

Field-Testing Transgenic American Chestnuts on Reclaimed Coal Mines¹

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Abstract: Prior to the introduction of a non-native fungal pathogen in the late 1800s, the American chestnut (*Castanea dentata*) was a dominant hardwood tree in eastern North America. Its abundant mast made it an important food resource for both animals and humans, and the rapid spread of the blight was devastating to eastern forest communities. Organizations including the American Chestnut Foundation have been using hybrid/backcross-breeding to introduce blight resistance from Chinese chestnut (*Castanea mollissima*), but while there have been some promising results, resistance levels in resulting offspring are variable. Recently, efforts to develop blight-resistant trees have involved genetic modification to insert candidate blight resistance genes from Chinese chestnuts. Bioassays of GM chestnuts have shown promise, but their viability in the field is still being assessed. From a regulatory perspective, reclaimed coalmines in need of reforestation provide an excellent locale for field-testing GM chestnuts, as there is little opportunity for genetic material (e.g. pollen) to escape the study site and pollinate compatible species. Over 2014 and 2015, we planted two cohorts of GM chestnuts comprised of multiple genetic constructs (background genotype x inserted genes) on a reclaimed coal mine in southwestern Virginia. The aim was to select promising candidates for increased production that could be planted in a larger scale study. To assess their viability, trees were phenotyped monthly during the growing season, including recording any visible indications of blight. Height and basal diameter were measured after planting and at the end of the growing season. Growth and survival varied among constructs. By 2016, a number of trees, both GM and non-GM, developed blight cankers, several of which resulted in stem dieback and resprouting, but some constructs appear to have more blight resistance. In spring 2018, we inoculated trees with a virulent strain of *C. parasitica* to a) assess canker development and b) quantify gene expression through RNA-sequencing to determine whether resistance genes are being expressed after inoculation. Inoculation data will not be available until spring 2019, but we hope to ascertain whether differences in resistance to natural blight infection correlate to differences in gene expression. ³

Additional Key Words: *Cryphonectoria parasitica*, *Castanea dentata*, genetic modification, reforestation.

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3. Work reported here was conducted near 36°59'9"N, 82°42'5"W.