Evaluation of Glyphosate and Bottom Heat on Nursery Tree Hardiness

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Significance to Industry: The economic cost to the U.S. nursery industry of bark cracking is conservatively estimated at $6.6M annually (or 2.5% of finished inventory) according to recent calculations. This estimate does not include the additional estimate of $14M in landscape tree failures due to bark cracking. The nursery cost estimates continue a pattern of strong and steady increased severity and frequency of bark cracking throughout the US nursery/landscape industry since 2004. Concurrently, (2001-05) consumer preference for faster working glyphosate products was driving the production and use of various surfactants to break down the cuticle of plants to increase the rate and amount of glyphosate uptake. However, in 2005 researchers at Ohio State University (OSU) speculated that bark cracking was *not solely* related to cold injury as was widely and previously accepted (Mathers, 2006) but that the absorption of glyphosate into thin or pigmented-bark was also a factor due to the reduction of cold hardiness. Exposure of an ornamental plant to glyphosate through green bark is considered a sub-lethal dose (Kuhns, 1992). There were two objectives to this study: 1) determine if glyphosate, tillage and sod cover can affect the cold hardiness of field grown trees; and, 2) evaluate the influence of bottom heat and glyphosate and non-glyphosate treatments on root growth in *Magnolia virginiana* and *Cornus kousa.* Previous research supports that hardiness should be reduced by sub-lethal dosing with glyphosate (Stasiak et al, 1991); however, this is the first conclusive study indicating glyphosate reduces root hardiness in *Cornus kousa* but not in *Magnolia virginiana*. This is also the first report of *Magnolia virginiana* roots expressing no root dormancy and producing significant biomass during the period of shoot dormancy. In contrast, *Cornus kousa* roots exhibited dormancy and even deteriorated when placed in elevated root zone temperatures of 17°C. This was interesting as the effect of glyphosate treatments was most pronounced on *Cornus* versus *Magnolia*, indicating species variability in susceptibility to glyphosate causing increased cold susceptibility via possible inhibition of root dormancy.

Materials and Methods: Sweetbay magnolia (*Magnolia virginiana)* and kousa dogwood (*Cornus kousa*) were planted in the field the week of May 21, 2007 on Waterman Farm at Ohio State University Columbus, OH. Trees were all one year old bare root plants with an average height of 42 cm and a caliper of 4.1 cm. The experimental design was a split plot design (fertilizer=main plot, treatment=sub-plot, species=sub-sub plot) in a randomized complete block with seven sub samples per species, per treatment, with five replications. Plants were spaced 2' x 9' within and between rows, respectively. Overhead irrigation was applied immediately following planting.

Field Treatments: Two fertilizer treatments of ammonium nitrate were applied on June 13, 2007: 125 pounds per acre and 250 pounds per acre. Additionally, Osmocote (14-14-14) was applied at a rate of 50 lbs/N/acre on August 1. Four herbicide or weed suppression treatments were subsequently applied on June 22, July 25, August 29, and October 2 (2007): Roundup Original Max, Roundup Pro, Kleenup Pro, and tillage. The herbicides were applied at a 5% solution, or 6.5 ounces/gallon. All herbicide treatments were applied using a five gallon backpack sprayer with a LFG 80^o nozzle.

Freezing Treatments: On December 3, five of the seven sub samples were dug out of the field. Plants were soaked overnight in water to loosen soil from roots. Roots were washed on December 4 and 5, 2007 and measured using a volumetric flask and with displacement to estimate root volume. This technique was repeated after bottom heat treatments were imposed for 70 days. Trees were placed in quart or pint size (depending on mass of roots) polyethylene bags and filled with a moistened 50-50 sand/perlite mixture to cover roots and tied with wire ties. Trees were then put inside a walk-in cooler set at 5^{o}C ambient temperature. Two-sided wooden boxes, measuring 3' x 10' were placed in the cooler with heating mats set at 8^o, 11^o, 14^o, and 17^{o}C under each box. Sawdust was packed around the bags containing trees in the boxes to facilitate even heating over the root surface. To assess cold hardiness, 1-3 millimeter segments of stems (new growth) and primary roots were removed. Two to three segments of roots and stems were put into test tubes and put into an ultra low freezer (Forma Scientific, Inc., Marietta, OH). Segments were frozen at a rate of -6^o C per hour at the following temperatures: no freezing, -6^o,-12^o,-18^o,-24^o, and -30^{o}C. Immediately after removing from freezer, 3 milliliters of distilled water was added to each test tube, and shaken overnight at 200 rpm. An initial baseline electrical conductivity (EC) reading was taken at this time for all temperature treatments. The tubes were then autoclaved at 121^o C for 20 minutes to completely kill all tissue. The tubes were then shaken again overnight at 200 rpm. After shaking, a final EC reading was obtained for all treatments. The baseline EC was subtracted from the final EC and recorded as the differential EC for reporting of cold hardiness. The assumption is that the higher the differential, the greater the cold hardiness of the tissue (Stergios and Howell, 1973)

Results and Discussion: EC readings decreased with all declining freezing temperatures with *Cornus* stems and after -6^o C for *Magnolia* stems (data not shown). Without exposure to short days and cool temperatures (ie. acclimation), *Malus* seedlings were hardy to approximately -7 °C (Magness, 1929). Our findings concur with *Magnolia* (non-dormant) but not *Cornus* (dormant). *Magnolia* shoots and roots did not exhibit dormancy in our study and thus were more susceptible to temperature fluctuations in contrast to our results with *Cornus,* which were similar to Magness (1929). These results also concur with the main effect of increasing bottom heat, which resulted in increased root growth (Fig. 1 and 2) in *Magnolia* (non-dormant) but not *Cornus* (dormant) (Fig. 1 and 3). Bottom heat also significantly increased hardiness for both species in roots and shoots (Fig. 1) versus the ambient control; however, the affect of bottom heat was greatest on *Cornus* shoots. The bottom heat by freezing temperature interaction for dogwood roots was also significant at α=0.05 (data not shown) and indicated shoot and root hardiness increase caused by bottom heat was primarily due to the tillage treatment at bottom heats of 14^o and 17^o C. A significant herbicide by freezing temperature interaction (α=0.05) also occurred with *Cornus* (Fig. 1) but not *Magnolia*. The tillage treatment significantly increased hardiness versus the glyphosate treatments or sod cover (Fig. 1). Mathers and Stushnoff (2005) indicated that non-acclimated tissue [not exposed < -7^o C (Magness, 1929)] cannot be used to distinguish treatment differences related to cold. The most pronounced treatments differences in our study were found at -18^o C where the glyphosate treatments showed less hardiness as compared to tillage, but not significantly different from sod cover. It is known that sod cover reduces hardiness via competition and inhibition of acclimation (Calkins and Swanson, 1998), and bare soil treatments have less cold injures (bark cracking/root injuries) versus sod cover. However, glyphosate treatments causing similar cold hardiness reduction to sod cover indicates a possible similar effect via acclimation inhibition. This occurs with the herbicide and freezing interaction that is evident with *Cornus* (dormant), but not with *Magnolia* (non-dormant).

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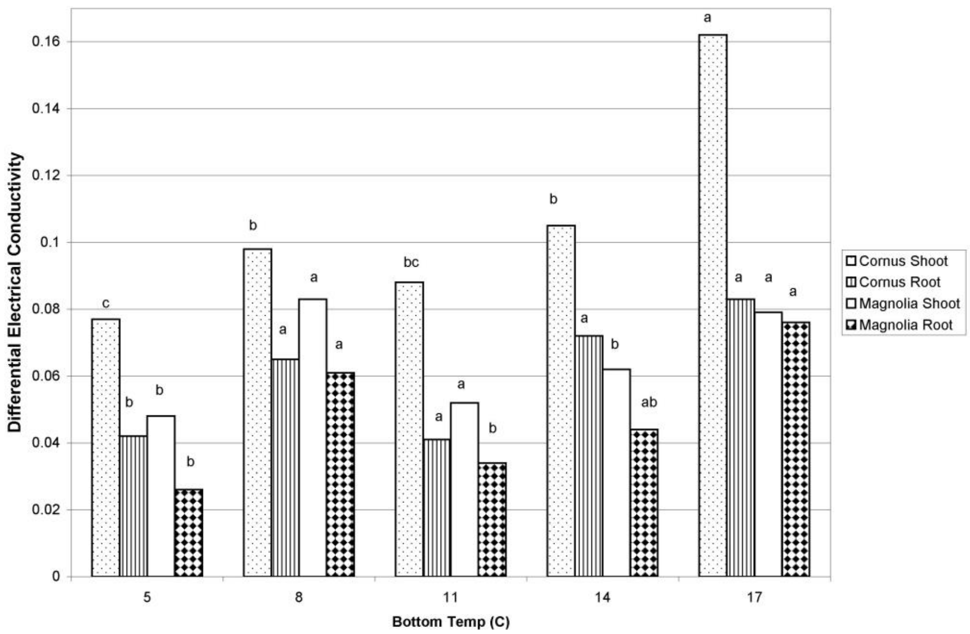

Fig. 1. Effect of freezing on *Cornus kousa* and *Magnolia virginiana* exposed to various amounts of bottom heat temperatures combined over freezing temperatures, herbicides, and fertility. Letters indicate significance differences between similar colored bars LSMeans α=0.05. 5º C was the ambient temperature. Differential=how determined baseline EC (after freezing)-final EC (after freezing and autoclaving).

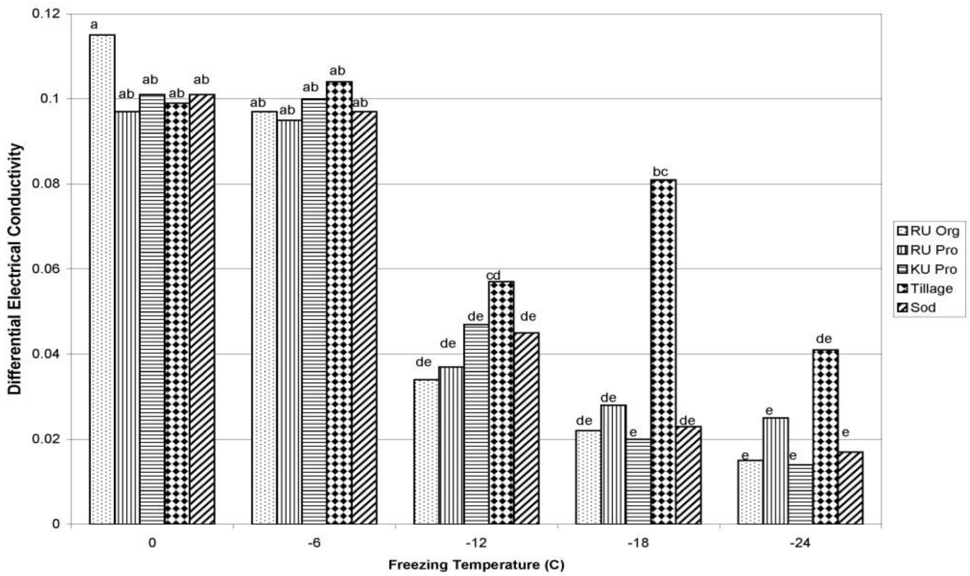

Figure 2. Herbicide by freezing temperature interaction of *Cornus kousa* roots subjected to 5 x 5 herbicide/temperature treatments. Letters indicate significance between similar colored bars LSMeans α=0.05. . RU Org=Roundup Original, RU Pro=Roundup Pro, KU Pro=Kleenup Pro, Till=Tillage, and Sod=Sod Cover. Differential=how determined baseline EC (after freezing)-final EC (after freezing and autoclaving).

Fig. 3a and 3b. *Cornus kousa* and *Magnolia virginiana* roots from bottom heat temperatures of 5^{o} C (ambient) and 17^{o} C treatments. At 17^{o} C treatment there was significant growth for *Magnolia* and deterioration for *Cornus.* A=*Magnolia at 5^{o}* C, B=*Cornus* at 5^{o} C, C=*Cornus* at 17^{o} C, and D=*Magnolia* at $17^{o\,C}$.