Figure 1. Emerald ash borer

www.ci.montgomery.oh.us

Regeneration of plants from *Fraxinus americana* hypocotyls and cotyledons

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

[1]Purdue University, Dept. of Horticulture and Landscape Architecture, Hardwood Tree Improvement and Regeneration Center (HTIRC), 715 West State St., West Lafayette, IN 47907, kpalla@purdue.edu

[2]USDA Forest Service, Northern Research Station, HTIRC, Purdue University, Dept. of Forestry and Natural Resources, 715 West State St., West Lafayette, IN 47907, ppijut@purdue.edu

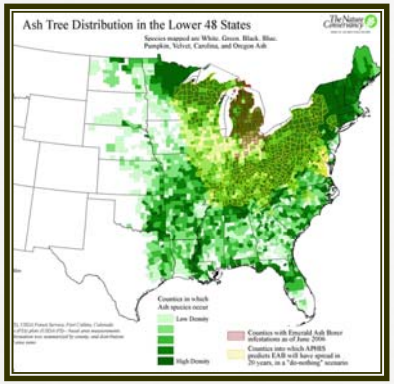
Figure 2. Damage by pest

www.treecanada.ca

Abstract:

Hypocotyls and cotyledons from freshly isolated white ash (*F.* americana) embryos were excised and cultured on Murashige and Skoog (MS) medium supplemented with 13.3 µM 6-benzylaminopurine (BA) plus 4.5 µM thidiazuron (TDZ). Sixty-four percent of hypocotyl segments and 10.4% of cotyledon segments produced adventitious shoots. The mean number of adventitious shoots produced per explant were 3.5 and 2.1 for hypocotyls and cotyledons, respectively. These shoots were established as proliferating shoot cultures upon transfer to MS basal medium with Gamborg B5 vitamins supplemented with 10 µM BA plus 10 µM TDZ. Early results show successful (77.1%) rooting of in vitro shoots from hypocotyls when placed on woody plant medium containing 4.9 µM indole-3-butyric acid plus 5.7 µM indole-3-acetic acid. This regeneration system provides a possible basis for developing *F. americana* resistant to the emerald ash borer using *Agrobacterium*-mediated genetic transformation.

Figure 3. The distribution of ash and emerald ash borer



www.nature.org

Introduction:

White ash (*Fraxinus americana*) trees provide both economical and ecological benefits. As an endemic tree to North America, it would adversely impact the environment to lose this species. The urban ash tree industry in the U.S. would suffer, as would the commercial industry, since the hardwood is used in the production of baseball bats, furniture, and cabinets. The emerald ash borer (EAB) is an invasive species that is fatal to a tree once it infests it, and there are no known means of complete eradication or of any innate resistance in *F. americana*. The threat from this pest becomes more urgent with each growing season, making the development of an in vitro plant regeneration system a valuable goal. Such a system would provide a basis for developing resistance through *Agrobacterium*-mediated transformation. As a first step towards this objective, a protocol for adventitious shoot regeneration and rooting was developed for white ash using seedling explants.

Materials and Methods:

Adventitious shoot induction from hypocotyls and cotyledons

The pericarps of *Fraxinus americana* seeds were removed and 2 to 3 mm opposite the radical was removed. Seeds were surface disinfected in 70% ethanol for 1 min, then immersed in a 20% commercial bleach solution for 18 min, followed by three thorough rinses in sterile-distilled water before being stored in sterile-distilled water overnight. The 0-day-old embryos were extracted and the excised hypocotyls and cotyledons were directly cultured on Murashige and Skoog (1962) (MS) 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 were incubated at 24 ± 2˚C under a 16h photoperiod (80 µmol m^{-2} s^{-1}) for 4 weeks.

Elongation of adventitious shoots from hypocotyls and cotyledons

Hypocotyls and cotyledons producing adventitious shoots were 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). Microshoots were continuously cultured for one month, with those elongating removed once they were approximately 2-3 cm long and placed on rooting media.

Rooting of elongated adventitious shoots

Elongated adventitious shoots were cultured on woody plant medium (WPM) (Lloyd and McCown, 1980) supplemented with 4.9 µM indole-3-butyric acid (IBA) plus 5.7 µM IAA to induce root formation.

Results:

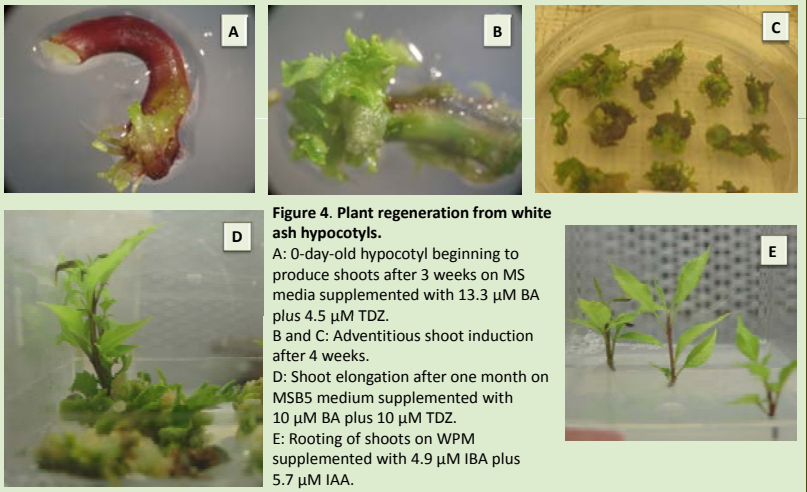


Figure 4. Plant regeneration from white ash hypocotyls.
A: 0-day-old hypocotyl beginning to produce shoots after 3 weeks on MS media supplemented with 13.3 µM BA plus 4.5 µM TDZ.
B and C: Adventitious shoot induction after 4 weeks.
D: Shoot elongation after one month on MSB5 medium supplemented with 10 µM BA plus 10 µM TDZ.
E: Rooting of shoots on WPM supplemented with 4.9 µM IBA plus 5.7 µM IAA.

Results:

Figure 5. Shoot regeneration by organ used

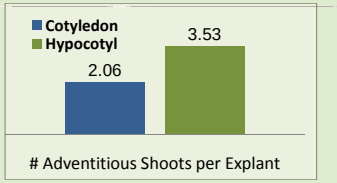


Table 1. Hypocotyl regeneration

Rep #	Callus Formation	Adventitious Shoot Formation	# Adventitious Shoot/Explant	Root Formation
1	100.00%	91.67%	4.90	85.71%
2	94.12%	52.94%	3.80	100.00%
3	93.33%	41.67%	2.55	45.45%
4	84.62%	69.23%	2.85	n/a
Avg.	**93.02%**	**63.88%**	**3.53**	**77.06%**
St.Dev.	0.06	0.22	1.06	0.28

Table 2. Cotyledon regeneration

Rep #	Callus Formation	Adventitious Shoot Formation	# Adventitious Shoot/Explant	Root Formation
1	16.67%	16.67%	4.00	0.00%
2	16.67%	16.67%	2.50	0.00%
3	66.67%	8.33%	1.75	0.00%
4	15.38%	0.00%	0.00	n/a
Avg.	**28.85%**	**10.42%**	**2.06**	**0.00%**
St.Dev.	0.25	0.08	1.66	0

Conclusions:

- Media previously successful in our lab with green ash (*F.* pennsylvanica) proved to regenerate white ash as well. Media was MS supplemented with 13.3 µM BA plus 4.5 µM TDZ.
- The average percent of hypocotyl segments regenerating was 63.9%, and the average percent of cotyledon segments regenerating was 10.4%.
- The average number of adventitious shoots elongated per segment on MSB5 medium supplemented with 10 µM BA and 10 µM TDZ was 3.5 for hypocotyls and 2.1 for cotyledons.
- Roots formed on 77.1% of shoots induced from hypocotyls on WPM containing 4.9 µM IBA plus 5.7 µM IAA, but no adventitious shoots from cotyledons were successfully rooted.

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.