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

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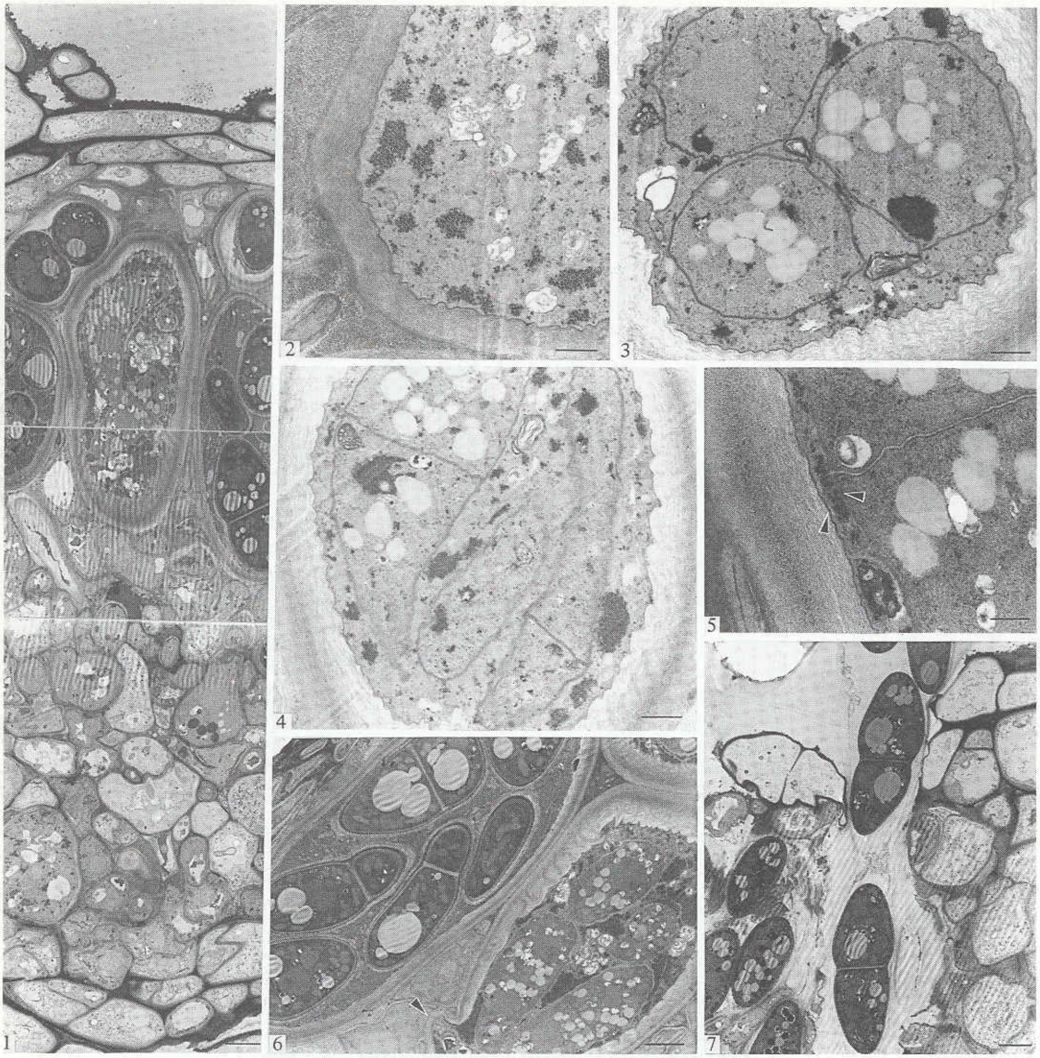

Figs 1–7. Ascus development and release in the teleomorph of *Phoma arachidicola* (bar = 1 μm).

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.