AGRICULTURAL RESEARCH FOUNDATION
INTERIM REPORT
FUNDING CYCLE 2020 – 2022

TITLE: Developing molecular markers to identify glyphosate-resistant weed seeds in cool-season grass seed lots

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EXECUTIVE SUMMARY: *L. multiflorum* has evolved resistance to many commonly used herbicides. In Oregon, *L. multiflorum* is a weed in many crops, including perennial ryegrass, tall fescue, wheat, orchardgrass, and annual ryegrass grown for seed. Recently, 60 resistant *L. multiflorum* populations were identified in Oregon, where some populations exhibited resistance to up to four different herbicide mechanisms of action. Resistance to acetolactate synthase (ALS), acetyl-CoA carboxylase (ACCase), and 5-enol-pyruvylshikimate-3-phosphate synthase (EPSPS) inhibitors was widespread. The widespread herbicide resistance in grass seed fields in Oregon poses a serious threat to the local agricultural communities, because of potential contamination of grass seed lots with herbicide resistant weed seeds. The seed lots are sold for many uses nationally and internationally, posing a potential source of dispersal of herbicide resistance genes. The objective of this research was to identify genes involved in glyphosate resistance in 16 *L. multiflorum* populations in order to develop genetic markers for the rapid identification of resistant biotypes. We have conducted a genotype-by-sequencing experiment to identify markers throughout the *L. multiflorum* genome and attempt to associate the markers with glyphosate resistance. To date, the genotyping and phenotyping have been concluded. The next step is to test statistical methods that best correlate the markers with glyphosate resistance and identify the genes by annotating the genomic regions identified.

OBJECTIVES: Identify genes involved with glyphosate resistance so that molecular markers can be developed for the rapid identification of resistance in plant tissue and grass seed lots

PROCEDURES: DNA from 288 *L. multiflorum* individuals was extracted, and sent to the CGRB at OSU for genotype-by-sequencing. Each individual plant was characterized for glyphosate resistance as follows. When plants reached the 23-BBCH growth stage, leaf tissue from the youngest fully expanded leaves were collected from the main tiller, immediately frozen in liquid nitrogen, and store in a -80 C freezer until further analysis. After tissue sampling, plants were subjected to a glyphosate application at 1456 g equivalent acid per hectare. Forty-eight hours after glyphosate application, the youngest fully expanded leaves from a different tiller was collected, weighed, and stored in Eppendorf tubes in a -80 C freezer until shikimate accumulation quantification was performed as described elsewhere. This method was implemented because glyphosate inhibits the EPSPS in the shikimate pathway, resulting in the accumulation of shikimate that can be used as a biomarker for glyphosate effects. This method relies on the oxidation of shikimate by periodic acid and quantification spectrophotometrically.

SIGNIFICANT ACCOMPLISHMENTS TO DATE: We have obtained the marker and glyphosate response datasets that will be necessary for the identification of glyphosate resistance. Future steps are to identify the appropriate statistical method for the association between the genetic markers and phenotypic data. We will test genome-wise association models, as well as FST outlier analysis.

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM: None.

FUTURE FUNDING POSSIBILITIES: USDA-NIFA-AFRI