Desiccation Tolerance of Green Ash and Sugar Maple Fine Roots[1]

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Abstract

Exposed fine roots are subject to desiccation, which may affect their survival as well as new root growth following bare root transplanting. Fine roots of dormant 1-year-old green ash (*Fraxinus pennsylvanica*) and sugar maple (*Acer saccharum*) seedlings, subjected to desiccation treatments of 0, 1, 2, or 3 hours in December and March, lost up to 82 percent of their water. Root electrolyte leakage, a measure of cell damage, tripled after three hours of desiccation. The increase was moderately, but significantly, greater in March for both species. Desiccation treatments had no effect on fine root survival. Growth of new roots (RGP) was also unaffected by desiccation treatments. RGP of maple was greater in March than December, but not ash.

Index words: root injury; root electrolyte leakage, root moisture content, root growth potential, bare root.

Significance to the Nursery Industry

Preventing desiccation of exposed roots during bare root transplanting is considered important to minimize loss of the fine roots and the resulting reduction of the newly planted tree's ability to absorb water. Just how much damage is caused by desiccation of fine roots is not well understood. This study showed that fine roots can survive serious desiccation and produce new root growth. In showing that fine roots do survive desiccation and are the source of most new root growth, this study provides even greater incentive to protect fine roots from desiccation during bare root transplanting.

Though fine roots subjected to desiccation treatments lost up to 81 percent of their water content and cell damage tripled, 50 to 85 percent of the new root growth originated from roots less than 1 mm (0.04 in) in diameter, and 85 to 97 percent on roots less than 2 mm (0.08 in) in diameter. Severe, but short-term, desiccation caused no reduction in fine root survival or new root growth. Fine roots may not be seriously harmed during normal bare root transplanting in the nursery or landscape, though keeping fine roots as moist as possible is always recommended.

Introduction

Bare root transplanting can subject fine roots to at least moderate desiccation under the best of conditions. Handling root systems under less than ideal conditions may subject fine roots to more severe desiccation. Desiccation is the most likely stress factor experienced by bare root trees before planting (20).

Exposure to the atmosphere for even a short time is a concern. Most moisture and nutrient absorption occurs in fine roots, and the greatest risk of damage from desiccation occurs in fine roots (2, 10). The majority of water loss from fine roots, and associated increase in root electrolyte leakage (REL) from cell damage, occurs within three hours of exposure at 11–15C (52–59F) (2, 17, 18) and as little as 60 minutes at 20C (68F) (15).

Most published studies on the effect of desiccation of roots focus on relating the amount of root tissue drying [root moisture content (RMC)] or physiological damage (REL) to post-planting survival and growth of seedlings (12). Attempts to develop these 'measures' as predictors of seedling survival have sometimes been successful (15, 17, 18, 21), but not always (8, 9, 21).

Root growth potential (RGP, also called root growth capacity) has frequently been used to assess viability of root systems after desiccation treatments (4, 7, 8, 10, 21). RPG has been defined as 'the capacity of roots or root systems to extend existing roots and initiate new ones' (23). The method used to quantify RPG varies (19), but typically involves some measure of new roots (number, length, weight), under specified conditions over a certain period of time. RGP varies with time of year (11). The traditional bare root planting seasons in temperate climates, late fall and early spring, correspond to periods of high RGP in deciduous species (8, 13, 14). Desiccation treatments have been shown to reduce RGP (4, 7, 8, 10, 21). A strong relationship between RGP and field performance has been demonstrated (3, 7, 8, 14, 19).

RGP is a measure of a root system's ability to produce new roots, but alone is not a direct measure of survival of existing roots, as initiation of new roots can occur on remaining live roots proximal to the dieback. Fine root removal resulted in a similar reduction of RGP as the desiccation treatment (3, 10), implying the loss of fine roots from desiccation.

The objective of this study was to determine the extent that desiccation can damage fine roots, and to understand how injury to fine roots will affect new root growth.

Materials and Methods

Seedlings were used for the study since many cultivars use seedlings as rootstock, and the adventitious root systems produced from cuttings could possess characteristics not typical of natural root systems. Dormant 1-year-old green ash (*Fraxinus pennsylvanica*) and sugar maple (*Acer saccharum*) seedlings were obtained from a commercial source (Lawyer Nurseries, Plains, MT). The primary roots were pruned to 15 cm (5.9 in) in length, and the seedlings were planted in plastic containers 35 cm (14 in) D × 15 cm (6 in) H to allow as much room for lateral root growth as possible. The containers filled with medium sand [predominantly 0.25–0.50 mm (0.01–0.02 in) particle size] to facilitate later removal of roots with minimum root damage. Seedlings were grown for one growing season in the greenhouse at ambient light and temperature conditions to establish a vigorous root system.

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All containers were moved to plastic covered hoop house shelters in October to induce dormancy before the December desiccation treatment date, and were kept there over winter. No supplemental heating was provided.

Desiccation treatments took place on separate groups of dormant plants in December and March to approximate conditions at the time of fall and spring dormant bare-root transplanting in the upper Midwest. For each species, of the original bundle of 100 seedlings purchased, the 80 most uniform seedlings were selected and grown for one season, as indicated above. Prior to the first treatment, the seedlings were graded by overall size and vigor and the most uniform 72 seedlings were selected and divided first into two groups of 36 for treatment month, and further into four groups of 9 replications for each of the four desiccation treatments (including control) so that each treatment group contained similar variability.

Root systems were carefully removed from the sand and gently washed. Each species and desiccation treatment combination (9 plants each) was implemented separately so that the roots would be out of the soil for a minimum of time before and after the desiccation treatment. Controls were removed from the soil and immediately replanted.

Methods reported for similar fine root desiccation work have ranged broadly from outdoor (7) or indoor (7, 10) ambient conditions, to precise control [8–32C (46–90F), 30–80% humidity, and 2–6 hours] in a growth chamber (2, 15, 17, 18). The method chosen for this study was intended to simulate exposure experienced by fine roots of bare root plants during local handling and planting process.

Plants were laid on a tabletop in the lab at room temperature [20C (68F)], 60 cm (24 in) from an overhead 500 watt halogen lamp for 0, 1, 2 or 3 hr, and turned at 30 minute intervals. Preliminary tests showed that these conditions produced levels of REL similar to other studies reported in the literature that reduced RGP (8).

A methylene blue staining technique shown to be effective in distinguishing newly regenerated roots from previously existing *Malus* roots was used (1). After desiccation treatments, root systems were stained by dipping in 1% (w/v) methylene blue for 15 seconds, and then placing them between moist paper towels for 10 min to allow excess dye to drain from the root system. The seedlings were then repotted in the same containers of sand. December treated plants were returned to the hoop house shelters for the winter. Following the March desiccation treatment, all plants were moved to the heated greenhouse with supplemental light to grow for 60 days. At the end of this time, the seedling roots were again carefully removed from the sand for analysis.

REL was used to compare the physiological status of roots after desiccation treatment following the method described by Shirazi and Fuchigami (22). Approximately 100 mg (0.0035 oz) fresh weight of fine roots (< 1 mm diameter) was sampled from three locations on each root system of the treated and untreated seedlings after treatment. Roots were rinsed in deionized water of known conductivity, placed in vials with 15 ml (0.51 oz) of the same water, shaken at 20C (68F) for 24 hr, and then the conductivity of the bathing solution was recorded. The roots were killed and all membranes ruptured by immersion in distilled water at 80C (176F) for 1.5 hr, shaken for 24 hr, and the final conductivity recorded. REL was expressed as a percentage of the heat killed value after subtracting conductivity of the distilled water.

Root moisture content (RMC) is the measure of desiccation often reported in the literature, and is expressed as a percent of oven-dry weight (9, 17, 18). RMC was determined using samples of similar size and location as for REL. The fine root samples were weighed after desiccation and again after oven-drying (80C) for 48 hr.

Root moisture loss (RML) was calculated in addition to RMC, as a more direct expression of the amount of water lost from the root tissue. Fresh weight of samples could not be measured directly before desiccation treatments, so it was estimated by calculating percent weight loss of similar samples from each tree. The average of fresh weight / oven-dry weight ratio of 27 subsamples (9 root systems × 3 samples) for each species and treatment combination were averaged and multiplied times the oven-dry weight of each individual desiccated sample to estimate the fresh weight equivalent (FWE). The water content of desiccated RMC samples was used for RML. RML was estimated by the formula: RML = (FWE – desiccated weight) / FWE × 100.

After the 60 days of active growth in the greenhouse, the seedling roots were again carefully removed from the sand for RGP analysis. All lateral roots on the upper ten centimeters of the primary root, were examined for new root growth (unstained roots). The data recorded for each new root were diameter of the 'parent root', origin of new root growth (new lateral or extension of a previously existing root tip), and length of each new fine root. RGP was expressed as the total length of all new roots.

Each species was considered a separate experiment. Dead trees were treated as missing data points for measurements taken on dates after death. REL, RMC, RML, RGP and fine root survival data were analyzed with two-way ANOVA with month and treatment time as main factors. When they were significant ($P \leq 0.05$), treatment × month interactions were presented. If interactions were not found to be significant, then the main effects of the treatments were tested. Where significant effects were found ($P \leq 0.05$), means were compared using Holm-Sidak method ($P \leq 0.05$) (SigmaStat 3.0, SPSS Science).

Results and Discussion

Assessment of treatment effects. REL, RMC and RML were used primarily as indicators of the severity of the desiccation treatments. Desiccation treatments did cause cell damage in fine roots, as indicated by an increase in REL. REL was affected by both drying time and month, with significant interaction in both species. Just one hour of desiccation increased REL significantly for both species, in both treatment months. In green ash, REL was significantly greater after two hours than after one hour in both December and March, and still greater after three hours in March. In sugar maple, REL increased after the second hour of drying in March, but no further increases in REL occurred after the first hour in December (Table 1).

After three hours, REL was higher after desiccation treatment in March for both species, but the effect of shorter desiccation times was not as consistent. REL was higher in December after one and two hours of desiccation in green ash. There was no difference between treatment months in sugar maple (Table 1).

Desiccation treatments significantly reduced RMC in both species. In green ash, there was a significant reduction in RMC after the first hour of desiccation, reaching a maxi-

Table 1. **Measurements used to determine effectiveness of desiccation treatments. Root Electrolyte Leakage (REL), Root Moisture Content (RMC), Root Moisture Loss (RML).**

Desiccation time (hr)	REL (%)		RMC (%)		RML (%)
	March	December			
Green ash					
0	23.0a[z]z[y]	26.4az	741.1a		0.0a
1	44.8bz	54.1by	232.5b		37.8b
2	64.5cz	77.4cy	121.2c		66.8c
3	89.6dy	78.7cz	102.2c		70.2c
Significance					
Time[x]	***		***		***
Month	NS		NS		NS
T × M	**		NS		NS
	March	December	March	December	
Sugar maple					
0	29.4ay	18.0az	521.9az	1156.9ay	0.0a
1	70.7bz	77.7bz	118.4bz	162.2bz	70.4b
2	79.0cz	76.9bz	83.3bz	151.3bz	78.3c
3	82.1cy	73.0bz	83.4bz	96.5bz	81.2c
Significance[x]					
Time	***		***		***
Month	*		***		NS
T × M	**		***		NS

[z]For each species, values in the same column followed by the same letter are not significantly different at $P \leq 0.05$ (Holm-Sidak).

[y]For each species, values in the same row followed by the same letter are not significantly different at $P \leq 0.05$ (Holm-Sidak).

[x]NS, *, **, *** indicate non-significant or significant at the 0.05, 0.01, or 0.001 levels, respectively. Main effects only (mean of March and December treatments) are presented when there was no interaction

mum after the second hour. Treatment month had no effect, and there was no interaction between treatment month and drying time (Table 1). In sugar maple, RMC was affected by both drying time and month, with significant interaction. RMC was reduced after the first hour of desiccation in both months, but no more with further drying time. Only in control was there a difference between months, with RMC lower in March than December. Lower RMC and higher REL in March in this species may be an indication that fine root damage not related to desiccation treatments occurred between December and March, possibly from cold winter temperatures.

RMC is a simple way to calculate an expression of moisture loss, but values are difficult to interpret since a value of 100 percent is considered low. RML, expressed as a percentage of the total water in the sample, provides a more understandable expression of the amount of water lost after the desiccation treatment. Drying time had a significant effect on both species. Roots lost up to 81 percent of their water content as a result of the desiccation treatments. The pattern of significant changes in RML is similar to RMC data, except that additional water loss was evident in sugar maple after the second hour of desiccation (Table 1). Month of desiccation treatment had no effect and there was no interaction between desiccation time and month.

The desiccation treatments produced REL and RMC values similar to those published for other deciduous species in which RGP, survival and performance after planting were reduced (8, 15, 17, 21, 26). Sugar maple often reached maximum levels after just one hour, compared to two hours or longer for green ash. The magnitude of the changes in sugar maple fine roots was nearly twice that of green ash in the first hour for all three measurements. This may be an indication of greater vulnerability to fine root damage in sugar maple. Based on these data, it was reasonable to expect that the desiccation treatments would cause fine root damage to both species.

Seedling survival. Seedling survival was generally very good. No ashes were lost. One of 9 maples was lost in the 2 hr drying treatment in March. December treated sugar maples had somewhat higher mortality rate with 4, 2, and 1 of 9 seedlings lost from the 0, 1, and 2 hr drying treatments, respectively. Since the most seedlings were lost from the control group, and no plants were lost from the most severely desiccated group, survival appears to have been unrelated to desiccation treatment.

Similar levels of REL and RMC resulting from desiccation treatments reduced survival of deciduous and conifer seedlings in previous studies (8, 17, 18, 26). Favorable growing conditions in the greenhouse may explain the high survival rates after the desiccation treatments compared to more stressful field conditions of reforestation plantings in the previous studies. One report showed better survival after desiccation treatments on more favorable sites with more than 100 mm (3.94 in) rainfall per month during spring compared to more stressful sites receiving less rainfall (15).

Fine root survival. Using new root growth as evidence that fine roots survived the desiccation treatment, drying time had no effect on survival of any size class of fine roots for either species. The only significant difference related to month of treatment was that more roots 1–2 mm (0.04–0.08 in) diameter produced new roots in March than December for both species. There was no difference in the smaller size class. This may have been due to a difference in the ability of these slightly larger roots to generate new roots, rather than to survival of roots. There was no interaction between drying time and month of treatment.

More new roots were produced from roots less than 1.0 mm (0.04 in) diameter than any of the larger size classes for all desiccation treatments and species. This indicates good fine root survival, but may also reflect an overall higher number of roots in this size class (Table 2). There was no difference in fine root survival between month of treatment for any desiccation treatment in green ash, and for all sugar maples except the untreated control group.

The only significant interaction between size class and month of treatment was for the sugar maple control group in which the number of live roots in the < 1.0 mm size class was greater in March than December, and greater than the 1–2 mm (0.04–0.08 in) and 2–3 mm (0.08–0.12 in) size classes in March. There was no difference among size classes in December. The larger numbers of sugar maple live roots in the smallest size class in March is inconsistent with higher levels of damage (REL and RMC) at the same time. An explanation for this was not apparent, but is not likely of consequence since it occurred only in the untreated control group.

Even the smallest fine roots survived severe desiccation quite well, though as much as 81 percent of their water was lost. Fine roots have been reported to survive periods of

Table 2. Average number of live fine roots by size class after desiccation treatments, as determined by the production of new root growth after treatment.

	Size class (mm)			Significance		
	< 1.0	1.0–2.0	2.0–3.0			
	# roots	# roots	# roots	Size class	Month	S × M
Sugar maple						
Desiccation time (hr)						
0 March	224a[z]z[y]	36ay	2ay	***	**	*
0 December	60bz	9az	2az			
0[x]	142a	23a	2a			
1	122az	20ay	3ay	***	NS	NS
2	103az	13ay	8ay	***	NS	NS
3	170az	35ay	11ay	***	NS	NS
Treatment month						
March	159a	31a	7a			
December	111a	15b	5a			
Significance[w]						
Desiccation time	NS	NS	NS			
Treatment month	NS	*	NS			
T × M	NS	NS	NS			
Green ash						
Desiccation time (hr)						
0	69az	35ay	11ax	***	NS	NS
1	76az	29ay	11ay	***	NS	NS
2	95az	44ay	5ax	***	NS	NS
3	105az	29ay	10ay	***	NS	NS
Treatment month						
March	77a	44a	11a			
December	95a	25b	8a			
Significance[w]						
Desiccation time	NS	NS	NS			
Treatment month	NS	**	NS			
T × M	NS	NS	NS			

[z]For each species, values in the same column followed by the same letter are not significantly different at P ≤ 0.05 (Holm-Sidak).

[y]For each species, values in the same row followed by the same letter are not significantly different at P ≤ 0.05 (Holm-Sidak).

[x]Significant interaction between month and size class in this group, main effects data listed for desiccation treatment comparison only.

[w]NS, *, **, *** indicate non-significant or significant at the 0.05, 0.01, or 0.001 levels, respectively. Main effects (mean of March and December treatments, or 0, 1, 2, and 3 hr desiccation time) are presented when there was no interaction.

drought by entering a dormant state (6, 16). This may help to explain how the fine roots survived after such severe desiccation.

New root growth. Fine roots survived in large numbers and most new root growth was initiated from them. New laterals accounted for 99 percent of all new root growth initiated by both species for all treatments. There was virtually no new root growth from the existing root tips. This is contrary to previous reports that almost all new root growth of bare-root seedlings was produced by extension of pre-existing root tips (3, 5). The previous reports were on conifers, but deciduous species roots are generally considered less sensitive to exposure (9). Since there was little growth from root tips in the control group as well, it is not likely that desiccation treatment damaged the root tips. It is possible that the dye treatment damaged the sensitive meristematic tissue in the root tip. The study from which this method was adapted did not include analysis of root tip growth after treatment (1).

Desiccation treatments had no effect on RGP in either species (Table 3). Since the desiccation treatments caused no fine root mortality, the lack of desiccation treatment effect on RGP is understandable. Month of treatment affected only

Table 3. The effect of desiccation time and treatment month on Root Growth Potential (RGP). Each species was considered a separate experiment.

	Root growth potential (mm)	
	Sugar maple	Green ash
Desiccation time (hr)		
0	5159a	5271a
1	5575a	4444a
2	3668a	6257a
3	6794a	5565a
Treatment month		
March	6378a[z]	5486a
December	4220b	5284a
Significance[y]		
Desiccation time	NS	NS
Treatment month	*	NS
T × M	NS	NS

[z]For each species, values in the same column followed by the same letter are not significantly different at P ≤ 0.05 (Holm-Sidak).

[y]NS, *, **, *** indicate non-significant or significant at the 0.05, 0.01, or 0.001 levels, respectively. Main effects (mean of March and December treatments, or 0, 1, 2, and 3 hr desiccation time) are presented when there was no interaction.

sugar maples, which showed increased RGP in March and is consistent with the greater number of live sugar maple fine roots in March. There was no interaction between drying time and month for either species.

The lack of reduction in RGP after desiccation treatment is also inconsistent with literature reports. Desiccation treatments resulting in similar levels of REL (8) or RMC (21, 26) did reduce RGP in previous studies. In all studies, including this one, RGP was assessed under similar growing conditions in greenhouses or growth chambers (4, 7, 8, 21). The reason for the different result in this study is not clear, though species differences cannot be ruled out.

Though fine roots subjected to desiccation treatments lost up to 81 percent of their water content (RML) and cell damage (REL) tripled, 50 to 85 percent of the new root growth originated from roots less than 1 mm in diameter, and 85 to 97 percent on roots less than 2 mm (0.08 in) in diameter. Severe, but short-term, desiccation caused no reduction in fine root survival or new root growth (RGP). Death of fine roots during normal bare root transplanting in the nursery or landscape may not be a serious concern, though keeping fine roots as moist as possible is always recommended.

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