Adventitious shoot regeneration and rooting of *Fraxinus americana*

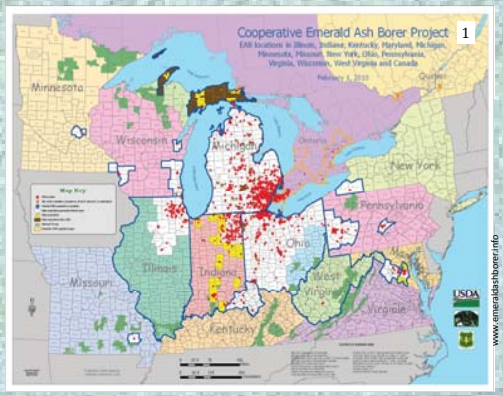
Kaitlin J. Palla[1] and Paula M. Pijut[2]

[1]Purdue University, Dept. of Forestry and Natural Resources, Hardwood Tree Improvement and Regeneration Center (HTIRC), 715 West State St., West Lafayette, IN and [2]USDA Forest Service, Northern Research Station, HTIRC.

ABSTRACT

The threat of the emerald ash borer (EAB) to white ash becomes more urgent with each growing season, making the development of an in vitro regeneration and transformation system an important goal. Best regeneration resulted from freshly isolated hypocotyls on MS medium supplemented with 13.3 µM BA plus 4.5 µM TDZ. Sixty-six percent of hypocotyl segments produced adventitious shoots, with a mean of 3.5 adventitious shoots induced per explant. Adventitious shoots from hypocotyls were established as proliferating shoot cultures following transfer to MSB5 medium supplemented with 10 µM BA plus 10 µM TDZ. For in vitro rooting trials, woody plant medium with IAA [2.9, 5.7, or 8.6 µM] plus 4.9 µM IBA were tested under 5-day and 10-day dark treatments followed by culture in the light. Early results show successful (75%) rooting of shoots exposed to a 5-day dark pulse on medium containing 4.9 µM IBA plus 8.6 µM IAA. This regeneration system provides a foundation for *Agrobacterium*-mediated genetic transformation of white ash for resistance to the EAB.

Fig. 1 Range of EAB as of February 1, 2010



INTRODUCTION

- White ash (*Fraxinus americana*) trees provide both economical and ecological benefits.
- White ash has high economic value as a commercial hardwood, in fact having the highest value of the various ash species.
- The wood is used in the production of furniture, flooring, doors, tool handles, baseball bats, and boats.
- The emerald ash borer is an invasive beetle that is fatal to a tree once it infests it.
- Developing a regeneration system is the basis for *Agrobacterium*-mediated genetic transformation, which could result in the development of trees resistant to EAB.

Fig. 2 Larval galleries

Fig. 3, 4 EAB larvae, EAB adult

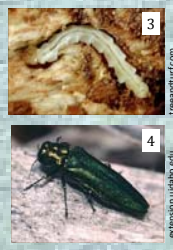


Fig. 5 Defoliation

MATERIALS AND METHODS

Adventitious shoot induction from hypocotyls

- Pericarps of *Fraxinus americana* seeds removed, along with 3 mm opposite the radical, then surface disinfected with 70% ethanol for 1 min, 20% commercial bleach solution for 18 min, rinsed three times in sterile-distilled water and stored overnight in sterile-distilled water
- Embryos extracted and the excised hypocotyls cultured on Murashige and Skoog (1962) (MS) medium supplemented with 13.3 µM 6-benzylaminopurine (BA) plus 4.5 µM thidiazuron (TDZ), 3% sucrose, and 0.7% Difco-Bacto agar (Du and Pijut, 2008)
- Cultures incubated at 24 ± 2°C under a 16h photoperiod (80 µmol m^{-2} s^{-1}) for 4 weeks

Elongation of adventitious shoots

- Explants producing shoots transferred to MS basal salt plus B5 vitamins (MSB5) medium supplemented with 10 µM BA plus 10 µM TDZ to induce shoot elongation (Kim et al., 1997)
- Elongated shoots removed when approximately 3 cm long and placed on rooting media

Rooting of adventitious shoots

- Elongated adventitious shoots cultured on woody plant medium (WPM) (Lloyd and McCown, 1980) supplemented with 4.9 µM indole-3-butyric acid (IBA) plus 2.9, 5.7, or 8.6 µM indole-3-acetic acid (IAA) to induce root formation
- Cultures given a 5-day or 10-day dark pulse before being incubated under a 16h photoperiod

RESULTS

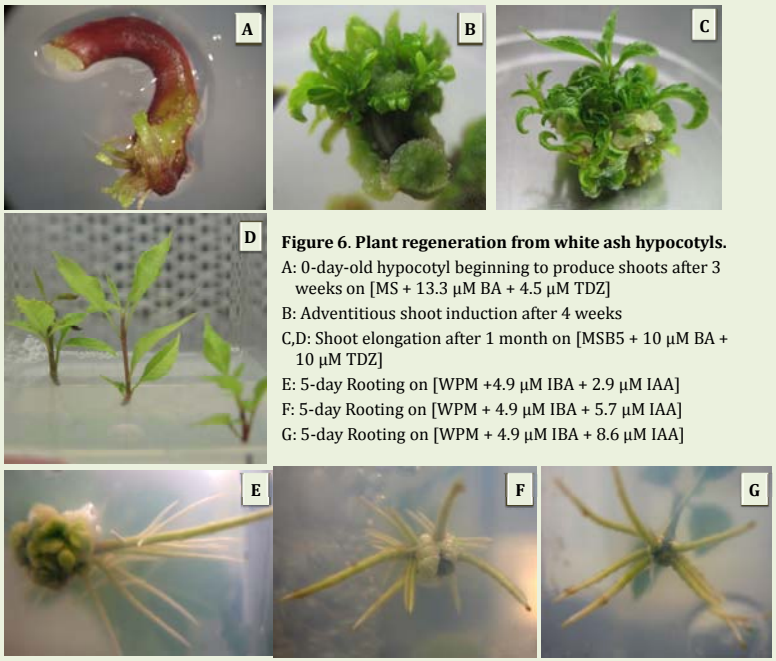


Figure 6. Plant regeneration from white ash hypocotyls.

A: 0-day-old hypocotyl beginning to produce shoots after 3 weeks on [MS + 13.3 µM BA + 4.5 µM TDZ]

B: Adventitious shoot induction after 4 weeks

C,D: Shoot elongation after 1 month on [MSB5 + 10 µM BA + 10 µM TDZ]

E: 5-day Rooting on [WPM +4.9 µM IBA + 2.9 µM IAA]

F: 5-day Rooting on [WPM + 4.9 µM IBA + 5.7 µM IAA]

G: 5-day Rooting on [WPM + 4.9 µM IBA + 8.6 µM IAA]

RESULTS

Table 1. Regeneration from hypocotyls

Rep #	Explants Forming Callus	Explants Forming Shoots	Avg. # Shoots per Explant
1	100.0%	91.7%	4.9 ± 1.1
2	94.1%	52.9%	3.8 ± 1.5
3	93.3%	41.7%	2.5 ± 0.7
4	84.6%	69.2%	2.8 ± 1.2
5	85.7%	75.0%	3.4 ± 1.4
Avg.	91.6%	66.1%	3.5
St.Dev	0.06	0.19	0.9

Table 2. Rooting of shoots after 10 days in light

Treatment	Shoots Forming Roots	Avg. # Roots per Shoot
5-day dark, IAA 0 µM	12.5%	2.0 ± 0.0
5-day dark, IAA 2.9 µM	50.0%	3.8 ± 1.7
5-day dark, IAA 5.7 µM	62.5%	2.8 ± 2.1
5-day dark, IAA 8.6 µM	75.0%	3.8 ± 2.5
10-day dark, IAA 0 µM	0.0%	n/a
10-day dark, IAA 2.9 µM	20.0%	1.0 ± 0.0
10-day dark, IAA 5.7 µM	33.3%	1.8 ± 1.0
10-day dark, IAA 8.6 µM	0.0%	n/a

CONCLUSIONS

- ✓ Media previously successful with green ash (*F. pennsylvanica*) proved to regenerate white ash. Media was MS supplemented with 13.3 µM BA plus 4.5 µM TDZ.
- ✓ The average percent of hypocotyl segments regenerating shoots was 66.1% ± 0.19.
- ✓ The average number of adventitious shoots elongated per segment on MSB5 medium supplemented with 10 µM BA and 10 µM TDZ was 3.5 ± 0.9.
- ✓ Preliminary rooting results show the most rooting (75%) on shoots exposed to a 5-day dark pulse on WPM supplemented with 4.9 µM IBA plus 8.6 µM IAA.
- ✓ This regeneration system will be used for *Agrobacterium*-mediated genetic transformation.

REFERENCES

Du, N., Pijut, P.M. 2008. Regeneration of plants from *Fraxinus pennsylvanica* hypocotyls and cotyledons. Sci. Hort. 118: 74-79.

Kim, M.S., Schumann, C.M., Klopfenstein, N.B. 1997. Effect of thidiazuron and benzyladenine on axillary proliferation of three green ash (*Fraxinus pennsylvanica* Marsh) clones. Plant Cell Tiss. Org. Cult. 48: 45-52.

Lloyd, G., McCown, B. 1980. Commercially feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot-tip culture. Proc. Int. Plant Prop. Soc. 30: 421-427 (Publ. 1981).

Murashige, T., Skoog, F. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant. 15: 473-497.