Trans. Brit. mycol. Soc. 47 (3), 393–401 (1964)
Printed in Great Britain

GENICULOSPORIUM SERPENS GEN. ET SP.NOV., THE IMPERFECT STATE OF *HYPOXYLON SERPENS*

By C. G. C. CHESTERS and G. N. GREENHALGH
Department of Botany, University of Nottingham

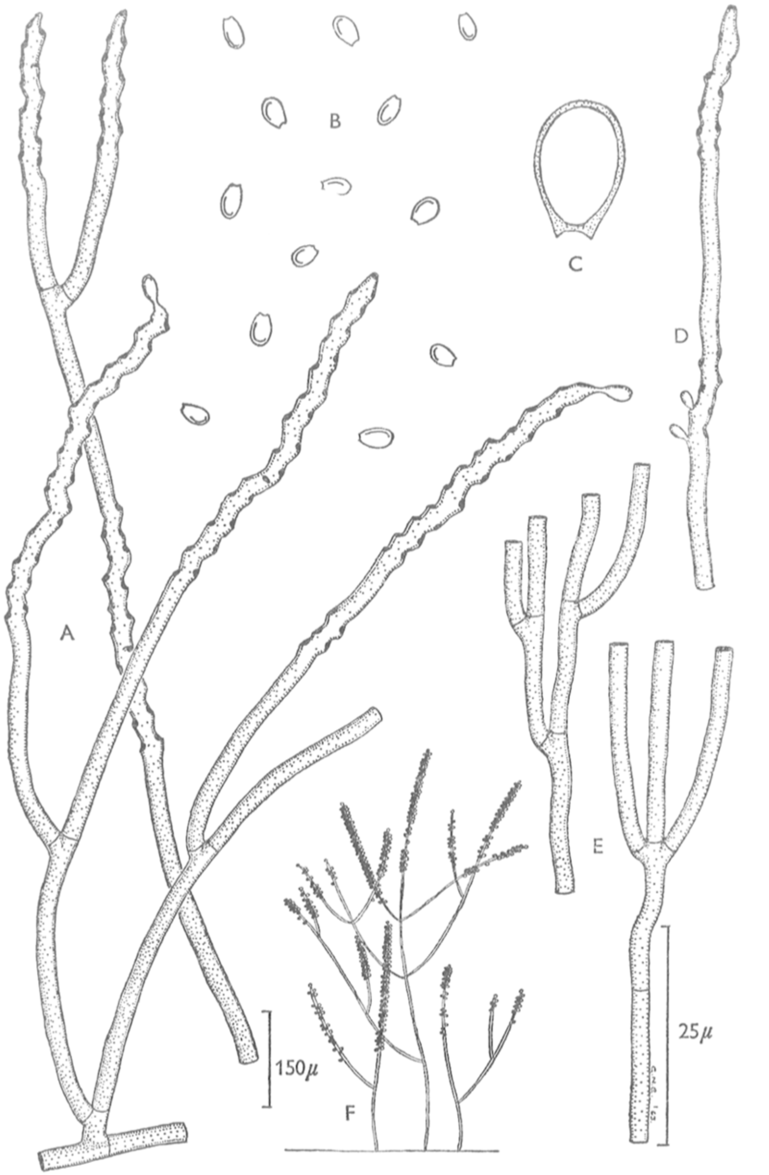
(With Plate 18 and 3 Text-figures)

A fairly common mould on decaying branches and trunks of forest trees, formerly incorrectly known as *Haplaria grisea* Link, is described and *Geniculosporium* gen. nov. is proposed to accommodate it, with *G. serpens* sp.nov. as type species. Its relationship with *Hypoxylon serpens* (Pers. ex Fr.) Kickx. is discussed and it is briefly compared with the imperfect states of other Xylariaceae.

Haplaria grisea (Link, 1809) is the name which has long been applied to a not uncommon mould found on decaying wood and on branches of various deciduous trees. Lindau (1907) described this mould, under this name, as having a widespread grey turf with compact conidiophores about 0·2 mm. high and 3·4 μ thick, septate, branched and with the branches mostly arising beneath a septum; they were also said to be bent 'back and forth' (i.e. geniculate). The conidia were acrogenous, becoming lateral by continued growth of the conidiophore, unicellular, almost spherical or rather more ellipsoid, and measuring about $4 \times 3\,\mu$. It was stated to occur on oak wood, rotting willow bark and dry leaves of various marsh plants such as *Typha* and *Sparganium*. The conidiophores were illustrated (Pl. 18, fig. 1).

Hughes (1958) reported that examination of the type material of *H. grisea* revealed it to be identical with *Botrytis cinerea* Pers. ex Fr. (1801). Therefore, since *H. grisea* is in effect *B. cinerea*, and as the former is the type of *Haplaria* that genus must be discarded. It therefore becomes necessary to find a name for the fungus described by Lindau, which, of course, is not *B. cinerea*.

Lindau's description and figure fit the imperfect state of *Hypoxylon serpens* very well, and examination of specimens of '*Haplaria grisea*' preserved at the Royal Botanic Gardens, Kew, and the Commonwealth Mycological Institute, Kew, has confirmed the identity. *Hypoxylon serpens* is a common pyrenomycete on decorticated wood, stumps and logs of various deciduous trees (Pl. 18, figs. 3 and 6). The conidial state is readily obtained in culture from single ascospores or groups of ascospores shot off from stromata collected in the right condition. Part of the stroma is fixed with vaseline to the inner surface of a Petri dish lid inverted over agar. It may be necessary to moisten the fruit body first. Cultures so obtained are greyish, occasionally somewhat brownish, generally with a low and floccose surface, and may be irregularly zonate. The intensity



Text-fig. 1. Conidial state of *H. serpens*. A, conidiophores; B, conidia; C, enlarged conidium (about 4μ long); D, portion of conidiophore showing two geniculate regions separated by length of undifferentiated conidiophore; E, showing type of branching of the conidiophore; F, habit of the conidiophore.

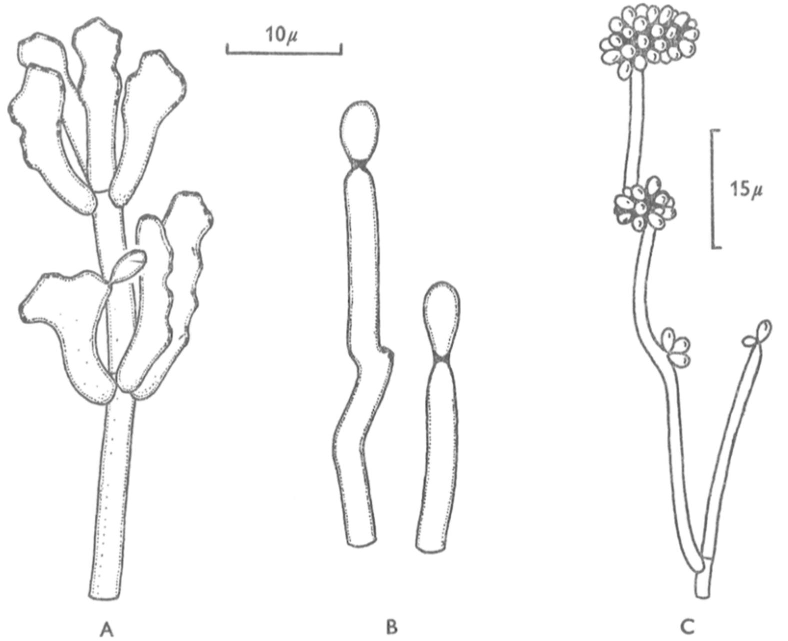
of colour depends on the degree to which spores are produced. Some isolates may produce very few conidiophores and such colonies are whitish. However, most isolates produce conidia in great quantity. The conidiophores reach a height of several hundred microns and branch several times, each branch arising just beneath a septum. The parent conidiophore may be slightly displaced to one side; then the branching may superficially appear to be dichotomous and often two or more branches arise beneath the same septum (Text-fig. 1 E); thus, there is no morphologically well-defined main axis. Conidiophores are smooth-walled, very regular in form and rather brownish except in the apical fertile regions where they are usually colourless. However, cultures inoculated on to culms of *Typha* produced conidiophores that were eventually almost uniformly pigmented right to the tips. Conidiophores are approximately 2–4μ diam. below, and 1·5–2μ in the apical region. The conidia are unicellular, hyaline, smooth, sub-globose to ovoid-ellipsoidal, and measure 3–5 × 2–3μ (Text-fig. 1 B). They are acrogenous and produced in continuous succession at the apex, the conidiophore continually extending from just beneath the terminal spore and pushing it into a lateral position (Text-fig. 1 F). When the spore is fully formed it is attached by a short neck, and on breaking away is seen to have a truncated base (Text-fig. 1 C) which is usually slightly concave. It leaves a circular scar on the conidiophore equal in area to the base of the conidium. The spore wall at its base and the conidiophore wall at the scar are both somewhat thickened. The scar itself generally has a refringent appearance. It is sometimes possible to see a narrow pore through the thickened base of the spore, and there is a corresponding pore through the scar on the conidiophore wall. The actual mechanism of dehiscence is obscure, but since the base of the spore tends to be concave, whilst the scar on the conidiophore tends to be flat, then just before the spore comes away it must be attached by a thin annulus of wall material around its base. This suggests that the wall material is dissolved away from within until only this annulus remains. The effect of this method of spore production on the morphology of the conidiophore is quite characteristic. The spores themselves are easily detached, exposing the scars on the conidiophore wall, and because of the continuous morphological changes of direction made by the conidiophore as it grows out from beneath each successive terminal spore, its final form is that seen in Text-fig. 1 A and Pl. 18, fig. 2. The fertile regions present an irregularly geniculate appearance. It must be borne in mind that the conidia are not in two ranks but lie in an irregular spiral around the conidiophore.

Brefeld (1891) in his work on Ascomycetes described this conidial form and illustrated it, though he referred to the perfect state under the name *Hypoxylon unitum* (Fr.) Nits.

Occasionally the conidiophore, after producing a series of conidia, resumes what may be called extension growth and then produces a new set of spores. There are then two geniculate regions separated by a length of conidiophore (Text-fig. 1 D). In such an instance one may find a series of conidia, presumably immature in that they do not fall away during the preparation of a squash mount, on the lower part of the conidiophore and all at the same stage of development. All the conidia in the more distal regions may have matured and fallen away. These 'secondary' spores appear to be produced through the scars left by the previous conidia.

Conidial *H. serpens* is often found on decaying wood, sometimes in proximity to the mature stromata, and has been collected on sawdust.

Although a lengthy process, production can be induced on host wood inoculated with cultures from germinating ascospores. Short lengths of wood are flamed or autoclaved and inserted on malt extract agar in the base of wide-mouthed, 500 ml. conical flasks. After several weeks the wood is removed, labelled by means of metal tags fixed with wire and placed out of doors in a protected position such as amongst long grass beneath and around bushes. By this method the conidial state has been produced on flamed alder (*Alnus*) wood, autoclaved hawthorn (*Crataegus*) and autoclaved beech (*Fagus*). On beech, the perfect state also developed



Text-fig. 2. A, Part of conidiophore system of *H. fragiforme*; B, tips of conidiophores of *H. argillaceum*; C, habit of conidiophore of *H. fuscum*.

to maturity. The imperfect state may take the form of an effuse grey mould extending over the wood surface, or may appear as discrete grey patches marking the position of development of subsequent stromata. In vertical sections perithecial initials may frequently be seen in the loose tissue beneath the mould. The conidial state may then disappear and the surface hyphae of the stroma become somewhat compacted giving a creamy grey appearance, or it may remain until the perithecia are quite well developed. It may also exist independently of, and at the same time as, the perfect state, both over the stromata and on the surface of the adjacent substratum.

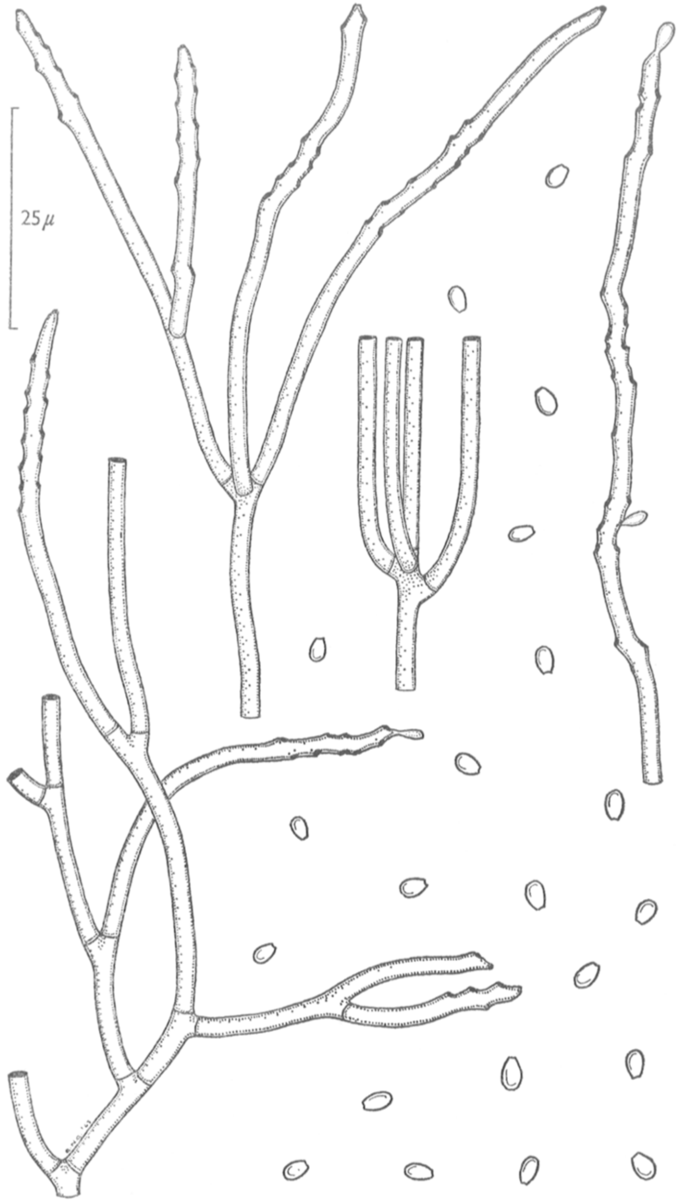
Having shown that '*Haplaria grisea*' sensu Lindau is actually the imperfect state of *Hypoxylon serpens*, it remains to find a genus within which it might be suitably accommodated. The imperfect states of many species of the Xylariaceae may be referred to *Nodulisporium* Preuss (1862) described as follows: 'Flocci erecti, septati, irregulariter ramosi; moduli verrucosi, apice subcapitati sen laterales; spores simplicibus, verrucosis, minatis, dein deciduis et hilo instructis. Hyphasma effusum.' Conidial *Daldinia concentrica* (Bolt. ex Fr.) Ces. & de Not. is a good example of the genus and Molliard (1904) after giving a description, included it as *N. tulasnei*. Miller (1961), describing the development of the stromata of *Hypoxylon* spp. refers to the formation of the conidial layer which covers the ectostroma before maturity is reached, stating that the conidia 'vary in size, usually from 3·5 μ in long axis, and are globose to elliptical and hyaline. They are borne singly or in clusters of several on ends of branched hyphae. The cluster is forced to occupy a lateral position by a branch arising directly under it and growing upward.' This description covers most of the variations found in the imperfect states of members of this genus though the range of conidial size sometimes exceeds that given. For example, cultures from ascospores of *H. argillaceum* Pers. ex Berk., an inconspicuous and not very common species found on ash, have produced conidia measuring 4·5–7·5 × 2·5–4 μ; they are ovoid to ellipsoid, hyaline and typically are produced singly at the apex of simple or branched conidiophores. Occasionally they are pushed to one side into a lateral position (Text-fig. 2B). A number of isolations of *H. rubiginosum* Pers. ex Fr., perhaps the commonest species of this genus, have produced conidia in culture which have dimensions falling into the range 3–8 × 2–4.5 μ. Rarely they may reach a length of 10 μ. Development follows the usual pattern but here several conidia are formed in close proximity, giving rise to a compact cluster. In the course of this the apex becomes swollen. Growth of the conidiophore usually recommences just beneath this cluster and pushes it to one side. In *H. fuscum* Pers. ex Fr., commonly found on hazel (*Corylus*) but not restricted to that host, development is very similar to that in *H. rubiginosum*. Here several spore clusters may be forced aside in turn, each separated by a short length of conidiophore. This can readily be demonstrated if a piece of bark bearing the fresh young stromata of this species is incubated in a moist chamber. Under these conditions conidia are produced in quantity and the conidiophores become quite long. They then present a noded appearance with the conidia clustered at each node. Because of this pattern of development the conidiophores may appear rather disjointed (Text-fig. 2C). In *H. rubiginosum* this character is less marked.

The type species of the genus is *H. fragiforme* (Pers. ex Fr.) Kickx. and here the conidiophores have reached a more complex state of development, branching several times and producing whorls of short lateral branches each of which produces several conidia. As a result of spore production, the distal portions of these lateral branches often become conspicuously swollen (Text-fig. 2A).

Therefore within this genus, and indeed within the Xylariaceae as a whole, the different forms taken by the conidiophores are the result of a series of variations on a basic and identical method of conidium production. In most species conidia are formed in the same way and are very similar, exhibiting only minor differences in shape. One exception is conidial *H. punctulatum* (Berk. & Rav.) Cke, described by Barnett (1957) under the generic name *Basidiobotrys*.

With the possible exception of conidial *H. argillaceum*, where the conidiophores have not been observed to develop swollen or capitate apices, all the conidial states mentioned above may be accommodated within *Nodulisporium*. However, this genus is plainly not an appropriate one in which to place conidial *H. serpens*, where the final morphology, although a direct result of the same basic method of spore production, is consistently different and quite characteristic. The lateral or apical swellings found, for example, in conidial *D. concentrica* are never formed. As far as is known, the only other existing genus which might take this fungus is *Hansfordia* Hughes (1951), in which the spores are produced in exactly the same way as in '*Haplaria grisea*'. The ultimate lateral branches form a series of sporogenous cells which become geniculate, and produce spherical or ovoid to fusoid, usually smooth conidia. In the type species, *Hansfordia ovalispora* Hughes, the conidiophores are 3–4 μ wide, brownish at the base and taper to a sterile apex which is hyaline to subhyaline and about 1 μ wide. Some of the lateral branches may also be drawn out into similar narrow sterile apices. However, it should be noted that some species were included in which the conidiophores are entirely fertile without sterile apices. The conidia are borne on small but conspicuous denticles. These two characters plus the fact that the conidiophores of *Hansfordia* are somewhat more organized, with a distinct central axis and with regular branching, would seem sufficient to preclude the inclusion of '*Haplaria grisea*' within that genus. The conidiophores of the latter are more indeterminate in arrangement and growth, the main axis losing its identity; insertion of the conidia is also different in that they are not borne on distinct denticles. These differences seem distinct enough to warrant the provision of a new generic name, *Geniculosporium*, for such states, with the type species *G. serpens*, imperfect state of *Hypoxylon serpens* (Pers. ex Fr.) Kickx. A culture derived from this imperfect state has been deposited at the Commonwealth Mycological Institute (**IMI** 101242).

Conidial *H. confluens* (Tode ex Fr.) West is of very similar morphology and should be included in the same genus (Text-fig. 3). This pyrenomycete has several similarities to *H. serpens* and is found on the same type of substratum, usually wet decaying oak wood (Plate 18, fig. 5). They were both classified by Miller (1961) in the same sub-section, Primocinerea, of the genus. This group is characterized by a stroma which is initially light grey to white, becoming dark brown or black later. *H. serpens* is in fact taken by Miller as the type species of the group. The two species differ mainly on stromal characters and ascospore shape. As the young ectostroma of a *Hypoxylon* is typically covered with a layer of conidia, then it might be reasonably inferred that the whitish colour of the young stromata of *H. confluens* is due to this conidial layer. In fact, though several specimens at this stage of development have been examined, no conidia or conidiophores have been located. The layer is apparently merely a tomentum of hyaline hyphae (Plate 18, fig. 4). *H. udum* Pers. ex Fr. is very similar to *H. confluens* in macroscopic stromal characters, but it has much larger ascospores of a different shape; they are ellipsoid to oblong with obtuse ends and measure 28–38 × 10–14 μ; they also possess a more or less oval, laterally placed and quite conspicuous germ pore.



Text-fig. 3. Conidiophores and conidia of *H. confluens*, from culture.

The ascospores of *H. confluens* are broadly ellipsoid fusoid, with the longitudinal germ slit characteristic of the Xylariaceae, and measure 16–22 × 8–12 μ. It would be of considerable interest to investigate the imperfect state of this species, but it is rare and, as far as the authors are aware, has not been collected in recent years.

Cultures grown from ascospores of *H. confluens* are quite different from those of *H. serpens*, being slow growing, white and conspicuously zonate. They remain sterile for a long time and only on two occasions have conidia been obtained. At this stage the colony becomes light or creamy brown, and then somewhat grey. The conidiophores are very similar to those of *H. serpens* (Text-fig. 3) and measure 1·5–4 × 1·5–2·5 μ.

Geniculosporium gen.nov.

Mycelium immersum vel superficiale. Conidiophora erecta, septata, non in coremia aggregata, laevia, regularia; vel semel vel saepius ramulata; stipes non plane distinctus, primo hyalinus, deinde inferne brunneus, ad apices hyalinus vel brunneus. Regiones apicales stipitis ramulorumque propter extensionem sympodialem geniculatae. Conidia acrogena, conidiophoris ab inferna parte continuo crescentibus ad alterum latus lateraliter dislocata, atque idcirco lateraliter secumdum conidiophorum in spiram irregularem vel alternatam compositionem inserta; non in conspicuis denticulis portata; sub-sphaerica usque ad ovoidea-ellipsoidea, continua, laevia, hyalina cum basi truncata.

Species typica: G. serpens Chesters & Greenhalgh (status imperfectus H. serpentis).

Geniculosporium serpens sp.nov.

Coloniae effusae griseae, vel griseo-fuscae. Mycelium immersum et superficiale, flexuosum, septatum, usque ad 3·5 μ latum, hyalinum vel brunneum. Conidiophora separata vel aggregata oriunda; usque ad 4·5 μ inferne et hyalina vel brunnea, et ad apices hyalina vel brunnea et circiter 2 μ lata; longitudinus indeterminatae, usque ad 300 μ vel amplius. Ramuli intervallis, plerumque saepius, nec nisi proxime sub septis oriundi. Cellulae fertiles usque ad 200 μ longae, sed plerumque minores, geniculatae, cum cicatricibus planis et conidialibus in spira irregulari compositis, hyalinae sunt, non numquam brunneae fiunt. Conidia acrogena, hyalina, laevia, continua, sub-sphaerica usque ad ovoidea, 3–5 × 2–3 μ, cum basi truncata. Habitat in ramis vel truncis arborum deciduarum mortuarum, corticatis vel decorticatis. Culturus in herbario **IMI** (101242) typus est.

The authors wish to express their thanks to Dr M. B. Ellis for taxonomic advice, and to W. R. Chalmers and G. R. Watson of the Classics Department, Nottingham University, for help in preparing the Latin diagnoses.

REFERENCES

BARNETT, H. L. (1957). *Hypoxylon punctulatum* and its conidial state on dead oak trees and in culture. *Mycologia*, **49**, 588–595.

BREFELD, O. (1891). *Untersuchungen aus dem Gesammtegebiete der Mykologie.* Vol. X. Ascomyceten II.

HUGHES, S. J. (1951). Studies on microfungi IX. *Calcariosporium, Verticicladium* and *Hansfordia* (gen.nov). *Mycol. Pap.* **43**, 1–25.

HUGHES, S. J. (1958). Revisiones hyphomycetum aliquot cum appendices de nominibus rejiciendis. *Canad. J. Bot.* **36**, 727–836.

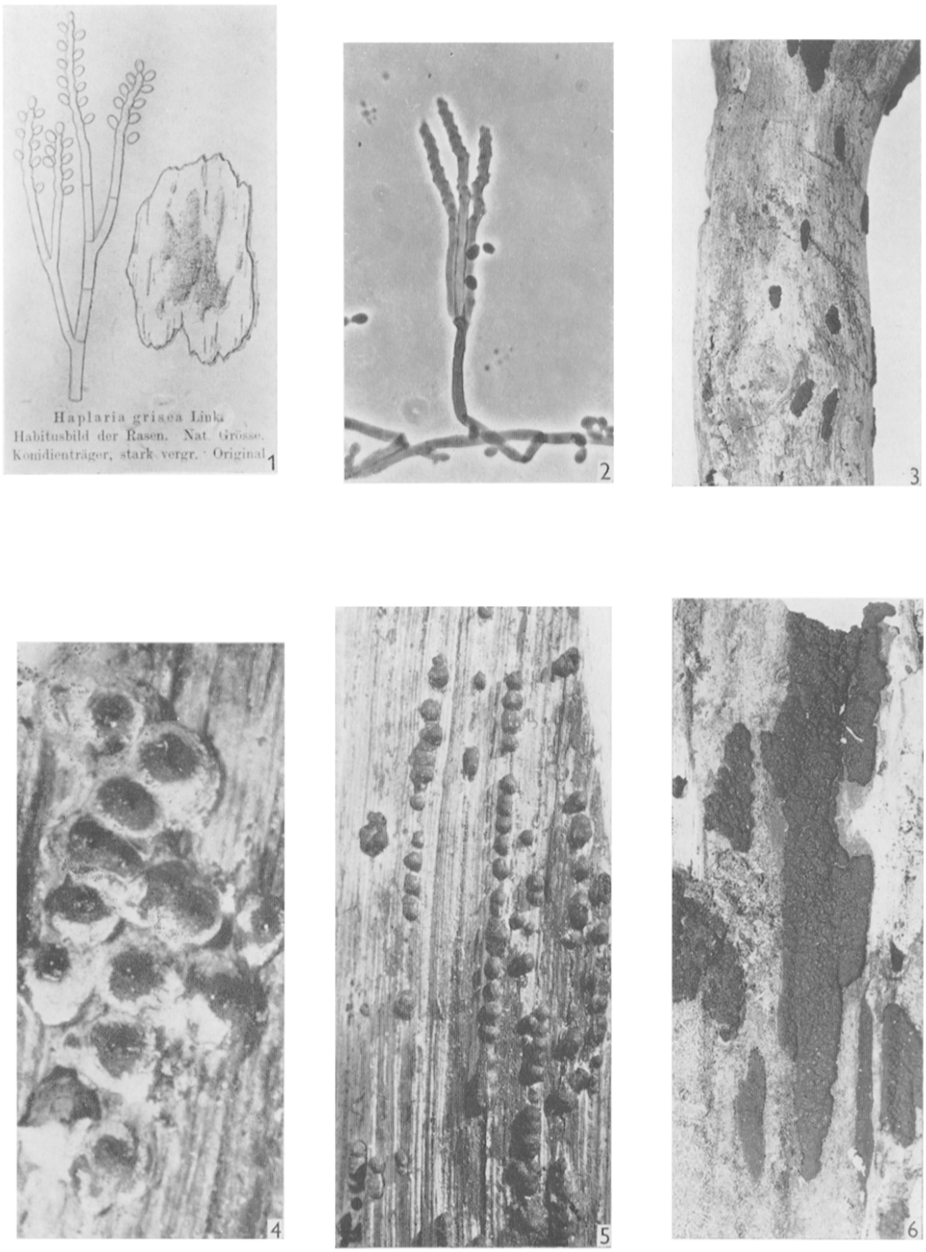
LINDAU, G. (1907). *Kryptogamen-Flora von Deutschland, Oesterreich und der Schweiz* (Rabenhorst), VIII, Fungi Imperfecti, p. 228. Leipzig.

LINK, H. F. (1809). Magaz. Ges. Nat. Fr. Berlin III, II.

MILLER, J. H. (1961). *A monograph of the world species of* Hypoxylon. University of Georgia Press, U.S.A.

MOLLIARD, M. (1904). Forme conidienne de *Daldinia concentrica. Bull. Soc. mycol. Fr.* **20**, 55–60.

PREUSS, C. G. (1862). Deutschlands Flora in Abbildungen nach der Natur. mit Beschreibungen. (Sturm J.), III. *Pilze Deutschlands*, **6**, 99. Nuremburg.


Haplaria grisea Link
Habitusbild der Rasen. Nat. Grösse.
Konidienträger, stark vergr. · Original
1
2
3
4
5
6

(*Facing p.* 401)

EXPLANATION OF PLATE 18

Fig. 1. Illustration of '*Haplaria grisea*' Link from Lindau (1907).
Fig. 2. Conidiophores produced in culture by an isolate from *Hypoxylon serpens*. × 500.
Fig. 3. Habit of mature stromata of *H. serpens*. × ½.
Fig. 4. Young stromata of *H. confluens* with white sterile tomentum still present. × 9·75.
Fig. 5. Typical habit of mature *H. confluens*. × 2.
Fig. 6. Mature stromata of *H. serpens*. × 1½.

(*Accepted for publication 2 March 1964*)