

Effect of Propagation Tray Design on Early Stage Root Development of *Acer rubrum*, *Quercus rubra*, and *Populus tremuloides*¹

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Abstract

This experiment investigated the effect of different plug-tray cell designs on root development of red maple (*Acer rubrum*), red oak (*Quercus rubra*), and quaking aspen (*Populus tremuloides*) seedlings. In April of 2015, seeds of each species were sown into three plug trays with different substrate volumes and grown for 17 weeks. Two trays had permeable walls for air-pruning, one with vertical ribs and one without. The third tray had impermeable plastic cell walls. Harvested seedlings were analyzed for root dry weight, length, volume, surface area and number of deflected roots. Root length per volume was highest in the impermeable-walled tray for red maple and quaking aspen. The total numbers of deflected root systems were higher for all species in the impermeable-walled tray. Seedlings grown in the air-pruning trays had smaller proportions of deflected root masses. Greater substrate volume did not influence root deflection development. The air-pruning tray without vertical ribs had the lowest total number of root masses with misdirected roots and lower proportions of root masses with misdirected roots for all species. These results indicate that improved root architecture in root-air pruning tray designs is achievable in tree propagation; however, vertical plastic structures in air-pruning trays can still cause root deflections.

Index words: Deflected roots, air-pruning, seedling, propagation, plugs, root architecture.

Species used in the study: red maple (*Acer rubrum* L.); red oak (*Quercus rubra* L.); quaking aspen (*Populus tremuloides* Michx.).

Significance to the Horticulture Industry

Root deflections are initiated at the propagation stage and misdirected structural roots makes trees less robust after transplanting, increasing tree mortality because roots are not placed advantageously for survival and establishment. Although manual correction (pruning or shaving) is possible for larger nursery stock sizes, root masses at the propagation stage are small and numerous, making it inefficient to manually shave the plugs. In propagation, air-pruning is an effective way to manage root growth and development using permeable-walled containers, which stop root growth at the wall-substrate interface by desiccating root tips. Plug-trays that have some air-pruning features in addition to plastic structures, for instance, are designed to direct roots (with either vertical or horizontal ribbing, lattices or strategically placed holes) still cause root deflections by forcing the roots to change direction (e.g. ascending, descending, circling or kinked roots). However, deflected roots can be greatly reduced by growing seedlings in propagation trays that minimize contact between the substrate and the tray cell walls.

Introduction

Improper nursery container and propagation tray design can significantly alter the root architecture in woody perennials, leading to substantial deformation (Gilman

2001). Unfortunately, root deflections are common in container-produced nursery stock and if left uncorrected can contribute to long-term tree growth problems in the landscape (Ortega et al. 2006). Deflected roots generally occur when the design of the container or propagation tray prevents the lateral roots from extending horizontally, forcing the roots to either circle within the container or grow vertically down to the bottom (Amoroso et al. 2010).

Nursery container technologies have evolved in an attempt to manage the incidence of deflected roots, promoting improved root architecture through the use of specialized container shapes, bottomless containers, woven or non-woven fabrics, mechanical deflection or chemicals to control root growth (Appleton and Whitcomb 1983, Marshall and Gilman 1998, Gilman et al. 2003, Gilman et al. 2010). Root growth and development can also be managed using permeable-walled containers, which stop root growth at the wall-substrate interface (Privett and Hummel 1992). As the roots grow towards the container walls, the openings expose the substrate and the lateral roots to air, resulting in desiccation of the tips (air-pruning) (Amoroso et al. 2010). As a result of the desiccated tips, branching occurs behind the root tip, causing more fine roots to develop in the inner part of the root ball, developing an even root distribution (Marler and Willis 1996). The structure of developing root systems is critical because increases in the absorptive length and metabolic activity in tree roots is positively correlated with the distal ends of the branching root system (Pregitzer et al. 1998, Pregitzer et al. 2002, Reich et al. 2008, Pregitzer 2008).

Some plug trays promote air-pruning or “directing” roots in propagation (Ortega et al. 2006). Once roots are deflected, even at the plug stage, their effects become challenging to correct downstream (Ortega et al. 2006, Blanus et al. 2007, Devine et al. 2009). However, information on the benefits of air-pruning within the

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Table 1. Description of the key features and dimensions of the plug tray cells tested at Vineland Research and Innovation Centre, Ontario from April to August 2015.

Tray Number	Container name/material	Cells per Tray	Ellepot ^z Dimensions Diameter	Substrate Volume (cm ³)	Description of Cell Features
Tray 1	PioneerPot™ PP60E	20	60 mm diameter 165 mm tall	466.53	Ellepot ^z (paper pots) are completely exposed except for four vertical ribs and an open support structure at the bottom.
Tray 2	Proprietary new technology	32	45 mm diameter 120 mm tall	159.04	Ellepot ^z (paper pots) are completely exposed except for horizontal structures (rings) at the distal ends of the cell.
Tray 3	SureRoots SR 50 Deep Bottomless	50	35 mm diameter 100 mm tall	115.45	Ellepot ^z (paper pots) are housed in solid-plastic walls with four interior vertical ribs that protrude inside each cell. Cells are open at the bottom.

^zEllepot paper is comprised of cellulose.

production system is difficult to parse. Due to the variety of configurations, structures and dimensions of tree seedling plug trays available, it is challenging to determine what factors are most pertinent for morphologically desirable seedling production. The objective of this trial was to evaluate three different propagation trays to better understand how cell volume and permeability can jointly influence early stage root development of woody perennials. According to Dominguez-Lerena et al. (2006), container volume influences plant morphology, and larger rooting volumes increase height and diameter in trees. Therefore, the objective of this study was to assess the root development response of three species of seedlings, red maple, red oak, and quaking aspen to cell permeability, cell structure and substrate volume in propagation trays. These species were chosen for their importance in the North American nursery sector. The United States Department of Agriculture (USDA) census on Horticulture (2014) reported that red maple, oak and poplar all ranked in the top ten for sales in the deciduous shade tree category. Additionally, these species were chosen for the study because of the differences in their characteristic root structure and root development. For instance, the prominent taproot of the oak (Devine et al. 2009), the shallow fibrous roots of red maple (Gilman et al. 2010) and the plasticity of root growth response of *Populus* spp. to resources (Friend et al. 1999) may develop differently in cells with different structures and/or volumes and dimensions.

Materials and Methods

The study was carried out in an experimental greenhouse at the Vineland Research and Innovation Centre located in Vineland Station (Ontario, Canada; lat. 43.19 N, long. 79.40 E). The substrate used was a proprietary grow mix containing peat, perlite, vermiculite (Fafard et Frères, Ltd., Bonaventure, QC), and vermicompost (TerraVesco®, Sonoma, CA) in Ellepot™ paper containers with a paper thickness of 0.0127 cm (0.05 in) (Ellegaard A/S, Esbjerg, DK). Three propagation trays with different cell features were tested. The study tested the root growth and development of tree seedlings propagated from seed in Ellepot™ paper containers placed into plastic plug trays. Tray 1 (PioneerPot™ PP60E, Stuewe & Sons, Tangent, OR) and Tray 2 (a new proprietary technology, Vineland Research and Innovation Centre, Vineland Station, ON) have air-pruning features on the sides of the cells and

bottom. Tray 1 has vertical ribs that hold the paper substrate containers whereas Tray 2 is without vertical structures that make contact with the substrate. Tray 3 (SureRoots® 50 Deep Bottomless, T.O. Plastics, Clearwater, MN) was only open on the bottom of each cell. The dimensions and key features of each tray are outlined in Table 1. Seeds of red maple, red oak and quaking aspen were sowed into the trays in April 2015 with four trays per treatment.

The trial ran for 119 days starting on April 20th 2015 and concluding on August 24th 2016. Monthly temperature overall averages for the greenhouse were 22.9 C (73.2 F), 20.9 C (69.62F), 24.1 C (75.4 F), and 23.3 C (73.9 F) for May, June, July and August, respectively. All plants were watered daily via an overhead irrigation system throughout the growing season. Fertilizer was applied to the irrigation water once a week. PlantProd® 11N-41P-8K (Master Plant-Prod Inc. Brampton, ON) at 200 ppm N was used for two applications, followed by PlantProd® 20N-8P-20K at 350 ppm N weekly for the remainder of the trial. The starting pH of the irrigation water was 7.56, the starting average pH of the media was 5.98, and the average starting EC of the media was 3.71 cm⁻¹. The trays were re-randomized monthly to reduce location error.

Between 25 and 35 plants per treatment and species were sampled and analyzed (five per tray). Due to the cell volume differences among the trays and the influence it had on shoot growth (Dominguez-Lerena et al. 2006), the sampling and analysis for this study focused solely on root parameters.

Root morphology was determined through destructive harvesting at the end of the growing season. Substrate was removed from the roots by washing. Roots were analysed using the WinRHIZO™ root scanner for evaluation (Regent Instruments Inc., Quebec City, QC) to determine root length (cm), surface area (cm²) and volume (cm³). The roots were examined for any deflections caused by the container (roots coming into contact with the container caused them to change direction i.e. circling, ascending, or descending). When a deflection was identified, it was manually separated at the deflection point. The roots were then dried at 75 C (167 F) until a constant weight was achieved. The total root weight was measured for each seedling. The total number of seedlings with at least one deflected root present by tray and species was counted. The percentage of seedlings with deflected roots present was then determined (*sensu* Amoroso et al. 2010) and then the

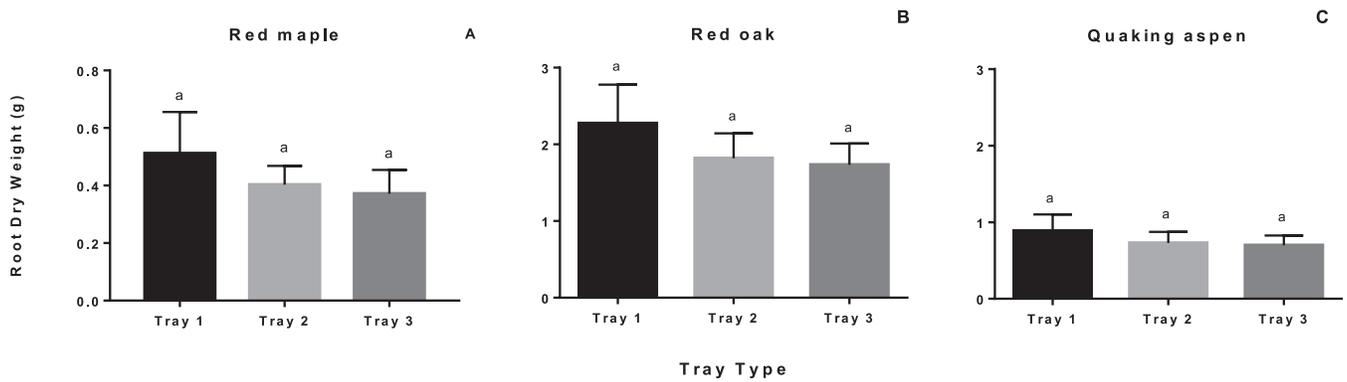


Fig. 1. Study conducted at Vineland Research and Innovation Centre, Ontario from April to August 2015 demonstrating mean root dry weight (g) for red maple, red oak and quaking aspen seedlings in three nursery plug trays. Error bars represent 95% confidence intervals. Different letters indicate a significant difference ($P \leq 0.05$) by ANOVA. The numbers of replicates are as follows: red maple Tray 1, $n = 25$; 2, $n = 30$, Tray 3, $n = 29$; red oak Tray 1, $n = 35$, Tray 2, $n = 33$, Tray 3, $n = 32$; quaking aspen Tray 1, $n = 34$, Tray 2, $n = 33$, Tray 3, $n = 33$. All trays hold Ellepots™ (paper pots) of varying sizes. Key tray cell features: Tray 1 has open cells except for 4 vertical ribs connected at the bottom; Tray 2 has open cells except for horizontal plastic rings at distal ends, and Tray 3 solid-plastic walled cells and an open bottom.

proportion of deflected root dry weight to total root dry weight was calculated for each seedling.

Statistical analysis. Data for root dry weight, length per volume, surface area and deflection proportions were subjected to one-way analysis of variance (ANOVA). The Tukey's multiple comparisons test was applied to perform a multiple means comparison of all pair-wise combinations of the means. Quaking aspen root dry weight data was transformed using the $Y = \text{Log}(Y)$ function and a Kruskal-Wallis test using Dunn's multiple comparison test was used for oak root length per volume, and quaking aspen and oak root deflection proportion analysis. Outlier data points were assessed in all data sets before conducting one-way ANOVAs using the ROUT Method with $Q = 1\%$. All data sets were analysed using GraphPad Prism version 6.0 software (GraphPad Software Inc., La Jolla, CA). All data were evaluated using a significance level of $P < 0.05$.

Results and Discussion

Although maple, red oak, and quaking aspen root dry weights were numerically higher in plants grown in Tray 1, results were not statistically different from that seen with Tray 2 or Tray 3 (Fig. 1). Generally, there were no differences in average root masses grown in the three trays within a species, despite the differences in cell permeability and substrate volume.

Average root length per substrate volume in Tray 3 was higher than Tray 1 and 2 across species except with respect to red oak where Tray 2 and Tray 3 did not differ (Fig. 2A-2C). The impermeable walls of Tray 3 compared to Tray 1 and 2 influenced root-length per volume by producing roots that were long and terminated at the bottom of the cells. According to Whitcomb (1985), as a root reaches the wall in impermeable plastic containers it will circle for half to one times around the container before reaching the bottom where it will continue to elongate for as much as five

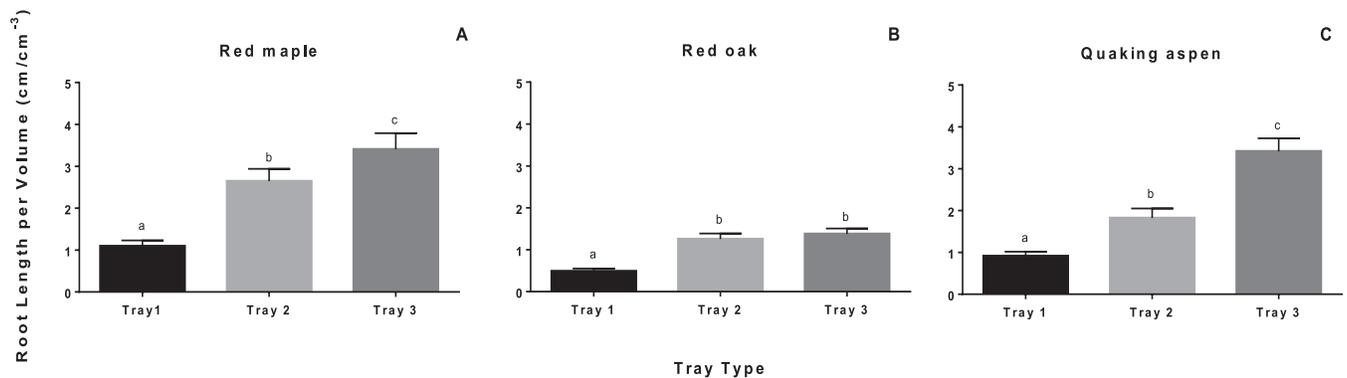


Fig. 2. Study conducted at Vineland Research and Innovation Centre, Ontario from April to August 2015 demonstrating mean root length (cm) per substrate volume (cm^3) for red maple, red oak and quaking aspen seedlings in three nursery plug trays. Error bars represent 95% confidence intervals. Different letters indicate a significant difference ($P \leq 0.05$) by ANOVA. The numbers of replicates are as follows: red maple Tray 1, $n = 33$, Tray 2, $n = 32$, Tray 3, $n = 31$; red oak Tray 1, $n = 33$, Tray 2, $n = 33$, Tray 3, $n = 32$; quaking aspen Tray 1, $n = 34$, Tray 2, $n = 34$, Tray 3, $n = 33$. All trays hold Ellepots™ (paper pots) of varying sizes. Key tray cell features: Tray 1 has open cells except for 4 vertical ribs connected at the bottom; Tray 2 has open cells except for horizontal plastic rings at distal ends, and Tray 3 solid-plastic walled cells and an open bottom.

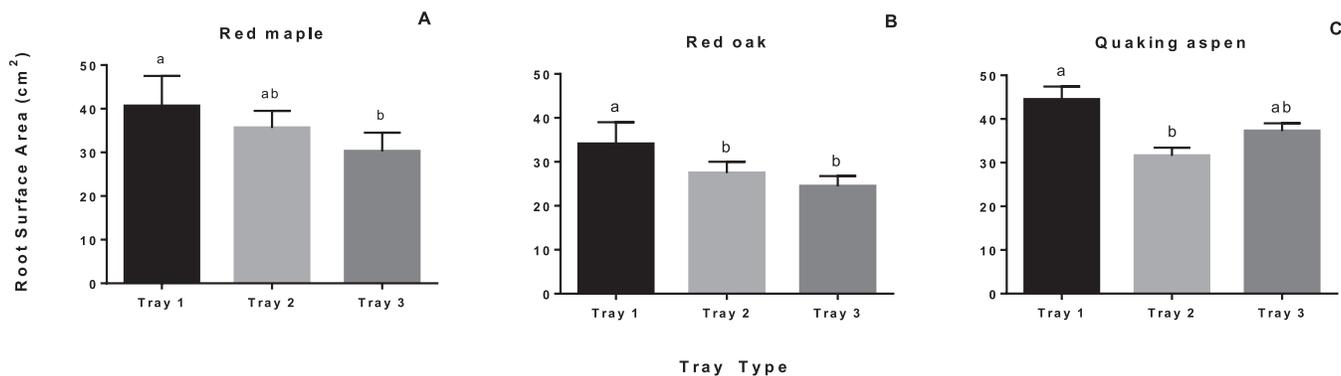


Fig. 3. Study conducted at Vineland Research and Innovation Centre, Ontario from April to August 2015 demonstrating mean root surface area (cm^2) for red maple, red oak and quaking aspen seedlings in three nursery plug trays. Error bars represent 95% confidence intervals. Different letters indicate a significant difference ($P \leq 0.05$) by ANOVA. The numbers of replicates are as follows: red maple Tray 1, $n=28$, Tray 2, $n=30$, Tray 3, $n=29$; red oak Tray 1, $n=35$, Tray 2, $n=33$, Tray 3, $n=32$; quaking aspen Tray 1, $n=34$, Tray 2, $n=34$, Tray 3, $n=33$. All trays hold Ellepots™ (paper pots) of varying sizes. Key tray cell features: Tray 1 has open cells except for 4 vertical ribs connected at the bottom; Tray 2 has open cells except for horizontal plastic rings at distal ends, and Tray 3 solid-plastic walled cells and an open bottom.

revolutions or more. Therefore, we attribute the increased root length per volume in Tray 3 to the impermeable container structure. Due to the closed wall design of this tray, no root pruning occurred, leading to a higher root length per substrate volume, despite the small cell volume of 115.43 cm^3 relative to the other trays. Ultimately, this lack of root pruning and increased root length resulted in roots that terminated at the drainage hole in the cells, creating a distribution of roots that has been found to diminish outplanting performance (Balisky et al. 1995, Dey and Parker 1997, Davis and Jacobs 2005, Dominguez-Lerena et al. 2006). At the time of planting, the root tips are positioned mostly at the bottom of the plugs, which decreases absorption of water and nutrients (Whitcomb 1985, Guo et al. 2008, Pregitzer 2008).

Red oak in Tray 1 had higher values than both Tray 2 and Tray 3 for root surface area (Fig. 3B). Root surface area was greater in Tray 1 than Tray 2 and Tray 3, but did not differ significantly between Tray 1 and 3 or Tray 2 and 3 for quaking aspen seedlings (Fig. 3C). Root surface area is an important measure of the absorptive capacity of a root system. Guo et al. (2008) found that first-order roots serve high absorptive functions and that anatomical traits associated with absorption occur mainly in first to third orders. The growth that occurs in the propagation stage is primarily of the first to third order class and therefore larger surface areas result in increased absorptive capacity. Pregitzer et al. (2002) found that the distal branches consisting of first- and second-order roots provide most of the surface area for resource uptake. Therefore, root surface area is a better measure of the functional capacity of a root system than root length. The branching that is encouraged by air-root pruning technology may increase root surface area. Cell permeability may be more important in root surface area development than cell volume for some species. For instance, there was no difference between Tray 2 and Tray 1 for root surface area of red maple seedlings, although Tray 1 has 270 more cm^3 of substrate than Tray 2. However, with respect to quaking aspen, the influence of cell permeability on surface area is not as clear-cut. Plants

grown in Tray 1 and Tray 3 had root surface areas that were not different though Tray 1 has permeable walls and has 351 cm^3 more substrate per cell than Tray 3. The additional substrate in Tray 1 for instance, did not provide a clear advantage with respect to root surface area.

There were differences in the percentage of seedlings with deflected roots present among trays and species (Figure 4). There were also differences in the average proportion of deflected root dry weight to total root dry weight among trays and species. Tray 3 had the most seedlings with root deflections for red maple, red oak and quaking aspen and had significantly higher average proportions of root deflections' dry weight to total root dry weight in red oak compared to Tray 2, but not Tray 1 and quaking aspen seedlings compared to both Tray 1 and 2 (Fig. 4A-4C). Tray 1 had a higher percentage of seedlings than Tray 2 with deflected roots present for red maple and quaking aspen (Fig. 4A and 4C). The percentage of red oak seedlings with deflected roots present was the same between Tray 1 and Tray 2 (Fig. 4B). However, red oak in Tray 1 had a much higher average proportion of deflected roots to total root dry weight when compared to Tray 2 (Fig. 4B). Based on our findings, the root deflections likely occurred at the early stages of root development, as the radicle emerged, and remained for the duration of the production cycle, which is consistent with findings from Devine et al. (2009). Dominguez-Lerena et al. (2006) reported that container diameter is the most important determinant in aboveground tree growth but found no direct relationship between root deflections and container dimensions. With respect to root deflections during the propagation stage, we found that even minimalist vertical structures can cause deflections in oak irrespective of cell dimensions and volume. Tray 1 had a cell diameter of 60 mm as compared to 45 mm and 35 mm of Tray 2 and Tray 1 respectively. However, Tray 2 had the smallest percentage of seedlings with deflected roots present and the lowest average proportion of root deflection dry weight to total root dry weight for all species (Table 2). Among the species grown in Tray 2, deflections were highest for red

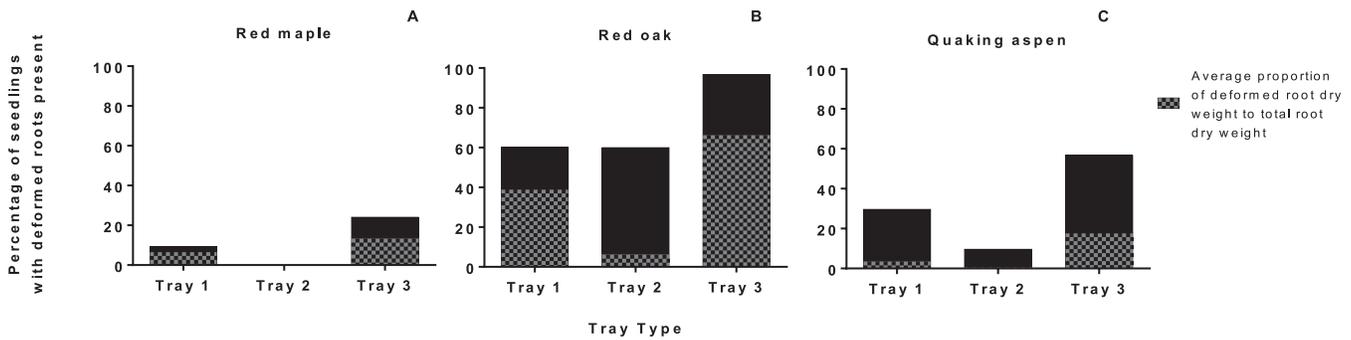


Fig. 4. Study conducted at Vineland Research and Innovation Centre, Ontario from April to August 2015 demonstrating average percentage of seedlings with deflected roots for red maple, red oak and quaking aspen in three trays. Checkered area within each bar indicates the average proportion of deflected root dry weight to total root dry weight among all seedlings with deflected roots present. The numbers of replicates are as follows: red maple Tray 1, $n=3$, Tray 2, $n=0$, Tray 3, $n=7$; red oak Tray 1, $n=21$, Tray 2, $n=18$, Tray 3, $n=31$; quaking aspen Tray 1, $n=10$, Tray 2, $n=3$, Tray 3, $n=17$. All trays hold Ellepots™ (paper pots) of varying sizes. Key tray cell features: Tray 1 has open cells except for 4 vertical ribs connected at the bottom; Tray 2 has open cells except for horizontal plastic rings at distal ends, and Tray 3 solid-plastic walled cells and an open bottom.

oak. This likely has to do with the horizontal structure (rings) at the base of the tray where the taproot root of the oak can become trapped, causing circling. This is demonstrated by the relatively low average proportion of deflected root dry weight to total root dry weight in red oak because the deflections only occurred at the distal end of the cells.

Amoroso et al. (2010) reported that air-pruning technology and mechanical impediments on the inside of container walls for 1-L and 3-L containers was effective for limiting root deflections in container-grown littleleaf linden (*Tilia cordata* P. Mill.). However, our study demonstrated that air-pruning technology absent of mechanical impediments on the inside of cell walls is an effective strategy for minimizing the occurrence of deflected roots. We hypothesize having cell walls absent of mechanical impediments

is critical at the earliest stage of root formation when the seminal root and first-order laterals are developing. For instance, we observed as the red oak germinated and the radicle emerged, it quickly reached the edge of the substrate. In the impermeable tray (Tray 3) and the tray with vertical ribbing (Tray 1) it was directed down by the plastic structures. The absence of vertical structures in Tray 2 appears to have reduced the incidence of deflections and the proportion of root mass that was deflected in red oak. Contrary to Amoroso et al. (2010), we found that wall structures increase the percentage of deflected roots that develop at the plug stage.

The results of the study suggest that cell typology influences tree seedling root architecture of red maple, red oak and quaking aspen during plug production. Seedlings grown in cells with solid-walls generally had higher percentages of deflected roots compared with seedlings grown in cells with minimal mechanical impediments to facilitate air-pruning. Poor root development and formation can decrease out planting survival of seedlings (Balisky et al. 1995, Dey and Parker 1997, Amoroso et al. 2010). Consequences of poor root formation also include reduced plant vitality as well as poor anchorage in urban soils (Gilman et al. 2010, Gilman and Wiese 2012). To produce red maple, quaking aspen and red oak seedlings with well-structured root systems, the use of air-pruning plug trays with structures that limit contact between cells and substrate should be employed in the nursery.

Table 2. Study conducted at Vineland Research and Innovation Centre, Ontario from April to August 2015 demonstrating mean proportions of deflected root dry weight to total root dry weight \pm SEM among all seedlings with deflected roots present. Significant treatment effects ($p < 0.05$) are shown in bold text for the differences among mean proportions among tray types. All trays hold Ellepots™ (paper pots) of varying sizes. Key tray cell features: Tray 1 has open cells except for 4 vertical ribs connected at the bottom; Tray 2 has open cells except for horizontal plastic rings at distal ends, and Tray 3 solid-plastic walled cells and an open bottom.

Species	Tray Type Comparison	Mean proportion of deflected root dry weight to total root dry weight \pm SEM	Difference
Red maple	Tray 1 vs. 2	0.73 \pm 0.03 vs. 0	N/A ²
	Tray 1 vs. 3	0.73 \pm 0.03 vs. 0.55 \pm 0.09	0.18
	Tray 2 vs. 3	0 vs. 0.55 \pm 0.09	N/A ²
Red oak	Tray 1 vs. 2	0.65 \pm 0.05 vs. 0.10 \pm 0.02	0.55
	Tray 1 vs. 3	0.65 \pm 0.05 vs. 0.68 \pm 0.03	-0.03
	Tray 2 vs. 3	0.10 \pm 0.02 vs. 0.68 \pm 0.03	-0.58
Quaking aspen	Tray 1 vs. 2	0.12 \pm 0.03 vs. 0.05 \pm 0.02	0.07
	Tray 1 vs. 3	0.12 \pm 0.03 vs. 0.31 \pm 0.04	-0.19
	Tray 2 vs. 3	0.05 \pm 0.02 vs. 0.31 \pm 0.04	-0.26

²Because no seedlings in Tray 2 possessed deflected roots, statistical analysis was not possible.

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