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GRAMINICOLOUS PYRENOMYCETES. V. CONIDIAL STATES OF *LEPTOSPHAERIA MICHOTII*, *L. MICROSCOPICA*, *PLEOSPORA VAGANS* AND THE PERFECT STATE OF *DINEMASPORIUM GRAMINUM**

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(With 5 Text-figures)

By means of cultures the connexions between the following Pyrenomycetes and Fungi Imperfecti have been demonstrated: *Leptosphaeria michotii* and *Coniothyrium scirpi*, *Leptosphaeria microscopica* and *Phaeoseptoria festucae*, *Pleospora vagans* and a species of *Hendersonia*. The perfect state of *Dinemasporium graminum* is described as *Phomatospora dinemasporium* sp.nov. The synonymy of some of these fungi is discussed.

The task of connecting Fungi Imperfecti with their perfect states is important, but fraught with many difficulties. Where type material of the conidial form has been preserved, there is the difficulty of comparing it with cultures, which may show greater variability than collections on the host plant. Where type or authentic material is no longer preserved the task becomes hazardous, since comparison is then made between often inadequate descriptions and cultures. It has also been shown that unrelated fungi may have conidial states which are distinguishable only with difficulty (see, for example, Simmons, 1952). In comparatively few cases can one be satisfied in reducing a name in the Fungi Imperfecti to synonymy. In the remaining cases it seems wiser merely to describe the conidial form obtained in culture, to compare it where possible with material collected on the host, and to do no more than suggest the names in the Fungi Imperfecti which might be considered as synonyms.

Cultures have been prepared from collections of perithecia and conidia of fungi on grasses, and the present paper describes the results of these isolations. The cultures were prepared by crushing a single perithecium or pycnidium in a drop of sterile water on a slide, transferring part of the spore suspension to agar plates with a fine pipette. The remaining spore suspension was mounted in cotton blue in lactic acid, and the slide preserved. The measurements in the following tables are taken from these 'isolation slides' and thus do not represent the whole range of variation of spore size in the collections. Cultures were prepared from single spores or asci, or occasionally from groups of spores, and when the hyphae from germinating spores had grown free from bacteria, transfers were made to

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oat agar slopes (6 × 1 in. boiling tubes containing about 20 ml. of medium). The cultures were incubated at room temperature in diffuse light or in direct light from a window, and in most cases examined within 2 months.

(1) **Leptosphaeria michotii** (Westend.) Sacc. (Fig. 1)

The description below is drawn up from material on *Dactylis glomerata* (Mycological Herbarium of the University of Sheffield, no. 1020). *Perithecia* small, globose, black or dark brown, with a small papillate ostiole, arising from a submerged mycelium, hyaline to pale brown in colour, septate, 2–3 μ wide, penetrating both lignified and parenchymatous tissues; the perithecia measure 130–250 μ in diameter and occur on the inflorescence axis, the internode, nodes and leaf sheaths, of the current or previous year. The perithecial wall is 20–30 μ thick and is made up of dark brown polygonal, thin-walled cells, about 10–20 μ across. *Asci* numerous cylindrical or club-shaped, sessile, rounded at the apex, possessing a double wall, 8-spored, 62–100 × 12–14 μ; 'paraphyses' about as long as the asci, septate, cylindrical 2–4 μ in width. *Ascospores* biseriate, or more closely packed within the ascus, cylindrical, 2–3 septate, slightly constricted at the septa, end cells rounded, brown in colour, becoming darker with age 14–22 × 4–5 μ. On germination a single germ-tube is usually produced from one of the end cells, occasionally from both, rarely from the central cell.

Conidial stage. Cultures were prepared from single ascospores. Transfers were made to oat agar and potato-dextrose agar. On these media the fungus grew fairly rapidly, forming a submerged brown mycelium and a small amount of white aerial mycelium. When observed 6 weeks later perithecia and pycnidia were found in cultures on both media (Fig. 1 C). The pycnidia were globose, brown, thin-walled bodies (wall 15–20 μ thick) with a small papillate ostiole, occurring either singly or grouped together, and measuring 110–250 μ in diameter. They contained numerous dark brown oval spores (Fig. 1 E), measuring 6–10 × 3–5 μ (usually 7–9 × 4–4·5 μ).

The pycnidia belong to the form genus *Coniothyrium* and match the description of *C. scirpi* Trail (1889). Pycnidia have also been found on *Dactylis*, having the same distribution as the perithecial stage and also occurring occasionally on the glumes. A few collections have been made of the perithecial and pycnidial stages occurring together (Fig. 1 A). On *Dactylis* the pycnidia are small, dark brown, globose, with a small projecting papilla, measure 90–170 μ in diameter, and contain dark brown oval spores measuring 5–10·5 × 4–5 μ (usually 7–10 × 4–4·5 μ).

The type of *Leptosphaeria michotii* (Herb. Hort. Bot. Bruxellensis no. 1218) has been examined by kind permission of Prof. W. B. Robyns of the Brussels Botanic Garden. It consists of a single stem fragment of *Juncus squarrosus*. Beneath the epidermis numerous perithecia were present. A few of these were mounted, but none contained ripe asci of *L. michotii*. However, occasional ascospores were found (Fig. 1 D). They are brown, 2-septate, the central cell slightly swollen, the end cells rounded; they measure 17–21 × 4–6 μ. Since these ascospores match sufficiently closely the original description no further search was made, in order to conserve

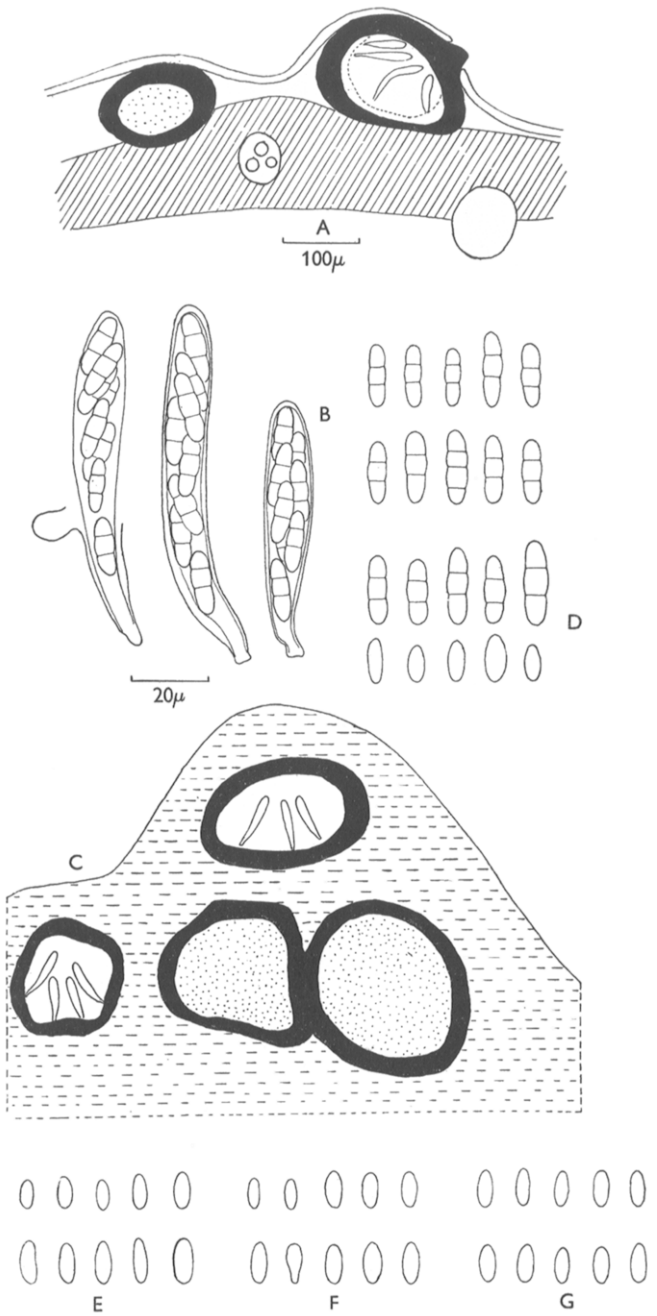

Fig. 1. *Leptosphaeria michotii*. A, T.S. stem with a perithecium and a pycnidium (stippled); B, asci and ascospores; C, section of oat agar culture derived from a single ascospore showing perithecia and pycnidia; D, ascospores and conidia from the type specimen of *L. michotii*; E, pycnospores from culture; F, pycnospores from *Dactylis*; G, pycnospores from type specimen of *Coniothyrium scirpi*. A and C to same scale; B, D, E, F and G to same scale.

the type specimen. The specimen also contained pycnospores which matched those described above, but showing a slightly greater range of size, measuring 7–13·5 × 3·5–5·5 μ.

I have also examined a slide, probably prepared from the type specimen of *Coniothyrium scirpi* Trail, preserved in the University of Aberdeen. According to Prof. Matthews (*in litt.*) there is no other material of *Coniothyrium* on *Eleocharis palustris* in the Trail Herbarium. The slide is labelled '*Coniothyrium scirpi* sp.n. from Scirpus palustris. Loch Acray, Perthshire. 15/9/88, Cly.' It appears to be a lactophenol mount of a crushed pycnidium, ringed with Canada balsam but now somewhat dried out. Few details of the structure of the pycnidium could be elucidated, but numerous spores were present; they were pale yellow to brown in colour, smooth, elliptical in shape, and measuring 9–11 × 3·5–4·5 μ (mostly 9–10 × 4 μ)—see Fig. 1 G.

I regard the material and cultures from *Dactylis* as matching type material of both *Leptosphaeria michotii* and *Coniothyrium scirpi*.

Perithecia have been previously recorded from a wide range of monocotyledonous hosts (see, for example, Oudemans, 1919), and I have made collections on *Agropyron repens*, *Deschampsia caespitosa*, *Festuca rubra*, *Nardus stricta* and *Typha latifolia*, in addition to the numerous collections on *Dactylis*. Pycnidia have been found on the following hosts: *Agropyron repens*, *Brachypodium pinnatum*, *Calamagrostis epigeios*, *Deschampsia caespitosa*, *Holcus lanatus*, *Melica uniflora*, *Nardus stricta* and *Phalaris arundinacea*. Cultures made from single pycnospores from several of these collections matched those derived from ascospores.

(2) **Leptosphaeria microscopica** Karst. (Fig. 2)

L. microscopica was described by Karsten (1872) from leaves of various grasses. *L. culmorum* Auersw. is generally accepted as a synonym, but since this name was published without a description various authors (e.g. Saccardo, 1883; Bisby & Mason, 1940) regard *L. culmorum* as a nomen nudum, and take *L. microscopica* as the valid name. Others (e.g. Winter, 1887; Muller, 1950) have accepted the name *L. culmorum*. Unfortunately, Karsten's specimens are not available (H. Roivainen, *in litt.*).

The fungus is widely distributed on numerous Monocotyledons, especially grasses (see Oudemans, 1919). I have collected perithecia on the following hosts: *Agropyron junceum*, *A. repens*, *Ammophila arenaria*, cultivated barley, *Calamagrostis epigeios*, *C. lanceolata*, *Cynosurus cristatus*, *Dactylis glomerata*, *Deschampsia caespitosa*, *Elymus arenarius*, *Festuca arundinacea*, *F. pratensis*, *Holcus lanatus*, *H. mollis*, *Lolium perenne*, *Melica uniflora*, *Nardus stricta*, *Phalaris arundinacea*, *Phragmites communis* and cultivated wheat. The following description is based on material from *Dactylis glomerata*.

Mycelium immersed in the tissues of the internodes, nodes, leaf sheaths, and inflorescence axis, confined mainly to parenchymatous tissues but occasionally penetrating lignified tissue, branched, septate, pale yellow to brown in colour, 2–4 μ wide. *Perithecia* occur in rows between the vascular bundles, raising the epidermis and penetrating it by a short, blunt, black neck; they are black globose, often elongated along the long axis of the

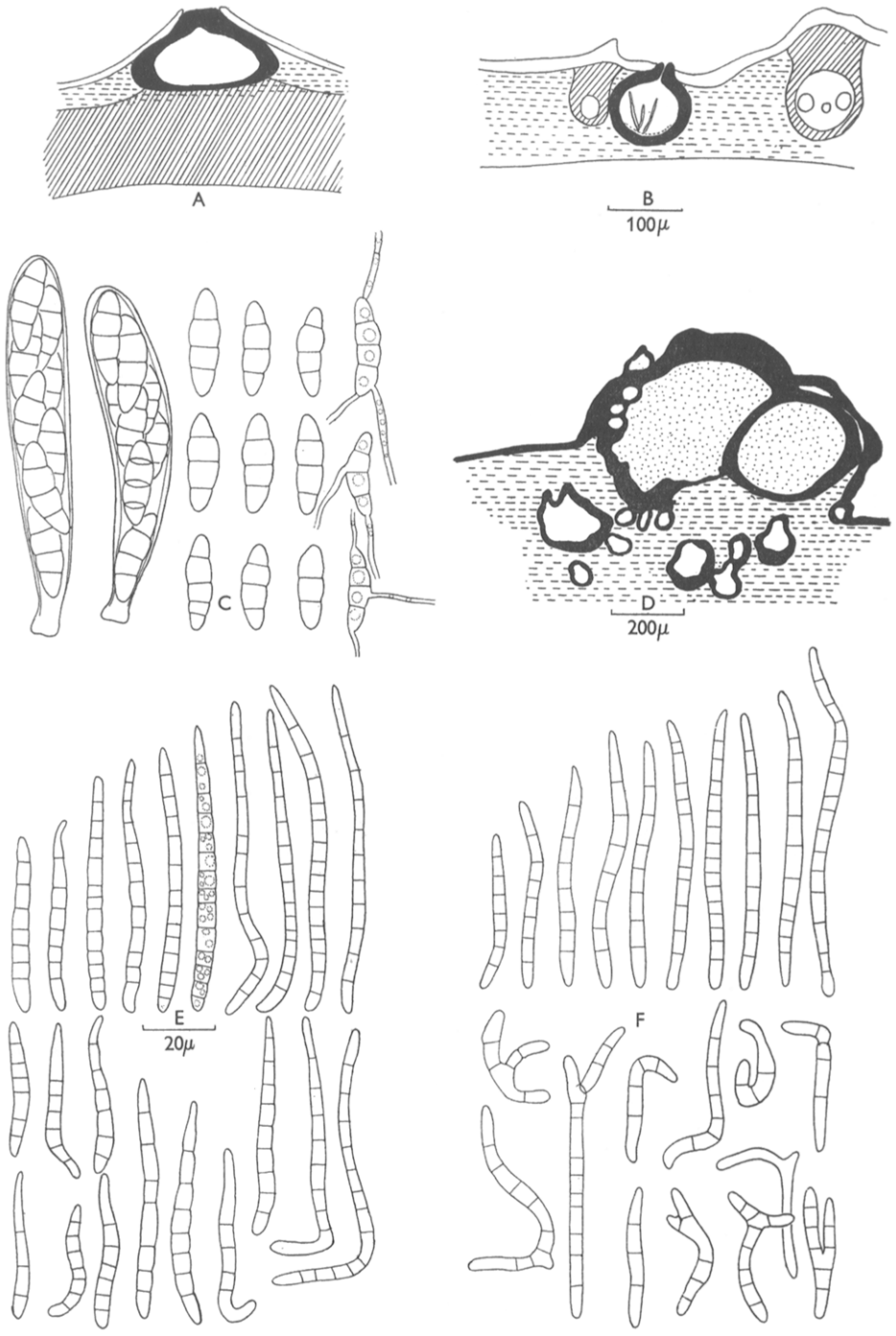

Fig. 2. *Leptosphaeria microscopica.* A, T.S. stem and perithecium; B, T.S. leaf sheath and pycnidium; C, asci and ascospores; D, section of oat agar culture derived from a single ascospore showing pycnidia (stippled) and perithecia; E, pycnospores from *Dactylis*; F, pycnospores from culture.

stem, and flattened below when growing on the stem. The wall is uneven in thickness (see Fig. 2 A) 15–20 μ thick above, 10–15 μ thick at the sides, sometimes almost absent below, and is composed of irregular brown polygonal cells 18–24 μ wide. *Asci* are numerous, broadly clubshaped, sessile, with double membranes, 8-spored and 68–104 × 15–18 μ; they are interspersed by septate branched 'paraphyses' 2–4 μ in width. *Ascospores* are biseriate in the upper part of the ascus and uniseriate below, pale yellow to golden in colour, most frequently 3-septate (but occasionally 4-septate) broadly fusoid, inequilateral, constricted at the septa but especially at the median septum, the second cell often more swollen than the rest, and 22–28 × 7–8·5 μ. On germination a germ tube is usually put out from one or both end cells, and also occasionally from a central cell (Fig. 1 C).

Conidial stage. Cultures were prepared on various media from asci and ascospores. On oat agar fruit-bodies were observed after 3 months. At the surface of the medium black stromatic masses up to 2 mm. in diameter were formed. When these were sectioned they were found to be composite structures containing large pycnidia, and smaller perithecia (Fig. 2 D). Perithecia were found in cultures derived from a single ascospore. The perithecia varied in diameter from 200 to 400 μ and contained asci and ascospores closely resembling those described above. The pycnidia, which were up to 600 μ in diameter, had dark walls up to 60 μ thick. The contents of the pycnidia consisted of large numbers of brown elongate pycnospores produced from sporogenous cells lining the wall of the pycnidium. The pycnospores showed great variety in shape and size (see Fig. 2 F), but the more typical spores were cylindrical or tapering towards the upper end, more or less curved, with 3–15 septa, slightly constricted at the septa, and 30–96 × 4–5·5 μ.

Pycnidia resembling those described were also found on *Dactylis*. The pycnidia on the host occur most frequently on the upper leaf-sheaths and internodes during August and September. They are visible as small black dots beneath the epidermis, between the vascular bundles (Fig. 1 B). They are globose, brown in colour, with a wall 10–15 μ thick, composed of brown polygonal cells up to 18 μ in width, but the wall is thicker and darker near the ostiole; ten pycnidia measured 120–190 μ in diameter. The pycnospores are produced from the inner wall cells on the lower half of the pycnidium; they are rather more regular than those produced in culture; yellow to brown in colour, cylindrical or tapering, 3–15 septate, frequently constricted at the septa, and each cell often contains several small guttules; they measure 44–95 × 4–5·5 μ (Fig. 2 E).

Cultures have also been prepared from the collections of perithecia or conidia from various hosts. Table 1 shows the results of these isolations, and measurement of conidia, and ascospores on the host and in culture.

Table 1 shows that there is some variation both in the size of ascospores and conidia. In the material listed ascospores vary in size from 22–28 × 6–8·5 μ on grasses and 19–32 × 5–8 μ in cultures on oat agar. A proportion of four (or rarely five) septate spores occurs in most collections and cultures. The conidia vary in size from 36–82 × 4–6 (usually 4–4·5) μ on the host, and appear to have a basic number of seven septa, although

additional septa may be laid down in certain cells so that spores with up to eleven septa are occasionally found. In culture the conidia range in size from 30–96 × 3·5–6 (usually 4–4·5) μ and contain 3–15 septa, although spores with fewer than seven septa are probably immature. It is interesting to note the behaviour of different cultures. Starting from ascospores or conidia it is possible to obtain both perithecia and pycnidia in culture. However, cultures started from ascospores seem to produce perithecia more readily than pycnidia, whilst the converse is true for cultures started from conidia.

Table 1. *Dimensions of ascospores and conidia of* Leptosphaeria microscopica *on various hosts and in 2-month-old cultures on oat agar*

Herb. no.	Host	Type of fruit-body produced in culture	Dimensions on host (μ)		Dimensions in culture (μ)	
			Ascospores	Conidia	Ascospores	Conidia
Perithecia						
1188	*Ammophila arenaria*	per. & pyc.	24–28 × 6–7	—	20–26 × 5–6	50–60 × 3·5–4
1195	*Cynosurus cristatus*	per.	23–26 × 6–8	—	24–27 × 6–7	—
1353	*Nardus stricta*	per.	24–28 × 6–8	—	—	—
1366	*Phalaris arundinacea*	per. & pyc.	23–24 × 6–7	—	24–32 × 6–8	40–60 × 3·5–5
1368	*P. arundinacea*	per.	24–27 × 7–8	—	24–26 × 6–7	—
1373*a*	*Festuca arundinacea*	per.	22–26 × 6·5–8	—	22–25 × 6–7	—
1378	*F. pratensis*	per.	24–28 × 7·5–8·5	—	—	—
1352	*Holcus lanatus*	per.	24–28 × 6·5–8	—	19–26 × 6–8	—
1354*a*	*Deschampsia caespitosa*	per.	24–26 × 6–8	—	21–26 × 6–7	—
1604	*Agropyron repens*	per. & pyc.	—	—	24–26 × 7–8	—
1093	*Dactylis glomerata*	per. & pyc.	22–28 × 7–8·5	—	22–26 × 7–8	30–96 × 4–5·5
Conidia						
1196	*Cynosurus cristatus*	pyc.	—	44–60 × 4–4·5	—	32–68 × 3·5–4
1200	*Deschampsia caespitosa*	per. & pyc.	—	40–72 × 4–4·5	24–26 × 6–7	46–60 × 3·5–4
1205	*Alopecurus pratensis*	pyc.	—	40–60 × 4–4–5	—	46–80 × 4–6
1207	*Poa pratensis*	pyc.	—	36–56 × 4–5	—	40–90 × 4–5
124	*Lolium perenne*	pyc.	—	46–76 × 4–4·5	—	54–88 × 4–4·5
1373*b*	*Festuca arundinacea*	pyc.	—	40–82 × 4·5–6	—	42–74 × 4–5
1350	*Holcus lanatus*	pyc.	—	48–72 × 4–4·5	—	48–74 × 3·5–4
1478*b*	*Phragmites communis*	per.	—	42–58 × 3·5–4	24–28 × 6–8	—

The conidia are referable to the form-genus *Phaeoseptoria* Speg. Dr R. Sprague has determined material of *Leptosphaeria microscopica* conidia sent to him (Herb. Sheffield 1093) as *Phaeoseptoria festucae* Sprague described (Sprague, 1943*b*) from 'languishing leaves of *Festuca rubra*', with 8–11 septate pycnospores, measuring 50–85 × 2·8–4·8 μ. Various other species of *Phaeoseptoria* have been distinguished by Sprague, and it is possible that some of these may represent conidia of *Leptosphaeria microscopica* on different hosts. In particular *Phaeoseptoria airae* (Grove) Sprague from *Deschampsia caespitosa* with spores 9–10 septate, 51–56 × 3·0–3·5 μ (later (Sprague, 1950) given as 60–75 × 2·5–3 μ), falls within the range listed in Table 1, and it may be noted that cultures of conidia from *Deschampsia caespitosa* yielded perithecia of *Leptosphaeria microscopica*.

Both perithecia and pycnidia of *L. microscopica* are found most commonly on the upper leaf sheaths of grasses in late summer as the leaves become moribund. On *Dactylis* perithecia persist throughout the winter, but pycnidia are difficult to find after October (see Webster, 1956).

There is a striking similarity between pycnospores obtained from cultures of *Leptosphaeria microscopica* and those described by Hughes (1949) from cultures of *L. nigrans*. This was confirmed by comparison of single-ascospore cultures of *L. nigrans* from *Dactylis glomerata* isolated on 30 May 1952. Transfers were made to oat agar, potato-dextrose agar and to steam-sterilized *Dactylis* stems. After 2 months pycnidia were formed, but only on the first of these media. Hughes reported the production of pycnidia in cultures of *Leptosphaeria nigrans* from wheat on potato-dextrose agar and on steamed wheat leaves. The pycnidia formed in the present investigations resemble those described by Hughes.

The fungus on oat agar forms at the surface of the medium a close felt of grey aerial mycelium; in the medium intense black colour is produced due to the dark pigmentation of the submerged hyphae, which are branched and septate, 2–3 μ wide. The pycnidia are formed at the surface and are black, globose with a small flattened papilla-like neck, and measured 80–170 μ. The walls of the pycnidium are about 20 μ thick, and made up of dark polygonal cells 5–8 μ wide. Pycnospores are produced from cells on the inner wall of the pycnidium. The pycnospores are dark brown in mass, pale brown or yellow when viewed singly, variable in length, measuring 32–88 × 3·5–5 μ, with 3–14 transverse septa, not constricted, wider at the base, tapering to the apex, straight or curved, ends rounded. Hughes gives measurements of pycnospores as 49·5–97·2 × 3–5 μ from agar cultures, with 7–16 transverse septa. 'Pycnospores with less than seven transverse septa were probably immature.'

Hughes has compared the pycnidia obtained in cultures with *Septoria alopecuri* var. *calamagrostidis*. Pycnospores from the type specimen are figured in Fig. 3 D. The similarity with well-developed pycnospores of *Leptosphaeria nigrans* is most striking. The dimensions of pycnidia and pycnospores of these two species of *Leptosphaeria* in culture on oat agar are compared below.

Table 2

	Diameter of pycnidia (μ)	Pycnospores	
		Size (μ)	Septa
L. microscopica	Up to 600	30–96 × 4–5·5	3–15
L. nigrans	80–170	32–88 × 3·5–5	3–14

In spite of the resemblance, the size of the pycnidia and the difference in conidial width, together with perithecial formation in *L. microscopica* and other cultural characters form a satisfactory basis for separating these two forms in culture. However, the similarity of the conidia of these two species indicates the difficulties in linking together names for conidial and ascigerous states in these fungi.

Grove (1937, p. 77) stated that *Hendersonia culmicola* var. *minor* Sacc. 'is no doubt the pycnidial stage of *Leptosphaeria microscopica*, which continually occurs with it'. This statement is not supported by cultural evidence. Various isolates of *Hendersonia culmicola* var. *minor* have been made, but bear no comparison to those of *Leptosphaeria microscopica*.

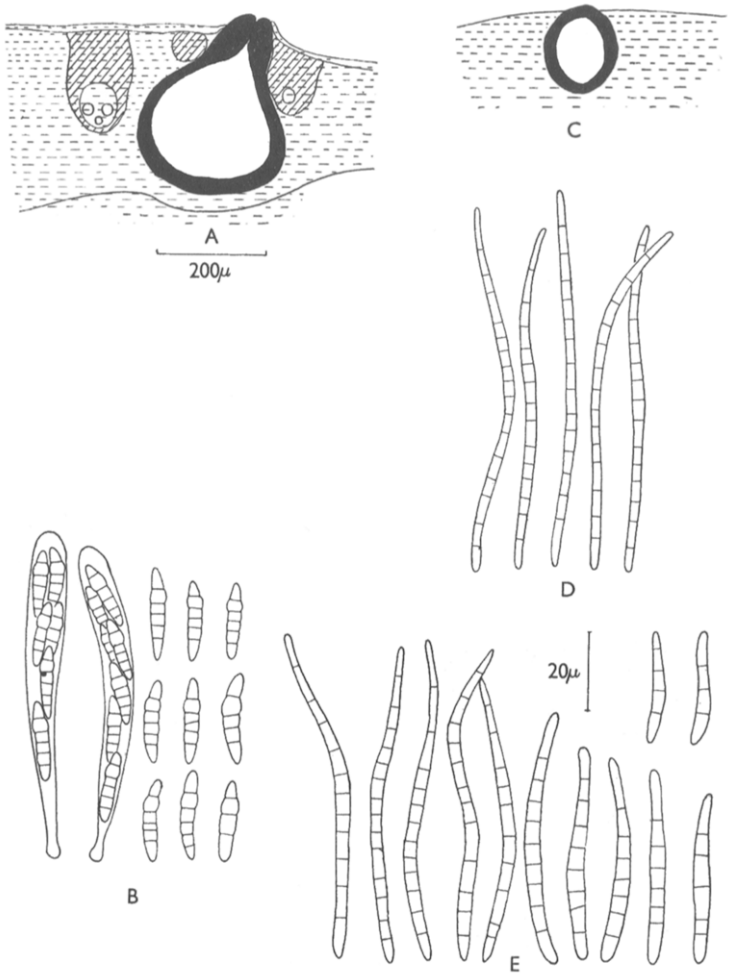

Fig. 3. *Leptosphaeria nigrans*. A, T.S. leaf sheath and perithecium; B, asci and ascospores; C, section of culture showing a pycnidium; D, pycnospores from the type specimen of *Septoria alopecuri* var. *calamagrostidis*; E, pycnospores from culture.

(3) **Pleospora vagans** Niessl. (Fig. 4)

This fungus was described by Niessl (1876), who distinguished three forms: (*a*) arenaria, (*b*) pusilla and (*c*) Airae. Berlese (1900) added *P. vagans* var. *sparganii* based on *Pleospora sparganii* Cooke. Perithecia of *P. vagans* have since been collected on numerous grasses and other Monocotyledons (see Oudemans, 1919; Wehmeyer, 1949). I have collected perithecia on the following hosts: *Agropyron repens*, *Agrostis* sp., *Alopecurus pratensis*, *Ammophila arenaria*, *Anthoxanthum odoratum*, *Arrhenatherum elatius*, *Brachypodium sylvaticum*, *Bromus ramosus*, *Calamagrostis epigeios*, *C. lanceolata*, *Dactylis glomerata*, *Deschampsia caespitosa*, *Elymus arenarius*, *Festuca arundinacea*, *F. pratensis*, *F. rubra*, *Holcus mollis*, *Hordeum murinum*, *Lolium perenne*,

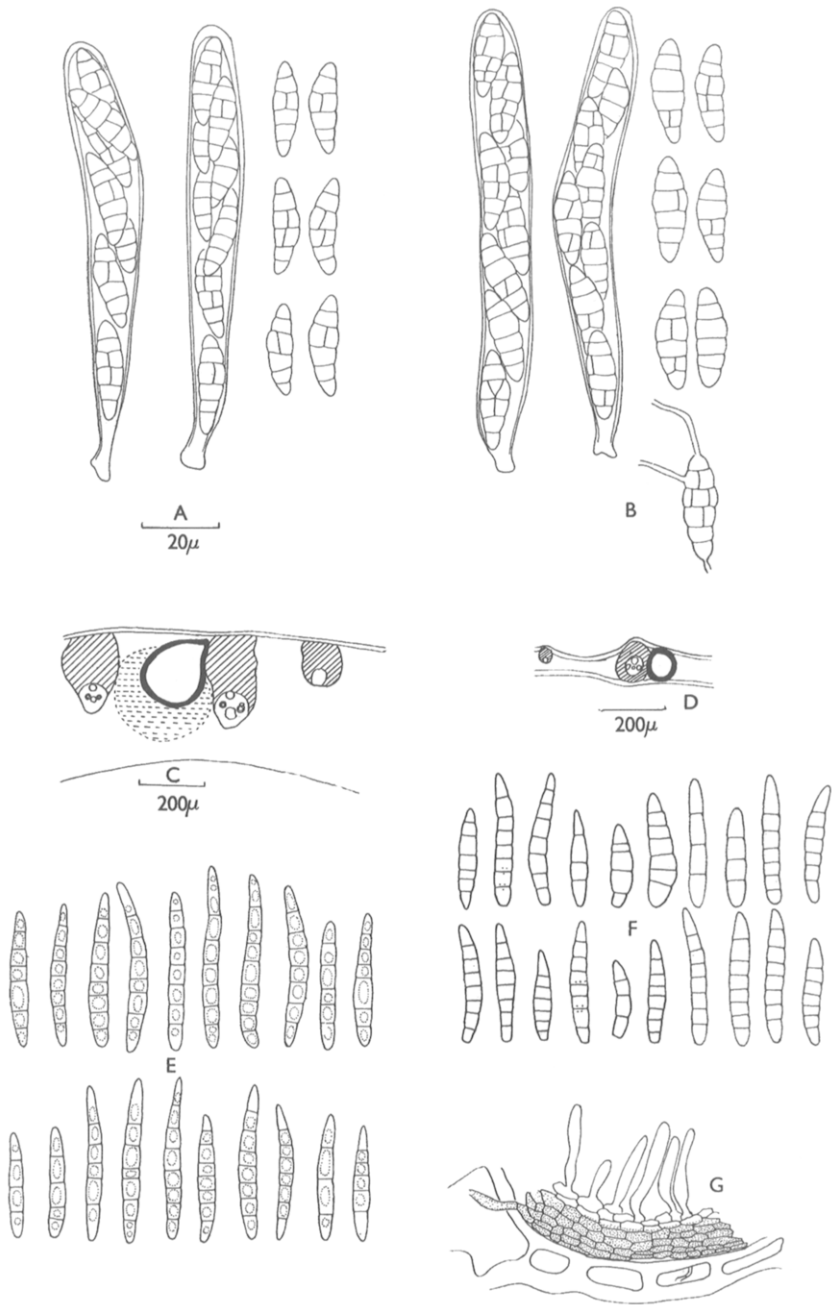

Fig. 4. *Pleospora vagans*. A, asci and ascospores formed in culture on oat agar derived from a single pycnospore; B, asci and ascospores from *Dactylis*; C, T.S. leaf sheath of *Dactylis* with a perithecium; D, T.S. leaf sheath and pycnidium; E, pycnospores formed in culture on sterilized grass stems derived from a single ascospore; F, pycnospores from *Dactylis*; G, a portion of the pycnidial wall in section showing developing pycnospores.

Phalaris arundinacea, *Phragmites communis* and *Typha latifolia*. The specimen described below was collected on *Dactylis glomerata*. It does not match exactly any of Niessl's varieties, but as Wehmeyer (1949) has shown, intergrading forms between the varieties exist.

Mycelium immersed in the parenchymatous tissues of the leaf-sheath, stem and nodes, hyaline to pale brown in colour, branched, septate hyaline, 3–4μ wide. *Perithecia* produced between the vascular bundles (see Fig. 4 C) and are thus often arranged in rows: raising the epidermis slightly, with the small inconspicuous conical necks projecting; the perithecial wall is brown, about 15–20μ thick, composed of brown-walled flattened polygonal cells, 8–22μ wide, and bearing numerous hyphae at the base. Mature perithecia measure 180–220μ in diameter. *Asci* numerous arising at the base of the perithecium, expanding between the cylindrical septate 'paraphyses' which are 2–4μ in width; mature asci club-shaped or cylindrical, shortly stalked, rounded at the apex, with two membranes, 8-spored and measure 92–124 × 14–16μ. *Ascospores* yellowish brown, biseriate in the upper portion of the ascus, uniseriate below, broadly fusoid, inequilateral, divided by five (or occasionally six) transverse septa, and slightly constricted at the septa. The third cell from the apex is rather wider than the rest, the end cells are rounded; one or more of the four central cells are divided by longitudinal septa, but occasionally spores without longitudinal septa may be found; the ascospores measure 22–28 × 7·5–9·5μ.

The ascospores germinate within a few hours in water producing germ-tubes apparently from any cell. Cultures were prepared from single ascospores on several occasions.

Stems of *Dactylis* sterilized by exposure to propylene oxide vapour and incubated at room temperature in boiling tubes containing sterilized damp sand, and *Sphagnum* were inoculated with single ascospore cultures on 13 November 1950. When examined on 24 January 1951 a few brown pycnidia had formed on the surface mycelium covering some of the stems; the pycnidia contained brown, elongate, 7-septate pycnospores. The conidial stage belongs to the form-genus *Hendersonia*. A similar pycnidial form is found associated with *Pleospora vagans*, on *Dactylis*. In order to confirm the observation that this *Hendersonia* is the conidial stage of *Pleospora vagans*, cultures were made from single ascospores and single pycnospores from material on *Dactylis* on various media and on sterilized grass stems on 15 December 1951, incubated in diffuse light at room temperature and examined on 9 February 1952. The cultures of *Pleospora vagans* and *Hendersonia* on oat agar were identical in growth and appearance, and in both cultures ripe perithecia of *Pleospora vagans* were found. Perithecia were also found in cultures of both isolates on sterilized grass stems. We may therefore conclude that *P. vagans* is homothallic and that its conidial state is a *Hendersonia*.

The pycnidia of *Pleospora vagans* on the host have a similar distribution to the perithecia, occurring between the veins most frequently on the upper internodes and nodes; they are however smaller, measuring 95–110μ in diameter, globose or somewhat flattened with a small papillate ostiole; the wall is brown, 12–15μ thick, and composed of polygonal cells. The

inner layers of the wall are hyaline and bear the pycnospores; individual cells appear to elongate to form the cylindrical body of the spore (Fig. 4 G), which becomes divided by transverse septa. Mature pycnospores are pale yellow in colour, 3–7 septate, faintly constricted at the septa, straight or slightly curved, tapering above, rather abruptly truncate below; each cell contains several small guttules which are grouped together near the septa (Fig. 4 F): the pycnospores measure 24–46 × 4–6 μ. They germinate by producing germ tubes, apparently from any cell.

The cultures on oat agar slopes are grey to black in colour with a limited aerial mycelium with white or pale rose tints; at the upper end of the tube the mycelium in the substratum is orange to brown in colour with small black stromatic masses. Perithecia only were observed in these cultures, for the most part embedded in the medium. These resembled those formed on the host; the asci measured 112–122 × 14–16 μ; the ascospores were slightly narrower than those on the host, measuring 23–28 × 7–8 μ. The pycnidia formed in culture on the sterilized grass stems were superficial, dark brown, depressed globose, rather larger than those on the host 100–150 μ in diameter; the pycnospores were yellow to brown in colour 3–7 septate, usually with a single large guttule in each cell; they measured 27–48 × 4–5 μ.

Cultures have also been prepared from conidia and ascospores from various hosts, and the dimensions of these collections and the behaviour of the cultures are tabulated in Table 3. The conidia obtained in some of these cultures show greater variation than those described in cultures from *Dactylis*. For example, conidia varying in size from 34–60 × 4·5–6 μ containing 3–14 septa were found in cultures started with ascospores from *Anthoxanthum odoratum* (1192*b*), and conidia varying from 36–64 × 4–6 μ with 7–10 septa have been found in cultures started with conidia from *Melica uniflora* (1361). The conidia on the host generally show less variation in size; conidia usually measure 26–40 × 3·5–5 μ. I have collected conidia on the following hosts: *Anthoxanthum odoratum*, *Calamagrostis epigeios*, *Cynosurus cristatus*, *Dactylis glomerata*, *Deschampsia caespitosa*, *Elymus arenarius*, *Festuca arundinacea*, *F. rubra*, *Helictotrichon pubescens*, *Holcus lanatus*, *Hordeum murinum*, *Melica uniflora*, *Phalaris arundinacea*, *Phragmitis communis* and cultivated barley.

Some of the collections of perithecia listed in Table 3 probably correspond with Niessl's varieties: e.g. var. *arenaria* described on *Elymus arenarius* is probably represented by collections 1210*a* and 1393, whilst var. *Airae* described from *Deschampsia caespitosa* is probably represented by collection 1607.

Hendersonia crastophila Sacc. is a name which should be considered as a possible synonym of *Pleospora vagans*. Saccardo's (1879) description of this fungus from *Phragmites communis* gives 'stylosporis bacillari-fusoideis, utrinque rotundatis, 35 × 5·5, 7–8 septatis, eguttulatis, fuligineis'. Unfortunately, there is no type material available for study. Sprague (1943*a*, *b*) discussed this species, and later (1950) gave a more extensive account with a list of grass hosts, citing *Wojnowicia graminis* (McAlp.) Sacc. et Sacc. as a synonym. However, in more than 150 isolations of *W. graminis* from wheat and grasses Sprague (1935) did not report perithecia, which were

readily obtained in 2-month-old oat agar culture started from pycnospores in the present study. I have studied material and cultures of *W. graminis* from *Agropyron pungens* and wheat stubble, but this fungus is different from *Pleospora vagans*. In the absence of type material of *Hendersonia crastophila*, it is difficult to know whether Sprague is correct in regarding *H. crastophila* and *Wojnowicia graminis* as synonyms. However, *W. graminis* is not related to *Pleospora vagans*.

Table 3. *Dimensions of ascospores and conidia of* Pleospora vagans *on various hosts and in 2-month-old cultures on oat agar*

Herb. no.	Host	Type of fruit-body produced in culture	Dimensions on host (μ)		Dimensions in culture (μ)	
			Ascospores	Conidia	Ascospores	Conidia
1187	*Ammophila arenaria*	per.	22–30 × 7–10	—	24–28 × 6–8·5	—
1192*b*	*Anthoxanthum odoratum*	per. & con.	23–28 × 7–9	—	24–28 × 6·5–8	34–60 × 4·5–6
1210*a*	*Elymus arenarius*	per. & con.	22–28 × 7–9	—	20–28 × 7–10	40–56 × 4·5–5
1383	*Agropyron repens*	per.	22–28 × 7–10	—	22–26 × 7–9	—
1393	*Elymus arenarius*	per. & con.	24–30 × 8·5–11	—	25–30 × 8–10	30–32 × 4–4·5
1478*a*	*Phragmites communis*	per. & con.	22–30 × 7–10	—	22–28 × 7–9	33–50 × 3·5–4·5
1607	*Deschampsia caespitosa*	per.	24–28 × 7·5–8·5	—	24–27 × 7–8	—
1627	*Typha latifolia*	per.	22–27 × 7–8·5	—	22–28 × 6·5–8	—
1190	*Phragmites communis*	con.	—	36–44 × 3·5–4	—	32–38 × 4–4·5
1192	*Anthoxanthum odoratum*	con.	—	26–38 × 4–5	—	30–56 × 4–5
1199	*Helictotrichon pubescens*	per.	—	28–42 × 3·5–4·5	20–24 × 7·5–10	—
1352*a*	*Holcus lanatus*	per.	—	26–38 × 4–4·5	24–28 × 8–10	—
1361	*Melica uniflora*	per. & con.	—	27–37 × 4·5–6	24–28 × 7–8	36–64 × 4–6
1209*b*	*Arrhenatherum elatius*	per.	—	26–36 × 4–5	22–26 × 7–8	—
1477	*Phragmites communis*	con.	—	31–64 × 4–6	—	28–52 × 4–6
1606	*Deschampsia caespitosa*	per.	—	32–49 × 4·5–6	23–29 × 7–8·5	—

Wehmeyer (1949) cites *P. fuegiana* Speg. and *P. forsteri* as synonyms of *P. vagans*. During examination of specimens in Herb. Kew it became evident that *Sphaeria clara* Auersw. ex Cooke is also a synonym. The name dates from the publication by Cooke (*Grevillea*, 1877, **5**, 121) of '*Sphaeria clara* Awd. Perithecia scattered, seated beneath the cuticle, which in consequence is darkened above them; asci clavate; sporidia lanceolate, yellowish, 4–5 septate, with one of the cells longitudinally divided. On *Sparganium*, N. Wootton. Appears to be the same as specimens distributed by Dr Winter under this name, although we have seen no description. Sporidia 0·035 × 0·01 mm., the divided cell usually broadest.'

There is no record of the publication of a description by Auerswald. Saccardo (1883) compiled Cooke's description as *Leptosphaeria clara* (Cooke & Auersw.) Sacc., although he noted in his description 'loculo uno longitudinalita diviso (teste Cooke)'. He also observed that specimens sent by Winter under the name *L. clara* contained a *Didymella*, which was confirmed by Berlese (1894). The Winter specimen referred to by Cooke is

in Herb. Kew labelled 'Herbarium von Georg Winter. Leptosphaeria clara Awd. Leipzig: Corewitz. Carex acuta. 7.7.1866. leg Auerswald', and also bearing the stamp of Cooke's Herbarium. Unfortunately, preparation revealed only immature specimens and no spores.

There is no specimen on *Sparganium* in the *Leptosphaeria clara* folder in Herb. Kew. However, there are various other specimens bearing Cooke's Herbarium stamp:

1. *Sphaeria clara* Awd. on glumes of *Festuca*, Neatishead. Dec. /76, labelled in Cooke's handwriting. This specimen contained perithecia of *Pleospora vagans*.

2. Herbarium von Georg Winter. *Leptosphaeria clara* Awd. Leipzig. Mai 1871. leg. G. Winter. Perithecia of *P. vagans* were also found on this specimen.

3. *Sphaerella microspora*. Rab. F. E. 1646 in Cooke's handwriting. This specimen bears an ink drawing of four spores each with 5 transverse septa and one with a single longitudinal septum. *Pleospora vagans* perithecia were found on this specimen.

In the same folder are two loose drawings by Cooke, one of four spores, each with five transverse and a single longitudinal septum labelled 'clara' and another with four 2-celled spores of the *Didymella* type labelled 'clara' Winter.

It thus seems likely from Cooke's description, drawings and specimens that his conception of *Sphaeria clara* Auersw. was *Pleospora vagans* Niessl, which antedates Cooke's description. It is possible that this was not the fungus that Auerswald intended to name, for according to Saccardo, Berlese and Cooke's own drawings a *Didymella* was present on the Winter specimens. However, since Auerswald's name was not validly published, the specimen on *Sparganium* from North Wootton is the type specimen. It is unfortunate that this specimen is missing, but the specimen on *Festuca* is authentic. It is therefore proposed that the name *Leptosphaeria clara* (Auersw. ex Cooke) Sacc. be rejected as a synonym of *Pleospora vagans* Niessl.

It is interesting to note that in the *P. sparganii* folder (labelled by Cooke) in Herb. Kew is a specimen labelled in Cooke's handwriting of 'Sphaeria on *Sparganium* N. Wootton 19'. This is possibly the type specimen of *Pleospora sparganii* Cooke (*Grevillea*, **19**, 8), and later named *P. vagans* var. *sparganii* by Berlese, 1900. Examination of the specimen confirms Berlese's disposition of the fungus.

(4) **Phomatospora dinemasporium** sp.nov. (Fig. 5)

Several collections have been made of a *Phomatospora* which is the perfect state of *Dinemasporium graminum* (Lib.) Lév.

The description below is of a collection on *Agrostis stolonifera* (Herb. Sheffield no. 1163):

Mycelium hyaline to pale brown, 1–2 μ wide, penetrating parenchymatous and lignified tissues, associated with a conspicuous darkening of the epidermis around the perithecia, which becomes raised, black and shining. *Perithecia* subepidermal, on leaf sheaths and stems, singly or in groups, between the veins, globose or elongated parallel to the stem,

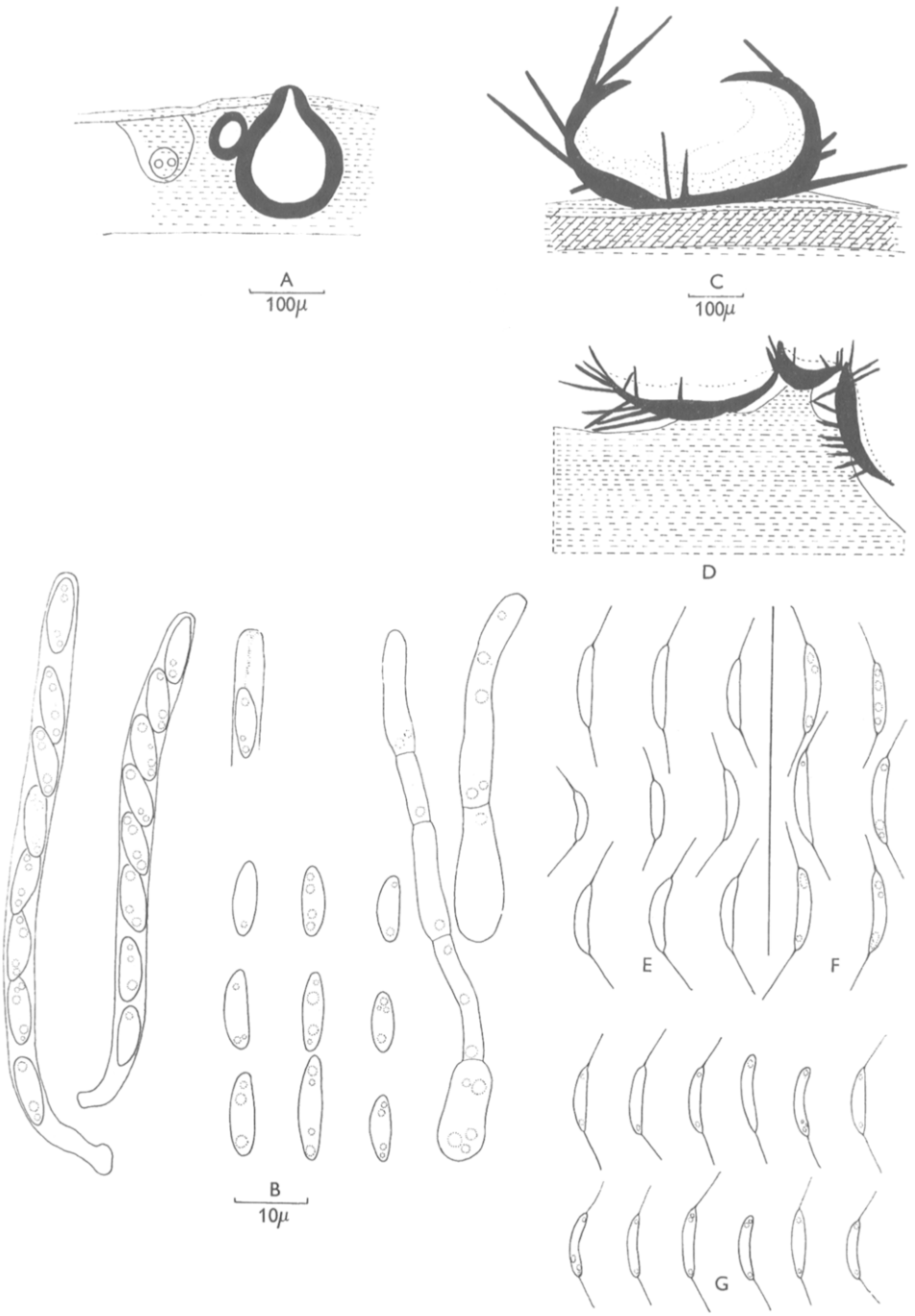

Fig. 5. *Phomatospora dinemasporium*. A, T.S. leaf sheath and perithecium; B, asci and ascospores; C, T.S. stem and conidial pustule; D, section of oat agar culture derived from a single ascospore, with conidial pustules; E, conidia from culture; F, conidia from host; G, conidia from Libert's Pl. Crypt. Ardeunn. Exs. no. 348. C, D to same scale; B, E, F, G to same scale.

120–300μ in diameter, wall 6–15μ thick composed of brown elongate polygonal cells; ostiole inconspicuous. *Asci* 4–8-spored, narrow, cylindrical, short-stalked, rounded above, wall single, 68–84 × 4–5μ. *Ascospores* typically uniseriate (rarely sub-biseriate) narrowly elliptical to spindle-shaped or slightly inequilateral, hyaline, smooth, biguttulate 9–16 × 3–4μ. Paraphyses were not seen.

The ascospores germinated readily on the surface of maize extract agar or in water drops on a microscope slide. Appressoria were not seen. Germinating ascospores were transferred to oat agar slopes and produced orange-brown colonies with little aerial mycelium. In 2-month-old cultures black setose pustules were found, bearing conidia. The pustules are single or in clusters, and may be up to 2 mm. in diameter. Each pustule consists of a saucer-shaped receptacle composed of dark thick-walled cells about 5μ wide, surrounded by thick-walled septate, tapering, pointed black bristles up to 250μ long and 4–10μ wide at the base. The receptacle is lined by a palisade of hyaline conidiophores, branched or unbranched, ending in phialides. The conidia are allantoid, hyaline, smooth, and bear a single bristle at each end, measuring up to 10μ long. The body of the conidium measures 8–12 × 1·5–2μ. Bristles were not found on conidia still attached to their conidiophores, and are possibly formed later.

Similar conidial pustules were collected with the perithecia, growing on the nodes, leaf sheath or stem single or in clusters, varying in size from 0·2 to 1·5 mm. They develop from a mycelium completely filling the host tissues beneath the pustule, then forming a surface hyaline mat, from which the black setae arise. The conidia resemble those described above and measure 8–13 × 1·5–2μ. Cultures prepared from single conidia matched very closely the cultures described above derived from ascospores.

Table 4. *Dimensions (in μ) of asci, ascospores and conidia of* Phomatospora dinemasporium

Herb. Sheffield no.	Date	Host	Asci	Ascospores	Conidia from culture
237*a*	18. iii. 50	*Dactylis glomerata*	66–80 × 4–5	9–10 × 2–2·5	—
242	26. vi. 49	*Phalaris arundinacea*	70–92 × 4–5	10–12 × 2·5–3	—
246	31. v. 49	*Agropyron repens*	100–122 × 4–5	11–16 × 2–3·5	7–14 × 1–2
393	29. v. 50	*Poa trivialis*	60–80 × 4–5	9–12 × 2·5–3	—
1163	5. iii. 54	*Agrostis stolonifera*	68–84 × 4–5	9–16 × 3–4	8–12 × 1·5–2
1172	18. x. 52	*Arrhenatherum elatius*	50–74 × 4–6	9–12 × 2·5–3	—
1176	17. v. 54	*Festuca rubra*	70–78 × 4–5	8–10 × 2–3	—
1221	28. x. 50	*Dactylis glomerata*	64–80 × 4–5	9–12 × 2·5–3	—
1311	12. ii. 51	*D. glomerata*	60–75 × 4–4·5	9–12 × 2–3	8–12 × 1·5–2
1337*a*	16. iv. 52	*D. glomerata*	62–88 × 4–5	8–10 × 3–4	—
1377	28. viii. 54	*Phalaris arundinacea*	64–94 × 4–5	8–13 × 2·5–3	8–14 × 1·5–2
1391	16. ix. 54	Cereal stubble	80–84 × 4–5	10–13 × 3–4	6–12 × 2–2·5
1449	26. x. 54	*Zerna erecta*	66–76 × 4–5	8–12 × 2–2·5	—
1450	26. x. 54	*Agropyron repens*	50–70 × 4–5	8–12 × 2·5–3	—

Perithecia have been collected on various grasses, and appear to be most common on old fallen stems lying on the ground in damp places. Table 4 shows the range of size of asci and ascospores, and of conidia produced in cultures on oat agar from some of these collections.

The conidia of the fungus belong to the form genus *Dinemasporium*, and many collections have been made of the conidial state on various grasses, sometimes in association with the perithecia. Conidia have been collected on the following hosts: *Agropyron repens*, *Agrostis stolonifera*, *Alopecurus pratensis*, *Arrhenatherum elatius*, barley stubble, *Brachypodium pinnatum*, *Dactylis glomerata*, *Deschampsia caespitosa*, *Festuca rubra*, *Holcus mollis*, *Juncus maritimus*, *Molinia caerulea*, *Phalaris arundinacea*, *Phleum pratense*, *Phragmites communis*, *Poa* sp. and wheat stubble, and are found throughout the year. They are most common on stem bases and fallen stems in damp places, and appear to colonize both freshly dead and old tissues.

Most of these collections bear conidia 8–14 × 1·5–2 μ, but the overall dimensions are 5–16 × 1–2·5 μ. In some collections (1221*b* on *Dactylis glomerata*, 1176*b* on *Festuca rubra*, 1448*a* on *Brachypodium pinnatum*) individual pustules were found in which the conidia lacked bristles. However, an examination of two adjacent pustules of specimen no. 1448 showed that one pustule contained spores bearing bristles, whilst the other bore conidia of the normal type. In another collection (1447 on *Dactylis glomerata*) 38 out of a sample of 116 spores examined (30·5 %) lacked bristles.

The conidia obtained from the *Phomatospora* ascospores, and the collections of conidia discussed above match descriptions of *Dinemasporium graminum* (Lib.) Lév. Léveillé (1847) erected the genus *Dinemasporium* with *D. graminum* as the type, citing the following synonyms and exsiccati: '*Peziza strigosa*, Fr. *Obs. myc.* 2, p. 304. S.M. 24, p. 103.—*Vermicularia graminum*, Lib. exsicc., No. 348—*Excipula graminum* Corda, *Icon. fung.* 3, p. 29. tab. 5, f. 79.' An example of Libert's exsiccatum no. 348 is preserved in the Herb. Kew and consists of three pieces of grass leaves bearing the characteristic black saucer-shaped pustules containing hyaline and typically biciliate spores measuring 8–11 × 1·5–2 μ, with bristles 6–10 μ long (see Fig. 5 G). Libert's specimen thus matches the collections and cultures described above.

Fries (*Systema mycologicum*, **2**, 103) cites *Exsiccatum* no. 136 as an example of *Peziza* (*Excipula*) *strigosa*. Examination of this exsiccatum from the Herb. Kew (Scleromycetae Sueciae no. 136) confirms Léveillé's citation of this fungus as synonymous with Libert's specimens no. 348. I regard both these specimens as typical *Phomatospora* conidia.

Karsten (1884) described *D. graminum* s.s. *strigosulum*, 'A typo recedit spermatiis minoribus (longit 9–12 mm.) crassit 2–3 mm. setulisque 6–8 mm. longis utrinque acutis. In culmis foliisque emortuis *Secalis cerealis*, *Poae* et *Phragmitis communis* circa Mustiala non rarum.'

Three specimens which are probably those cited by Karsten were examined:

1. Specimen labelled in Karsten's handwriting—*Dinemasporium strigosulum*. Mustiala ad caul. Secal. cereal. 4 Jan. 66.

The packet has been relabelled = *Dinemasporium* Lév. var. *strigosulum* Karst. and bears a sketch of a few spores, but these show no bristles. The specimen consists of four pieces of straw bearing dark flattened pustules with black margins and pale yellow disks. No setae were found around the pustules. The hymenium contains numerous branched sporophores

bearing cylindrical hyaline spores with rounded ends, but lacking bristles, measuring 8–12 × 2 μ.

This specimen is clearly not a *Dinemasporium*.

2. Specimen labelled—*Polynema strigosum* Fr. f. *Arundinis*. Mustiala ad Phragm. P. A. Karsten 22 May 1866.

The packet has been relabelled *Dinemasporium graminum* Lib. (Lév). Sacc S. F. xi. 560, and also bears a sketch of two typical *Dinemasporium* spores. The specimen consists of pieces of *Phragmites* stem and leaf sheaths bearing *Dinemasporium* pustules, with spores 12–17 × 2 μ, and bristles up to 15 μ long. It is probably best referred to *D. graminum*.

3. Specimen labelled—*Polynema strigosum* Mustiala 15 Apr. 1866.

The packet has been relabelled *Dinemasporium graminum* Lév. W.N., bears drawings of spores lacking bristles. The specimen consists of numerous pieces of grass leaf bearing black pustules not surrounded by setae. The spores are subhyaline, cylindrical, tapering at the ends, but lacking bristles. The specimen is not a *Dinemasporium*.

From an examination of these specimens from the Karsten Herbarium there is no foundation for maintaining *D. graminum* var. *strigosulum*. The only specimen which can be certainly identified with this name is not even a *Dinemasporium*. The collection on *Phragmites* (2) whilst undoubtedly a *Dinemasporium*, bears spores which are slightly larger than the Libert specimen cited by Léveillé. It is probable that collections cited under this name should be identified as *D. graminum*.

Phomatospora dinemasporium sp.nov. (Fig. 5)

Mycelium hyalinum vel brunneum, 1–2 μ latum, partes parenchymaticas et ligneas penetrans et epiderma nigrificans circa perithecia, atque ita prominens et polita fit. Perithecia sub epidermida crescentia, in culmis vaginisque, aut singula aut plura, inter venas, globosa vel elongata, 120–300 μ diametra quorum muri 6–15 μ lati sunt, et ex cellis elongatis brunneisque compositi, ostiola inconspicua. Asci 4–8 sporas continentes, angusti, cylindricales, cum brevibus stipitibus, membranis unis, supra rotundati, 68–84 × 4–5 μ.

Ascosporae uniseriatae, plerumque (nonnumquam sub-biseriatae) tenuatim ellipticales, vel fusoideae vel inequilaterales, hyalinae, glabrae, biguttulatae, 9–16 × 3–4 μ. Paraphyses non visae sunt.

Status conidicus *Dinemasporium graminum* est. In culmis multorum graminum. Specimen No. 1163 Mycological Herbarium, University of Sheffield typus est.

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