Histopathology of Resistant and Susceptible Sugar Beet Roots Inoculated with Rhizoctonia solani

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ABSTRACT

Resistant and susceptible sugar beet cultivars were penetrated by *Rhizoctonia solani* directly by individual hyphae or by infection cushions. Lesion diameter and depth were greater in susceptible roots than in resistant roots. Necrosis and some tissue degeneration preceded hyphal advance in all roots after penetration. Hyphae in resistant roots usually were observed only in the periderm or outer secondary cortex, whereas in susceptible roots the hyphae often transected several vascular rings. Hyperplasia of cortical cells occurred at the margin of necrotic and healthy tissue, but no wound periderm or cicatricelike cell layers were evident in resistant roots. Resistance in sugar beet to *R. solani* was not found to be due to mechanical barriers to the pathogen.

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Root rot of sugar beet (*Beta vulgaris* L.) incited by *Rhizoctonia solani* Kuehn (*Thanatephorus cucumeris* [Frank] Donk) is a serious disease in many sugar beet-growing areas of the USA. Symptoms of the disease, described by Pammel (14) and Richards (19), consist of a brown, relatively dry, spongy decay with a distinct margin between healthy and diseased tissue. Unequal root growth frequently causes open cankers or splits through rotted areas. The fungus can penetrate and infect all portions of the crown and root.

The anatomy of a sugar beet root described by Artschwager (2) is somewhat unique. Secondary growth in the taproot consists of secondary vascular tissue alternating with bands of parenchyma (each called a "secondary cortex"). Growth is centrifugal, so the youngest rings of vascular tissue are toward the outside. When the seedling has about five pairs of leaves, the primary cortex of the root begins to slough off, and cells of the pericycle become meristematic forming the phellogen or cork cambium. Reciprocal division of the phellogen produces cork cells to the outside and phelloderm (cork parenchyma) cells to the inside. The cork cells, phellogen, and phelloderm comprise the periderm. The above terminology is used in this study.

In 1966, Gaskill (9) released two sugar beet cultivars with substantial resistance to *R. solani*. The availability of cultivars with varied degrees of resistance prompted the present study to (i) determine whether differences in susceptibility could be explained through histological examination of roots; and (ii) to provide a basis for investigating the nature of resistance to *R. solani*.

MATERIALS AND METHODS.—Susceptible sugar beet cultivars GW 674-56C and C 817, and resistant cultivars FC 701/2 and FC 702/2, were grown individually in 15-cm-diam pots of steam-treated soil. Cultivar FC 701/2 is a selection from GW 674-56C, whereas FC 702/2 was selected from C 817 (9). When the plants were 10 weeks old, soil was carefully removed from one side of the taproots. A 3-mm^2 piece of mycelium-agar from a 3-week-old culture of *R. solani* (isolate RR-9) growing on potato-dextrose agar was placed against each taproot about 2 cm below the soil surface and several cm above any secondary roots. The inoculum was placed on a smooth area of the root between the vertical, lateral-root grooves. The soil was replaced and the pots were irrigated immediately. Irrigation thereafter

was done as needed. Noninoculated controls of each cultivar were included. The plants were kept in the greenhouse at 24 to 28 C with supplemental fluorescent light at night. Isolate RR-9 isolated from a rotting sugar beet root has been used for initiating epidemics of root rot in breeding nurseries at Fort Collins, Colo., for several years. This isolate is representative of *R. solani* as typified by species criteria outlined by Parmeter et al. (15).

At 1, 2, 4, 8, and 16 days after inoculation, 5-mm^3 blocks of tissue that included the site of inoculation were excised from three inoculated roots of each cultivar. Comparable blocks from noninoculated roots were taken on the 16th day. Blocks were killed and fixed in FAA (10 ml 40% formaldehyde, 5 ml glacial acetic acid, 50 ml 95% ethyl alcohol, 35 ml water) for 96 hr (including 1 hr of aspiration at the onset of fixation), dehydrated in tertiary butyl alcohol, and infiltrated with paraffin (20). Longitudinal and transverse serial sections 10 μ thick were made with a rotary microtome. The sections were mounted in Haupt's adhesive and stained with safranin-fast green. Phase microscopy facilitated the detection of fungal hyphae within the cells.

RESULTS.—*One and 2 days after inoculation.*—There were no obvious differences among the resistant and susceptible cultivars. All roots appeared normal, and no necrosis was evident. The only histological evidence of infection was the presence of an occasional hypha within or between external cork cells, with some accompanying dissolution of middle lamella.

Four days after inoculation.—A small, depressed necrotic lesion was evident in most roots at the site of inoculation. Lesions in resistant roots were 2 mm or less in diam; those in susceptible roots, 2 to 10 mm in diam. Lesions were largest in susceptible cultivar GW 674-56C.

Penetration of root tissue by *R. solani* was inter- and intracellularly by infection cushions (1, 10) or individual hyphae (12). The fungus spread tangentially within the periderm (cork, cork cambium, cork parenchyma), and outer layers of the secondary cortex where hyphae were primarily intercellular in the advanced portions of lesions, and intracellular nearer the area of penetration. In susceptible cultivars, especially GW 674-56C, intercellular growth of hyphae carried the fungus deeper into the outer secondary cortex to the youngest cambial-vascular ring. Dissolution of middle lamella between parenchyma cells of susceptible roots, followed by the collapse of the cells, often created pockets of degenerated tissue a short distance in advance of the hyphae. Apparent hyphal advance in resistant cultivars was limited to the periderm.

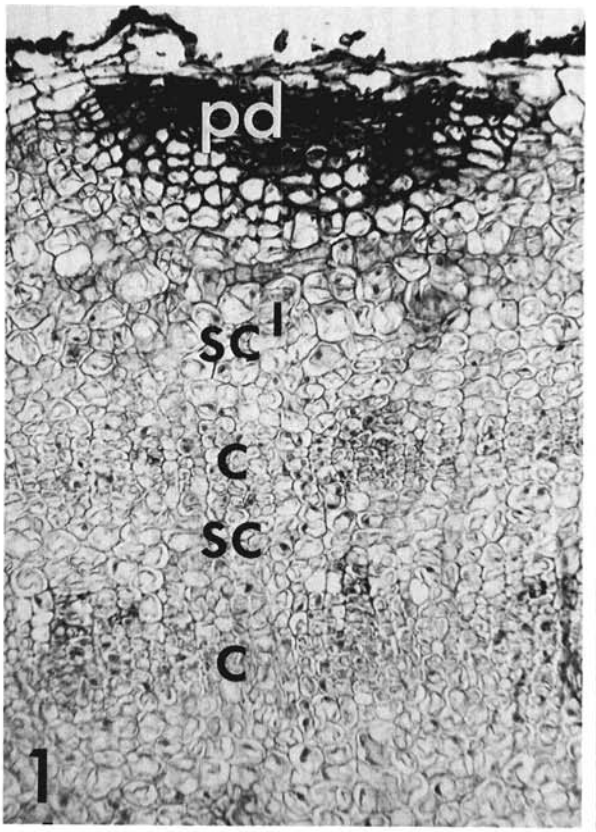
Eight days after inoculation.—Lesions were evident in all inoculated roots. The lesions ranged from 1 to 7 mm in diam in resistant cultivars, and from 2.5 to 13 mm in susceptible roots. Several additional lesions were evident in noninoculated portions of susceptible cultivars, suggesting spread of the fungus along the surface of the roots. Depth of necrosis in susceptible cultivars varied from 1.3 to 2.5 mm, as compared with 0.2 to 0.8 mm in resistant roots. But necrotic areas in resistant roots were considerably more dense and delimited than those in susceptible roots. Cells in necrotic regions in resistant roots were occluded with a deep-staining material; cell walls also were thicker and deeply stained with safranin (Fig. 1). In susceptible roots, necrosis was more uniformly spread throughout the periderm, secondary cortex bands, and youngest vascular rings (Fig. 2).

Necrosis preceded the hyphae in all roots. Hyphae were observed in parenchyma and medullary ray cells of the youngest three vascular rings of susceptible roots, and in undifferentiated xylem vessels of the first ring (Fig. 3). No hyphae could be detected inward from the cells of the outermost secondary cortex in resistant roots. Tangential spread of hyphae after initial penetration of the roots appeared to be more rapid than radial progress, and vascular cells with normally thick secondary walls seemed to retard fungus advance. Hyperplasia of secondary cortex cells occurred at the margin of necrotic and healthy tissue in resistant roots (Fig. 1). There also was some evidence of hyperplasia in susceptible roots, but rapid tissue degeneration occurring between the 4th and 8th day precluded detection of actual cell divisions. No secondary phellogen developed and, therefore, no wound periderm or cicatricelike cell layers as was described by several authors (4, 6, 7, 21) were evident in any root.

Sixteen days after inoculation.—Most roots of the resistant cultivars exhibited small, circular, isolated lesions at and around the point of inoculation, whereas 25 to 75% of the surface of susceptible roots was rotted. Necrosis in susceptible roots was 1 to 5 mm deep, whereas in resistant roots it was less than 1 mm. Extensive cankers were evident in many susceptible roots.

Internally, necrosis still preceded the hyphae. Hyphae were observed much deeper in susceptible roots (up to 4 mm) than in resistant roots where hyphae were detected only in the periderm and outer secondary cortex. In severely diseased roots of the susceptible cultivars, the periderm and cortex were

Fig. 1-4. Histopathology of sugar beet roots infected with *Rhizoctonia solani*. **1, 2, 3)** Eight days after inoculation. **1)** Transverse section through resistant cultivar FC 702/2 showing delimitation of necrosis to periderm (pd) and outermost secondary cortes (sc^1) (× 124). **2)** Longitudinal section through susceptible cultivar GW 674-56 C showing general necrosis through youngest vascular rings (× 124). **3)** Longitudinal section showing hyphae (h) in undifferentiated vessel cells (uv) of first vascular ring in cultivar GW 674-56 C (× 544). **4)** Transverse section through the sixth vascular ring of susceptible cultivar C 817 showing hyphae colonizing mature vessels (mv) 16 days after inoculation (× 544). Figures 2 and 3 rotated 90° from normal. c = cambium.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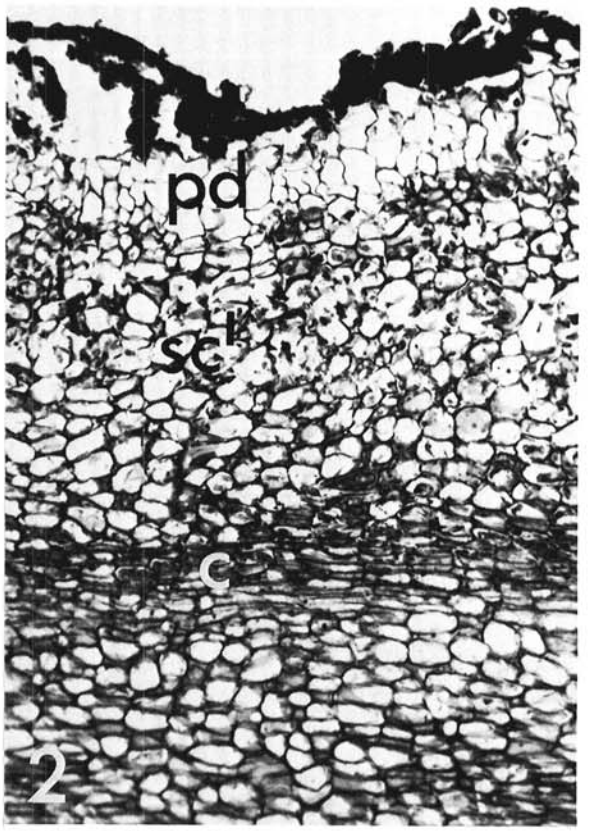
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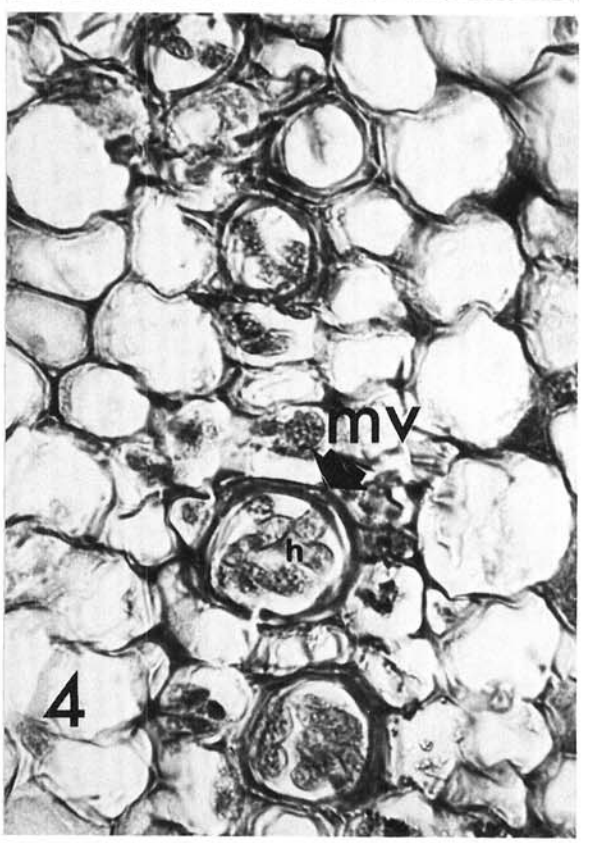
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almost completely degenerated. Hyphae occurred in mature xylem vessels in the youngest six vascular rings of susceptible, but were absent from xylem vessels in resistant roots (Fig. 4).

DISCUSSION.—Penetration of roots of susceptible and resistant sugar beet cultivars by *R. solani* was similar to that reported in several other hosts as reviewed by Dodman & Flentje (5). No host tissue discoloration or damage was observed before penetration, although several reports (3, 11, 13, 18) indicate that substances produced by some isolates of *R. solani* can cause disintegration of host tissue before penetration. Since the external cell layers of older sugar beet roots consist of dead cork cells (2), the reaction here to fungus-produced toxins or enzymes presumably would differ from that of a living epidermis. Seedling sugar beets, which have a living epidermis, apparently are invaded much more rapidly by *R. solani* than are older roots (17). Gonzalez & Owen (10) found no evidence for prepenetration damage caused by cultural filtrates of *R. solani* in tomato fruits having a thick cuticle. The nature of the tissue under attack undoubtedly governs the type of host response to the fungus or its metabolites.

After infection, cortical cell walls in lesions of resistant sugar beet roots became thicker and had a greater affinity for safranin than did comparable cells in susceptible roots, which may indicate a concentration or localization of suberin in the affected tissues. Such a host response might be classed as a hypersensitive reaction. It is unlikely, however, that suberization alone could account for restricted hyphal advance of *R. solani* because the fungus readily penetrates the suberized outer cork cells of the root. Also, it is uncertain whether the hyphae failed to penetrate the deeper tissues in resistant roots, or whether they penetrated and were lysed within the tissues. Lysis of the hyphae would indicate that a metabolic defense mechanism may exist in resistant sugar beet cultivars. A phytoalexin type of resistance mechanism has been reported for a Rhizoctonia disease of bean (16). A similar mechanism may have been responsible for the hypersensitive response of lettuce to *R. solani* reported by Flentje (8). Since no wound periderm or other mechanical barrier was observed to have developed in advance of the fungus, further studies on the fate of hyphae within resistant roots would serve as a prelude to physiological and biochemical investigations on the nature of resistance to *Rhizoctonia* in sugar beet.

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