ALTERNARIA PHRAGMOSPORA NOV. SPEC.

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SUMMARY

A new species of *Alternaria*, *A. phragmospora*, is described and illustrated. Observations on the fungus are recorded and a comparison with *Alternaria raphani* Groves and Skolko is made.

INTRODUCTION

In the course of a study on the fungal flora in one of the Zuider Zee polders we repeatedly isolated a dematiaceous hyphomycete characterized by the production of acropetal chains of pigmented, transversely septate porospores and of multicellular chlamydospores.

Although the fungus by its macroscopic appearance as well as by its microscopic characters strongly suggested affinity with *Alternaria* Nees, we at first did not think it should be classified as a species of that genus, because no dictyospores were observed.

Since we could not place it in any other genus we sent several isolates to Dr. M. B. Ellis at the Commonwealth Mycological Institute. Dr. Ellis, together with Dr. E. G. Simmons, who happened to be working at C. M. I. during the summer of 1969, kindly examined our fungus. They concluded that the fungus in question should be considered as a new species of *Alternaria* and encouraged us to describe it.

In the meantime we have examined many more isolates and we saw thousands of conidia; in some of the strains we have found a few spores with one longitudinal septum each (*plate 1d*). Having established the capacity of the fungus to produce muriform spores, although exceptionally, we felt free to classify it as a species of *Alternaria*, and because of the phragmosporous nature of the vast majority of the conidia we decided on the specific epithet "*phragmospora*".

Alternaria phragmospora nov. sp.

Coloniae in agaro malto initio olivaceo-atrae deinde brunneo-atrae, celeriter crescentes. Mycelium septatum, hyphis brunneis, 3–4 μ crassis, interdum funiculosis et anastomosantibus. Conidiophora plerumque perpendiculariter lateraliter e hyphis oriunda, brunnea, interdum aspera, septis crassis, ad 3–5,3 μ crassa, plerumque brevia, simplicia nonnunquam uno geniculo subapicali praedita. Conidia in catenis acropetalibus – nonnumquam ramosis – nata, obclavata, brunnea, episporio levi vel minime verruculoso, plerumque rostro brevi ex 1–3 cellulis subhyalinis composito praedita, (20–) 30–50 × (6–) 8–10 (–12)μ, septis transversalibus (1–) 3–5 (–9) poris centralibus perforatis, septis

longitudinalibus rarissimis, Chlamydosporae numerosae, et in conidiis et in mycelio oriundae, multicellulares, micro-sclerotiis similes.

Habitat in terra. Typus CBS 274.70 vivus et exsiccatus in Centraal Bureau voor Schimmelcultures, Baarn preservatus.

Single spore cultures on malt agar reach a diameter of 60 mm in 2 weeks at 15 °C. Colony flat, margin regularly circular, greenish grey in incident light, green in transmitted light. Exudate colourless, abundantly produced as small droplets in the aerial mycelium. Reverse brownish grey, yellowish towards the margin. Mycelium thin and hyaline when young; thick and pigmented when older, often aggregated into hyphal ropes with frequent anastomoses between hyphae. Conidiophores mostly arising as short branches on the mycelium, sometimes terminating a hypha, aseptate or septate, unbranched, generally smooth, occasionally roughened, slightly swollen towards the apex, most often with one apical scar, occasionally geniculate with one scar below the apex. Conidia pigmented porospores (20–) 30–50 × (6–) 8–10 (–12)μ, borne solitary or more often in branched or unbranched acropetal chains of up to 15 spores, slenderly obclavate, rarely almost narrowly elliptical, sometimes irregularly bent, with (1–) 3–5 (–9) transverse septa (longitudinal septa were occasionally observed), mostly with a short beak consisting of 1–3 hyaline cells, epispore smooth or slightly verruculose. Chlamydospores many-celled, abundantly produced in the submerged mycelium but also in the aerial mycelium and in conidia, sometimes equalling microsclerotia in size.

Type material is deposited in Centraal Bureau voor Schimmelcultures, Baarn under no. 274.70.

OBSERVATIONS ON *Alternaria phragmospora*

Conidiophores

The conidiophores either arise as short branches of mycelial threads or terminate a hypha. In the latter case they are poorly defined since there is a gradual transition from the hypha into the conidiophore. The number of septa in the conidiophore usually varies from 0–3; the septa are usually thicker than those in the mycelium. The width of the conidiophore is only slightly greater than that of the hypha on which it is borne; the terminal cell is sometimes slightly inflated. Conidiophores are unbranched and rarely show one geniculation.

Conidia

The conidia are porospores, acropetally produced in branched or unbranched chains of varying length. Branching of chains occurs by a cell of a conidium putting out a short branch which immediately starts producing a porospore. Such a branch most often arises from the basal cell of a conidium (*plate 1f*), less frequently from apical cells (*plate 1 g*), while branching from other than apical or basal cells is rare (*plate 1 h*). Although the chains usually mature acropetally, exceptionally an apparently immature conidium is found below a dark coloured one (*plate 1 e*). The question arises whether the apparently immature conidium was arrested in its development or whether there was the intercalary formation of a new porospore at the same site. In some isolates we observed short chains of conidia the basal ones of which were clearly porospores, while those situated apically were broadly attached (*plate 1, n, o, p, q*). These short chains are reminiscent of some of the spore chains in

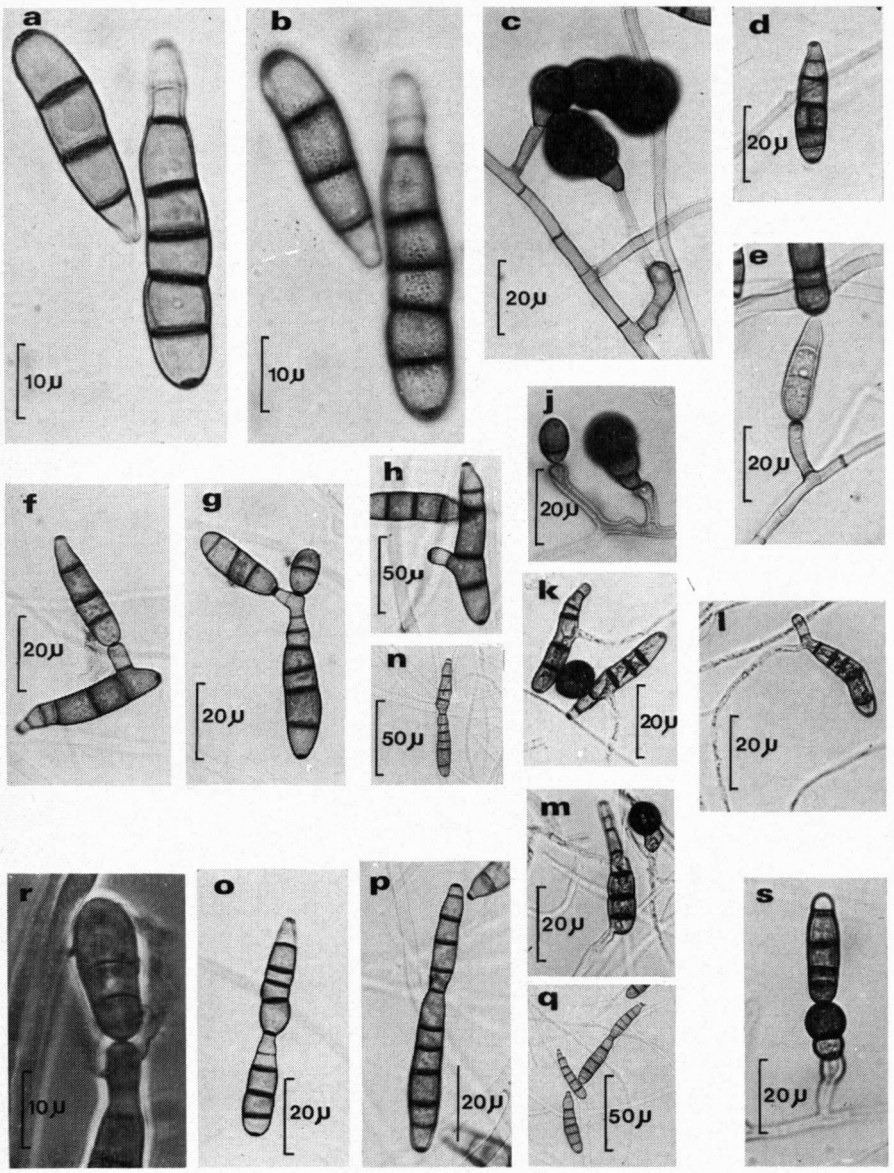


Plate. 1. *A. phragmospora*, a, b. conidia; c. chlamydospore broadly attached; d. conidium with longitudinal septum; e. apparently juvenile conidium between conidiophore and mature conidium; f–h. branching of conidia; j. chlamydospore originating from a pore; k–m. germinating conidia; n–q. broadly attached conidia; r. juvenile conidium with shrivelled sheath; s. chlamydospore producing conidium through a pore.

Dendryphiella Bub. & Ranoj. as depicted by REISINGER (1968, fig. 1A p. 33).

At room temperature germination of conidia (and chamydospores) begins within 6 hrs after a suspension is spread on tapwater agar. When conidia germinate the single germ tube may originate from any cell (*plate 1, k, l, m*). Not infrequently an apical conidium appears to be enveloped in a shrivelled sheath which also covers part of the subapical one (*plate 1 r*). Shrivelled sheaths have also been observed in mounts of *Alternaria raphani* Groves & Skolko, *Ulocladium consortiale* (Thüm.) Simmons, and *Dendryphion penicillatum* (Cda) Fr. var. *sclerotiale* Meffert. Their nature is unknown and has not yet been investigated.

The shape of the conidia is most often slenderly obclavate, but occasionally narrowly elliptical ones may be found, especially in cultures grown at low tempetures.

The beak is either short or absent; beaks of more than three cells were not seen. NEERGAARD (1945 p. 27) mentions 3 criteria for the beak:
- the beak should be lighter than the body,
- the sides of the beak are to be parallel or nearly so,
- the distance between the transversal septa should be greater in the beak than in the body.

In our fungus these requirements are hardly ever met and according to NEERGAARD the conidia should be regarded as beakless. If, however, we follow JOLY (1964) in his description of the conidia in *Alternaria oleracea*, we would also recognize beaks of 1–3 cells in most conidia of *A. phragmospora*.

When studied under an oil immersion lens the surface of the cell wall shows a pattern of dark spots (*plate 1 b*). In the young conidia this appears to be due to areas of greater density within the wall, as is suggested by *plate 1 a*.

Chlamydospores

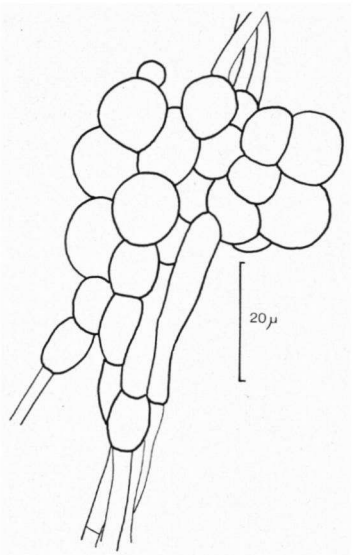
Following GROVES & SKOLKO (1944), JOLY (1964) and SIMMONS (1967) this name is applied to the multicellular bodies consisting of few to many spherical cells. The chlamydospores are produced in different ways. Sometimes several aggregated hyphae simultaneously produce intercalary chlamydospores which aggregate to form one large multicellular body (*fig. 1*).

Chlamydospores can also arise terminally on short branches of hyphae (*fig. 2*); they may then closely resemble the spores of *Acrospeira*. They can also be produced by proliferation of conidia. Terminal chlamydospores are usually broadly attached (*plate 1 c*). Sometimes, however, it is evident that they are formed in the manner of a porospore (*plate 1 j* and *fig. 2*) arising at the apex of a conidiophore or a conidium. Conversely, chlamydospores may produce conidia through an apical pore (*plate 1 s*). Chlamydospores blown out from a pore may be considered as transformed conidia.

Influence of media and temperature

Growth was compared on the following media: potato-dextrose, malt, oatmeal, cherry, Czapek-Dox, carboxymethyl-cellulose and hay extract agar.

Fig. 1. *A. phragmospora*, chlamydospore produced by several hyphae.



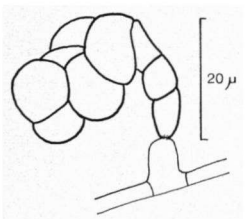


Fig. 2. *A. phragmospora*, chlamydospore produced from a pore.

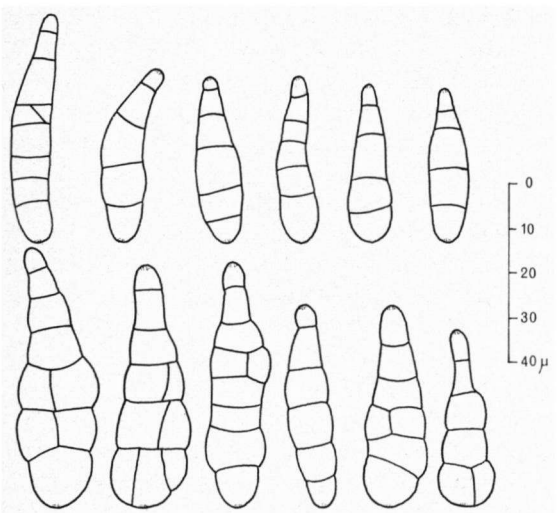


Fig. 3. top row: conidia of *A. phragmospora;* bottom row: conidia of *A. raphani.*

The fungus grew equally well on all media, except on cherry agar where growth was somewhat slower.

The influence of temperature on growth rate was studied on malt agar. At temperatures of 5, 15, 20, 25 and 35 °C colonies grew from a 5 mm inoculum to a diameter of 12, 40, 62, 76 and 40 mm respectively in 7 days. At 10 and 15 °C conidia are produced in abundance on all media. At 20 °C and above, however, conidial production is very sparse, except on hay extract agar where conidia were produced even at 25 °C. Chlamydospore production shows the

opposite trend; at 5°C and at 10°C chlamydospores are few and at 20°C and above they are abundant.

If an isolate is subcultured at a range of temperatures conidial length decreases and conidial width increases with increasing temperature. We calculated the following averages for conidia grown on hay extract agar.

Temperature	Average length	Average width	Average number of septa
5 °C	41,3	5,9	4,2
10	39,7	8,3	4,8
15	34,2	8,8	4,2
25	32,8	9,8	3,8

Influence of light

Daylight and near U.V. light promoted sporulation to some extent, but not enough to alter the pattern outlined above.

DISCUSSION

Generic disposition

All authors writing on *Alternaria* in this century, ELLIOTT (1917), BOLLE (1924), WILTSHIRE (1933), NEERGAARD (1945), JOLY (1964) and SIMMONS (1967) mention the production of muriform conidia. None of the well known keys such as LINDAU (1910), CLEMENTS & SHEAR (1931), BESSEY (1951), BARNETT (1955), GILMAN (1957), SMITH (1960), VON ARX (1967) and BARRON (1968) will lead to *Alternaria*, unless the potential to produce muriform conidia is assumed. The appearance of a few conidia with longitudinal septa in some of the isolates proves this potential in our fungus.

The sometimes slender and hardly obclavate conidia of *A. phragmospora* resemble those of *Dendryphiella* Bub. & Ranoj., but this genus cannot accomodate our fungus since an important diagnostic feature of *Dendryphiella*, production of several chains of conidia on swellings of the fertile cells, is lacking in *A. phragmospora*.

Comparison with *Alternaria raphani* Groves & Skolko

A. phragmospora is to be compared with *A. raphani* because this latter species also produces multicellular chlamydospores. The fungi differ in colony habit and growth rate (*plate 2 a*), in habit and size of conidia, in reaction to temperature with regard to conidial production, and in pathogenity.

A. phragmospora grows more rapidly than *A. raphani*. The colonies are less darkly pigmented and have an even circular outline whereas those of *A. raphani* are irregular in outline.

While *A. phragmospora* produces very few dictyospores, comparable cultures of *A. raphani* show more than 25% muriform conidia. The conidia of *A. raphani* are more strongly constricted at the septa than those of *A. phragmospora* and the apical portion is generally considerably narrower than the main body; the difference in habit is illustrated in *fig. 3*.

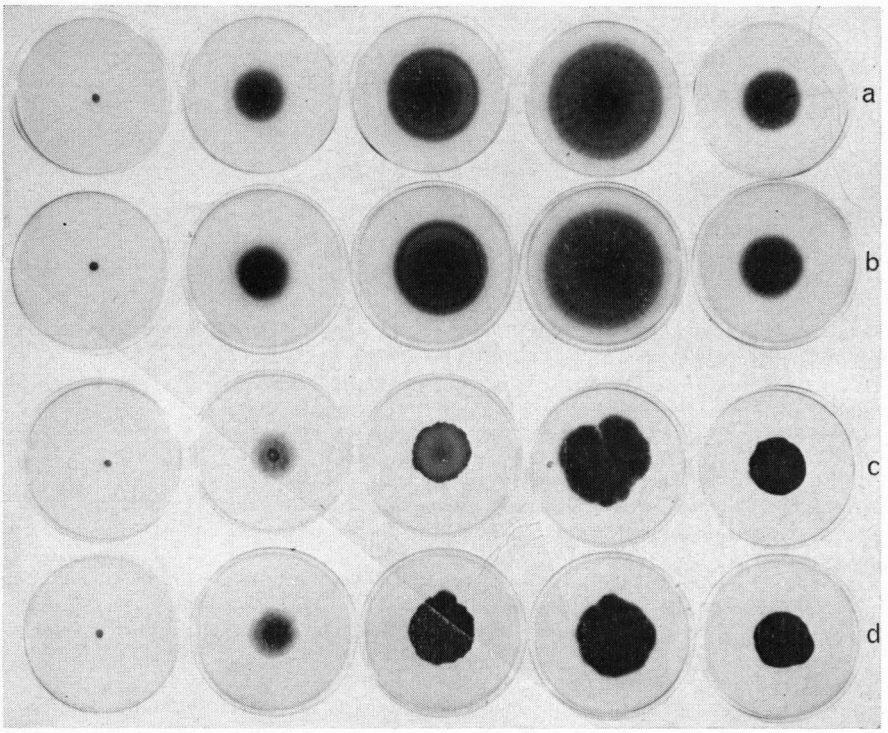


Plate. 2. *A. phragmospora* and *A. raphani*, one week old cultures on malt agar grown at 5-10-20-25-35 °C. row a: *A. phragmospora* front; row b: *A. phragmospora* reverse; row c: *A. raphani* front; row d: *A. raphani* reverse.

For *A. phragmospora* we calculated an average of 32.9 ± 0.76 × 9.6 ± 0.47 μ while for *A. raphani* grown under the same conditions the average measurements were 41.1 ± 1.44 × 12.4 ± 0.47 μ. When both fungi were inoculated onto leaves of radish seedlings the average measurements were 33.0 ± 0.84 × 7.3 ± 0.14 μ for *A. phragmospora* and 48.2 ± 1.85 × 13.9 ± 0.59 μ for *A. raphani*; the latter fungus is apparently more strongly stimulated in the presence of a host plant.

Production of conidia is maintained in *A. raphani* when the fungus is grown at higher temperatures, whereas in *A. phragmospora* production of conidia is severely reduced above 20 °C.

Pathogenity of the two fungi was compared on seedlings of *Brassica cernua* (chinese cabbage) and of *Raphanus sativus*. The plants were grown under sterile conditions on agar without an organic carbon source, under a day-night period of 14/10 hrs and at 23/17 °C.

Radish was more sensitive to both fungi than Chinese cabbage. Of the two fungi *A. raphani* was the more pathogenic one. *A. phragmospora* caused hardly any damage to the seedlings of radish, whereas *A. raphani* was capable of killing them.

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