Histopathology of Resistant and Susceptible Tomato Fruit Infected with *Rhizoctonia solani*

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ABSTRACT

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Histopathological and anatomical differences were detected in the carpel walls of tomato fruit of PI 193407, a plant introduction reported to be resistant to soil rot caused by *Rhizoctonia solani*, and Campbell-28 (C-28), a susceptible cultivar. Cells of the epidermal layer, subepidermal layer, and underlying parenchyma were larger and more consistent in size and shape in C-28 than in PI 193407. *R. solani* penetrated C-28 fruit by means of numerous infection pegs emanating from infection cushions, whereas in PI 193407 penetration was by individual hyphae. The establishment of *R. solani* in both hosts involved inter- and intracellular growth and the subsequent destruction of epidermal and subepidermal layers of cells. However, advancement of the pathogen in all tissues was slower in the resistant than in the susceptible host. Infected resistant parenchyma tissues stained more intensely with safranin O than did those of the susceptible host. This suggested that a chemical substance(s) may be responsible for "walling-off" the organism or slowing lesion development in the resistant host. In addition, the cell walls in PI 193407 dissolved in advance of hyphae of the pathogen, which resulted in microcavities in the carpel wall.

Tomato (*Lycopersicon esculentum* Mill.) fruit rots caused by soilborne organisms are so prevalent in the southern USA that no stable production of nonstaked processing tomatoes has been possible on a commercial scale. *Rhizoctonia solani* Kühn is the single most important cause of this problem (3,10). Conventional spray programs have not provided economic control. Resistant cultivars are a possible solution to the problem.

Differences in susceptibility to *R. solani* have been noted among tomato cultivars and plant introductions. Barksdale (2) suggested that resistance in tomato to *R. solani* was controlled by several genes. Johnson (9) used F_1, BC_1, and F_2 fruit from crosses between susceptible and resistant tomatoes and determined that PI 193407 was partially resistant and that resistance was expressed as a delay in infection and slow lesion development. He also showed that resistance was heritable through the F_3 generation and was controlled by a few genes, or possibly one gene with modifiers. The mechanism(s) responsible for this resistance have not been determined.

In the present study to determine the mechanism(s) controlling resistance in tomato fruit to *R. solani*, the host-parasite relationship was examined histologically in a susceptible cultivar and a resistant plant introduction line.

MATERIALS AND METHODS

Fruits of Campbell-28 (C-28), a tomato cultivar susceptible to soil rot caused by *R. solani*, and PI 193407, a plant introduction with partial resistance, were inoculated with *R. solani* (isolate R-23) and compared histologically. Inoculum was prepared by blending a 7-day-old potato-dextrose agar culture of R-23 with 50 ml of water in a Waring Blendor. Twenty-five milliliters of this suspension was placed on 300 cm^3 of a perlite/cornmeal/Czapek's medium (10:1:2) in a 75 × 100-mm storage dish and incubated at 25 C for 14 days. One and one-half grams of this inoculum were incorporated near the surface of steam sterilized Marietta clay loam soil in standard greenhouse flats (33 × 45 cm) and placed in temperature control chambers. The chambers were programmed for 8-hr each at 21 C and 32 C separated by a 4-hr transition period within each 24-hr period (9). The soil was kept wet and the relative humidity high by frequent waterings.

Seeds of the two lines were planted directly in the field during June 1975. C-28 was obtained from a commercial source and PI 193407 was furnished by the North Central Plant Introduction Center, Ames, Iowa. Mature green tomato fruit were randomly selected from the field, surface sterilized with 0.5% sodium hypochlorite, and placed on the artificially-infested soil. The length of the incubation period varied for the two cultivars. Due to slower lesion development on PI 193407 (9), selection of specimens for the sectioning process was conducted over a period of several days.

Carpel-wall samples (1.5 × 0.5 cm) of diseased and healthy fruit were placed in a killing-fixing solution (formalin/acetic acid/alcohol) and aspirated 10 min. The samples were embedded in paraffin and 15-μm-thick sections were cut along transverse and longitudinal planes (8). Sections were stained with a safranin/aniline blue/Delafield's hematoxylin combination as described by Popham et al (14). Morphological characteristics of the carpel wall were compared relative to the degree of infection on C-28 and PI 193407. Photomicrographs were made from slides of mounted sections of healthy fruit, fruit at varying times following exposure to *R. solani*, and fruit with various stages of lesion development.

Samples of both cultivars were studied with a Hitachi HHS-2R scanning electron microscope to determine modes of penetration. Epidermal tissue, 8 mm in diameter, was fixed in 4% glutaraldehyde in Millonig's phosphate buffer at room temperature, pH 7.2, for 1 hr. The tissue was dehydrated in an ascending series of concentrations to absolute ethanol, then passed through an ethanol-amyl acetate series (30, 50, 70, 90, 100, 100%: 30 min each) and dried by the critical point method (1). The dried tissue was mounted on stubs and coated with gold palladium over carbon in a vacuum evaporator.

RESULTS

Epidermal cells of C-28 fruit were larger and more uniform in size and shape than those of PI 193407 and subepidermal cells of the latter were smaller and more densely packed than those of C-28. Cells of underlying parenchymatous tissue of C-28 were larger

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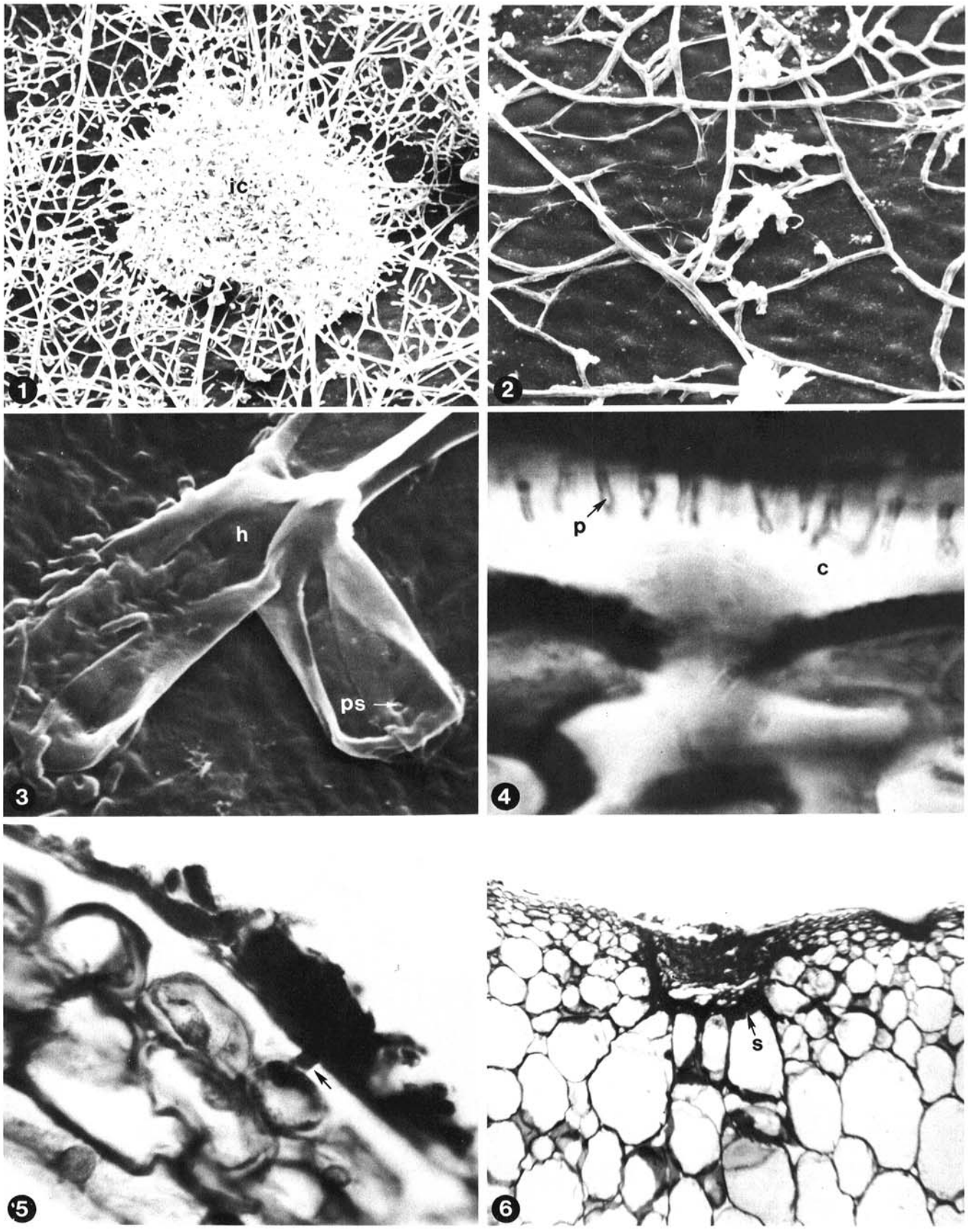

Fig. 1-6. Infection of tomato fruit by *Rhizoctonia solani*. **1**) Infection cushion (ic) of *R. solani* on surface of C-28 (susceptible cultivar) fruit (×85). **2**) Hyphae of *R. Solani* on surface of PI 193407 (resistant cultivar) fruit (×875). **3**) Hypha (h) of *R. solani* on fruit surface of PI 193407 showing possible site (ps) of penetration (×8500). **4**) Infection pegs (p) of *R. solani* penetrating cuticle (c) of C-28 (×1000). **5**) An individual hypha of *R. solani* (arrow) penetrating cuticle and epidermal cell of PI 193407 (×500). **6**) Transverse section of PI 193407 fruit through soil rot lesion revealing a greater concentration of safranin O (s) in infected tissue and *R. solani* hyphae occluded by the host (×100).

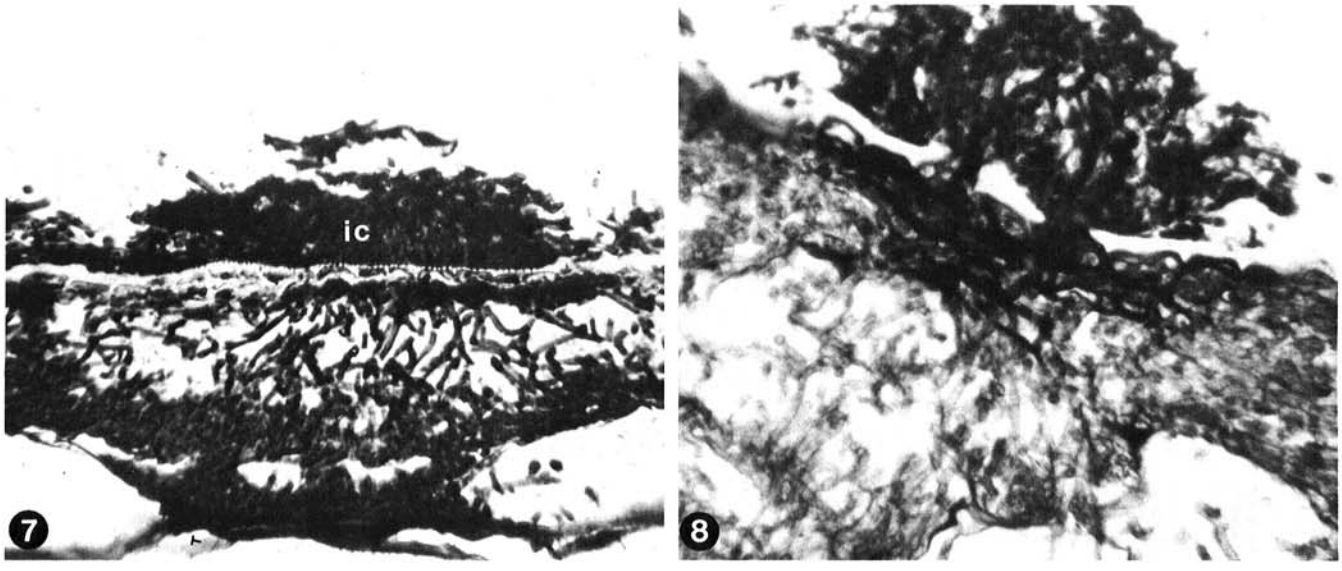

Fig. 7-8. Soil rot lesions caused by *Rhizoctonia solani* in tomato fruits. **7)** Transverse section of cultivar C-28 fruit through a soil rot lesion showing infection cushion (ic), infection pegs, and development of hyphae in underlying cells (×100). **8)** Longitudinal section through an advanced soil rot lesion of a PI 193407 fruit showing penetration by several hyphae and eventual destruction of host tissue (×300).

than those of PI 193407. The carpel wall of PI 193407 was much thinner than that of C-28.

Lesions on C-28 fruit were 2–5 mm in diameter within 24–48 hrs after exposure to *R. solani* and progressively enlarged with time; those on PI 193407 fruit reached 2 mm in diameter only after exposure to *R. solani* for 48–72 hr and usually enlarged slightly or not at all. Therefore, it was necessary to incubate material of PI 193407 longer to obtain lesions comparable in size to those on C-28. In some cases, lesions did not advance beyond a "fleck reaction" on the resistant host.

Scanning electron microscopy revealed extensive mycelial growth and probable infection cushion formation (Fig. 1) on the surface of samples taken from C-28 fruit which had been in contact with infested soil for 24–48 hr. Hyphal development on the surface of PI 193407 fruit (Fig. 2) was sparse compared to that on C-28, and extensive searching revealed only one probable penetration site at the end of a hypha (Fig. 3). Light microscopy revealed that penetration in C-28 was by multiple infection pegs (Fig. 4) which formed under infection cushions, whereas penetration in PI 193407 was by individual hyphae (Fig. 5).

Subsequent fungal growth into the epidermal and subepidermal layers occurred intercellularly in both hosts and was accompanied by an accumulation of safranin O stain. The greater stain concentration was especially apparent outside the cell walls and the substance with affinity for safranin O stain appeared to arise from the subepidermal region near the outer wall of the underlying parenchymal cells (Fig. 6). A greater concentration of stain accumulated in the resistant host and this heavily stained material appeared to wall-off or restrict growth of *R. solani* (Fig. 6). No successful walling-off was observed in C-28 and inter- and intracellular mycelial development was extensive (Fig. 7).

Although *R. solani* was walled-off temporarily in PI 193407, eventual destruction of some fruit was caused by hyphae growing intercellularly and intracellularly through the fruit tissue (Fig. 8).

Dissolution of host tissue occurred in advance of penetrating hyphae in both hosts. However, less cell wall dissolution occurred in the susceptible host. A cavity often developed between the fungus and the apparently uninvaded area wherever infection advanced beyond the subepidermal layer in the resistant host.

DISCUSSION

The resistance of PI 193407 was characterized by a delay in infection and slow lesion growth and is in agreement with the results of Johnson (9). Histological examination of carpel wall segments revealed that susceptible and resistant fruit differed in the size and configuration of epidermal and subepidermal cells. The densely packed epidermal cells of the resistant fruit may be a barrier that makes penetration relatively more difficult. A thickened cuticle, although not consistently apparent in this study, has been suggested as a possible barrier in other host-parasite associations (11,12,13).

The increased amount of mycelia on the surface of C-28 fruit during the prepenetration phase seems to indicate a stimulation of *R. solani* growth on the susceptible fruit. Whether this preference was caused by surface or exudate phenomena was not determined in this study.

Histological studies indicated a difference in the mode of penetration of susceptible and resistant fruit. There is evidence that infection of susceptible fruit by *R. solani* is associated with the development of dome-shaped infection cushions or lobate appressoria prior to penetration of the host (4,6,7). The failure of *R. solani* to form infection cushions on resistant fruit may be an important factor in the resistance of PI 193407 to Rhizoctonia soil rot. The absence of direct penetration by infection cushions and infection pegs, although not fully understood, has been speculated as a possible means of host resistance (5).

Although penetration of resistant fruit was achieved by individual hyphae, a walling-off occurred in the subepidermal region and *R. solani* was slowed for a period or stopped entirely.

The resistance of PI 193407 appears to be a composite of phenomena active before and after penetration and at sites on the fruit surface and within the tissue. More detailed studies are presently underway to ascertain the effects of surface and exudate characteristics on the resistance of PI 193407 to *R. solani*.

LITERATURE CITED

1. ANDERSON, T. F. 1951. Techniques for the preservation of three-dimensional structure in preparing specimens for the electron microscope. N. Y. Acad. Sci. 13:130-134.
2. BARKSDALE, T. H. 1974. Evaluation of tomato fruit for resistance to Rhizoctonia soil rot. Plant Dis. Rep. 58:406-408.
3. BATSON, W. E., Jr. 1973. Characterization and control of tomato fruit rots in Mississippi. (Abstr.) Phytopathology 63:440.
4. DODMAN, R. L., K. R. BARKER, and J. C. WALKER. 1968. A detailed study of the different modes of penetration by Rhizoctonia solani. Phytopathology 58:1271-1276.
5. DODMAN, R. L., and N. T. FLENTJE. 1970. The mechanism and physiology of plant penetration by Rhizoctonia solani. Pages 149-160

in: Parmeter, J. R., ed. Rhizoctonia solani, Biology and Pathology. University of California Press, Berkeley. 255 pp.
6. FLENTJE, N. T. 1957. Studies on Pellicularia filamentosa (Pat.) Rogers. III. Host penetration and resistance, and strain specialization. Trans. Br. Mycol. Soc. 40:322-336.
7. GONZALEZ, L. C., and J. H. OWEN. 1963. Soil rot of tomato caused by Rhizoctonia solani. Phytopathology 53:82-85.
8. JOHANSEN, D. A. 1940. Plant Microtechnique. McGraw-Hill Book Company, New York and London. Pages 64, 126-154. 523 pp.
9. JOHNSON, C. E. 1975. Inheritance of resistance to soil rot caused by Rhizoctonia solani Kuehn in tomato (Lycopersicon esculentum Mill.) MS Thesis, Mississippi State University, Mississippi State, MS. 28 pp.
10. JONES, C. W., and S. M. McCARTER. 1974. Etiology of tomato fruit rots and evaluation of cultural and chemical treatments for their control. Phytopathology 64:1204-1208.
11. LOUIS, D. 1963. Les modalités de la penetration du Botrytis cinera Pers. dans les plantes. Annales Epiphyt. 14:57-72.
12. NUTMAN, F. J., and F. M. ROBERTS. 1960. Investigations on a disease of Coffea arabica caused by a form of Colletotrichum coffeanum. I. Some factors affecting infection by the pathogen. Trans. Br. Mycol. Soc. 43:489-505.
13. PERIES, O. S. 1962. Studies on strawberry mildew, caused by Sphaerotheca macularis (Walls, ex. Fries) Jaczewski. II. Host parasite relationships on foliage of strawberry varieties. Ann. Appl. Biol. 50:225-233.
14. POPHAM, R. A., T. J. JOHNSON, and A. P. CHAN. 1948. Safranin and anilin blue with Delafield's hematoxylin for staining cell walls in shoot apexes. Stain Technol. 23:185-190.