

ADVENTITIOUS SHOOT REGENERATION OF *FRAXINUS NIGRA* MARSH.

Rochelle R. Beasley and Paula M. Pijut

Purdue University (RRB), Department of Forestry and Natural Resources, Hardwood Tree Improvement and Regeneration Center (HTIRC), 715 West State St., West Lafayette, IN 47907 and U.S. Forest Service (PMP), Northern Research Station, HTIRC, 715 West State St., West Lafayette, IN 47907.

PMP is corresponding author; to contact, email at ppijut@purdue.edu. or ppijut@fs.fed.us.

Fraxinus nigra Marsh. (black ash) is a native ash species occurring in Newfoundland west to Manitoba and south to Iowa, Illinois, West Virginia, and Virginia. Although it is not a commercially important species, it has significant ethnobotanical importance to Native American tribes of the eastern United States. The seeds are important forage for wildlife, the wood is preferred for making splints for basketry, and the wood is also used for flooring and furniture. *F. nigra* have immature embryos at seed set combined with complex stratification requirements, making the species difficult to regenerate naturally from seed. Because of this difficulty, an in-vitro adventitious shoot regeneration system would be beneficial for mass propagation and improvement of this species. The development of such a system will also provide the basis for an *Agrobacterium*-mediated transformation system for emerald ash borer resistance. To date, no in-vitro regeneration protocol for black ash has been developed.

An adventitious shoot regeneration system for *F. nigra* is currently being optimized using hypocotyls extracted from aseptic seeds. Hypocotyls were cultured on a Murashige and Skoog (MS) medium containing 13.3 μM 6-benzylaminopurine (BA) plus 4.5 μM thidiazuron (TDZ) for callus and shoot induction. Shoots were successfully regenerated using a combination of MS medium with Gamborg B5 vitamins (MSB5), 10 μM BA, and 10 μM TDZ for 4 weeks, and then they were transferred to MSB5 medium with 6.7 μM BA, 1 μM indole-3-butyric acid (IBA), and 0.29 μM gibberellic acid (GA_3) for shoot elongation. Once elongated, the shoots were successfully micropropagated using MSB5 medium with 13.3 μM BA, 1 μM IBA, 0.29 μM GA_3 , and 0.2 g L^{-1} casein hydrolysate. Rooting of elongated microshoots was successful using woody plant medium containing 4.5 μM IBA plus 5.7 μM indole-3-acetic acid with a 10-day dark incubation, and rooted shoots are undergoing acclimatization.