

Immunohistochemical Investigation of the Infection of Hemp Sesbania (*Sesbania exaltata*) by the Fungal Biocontrol Agent *Colletotrichum gloeosporoides*

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Colletotrichum gloeosporioides belongs to a genus of fungi which cause anthracnose diseases of a wide range of plant species. This fungus has been found to be a good candidate for use as a biocontrol agent of several different species of weeds, including hemp sesbania (*Sesbania exaltata*). Hemp sesbania is a problematic leguminous weed in many areas of the southern U.S. and is especially bothersome in soybean (*Glycine max*) production in much of the mid-southern U.S. The need to develop novel weed management tools and strategies is becoming increasingly more acute due in large part to environmental concerns and rising petroleum costs associated with the production and use of synthetic herbicides.

Fungi are known to produce a broad array of cell wall degrading enzymes during the course of infection of plants. Invasion by plants by fungi trigger a complex series of host defence responses. Plants can alter the composition of their cell walls to make them more resistant to penetration by the invading fungus. While traditional biochemical approaches to characterize these cell wall changes are extremely challenging, immunohistochemical approaches can be used very effectively to characterize the infection of plants by fungi and parasitic plants. In this study, we take advantage of the increased variety of immunocytochemical probes currently available to investigate wall modifications during the invasion of the important biocontrol fungus *Colletotrichum gloeosporioides* (Penz.) as it invades stems of the pernicious weed hemp sesbania (*Sesbania exaltata*).

Sesbania plants 8-10 inches tall were sprayed in the field with an “invert” formulation of spores of the fungus *Colletotrichum gloeosporioides* and the plants were harvested 6-9 days post-treatment. Stem sections with lesions were fixed in 3% GA, embedded in LR white, and sectioned. Sections were treated with various anti-polysaccharide antibodies obtained from the Complex Carbohydrate Research Center (CCRC), Athens, GA and PlantProbes, Leeds, UK. Sections were treated with a secondary antibody-colloidal gold conjugate, silver-enhanced, and imaged with brightfield illumination on a compound microscope.

We found that homogalacturonans with a wide range of methyl-esterification states were degraded by the invading fungus, as evidenced by the loss of binding of both the JIM5 and JIM7 antibodies in the 10-15 epidermal cells and subtending cortical cells just flanking the lesion (Figure 1, A and B). Furthermore, both the galactan and the arabinan side-chains of RGI (LM5- and LM6-reactive, respectively) were largely absent in the same regions (Figure 1, C and D), as was the RGI epitope recognized by CCRC-M2 (Figure 1E).

Furthermore, the host plant was found to produce a reaction zone/cork layer between the site of infection and the vascular system. The walls of the cells comprising this nascent layer were found to be enriched in AGPs (JIM8- and JIM13-reactive; Figure 2A and 2B), xyloglucan (both LM15- and CCRC-M1-reactive; Figure 2C and 2E), and possibly extensin (JIM19-, JIM20- and LM1-reactive; data not shown) compared with neighboring cells.

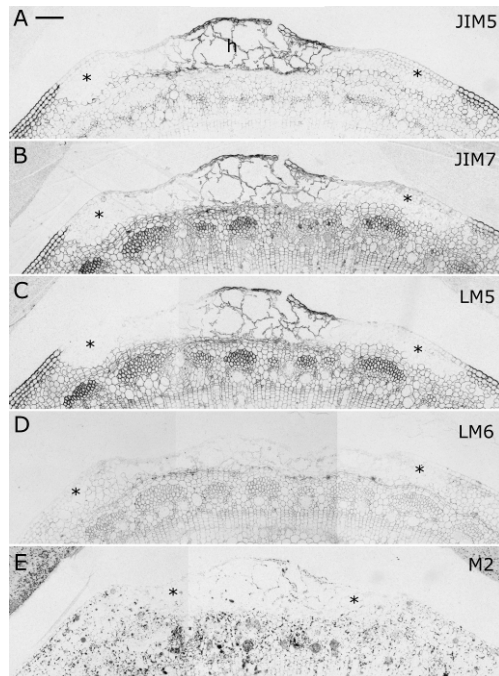


Figure 1: Cross-sections of a hemp sesbania stem lesion labeled with anti-polysaccharide antibodies. Several different classes of polysaccharide epitopes are lost or degraded from the tissue directly surrounding the lesion. Scale bar = 100 microns.

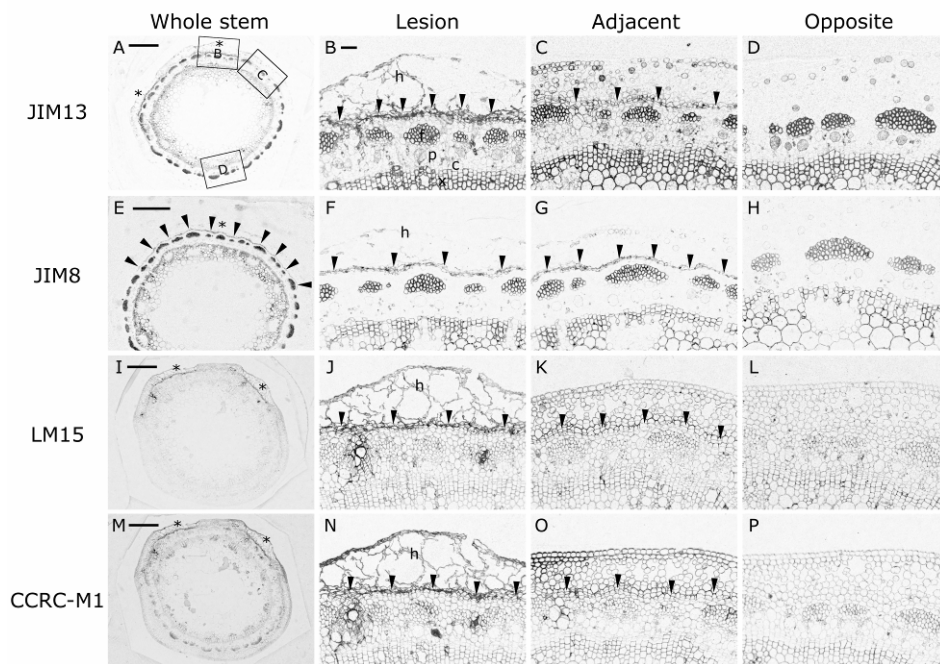


Figure 2. Cross-sections of hemp sesbania stem with anthracnose lesions. Antibodies to xyloglucan (LM15 and CCRC-M1) and AGPs (JIM13 and JIM8) recognize areas of apparent new cell wall deposition which occur between the infection lesion and the host vascular tissue. h = hyphae; f = fibers; p = phloem; c = cambium; x = xylem; * = lesion; arrowheads point to newly-synthesized “protective layer”. Scale bars in first column = 0.5 mm; other columns = 50 microns.