bears some resemblance to *L. maculans* (Desm.) Ces. & de Not. (Holm, 1957; Smith & Sutton, 1964) in size and septation of ascospores, and in the blackening of the infected tissue. However, the ascocarps of *L. narmari* are generally larger than those of *L. maculans*, and no conidial stage has been found for *L. narmari*, whereas *L. maculans* produces pycnidia of its conidial stage, *Plenodomus lingam* (Tode ex Fr.) Höhn. both on the host and in culture (Boerema & van Kesteren, 1964; Smith & Sutton, 1964).

Holm (1957) has mentioned and figured the order in which the cross walls in the ascospores are formed for several species. *L. narmari* and *L. korrae* are different in this respect. In *L. narmari*, immature spores show first a central septum. Later septa develop in pairs, one on either side of the central septum and progressively further away from it. The spores of *L. narmari* are thus (1–3)–5–(7) septate. In *L. korrae* the central septum is again produced first. The two cells so formed then develop one septum each, forming four cells. Each of these then develops a septum, and subsequently the seven cells so formed may each develop another septum. The spores of *L. korrae* are thus (1–3)–7–(15) septate. Departures from these patterns are common in both species. The sequence of cross-wall formation in spores should perhaps be investigated further as a character of possible taxonomic use in this and other groups.

L. narmari and *L. korrae* can cause severe disease in turf grasses and, under artificial inoculation, in the cereals wheat, oats, barley and rice (A. M. Smith, unpublished data). *L. korrae* has been found only in the Sydney Metropolitan Area, but *L. narmari* occurs in many parts of New South Wales, including cereal-growing areas. It is possible that these and related fungi may form part of the complex of brown sterile fungi often isolated from roots and crowns of cereals. On symptoms alone, the diseases caused by *L. narmari* and *L. korrae* on cereals cannot be distinguished from take-all caused by *Gaeumannomyces graminis* (Sacc.) Arx & Olivier and its varieties (Walker, 1972), but they are distinguishable in fruiting body and cultural characters.

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EXPLANATION OF PLATES 43 AND 44

PLATE 43

Fig. 1. *Leptosphaeria narmari*, part of holotype (**DAR** 16020), showing blackening of infected stolons and fruiting bodies. Scale in mm.

Fig. 2. *L. narmari*, part of holotype showing erumpent pseudothecia. Scale in mm.

PLATE 44

Fig. 3. *L. narmari*, section of pseudothecium, from holotype (× 160).

Fig. 4. *L. narmari*, ascus with spores, from holotype (× 400).

Fig. 5. *L. narmari*, two ascospores, from holotype (× 1000).

Fig. 6. *L. korrae*, section of pseudothecium, from holotype (**DAR** 13723) (× 160).

Fig. 7. *L. korrae*, ascus with spores, from holotype (× 600).

Fig. 8. *L. korrae*, ascospore, from holotype (× 800).

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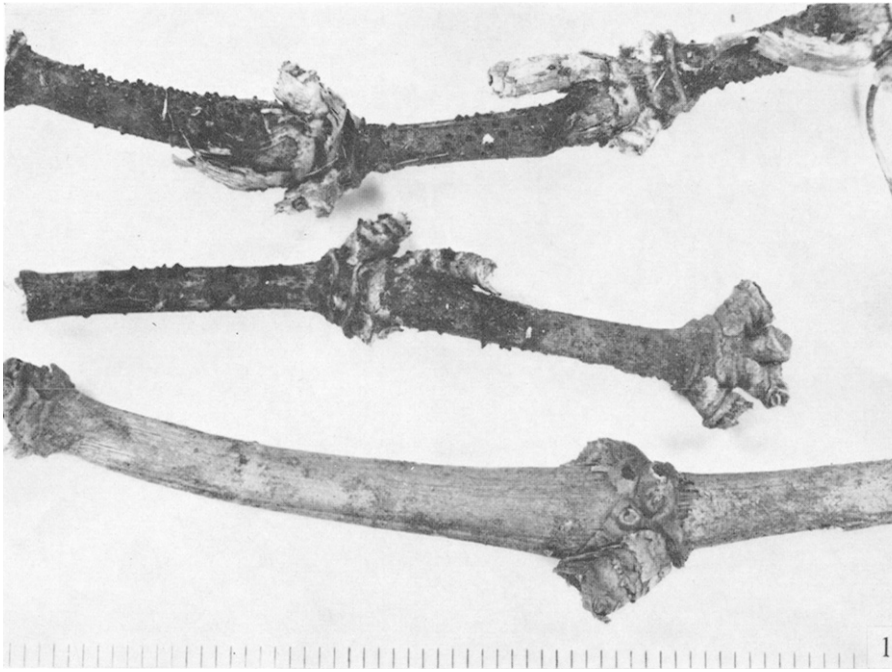

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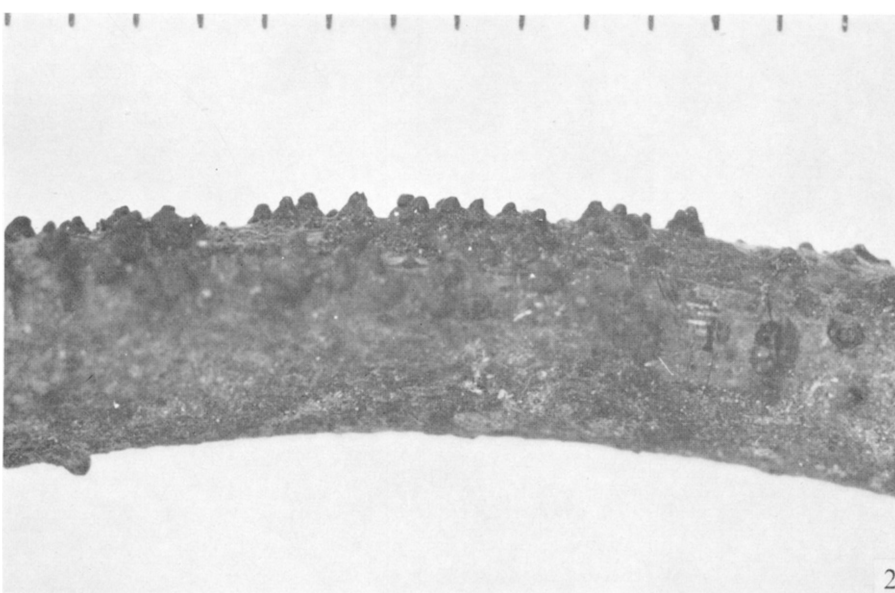

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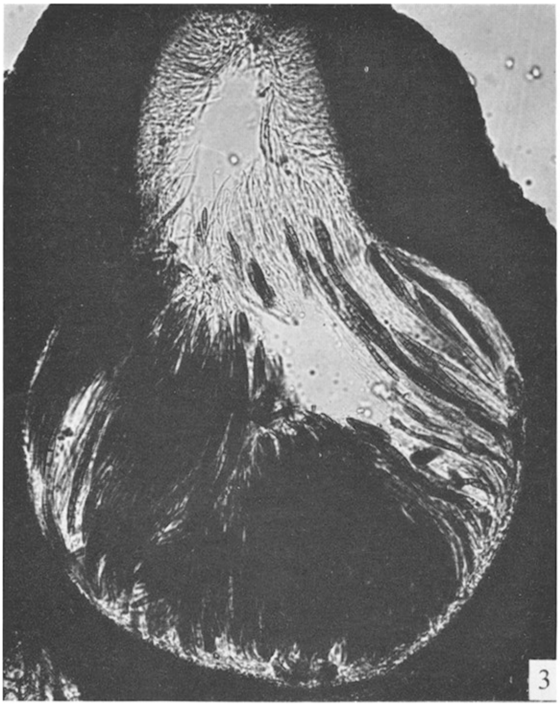
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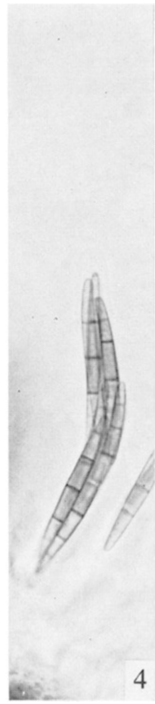
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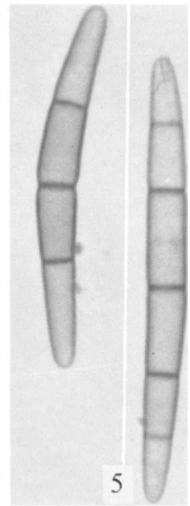
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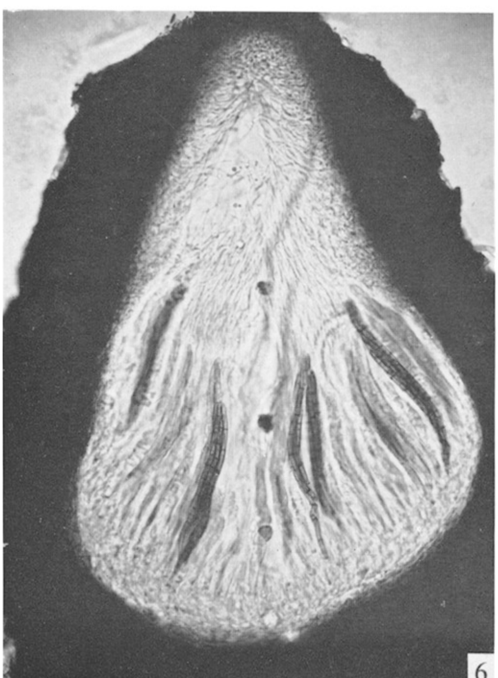
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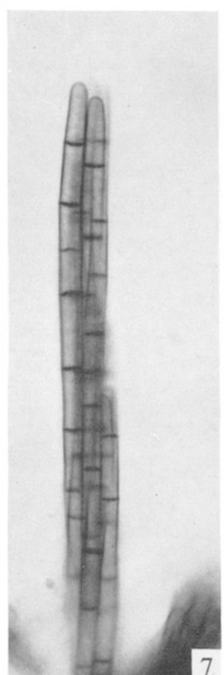
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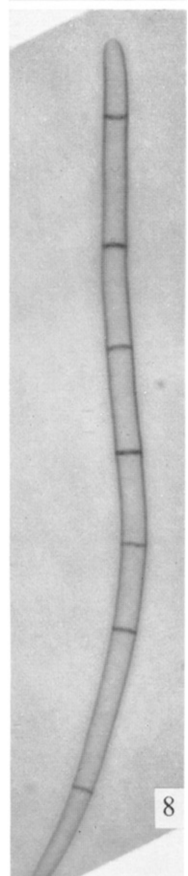
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