NAGENDRAN, SUBASHINI[1], and JOHN M. HALLOIN[1,2,*]. [1]Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824; and [2]USDA, ARS, SBRU, East Lansing, MI 48824. **Induction of chitinase activity during the defense response of sugarbeet tap roots.**

ABSTRACT

Previous work in our laboratory showed that sugarbeet tap roots produce phenolic compounds (PCs) as part of their defense response against *Rhizoctonia solani* (RS) AG2-2. These compounds are produced at cool (<15C), but not at warm (>25C) temperatures, are formed approximately 48 hours after inoculation, and can be induced by abiotic elicitors, but not by water. Plants typically produce a group of compounds known as pathogenesis-related (PR) proteins in association with their defense responses. Chitinase is one of these PR proteins, and is presumed to act through digestion of fungus cell walls. Sugarbeet leaves produce chitinases as PR proteins in response to infection by *Cercospora beticola*. We wished to determine if chitinase activity also is increased during the defense response of tap roots, and if it's production parallels PC production in time and sensitivity to temperature..

Holes 1cm (deep) X 3mm (diameter) were drilled into pieces of sugarbeet tap roots, and filled either with water (check), RS inoculum, or a 100ppm solution of chitosan (abiotic inducer); water and chitosan solutions were absorbed into the surrounding tissue within 20 min. Tissue pieces then were incubated at either 10 or 28°C for periods of 6 to 144 hr. Samples collected were 2mm thick cylinders of tissue surrounding the drilled holes; these were freeze dried for storage, and subsequently were ground and extracted with pH 6 phosphate buffer, and "native" proteins in the extract were separated by electrophoresis on glycochitin-containing polyacrylamide gels at pH 8.9. Chitinase activity was detected by enzymic digestion of glycochitin in the gels at pH 5.0. Residual glycochitin in gels was stained with fluorescent brightener dye at pH 8.9 and observed under UV light.

Both RS and solutions of chitosan elicited production of 2 discrete bands of chitinases within 12 hr., and this activity increased through at least 48 hr. post-treatment. Maximum production occurred within 48 hr, in tissue pieces incubated both at the cool and at the warm temperature. Additionally, production of chitinases occurred, albeit at a reduced rate, in the water-treated tissues.

Chitinases are produced in sugarbeet tap roots as part of their overall defense system. However, production of chitinases at both cool and at warm temperatures clearly differentiates this response from PC production and from observed defense against *R. solani*. Additionally, chitinase production occurs more quickly than PC production. Formation of chitinases within water-treated tissues indicates that this is a wound response, rather than a disease-related response. Chitinases may provide useful PR protein markers for future studies on the molecular basis of the defense response in sugarbeet roots.