PERSOONIA
Volume 18, Part 2, 153–161 (2003)

CONTRIBUTIONS TOWARDS A MONOGRAPH OF PHOMA (COELOMYCETES) – X
Section Pilosa (taxa with a Pleospora teleomorph) and nomenclatural notes on some other taxa

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The two anamorphs classified so far in *Phoma* sect. *Pilosa* ('hairy' pycnidia in vitro) are both metagenetically related to species of the ascomycetous genus *Pleospora*. In addition to their characteristics in vitro and in vivo short comments on the hosts, literature data, ecology and distribution are given.

The notes include additional documentation of the type specimens (dried cultures) of the recently described *Phoma schneiderae* Boerema et al., *Phoma pimpinellae* Boerema et al. and *Phoma leveillei* var. *microspora* de Gruyter & Boerema. Some excluded species are discussed as new proposed taxa: *Asteromella ulmi* nom. nov. (teleom. *Mycosphaerella ulmi* Kleb.), *Microsphaeropsis fuckelii* (Sacc.) Boerema comb. nov. (teleom. *Leptosphaeria coniothyrium* (Fuckel) Sacc.) and *Microsphaeropsis glumarum* (Ellis & Tracy) Boerema comb. nov.

This paper is the final one of a series of 'Contributions' to be included in a monographic treatment of the anamorphic genus *Phoma* based on cultural characteristics. To facilitate the identification the genus has been divided into nine sections; see Boerema, 1997 for their differentiating characteristics and keys.

Those previously treated are the sections *Phoma* (De Gruyter & Noordeloos, 1992; De Gruyter et al., 1993, 1998), *Peyronellaea* (Boerema, 1993), *Plenodomus* (Boerema et al., 1994, 1996; Boerema & de Gruyter, 1999), *Heterospora* (Boerema et al. 1997, 1999), *Sclerophomella* (Boerema & de Gruyter, 1998), *Phyllostictoides* (Van der Aa et al., 2000; De Gruyter et al., 2002), *Paraphoma* (De Gruyter & Boerema, 2002) and *Macrospora* (De Gruyter, 2002).

Phoma sect. *Pilosa* Boerema et al. (Boerema, 1997) treated in this paper, is based on *Phoma betae* A. B. Frank, anamorph of *Pleospora betae* (Berl.) Nevod., well known as a seedborne pathogen of *Beta vulgaris* and other Chenopodiaceae (Boerema et al., 1987). The section name refers to the 'hairy' or pilose appearance of the globose pseudoparenchymatous pycnidia of this anamorph in vitro (Fig. 1A). They are initially closed, with late development of a pore (sometimes V-shaped according to Monte & Garcia-Acha, 1988a: 236), instead of a predetermined ostiole. In vivo the pycnidia usually develop beneath the cuticle and look glabrous with a flush circular central opening (Shoemaker & Bissett, 1998). The subglobose-ellipsoidal conidia of *P. betae* are one-celled, but, when germination is initiated, the conidium sometimes becomes 1-septate (Monte & Garcia-Acha, 1988c). Electron microscope observations on the conidiogenesis (Monte & Garcia-Acha, 1988b) show a conidial ontogeny similar to that in other species of *Phoma*. Only the intermediate layer in the papilla preceding the initiation of the first conidium (Boerema, 1997) could not be observed.

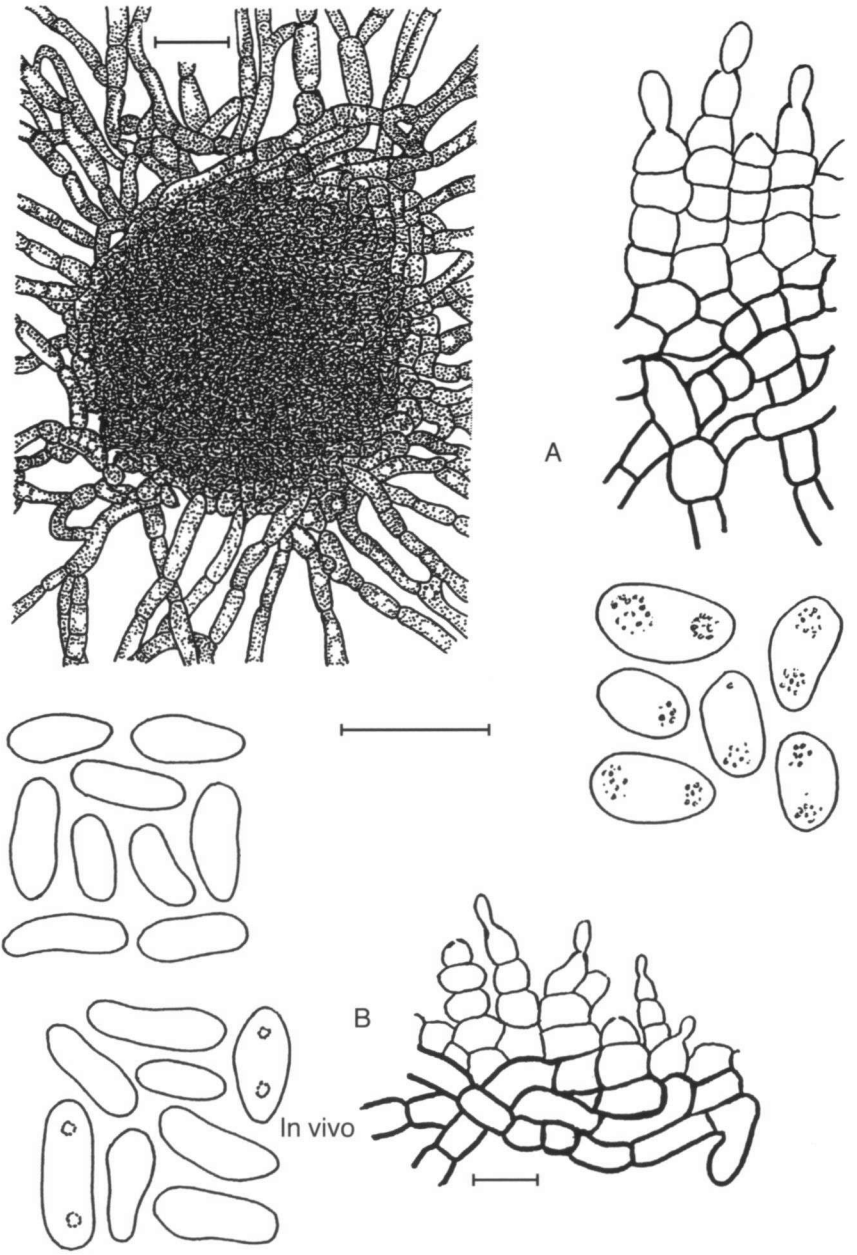

Fig. 1A. *Phoma betae*, type species of section *Pilosa*. 'Hairy' pycnidium in vitro, detail of pycnidial wall and conidia both in vitro. — B. *Phoma typhina*. Conidia in vitro and in vivo and detail of the wall of a small pycnidium in vitro. (Bar: pycnidium 50 µm, pycnidial walls and conidia 10 µm.)

So far this section includes only two taxa. The other species with 'hairy' pycnidia in vitro occurs on *Typha* spp. In its life cycle also a teleomorph of the genus *Pleospora* Rabenh. ex Ces. & De Not occurs.

The methodology is the same as used in the preceding Contributions.

KEY TO PHOMA SPECIES OF SECTION PILOSA

1a. Conidia relatively broad, globose to subglobose/ellipsoidal, mostly 4–6.5 × 2.5–4 µm ..... 1. *P. betae*
teleomorph *Pleospora betae*

b. Conidia smaller, ellipsoidal to subcylindrical or allantoid, mostly 4–6 × 2–2.5 µm
2. *P. typhina*
teleomorph *Pleospora typhicola*

HOST-FUNGUS INDEX

Chenopodiaceae	
e.g. *Beta vulgaris*	*P. betae* (1)
Spinacia oleracea	teleomorph *Pleospora betae*
Typhaceae	
e.g. *Typha angustifolia*	*P. typhina* (2)
Typha latifolia	teleomorph *Pleospora typhicola*

DESCRIPTIVE PART

1. **Phoma betae** A.B. Frank — Fig. 1A

Teleomorph: *Pleospora betae* (Berl.) Nevod.

Phoma betae A.B. Frank, Z. Rübenzucker-Ind. 42 (1892) 904, tab. 20, nom. cons. prop. (Shoemaker & Redhead, 1999) [often erroneously listed as '(Oud.) Frank' or 'Rostr.'].

Phyllosticta betae Oudem., Ned. kruidk. Archf II, 2 (3) (1877) 181.

Phoma sphaerosperma Rostr., Tidsskr. Landfkon. V, 8 (1889) 746; not *Phoma sphaerosperma* P. Karsten, Hedwigia 24 (1885) 74; not *Phoma sphaerosperma* Fuckel, Fungi rhen. Suppl. Fasc. 5 (1867) No. 1945.

Phyllosticta tabifica Prill., Bull. Soc. mycol. Fr. 7 (1891) 19, nom. rej. prop. (Shoemaker & Redhead, 1999), in Sylloge Fung. 10 (1892) 180 listed as '*Phoma tabifica* Prill.' [interpreted as an unintentional error of citation (Boerema & Dorenbosch, 1973), but see Shoemaker & Redhead l.c.]; not *Phoma tabifica* Kesteren, Gewasbescherming 2 (1971) 74 [= *Phoma telephii* (Vestergr.) Kesteren, sect. Phyllostictoides, see De Gruyter et al., 2002].

Phyllosticta spinaciae H. Zimm., Verh. naturf. Ver. Brünn 47 ['1908'] (1909) 87.

Phoma spinaciae Bubák & Willi Krieg. in Bubák, Annls mycol. 10 (1912) 47.

Gloeosporium betae Dearn. & E.T. Barthol. in Dearness, Mycologia 9 (1917) 356.

Selected literature. Booth (1967), Boerema & Dorenbosch (1973), Boerema et al. (1987), Shoemaker & Bissett (1998).

Description in vitro

OA: growth-rate 50–60 mm after 7 days, regular to slightly irregular, with finely floccose, pale olivaceous grey/mouse grey to grey olivaceous aerial mycelium; colony greenish olivaceous/grey olivaceous to dull green; reverse similar.

MA: growth-rate 60–75 mm after 7 days, regular to slightly irregular, with velvety to finely woolly, pale olivaceous grey to pale grey olivaceous aerial mycelium; colony greenish olivaceous to olivaceous grey/olivaceous black; reverse olivaceous black, olivaceous near margin.

CA: growth-rate 30–45 mm after 7 days, irregular, with felty to finely floccose, pale olivaceous grey aerial mycelium; colony grey olivaceous to dull green; reverse similar, with olivaceous black at centre.

Pycnidia densely covered by mycelial hairs, 100–200(–350) µm in diameter, globose to subglobose, solitary or confluent, with 1(–2) inconspicuous non-papillate pore(s), olivaceous to olivaceous black; with an outer wall formed of 2–3(–4) layers of dark polygonal cells, and an inner wall of 1–3(–4) layers of thin walled, often radially arranged hyaline cells; with milky white, later rosy buff to ivory exuded conidial masses; scattered, both on and in the agar. Conidiogenous cells 4–9 × 5–9 µm, globose to bottle-shaped. Conidia aseptate, (2.5–)4–6.5(–9.5) × (1.5–)2.5–4(–5.5) µm, av. 5.6 × 3.3 µm, Q = 1.1–2.4, av. Q = 1.7, globose to subglobose/ellipsoidal, biguttulate or with two polar concentrations of many small guttules.

Chlamydospores absent (but see Note), polymorphic swollen cells commonly occur on all media.

NaOH-spot test: positive, a greenish/bluish discolouring occurs immediately on OA and MA, soon changing to red (E+ reaction).

With Lugol's iodine the entire outer cells of the pycnidial wall become red (different from the blotting paper effect of the cell walls in sect. *Plenodomus*, Boerema et al., 1994).

On MA very fine, needle-like crystals are produced.

Note. Monte & Garcia-Acha (1988a) obtained additional vegetative structures on other media: "Sclerotium-like structures" (retarded development of the pycnidial cavity, 'pycnosclerotia' especially characteristic of species in sect. *Sclerophomella*, Boerema & de Gruyter, 1998).

"Holdfasts" (clusters of swollen cells at the tips of hyphae in contact with the bottom of the Petri dish).

"Arthrospores" (schizolytical fragmentation of hyphae).

"Intercalated chains of globose, somewhat elongated chlamydospores" (obtained in poor culture media with restricted carbon and nitrogen sources).

Description in vivo (especially on *Beta vulgaris*)

Pycnidia (immersed in necrotic tissue of seedlings, leaves, stems and roots; occasionally also in seed clusters) subglobose, up to 250 µm in diameter, glabrous with a flush circular central pore. Conidia as in vitro, subglobose-ellipsoidal, arising from an irregular layer of doliiform conidiogenous cells. These cells sometimes develop alternately or even in a zigzag pattern.

Pseudothecia (occasionally found immersed in necrotic tissue of overwintered seed stalks) subglobose, becoming depressed cupulate, with a short papilla, 200–400 µm in

diameter. Asci cylindrical-clavate, 70–80(–120 when free) × (14–)15–16(–17) µm, 8-spored, overlapping biseriate; separated by numerous paraphyses. Ascospores ellipsoidal to inequilaterally ovoid, 3-septate, with 1 longitudinal septum in the two central cells, constricted at the septa, (18–)20–22(–27) × (7–)8–9(–11) µm. (For detailed descriptions and illustrations see Booth, 1967 and Shoemaker & Bissett, 1998.)

Ecology and distribution. World-wide on beet (*Beta vulgaris*) and spinach (*Spinacia oleracea*). Also recorded from various wild Chenopodiaceae in Europe. Black Leg, Damping-off in seedlings, Root Rot, Storage Rot and Leaf Spot. Mainly seed-borne.

The disease symptoms may be confused with those caused by *Ascochyta caulina* (P. Karst.) Aa & Kesteren, teleomorph *Pleospora calvescens* (Fr.) Tul., see Boerema et al., 1987.

Representative cultures. CBS 523.66 (IMI 173140) and CBS 109410 (PD 77/113) ex *Beta vulgaris* (Chenopodiaceae): both from the Netherlands.

2. **Phoma typhina** (Sacc. & Malbr.) Aa — Fig. 1B

Teleomorph: *Pleospora typhicola* (Cooke) Sacc.

Phoma typhina (Sacc. & Malbr.) Aa, in:Van der Aa & Vanev, Revision Phyllosticta, CBS (2002) 468. — *Phyllosticta typhina* Sacc. & Malbr., in: Saccardo, Michelia 2 (1880) 88.

Phoma typharum Sacc., Sylloge Fung. 3 (1884) 163. — *Phyllosticta typharum* (Sacc.) Allesch., Rabenh. Krypt.- Flora [ed. 2], Pilze 6 [Lief. 61] (1898) 166 [vol. dated '1901'].

Phyllosticta renouana Sacc. & Roum., Revue mycol. 6 (1884) 32.

Phoma typhae Pass., in: Brunaud, Acta Soc. Linn. Bordeaux 40 (1886) 20. — *Phyllosticta typhae* (Pass.) Allesch., Rabenh. Krypt.- Flora [ed. 2], Pilze 6 [Lief. 61] (1898) 166 [vol. dated '1901'].

Phoma typhicola Oudem., Versl. gewone Vergad. wis- en natuurk. Afd. K. Akad. Wet. Amst. 9 (1900) 298; Ned. kruidk. Archf III, 2 (1) (1900) 246.

Phyllosticta coralliobola Bubák & Kabát, Hedwigia 44 (1905) 350.

Selected literature. Webster & Lucas (1959), Van der Aa & Vanev (2002).

Description in vitro

OA: growth-rate 70–80 mm after 7 days, regular, with floccose to woolly, white to pale olivaceous grey aerial mycelium; colony grey olivaceous/olivaceous to dull green; reverse olivaceous/olivaceous grey to greenish grey.

MA: growth-rate 75–80 mm after 7 days, regular, with woolly, white to (pale) olivaceous grey aerial mycelium; colony olivaceous buff/honey to olivaceous, citrine near margin; reverse similar, partly with olivaceous black.

CA: growth-rate c. 80 mm after 7 days, regular, with woolly, pale olivaceous grey aerial mycelium; colony grey olivaceous/olivaceous grey to dull green; reverse similar.

Pycnidia surrounded by short dark hyphae, 80–200 µm in diameter, globose to subglobose, solitary or confluent, with 1, often indistinct non- or slightly papillate pore (usually only visible as a light spot in crushed pycnidia), honey/sienna, later olivaceous to olivaceous black; wall thin to rather thick in old cultures (as with sect. *Sclerophomella*), composed of 1–8 outer layers of dark brown rounded or isodiametric cells and 1–10 inner layers of smaller, often radially arranged cells, which may become thick-walled; with white to salmon exuded conidial masses; scattered, both on and in the agar. Conidiogenous

cells 3–7 × 4–7 µm, globose to bottle shaped. Conidia aseptate, (3.5–)4.5–7(–9) × 1.5–2.5 (–4) µm, av. 5.2 × 2.3 µm, Q = 1.6–3.0, av. Q = 2.3, subglobose to ellipsoidal/allantoid, eguttulate or with some small guttules.

Retarded development of the pycnidial cavity may occur, resulting in 'pycnosclerotia', similar to those found in cultures of some species in sect. *Sclerophomella*.

Chlamydospores absent, but dark swollen cells may occur.

NaOH-spot test: negative.

With Lugol's iodine the contents of the cells in the peridium of the pycnidia usually become red.

Crystals absent.

Note. The wall structure of the poroid pycnidia and the pycnosclerotia of this anamorph are characteristic of species in sect. *Sclerophomella* (Boerema & de Gruyter, 1998). However, due to its connection with a teleomorph in *Pleospora* (members of sect. *Sclerophomella* are related to *Didymella*) and the hairy appearance of the pycnidia in vitro, we have classified it in sect. *Pilosa*.

Description in vivo (especially on *Typha latifolia*)

Pycnidia (subepidermal, then half-free on leaf spots and decayed leaves, leaf sheaths and stems) globose or depressed globose, variable in size, but commonly 150–200 µm in diameter, glabrous with flat ostiole. Conidia as in vitro, but more variable in shape and size (Fig. 1B), arising from a layer of small hyaline cells lining the cavity.

Pseudothecia (also subepidermal and later almost superficial, on dead leaf sheaths and stems; often occurring together with pycnidia) globose or irregularly globose, 280–480 µm diam. Asci cylindrical or broadly clavate, 200–240 × 44–48 µm, 8-spored, irregularly biseriate; separated by numerous paraphyses. Ascospores oblong, rounded at the ends, 3-septate with 1 longitudinal septum, running down the length of the spore in one plane, 46–56 × 18–24 µm, strongly constricted at the septa; each cell of the spore is rounded and may separate slightly from adjacent cells. Mature spores are surrounded by a gelatinous sheath (for detailed description and illustration see Webster & Lucas, 1959).

Ecology and distribution. In Europe recorded in association with leaf spots and on dead leaves, leaf sheaths and stems of *Typha latifolia* and *Typha angustifolia*. Probably occurring everywhere the hosts are growing.

The occurrence of thin-walled and thick-walled pycnidia initially made us believe there were two different species, see Van der Aa & Vanev (2002). However, a comparative study of the original material of Webster & Lucas (1959) with Dutch collections in vivo and in vitro, proved that only one fungus was involved.

Representative cultures ex *Typha angustifolia* (Typhaceae). CBS 132.69, the Netherlands and SHEFF 2265a (dried), Great Britain.

NOMENCLATURAL NOTES ON SOME OTHER TAXA

i. The type specimens of three new taxa recently described in this Contribution series still need to be documented according to Art. 37.3:

Phoma schneiderae Boerema, de Gruyter & van de Graaf, Persoonia 17 (2) (1999) 282.
Type specimen L 998.099-105, dried culture on MA, dated 14-07-1998, made by J. de Gruyter, Plant Protection Service (PD) Wageningen, the Netherlands from living culture CBS 101.494 (ADAS AR 98/11 = PD 98/5247) isolated from leaf spot on *Lupinus albus*, Mepal-Ely, Cambridgeshire, Great Britain, April 1998.

Annotation by Boerema, de Gruyter & van de Graaf

Phoma pimpinellae Boerema & de Gruyter, Persoonia 17 (2) (1999) 278.
Type specimen L 992.163-138, dried culture on MA, dated 01-04-1999, made by J. de Gruyter, Plant Protection Service (PD) Wageningen, the Netherlands from living culture CBS 10637 isolated from a single ascospore of *Leptosphaeria pimpinellae* Lowen & Sivanesan (1989) on stem of *Pimpinella anisi*, collected at Mt Carmel, Beit Oren Forest, Wadi near Kibbutz Oren, Israel.

Annotation by Boerema & de Gruyter

Phoma leveillei var. ***microspora*** de Gruyter & Boerema, Persoonia 17 (4) (2002) 553.
Type specimen L 999.2 42399, dried culture on MA, dated 15-08-2000, made by J. de Gruyter, Plant Protection Service (PD) Wageningen, the Netherlands from living culture CBS 102876, isolated from water, Lake of Skadar, Yugoslavia (Montenegro).

Annotation by De Gruyter & Boerema

ii. In our study on the identity of the *Phoma*-like anamorphs described by Wollenweber & Hochapfel (Boerema & Dorenbosch, 1973) it was noted that one of the species listed represented a spermatial state and fits into the genus *Asteromella* Pass. & Thüm. However, the new combination proposed at that time, appeared to be an invalid later homonym and must be replaced:

Asteromella ulmi Boerema nom. nov.
≡ *Asteromella bellulensis* (Martelli) Boerema & Dorenb., Stud. Mycol. 3 (1973) 5 [replaced synonym]; not *Asteromella bellulensis* Syd., Annls mycol. 30 (1933) 397.
≡ *Phyllosticta bellulensis* Martelli, Nuovo G. bot. Ital. 20 (1888) 395.
Teleomorph: *Mycosphaerella ulmi* Kleb.
Conidial anamorph: *Phloeospora ulmi* (Fr.: Fr.) Wallroth.
In Europe a common leaf spot fungus of elms, *Ulmus* spp., see the experimental study by Klebahn (1905).

iii. *Phoma*-like phialidic pycnidia containing small brown to brown-black conidia at maturity, formerly classified in *Coniothyrium* Corda, are better placed in *Microsphaeropsis* Höhn. on account of conidium ontogeny[1] (Sutton, 1980).
The conidia of *Microsphaeropsis* species are initially subhyaline, which means that collections and cultures with young pycnidia may be easily mistaken for *Phoma*.

1) The conidiogenous cells of the type- and related species of *Coniothyrium* show annellations (annellidic appearance) or an annulated collar (Sutton, 1971; electron microscopic study by Reisinger et al., 1977), comparable with our observations on *Ascochyta* species (Boerema & Bollen, 1975). The conidial ontogeny of the type- and related species of *Microsphaeropsis* agrees with that of *Phoma* (electron microscopic study by Jones, 1976): conidiogenous cells with a collarette, i.e. the basal part of the papilla preceding the initiation of the first conidium.
In both genera the pigmentation and differentiation of the conidium wall into an outer and an inner layer by diffuse wall-building occur after conidial secession: maturation asynchronous with conidium ontogeny (Reisinger et al., 1977; Jones, 1976).

Two examples:

Microsphaeropsis fuckelii (Sacc.) Boerema, comb. nov.

- *Coniothyrium fuckelii* Sacc., Michelia 1 (2) (1878) 207 [basionym].

Teleomorph: *Leptosphaeria coniothyrium* (Fuckel) Sacc.

Isolates of this cosmopolitan pathogen of trees and shrubs (Boerema & Verhoeven, 1972) have frequently been sent to the Dutch Plant Protection Service as suspected *Phoma* species.

Microsphaeropsis glumarum (Ellis & Tracy) Boerema, comb. nov.

- *Phoma glumarum* Ellis & Tracy in Ellis & Everh., J. Mycol. 4 (1888) 123 [basionym].
- *Phyllosticta glumarum* (Ellis & Tracy) I. Miyake, J. Coll. Agric. imp. Univ. Tokyo 2 (1910) 252.
- *Sphaeropsis glumarum* (Ellis & Tracy) Kuntze, Revisio Gen. Pl. 3 (2) (1898) 525.

An anamorph recorded on dark glumes of poorly developing or aborted kernels of rice in the USA and China (incl. Taiwan). This fungus has been confused with the plurivorous *Phoma sorghina* (Sacc.) Boerema et al., even by S.M. Tracy, one of its original authors. See the discussion in Boerema, Dorenbosch & Van Kesteren (1973: 136).

ACKNOWLEDGEMENTS

Thanks are due to Dr. M.E. Noordeloos and Dr. A. Lyon of the Universities at Leiden and Sheffield for their help in obtaining original material of *Phoma typhina* and *Pleospora typhicola*. Dr. R.T.A. Cook kindly improved the English of this paper.

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