AGRICULTURAL RESEARCH FOUNDATION
INTERIM REPORT
FUNDING CYCLE 2016 – 2018

TITLE: Test Case for Improving Disease Resistance Breeding in Trees

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COOPERATORS: none

SUMMARY: Typically, hybrid poplar tree farms must grow for ~15 years before the trees are large enough to be harvested. Severe outbreaks of this disease have historically prevented trees farms from reaching a harvestable age (Newcombe et al. 2001). A variety of methods have been tested to manage Septoria leaf spot and stem canker ranging from chemical to cultural control (Newcombe et al. 2001). The results of these trials have indicated that the development and deployment of disease resistant varieties is the most economical and ecologically sound means of managing this disease (Newcombe et al. 2001). Tree breeding is a slow time consuming process making it extremely difficult to prove that varieties with this gene present in their genetic code are resistant to Septoria leaf spot and stem canker using traditional plant breeding approaches. As a result, I would like to short cut this process by genetically transforming susceptible hybrid poplar with the resistance genes we have identified in collaboration with scientists at the Department of Energy.

OBJECTIVES: (1) Genetically transform susceptible varieties of hybrid poplar with a resistance gene to Septoria leaf spot and stem canker; (2) test the levels of resistance of transformed trees by exposing them to the fungus that causes Septoria leaf spot and stem canker; and (3) develop a tool to identify resistant trees based on their genetic code.

PROCEDURES: Objective 1: Genetic transformation of susceptible varieties of hybrid poplar with a resistance gene to Septoria stem canker. In order to test whether or not the gene we have identified does in fact confer resistance to this pathogen we will first clone (copy) and subsequently transform (insert) the resistance gene into the susceptible hybrid poplar variety. The methodology to genetically transform hybrid poplar, called Agrobacterium – mediated transformation, is well developed in the literature (Howe et al. 1994). I will employ a similar methodology in this project. Once the successful transformation of the trees has been confirmed we will then conduct a greenhouse experiment to test that we have in fact made the susceptible variety resistant.

Objective 2: Greenhouse experiment to test the resistance of the transformed plants. The greenhouse experiment will be conducted by propagating several plants from the resistant variety (n = 20); the susceptible variety (n = 20); and the susceptible variety that has been transformed with the resistance gene (n = 20). Hybrid poplar varieties are easily propagated by collecting 3-inch branch cuttings and placing those cuttings in soil with approximately 1/8 inch of the cutting exposed above the soil surface. Within three months the trees will be of sufficient size that they can be used in the experiment described below.

Objective 3: Tool development to identify resistant trees based on their genetic code. Once we have confirmed that the gene we have identified confers resistance to Septoria leaf spot and canker it will be possible to develop markers that can be used to screen hybrid poplar varieties for the presence/absence of the resistance gene. This is achieved by extracting DNA from the trees

and checking for the presence of the marker using a procedure called PCR. These markers can then be used to rapidly screen varieties that are currently part of hybrid poplar breeding programs in the US. Without this tool hybrid poplar varieties are screened in both the greenhouse and field requiring a significant number of resources to care for and maintain the trees. This is both time consuming and expensive in particular if large numbers of varieties are screened at one time. The ability to preselect trees that have this resistance gene, using their genetic code, will reduce the resources expended in the greenhouse screening methodology and allow breeding programs to focus their on those varieties which have the resistance gene.

SIGNIFICANT ACCOMPLISHMENTS TO DATE: Work on this project was initiated in the fall of 2016 following the recruitment of an undergraduate to conduct the work. To date we have successfully placed 10 new genotypes of *Populus trichocarpa* into tissue culture. This is the first and necessary step to complete prior transformation. We are currently, testing the transformation efficiency of these genotypes. The ability to put several *P. trichocarpa* genotypes into tissue culture and then transform them has already led the funding of one competitive grant. In addition we have submitted two letters of intent using this data and full proposals have been encouraged.

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM:
Department of Energy – Community Science Project: “RNAseq enabled metabolic modeling of disease resistance to Septoria canker in the DOE flagship *P. trichocarpa*.”

FUTURE FUNDING POSSIBILITIES:
Department of Energy – USDA Plant Feedstock Genomics for Bioenergy 2017 FOA.
USDA Biotechnology Risk Assessment Grant (BRAG) Program 2017 RFP.

1. Newcombe G, Ostry ME, Hubbes M, Perinet P, Mottet, MJ. Poplar diseases. In Poplar culture in North America (DI Dickman, JG Isebrands, JE Eckenwalder, J Richardson eds), NRC Research Press. Ottawa. 249–276. 2001.