

## **Advances in American Chestnut Somatic Seedling Production**

S.B. Johnson, H.T. Le, T. L. Kormanik and S.A. Merkle

Warnell School of Forestry and Natural Resources, University of Georgia, Athens, GA 30602

The American chestnut was one of the most important forest trees in the Appalachian Forest until the introduction of the chestnut blight fungus, which caused the death of virtually every mature American chestnut tree in the eastern United States. A system for mass propagation of blight-resistant material obtained through conventional breeding or gene transfer is still lacking. Thus, the goal of our project is to develop a high-frequency in vitro propagation system for American chestnut via somatic embryogenesis. Two bottlenecks in this approach are the low initiation rate of embryogenic cultures and the low production efficiency of plantlets (somatic seedlings) from the somatic embryos. To increase embryogenic culture initiation, we tested two plant growth regulators (2,4-D and picloram) at different concentrations and found that 2,4-D resulted in the highest frequency of embryogenesis (up to 3.5 %). This culture initiation experiment also demonstrated for the first time that highly productive embryogenic cultures could be initiated from immature seeds resulting from controlled crosses between known American chestnut parents. To increase plantlet production, we tested variations in cold (4° C) treatment duration (12, 15, and 18 weeks) and light quality (red, red + far red, and cool white fluorescent). For some genotypes, the longer cold treatments improved plantlet production and red light improved overall plantlet production frequency (up to 80% and 69%, respectively). Thus, by manipulating the cultural treatments, we were able to increase American chestnut somatic seedling production efficiency above the levels we previously reported. The first American chestnut somatic seedlings to be tested under nursery conditions were promising, growing up to 1.5 m in their first season. These advances in clonal propagation will aid in the restoration of the American chestnut to our forests.