Infection Cushion Development by *Rhizoctonia solani* on Cotton

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ABSTRACT

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Infection cushions produced by *Rhizoctonia solani* AG-4 form on seedling cotton hypocotyls 21 hr after inoculation. Hyphae align in grooves between epidermal cells and produce lateral branches. These branches often terminate in a "foot," or T-shaped branch. Tips from these branches elongate to become hyphae parallel to the first. Accumulations of axial hyphae and lateral branches form the cushion. A mucilage-like material is observed, which presumably allows hyphae of the fungus to adhere to the plant surface and to each other. Many hyphal tips form on the underside of the cushion for penetration of the hypocotyl. These developmental steps and the architecture of the cushion that results from them should be considered in interpretation of experimental studies regarding cushion formation.

Additional key words: infection structure, mucilage.

Rhizoctonia solani Kühn (= *Thanatephorus cucumeris* (Frank) Donk) is a widely distributed fungal plant pathogen that is represented by several anastomosis groups with different cultural characteristics and host specificities (1,6,18). These different anastomosis groups (AG) and varied isolates of *R. solani* attack a wide range of susceptible plants by a variety of means (7,8). One is by elaboration of a complex infection structure, the infection cushion, as an aid to quick penetration and colonization of the plant. This intricate structure has been the subject of many studies. *R. solani* or other *Rhizoctonia* species have been subjected to a variety of treatments and "cushions" have formed as a result (10,11,19). Such studies were conducted to study the factors that stimulate morphogenesis of the infection cushion on the plant surface. There has been, however, little elucidation of the nature of the stimulants, although they have been said to be present in exudates from the plant (10,16,19).

One difficulty in interpreting such studies is a lack of definition of what constitutes an "infection cushion". An understanding of the steps in development of the infection cushion (for the particular isolate under study) is an important preliminary to any study of cushion formation by *R. solani* under experimental conditions. For such a study to be valid, the "cushion" as seen after experimental manipulation should be the result of the same developmental sequence as observed on the plant surface.

In this study, we describe the development of infection cushions on seedling cotton hypocotyls inoculated with *R. solani*. A second study (2) will examine factors on the plant surface that may influence cushion formation.

MATERIALS AND METHODS

R. solani AG-4, (ATCC 60734) was obtained as isolate no. 21 from A. R. Weinhold, University of California, Berkeley. The fungus was maintained at 25 C on potato-dextrose agar (PDA) (Difco Laboratories, Inc., Detroit, MI). For inoculum production, *R. solani* was grown in still culture on liquid Medium A (20) with 0.1 M glucose, as previously described (3). A cork borer was used to cut disks (4 mm in diameter) of 7-day-old mats from these cultures for use as inoculum.

Cotton, *Gossipium hirsutum* L., cultivar Acala 4-42, was used for scanning electron microscopy, whereas the cultivar SJ-2 was used for light microscopy. No differences were observed in the behavior of *R. solani* on these two cultivars. Cotton seeds (delinted and untreated with fungicide) were surface-sterilized for 10 min with household bleach (10%, v/v) and rinsed with deionized water. They were then planted in UC mix (4) and placed in a growth chamber at 28 C under fluorescent illumination (GE cool white fluorescent bulbs: 60 $\mu Em^{-2} sec^{-1}$). The seedlings were used 6–7 days after planting, at which time they had fully expanded, green

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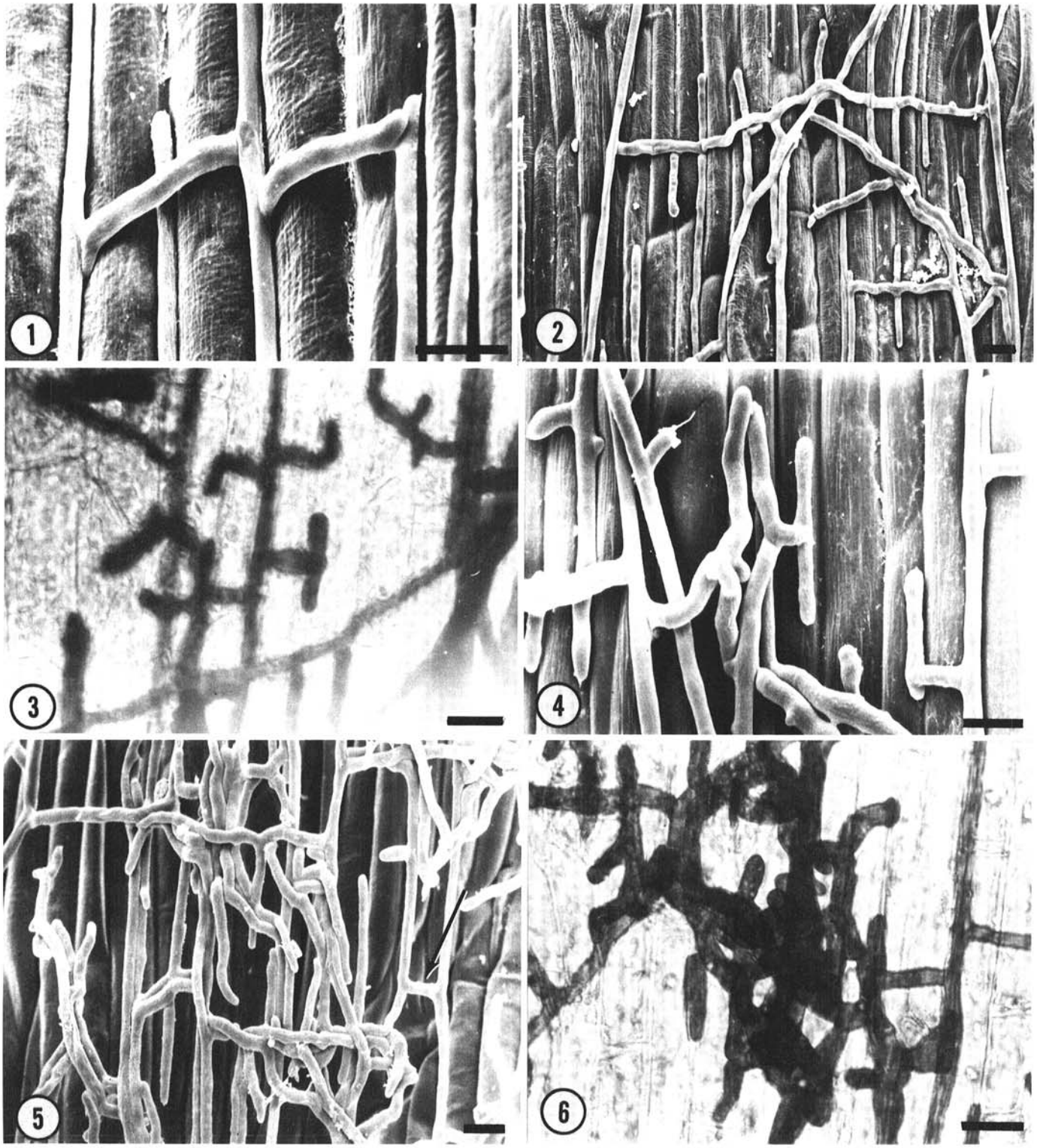
cotyledons.

Intact cotton seedlings were inoculated with *R. solani* for the scanning electron microscopy. The seedlings were rinsed with deionized water and transplanted into 6-oz styrofoam cups, where approximately 67 g of moist (deionized water) acid-washed silica sand supported the seedling so that the hypocotyl was exposed at the base. An inoculum disk of *R. solani* was placed adjacent to the base of the hypocotyl. The entire cup was covered with a plastic bag and placed in a growth chamber at 28 C, 95% relative humidity, and illuminated by fluorescent lights. The seedlings were collected at 15, 18, 21, or 24 hr after inoculation and prepared for scanning electron microscopy. The base of the hypocotyl was bathed with 2% glutaraldehyde in 0.05 M sodium cacodylate buffer, pH 7.4. A double-edged razor blade was used to cut the hypocotyl, with attached mycelium, free. Sections of the hypocotyl were fixed overnight at room temperature in glutaraldehyde, post-fixed with 2% osmium tetroxide in 0.05 M sodium cacodylate buffer for 2 hr, then dehydrated in an acetone series. After critical-point drying in an Omar SPC-1500 critical-point dryer, the specimens were coated with gold (150 Å) in a Hummer V sputter-coater and viewed with



Figs. 1–6. Early development of *Rhizoctonia solani* on the surface of cotton seedling hypocotyls, scanning electron micrographs (SEM) or light micrographs (LM); bar = 10 μm. **1,** Alignment of hyphae in grooves between epidermal cells (SEM). **2,** Perpendicular (to axis of plant) hypha with bidirectional branches (SEM). **3,** Internode shortening and foot formation by aligned hypha (LM). **4,** Feet formed by aligned hyphae (SEM). **5,** Axial hyphae with overlaid branches; arrow shows "H" formation, which could be mistaken for an anastomosis (SEM). **6,** Axial hyphae with accumulation of branches (LM).

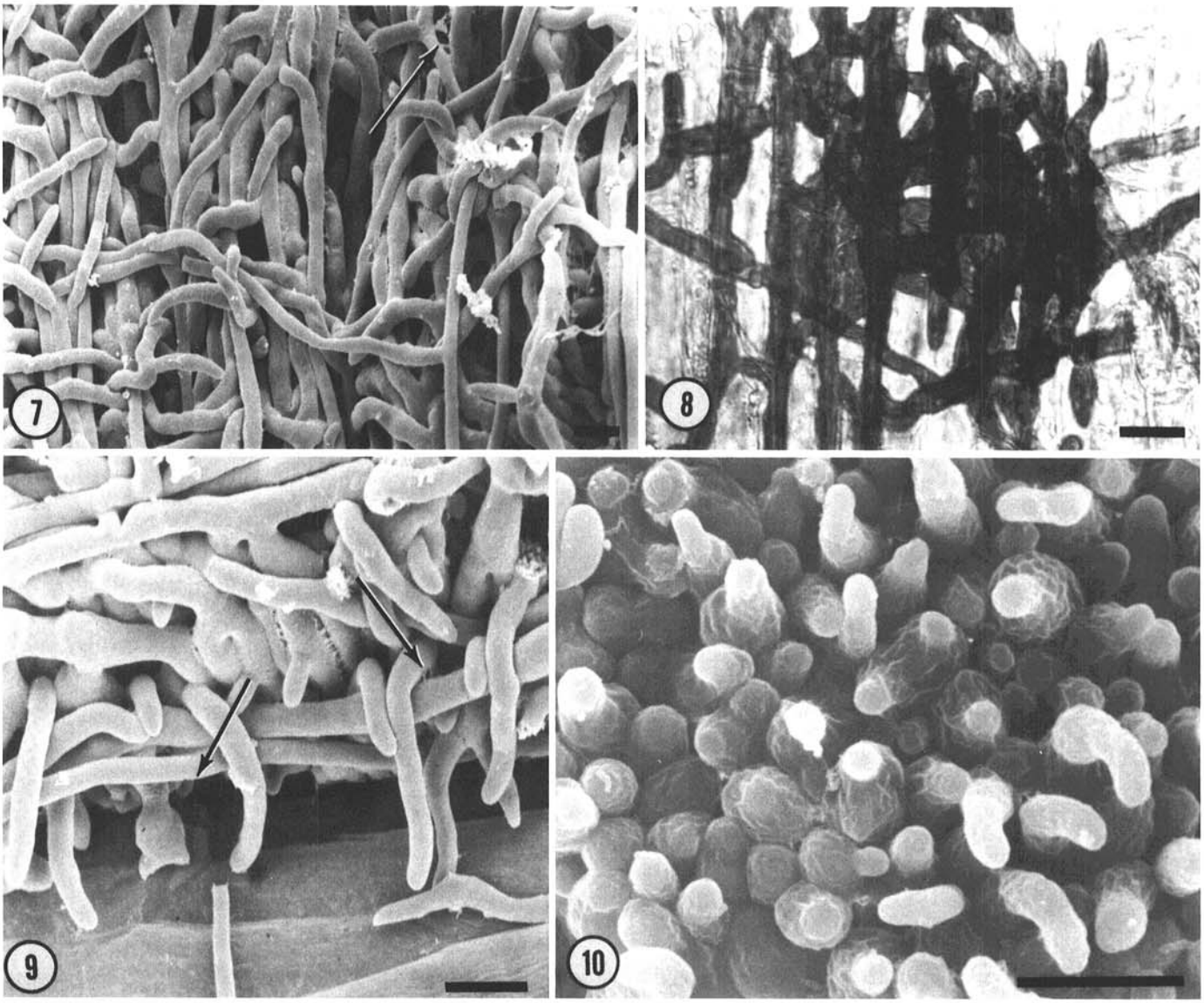
an AMR-1000 A scanning electron microscope.

Detached hypocotyls were inoculated with *R. solani* for light microscopy. The seedlings were grown as described, washed free of soil, and rinsed with deionized water. Segments (2–4 cm) were excised with a razor blade and the cut ends immediately dipped in paraffin. The hypocotyl segments were surface-sterilized for 3 min in 10% (v/v) bleach, washed in sterile deionized water, then aseptically placed in a sterile moist chamber (Whatman No. 1 filter paper in 14.5-cm petri plates) and inoculated with disks of *R. solani*. The chambers were sealed with Parafilm (American Can Co., Greenwich, CT.) and incubated at 25 C without illumination. After incubation, the hypocotyls were immersed in 0.1% (w/v) trypan blue in 50% (v/v) acetic acid for 10 min, then rinsed in distilled water to remove excess stain. A cut was made across the hypocotyl, then the epidermis, together with attached fungal mycelium, was lifted off with a pair of forceps. Epidermal peel preparations were photographed by transmitted light with a Zeiss Standard 14 compound microscope.

RESULTS

The general progress of infection cushion formation on cotton seedlings by *R. solani* was similar to that described by Weinhold and Motta (21). By 12 hr after inoculation, relatively unbranched hyphae of the fungus could be observed growing on the surface of the hypocotyl. Branching in limited areas of the hypocotyl surface was seen by 15 hr; by 18 hr this branching was more pronounced and there were dense accumulations of hyphae in some areas. The infection cushions were well-formed by 21 hr after inoculation, and were accompanied by a lesion, with discoloration and maceration of the hypocotyl, by 24 hr after inoculation. There was no difference in development of the infection cushions on intact cotton seedlings and excised hypocotyls.

Cushions of *R. solani* on cotton had a distinctive architecture as a result of the specialized pattern of growth and branching by the fungus on the hypocotyl surface. First, hyphae came into contact with the hypocotyl surface and appeared to adhere tightly to it, often aligned with the grooves formed by the anticlinal walls of epidermal cells (Figs. 1 and 2). They elongated rapidly in a direction parallel to the plant axis, then began to form closely spaced lateral branches. These branches sometimes extended to form a hypha that runs over the surface of the hypocotyl perpendicular to its axis (Fig. 2). Often, however, the branch tips grew only as far as the next groove between cells, where each tip formed a T-shaped branch, or "foot" (Figs. 3 and 4). The foot consisted of two new hyphal tips, each of which then elongated along the epidermal groove to form a new hypha parallel to the axis of the hypocotyl. Lateral branches from this hypha again formed



Figs. 7–10. Infection cushions of *Rhizoctonia solani* on the surface of cotton seedling hypocotyls, scanning electron micrographs (SEM) or light micrographs (LM); bar = 10 μm. **7,** Cushion surface (SEM); note accumulation of axial hyphae and branches; arrow points to mucilage-like material. **8,** Small cushion (LM); note orientation of hyphae with respect to the epidermal cells. **9,** Side view of cushion (21 hr after inoculation); arrows point to mucilage-like material adhering to hyphae (SEM). **10,** Underside of cushion, showing numerous hyphal tips from bulbous cells (SEM).

feet, which in turn originated new axial hyphae parallel to the parent hypha. Perpendicular running hyphae formed bidirectional branches similar to feet at numerous locations along their lengths; these also elongated to become hyphae parallel to the plant axis and one another (Fig. 2). Hyphal tips of lateral branches sometimes reached a groove or an axial hypha and turned to grow along the same axis. Repeated branching and foot formation led to a patterned accumulation of axial hyphae with lateral connections (Figs. 5 and 6), and finally to a cushion (Figs. 7 and 8). Hyphal tips now turned toward the surface of the hypocotyl, so that the plant surface was penetrated by a large number of tips (Figs. 9 and 10). Penetration hyphae were formed at the underside of the cushion from bulbous cells (Fig. 10). A mucilage-like material was sometimes observed between a hypha and the plant surface, or between hyphae (Fig. 9).

DISCUSSION

R. solani is a ubiquitous causal agent of many different plant diseases, and the infection structures that this fungus forms to penetrate susceptible plants have been the subject of many descriptive studies. As has been noted (7–9,17), different modes of penetration are employed, depending on the isolate, the AG, the plant species, and the plant part on which the infection structure is formed. Direct penetration by hyphae, especially through stomates, is rarely observed (8,17); more often, lobate appressoria are formed, in which an infection peg is produced beneath a swollen hyphal tip (8,9,15,17). However, the dome-shaped infection cushion is the infection structure that is considered "typical" of *R. solani*, and it is this structure that has received the most attention.

The infection cushion formed by *R. solani* is generally described as a dome-shaped aggregation of hyphae from which multiple infection pegs are produced for penetration of the plant surface. However, descriptions of the developmental steps in the morphogenesis of this complex structure vary a great deal. Long, leading hyphae often follow the lines of the anticlinal walls of epidermal cells (8,9,13,15,22), but not always (8,17). Side branches form; these are often short and stubby (8,13) and are sometimes shown as bilobed or with short dichotomous branches (8,14). Stubby side branches, in several accounts, curl back on themselves, with proliferation and aggregation of these branches leading to cushion formation (8,13,17,22). A cushion may originate from one or many hyphae (8,13,17). Sometimes it is said that cushions arise by anastomosis of hyphae (14,17,22). The infection cushions produced by all these various processes resemble one another, but are not identical in appearance. Dodman et al (8) remarked that infection cushions produced by one isolate on bean were compact and regularly oriented with the axis of the hypocotyl, but loose and irregular with regard to orientation on radish. Gladders and Coley-Smith (11) found that an isolate of *R. tuliparum* Whetzel & Arthur formed cushions with a radial pattern on tulip leaves, but implied that the cushions formed on narcissus were longer and more rectangular.

Our observations of infection cushion formation by *R. solani* on cotton differ somewhat from previous descriptions of infection cushion formation by *R. solani*. Alignment of hyphae with the anticlinal walls of epidermal cells was a consistent feature of cushion formation. These hyphae displayed shortened intervals between lateral branches (Fig. 3). Such internode shortening has not been remarked on in other studies. It has been measured quantitatively and shown to be an important change in the branching characteristics of the fungus when on the plant surface (2). Local proliferation of side branches did not appear to be important in production of hyphal aggregations. Instead, feet formed by short lateral branches and bidirectional branches from lateral running hyphae elongate to produce long axial hyphae, which in turn produce more lateral branches. This pattern of development results in an infection cushion with a well-defined and characteristic structure of interwoven hyphae (Figs. 7 and 8) rather than in an amorphous hyphal aggregation. Anastomoses of hyphae were not observed. However, the short bridge (Fig. 5) between hyphae that are derived from elongation of feet on short lateral branches could be mistaken for an anastomosis if the developmental steps leading to this configuration were not understood. The infection cushion is a specialized structure for rapid penetration of a plant surface. The numerous infection pegs (Figs. 9 and 10) presumably are able to push forcefully through the cuticle with the aid of the mechanical support of the hyphal mass behind them. This mechanical advantage would be enhanced by the presence of a mucilage, or extracellular adhesive substance, which would enable the fungus to hold fast to the cuticle. A mucilage-like material was observed in this study (Fig. 9) and has been described by other observers as well (9,12), though not consistently (15). *R. solani* produces cell-wall degrading enzymes, notably polygalacturonase (5), which presumably aids in penetration; pectic substances are lost from walls, and the tissue of the lesion beneath the infection cushion is finally macerated (12,21).

An attempt to draw generalizations with regard to infection cushion formation on various crops by the many AG and isolates of *R. solani* is a hazardous undertaking. These fungi may be expected to show a good deal of variation in their pathogenic mechanisms (1). Indeed, differences in infection cushion formation among them have been observed (7–9,17). Nevertheless, the complexity of the infection cushion and the wide distribution and importance of the fungus have been inducements to speculate on the mechanisms of cushion development (10,19,23). Any study of factors that govern the morphogenesis of the infection cushion should be based on a clear description of the development of the mature cushion in the isolate under study.

We have described the development of the infection cushion of *R. solani* on seedling cotton hypocotyls. In a second study (2), we will examine the contribution of aspects of the surface of the cotton hypocotyl, such as its topography and the presence of plant exudates, to infection cushion morphogenesis.

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