

PERFORMANCE OF TWO CULTIVARS OF RED MAPLE FROM TISSUE-CULTURED VERSUS BUDDED PROPAGATION

by Jeff L. Sibley, D. Joseph Eakes, Charles H. Gilliam, Gary J. Keever, and William A. Dozier, Jr.

Abstract. 'Franksred' and 'October Glory' red maple (*Acer rubrum*) from tissue culture were compared in an existing field study to their budded counterparts to determine differences in annual growth rate, leaf morphology characteristics, leaf greenness, initiation of fall color, and gas exchange capacity. There were no differences between propagation methods for the two cultivars in height or caliper increases, fall color patterns, or gas exchange capacities.

Current methods of propagation for cultivars of red maple include softwood cuttings (7,10,12), tissue culture (1,9,13) and budding onto seedling understocks (8). Losses exceeding 50% in the first year and an additional 10 to 20% in the second year as a result of bud union incompatibility have been reported for selections of the species (2,7,10). Bud union incompatibility occurs where there is an initial "take" between stock and scion, but poor growth of the scion or actual breakage at the bud union occurs early in production or after some years in the field (8). In a previous red maple evaluation conducted at the Piedmont Alabama Agricultural Experiment Substation (2), bud union failure was evident in eight of nine cultivars tested within three years after planting. All trees were budded onto seedling understocks. Ten years after planting the only cultivar without visible compatibility problems was 'Franksred' (Red Sunset™). 'October Glory' was not included in these previous evaluations.

Limited data are available for red maple where budded and tissue-cultured microplantlets were compared. The objective of this study was to determine the influence of tissue culture and budded origins on two selections of red maple. Specific characteristics evaluated included mor-

tility, annual growth, leaf morphology characteristics, leaf greenness, initiation of fall color, and gas exchange capacities. Gas exchange was measured to devise a rapid screening technique whereby stress from bud union failure might be detected prior to physical evidence.

Materials and Methods

'Franksred' and 'October Glory' were obtained in March 1988 from A. McGill & Son, Fairview, OR as tissue-cultured microplantlets and as budded trees on seedling rootstocks. Trees were containerized in 2.8 liter pots in an amended 6:1 (v:v) pinebark/sand medium and grown in a double layer polyhouse for three months, then moved outdoors under overhead irrigation for the remainder of the growing season. In 1989, trees were transplanted to 9.5 liter containers for another 12 months. Trees ranged from 1.2 to 1.5 m in height when transplanted in March 1990 into a Cecil gravelly sandy loam soil at the Piedmont Substation, Camp Hill, AL (lat. 32° 83' N, long. 85° 65' W). Selections were interplanted within a cultivar trial with 12 other red maple selections in a randomized complete block design with 5 blocks of 2 plants each. Trees were planted on a 9.1 x 10.7 m spacing and fertilized with 59 g of nitrogen (N), as 13N-5.6P-10.8K (13-13-13), per 2.5 cm of caliper measured 30 cm above ground level, at planting and annually in March prior to bud break. Drip irrigation was supplied to each tree based on 100% replacement of net evaporation from a class A pan. Height and caliper increases were determined by differences in current and the

previous year measurements through 1994.

Ten leaves from the midpoint of current season's growth were harvested at random from each tree monthly May through September, 1993 for total leaf area and petiole length determinations. Leaf area was determined with a leaf area meter, (LI-3050A, LI-COR Inc., Lincoln, NE).

Similar leaf samples were collected from each treatment within one block in August and September, 1993. Stomatal density was calculated using an eyepiece reticle with an area of 0.0156 mm² at 40x magnification. Means were derived for each cultivar from five leaves per tree from each propagation method, with 4 fields per leaf, for a total of 20 observations per cultivar per propagation method.

A paper hole punch was used to remove 25 discs (0.31 cm²/disc) from each of six leaves of each red maple selection (150 total disc/tree) collected in August, 1993. This work was repeated in September using three leaves. A SPAD-502 Chlorophyll Meter (Minolta Camera Co., Ltd., Japan) was used to assess leaf greenness. Chlorophyll concentrations were determined and averaged for each of the discs immediately after they were excised with a hole punch. A total of 600 SPAD-502 measurements per cultivar were taken.

Foliar nitrogen (N) levels were determined in August and September 1993 on discs from chlorophyll determinations with a LECO CHN-600 Analyzer (LECO Corp., St. Joseph, MI). Discs were dried at 80°C, ground in a cyclone mill to pass a 0.5 mm sieve, weighted, and analyzed by combustion.

Foliar fall color patterns and defoliation rates were evaluated twice weekly, from September through December in 1992, 1993 and 1994. Fall color patterns were established by the following guidelines: 1) Color initiation was considered to be the point when all trees of a cultivar had at least 1% color or when the average color for a cultivar was greater than 5%. 2) Color cessation occurred at the point when color was 0% in over 50% of samples or when the average defoliation of the cultivar exceeded 85%. Defoliation ratings began when 30% of the cultivar samples exhibited some defoliation and was considered complete when the average defoliation for the cultivar exceeded

95% or when 70% of the trees were completely defoliated.

Methods for measurement of CO₂ exchange rate followed Jurik (5), who reported that CO₂ exchange rates of sugar maple leaves at light saturation increased to a maximum near the completion of leaf expansion in early June. Gas exchange rates were then constant until mid-September, when they declined rapidly until leaf death. Gas exchange measurements (net photosynthesis (P_n), stomatal conductance (C_s), and transpiration (E)), were taken from 8:00 AM until 2:00 PM CST at an average photosynthetically active radiation (PAR) level of 1474 μmol/m²/s. Evaluations were repeated under similar PAR levels in June and September 1992, and June and August 1993. Gas exchange measurements were made with a LI-6250 Portable Photosynthesis System (LI-COR Inc., Lincoln, NE) in a closed mode (6), allowing a leaf to decrease the CO₂ concentration in the one-liter chamber over a 20 second period. Three gas exchange measurements were made on each tree within each replication. Measurements were made on attached, mature leaves growing in full sun at the mid-point of current seasons growth and tree canopy. Assimilation rates were measured over a 45 minute period within each replication with CO₂ concentrations ranging from 320 to 390 mg/liter at near constant leaf temperatures of 32°C.

Night respiration rates were determined in July and August 1993, on consecutive nights between 10:00 PM and 2:00 AM. Night respiration rates were determined in the same manner in which P_n rates were measured in the day. Measurements were made under full moon light with supplemental light only from the diode on the LI-COR monitor.

All data, with the exception of fall color evaluations, were subjected to analysis of variance. Differences between propagation methods for each cultivar were determined by Duncan's Multiple Range Test at *P* = 0.05.

Results and Discussion

During the first five growing seasons in the field, there were no differences in annual mean height increases between tissue cultured and budded plants for either cultivar. Mean height growth

attained for budded 'Franksred' was 21 cm greater annually in this study than that reported from previous evaluations at the same substation (2). Differences may be attributed to trickle irrigation for the current study, and no supplemental irrigation in the previous evaluations. Final mean height for 'Franksred' from tissue culture and budded propagation were 481 cm and 465 cm, respectively. No 'Franksred' trees have been lost to date from bud union incompatibility. However, variability in total final height for individual budded trees had a range of 145 cm from the smallest to the largest tree. The difference in total final height for tissue cultured trees was 100 cm from smallest to largest.

Final mean height for 'October Glory' from tissue culture and budded propagation were 510 cm and 492 cm, respectively. One 'October Glory' was lost in July 1993 to bud union incompatibility. No physiological or physical evidence of bud union problems were evident prior to the tree breaking off during heavy winds at ground level. Variability in total final height for individual budded trees was greater than 204 cm from the smallest 'October Glory' (360 cm) to the largest tree (564 cm). Difference in total final height for individual tissue cultured trees was 104 cm.

Annual increases in mean caliper were not different for tissue cultured versus budded plants for either cultivar with the exception of 1993 increases for 'Franksred'. Tissue cultured 'Franksred' trees had 21% more mean caliper growth than

budded trees in 1993. Increases seen in the mean caliper growth for budded 'Franksred' were again greater annually under irrigation than those reported in earlier evaluations (2). Final caliper for 'Franksred' from tissue culture and budded propagation were 9.9 cm and 9.2 cm, respectively. Final mean caliper for 'October Glory' from tissue culture and budded propagation were 11.9 cm and 10.7 cm, respectively.

Additional evaluations were made in an effort to detect differences that might indicate bud union incompatibility. The only difference noted for 'Franksred' was a greater stomatal density on the trees from tissue cultured origin (76,400 per cm²) compared to those budded onto seedling rootstocks (70,200 per cm²). However, 'October Glory' had differences in leaf area, petiole length, stomatal density, and leaf greenness (Table 1). There were no differences in foliar N levels for the two cultivars when propagation methods were compared (data not shown). Foliar N levels for 'Franksred' were similar to reports by others (4).

For cultivars grown under the same conditions, the SPAD-502 Chlorophyll Meter can be a useful tool in ranking differences in leaf greenness (11). Often leaf greenness is considered to be highly correlated with foliar N levels. However, in this study, 'Franksred', generally accepted among growers to have the deepest green color of the red maple cultivars, had a lower N level (2.18%) than 'October Glory' (2.52%), while having higher SPAD-502 values (Table 1).

Table 1. Leaf characteristics^z of select *Acer rubrum* cultivars from tissue cultured versus budded propagation.

	Average leaf area (cm ²)	Petiole length (cm)	Stomatal no./ cm ²	Chlorophyll level (SPAD)
Franksred				
Tissue cultured	53.37 a ^y	9.26 a	76,410 a	52.0 a
Budded	53.83 a	9.79 a	70,192 b	52.5 a
October Glory				
Tissue cultured	61.87 a	17.09 a	64,423 b	43.2 b
Budded	57.55 b	15.52 b	73,076 a	44.0 a

^z Means by column derived from: 80, 80, 40, 1200 leaf samples, respectively.

^y Means separation by cultivar (tissue cultured versus budded) within columns by Duncan's Multiple Range Test, *P* = 0.05.

Values of leaf greenness were basically the same for each cultivar from both propagation methods. There was a gradual increase in greenness over the summer for both cultivars (data not shown). 'Franksred' with a mean SPAD value of 47.4 in May, had a mean SPAD value of 52.3 in September. Mean SPAD values for 'October Glory' increased from 35.7 in May to 44.6 in September.

Fall color initiation for 1992 became evident on both cultivars about October 20. There were no differences in fall color patterns, duration, or intensity from either propagation method (data not shown). The longest duration of fall color for 1992 was on 'October Glory' with about 35 days of color, while 'Franksred' had about 27 days of color. Fall color patterns began earlier in 1993 for both cultivars. 'Franksred' began to show fall color by October 6 and color extended about 34 days. Notable fall color development began on 'October Glory' about October 15, extending for about 34 days. Fall color patterns for 1994 were similar to previous years for both cultivars. 'Franksred' began to show fall color by October 6 and color extended about 18 days. Notable fall color development began on 'October Glory' about October 20, extending for about 30 days.

There were no differences in daily gas exchange capacities for either cultivar (data not shown). There were no differences in night respiration rates for each cultivar when analyzed independently (data not shown). At this point, no rapid screening technique for monitoring bud union incompatibility has been developed due to a lack of bud union failures.

Either method of propagation appears to be suitable for 'Franksred' and 'October Glory'. In this study losses have been minimal to this point. Therefore, selecting a propagation method based on production costs of a particular method is justified for these two cultivars. Bracken (1), Flemer (3), and Schwab (10) address economic concerns for selecting one propagation method over another. In many nurseries the sequencing of production schedules determines the merit of selecting propagation methods (1). Although many red maple cultivars have exhibited bud union problems, serious concern for incompatibility problems in

'Franksred' and 'October Glory' cannot be substantiated from this study at this time. There are many advantages for growers who bud or graft as a standard means of propagation in a number of species, however, the variability of trees from budded production can be difficult to handle profitably for these two cultivars.

Literature Cited

1. Bracken, M. 1988. *Pros and cons of trees from tissue culture*. Proc. Int. Plant. Prop. Soc. 38: 451-453.
2. Fare, D.C., C.H. Gilliam, and H.G. Ponder. 1990. *Acer rubrum cultivars for the south*. J. Arboric. 16: 25-29.
3. Flemer, W.E., III. 1989. *Why we must still bud and graft*. Proc. Int. Plant. Prop. Soc. 39: 516-522.
4. Gilliam, C.H., S.M. Still, S. Moor, and M.E. Watson. 1980. *Effects of three nitrogen levels on container-grown Acer rubrum*. HortScience 15: 641-642.
5. Jurik, T.W. 1986. *Seasonal patterns of leaf photosynthetic capacity in successional northern hardwood tree species*. Amer. J. Bot. 73: 131-138.
6. Mitchell, C.A. 1992. *Measurement of photosynthetic gas exchange in controlled environments*. HortScience 27: 764-767.
7. Moller, G.M. 1985. *How one Oregon grower produces trees from softwood cuttings*. Amer. Nurs. 162(5): 68-69.
8. Santamour, F.S., Jr. 1992. *Predicting graft incompatibility in woody plants*. Proc. Int. Plant. Prop. Soc. 42: 131-134.
9. Schilpzand, R.T. 1989. *How tissue culture can be the answer*. Proc. Int. Plant. Prop. Soc. 39: 523-526.
10. Schwab, B.W. 1979. *New techniques for growing west coast trees*. Amer. Nurs. 149(9): 70-72.
11. Sibley, J.L., D.J. Eakes, C.H. Gilliam, and W.A. Dozier, Jr. 1994. *Potential of SPAD-502 chlorophyll meter for determination of leaf greenness*. SNA Res. Conf. 39:(in press).
12. Still, S.M. and B.H. Lane. 1984. *Influence of extended photoperiod and rooting media fertility on subsequent growth of Acer rubrum L. 'Red Sunset'*. Scientia Horticulturae 22: 129-132.
13. Suttle, G.R.L. 1992. *Micropropagation of select deciduous trees and shrubs*. Com. Proc. Int. Plant Prop. Soc. 42: 415-416.

Department of Horticulture
Auburn University
Auburn University, AL 36849