Bothalia 9, 1:183–194.

The Identity of a Pea Blight Fungus in South Africa*

by

K. T. van Warmelo

Plant Protection Research Institute, Pretoria

ABSTRACT

The perfect stage of *Ascochyta pinodes* (Berk. & Blox.) Jones, a cause of pea blight in Natal, was compared with type material of *Sphaeria pinodes* Berk. and Blox., *Mycosphaerella pinodes* (Berk. & Blox.) Stone, and *Didymella pinodes* (Berk. & Blox.) Petrak and the development of its ascocarps studied.

Two types of ascocarp were found on the material of *Didymella pinodes*, one perithecial and the other ascolocular in structure. The ascocarp of the South African fungus was typically ascolocular in development and construction and similar to that of other species of *Mycosphaerella*. These ascocarps were identical to those of *Sphaeria pinodes* and *Mycosphaerella pinodes* and the ascolocular ascocarps of the *Didymella pinodes* material.

In development and morphology this fungus agrees more closely with the original generic concepts of the genus *Mycosphaerella* Joh. than with *Didymella* Sacc. and should thus be named *Mycosphaerella pinodes* (Berk. & Blox.) Stone.

INTRODUCTION

While investigating pea blight in Natal, it was noticed that an anomaly existed concerning the identity of *Mycosphaerella pinodes* (Berk. & Blox.) Stone, one of the three causal organisms listed by Doidge, Bottomley, van der Plank and Pauer (1952). No paraphyses could be seen in the ascocarps found on overwintered straw but Müller and von Arx (1950, 1962) followed Petrak (1924) who transferred this fungus to *Didymella* Sacc. This latter genus differs from *Mycosphaerella* Joh. by the presence of interascal paraphyses (Saccardo, 1882b).

Although Müller and von Arx (1950, 1962) do not regard the presence or absence of paraphyses as an important character in the taxonomy of this group of Ascomycetes, Miller (1949) and Luttrell (1951, 1955) showed that in the Dothidea-type ascocarp, as found in *Mycosphaerella*, the centrum is composed of pseudoparenchyma which is squashed as the asci develop. This results in the formation of a locule occupied by a cluster of aparaphysate asci. In the Pleospora-type ascocarp, as found in *Didymella* (Gäumann, 1952), the centrum consists of pseudoparaphyses which, according to Luttrell (1955), originate as separate paraphysis-like hyphae prior to formation of the asci. The asci later develop among them. The pseudoparaphyses are thus not remnants of interthecial tissue. Luttrell (1951, 1955) believed that these differences afford an adequate basis for the separation of orders in the Ascomycetes.

Müller and von Arx (1950) considered that the presence or absence of pseudoparaphyses can not be used as a criterion for the separation of genera. They maintain that pseudoparaphyses are remnants of interthecial tissue which may be eventually completely absorbed. The presence or absence of pseudoparaphyses is thus a variable condition depending on the stage of maturity of the ascocarp. These two authors (1962) do, however, employ the presence or absence of pseudoparaphyses in their key to separate *Mycosphaerella* and *Didymella*.

* Based on a thesis submitted to the University of Natal, Pietermaritzburg, in partial fulfilment of the requirements for the degree of M.Sc. Agric.

Petrak (1924) considered that all species of *Mycosphaerella* which possess *Ascochyta* imperfect stages should be placed in *Didymella*. This was accepted by Müller and von Arx (1950, 1962) who stated that the most important distinction between *Mycosphaerella* and *Didymella* was the shape of the ascospores. In *Mycosphaerella* species the ascospores have a length: breadth ratio of 3: 1 or more, whereas this ratio is lower in the ascospores of *Didymella* species.

Berkeley and Bloxam in 1861 first described *Sphaeria pinodes*, a fungus producing hyaline, two-celled ascospores in single perithecia (Müller and von Arx, 1962). This name was changed to *Sphaerella pinodes* by Niessl (Saccardo, 1882a) who accepted the genus *Sphaerella* proposed by Cesati and de Notaris in 1861 (von Arx, 1949). Johanson (Grove, 1912) showed in 1884 that *Sphaerella* was not a valid name and substituted *Mycosphaerella* which, according to Saccardo (1891) did not possess paraphyses (pseudoparaphyses sensu Luttrell).

Stone (1912) transferred *Sphaerella pinodes* (Berk. & Blox.) Niessl to *Mycosphaerella pinodes* (Berk. & Blox.) Stone. In his detailed description of *M. pinodes*, Stone did not mention paraphyses which must, therefore, be assumed to be absent. He also considered the imperfect form of *Mycosphaerella pinodes* to be *Ascochyta pisi* Libert. Linford and Sprague (1927) showed that the imperfect stage of *M. pinodes*, named *Ascochyta pinodes* by Jones (1927), differed from *Ascochyta pisi* Lib., thus disproving Stone's assumption. Jones (1927) was unable to find the perfect stage of *Ascochyta pisi* Lib.

Von Höhnel (1918) transferred *Mycosphaerella pinodes* (Berk. & Blox.) Stone to *Didymellina*, a genus which Petrak (1923) later considered to be invalid.

Petrak (1924) examined fresh material of a fungus he identified as *Didymellina pinodes* (Berk. & Blox.) v. Höhn. and published a detailed description of the ascocarp. He observed that the centrum was filled with paraphysoids (pseudoparaphyses) which consisted of pseudoparenchymatous cells. Eventually the pseudoparaphyses formed an indefinite fibrous matrix in which the asci were embedded and difficult to remove. Petrak then reclassified this fungus as *Didymella pinodes* (Berk. & Blox.) Petr. on the basis of the morphology of the ascocarp and asci and the presence of the *Ascochyta* imperfect stage.

As a result of these conflicting views about the morphology and taxonomy of this ascocarpic pea blight fungus it was decided to investigate the morphology of the ascocarp and to establish the identity of the South African fungus by examination of type and authenticated specimens of the fungi concerned.

Materials and Methods

The South African fungus studied here was found on pea straw from Pietermaritzburg, Natal. Type material of *Sphaeria pinodes* Berk. & Blox., *Mycosphaerella pinodes* (Berk. & Blox.) Stone and *Didymella pinodes* (Berk. & Blox.) Petr. was obtained for comparison.

Ascocarps from herbarium material were softened in a 1: 1: 2 mixture of glacial acetic acid, lactic acid and water. This allowed satisfactory squash preparations showing the internal structures to be made. Squash preparations were mounted in lactophenol.

The fungus was cultured on 3 per cent malt agar to which barley kernels, sterilized by dry heat at 160°C for 2 hours, were added. Ascospore or pycnospore suspensions were used as inoculum for subcultures.

The development of the ascocarp was studied from microtome sections cut at 16μ. Ascocarps were fixed in formal-acetic-alcohol (Riker and Riker, 1936) for 24 hours, dehydrated in Cellosolve (2-Ethoxy-ethanol) (Gurr, 1956) and embedded in paraffin wax of 55°C melting point.

The ribbon was mounted with Haupt's adhesive (Gurr, 1956). Sections were stained in Pianeze IIIb (Conn and Darrow, 1946) for one hour, differentiated in acid alcohol, washed in alcohol and mounted in Canada Balsam.

Descriptions

Sphaeria pinodes *Berkeley and Bloxam*, in Ann. Mag. Nat. Hist., ser. 3, 7: No. 981 (1861). (Müller and von Arx, 1962. p. 363).

Material examined: Berkeley Herb. No. 1879 in Herb. Royal Botanic Gardens, Kew, England.

Figs. 1, 11, 15.

Ascocarps caulicolous, scattered over the surface, not in distinct lesions, subepidermal becoming partially erumpent, opening by central ostiole, brown, 100–180μ in diameter; wall of ascocarp stromatic; centrum composed of thin-walled, hyaline, pseudoparenchymatous cells; asci cylindrical to cylindrical-clavate, bitunicate, 45 — 65 × 11 — 15μ; spores eight, irregularly biseriate, hyaline, ellipsoid, two-celled, slightly constricted at the septum, upper cell somewhat larger, 12·5 — 16 × 5 — 7μ.

No indication as to the host is given on the specimen mount.

Mycosphaerella pinodes (*Berk. & Blox.*) *Stone*, in Ann. Myc. 10: 564–592 (1912).

Material examined: From Plant Pathology Herbarium, Cornell University, United. States of America. (CUP-A).

Figs. 2, 12, 16.

Ascocarps caulicolous, scattered over the surface, not in distinct lesions, subepidermal, becoming partially erumpent, opening by central ostiole, brown, 100–200μ in diameter; wall of ascocarp stromatic, one to three cell layers thick; centrum composed of thin-walled, hyaline, pseudoparenchymatous cells; asci cylindrical-clavate, bitunicate, not all maturing at the same time, 60–80 × 12–16μ; spores eight, irregularly biseriate, hyaline, elliptical-obovate, two-celled, slightly constricted at the septum, upper cell usually somewhat larger, 12–15·5 × 4–6μ.

In this material a large number of ruptured asci were found in each ascocarp. The walls of these asci were not always easily visible while the central lumina often appeared as dark lines (Fig. 16) which could be mistaken for pseudoparaphyses.

On *Pisum sativum* L.

Didymella pinodes (*Berk. & Blox.*) *Petrak*, in Ann. Myc. 22: 16–18 (1924).

Material examined: Mycotheca Generalis No. 428. Two distinct types of ascocarp were present on this material.

Type A

Figs. 3, 13.

Ascocarps caulicolous, scattered over the surface, not in distinct lesions, subepidermal becoming partially erumpent, opening by central ostiole, brown, 90–180μ in diameter; wall of ascocarp stromatic, one to three cell layers thick; centrum composed

of thin-walled, hyaline, pseudoparenchymatous cells which are compressed by the developing asci; asci cylindrical to cylindrical-clavate, bitunicate, not all maturing at the same time, 60–75 × 12–16μ; spores eight, irregularly biseriate, hyaline, ellipsoid to elliptical-obovate, two-celled, slightly constricted at the septum, upper cell usually somewhat larger, 12·5–16 × 4·5–5·5μ.

On *Pisum sativum* L.

TYPE B

Fig. 17.

Ascocarps caulicolous, scattered over the surface, not in distinct lesions, subepidermal becoming erumpent, opening by central ostiole, brown to black, 130–250μ in diameter; wall of ascocarp hard; asci cylindrical-clavate, 70–95 × 18–24μ, immature, interspersed with slender septate paraphyses with free apices, 65–100 × 3–4μ. Spores not found.

On *Pisum sativum* L.

Mycosphaerella pinodes (*Berk. & Blox.*) *Stone.*

Material examined: Mycological Herbarium, Nat. Herb., Pretoria. PRE 42549.

Fig. 4, 5, 6, 7, 8, 9, 10, 14.

Ascocarps caulicolous and fructicolous, scattered over the surface, not in distinct lesions, subepidermal becoming partially erumpent, opening by central lysigenous ostiole, brown, 80–180μ in diameter; wall of ascocarp stromatic, one to three cell layers thick; centrum composed of thin-walled, hyaline, pseudoparenchymatous cells which are compressed by the developing asci, leaving strands of tissue which may disappear completely; asci cylindrical to cylindrical-clavate, bitunicate, not all maturing at the same time, 65–80 × 12–15μ; spores eight, obliquely uniseriate to irregularly biseriate, hyaline, elliptical-obovate, two-celled, slightly constricted at the septum, upper cell somewhat larger, 13–15·5 × 4·5–6μ.

Pycnidial stage: *Ascochyta pinodes* (Berk. & Blox.) Jones.

Fig. 18.

Pycnidia caulicolous and foliicolous, scattered in irregular purplish lesions, subepidermal becoming partially erumpent, opening by central ostiole, brown appearing black under reflected light, globose with papillate rostrum, 70–180μ in diameter; pycnidial wall pseudoparenchymatous; spores hyaline, ellipsoid, straight or sometimes somewhat curved, two-celled, slightly constricted at the septum, 11–20 × 2·5–6·0μ.

On *Pisum sativum* L.

Development of the ascolocule. The ascostromata were produced singly, each stroma forming one locule only.

Locule development was initiated in young growing ascostromata. Stromal cells in the centre of the globose ascocarp became thin-walled and larger while the outer two to three layers of cells became thick-walled and dark in colour. No ascogonia were observed but it is probable that the ascogenous hyphae arose in this less dense, pseudoparenchymatous, stromal tissue (van Warmelo, 1966) (Fig. 5). The asci developing from the ascogenous hyphae grew into this pseudoparenchyma, crushing it and so forming the locule (Fig. 6). Lateral growth of the asci resulted in strands of compressed cells (Fig. 7) which finally disappeared leaving a locule completely

filled with aparaphysate asci in various stages of maturity (Fig. 8). A lysigenous, aperiphysate ostiole was formed (Fig. 9). As the asci matured they elongated through the ostiole, ruptured to release their spores and collapsed completely. Old ascocarps could, therefore, be found only partially filled with asci or completely empty (Fig. 10).

The development described above is similar to that observed in several other species of *Mycosphaerella* (Barr, 1958; Higgins, 1920, 1929, 1936; Jenkins, 1930, 1938, 1939).

Discussion

From the above descriptions it is evident that the fungi collected by Berkeley and Bloxam, Stone, Petrak (type A) and the author are markedly similar in location, size, shape and morphology of the ascocarps. Their asci are also similar in shape as can be seen from figures 11, 12, 13 and 14. The small variation in their size was ascribed to the desiccation caused by long storage. Only slight differences in the sizes of the ascospores were observed, but none in their shape. These four fungi are thus considered identical. The specific epithet "*pinodes*" can thus be applied to the South African species.

The two types of ascocarp found on the material obtained from Dr. Petrak showed marked differences in centrum structure. In type A ascocarps the centrum consisted of hyaline, isodiametric, thin-walled, pseudoparenchymatous cells, strands of which sometimes remained between the asci (Fig. 13). There was no evidence of the presence of pseudoparaphyses. The centrum was thus typically ascolocular and identical with that of *Mycosphaerella pinodes* (Berk. & Blox.) Stone. In the type B ascocarps slender and septate true paraphyses with free apices could be distinguished (Fig. 17). The asci were similar in shape to those in type A but were larger. In the absence of ascospores the genus could not be determined. The presence of true paraphyses does, however, show that this fungus possesses a true perithecium and is thus not a species of *Didymella* Sacc. which has ascolocular ascocarps. In view of the differences shown to exist between the ascocarps in Petrak's material, it would appear that the description of *Didymella pinodes* (Berk. & Blox.) Petr. was based on a mixed collection.

The average length: breadth ratio of the ascospores of the four fungi examined is 2·7 which is less than the ratio for *Mycosphaerella* quoted by Müller and von Arx (1950). It must, however, be noted that in the descriptions of *Mycosphaerella* and *Didymella* species by Müller and von Arx (1962) the length: breadth ratios are not constant for all species of these two genera.

Luttrell (1951) and Müller and von Arx (1950) agree that the development and morphology of the ascocarp and of the asci and ascospores are the criteria on which taxonomic divisions should be based. Müller and von Arx (1950) stated that characteristics of the vegetative phase may only be considered to the extent of their direct relation to the sexual stage.

Petrak (1924) and Müller and von Arx (1962) stated that species with *Ascochyta* imperfect stages should be classified in *Didymella*. The perfect stage of *Ascochyta pinodes* has been shown here to lack certain morphological structures found in the type and other species of *Didymella*. These structures are regarded as most important in the classification of Ascomycetes by Luttrell (1951, 1955) and Müller and von Arx (1950, 1962). Furthermore, species of both the genera *Phoma* and *Phyllosticta* are known to have ascocarpic forms in both the genera *Didymella* and *Mycosphaerella* (Müller and von Arx, 1962; Wolf, 1940; Brooks, 1953). The restriction of the perfect stages of *Ascochyta* species to the genus *Didymella* thus appears unjustified.

The development of the ascolocule in the South African fungus has been shown to follow that of a typical Dothidea-type, sensu Luttrell (1951). Pseudoparaphyses were not observed at any stage of development. In view of the similarity of this fungus

with those of Berkeley and Bloxam, Stone and Petrak (type A) it must be assumed that pseudoparaphyses were not present at any stage of development of these older collections, of which the development could not be examined.

It is thus evident that the perfect stage of the South African pea blight fungus, *Ascochyta pinodes*, agrees much more closely with the concept of *Mycosphaerella* Joh. than with *Didymella* Sacc. and should, therefore, be known as *Mycosphaerella pinodes* (Berk. & Blox.) Stone.

Acknowledgements

The author is indebted to The Director, Royal Botanic Gardens, Kew, England; The Curator, Plant Pathology Herbarium, Cornell University, Ithaca, United States of America and Dr. F. Petrak, Naturhistorisches Museum, Vienna, Austria for the loan of the type and authentic material of *Sphaeria pinodes* Berk. and Blox.; *Mycosphaerella pinodes* (Berk. & Blox.) Stone and *Didymella pinodes* (Berk. & Blox.) Petr. respectively.

References

ARX, J. A. VON, 1949. Beiträge zur Kenntnis der Gattung *Mycosphaerella*. Syd. Ann. Myc. 3: 28–100.

BARR, MARGARET, E., 1958. Life history studies of *Mycosphaerella tassiana* and *M. typhae*. Mycologia 50: 501–513.

BROOKS, F. T., 1953. Plant diseases. Oxford Univ. Press. London, 457p.

CONN, H. J. & DARROW, MARY A., 1946. Staining procedures used by the Biological Stain Commission. Biotech Publications, Geneva, N.Y.

DOIDGE, E. M., BOTTOMLEY, A. M., VAN DER PLANK, J. E. & PAUER, G. D., 1952. A revised list of plant diseases occurring in South Africa. Science Bull. No. 346. Botany and Plant Pathology Series No. 16, 122p.

GÄUMANN, E. A., 1952. The fungi. A description of their morphological features and evolutionary development. Hafner Publ. Co. New York. 420p.

GROVE, W. B., 1912. *Sphaerella* v. *Mycosphaerella*. Journ. of Bot. 50: 89–92.

GURR, E., 1956. A practical manual of medical and biological staining techniques. Leonard Hill Ltd. 320p.

HIGGINS, B. B., 1920. Morphology and life history of some Ascomycetes with special reference to the presence and function of spermatia. Amer. Journ. Bot. 7: 435–444.

HIGGINS, B. B., 1929. Idem. II. Ibid. 16: 287–296.

HIGGINS, B. B., 1936. Idem III. Ibid. 23: 598–602.

HÖHNEL, F. VON, 1918. Mycologische Fragmente CCVIII. Über *Sphaeria* (*Depazea*) *phaseolicola* Roberge. Ann. Myc. 16: 66–67.

JENKINS, W. A., 1930. The cherry leafspot fungus, *Mycosphaerella cerasella* Aderh., its morphology and life history. Phytopathology 20: 329–337.

JENKINS, W. A., 1938. Two fungi causing leaf spot of peanut. Journ. Agr. Res. 56: 317–332.

JENKINS, W. A., 1939. The development of *Mycosphaerella berkeleyii*. Ibid. 58: 617–620.

JONES, L. K., 1927. Studies of the nature and control of blight, leaf and pod spot, and footrot of peas caused by species of *Ascochyta*. New York State Agr. Exp. Sta. Bull. 547. 46p.

LINFORD, M. B. & SPRAGUE, R., 1927. Species of *Ascochyta* parasitic on the pea. Phytopathology 17: 381–397.

LUTTRELL, E. S., 1951. Taxonomy of the Pyrenomycetes. Univ. Missouri Studies 24 (3): 1–120.

LUTTRELL, E. S., 1955. The ascostromatic Ascomycetes. Mycologia 47: 511–532.

MILLER, J. H., 1949. A revision of the classification of the Ascomycetes with special emphasis on the Pyrenomycetes. Mycologia 41: 99–127.

MÜLLER, E. & VON ARX, J. A., 1950. Einige Aspekte zur Systematik pseudosphärialer Ascomyceten. Ber. Schweiz. Bot. Ges. 60: 329–397.

MÜLLER, E. & VON ARX, J. A., 1962. Die Gattungen der didymosporen Pyrenomyceten. Beitr. Kryptogamenflora Schweiz 11: 1–922.

PETRAK, F., 1923. Mykologische Notizen. V. No. 199. Über *Didymella, Mycosphaerellopsis* und verwandte Gattungen. Ann. Myc. 21: 19–30.

PETRAK, F., 1924. Mykologische Notizen. VII. No. 305. Über *Didymellina pinodes* (Berk. & Blox.) v. Höhn. Ibid. 22: 16–18.

RIKER, A. J. & RIKER, REGINA S., 1936. Introduction to research on plant diseases. Publ. by authors. Univ. Wisconsin. 117p.

SACCARDO, P. A., 1882a. *Sphaerella pinodes* (B. et Blox.) Niessl. Syll. Fung. 1: 514.

SACCARDO, P. A., 1882b. *Didymella* Sacc. Ibid. 1: 545.

SACCARDO, P. A., 1891. *Mycosphaerella* Johans. Ibid. 9: 659.

STONE, R. E., 1912. The life history of *Ascochyta* on some leguminous plants. Ann. Myc. 10: 564–592.

WARMELO, K. T. VAN, 1966. The cytology of *Mycosphaerella pinodes*. Bothalia (in press).

WOLF, F. A., 1940. A leafspot fungus on *Nyssa*. Mycologia 32: 331–335.

EXPLANATION OF FIGURES

FIGS. 1–4.—Host material showing ascocarps. 4.3x.

FIG. 1.—*Sphaeria pinodes* (Berk. Herb. 1879).

FIG. 2.—*Mycosphaerella pinodes* (CUP–A).

FIG. 3.—*Didymella pinodes* (Myc. gen. 428).

FIG. 4.—*Mycosphaerella pinodes* (PRE 42549).

FIGS. 5–10.—Development of an ascolocule in *Mycosphaerella pinodes* (PRE 42549). 500x.

FIG. 5.—Young ascostroma showing less dense central tissue.

FIG. 6.—Stroma showing developing asci growing into the pseudoparenchyma.

FIG. 7.—Stroma showing advanced locule formation. Pseudoparenchyma still noticeable.

FIG. 8.—Mature locule with open ostiole.

FIG. 9.—Mature locule with asci emerging from the ostiole.

FIG. 10.—Mature locule with copious ascospore liberation.

FIGS. 11–16.—Preparations showing asci and ascospores. 500x.

FIG. 11.—*Sphaeria pinodes* (Berk. Herb. 1879).

FIG. 12.—*Mycosphaerella pinodes* (CUP–A).

FIG. 13.—*Didymella pinodes* (Type A) (Myc. gen. 428). Note the remaining pseudoparenchyma.

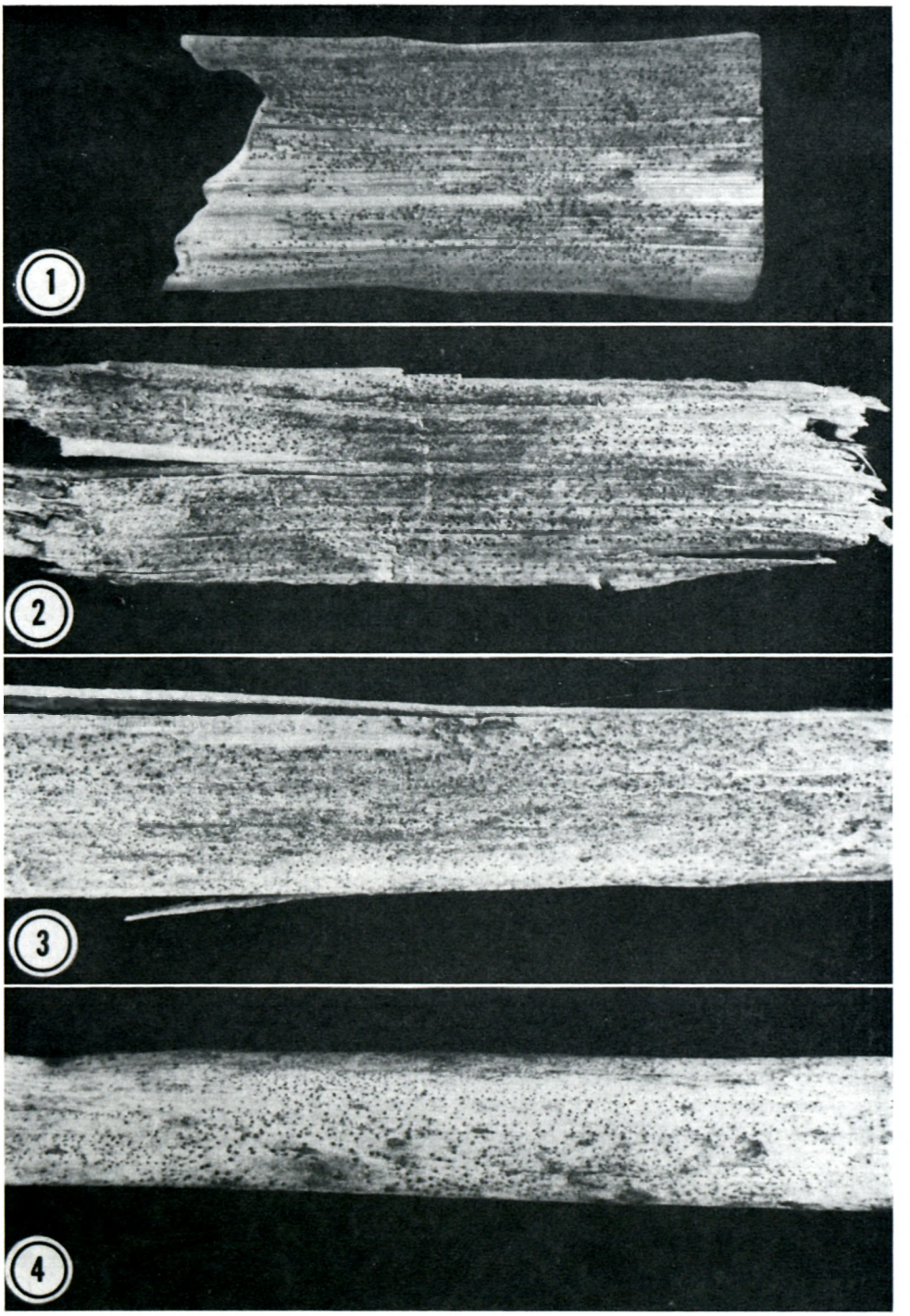
FIG. 14.—*Mycosphaerella pinodes* (PRE 42549).

FIG. 15.—*Sphaeria pinodes* (Berk. Herb. 1879). Note the remaining pseudoparenchyma.

FIG. 16.—*Mycosphaerella pinodes* (CUP–A). Note the ruptured asci giving the appearance of pseudoparaphyses.

FIG. 17.—Preparation of *Didymella pinodes* (Type B) (Myc. gen. 428) showing immature asci and true paraphyses with free apices. 500x.

FIG. 18.—Section through pycnidium of *Ascochyta* pinodes (PRE 42549) showing pycnospores. 500x.



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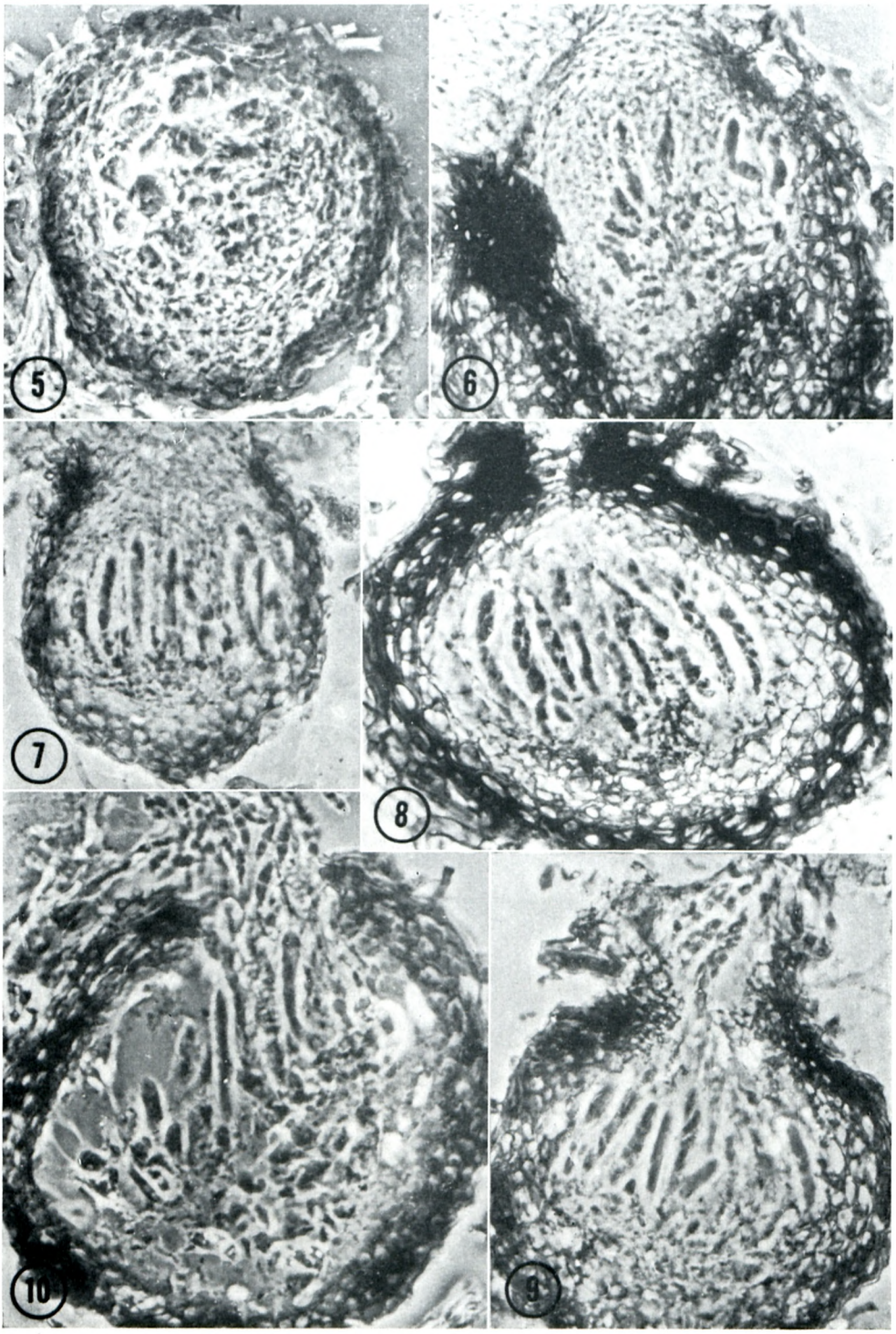
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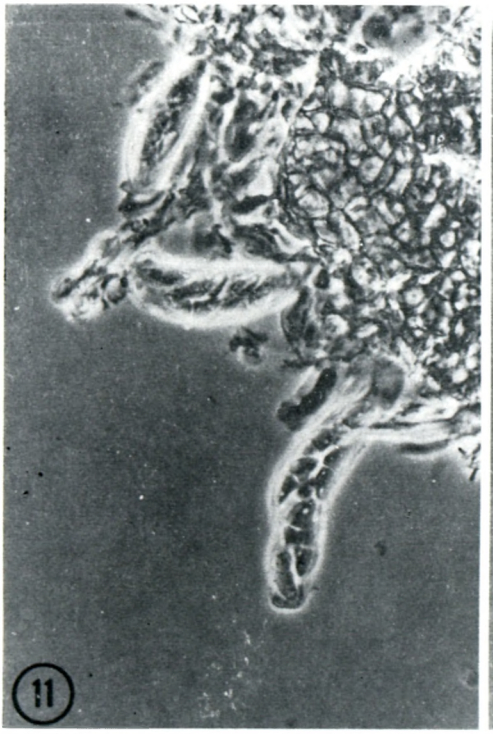
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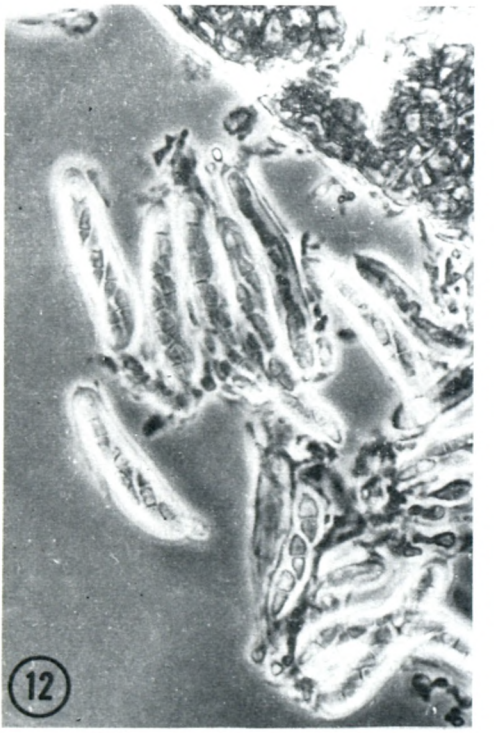
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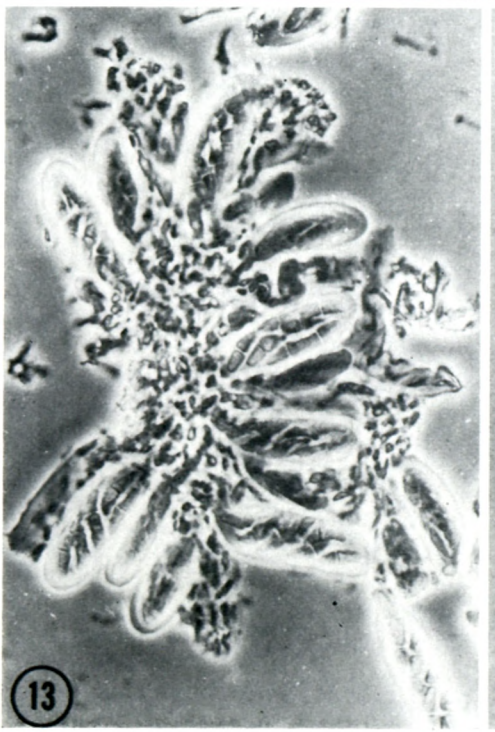
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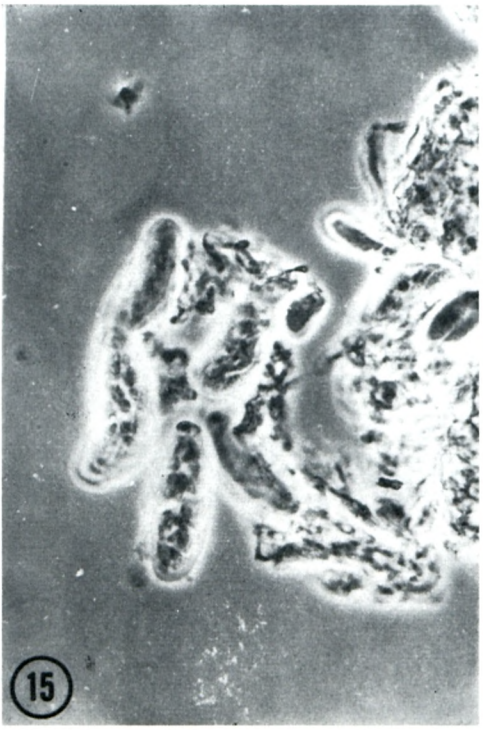
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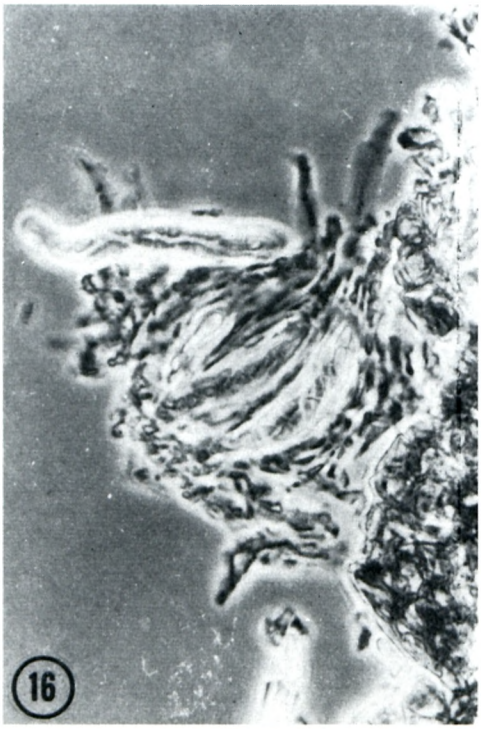
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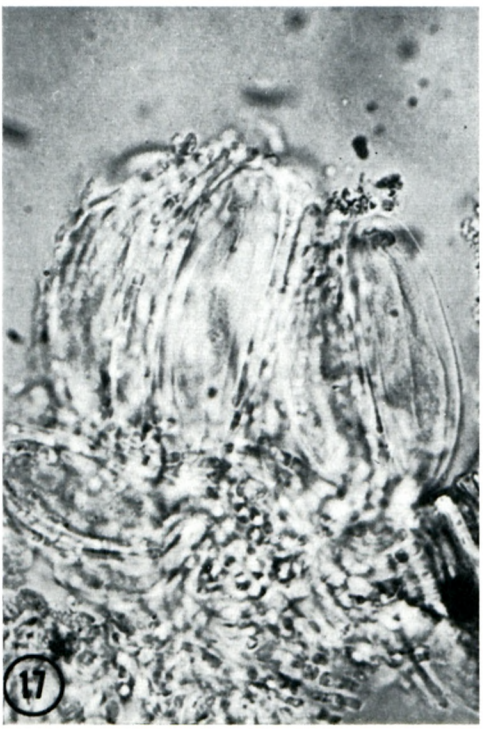
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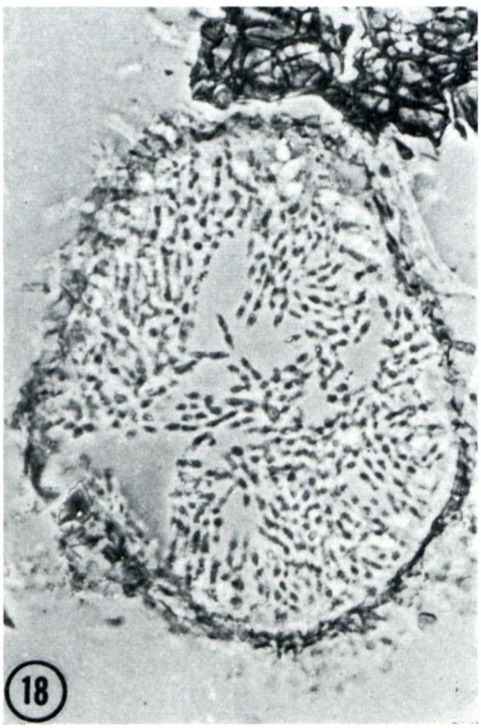
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