

## Reducing Drought Stress in Transplanted Trees Using Mycorrhizae<sup>©1</sup>

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**High mortality rates among most species of nursery trees after transplanting is generally attributed to water stress imposed by a range of soil and other environmental conditions. This study examined the efficacy of a consortium of mycorrhizae (Root Rescue Landscape Powder), comprised of 20 species of both endo- and ecto-mycorrhizae, in mitigating water stress when inoculated into the root zone of recently transplanted trees [*Thuja occidentalis* ‘Smaragd’ (emerald pyramidal cedar) and *Acer rubrum* ‘Brandywine’ (Brandywine red maple)]. The water status of the trees was monitored with automated stem psychrometers measuring stem water potential ( $\Psi$ ) at 30-min intervals for at least 2 weeks after transplanting. Treated trees exhibited a significant reduction in mid-day water stress and enhancement of overnight rehydration, relative to control trees, when inoculated with the consortium of mycorrhizae, as shown by diurnal patterns of water stress and recovery.**

### INTRODUCTION

Irrigation of nursery plants in Canada consumes approximately 180 million m<sup>3</sup> of water annually. This represents a significant portion of the water, not allowing for the recycled portion, used by the ornamental horticulture sector (Deloitte and Touche, 2009). The majority of this nursery irrigation water is applied using overhead sprayers, which remains one of the least efficient forms of irrigation for above ground [containerized] crops (Howell, 2003). With ever increasing pressure on water resources the nursery industry is an obvious candidate for continued development of more efficient water management practices.

There are few technologies that can reliably measure the effects of water management practices on nursery crops (Jones, 2004). This technology vacuum represents a significant challenge to environmental stewardship in the perennial nursery industry. Conventional agronomic measurements such as caliper, foliage density, plant height, and qualitative assessments of plant vigour are insufficient in providing data appropriate for interpreting water management strategies in a timely manner (Jones, 2008). Other factors which must be accounted for in the water management strategy include contributions from precipitation events; leaching fraction for container grown plants; applications of pesticides, herbicides, and nutrient fertilizers; and control of run-off.

It has long been understood that the plant is the most reliable “sensor” of its environment. Plants routinely and continuously integrate the effects of all the environmental variables to which they are exposed. Significant changes in any of these variables (e.g., temperature, humidity, light, CO<sub>2</sub>, nutrients, and water) are ultimately reflected in the water status of plants. Reliable plant water status data, in addition to other currently used metrics, could offer significant improvements in water use efficiency through improved irrigation scheduling (Howell, 2007).

The study of plant water relations has produced a number of techniques to measure total

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<sup>1</sup> The presented data is a subset of a larger data set presented elsewhere.

plant water potential ( $\Psi$ ) or the value of the suction forces in the water conducting tissues of a plant in response to evaporative and osmotic forces. The least invasive and most reliable technique in measuring  $\Psi$  is arguably the thermocouple psychrometer (Dixon and Tyree, 1984). Many psychometric-based systems have been developed and evaluated in the literature (Boyer, 1972; Brown and Tanner, 1981; Campbell and Campbell, 1974; Dixon and Tyree, 1984; McBurney and Costigan, 1982; Millar, 1974; Neumann and Thurtle, 1972); however, the instrument described by Dixon and Tyree (1984) has emerged as the most successful and reliable technique in the field (Dixon et al., 1988; Lee et al., 1989; Edwards and Dixon, 1995a, b; Coffey et al., 1997; Chamberlain et al., 2003). The most recent version of this device (ICT International Pty. Ltd., Armidale, NSW, Australia) was used in the current study to evaluate mycorrhizae inoculation efficacy in the mitigation of drought effects on ornamental tree species.

Root Rescue Landscape Powder (RRLP) is a proprietary compound comprised of 20 species of both endo- and ectomycorrhizae, which can be applied as a water-based root zone inoculum. There is ample evidence in the literature that supports the benefits of mycorrhizal inoculation in a number of applications, such as tree nursery and horticultural production (Marx et al., 1989; Davies et al., 1996), as well as field crops and turf grass management (Fini et al., 2011; Lehto and Zwiazek, 2011; Balakrishna et al., 2006; Auge, 2001). Numerous studies have attempted to quantify the beneficial effects of associating mycorrhizal fungi with the root zones of various plants, especially in relation to drought stress conditions (Al-Karaki, 1998; Abdel-Fatah et al., 2002), but all have lacked suitable evaluation of water potential in response to a fluctuating environment. This underscores the inherent technical difficulty and interpretation problems associated with many attempts to measure water potential in the field. As the dominant physiological variable in assessing plant-environment interactions, it is clear that better temporal resolution and more reliable measurement techniques are required if these interactions are to be reliably and correctly interpreted.

The objective of this study was to evaluate the efficacy of a custom mycorrhizae inoculum for the amelioration of drought stress in commercially significant nursery tree species following transplanting. The course of stem water potential responses was monitored for at least one extended drought period (4-7 days) for each species during the season to determine if there were differences in average plant water status responses between the mycorrhizae treated trees and untreated control trees. The study further served to evaluate the field performance of a fully automated in situ stem psychrometer equipped with wireless data telemetry.

## **MATERIALS AND METHODS**

### **Site Description**

The field trials took place between June and September, 2012 at Connon Nurseries NVK Holdings Ltd., located in Dundas, Ontario (43°21'07.90"N, 79°54'36.86"W). Six species of ornamental trees were planted in a recently constructed berm that comprised of a mix of soil and subsoil from a recently excavated holding pond. It was reasoned that this substrate would be a reasonable facsimile for housing development sites in urban areas and roadside plantings where the majority of these species would typically be planted. Trees were planted approximately 3 m apart and grouped by species. Treatments were randomly assigned within each species block within the field plot. Tree planting was conducted by Connon Nursery staff and was split up into two plantings, one occurring on 14 June 2012 and the other on 7 Aug. 2012.

### **Plant Material and Mycorrhizae Inoculation**

In the spring and summer of 2012, potted trees ready for transplanting were selected and planted in a specially prepared site at Connon Nurseries NVK Holdings Ltd. (Dundas, Ontario, Canada). Although eight species were planted this report will focus on two of them representing both an evergreen and deciduous species [*Thuja occidentalis*

‘Smaragd’ (emerald pyramidal cedar) and *Acer rubrum* ‘Brandywine’ (Brandywine red maple)]. The trees varied in height from 2-3 m and from 0.2-0.3 m in stem caliper at the site of installation of the stem psychrometers. There were 16 trees of each species, which were divided into two groups ( $n=8$ ). One treatment comprised a one-time drench inoculation of the rootball during transplant at the field site. The inoculum consisted of 6 g of Root Rescue Landscape Powder (RRLP) suspended in 10 L of water (Root Rescue Environmental Products Inc., Waterdown, Ontario, Canada). The control group was untreated and both treatment and control trees were provided with the same volume and frequency of manual irrigation and natural rain events throughout the season.

For the purposes of this presentation the results of one representative deciduous species (*A. rubrum*) and one conifer (*T. occidentalis*) will be used to illustrate the water status responses of the transplanted trees to the mycorrhizal inoculations.

### **Water Potential Measurements**

To assess the response to repeat cycles of drought and recovery of the trees in this field setting, each tree was fitted with an in situ stem psychrometer (Dixon and Tyree, 1984) and wireless datalogger system provided by ICT International Pty. Ltd., (Armidale, NSW, Australia). The instruments were installed as shown in Figure 1 and each of two species (32 trees) were monitored simultaneously for periods of up to 2 weeks during the summer. The psychrometer installation procedure is also outlined in detail at: [http://www.ces.uoguelph.ca/psychrometer\\_media.shtml](http://www.ces.uoguelph.ca/psychrometer_media.shtml).

### **Design and Statistical Analyses**

The experiment was laid out as a completely randomized design. Water potential data was analyzed using SAS PROCMixed with repeated measures (SAS Institute Inc., Cary, North Carolina, USA). Stress events were defined as the period immediately after a rain or irrigation event through to the next watering event, provided that the interim period was sufficiently dry to induce water stress as directly measured by the in situ stem psychrometer. Periods that exhibited, or were subjected to, the most stress were designated as “stress phases” and ran from 00:00 HR of the first stress day to 23:59 HR of the last stress day. A diurnal, negative peak in water potential, typically plateauing between 13:00 and 17:00 HR, characterized the maximum stress phase. Conversely, between 01:00 and 05:00 HR the trees typically displayed a recovery phase characterized by an increase in water potential, reaching a plateau sometime within this time frame. These peak stress and recovery periods (4 h) were used as the base data for the repeated measures analysis; mean peak separation was evaluated for each daily stress and recovery plateau. The 4 h stress and recovery means were based on a minimum of five instruments (one instrument per tree) collecting a water potential measurement every 30 min. No comparisons between species were made.

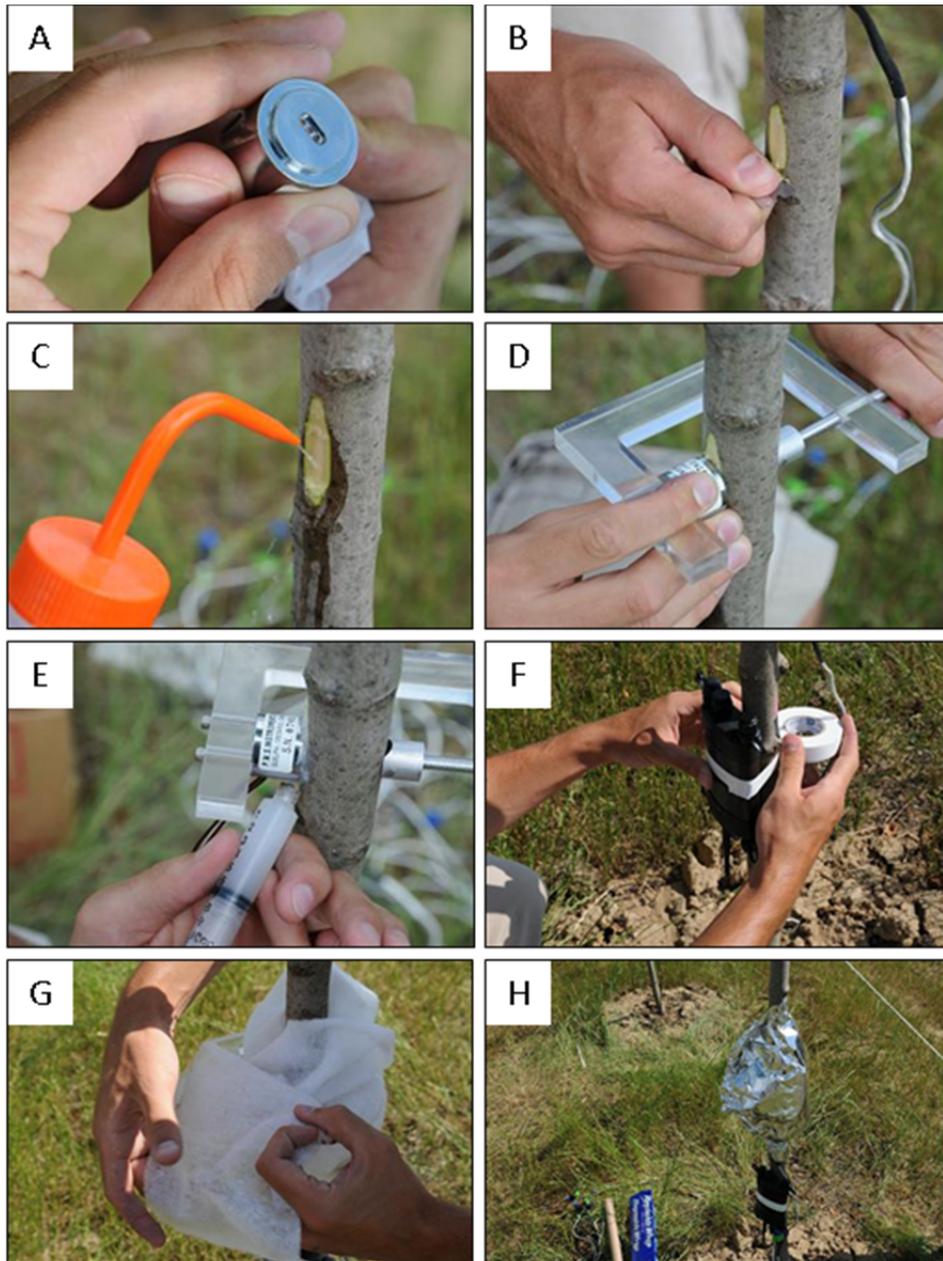


Fig. 1. Typical installation of an automated stem psychrometer for the evaluation of water stress in transplanted ornamental nursery trees: (A) Make sure sensor is clean and thermocouples are intact; (B) Expose plant sapwood tissue; (C) Use deionized water to wash away residual plant tissue; (D) Secure sensor flush against plant using clamp; (E) Apply silicone grease for a gas-tight seal around instrument; (F) Attach automated PSY data logger to plant; (G) Insulate instrument; (H) Wrap installation in aluminum foil.

## RESULTS

### *Acer rubrum* ‘Brandywine’

Figure 2 shows the season long results of the RRLP treatment for *A. rubrum* following a particularly dry summer during which the treated tree exhibited a much healthier and

robust appearance than the untreated tree. The water relations data in Figure 3 showed a clear separation between control and treated tree groups. After an initial rain event just prior to the period shown in Figure 3, the mean peak separation increased each day, culminating in another watering event that brought the water potential back to a similar level in both groups during the following night recovery phase (not shown in Fig. 3). The treated trees maintained a higher (less negative) water status both in the daily stress phases and in overnight recovery phases. The cumulative effects of this water status differential between the control and treated plants are again clearly demonstrated in Figure 2.

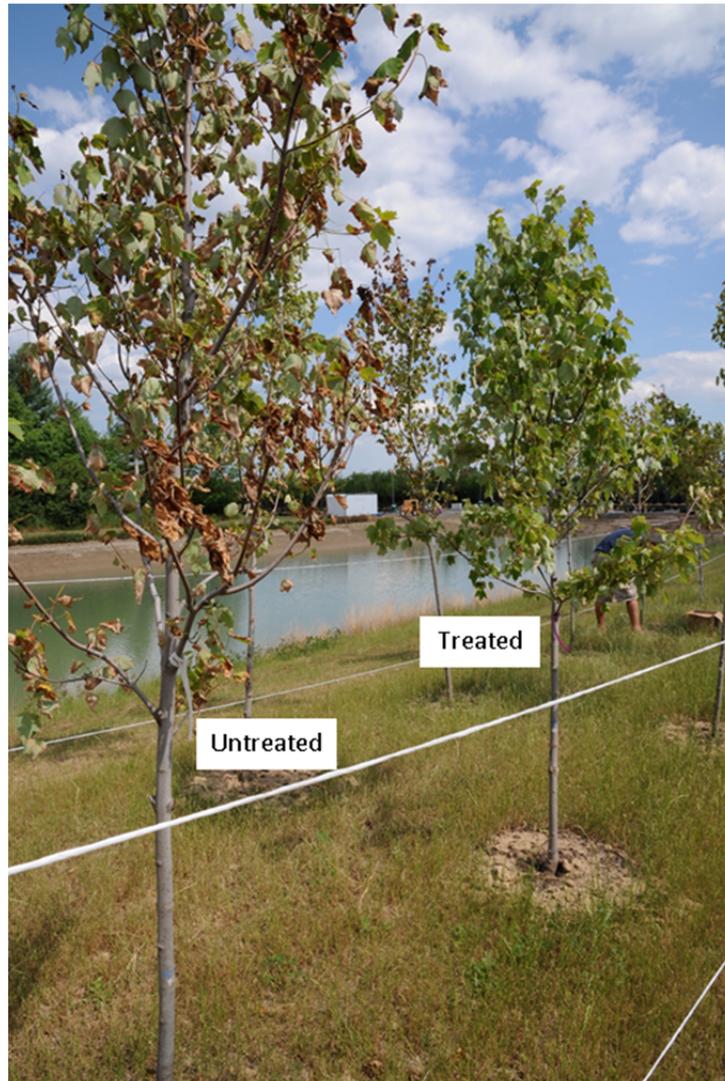


Fig. 2. Comparison of treated (background) and untreated (foreground) transplanted *Acer rubrum* trees in the experimental plot at Connon Nurseries NVK Holdings Ltd.

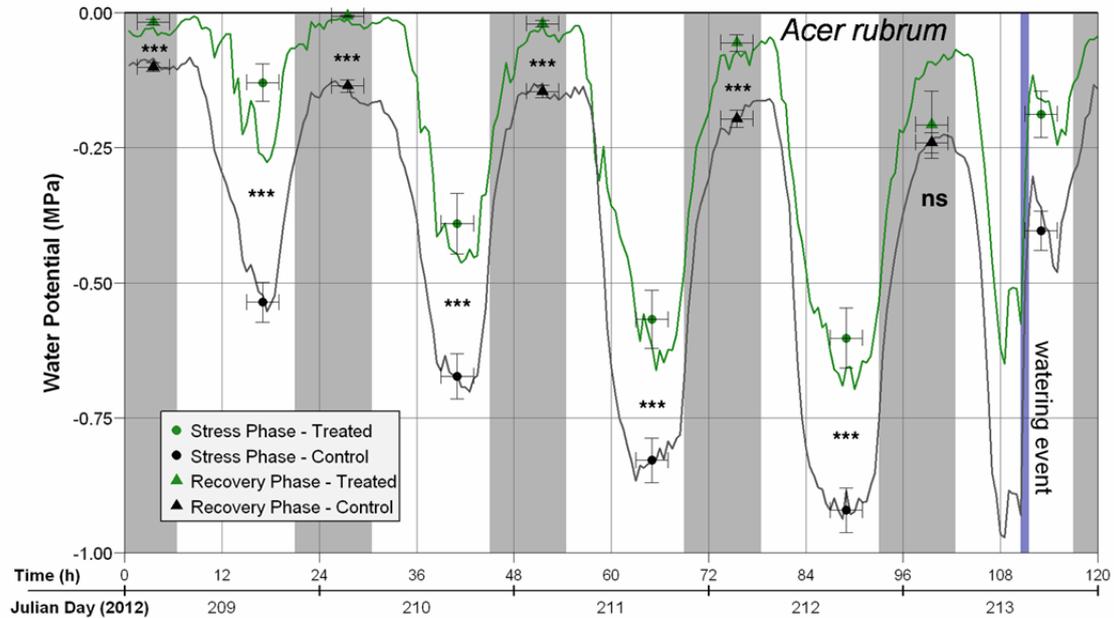


Fig. 3. Five-day drought time course of water potential (MPa) measurements comparing groups of *Acer rubrum* transplanted trees treated with a mycorrhizae inoculum and untreated controls. Lines are a local regression smoothing (loess) of all the data collected at 30 min intervals for each treatment over the 5-day drought-monitoring period. Grey shaded vertical bars represent sunset to sunrise periods. Specific stress (circles) and recovery (triangles) data points are the means of eight consecutive readings during the stress and recovery plateau periods. Vertical error bars are  $\pm$ SEM; horizontal bars are the time range from which means were calculated. Daily stress and recovery mean difference significance levels are represented by: \*, \*\*, \*\*\*, ns, corresponding to  $P \leq 0.05$ , 0.01, 0.001 and  $P \geq 0.05$ , respectively.

### *Thuja occidentalis* ‘Smaragd’

Figure 4 outlines the clear differences in stem water potential between treatment groups, with consistently higher water potential readings for the treated group in both the stress and recovery phases; differences between the recovery and the stress phases become more pronounced over time. According to Dixon et al. (1984), the critical threshold for stomatal closure for *T. occidentalis* is approximately -2.0 MPa. At that point, plants were found to close their stomata and decrease transpiration in order to conserve water until more favourable conditions returned. Figure 4 shows that the control group consistently fell below the threshold for stomatal closure while the treated group remained above it, suggesting that treated trees were able to remain photosynthetically active for greater periods of the day than the control trees.

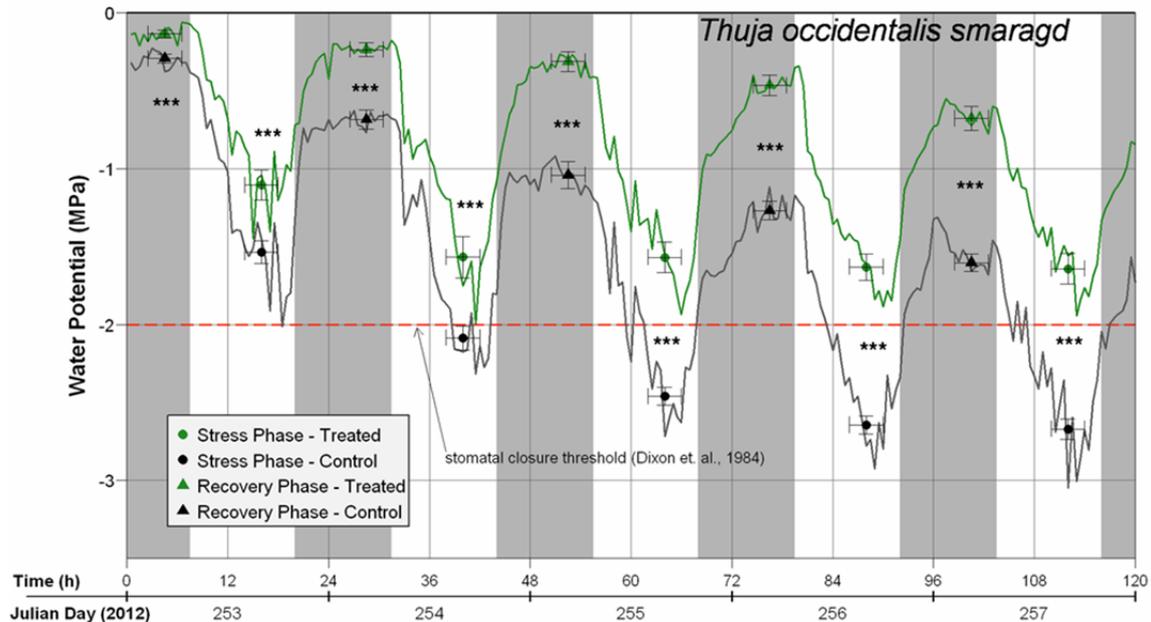


Fig. 4. Five-day drought time course of water potential (MPa) measurements comparing groups of *Thuja occidentalis* 'Smaragd' transplanted trees treated with a mycorrhizae inoculum and untreated controls. Lines are a local regression smoothing (loess) of all the data collected at 30 min intervals for each treatment over the 5-day drought-monitoring period. Grey shaded vertical bars represent sunset to sunrise periods. Specific stress (circles) and recovery (triangles) data points are the means of eight consecutive readings during the stress and recovery plateau periods. Vertical error bars are  $\pm$ SEM; horizontal bars are the time range from which means were calculated. Daily stress and recovery mean difference significance levels are represented by: \*, \*\*, \*\*\*, ns, corresponding to  $P \leq 0.05$ , 0.01, 0.001 and  $P \geq 0.05$ , respectively.

## CONCLUSIONS

The time courses of detailed water potential measurements from the 2012 field trials demonstrated that RRLP was generally effective in mitigating the effects of drought stress on ornamental transplanted trees. The generally higher (less negative) water potential exhibited by the treated plants suggested that, in this case, the mycorrhizae had a direct positive effect on water uptake. The water potential trends in both the control and treated samples were negative (progressively more negative daily peak water potentials) over the course of a drying event, which was expected. Although the trend was negative, the treated plants maintained higher, more favourable overall water potentials over the drought stress period relative to the controls.

In conclusion, RRLP mycorrhizae inoculations improved overall drought tolerance in *A. rubrum* and *T. occidentalis*. The use of in situ stem psychrometers, in numbers that gave statistically reliable data, allowed for a unique look at water relations in nursery woody perennial species. The combination of in situ water potential monitoring and drought mitigating mycorrhizae inoculation, as demonstrated, may provide new mechanisms for managing irrigation water use in the nursery sector.

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