ULTRASTRUCTURE OF ASCUS DEVELOPMENT IN THE TELEOMORPH OF *PHOMA ARACHIDICOLA*

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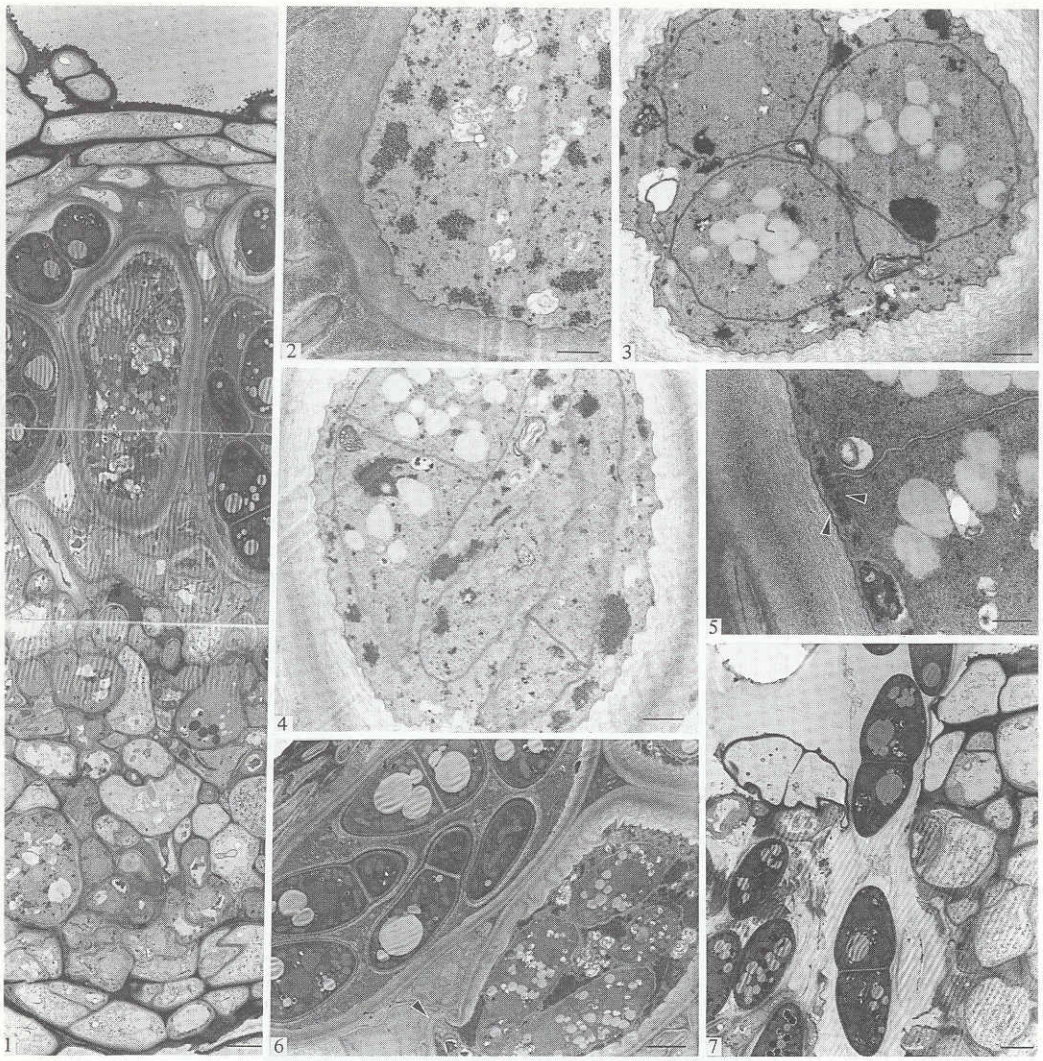
Ascus development in the teleomorph of *Phoma arachidicola* was examined using light and electron microscopy. Ascus initials developed from pseudoparenchymatous elements in the pseudothecium and ascospores were delimited by a double-layered membrane produced independently of the plasmalemma. Ascospores were hyaline and became dark after discharge. No pseudoparaphyses were observed but threadlike strands, apparently remnants of the pseudoparenchymatous centrum compressed by developing asci, were interspersed among the asci. On the basis of these findings, the teleomorph of *P. arachidicola* cannot be accommodated in *Didymella*, *Didymosphaeria* or *Mycosphaerella*.

Web blotch of peanuts (*Arachis hypogaea* L.) was first described from South Africa in 1974 (Marasas, Pauer & Boerema, 1974). On the basis of developmental and morphological features of the anamorph, the causal fungus was named *Phoma arachidicola* Marasas, Pauer & Boerema. The teleomorph of *P. arachidicola* was not observed by Marasas *et al.* (1974). Pseudothecia have subsequently been found on infected peanut leaves in South Africa and have also been induced to form in culture. Production of pseudothecia in a South African isolate of *P. arachidicola* has also been reported by Taber, Pettit & Philley (1984).

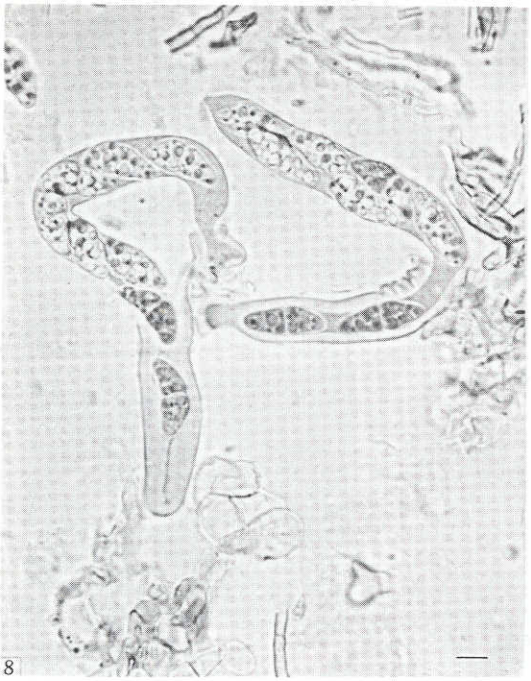
An appropriate name for the teleomorph of *P. arachidicola* has been the subject of considerable controversy. Several names, including *Mycosphaerella arachidicola* Chochrjakov, *M. argentinensis* Frezzi, *Didymella arachidicola* (Chochrjakov) Tomilin, *D. arachidicola* (Chochrjakov) Taber, Pettit & Philley, and *Didymosphaeria arachidicola* (Chochrjakov) Alcorn, Punithalingam & McCarthy, have been used (Alcorn, Punithalingam & McCarthy, 1976; Taber *et al.*, 1984; Sivanesan, 1984). In a recent monograph of the bitunicate ascomycetes, Sivanesan (1984) accepted *Didymosphaeria arachidicola* as the correct name of the teleomorph of this fungus.

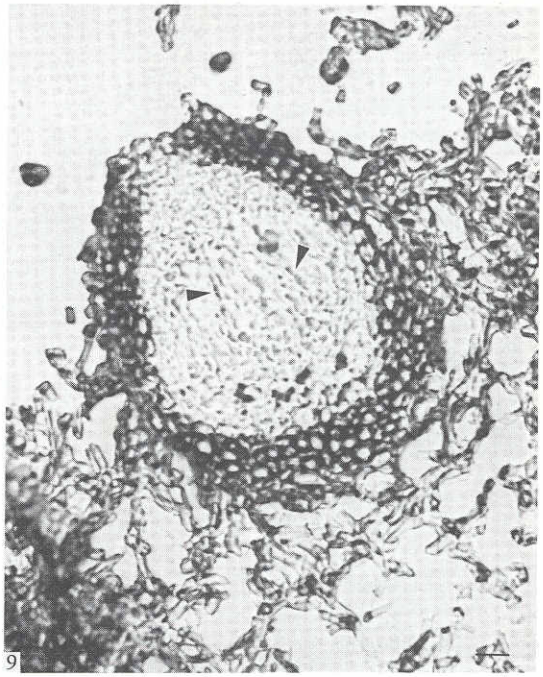
The ultrastructure of ascus development in the teleomorph of *Phoma arachidicola* is presented for the first time in this report. Special attention is given to the presence or absence of pseudoparaphyses in this fungus. Isolate Ph47M from infected peanut leaves was incubated on wheat-bran-enriched (30 g/l) water agar under continuous white/near ultraviolet illumination at 20 °C for 10 d. Pseudothecia on agar cultures were fixed in 3% glutaraldehyde in 0·1 M phosphate buffer (pH 7·0) for 4 h. After three rinses in 0·1 M phosphate buffer, material was post-fixed for 4 h in 2% osmium tetroxide in 0·1 M phosphate buffer. The material was rinsed in buffer, dehydrated in a graded series of ethyl alcohol and embedded in epoxy resin. Impregnation under vacuum was followed by polymerization at 70° and sections were cut on a LKB Ultrotome III using a diamond knife. Sections, approximately 60 nm, were stained for 25 min in a saturated solution (*ca* 6%) of uranyl acetate and in lead citrate for 10 min, respectively, and viewed using a Philips EM 300 TEM. Sections for light microscopy were cut approximately 600 nm thick, mounted on glass slides and stained with toluidine blue–pyronin–borax mixture.

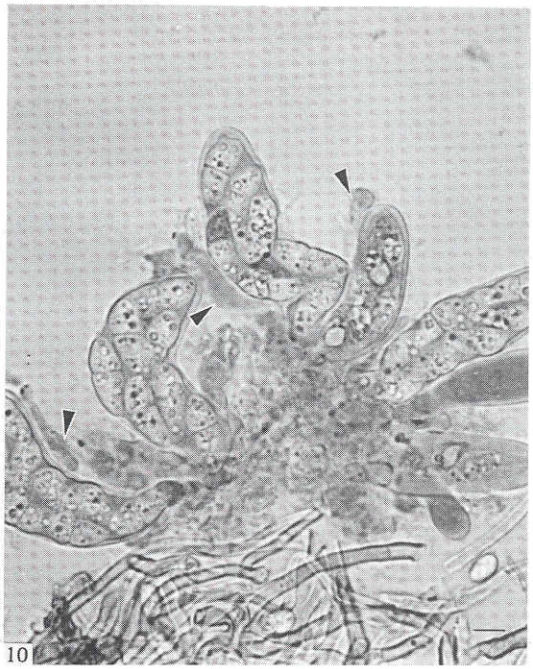
The pseudothecium originates as a localized pseudoparenchymatous body, delimited by thick-walled elements (Fig. 1). The elements present in the pseudothecium from top to bottom (Fig. 1) include two to three layers of flattened, thick-walled, melanized cells. The upper half of the



Figs 1–7. Ascus development and release in the teleomorph of *Phoma arachidicola* (bar = 1 μm).
Fig. 1. Pseudothecium composed of pseudoparenchymatous elements and developing asci surrounded by thick-walled elements.
Fig. 2. Differentiating pseudoparenchymatous element with granular contents.
Fig. 3. Cross-section through a developing ascus. The outer layer of the wall has thickened to about × 12 original size. Delimiting membranes of ascospores are produced independently of each other and the plasmalemma. Note accumulation of lipid bodies in delimited areas.
Fig. 4. Longitudinal section through a developing ascus. Delimiting membranes and septa of the ascospores are produced simultaneously. Note the double-layered membrane of the young ascospore.
Fig. 5. Independence of the double-layered membrane of a developing ascospore and the plasmalemma (arrowheads).
Fig. 6. Attachment of a developing ascus to undifferentiated pseudoparenchymatous elements (arrowhead).
Fig. 7. Mature ascospores within the inner wall layer of the ascus through the pseudothecium wall. Note the fragile inner wall layer (plasmalemma) and the disintegration of the pseudothecium cell wall to produce a canal.







Figs 8–10. Teleomorph of *Phoma arachidicola* – light microscopy (bar = 10 μm).
Fig. 8. Release of ascospores within the fragile inner wall layer (plasmalemma) of a bitunicate ascus.
Fig. 9. Threadlike structures in a pseudothecium (arrowheads).
Fig. 10. Threadlike structures (arrowheads) among asci in a squash mount.

pseudothecium contains a layer of asci at different stages of development and compressed pseudoparenchymatous elements whereas the lower half consists only of a layer of pseudoparenchymatous elements. Certain pseudoparenchymatous elements act as ascus initials and differentiate (Fig. 2). The walls become thicker (Fig. 3) and lipid bodies accumulate within the ascospore initials (Figs 3, 4). The ascospores are delimited by a double-layered membrane and the septum is laid down at the same time as the outer layer of the ascospore wall (Fig. 4). This delimiting double-layered membrane system is produced independently of the plasmalemma (Figs 4, 5).

The irregular outer surface of the ascospore wall, in contrast to the smooth inner surface (Fig. 6), suggests that these wall layers are produced from the outside. The complete wall consists of an electron dense outer layer and an inner layer which is less dense (Fig. 7). The ascus wall in the early stages of development is similar to that described by Reynolds (1971) in the bitunicate ascus of *Limacinula theae* Syd. & Butl. When the ascospores are nearly mature, the ascus wall decreases in thickness but the ascus remains attached to the remaining undifferentiated cells (Fig. 6). Differentiation of the ascus wall results in the formation of a fragile inner wall layer which appears to consist only of the original plasmalemma (Figs 7, 8).

The section of the pseudothecium above the mature asci disintegrates, presumably by enzymatic activity, resulting in a pore through which ascospores are released (Fig. 7). The outer wall layer apparently remains attached to the pseudoparenchymatous layer at the base of the pseudothecium.

Pseudoparaphyses as defined by Luttrell (1965) were not observed in the pseudothecia. However, threadlike structures (Figs 9, 10) among young developing asci stained intensely and were obvious using light microscopy. These threadlike structures appear to be remnants of the original pseudoparenchymatous centrum compressed by developing asci. Compressed elements could also be seen in ultrathin sections (Figs 1, 6). Pseudoparaphyses are vertical outgrowths from the overlying stroma that are ultimately attached at both ends and formed before the asci (Luttrell, 1965). The threadlike structures, apparently produced secondarily as a result of ascus enlargement seem more likely to be interthecial elements (Luttrell, 1965) or remnants of the centrum. Interthecial elements, characteristic of the *Pseudosphaeria* type centrum (Luttrell, 1951, 1973), are fundamentally different from pseudoparaphyses. It is probable that the threadlike structures have been incorrectly referred to as pseudoparaphyses (Alcorn *et al.*, 1976; Sivanesan, 1984; Taber *et al.*, 1984).

Difficulties in differentiating between *Mycosphaerella* Johansen, *Didymella* Sacc., and *Didymosphaeria* Fuckel have resulted in confusion. Corlett (1981) and von Arx (1983) have defined the distinguishing characteristics between *Didymella* and *Mycosphaerella*. Based on these characteristics, the teleomorph of *P. arachidicola* can clearly not be accommodated in *Mycosphaerella*. The choice as to whether to place it in *Didymella* or *Didymosphaeria* is more difficult. The absence of pseudoparaphyses suggests that neither genus is appropriate. Moreover, the ascospores are hyaline but become dark after discharge (Alcorn *et al.*, 1976), whereas ascospores of *Didymella* are usually hyaline and those of *Didymosphaeria* are usually dark. Further developmental studies are necessary to determine the correct generic position of the teleomorph of the peanut web blotch fungus.

We thank Ruth A. Taber for reviewing the manuscript.

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