

"BLACK ROOT ROT" IN CUCURBITACEAE CAUSED BY  
*PHOMOPSIS SCLEROTIOIDES* NOV. SPEC.<sup>1</sup>

*Zwartwortelrot bij Cucurbitaceae, veroorzaakt door*  
*Phomopsis sclerotioides nov. spec.*

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Amplifying an earlier report on black root rot of Cucurbitaceae, a full description is given of the disease symptoms – a black root rot resulting in wilting – and of the causal organism. On the basis of pycnidia produced *in vitro* it is proposed to name the fungus *Phomopsis sclerotioides* nov. spec. On the plants only pseudomicrosclerotia and pseudostromata are found ("*Sclerotium* spec.").

INTRODUCTION

In a short communication (VAN KESTEREN, 1965) wilting symptoms were reported in cucumbers and gherkins, caused by a sclerotia-producing fungus (*Sclerotium* spec.). This fungus attacks the roots, which become discoloured a striking brownish-black.

To distinguish the disease from other cucumber and gherkin diseases with attendant wilting symptoms, it is designated in this publication as "black root rot" (compare Fig. 1).

DISEASE SYMPTOMS

The brownish-black discoloration of the roots of infected Cucurbitaceae arises from the coalescence of somewhat sunken uniform greyish-black spots, enclosed by distinct dark lines or sheets (Fig. 2) and black speckled, greyish spots with no sharply defined margin (Fig. 5).

The black bordered spots are often located mainly at the places of attachment of lateral roots and hair roots and are then more or less ring-shaped (Fig. 4). If a spot girdles a root completely, the portion down to the lesion becomes brown and dies. The attack may be restricted to a few roots; it is only in serious cases that all the roots of the plants are affected. In the last stage of the disease the cortical tissue of the roots rots away entirely so that only the vascular bundles are left.

Symptoms of wilting usually do not manifest themselves until the plants are about to bear their first fruits. The extent of wilting is determined by the degree of root infection. The weather conditions are also of great importance; in sunny periods (high evaporation, low air humidity) even slightly infected plants may suddenly wilt. In fully wilted plants the stem bases generally also show rotting symptoms. These start as watery spots, later becoming light-brown and sunken and extending gradually. Sometimes these stem lesions also become discoloured black and there may be amber-coloured gummy exudations. Inoculation experiments have also established that the fungus can cause a black fruit rot, though this has not been seen in the field (BOEREMA, VAN KESTEREN & DORENBOSCH, 1965).

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## OCCURRENCE

The disease was observed for the first time in gherkins (*Cucumis sativus*) in 1964 in the gherkin-growing centre near Venlo. In the same year an outbreak was confirmed in cucumbers (also *C. sativus*) and it appeared that the disease had already been reported on a vegetable nursery in the province of Drenthe in 1962. Pathogenicity tests with the isolated fungus reproduced the disease symptoms in both crops (VAN KESTEREN, 1965). In 1965 and 1966 it appeared that the disease caused serious damage to gherkin and cucumber growing under glass on a great many nurseries. In the same period black root rot symptoms were also often observed in melons (*Cucumis melo*). Comparative inoculation experiments revealed that this was the same disease.

Various factors may affect the incidence of this disease. First it may be noted that tests have shown that even a very small quantity of inoculum may cause a serious root rot and wilting of the plants. Another factor is that recent intensification of the cropping programme has stopped or at least diminished crop rotation. Furthermore, the soil of many new glasshouses has not been steamed or not been sufficiently steamed. Lastly, in cucumber growing most plants are no longer grafted and are often bred elsewhere. The rootstock *Cucurbita ficifolia*, which was generally used a few years ago, is more or less insusceptible to black root rot (Fig. 1). Originally this rootstock was supposed to be entirely resistant, but in recent years root infections have been observed on it.

## IDENTITY OF THE FUNGUS

*In vivo* the black root rot fungus is characterized by:

1. Black-brown sheets or lines ("zone lines") of thick-walled fungus cells, the so-called pseudostromata (Figs. 2-4). These stroma-like structures enclose necrotic root tissue and are mostly oriented in the longitudinal axis of the root. They are also often found surrounding the places of attachment of lateral roots and hair roots.

2. Small black-brown pseudo-sclerotia distributed in the cortical cells (Fig. 5). The dimensions and forms of these pseudo-sclerotia vary in conformity with the dimensions and shape of the cortical cells. In other cortical cells dark-coloured mycelium often grows prolifically (Fig. 8). *In vitro* on agar media the fungus generally forms a thin dull grey to greyish-brown mycelial mat. This mycelium (Figs. 6-7) consists of a mixture of thin hyaline hyphae (2-4.5  $\mu$  in diameter) and relatively thick hyaline to dark-brown coloured hyphae (10-17.5  $\mu$  in diameter). After some time in culture superficial or subimmersed sclerotia are produced, which may vary greatly in size. Most are very small and more or less flattened, spherical in shape and 0.1-1 mm in diameter. Others, on the contrary, are relatively large, usually elongated and flattened (3-10  $\times$  2-5 mm). The large sclerotia consist of a loose tissue of branched hyphae surrounded by a layer of dark thick-walled cells. On certain culture media, e.g. cherry agar and sterilized beanpods, the fungus produces not only sclerotia but also pycnidia.<sup>2</sup> These pycnidia (Fig. 9 A-G) are mostly subglobose or spherical, but may also be of very irregular form. They are often of a stromatic structure with

<sup>2</sup> It must be noted that the pycnidia have never been found on the plants, so that they probably play no role in the spread of the disease.

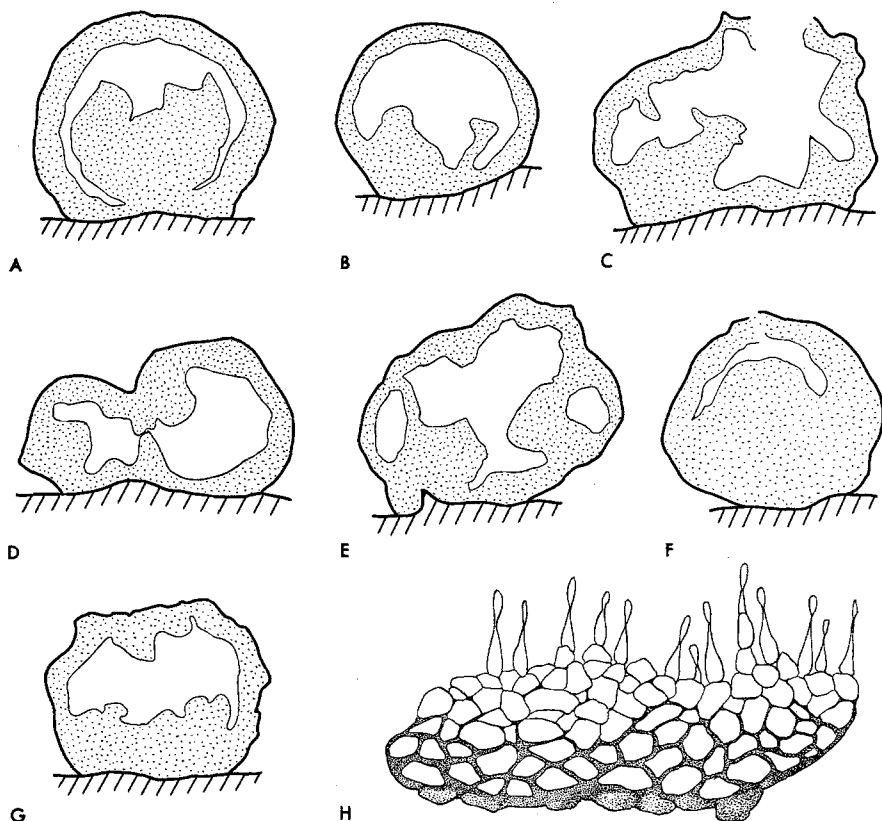


FIG. 9. A-G. Variation in shape of the pycnidia of *Phomopsis sclerotioides*.

*Variatie in vorm van de pycniden van Phomopsis sclerotioides.*

FIG. 9. H. Detail of wall with sporogenous cells.

*Detail van de wand met sporevormende cellen.*

a thick wall, brown or blackish towards the outside, without a distinct ostiole. The internal cavity is often divided by protrusions of the proliferous layer. The spores are hyaline, mostly ellipsoidal to ovoid with subacute to oblong extremities, often with two or more guttules ( $7.5-10.5 \times 2.5-4.5 \mu$ ). They are produced by a repetitive budding process on ventricose, occasionally septate sporophores ( $12.5-15 \times 2.5-5 \mu$ ) (Fig. 9 H).

The characteristics described above would place this fungus in the form-genus *Phomopsis*, as a species with "alpha-spores" only. No data have been found in the literature about a similar fungus. A *Phomopsis* species causing fruit- and stemrot of Cucurbitaceae has been described, viz. *P. cucurbitae* McKeen (McKEEN, 1957). Examination of the original material of this fungus, however, obtained from the Plant Research Institute in Ottawa, Canada, showed that it is completely different from the black root rot fungus found in the Netherlands. *P. cucurbitae* has both alpha- and beta-spores and produces no

sclerotia. Dr. C. McKEEN (private communication) also concluded that the two fungi are not identical. The black root rot fungus is therefore described here as a new species:

*PHOMOPSIS SCLEROTIOIDES* Kest. spec. nov.

*In radicibus hospitis:* Pseudostromata texturam necroticam secundum radices axem longitudinalem productam circumdantia; collectus cellularum membranis crassis dicti "pseudo-microsclerotia"; hospitis cellulae corticales mycelio congestae.

*In vitro:* textura densa mycelii cum sclerotiis atque pycnidiiis. Mycelium hyphis tenuibus hyalinis ( $2-4.5\ \mu$ ) et latis fuscis ( $10-17.5\ \mu$ ) compositum. Sclerotia subimmersa vel superficialia, depresso-sphaeroidea  $0.1-1\ \text{mm}$  diameter, interdum oblonga complanata  $3-10 \times 2-5\ \text{mm}$ , cum cortica atro distincto intus albo-medullosa, texturam hypharum ramosarum laxam. Pycnidia subglobosa vel irregularia, membranis stromaticis, crassis, fuscis, sine ostiolo distincto. Sporae hyalinae, ovatae vel ellipsoideae, apicibus subacutis vel oblongis, saepe guttulis  $7.5-10.5 \times 2.5-4.5\ \mu$ , gemmatione repetita formatae in sporophoris ventricosis interdum septatis  $12.5-15 \times 2.5-5\ \mu$ .

Habitat in radicibus Cucurbitacearum, putrefaciens radices, quo plantae exsiccant. Observata in Neerlandica. Typus in Herbarium Lugdunense Batavorum (L.) depositus. Cultura typica in "Centraalbureau voor Schimmelcultures" (CBS), Baarn (CBS 296.67) est.

Producing in the roots of the hostplants pseudostromata, mostly oriented in the longitudinal axis of the roots and often enclosing necrotic tissue; also complexes of thickwalled cells, "pseudo-microsclerotia", distributed in the cells of the cortex as well as abundant free hyphae in other cortical cells.

In culture producing a dense mycelial mat with sclerotia and pycnidia. Mycelium being a mixture of thin hyaline hyphae ( $2-4.5\ \mu$  diam.) and thick dark brown hyphae ( $10-17.5\ \mu$  diam.). Sclerotia sunken to superficial, flattened-spherical  $0.1-1\ \text{mm}$  diam., sometimes oblong  $3-10 \times 2-5\ \text{mm}$  with a distinct outer layer of dark-coloured cells and inside a loose tissue of branched hyaline hyphae.

Pycnidia subglobose to irregular, stromatic with a thick dark-celled wall and without a distinct ostiole. Spores hyaline, ellipsoidal to ovoid with subacute to oblong extremities, often guttulate ( $7.5-10.5 \times 2.5-4.5\ \mu$ ), produced by a repetitive budding process on ventricose, sometimes septate, sporophores ( $12.5-15 \times 2.5-5\ \mu$ ).

Occurring in the roots of Cucurbitaceae, causing a black rot and wilting of the plants. Type deposited at the Rijksherbarium at Leyden (L). A culture of the type is deposited at the Centraalbureau voor Schimmelcultures (CBS) at Baarn (CBS 296.67).

#### SAMENVATTING

Aansluitend op een vorige publikatie wordt een uitvoerige beschrijving gegeven van het zwartwortelrot bij *Cucurbitaceae*, een ziekte die uiteindelijk resulteert in een verwelken van de planten. De schimmel – vanwege de op aangetaste wortels gevormde pseudo-microsclerotiën en pseudostromata bekend als *Sclerotium* spec. – produceert in cultuur ook pycniden. Op grond van dit stadium is de schimmel hier beschreven als een nieuwe soort, *Phomopsis sclerotioides* spec. nov.

#### ACKNOWLEDGEMENTS

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and to Dr. J. A. PARMELEE, Curator of the Mycological Herbarium of the Plant Research Institute Ottawa, Canada, for supplying herbarium material of *P. cucurbitae*. Drs. P. A. A. LOOF kindly provided the Latin diagnosis.

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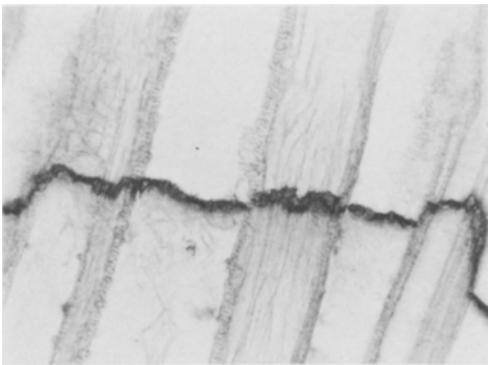
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FIG. 1. Grafted cucumber plant; cucumber roots completely rotted by *Phomopsis sclerotioides*, those of the rootstock *Cucurbita ficifolia* healthy.

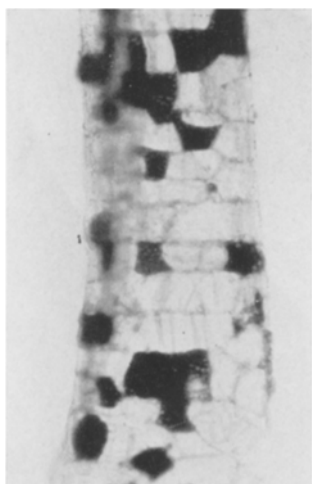
*Geënte komkommerplant; wortels van de komkommers geheel verrot ten gevolge van Phomopsis sclerotioides, die van de onderstam Cucurbita ficifolia gezond.*

FIG. 2 and 4. Roots of cucumber attacked by *P. sclerotioides*. Brownish-black sheets or lines, pseudostromata, enclosing necrotic tissue.

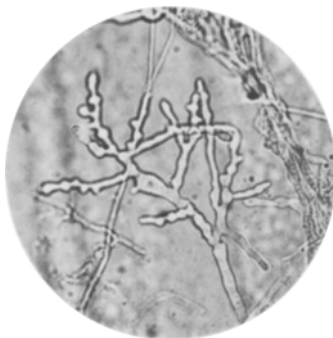
*Komkommerwortels, aangetast door P. sclerotoides. Bruinzwarte banen of lijnen, pseudostromata, die necrotisch weefsel omsluiten.*

FIG. 3. Cross section of a part of a pseudostroma.

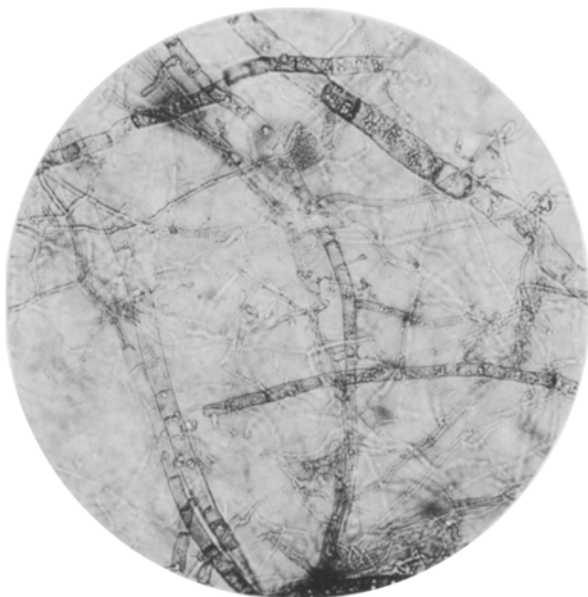
*Dwarscoupe van een deel van een pseudostroma.*



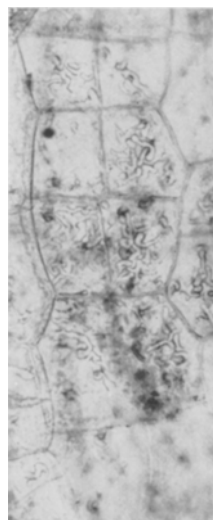
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FIG. 5. Small brownish-black pseudo-microsclerotia of *Phomopsis sclerotioides* distributed in the cortical cells.

*Verspreid voorkomende kleine bruinzwarte pseudo-microsclerotiën van Phomopsis sclerotioides in de schorscellen.*

FIG. 6. Typically crenate aerial mycelium produced by *P. sclerotioides* in culture.

*Typisch gegolfd luchtmycelium van P. sclerotioides in cultuur.*

FIG. 7. Submerbed mycelium of *P. sclerotioides* in culture.

*Ingezonken mycelium van P. sclerotioides in cultuur.*

FIG. 8. Masses of dark-coloured mycelium of *P. sclerotioides* in the cortical cells.

*Woekeringen van donker gekleurd mycelium van P. sclerotioides in de schorscellen.*