Depletion of the Galacturonic Acid Content in Bean Hypocotyl Cell Walls during Pathogenesis by Rhizoctonia solani and Sclerotium rolfsii

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ABSTRACT

Cell walls prepared from infected hypocotyl tissues of 8-day-old bean (*Phaseolus vulgaris* 'Red Kidney') seedlings inoculated with *Rhizoctonia solani* or *Sclerotium rolfsii* exhibited a marked and rapid reduction in galacturonic acid content. The galacturonic acid content of cell walls prepared from diseased and healthy tissues was estimated with uronic acid dehydrogenase following enzymatic hydrolysis of cell wall samples. At the time of inoculation, cell walls from hypocotyl tissue contained about 8.3% galacturonic acid. The galacturonic acid content of cell walls from hypocotyl tissues infected with *R. solani* and *S. rolfsii* decreased to 1.7 and 1.9%, respectively, within 48 hr after inoculation, whereas cell walls prepared from comparable healthy tissues contained 6.2-7.2% galacturonic acid. Phytopathology 60:1846-1847.

The environment in which some plant pathogens grow influences the types and quantities of cell wall degrading enzymes they produce (1, 7, 10, 12). For example, the pectic enzymes produced in vitro by *Rhizoctonia solani* Kuehn differ from those obtained from *R. solani*-infected tissues (2). In addition, Hancock (11) has demonstrated that although *Sclerotinia sclerotiorum* d By. produces a xylanase in infected sunflower hypocotyls, the xylan content of the infected host is not greatly altered. Thus, it appears desirable to demonstrate the occurrence of a particular cell wall degrading enzyme in diseased tissue as well as to demonstrate alteration or degradation of its substrate during pathogenesis before it is assigned a role in disease development.

Both *R. solani* and *Sclerotium rolfsii* Sacc. during pathogenesis produce pectic enzymes that have been assigned a functional role in disease development (2, 6). The purpose of this study was to determine if production of pectic enzymes by these pathogens in infected bean hypocotyls is associated with a depletion of the galacturonic acid content in the cell walls of invaded tissue.

Bean (*Phaseolus vulgaris* L. 'Red Kidney') was used as the suscept for both pathogens. Seeds were planted in steamed soil and placed in a greenhouse at 27 ± 3 C. Seedlings were permitted to develop for 8 days and then inoculated with either *R. solani* (isolate RB) or *S. rolfsii* (isolate 14).

Sclerotium rolfsii was grown on autoclaved moist wheat seed for 1 week at 30 C, and these seed, bearing the fungus, served as inoculum. One infested seed was placed beside each bean hypocotyl at the soil line. At 0, 24, 48, and 72 hr after inoculation, bean hypocotyls were harvested. Sections bearing lesions of diseased hypocotyls were obtained and pooled. *Rhizoctonia solani* inoculum was produced and applied to plants in the manner previously described (14). At 0, 48, and 72 hr after inoculation, the lesioned areas of diseased hypocotyls were cut out and pooled. Comparable healthy tissue was harvested at each harvest date from noninoculated plants to serve as controls.

Diseased and healthy hypocotyl tissues were dried at 70 C for 24 hr, then ground in a Wiley mill to pass a 60-mesh screen, and stored in stoppered flasks. Cell walls were prepared from 250 mg of ground healthy or diseased tissue by a procedure previously described (7).

The galacturonic acid content of the cell walls was determined enzymatically. Ten-mg samples were added to 20-ml test tubes, and moistened with 2 drops of 95% ethanol. One ml of ethylenediaminetetraacetic acid (EDTA), adjusted to pH 11, was added to each sample which was incubated for 30 min at 30 C. Then each sample received 0.5 ml of 0.5 M acetate buffer (pH 4.0) followed by 1.0 ml of a 2% solution of crude dialyzed freeze-dried enzyme produced by *S. rolfsii* grown on bean hypocotyls for 10 days at 30 C. This enzyme preparation degrades the major known plant cell wall polysaccharides (4, 9, 13) and contains both endo- and exopolygalacturonases (5) which convert the galacturonic acid polymers in the walls to galacturonic acid. Test tubes containing reaction mixtures received two drops of toluene, and were capped and incubated for 20 hr at 30 C. After the digestion period, debris in the reaction mixtures were removed by centrifugation for 15 min at 20,000 *g*. Two-tenths-ml aliquots of the supernatants were assayed for free galacturonic acid using uronic acid dehydrogenase (UAD) (8). Assays were replicated in each experiment, and experiments were repeated once.

Plants inoculated with *R. solani* developed lesions which passed through the characteristic three stages (young, intermediate, and mature) of development described by Van Etten et al. (14). The galacturonic acid content of cell walls on a dry wt basis at the time of inoculation was 8.3%. By the time lesions had reached the intermediate stage of development (48 hr after inoculation), the galacturonic acid content of the cell walls from lesions was only 1.7% as compared to 6.2% in control tissue. At the mature stage of lesion development (96 hr after inoculation), cell walls prepared from lesions and healthy tissues contained, respectively, 1.5 and 6.6% galacturonic acid. Lesions at the intermediate stage of development contained maximum polygalacturonase activity (14).

Lesions caused by *S. rolfsii* developed rapidly and had less discrete margins than those caused by *R. solani*. Based on the water-soaked appearance of hypocotyl cross sections bearing lesions, it was estimated

that approximately 30, 75, and 100% of the harvested tissue contained the pathogen at 24, 48, and 72 hr, respectively, after inoculation. Cell walls from hypocotyls at the time of inoculation contained 8.4% galacturonic acid. The galacturonic acid content of cell walls from hypocotyl cross sections bearing lesions was 4.7, 1.9, and 0.3% at 24, 48, and 72 hr after inoculation, respectively, and the galacturonic acid content of control tissue was 7.9, 7.2, and 6.1%, respectively. Infected bean hypocotyl tissues contain polygalacturonase (6).

The results reported here confirm the previous conclusions that pectic enzymes play a significant role in the diseases caused by *R. solani* and *S. rolfsii* (2, 6). The galacturonic acid content of cell walls in bean hypocotyls is rapidly decreased soon after either of these pathogens invades the tissue. Pectic enzymes produced by these fungi during pathogenesis can account for the soft macerated nature of invaded tissues (3, 6, 14) as well as the depletion of the galacturonic acid content of cell walls in diseased tissues.

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