Scanning Electron Microscopy of the Infection Process of *Rhizoctonia solani* in Leaf Sheaths of Rice Plants

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ABSTRACT

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Rhizoctonia solani, the causal fungus of sheath blight of rice plants, penetrates plants through the inner surfaces of leaf sheaths. The infection process of an isolate of *R. solani* was studied on the inner surfaces of leaf sheaths of rice plants. Hyphae from the inoculum grew upward and produced numerous side branches at the site 5–6 mm from the growing tips of mycelia. The side branches continued to proliferate in a localized area and resulted in infection cushions, which were closely appressed to the epidermis with mucilagelike material. Penetration from infection cushions was effected by slender infection pegs that developed from flattened cells at the base of the cushion. Numerous penetration pegs and pores were observed beneath the cushions. Multiple invasion from the epidermal cell lumen was observed. At the penetration site on the epidermis, the peg became slender, but it swelled and resumed normal diameter when it reached the cell lumen. The edges of the penetration pores on the epidermis were smooth.

Rhizoctonia solani Kühn (perfect state: *Thanatephorus cucumeris* (Frank) Donk (= *Pellicularia filamentosa* (Pat.) Rogers) is one of the most polyphagus plant pathogenic fungi, having an extremely wide host range (1). Ullstrup (24) showed that certain isolates of *P. filamentosa* enter leaves of some plants only through stomata. Nakayama showed that direct penetration through the cuticle of epidermal cells of cotton occurs beneath appressoria consisting of clumps of hyphae termed infection cushions (22). Flentje showed that many strains of *P. filamentosa* invade hypocotyls of several host plants directly through the cuticle after infection cushion formation and not via stomata (8). The rice sheath blight fungus, *R. solani*, grows on the inner surface of leaf sheaths, forms infection cushions, and penetrates the epidermal cells directly or via stomata from infection cushions (17). Thus, the penetration process apparently varies among different plants and host organs, and different isolates of the fungus (2–12,15,17, 19,21,22,24–26). Although there are some studies on the fine structure of the infection process of the fungus (11,14,19,20), most of the above-mentioned studies have been performed with light microscopy, and the mechanism by which *R. solani* penetrates the host surface, especially the epidermis of rice leaf sheaths, is still poorly understood. This study by scanning electron microscopy (SEM) documents the infection process of *R. solani* on the inner epidermis of rice leaf sheaths.

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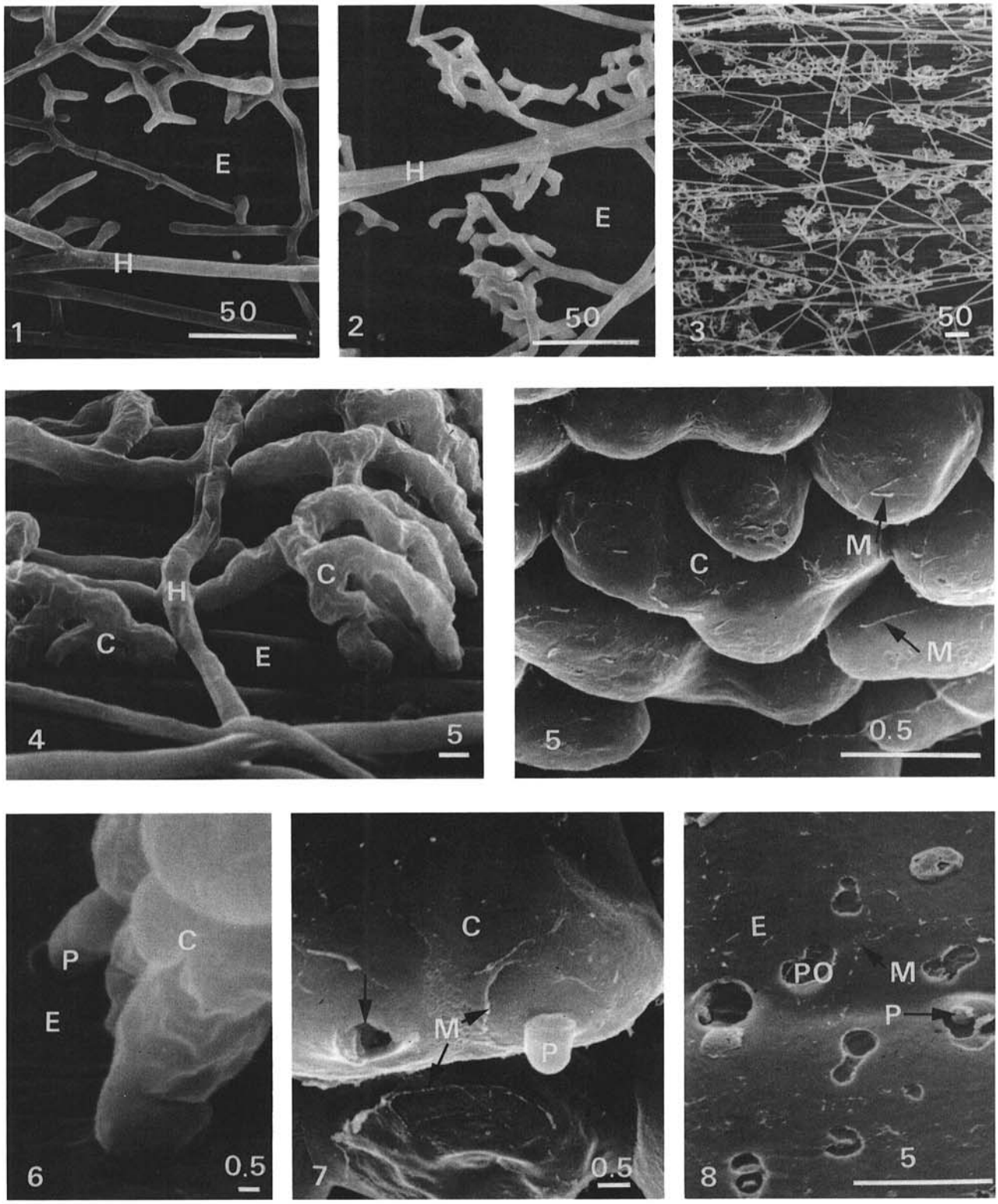
MATERIALS AND METHODS

Fungus, cultivation, and inoculation. *R. solani*, isolate TKF-44, anastomosis group (AG)-1 (23), was cultivated on Czapek agar medium for 2 days at 28 C. Detached leaf sheaths, about 7 cm long, were obtained from 7-wk-old rice plants, cultivar Nakate Shinsenbon, and thrust into Czapek agar medium in petri dishes near the advancing margins of colonies of *R. solani*. The dishes were kept in a moist chamber at 28 C for 12–48 hr.

Observation by SEM. The leaf sheaths were cut into pieces, 5 mm long, immersed in 2% glutaraldehyde for 3 hr, washed with deionized water, dehydrated by passage through a graded ethyl alcohol series, then rinsed in a graded ethyl alcohol-iso-amyl acetate series, and finally placed in 100% iso-amyl acetate. They were then dried in a critical-point dryer (Hitachi HCP-2) and coated with gold using an ion-coater (Eiko IB-3). Infection cushions were turned contact side up using a micromanipulator or adhesive cellophane tape to reveal penetration pegs and expose the tracks of infection pegs. To observe penetration hyphae in the lumena of epidermal cells, adhesive cellophane tape was pressed



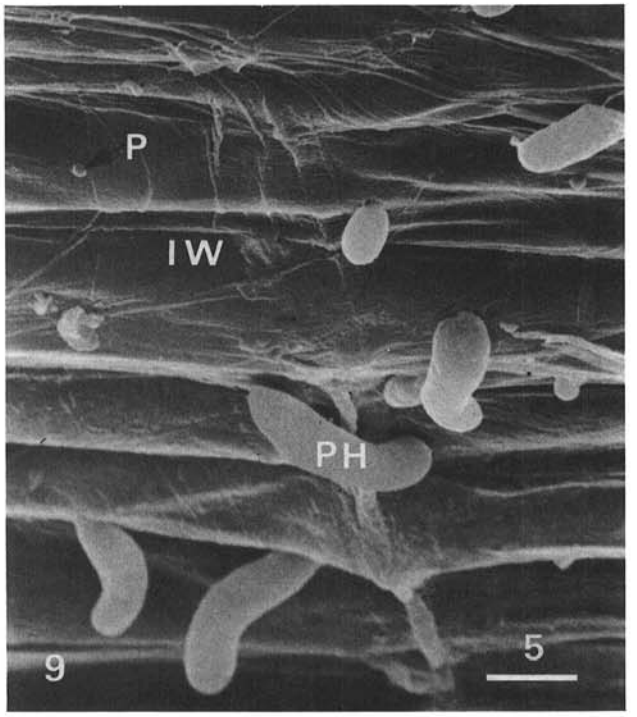
Figs. 1–8. Scanning electron micrographs of infection cushion formation and infection process of *Rhizoctonia solani* on epidermis of leaf sheaths of rice plants. Infection cushion formation of *R. solani* at: **1,** Formation of side branches at a site 3–4 mm from the growing tips of mycelia, **2,** An early stage of infection cushions at a site 5-6 mm from the growing tips, and **3,** A later stage of infection cushions at a site 10–12 mm from the growing tips of mycelia. **4,** Hyphae of infection cushions growing over epidermis of a leaf sheath along the sunken lines of the epidermal cells. **5,** Hyphal tips of infection cushions attached to the sheath epidermis. Mucilagelike material was observed around the hyphae. **6,** Penetration pegs extended from the basal part of infection cushions into epidermis of a leaf sheath. **7,** Development of fine penetration pegs from the flattened cells at the base of the infection cushion. An arrow indicates a broken penetration peg. **8,** Marks of the tips of penetration pegs and pores on the surface of a leaf sheath. Mucilagelike material was observed around infection pores. An arrow indicates a broken penetration peg. Abbreviations: C, infection cushion; E, epidermis of the inner surface of the rice sheath; H, hyphae; IW, inner wall of epidermal cell (lumen side); M, mucilagelike material; P, penetration peg; PH, penetration hypha; and PO, pore of penetration peg. Calibration bars are in micrometers.

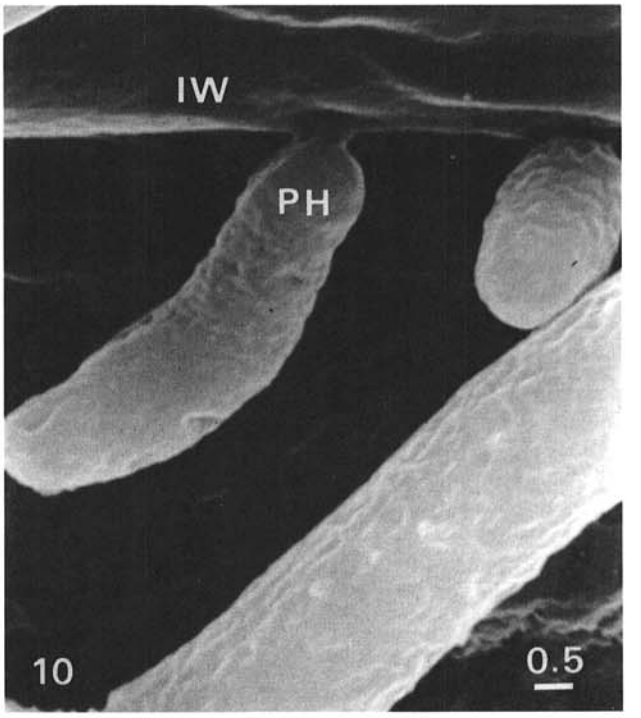
firmly onto the epidermis, peeled off, placed on stubs, and coated with about 20 nm of gold using the ion-coater. The specimens were observed with SEM (Hitachi S-430) at 20 kV accelerating voltage.

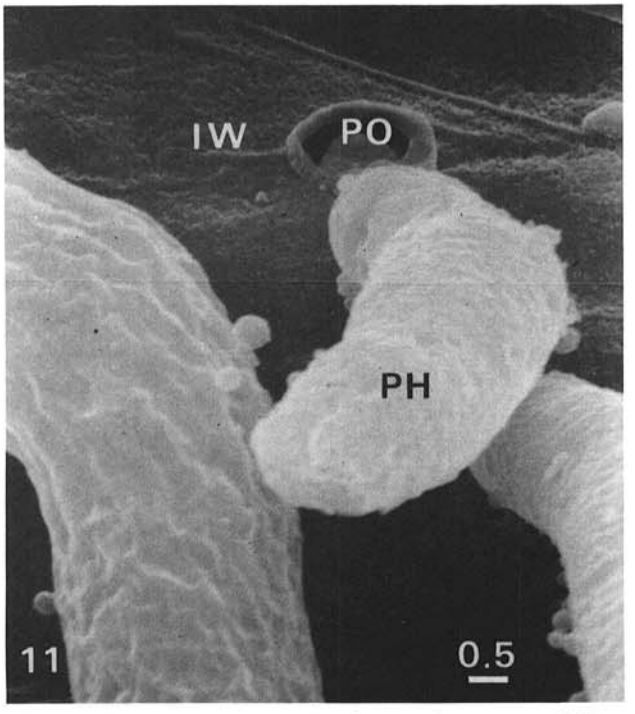
RESULTS

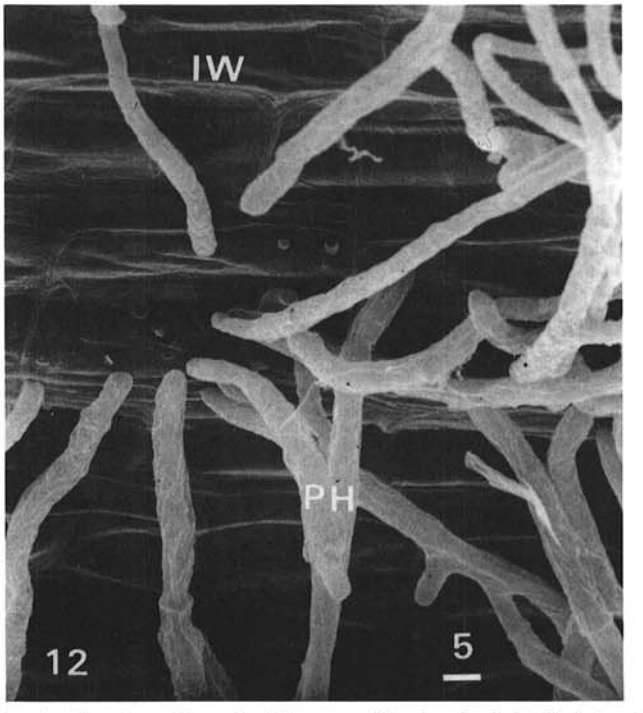
Development of infection cushions on the inner epidermis of rice leaf sheaths. Figures 1–4 present the process of infection cushion formation of *R. solani*. Mycelia grew from the inoculum on the surface of the inner epidermis of leaf sheaths at the rate of about 1.5 cm in 24 hr. Examination of epidermal strips of the leaf sheaths from inoculated plants revealed that hyphae appressed to the epidermis grew up on the inner surface of the leaf sheaths. After growing for some distance, they produced primary branches (Fig. 1). Repeated branching and development of resultant short hyphal cells in a localized area resulted in infection cushions (Figs. 2–4). The tips of the hyphae of infection cushions expanded the host surface and tended to orient along the sunken lines of epidermal cells (Fig. 4).

Overview of penetration site on the epidermis. Infection









Figs. 9–12. Scanning electron micrographs of penetration process of *Rhizoctonia solani* into the epidermal cell lumena of rice sheaths. **9,** Multiple invasion of penetration pegs into the epidermal cell lumen. Cell lumena were disclosed by pealing off the epidermal cell wall with cellophane adhesive tape. **10,** Infection hyphae in a cell lumen at the early stage of invasion. The penetration peg is slender at the site of invasion. **11,** Penetration pores on the inner side of the epidermal cell wall. **12,** Infection hyphae in a cell lumen. Infection hyphae formed branches a short distance from the intruded points. Abbreviations: IW, inner wall of epidermal cell (lumen side); P, penetration peg; PH, penetration hypha; and PO, pore of penetration peg. Calibration bars are in micrometers.

cushions are closely appressed to the epidermis of the leaf sheaths (Fig. 4), and it appeared that individual hyphae became attached to the epidermis by means of a mucilagelike material (Figs. 5, 7, 8). Infection cushions were easily lifted up with a manipulator and adhesive cellophane tape without destroying the epidermis (Fig. 8). It was most commonly observed that numerous fine penetration pegs developed from the flattened cells at the base of the infection cushions that were in contact with the epidermis (Fig. 7), and penetration was effected by these pegs (Figs. 6–8). Many penetration pores and pegs 1–2 μm in diameter were observed beneath the infection cushions after they were removed from the epidermis (Fig. 8). Penetration through stomata on the inner side of the sheath was infrequent.

Penetation from the epidermal cell lumen. From the undersurface of infection cushions multiple penetration pegs arose and invaded the epidermal cells. When penetration from the epidermal cell lumen was observed, multiple invasions were clearly revealed (Fig. 9). At the penetration site in the epidermal cell wall, the peg became slender, about 1–2 μm in diameter, but as soon as it reached the cell lumen it swelled and resumed normal diameter (3–4 μm), giving rise to so-called infection hyphae (Fig. 10). The edges of the pores from which penetration pegs appeared on the inner side of the epidermal wall were smooth (Fig. 11). The hyphae continued growing in the lumen of the epidermal cell, forming a branch a short distance from the point of penetration (Fig. 12).

DISCUSSION

The growth habit of *R. solani* hyphae observed along the anticlinal walls of epidermal cells of rice plants was similar to the observations reported by other workers (4,8,15,22). The tips of the hyphae of infection cushions tended to orient along the sunken lines of epidermal cell walls. This pattern of growth was observed on replicas of hypocotyl (7) but not on replicas of rice sheath surfaces (20). It is unknown whether the stimulus for infection cushions is chemical or physical.

The process of penetration into plants by *R. solani* has been reported by many workers. It now seems clear that penetration is commonly effected by means of infection cushions, which usually result from repeated branching and development of resultant short hyphal cells in a localized area. Infection cushions are usually closely appressed to the host surface. Flentje suggested that individual hyphae of infection cushions become attached to the host with mucilagenous materials (8). Fukutomi and Takada (11), using electron microscopy, reported that hyphae of infection cushions attached to the epidermis of cucumber hypocotyls by mucilagenous material, and Kenning and Hanchey (14) observed that hyphae within infection cushions were surrounded by a moderately dense material described as mucilagenous. However, Marshall and Rush (19) did not observe mucilagenous material around the tips of infection cushions on the sheath surface of rice plants. In the present study, the mucilagelike material was observed around the tips of hyphae of infection cushions and sheath surfaces. Because the infection cushions were moved and lifted up without destroying the epidermis of leaf sheaths, attachment of infection cushions seemed to be weak. Weak attachment of infection cushions to the surface of tomato fruits was reported by Gonzalez and Owens (12).

It has not been established whether penetration pegs from infection cushions penetrate primarily by mechanical pressure (4,8,15,22), by enzymatic destruction of host constituents (2,13,14,16,18,21), or both. It is not certain whether the smooth edges of penetration pores on the epidermis and on cell lumena of rice sheaths resulted from mechanical force or enzymatic means. Further investigation of these aspects is needed.

LITERATURE CITED

1. Baker, K. F. 1970. Types of *Rhizoctonia* diseases and their occurrence. Pages 125-148 in: *Rhizoctonia solani*: Biology and Pathology. J. R. Parmeter, Jr., ed. University of California Press, Berkeley. 255 pp.
2. Bateman, D. F. 1970. Pathogenesis and disease. Pages 161-171 in: *Rhizoctonia solani*: Biology and Pathology. J. R. Parmeter, Jr., ed. University of California Press, Berkeley. 255 pp.
3. Boosalis, M. G. 1950. Studies on the parasitism of *Rhizoctonia solani* Kühn on soybeans. Phytopathology 40:820-831.
4. Christou, T. 1962. Penetration and host parasite relationships of *Rhizoctonia solani* in the bean plant. Phytopathology 52:381-389.
5. Chi, C. C., and Childers, W. R. 1963. Penetration and infection in alfalfa and red clover by *Pellicularia filamentosa*. Phytopathology 54:750-754.
6. Dodman, R. L., and Flentje, N. T. 1970. The mechanism and physiology of plant penetration by *Rhizoctonia solani*. Pages 149-160 in: *Rhizoctonia solani*: Biology and Pathology. J. R. Parmeter, Jr., ed. University of California Press, Berkeley. 255 pp.
7. Downer, A. J., and Armentrout, V. N. 1983. The effect of host exudates on infection cushion morphogenesis of *Rhizoctonia solani*. (Abstr.) Phytopathology 73:958.
8. Flentje, N. T. 1957. Studies on *Pellicularia filamentosa* (Pat.) Rogers. III. Host penetration and resistance, and strain specialization. Trans. Br. Mycol. Soc. 40:322-326.
9. Flentje, N. T. 1959. The physiology of penetration and infection. Pages 76-87 in: Plant Pathology: Problems and Progress, 1908-1958. C. S. Holton, G. W. Fischer, R. W. Fulton, H. Hart, and S. E. A. McCallan, eds. The University of Wisconsin Press, Madison. 588 pp.
10. Flentje, N. T., Dodman, R. L., and Kerr, A. 1963. The mechanism of host penetration by *Thanatephorus cucumeris*. Austr. J. Biol. Sci. 16:784-799.
11. Fukutomi, M., and Takada, H. 1979. Ultrastructure of infection process of *Rhizoctonia solani* Kühn in cucumber hypocotyls. Ann. Phytopathol. Soc. Jpn. 45:453-462.
12. Gonzalez, L. C., and Owen, J. H. 1962. Soil rot of tomato caused by *Rhizoctonia solani*. Phytopathology 53:82-85.
13. Isaac, P. K. 1964. Metabolic specialization in the mycelium of *Rhizoctonia solani* Kühn. Can. J. Microbiol. 10:621-623.
14. Kenning, L. A., and Hanchey, P. 1980. Ultrastructure of lesion formation in *Rhizoctonia*-infected bean hypocotyls. Phytopathology 70:998-1004.
15. Khadga, B. B., Sinclair, J. B., and Exner, B. B. 1963. Infection of seedling cotton hypocotyl by an isolate of *Rhizoctonia solani*. Phytopathology 53:1331-1336.
16. Köller, W., Allan, C. R., and Kolattukudy, P. E. 1982. Role of cutinase and cell wall degrading enzymes in infection of *Pisum sativum* by *Fusarium solani* f. sp. *pisi*. Physiol. Plant Pathol. 20:47-60.
17. Kozaka, T. 1961. Ecological studies on sheath blight of rice plant caused by *Pellicularia sasakii* (Shirai) S. Ito, and its chemical control. Bull. Chugoku Agric. Res. 20:1-133.
18. Linskens, H. F., and P. Haage. 1963. Cutinase-Nachweis in Phytopathogen Pilzen. Phytopath. Z. 48:306-311.
19. Marshall, D. S., and Rush, M. C. 1980. Relation between infection by *Rhizoctonia solani* and *R. oryzae* and disease severity in rice. Phytopathology 70:941-946.
20. Marshall, D. S., and Rush, M. C. 1980. Infection cushion formation on rice sheaths by *Rhizoctonia solani*. Phytopathology 70:947-950.
21. Matsumoto, T. 1921. Studies in the physiology of the fungi. XII. Physiology specialization in *Rhizoctonia solani* Kühn. Ann. Mo. Bot. Gard. 8:1-62.
22. Nakayama, T. 1940. A study of the infection of cotton seedlings by *Rhizoctonia solani*. Ann. Phytopathol. Soc. Jpn. 10:93-103.
23. Ogoshi, A. 1972. Grouping of *Rhizoctonia solani* Kühn with hyphal anastomosis. Ann. Phytopathol. Soc. Jpn. 38:117-122.
24. Ullstrup, A. J. 1963. Leaf blight of China aster caused by *Rhizoctonia solani*. Phytopathology 26:981-990.
25. Van Etten, H. D., Maxwell, D. P., and Bateman, D. F. 1967 Lesion maturation, fungal development, and distribution of endo-polygalacturonase and cellulase in *Rhizoctonia*-infected bean hypocotyl tissues. Phytopathology 57:121-126.
26. Weinhold, A. R., and Motta, J. 1972. Initial host responses in cotton to infection by *Rhizoctonia solani*. Phytopathology 63:157-162.