WEBB, KIMBERLY M.[1]* and COREY BROECKLING[2], [1]USDA-ARS, Sugar Beet Research Unit, 1701 Centre Ave., Fort Collins, CO 80526 and [2] Colorado State University, Proteomics and Metabolomics Facility, 2021 Campus Delivery, Fort Collins, CO 80523. **Metabolomic differences in a susceptible and resistant *Beta vulgaris* germplasm during early responses to *Rhizoctonia solani* AG 2-2 IIIB.**

ABSTRACT

Sugar beet can be significantly impacted by Rhizoctonia crown and root rot caused by *Rhizoctonia solani* AG 2-2 IIIB. The molecular processes that mediate sugar beet resistance to *R. solani* are largely unknown and identifying the metabolites associated with *R. solani* infection may provide novel targets to utilize in breeding programs for enhanced resistance. The metabolic changes that occurred during susceptible (FC901) and resistant (FC709-2) *R. solani* interactions were compared with mock inoculated treatments and characterized using a non-targeted metabolomics workflow spanning primary and secondary metabolism products. Plants of both germplasm were grown in a greenhouse until 10 weeks after sowing. Hulless barley was infested with *R. solani* AG 2-2 IIIB isolate "R-9", air dried, ground, and then used to inoculate 12 pots, each containing 3 plants, for each germplasm. 12 pots of each germplasm, also containing three plants each, were inoculated with ground un-infected barley as a "mock" infected control. At 0, 3, 5, and 7days after infection (dai) or mock inoculated root and leaf tissue were taken from 3 pots for each of the two sugar beet lines, flash frozen with liquid nitrogen and then ground finely with a mortar and pestle. Metabolites were extracted with methanol:water (80:20) and detected using non-targeted reversed-phase UPLC-MS and GC-MS workflows.

Non-targeted mass spectrometry of sugar beet roots detected more than 900 compounds, of which 143 were annotated, including glycerolipids, primary metabolites, dipeptides, phenolics and conjugates, fatty acids, flavonoids, and terpenoids. Primary differences between germplasm were detected by PCA analysis using both LC-MS and GC-MS and statistical interrogation of the datasets revealed clear distinction between tissue type and genotype, and more subtle changes in response to inoculation that was dependent on genotype.

During these experiments we focused on very early interactions during the sugar beet/*R. solani* pathosystem (0 to 7dai). Here we saw very little influence of inoculation with *R. solani* at the earliest time points, but did see a slight change of metabolite profiles by the last day of the study (7dai) which could possibly be explained by the natural infection cycle of the pathogen. The time and extent of this process depends on the specific isolate of *R. solani* as well as the host that it is infecting and therefore full colonization of the cortex has been reported to take up to 3-4 days after infection is initiated. Metabolite profiles by both LC-MS and GC-MS clearly distinguish the two genotypes tested in both root and leaf tissues and these profiles differ depending on tissue type. LC-MS more clearly separated the metabolite profiles of the two genotypes better than GC-MS therefore the genotypes differ more in their secondary metabolites than in their primary metabolites.