FIELD PERFORMANCE OF IN VITRO PROPAGATED WHITE ASH MICROPLANTS

J.W. Van Sambeek, John E. Preece, and James J. Zaczek[1]

Abstract—White ash (*Fraxinus americana* L.) can routinely be propagated by in vitro axillary shoot proliferation and in vitro rooting of microshoots; however, no reports exist on performance and clonal variation following field planting of microplants. To obtain preliminary estimates of among and within clonal variations, we established a small planting with twelve white ash clones in 1992. Ten non-stratified seed from fifteen individual tree collections were cut and germinated in vitro on a medium consisting of Murashige and Skoog (MS) salts and organics, thidiazuron, 6-benzyladenine, indole-3-butyric acid (IBA), 2 to 3 percent sucrose, and 0.7 percent agar. In vitro germinants exhibiting high axillary shoot proliferation rates were repeatedly subcultured to produce microshoots for in vitro rooting experiments.

Microshoots were pulsed for 1 week on quarter-strength MS medium supplemented with various combinations of 1 to 5 uM of IBA and naphthaleneacetic acid. Auxin-pulsed microshoots were then transferred to 0.25X MS medium without plant growth regulators where most microshoots produced up to seven adventitious roots within 1 to 2 weeks. When 6 to 8 weeks old, microplants were transplanted to soil in rootrainers and acclimatized under mist to greenhouse conditions. In the greenhouse, most microplants showed one flush of new shoot growth and extensive root development from the adventitious roots produced during the in vitro rooting phase. Microplants were overwintered in a cooler, then moved to a shade house for a month, and planted as in-leaf containerized stock in June 1992 at the SIU Tree Improvement Center in Jackson County, Illinois.

Overall the microplants averaged 2.7 adventitious roots per microshoot following in vitro rooting. Microplants from Family 14 averaged more than 3.2 adventitious roots compared to only 1.4 adventitious roots for Family 13 with the fewest roots. Average height for the original in-leaf planting stock ranged from 5 to 50 cm following field planting. Most microplants produced little additional height growth in the field the first growing season. Early in the third growing season, many microplants produced abnormal leaves and lateral shoots presumably in response to herbicide drift that resulted in multiple short shoots from the axillary nodes and narrow leaflet blades on short leaves. Net height growth averaged less than 20 cm for the third growing season. Severity of symptoms declined in the fourth growing season and most trees had recovered by the fifth growing season. Net height growth is averaging more than 70 cm per year and individual tree heights range from 0.3 to 5.0 m after five years. Multiple regression analysis of fifth year height indicated that the number of adventitious roots and severity of deer browse damage were not related to fifth year tree height; however, putative herbicide damage negatively affected growth.

Most clones have retained similar height rankings from the second growing season through the sixth growing season. Exceptions are Clone 14-10, one of the shortest clones at planting and now one of the tallest after six years, and Clone 15-2, the tallest clone at planting and now in the shortest one-third of the clones after six years. Clone 99 was one of the shortest clones at planting and is now above average in height. Interestingly, Clone 99 originated from organogenic callus, produced thin microshoots in vitro, and had a low in vitro rooting percentage. Initially, variation for height among clones was more than six times that of within clonal variation. After the six years, variation among clones was only twice that of the within clone variation.

Survival of the microplants of all clones after six years was between 70 and 100 percent except for Clone 6-3 where all microplants had died. Microplants of Clone 6-3 produced the fewest adventitious roots during the in vitro rooting period and had the lowest survival during greenhouse acclimatization. In 1997 the basal stem diameter averaged 6.3 cm for all the microplants. Microplants from clones of Family 2, 6, and 14 averaged 7.8 cm in diameter and were 3.8 cm larger than the microplants from Family 5 and 13, the families with the slowest growing microplants. Substantial variation existed among the clones within some families. For example, the basal diameter of Clone 6-1, one of the fastest growing clones, averaged 9.9 cm compared to only 5.7 cm for Clone 6-6 both from Family 6. Likewise, the basal diameter for Clone 4-6, another one of the fastest growing clones, averaged 7.8 cm compared to 4.3 and 4.7 cm for Clones 4-4 and 4-7 from the same single tree collection.

In conclusion, in vitro propagated microplants of white ash planted as in-leaf containerized stock can be successfully established in field plantings. Within clone variation for growth was significantly reduced when compared to among clonal variation. Additional field plantings using microplants from white ash exhibiting a wider range of in vitro axillary shoot proliferation rates and more clones per family are needed to test these preliminary results.

[1] Research Plant Physiologist, USDA Forest Service, North Central Forest Experiment Station, Univ. of Missouri, Columbia, MO 65211-7260; Professor, Department of Plant, Soil, and General Agriculture, Southern Illinois Univ., Carbondale, IL 65201-4415; and Assistant Professor, Department of Forestry, Southern Illinois Univ., Carbondale, IL 62901-4411, respectively.

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