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Scanning Electron Microscopy of the Infection of Beans by *Rhizoctonia solani* Propagules

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ABSTRACT

Germination, hyphal spread, and formation of infection cushions and lesions on bean hypocotyls by infective large propagules of *Rhizoctonia solani* were studied with the scanning electron microscope. The surface of old, but not of young, hyphae appeared typically wrinkled and shrunken. Infection cushions appeared as a network of several layers of crossed anastomosing hyphae. These features could not be observed under the light microscope. No differences in the surfaces of infective and non-infective small propagules could be observed. Non-infective propagules germinating on the hypocotyls gave rise to spreading hyphae but did not develop infection cushions.

INTRODUCTION

The fungus *Rhizoctonia solani*, which causes damping off in many plants, attacks plant organs by means of its mycelial propagules. The inoculum quality of these propagules was studied by Henis and Ben-Yephet (1970) who found that while propagules smaller than 150 μm were non-infective to bean hypocotyls, larger propagules were infective. The importance of propagule size for successful infection has also been demonstrated with *R. crocorum* (Whitney, 1954) and *Fomes lignosus* (Altson, 1953). It is also well established that the production of cell wall degrading enzymes is essential for infection (Bateman and Millar, 1966). It was previously shown (Lisker, Katan, and Henis, 1974) that small propagules of *R. solani* produce less polygalacturonase (PG) and cellulase than the large ones when grown in relatively poor media.

Dodman and Flentje (1970) described the various stages in *R. solani* development during plant infection. Weinhold and Bowman (1974) reported that interference in infection cushion formation prevented disease development even when PG production was not blocked. Since infection cushions are produced through hyphal anastomosis, excessive hyphal branching in a localized area may result in their formation (Dodman and Flentje, 1970). Lesion formation by *R. solani* is the final stage in a series of sequential events in which the formation of infection cushions is of great importance (Dodman and Flentje, 1970; Weinhold and Motta, 1973).

The morphologically-different stages in the process of plant infection by *R. solani* have been documented using light microscopy (Christou, 1962; Dodman and Flentje, 1970; Weinhold and Motta, 1973). In the present study we followed this process with the scanning electron microscope (SEM) which has higher magnification and resolution capacities.

MATERIALS AND METHODS

A virulent isolate of *Rhizoctonia solani* Kuehn grown in yeast extract (YE) medium was used for propagule preparation (Lisker *et al.*, 1974). The resulting mycelial mats were

homogenized for 1 min with tap water in a Waring blender and the propagules were separated by sieves. The fractions retained between sieves of 590–400 μm and 250–150 μm are referred to as large and small propagules, respectively. Sclerotia were collected from the edges of the flask of YE medium in which the fungus was grown.

Seeds of bean (*Phaseolus vulgaris* L., cv Brittle-Wax) were germinated in plastic containers. After 4–5 days, when hypocotyls were about 2 cm long, 2·5 μl of distilled water were placed on the hypocotyl, which was then inoculated with one propagule and maintained in moistened closed plastic containers. After 36–48 h typical lesions were observed on the hypocotyl.

The material to be examined by SEM was fixed for 2–3 h in 5 per cent glutaraldehyde in 0·1 M phosphate buffer (pH 7), rinsed twice with the same buffer, and then dehydrated by immersing in increasing concentrations of ethanol (10–100 per cent, in 10 per cent increments, 15 min in each concentration). The fractioned mycelia were attached with collodion on stubs and immediately immersed again in liquid nitrogen for 20 min before coating with 20 μm (200 Å) thick gold in a Spatering Colting Unit E 5000 (Polaron Equipment Ltd., England) under vacuum. The material was then examined in a Cambridge Stereoscan electron microscope S4 at 20 kV.

RESULTS

Large infective propagules of *R. solani* in the SEM micrographs generally appeared as a homogenous mass of hyphae (Plate 1A) of uniform diameter with broken hyphal cells (Plate 1B). However, some of these cells were closed and showed protruding pore caps (Plate 1C).

After inoculation of bean hypocotyls with infective propagules, germination was observed in 2 h. Four hours after inoculation the emerging hyphae were still non-branched (Plate 1D). After 8 h the hyphae became branched in a typical L or V form (Plate 1E); the broken empty cells did not germinate (Plate 1F). At a later stage the hyphae appeared shrunken, with wrinkled, rough surfaces, as can be seen in Plate 1F. These wrinkles were also observed in samples fixed by other methods (i.e. 24 h fixation with glutaraldehyde, or 2 h fixation with glutaraldehyde followed by 2 h fixation with osmium tetroxide). The hyphae emerging from the propagules grown on the hypocotyl did not necessarily run along the lines of junction of underlying anticlinal cell walls (Plate 2A) or penetrate the stomata (Plate 2B). Anastomosis occurred between both distant and closely running hyphae (Plates 2C and D). The ends of some hyphae were extensively branched (Plate 2E), the apical cells of the individual branches being short, swollen, stubby cells (Plate 2F). These terminal cells differed morphologically from ordinary branching tips (Plates 1E and 2G). Such extensive branching was followed by formation of the infection cushions (Plate 3A). The cracked host cuticle observed under the infection cushions could have resulted from collapse of underlying epidermal cells. Higher magnifications revealed that the infection cushions consisted of several layers of profusely-anastomosing hyphae which gave rise to typical round or square-shaped patterns (Plate 3B). Finally, a concave lesion with a broken surface was formed on the hypocotyl at a distance of a few mm from the propagule (Plate 3C).

Except for their size, ungerminated small, non-infective propagules did not differ morphologically from ungerminated large ones. However, both germination of, and hyphal development from, the non-infective propagules on the bean hypocotyl surface were considerably less than from the large ones (Plate 3B). Only in rare cases did the inoculation of bean seedlings by small propagules result in damage to the hypocotyl surface, but this could not be detected at the light microscope level.

When observed under the SEM, sclerotia of *R. solani* resembled propagules (Plate 4A). At higher magnifications, however, an amorphic layer covering the surface was seen in

the sclerotium (Plate 4B) but not in the propagules. When this cover was removed by a fine needle, a loose arrangement of barrel-shaped cells was observed (Plate 4C ,D). These features were not shown in the scanning electron micrographs of the surface of the mature sclerotium of *R. solani* presented by Willets (1969).

DISCUSSION

Our SEM studies of the initial stages of infection of bean hypocotyls by *R. solani* propagules corroborate the findings of other authors who used light microscopy (Christou, 1962; Dodman and Flentje, 1970; Weinhold and Motta, 1973). However, the increased resolution and magnification capacities of the SEM gave us better insight into the various structures involved in pathogenesis.

Non-infective propagules have a reduced capacity in producing pectolytic enzymes (Lisker *et al.*, 1974) and do not form infection cushions. SEM observation of the infection cushion produced by infective propagules revealed a structure completely different from the typical compact one usually observed under the light microscope (Christou, 1962; Dodman and Flentje, 1970). The genetic and pathogenic significance of the anastomosis involved has still to be clarified (Bolkan and Butler, 1974; Flentje, 1970). Galun (1971) who carried out a SEM study of *Trichoderma viride*, suggested that in this fungus the 'tunnel system' consisting of fusing hyphae is of both genetic and morphogenetic significance.

As with many soil-borne pathogens, successful infection by *R. solani* requires a certain level of inoculum potential (Garrett, 1970). The consistent formation of the lesion at a distance from the propagule might reflect the requirement of both fungal and plant substances at certain concentrations for the formation of infection cushions, and/or the presence of substances in the propagules which inhibit the immediate formation of infection cushions. The mycelium which advances from the propagule is not directed or restricted to certain sites such as stomata or lines of junction of the epidermal cells, as has been shown in some cases (Dodman and Flentje, 1970).

Unlike the young hyphae of the pathogen, the older ones showed occasional surface wrinkling, as is also seen in *T. viride*. Such a change could either be an artifact caused by the preparation procedures in all the methods used, or it could result from structural and/or chemical changes in the ageing hyphae. Indeed, our observations of stained old and young hyphae under the light microscope revealed that young hyphae contain more cytoplasm than old ones. This is in agreement with the extensive vacuolation observed in old hyphae by the transmission electron microscope (Butler and Bracker, 1970).

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EXPLANATION OF PLATES

PLATE 1

A–C. Surface structure of infective (590–400 μm) propagule.
- A. Densely interwoven hyphae. ×160.
- B. Hyphae of uniform diameter that constitute the propagule. ×640.
- C. Broken hyphal cells, either empty (E) or closed with septal pore apparatus (PC). ×2000.

D–F. Germinating infective propagule.
- D. After 4 h, only unbranched hyphae are observed. ×500.
- E. After 8 h, short-branched hyphal tips are observed. ×950.
- F. Young (smooth) and old (WS) cells. Empty cells (E) are also visible. ×1400.

PLATE 2

A–D. Hyphal development and anastomosis.
- A. Spreading hyphae crossing junction lines (J) of epidermal cells. ×650.
- B. Hyphae running between stomata (S), without penetrating them. ×200.
- C. Anastomosis (An) between distant running hyphae. Hypocotyl bean hair (H) and hyphae with wrinkled and shrunken surface (WS) are visible. ×430.
- D. Anastomosis between closely running hyphae. ×4000.

E–G. Formation of infection cushions.
- E. Initiation of infection cushion. Typical short, branching hyphae are observed. ×220.
- F. Close-up of a short branching hyphae formed during the initiation of an infection cushion. ×3800.
- G. Branching of a running normal hyphae. Note the difference in morphology as compared to F. ×3400.

PLATE 3

- A. General view of an infection cushion: collapsed hyphae and cracked host surface (C) are clearly observed. ×300.
- B. Close-up of an infection cushion, showing typical cross anastomosis and several hyphal layers (arrows). ×1000.
- C. General view of an infective propagule (P), spreading hyphae, infection cushion, and an underlying concave lesion (L) with cracked (C) cuticle, 48 h after inoculation. ×55.
- D. A non-infective propagule (P) with a few emerging spreading hyphae, 48 h after inoculation. ×60.

Scanning Electron Microscopy of Infection by Rhizoctonia solani *Propagules*

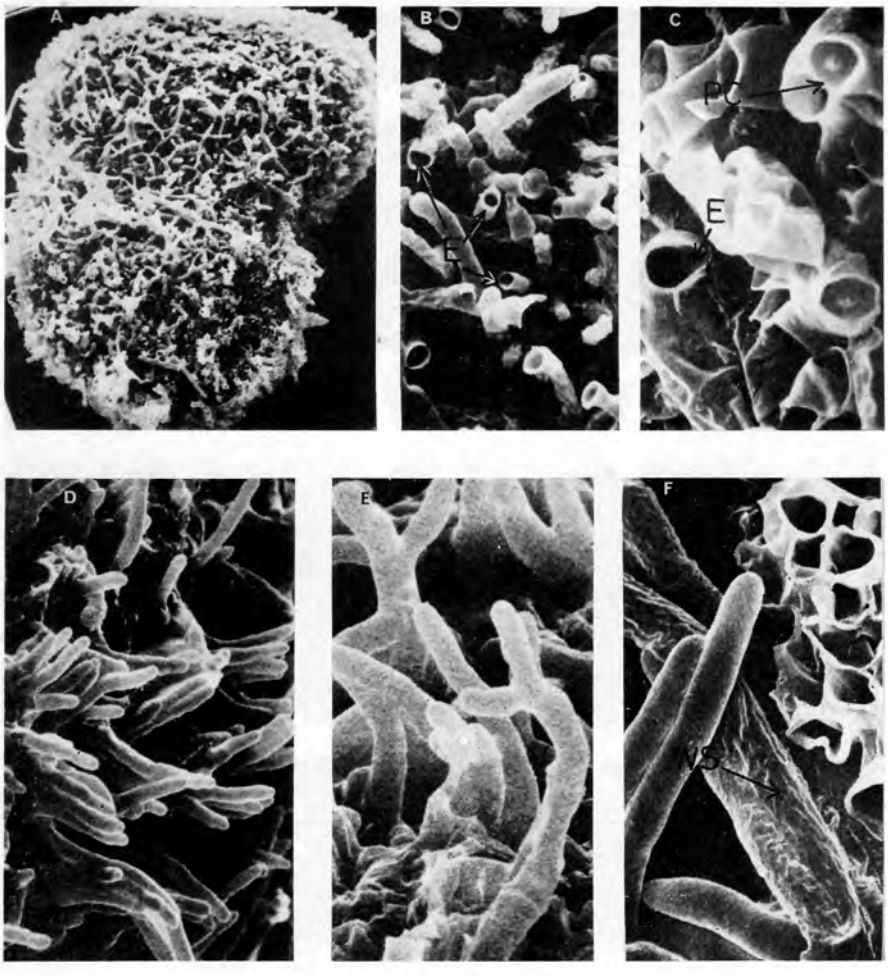

PLATE 1

(*facing p. 628*)

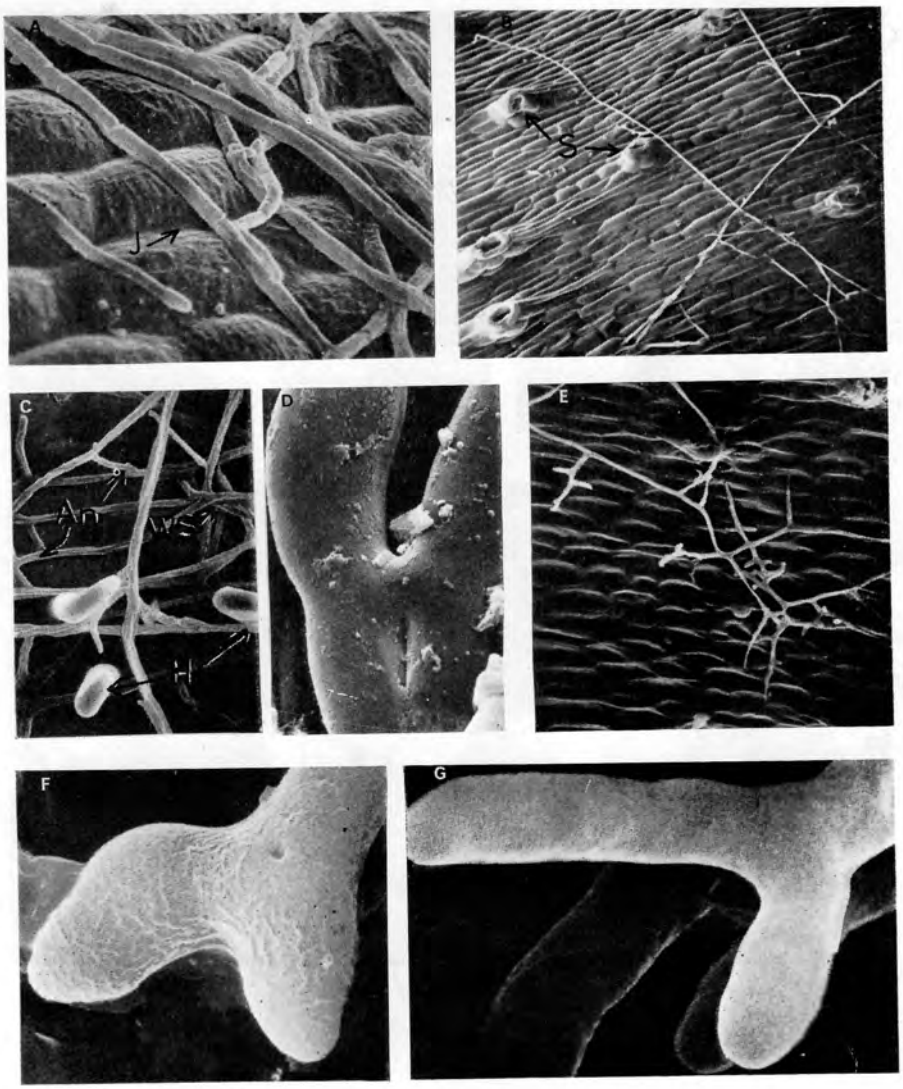

PLATE 2

Scanning Electron Microscopy of Infection by Rhizoctonia solani *Propagules*

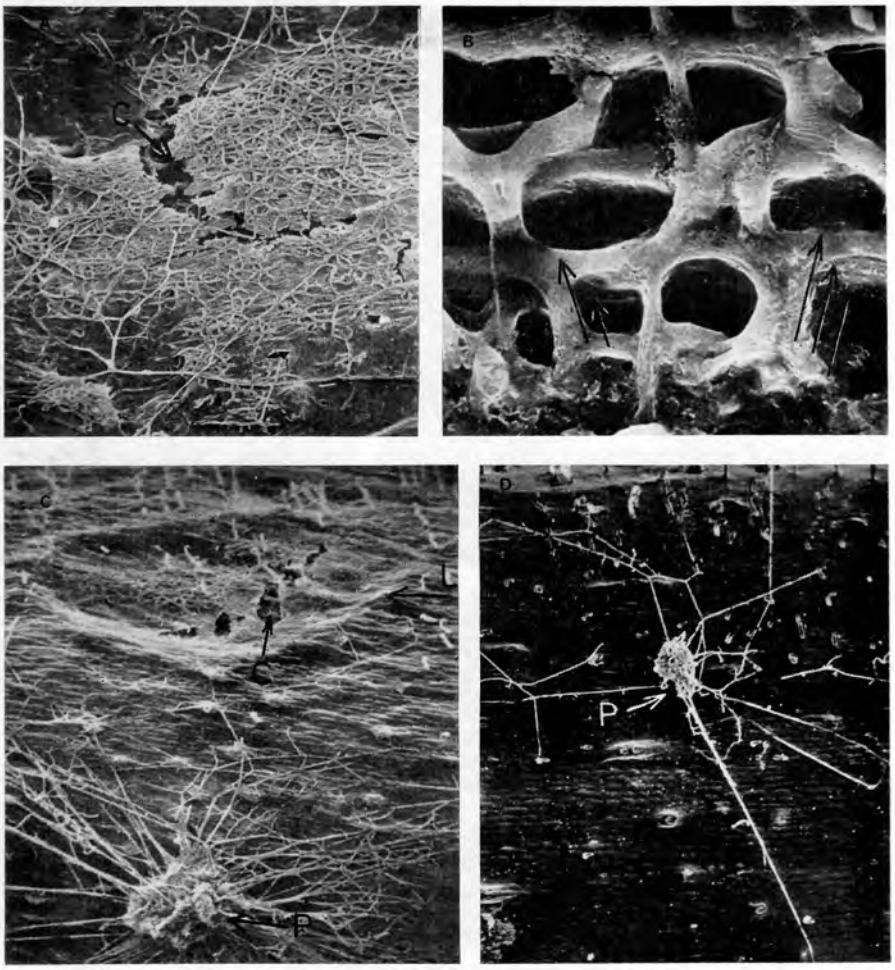

PLATE 3

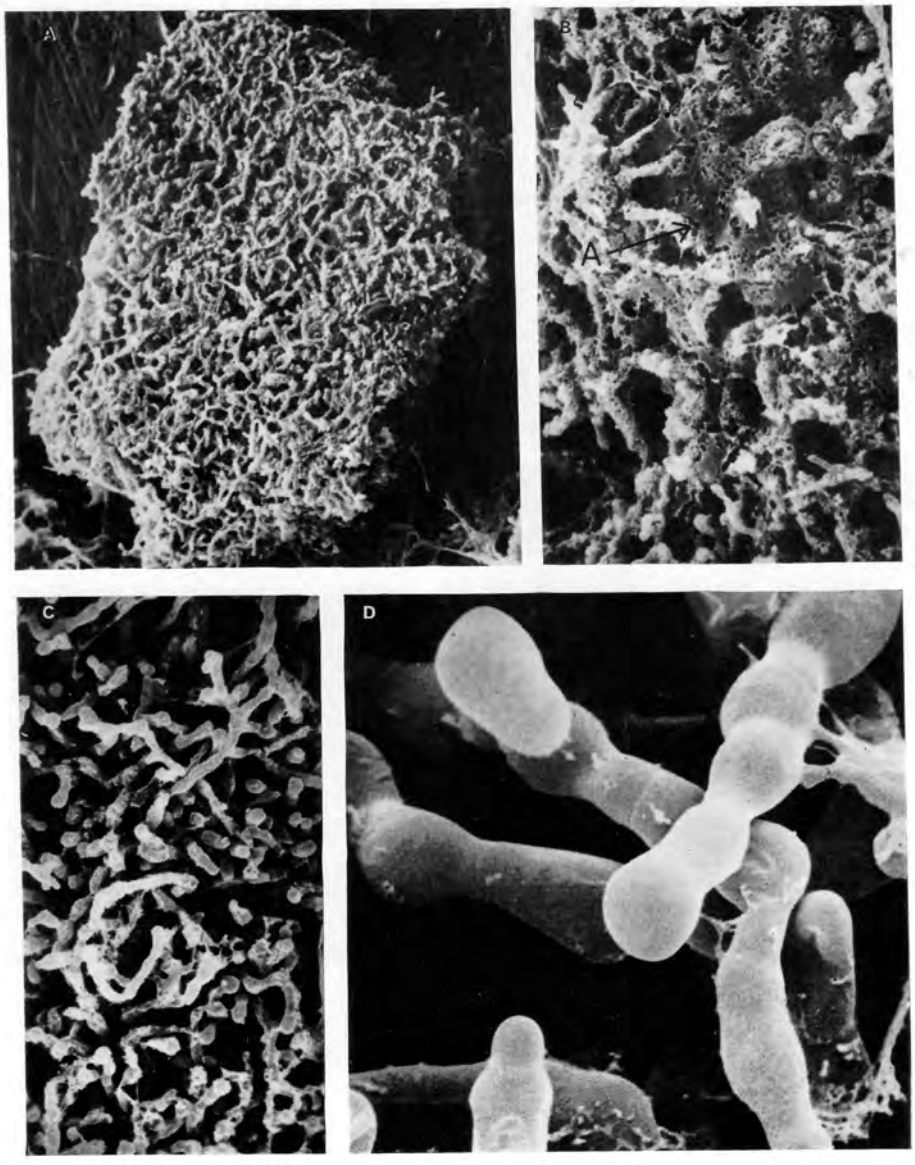

PLATE 4

PLATE 4

A–D. Sclerotium of *Rhizoctonia solani*.

A. General view of a sclerotium. ×120.

B. Surface of a sclerotium showing hyphae and an amorphous layer (A). ×380.

C. Surface of a sclerotium after removing the amorphous layer. ×400.

D. Typical barrel-shaped cells from the surface of the sclerotium. ×2400.