PROJECT SUMMARY

Title: CRISPR-Cas mutagenesis for genetic containment of forest trees: Field evaluation of efficacy, stability, and pleiotropy

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The dispersal of transgenes from genetically engineered plants presents substantial challenges to biotechnology regulatory bodies. Because most forest trees are weakly domesticated, have wild relatives, and pollen or seeds can spread widely, they are especially problematic. However, plantation trees are often vegetatively propagated, making fertile flowers unnecessary for commercial use. Thus, genes whose mutation causes complete sterility could provide strong and fully reliable containment, simplifying regulatory decisions. We propose to establish new means for CRISPR-Cas-based editing of floral genes to produce sterile *Populus* and *Eucalyptus* trees—two tree genera of value for forest, environment, and energy industries.

We propose three activities: First, we will conduct greenhouse and field tests of CRISPR-Cas gene-edited poplars and eucalypts to determine their floral and vegetative phenotypes, including their genetic stability over years in the field. Second, we will develop new, multiplex CRISPR constructs and test their ability to produce eucalypt trees that are sterile, but grow well and produce nutritive floral tissues (pollen, nectar) that support its diverse pollinators. Third, we will develop and test the mutagenesis efficiency of a system for asexual excision of CRISPR-Cas loci during *in vitro* plant regeneration.

The results of the project will include a field demonstration of the reliability of gene editing for genetic containment, new gene editing technology that mitigates the ecological impacts of containment genes, and a system for the somatic excision of gene editing machinery after mutagenesis. These tools and results are expected to simplify regulatory decisions and improve long term stability of gene-edited trees.