Improved Forest Tree Seedling Production Guidelines for Haiti

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Abstract

Haiti, a country who's land mass was at one time covered by 60% forests has suffered great losses from deforestation, with less than 2% of its forests surviving today. Current reforestation efforts in Haiti focus on seedling quantity rather than quality. Working with partners in Kenscoff, Haiti, we developed a study to determine some of the factors limiting the ability to produce high-quality seedlings in the nursery. Using the endemic Hispaniolan pine (*Pinus occidentalis*) as a model species, there were two general objectives of this study. The first objective was to explore the possibility of producing high quality seedlings utilizing economically feasible nursery practices.

More specifically, container type and growing media were evaluated for influence on seedling physiological and morphological parameters. The second objective was to examine the post-transplant effect of growing media on seedling growth and photosynthesis through drought and non-drought simulated field conditions. Results indicate that *Pinus occidentalis* had greatest overall growth in compost-based media types but that germination of this species was greatest in peat-based media. Following transplantation media type had less influence on seedling growth and photosynthesis. Suggesting that nursery cultural practices were less influential once seedlings were outplanted. Seedlings during this phase were affected more by irrigation treatments. Over time, drought treated seedlings showed significant declines in photosynthesis and had overall lower growth than those irrigated regularly.

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Table of Contents

Authorization to submit thesis ii

Abstract iii

Acknowledgements iv

Table of contents v

List of Tables vii

List of Figures viii

Chapter 1 Media as well as container type influence *Pinus occidentalis* seedling development

Abstract 1

Introduction 2

Methods 12
- *Experimental Design and Production 12*
- *Sampling 14*
- *Statistical Analysis 16*

Results 17
- *Physical Characterization of Growth Media 17*
- *Seedling Morphology 17*
- *Plant Tissue 19*
- *Germination 19*

Discussion 20
- *Media Physical Characterization 20*
- *Media and Plant Tissue Nutrient Concentration 21*
- *Seedling Morphology 23*
- *Germination 23*

Conclusions and Future Directions 25

Literature Cited 26

Tables 31

Figures 33

Chapter 2 Effects of plant moisture stress on growth and photosynthesis of *Pinus occidentalis*

Abstract 36

Introduction 37

Methods 40

Nursery Culture ... *40*
Experimental Design and Treatments ... *41*
Sampling ... *41*
Statistical Analysis ... *43*

Results ... 44
Photosynthesis and Plant Moisture Stress ... *44*
Morphological Measurements ... *45*

Discussion ... 47
Photosynthesis and Plant Moisture Stress ... *47*
Morphological Measurements ... *49*

Conclusions ... 51

References Cited ... 52

Tables ... 55

Figures ... 56

List of Tables

Table 1.1 Morphological growth characteristics of *Pinus occidentalis* seedlings following nursery culture 31

Table 1.2 Plant tissue nutrient concentration following nursery culture 32

Table 2.1 Morphological Characterization of *Pinus occidentalis* seedlings........ 55

List of Figures

Figure 1.1 Mean bulk density 33
Figure 1.2 Linear regression of soil bulk density and water holding capacity........ 34
Figure 1.3 Mean germination across media and container types........ 35
Figure 2.1 Mean pre-dawn and mid-day water potentials by date........ 56
Figure 2.2 Mean photosynthesis across treatment types........ 57
Figure 2.3 Mean photosynthesis across treatment types by date........ 58

Chapter 1

Media as well as container type influence *Pinus occidentalis* seedling development

Abstract

To evaluate possible alternatives to current nursery procedures, seeds of an endemic pine tree (*Pinus occidentalis*) were acquired. Seeds were directly sown into two container types (poly bag and D-40) in combination with five media types in a factorial design. Biomass (shoot, root and shoot-to-root ratio) and allometric (root collar diameter and height) variables were measured throughout the growing regime and/or at destructive sampling.

Media type was the main factor determining seedling growth. Seedlings grown in an 80:20 mixture of compost and grit had significantly greater heights, root collar diameters and root volumes than other treatments.

Given the significant increase in seedling characteristics associated with outplanting success, our findings suggest that by switching to a compost and grit mixture, two widely available products in Haiti, seedling quality can be improved. Additionally, nurseries that are able to acquire containers equipped to root train, may incur the extra benefits of improved root architecture.

Introduction

With continued wide-scale degradation and fragmentation of forest habitat around the world, forest restoration is becoming increasingly important (Kettle *et al*. 2008). Globally, deforestation accounts for approximately 17% of all anthropogenically generated greenhouse gas emissions and is seen as a relatively low-cost target for emissions reduction (Gorte and Sheikh 2010). Of deforestation-related greenhouse gas emissions, tropical ecosystems represent the largest contributor (Gorte and Sheikh 2010). Deforestation is also the root of a suite of other environmental and social issues including air and water quality, economic stability, and cultural preservation. Representing 44% of the world's forested area, tropical forests provide habitat for many of earth's terrestrial biota and are the basis for the livelihoods of approximately two billion people classified as rural poor across the globe (Kettle 2012). Reversing, or at least arresting, the trend in tropical forest loss and degradation through reforestation provides a major tool for global climate change mitigation, biodiversity conservation, and poverty alleviation.

Haiti, a tropical country whose landmass was at one time covered by approximately 60% forests, has suffered great losses from deforestation, with less than 2% forest cover today (Foxx 2012). Deforestation in Haiti is largely viewed as a result of centuries of political and economic strife (McClintock 2010), resulting in poor land management and subsistence agricultural and forestry practices. While it is clear that reforestation can prove to be greatly beneficial, there are several major hurdles to reforestation efforts in Haiti. These issues, including biological, cultural, and economic, must be addressed in a timely and connected manner to achieve reforestation success.

With an average per capita annual income of approximately US$480, most (93%) of the population rely on wood products for cooking (McClintock 2010). Often when trees reach a diameter of approximately 2 cm, branches and trunks are harvested for charcoal production (McClintock 2010). A combination of changes in land-use policies and practices, shifting from subsistence forestry and agriculture to a model of sustainable harvest, and community based initiatives have shown success in other regions and may serve as models for Haiti to adopt and adapt (Lamb *et al.* 2005).

Another formidable obstacle to reforestation in Haiti lies in its topography. Haiti is a mountainous country with 63% of the land sloping at a grade of 20% or greater (McClintock 2010). These steep hillsides are covered with visible erosion where seasonal monsoons wash away valuable topsoil. This is a problem for tree seedling establishment, as these plants focus their roots within topsoil where the majority of nutrients and beneficial microorganisms are present (Alameda *et al.* 2012). Additionally, subsoil layers oftentimes display high bulk densities causing great amounts of stress to newly developing root systems (Alameda *et al.* 2012).

While generating new forests may seem a daunting task, production of high quality seedlings grown specifically for reforestation is an imperative first step. Current nursery practices in Haiti, however, focus primarily on seedling quantity rather than quality. Multiple studies have shown that outplanting performance on reforestation sites correlates highly with seedling quality (Davis and Jacobs 2005; Grossnickle 2005; Haase *et al*. 2014). In a nursery system, seedling quality is often quantified via several morphological and physiological measurements. No single measurement can dependably predict outplanting performance; however, multiple studies suggest that seedling root-collar-diameter (RCD), shoot height, root volume, and root-to-shoot (R:S) ratio correlate highly with outplanting success (Mattsson 1997; Davis and Jacobs 2005a; Mexal *et al.* 2008).

Proper nursery culture will have the greatest impact on seedling quality, whether the nurseries are in Haiti or elsewhere. In today's modern forest seedling nurseries, growers are able to utilize high quality growth media in concert with cutting edge container technology and a wide array of fertilizers in a controlled environment. All of these factors assist growers in producing ideal seedlings. In contrast, the majority of nurseries located in areas of tropical deforestation, including Haiti, lack access to these nursery cultural tools. Nurseries in Haiti, as in other developing countries, must produce healthy seedlings using economically viable methods and resources, and focus expenditures on the most important aspects of growing healthy trees (Liegel and Venator 1987).

There are many variables that can influence seedling quality and outplanting success. Yet, access to moisture and nutrients are first and foremost. These two variables are easily manipulated in a nursery via proper growing medium and container selection (Landis and Morgan 2009; Wolken *et al*. 2010). In a study performed by Rose and Haase (1998) where Douglas-fir seedlings were grown in two different media types (traditional peat-based media and coconut coir) significant differences in seedling growth and nutrient uptake were measured. Seedlings grown in the peat-based media showed overall greater seedling quality than those grown in coconut coir. Yet, the authors point out that with further study of the use of coconut coir in a nursery setting some of the limitations of coir may be mitigated via changes in irrigation and fertilizer use, thus manipulation of moisture and nutrient access (Rose and Haase 1998). In a separate study, concerning seedling quality as it relates to container type, Aldrete and Mexal (2002) found that seedlings grown in polybag containers exhibited root systems that are associated with reduced seedling establishment, which has been associated with improper acquisition of moisture and nutrients.

Selection of the proper growing media is fundamental to effective nursery management and is an integral component to healthy root development. Growing media typically serves four major functions, that of: physical support, aeration, water holding capacity and mineral nutrient exchange (Landis *et al*. 2014). Beyond the chemical and physical properties of growing media, cost and sustainability are important considerations in dealing with an island-nation such as Haiti.

With proper root-to-medium contact, water and minerals within the media become increasingly available to seedlings (Tracy *et al.* 2013). However, variations in soil and medium type can lead to inconsistencies in root-to-medium contact. The root of these inconsistencies oftentimes lies in the overall amount of medium or soil within an area. This is where bulk density plays an important role in nursery seedling development. For growing medium and soils, bulk density is defined as the weight per volume of the medium in question. This may vary depending on the innate characteristics of medium components. Additionally, the mere filling of containers by machines or humans can highly affect bulk density thereby influencing the water and nutrient availability to seedlings. Ideal bulk densities will differ depending on the soil type and its texture (Pires *et al.* 1997). Additionally, different species will prefer varying bulk densities depending on the soil type (Tracy *et al*. 2013). However, the range of ideal bulk densities is relatively narrow; researchers have reported a normally distributed response curve of root growth to compaction, with growth being reduced at both the highest and lowest bulk densities (Tracy *et al*. 2013).

Severe soil compaction, or high bulk density, will decrease a plant's ability to uptake critical macro- and micronutrients as well as water. Jacobs *et al.* (2009) show that cation exchange capacity (CEC) is inversely related to soil bulk density. Soils with a high CEC generally have a larger reserve of available cationic mineral nutrients. Lack of access to moisture and nutrients as well as development of poor root structure pose two major setbacks to healthy plant growth and development. High bulk densities exhibit low water holding capacities leading to elevated amounts of runoff and leachate. Additionally, difficulty accessing essential nutrients may result in decreased photosynthesis as well as growth abnormalities (Bécel *et al.* 2011).

In Haiti, as in many developing countries, topsoil is the primary component of available potting media (Mexal 1996; Akpo *et al*. 2014). This option makes immediate sense to some, as it is what plants grow in naturally. However, container-grown systems are not effective when native topsoil is used, which often results in poor outplanting success (Landis 1995). For one reason, conditions in a container system are quite different from those in the field. The amount of growing medium available to plants is limited in a container resulting in inadequate availability of moisture and nutrients (Landis *et al*. 2014). Second, topsoil tends to be excessively heavy, compacts easily, and may drain poorly (Mexal 1996). Compacted media, exhibiting high bulk densities, often will force seedling roots to concentrate outside of the bulk media. Roots close to container walls are subject to spiraling and excessive damage during transport (Mexal 1996). Furthermore, topsoil is an unsustainable resource and is particularly valuable in heavily deforested regions already suffering from erosional soil losses (Mexal 1996).

Alternatively, depending on the region in which seedlings are being propagated, various substitute media sources may be utilized (Landis *et al*. 2014). Historically, some alternative media choices have included rice hulls, sand, compost, sawdust, pine bark etc. (Jacobs *et al*. 2009). When considering alternative media, the most important aspect to keep in mind is product availability. Industry standard media, which are typically composed primarily of peat, are of highest quality but also have high prices and shipping costs (Davis *et al*. 2009; Landis and Morgan 2009). Moreover, this type of media is essentially void of nutrients. While this quality can be greatly beneficial as it allows for easier manipulation of seedling growth via fertilizer application, the extra expense of extensive fertilizer application may not be economically feasible everywhere. Therefore, locating viable materials within each region is vital to success.

With proper attention, growers almost anywhere in the world can produce compost. While compost quality can vary greatly depending on content, utilization as a component of potting media can prove highly valuable (Avramidou *et al*. 2013; Landis *et al.* 2014; Akpo *et al*. 2014). Compost can greatly improve the medium's chemical and physical properties by enhancing water retention, porosity, and fertility (Kozlowski 1999). It has also been found that some composts are capable of suppressing seedborne and soilborne pathogens (Landis *et al*. 2014). In the tropics, mature compost can be produced in as little as 2 to 4 months. One form of compost in particular that is available to many Haitians is that of biosolid waste.

As of 2010, only 26% of Haitians had access to improved sanitation and no formal waste treatment facilities existed (Kramer *et al*. 2013). The installation of composting toilets has been providing a remedy to this deficit as well as an additional service. First, composting toilets provide a form of sewage treatment in areas suffering from the consequences of open sewers. Second, the product of these composting toilets can be employed to enrich the soil in a country where soil loss is of alarming concern. In 2006, the Sustainable Organic Integrated Livelihoods (SOIL) organization was funded to responsibly turn municipal waste into compost via composting toilets in Haiti. While the use of composted human waste can be somewhat controversial, research exists on the benefits of this type of compost and it has been successfully utilized in several instances (Lombard *et al*. 2010; Kramer *et al*. 2013; Avramidou *et al*. 2013).

Another source of commonly available and inexpensive growing amendments are sand, silt, and grit. These amendments generally offer little to no nutritional value to a container system but at the right size, may afford much needed aeration and drainage to plant root structures when added in appropriate ratios (Landis *et al*. 2014). Particles that are too small may increase bulk density within the container and inhibit root growth as well as lower CEC. In general, particle sizes .05-.25mm are ideal as they are less likely to plug container drainage holes or reduce aeration (Landis *et al*. 2014).

Besides media, a second factor that may lead to poor quality nursery grown seedlings is container type (Budy and Miller 1983; Amoroso *et al.* 2010). Forest seedling nursery containers must perform a combination of functions and the subsequent choice will vary by species (Aphalo and Rikala 2003). Container characteristics help to shape seedling growth in the nursery, such as various sizes and design features to mitigate root spiraling and further influence overall root architecture (Aldrete and Mexal 2002). The importance of quality root architecture is discussed below. Other important container traits are related to economic and management considerations both in the nursery and at the outplanting site (Landis 1990a).

A few elements are required for proper root growth in relation to containers. The first is appropriate volume. Shallow containers allow for root growth that may be appropriate for outplanting situations where drought is not an issue or soils are shallow. In areas where drought is likely, however, containers allowing for deep root growth are desirable (Liegel and Venator 1987; Pinto *et al.* 2012). Roots that are preconditioned to growing at greater depths have a higher likelihood of success in seeking out moisture when outplanted (Landis 1990a). Also attributed to container volume is the amount of time needed to store each seedling in the nursery. Nursery practitioners should be able to determine the appropriate container size and shape for each species without taking up unnecessary amounts of space while avoiding excessive amounts of root growth within each container (Liegel and Venator 1987; Landis 1990a).

Another important consideration of container traits is the potential for root training (Liegel and Venator 1987; Landis 1990a). In containers lacking internal ridges, lateral and taproots will often come into contact with the container wall and begin to grow horizontally. These roots will continue to do so and spiral continuously around the inner container; it is commonly referred to as root spiraling. Root systems that are allowed to spiral tend to focus energy on increasing the length of those roots rather than producing a higher abundance of lateral roots, thus increasing overall root surface area. In outplanting situations the resulting root spirals will likely fail to explore the surrounding soil and find appropriate nutrient and moisture sources (Landis 1990a; Aldrete and Mexal 2002; Amoroso *et al.* 2010).

In many developing countries, including Haiti, nursery growers typically use specialized polybags (i.e., plastic bags) as containers for growing tree seedlings. This container type is widely available and is lightweight and collapsible, which greatly reduces shipping costs. However, polybags have been associated with several plant growth and development issues, including root egression as well as malformation, which can greatly inhibit outplanting success (Landis 1990a; Aldrete and Mexal 2002). In spite of this, research suggests that poor establishment of seedlings grown in polybag nurseries must be assessed to determine whether or not proper shipping, handling and planting procedures were followed before determining that polybags are to blame (Mexal *et al.* 2008). This point is further illustrated by Burney *et al.* (2015) who observed that even after media and container type were changed in Mexican nurseries, seedling quality remained poor. This review found that despite the introduction of new and improved nursery technology, its successful use by nursery growers was still lagging. As a result, nursery practitioners in Mexico continued using old techniques with the new materials *(*Burney *et al.* 2015).

In addition to the above, when beginning any reforestation effort, selection of appropriate tree species for reforestation is critical. Globally, many reforestation efforts have focused on utilizing nonnative tree species. These species are generally chosen for their ability to grow quickly and mitigate soil erosion, whether or not they are native becomes irrelevant. Many scientists today would argue, however, that the advantages of choosing native over nonnative species may have advantageous long term effects (Shono *et al*. 2007; Davis *et* *al*. 2012). Planting native species has the potential to create an overall healthier and sustainable ecosystem for the long term.

Hispaniolan pine (*Pinus occidentalis*), a tree species native to the island of Hispaniola (comprising Haiti and the Dominican Republic), has been known as a species to scientists and foresters for almost 200 years but has received limited scientific attention. Little is known about the taxonomic variation of the species, its ecology, its wood properties, or its suitability for plantation forestry (Darrow & Zanoni 1990). The existing literature suggests that this endangered, indigenous species grows well at elevations ranging from 90 to 3200 meters and grows readily in various edaphic conditions including clay soils and acid gravels (Darrow & Zanoni 1990).

In Hispaniola, this species is highly valued. It is widely believed that this tree has served as a important timber species in the past and has historically been utilized for various medicinal purposes (Timyan 1996). Currently, there are several local nursery organizations in Haiti and the Dominican Republic propagating this species. Given the location of the remaining pine forests at high elevation, this species also represents a critical component of restoration programs aimed at conserving soil and reducing damage during heavy rains. As well, numerous associated species including a native strawberry and tortoise are only found in these remnant forests and hold potential future value for ecological study as well as economic gain through tourism.

Reforestation using high quality, nursery-produced seedlings is often the most efficient way to ensure successful establishment and achieve rapid growth following outplanting. The silvicultural combinations of proper container and growing media selection are two essential factors that contribute towards reforestation success and are likely treatments to increase the speed of reforestation in Haiti. The objective of this study was to gain a better understanding of the relative contribution of media and container type to the development of *Pinus occidentalis* via quantification of several seedling attributes. Attributes that were quantified included: RCD, shoot height (HT), root volume (RV), and root and shoot dry mass (RDM, SDM). These particular attributes correlate well with physiological processes linked to seedling quality. We hypothesized that seedlings grown in nutrient rich, (compost-based) media would exhibit morphological measurements correlated with higher seedling survival and greater early (outplanted) growth performance.

Methods

Experimental Design and Production

The experiment followed a randomized complete block design (RCBD) with a factorial structure (5 media treatments × 2 container types) containing 5 replicates per treatment. Randomization took place at the block level as each tray representing a container and media treatment combination was considered to be a block and containers within each block were randomized weekly. Each week, seedling trays were re-randomized to minimize effects from the nursery environment.

Five growth media were used in this study: (1) 100% peat-based [P] (SunGro® Metro Mix 167, Agawam, MA); (2) 100% topsoil [T] (NuLife Topsoil, Waupaca, WI); (3) 80:20 compost:grit [CG] (EKOCompost, Lewiston, ID) (Target® Forestry Nursery Grit, Burnaby, BC); (4) 80:20 compost:topsoil [CT]; (5) 70:20:10 compost:topsoil:grit [CTG]. Peat-based growing media is widely used in commercial forest tree seedling production nurseries in the US as well as other developed countries (Landis 1990b). Topsoil serves as an important media for observation as it is what is widely used in container nursery systems in developing countries, including Haiti (Liegel and Venator 1987; Mexal 1996; Akpo *et al.* 2014). The three latter mixtures serve as potential alternatives to currently utilized media types. Media types used covered a range of nutrient and physical properties (Table 1.1).

Pinus occidentalis seeds from a government funded seed bank in the Dominican Republic (Nigua Seed Bank, Santo Domingo, Dominican Republic, latitude 18.37629, longitude -70.06938, provenance unknown) were soaked in distilled water for 12 hours prior to sowing as recommended by the Nigua Seed Bank. One to two seeds per container were directly sown on 12 June 2014, at the University of Idaho's Center for Forest Nursery and Seedling Research in Moscow, Idaho (latitude 46.72552, longitude -116.95603). The seeds were sown into polybag (946 ml, 7.6 cm diameter, 19.1 cm height; Peaceful Valley Farm Supply, Grass Valley, CA) and D-40 (656 ml, 6.4 cm diameter, 25.4 cm height; & Stuewe & Sons Inc., Tangent, Oregon). Polybag containers were modified to the same volume of D-40 containers by using a heat sealer (Uline, Pleasant Prairie, WI) to close off excess container space while avoiding loss of container depth. Each of the five media types were premixed and used to fill 100 containers for each container type totaling 1000 containers. After direct sowing, containers were covered with Deluxe Seed Guard germination fabric (Dewitt Company Inc., Sikeston, Missouri) and irrigated using an overhead boom system three times per day until new germination ceased (approximately 2 weeks). Weekly, as well as final, germination counts were recorded.

After germination, seedlings were irrigated when block weights (one tray consisting of 5-20 seedlings) measured 80% of their weight at field capacity via the nursery manager method according to Dumroese *et al.* (2015). Using this method seedlings were irrigated approximately once per week. No fertilizer was added at any point throughout the growing regime.

Sampling

Germination counts were recorded starting 24 June and continued weekly until 22 August. On 11 November 2014 (154 days after sowing) each seedling's HT and RCD were measured for all seedlings. HT was measured from the cotyledon scar, at the root-collar, to the terminal end of the longest branch. RCD measurements were obtained at the root collar. Additionally, soil electrical conductivity (EC) and pH were measured approximately 1 hour after each irrigation event via the pour through method (Bilderback 2001) on a per weekly basis. The pour through method requires that after an irrigation event, media is allowed to drain freely until no longer dripping. At this point containers are suspended above a receptacle that captures a premeasured amount of water. Once all of the added water has drained through the potting media and into the receptacle, EC and pH measurements are obtained via the container leachate.

Destructive sampling occurred during the week of December 15th, 2014 for all seedlings. Measurements included morphological plant growth characteristics of HT, RCD, root volume (RV), root dry mass (RDM), and shoot dry mass (SDM). First, root systems were carefully washed clean of all media. Second, RV was determined by water displacement (Burdett 1979). Next, seedlings were severed at the root-collar, and roots and shoots were dried separately in paper bags at 70°C for 72 hours. Following drying, RDM and SDM were used to determine seedling root-to-shoot ratio (R:S). Tissue samples were collected at the time of destructive sampling from the entire shoot of each seedling and analyzed for foliar nutrition (N, P, K, S, Mg, Ca, Na, B, Zn, Mn, Fe, Cu, and Al) (A & L Great Lakes Laboratories, Fort Wayne, IN).

Each media type was analyzed for nutrient concentrations (N, P, K, Ca, Mg, Na, and NO_3N), EC, pH, and carbon:nitrogen, as well as physical properties (bulk density, water holding capacity) at the beginning of the growing season and again at the end (A & L Great Lakes Laboratories, Fort Wayne, IN). Nutrient concentrations were determined via the saturated media extract (SME) method, whereby media samples are saturated with distilled water and allowed to equilibrate for one hour. After equilibration, pH measurements are obtained directly from the media slurry. All other nutrient analyses are performed on the extracted leachate from the slurry obtained via a Buchner funnel lined with filter paper (Warnke 2009).

Media bulk density was approximated for each treatment. This was done using D-40 containers that were filled with five samples of each media type. Filling each D-40 container using the same technique as each of the actual treatment containers approximated the bulk densities. After filling, media was removed from each container and oven dried in paper bags at 100°C for 48 hours (Doran and Jones 1996) and weighed. Bulk density was calculated as:

$$\text{Bulk Density (g/cm}^3\text{)} = \text{Dry Soil Mass (g)} / \text{Soil Volume (cm}^3\text{)}$$

where dry soil weight is the mass in grams of soil after oven drying and soil volume equals the volume of the container.

Media water holding capacity (WHC) was calculated separately at the University of Idaho’s Soil laboratory. A high-range pressure system with ceramic plates was used to determine the water holding capacity for all five media types at two water potentials:

-0.033 MPa (field capacity) and -1.5 MPa (wilting point) were (Klute, 1986). Five samples of each media type were analyzed for field capacity and wilting point. Bulk density and the gravimetric water content of each sample were obtained. Gravimetric soil water content was calculated as:

$$SWC = (Db \times \theta m) / Dw$$

where Db = media bulk density, θm= gravimetric water content, and Dw = water density ($Dw = 1\ g/cm^3$). Once SWC was determined for each media type, both at field capacity and wilting point, WHC was calculated:

$$WHC = \text{field capacity } SWC - \text{wilting point } SWC$$

Statistical Analysis

Statistical analyses were completed using R (Version 3.1.1, The R Foundation for Statistical Computing, 2013). Analysis of variance (ANOVA) was carried out for each response variable (HT, RCD, SDM, RDM, RV, and R:S) after nursery culture and destructive harvest. The model included the main effects of media and container as well as their interaction. The data met all assumptions for normality and thus treatment comparisons were evaluated using the least significant difference of means; differences were deemed significant at $\alpha = 0.05$. When ANOVA indicated significant differences multiple comparisons were calculated using Tukey's Highly Significant Difference test (TukeyHSD) ($\alpha = 0.05$).

Results

Physical Characterization of Growth Media

Bulk density differed significantly ($P < 0.05$) across all media types ($P < 0.0001$) but not between container types ($P = 0.263$) and showed no interaction between container and media types ($P = 0.692$). CTG had the highest bulk density measuring 3.7× higher than P which exhibited the lowest bulk density followed by CG, then CT. Bulk density of T measured slightly higher (2.5×) than P (Figure 1.1).

Water holding capacity varied significantly ($\alpha = 0.05$) by media type but not container and there was no container × media interaction. P had the highest water-holding capacity at 62%. T and CT treatments were not significantly different from one another and had approximately half the water holding capacity of P. CTG had a 288% reduction in water content compared with P, whereas CG was 786% lower.

A regression analysis of media bulk density in relation to water holding capacity revealed a significant correlation between the two physical properties (Figure 1.2) ($R^2= 0.939$). Data showed a negative relationship between BD and WHC, as BD increased WHC decreased.

Seedling Morphology

For the morphological response variables measured (HT, RCD, RDM, SDM, RV), significant differences between container and across media types were present. For some of these variables, a significant interaction between both container and media type was also present (Table 1.1).

HT differed significantly across media treatments ($P = 2.003$ e-13) as well as between container types ($P = 3.653$ e-6) but no interaction was found ($P = 0.351$). Further analysis revealed that HT of seedlings grown in CG were 19% greater than the overall average across media treatments. Following CG in average HT across media treatments was CTG with an overall average of 3% greater than the mean. CT, T and P all displayed average HT below the overall mean. Seedling HT between container types was 10% greater in polybags than in D-40s.

RCD also differed significantly across media treatments ($P < 0.0001$) and between container types (P 0.0001) but showed no interaction between the two main effects ($P = 0.822$). Seedlings grown in CG displayed 35% greater overall RCD than seedlings grown in all other media types in this study. Seedlings grown in all other media types exhibited RCDs lower than the overall average with P displaying the lowest overall RCD measuring 11% lower than the overall average. Seedling RCD between container types was 10% greater in polybags than in D-40s.

No significant differences were observed in main effects or their interactions for seedling RV (Table 1.1).

RDM displayed significant differences across media types ($P < 0.0001$) but not between container types ($P = 0.086$) and no significant interaction was observed ($P = 0.069$) (Table 1.1). Similar to results from RCD and HT, seedlings grown in CG had the overall highest RDM measuring 93% greater than the overall mean. Again, seedlings grown in all other media types had lower RDMs than the mean with P having the overall lowest measurements for this variable, averaging 36% lower than the mean.

Analysis of SDM revealed significant differences across media ($P < 0.0001$) and between container types ($P < 0.0001$) but showed no interaction ($P = 0.9047$). Seedlings grown in

CG had overall greatest SDM, 109% greater than the mean, followed by CTG, CT, P and T. Seedling SDM between container types was 39% greater in polybags than D-40s. Seedling R:S differed significantly between media ($P < 0.0001$) as well as container types ($P = 0.0089$) with no interaction. Seedlings grown in T displayed the overall greatest R:S measuring 104% higher than seedlings in CG that measured overall lowest R:S. Seedling R:S between container types was 30% greater in D-40s than in polybags.

Plant Tissue

Plant tissue analyses revealed significant differences in nutrient absorption across media types (Table 1.2). No differences were detected between containers or as interactions between media and container types. Overall, nitrogen was present in lowest concentrations in P and T, measuring 30% and 51% lower, respectively, than CT exhibiting the highest overall nitrogen concentration. Phosphorus levels in CT were 18% higher than the second highest phosphorous concentration found in CTG. Phosphorous concentrations in P, T and CG were similar, differing by only a few percent. Highest potassium levels were observed in CT followed by T, CTG, CG and P.

Germination

Overall seedling germination was remarkably low, about 53% across all treatment combinations. Germination analyses revealed significant differences between container type ($P < 0.0001$), media type ($P < 0.0001$), and their interaction ($P < 0.0001$) (Figure 1.3). Differences in germination concerning media type occurred between P and all other media types as germination in P was 21% greater than the overall germination average. Germination in T was 7% greater than the overall germination average. All compost based media types (CT, CG, CTG) had lower germination than P or T with CT being the lowest.

Of the total germinants, 36% were in polybags and 64% in D-40 containers. D-40 containers yielded higher germination numbers than polybags ($p < 0.001$). Across media and between container types P germination was 45% higher in D-40s than in polybags, T germination was 59% higher in D-40s than polybags, CT germination was 18% higher in D-40s than polybags, CG was 117% higher in D-40s than polybags, and CTG showed no significant difference concerning container type.

Discussion

Media Physical Characterization

Soil bulk density and water holding capacity, greatly influence the amount of water available for plant uptake (Pires *et al.* 1997) as well as fine root proliferation. Soils that are highly compressed tend to lack the pore spaces necessary for holding water as well as air. These pore spaces are also necessary for plant roots to explore growing media (Tracy *et al.* 2013). Moreover, studies have shown that as bulk density increases available water holding capacity decreases (Davey 1996). The results of this nursery media research are congruent with these findings (Figure 1.2). Additionally, adequate pore space is essential to nutrient absorption as these spaces provide surface areas where moisture and nutrients are accessible by roots (Kozlowski 1999). Thus, limited pore space may lead to stunted growth (Tracy *et al.* 2013). None of our measured bulk densities are beyond that of root penetration, which is approximately 1.5 g/cm^3 (Brady and Weil 2004). Therefore, bulk density likely did not inhibit root penetration or hinder moisture and nutrient absorption during this study. Because bulk density did not inhibit root growth in this study, it may be inferred that RV and RDM were likely influenced by other treatment variables, including but not limited to media physical and nutritional properties.

The water holding capacity of each media type also varied significantly across treatments. Media types with higher water holding capacities will provide seedlings with increased moisture availability (Chirino *et al.* 2011). Therefore, a media with high water holding capacity would better serve most nurseries. Water holding capacity in this study was greatest in P and lowest in compost based media types. For compost-based media types, it may be advisable to add a water absorbing amendment such as sawdust or coconut coir to aid in increasing media water holding capacity (Davey 1996). Additionally, decreasing the amount of grit in these amended composts would increase the amount of organic material available to hold water as well as decrease bulk density.

Media and Plant Tissue Nutrient Concentration

According to Liebig's Law of the Minimums, growth is controlled not by the total amount of resources (nutrients, moisture, oxygen) available, but by the scarcest resource (Jacobs and Landis 2014). Considering that water and oxygen were available in adequate quantities throughout this study, nutrient analyses of plant tissues revealed some of what seedlings were lacking. Of the nutrients analyzed, seedlings grown in P and T were nitrogen deficient (Table 1.2). Nitrogen is a macronutrient essential to all plant physiological processes and lack of access to this nutrient may have contributed to the resulting overall lower morphological measurements in these seedlings. Lower morphological measurements indicate reduced physiological processes, including photosynthesis, which is essential for seedling establishment (Davis and Jacobs 2005b).

Seedlings grown in compost based media types (CT, CG, CTG) were all deficient in manganese (Table 1.2). Manganese has a wide range of roles but is essential for the synthesis of chlorophyll and also serves as an enzyme activator (Landis 1984). Consequently, seedling growth may have been inhibited by a lack of access to manganese. Nutrient analyses performed by the compost company used in this study (EKOCompost) reported average manganese concentrations of 280 ppm. A concentration of this magnitude indicates that manganese should have been adequate for plant growth. Research performed by Lombard *et al.* (2010), however, reported that biosolid compost used in their study also contained adequate manganese for plant absorption but that manganese absorption was inhibited by high concentrations of iron, calcium and aluminum. The nutrient analyses provided by EKOCompost support these findings, as concentrations of each of these minerals were present in large quantities (aluminum 4100 ppm, iron 6400 ppm, calcium 1.6%). Thus, perhaps, manganese absorption in this study was inhibited by these high concentrations.

A two-year study performed by Selivanovskya *et al.* 2006, that used MSW compost on outplanted *Pinus sylvestris* seedlings, showed similar results concerning seedling tissue nutrient concentrations. However, this study found no toxicity effects of high concentrations of macro or micro-nutrients (Selivanovskya and Latypova 2006). These findings highlight the fact that compost may be of varying quality and is widely ranging in nutrient concentrations, therefore seedling quality must be closely monitored in a nursery setting.

Seedling Morphology

Overall, seedlings grown in P as well as T exhibited the smallest morphological measurements. While it was expected that seedlings grown in T might exhibit lower morphological measurements, the fact it was not significantly different than seedlings grown in P was unexpected (Table 1.1). As mentioned before, P is essentially sterile. This is one of the qualities that make peat-based media desirable in some nurseries, as plant growth can be highly manipulated via fertilizers (Landis *et al.* 2014). T, however, exhibited overall higher nutrient concentrations than P. Thus, the physical structure of the soil most likely inhibited nutrient absorption by seedlings. Typically, topsoil does not provide adequate aeration, drainage or moisture retention to serve as a viable media type in a container nursery system (Sahin *et al.* 2005). The data from this study can neither support nor deny this assertion as in depth soil physical property analyses are absent. Alternatively, media treatments including compost performed better than those without. While CG performed best when considering both height and diameter, these measurements were also higher when compost was added to topsoil, as topsoil and compost plus topsoil mixtures differed significantly. Additionally, CTG outperformed T as well as P (Table 1.1). Because the physical properties of compost based media types did not appear to be more beneficial than other media types in this study, these advantages, most likely, can be attributed to higher nutrient concentrations.

Germination

Having performed a preliminary bioassay of each media type prior to sowing *Pinus occidentalis* seeds, significant differences in germination across both media and container types was not unexpected (Figure 1.3). This corroborates, however, anecdotal statements from nursery practitioners in Hispaniola that *Pinus occidentalis* seeds are notoriously difficult to germinate uniformly (P. Jules and W. Placido, personal communication, 2014). Nursery practitioners as well as seed bank employees in Hispaniola are still working towards improving germination protocols for this species. Furthermore, there is a general need for more studies focused on determining methods to break dormancy in tropical seeds (Baskin and Baskin 2005).

When considering the differences in germination across container types, variations in the microenvironments found between the germination cloth and container must have been present as all other factors were the same. Due to the flimsy nature of polybag containers it was difficult to obtain a uniform, flat layout of the germination cloth across multiple containers. In contrast, the D-40 containers, with their rigid sidewalls, made application of germination cloth flat, uniform, and easy. The absence of uniformity in germination cloth, across polybag containers may have contributed to differences in light exposure, varying humidity surrounding the seed, or uneven irrigation, reducing overall germination.

Many species of conifer depend on light as well as moisture conditions as a means to trigger germination (Li *et al.* 1994). Measured as a plant's ability to tolerate shade, light preference is highly variable among species. The use of germination cloth assists in maintaining adequate light levels while trapping enough moisture to avoid seed desiccation (Schmal *et al.* 2007). However, further investigation of microclimate conditions within different container types should be conducted to pinpoint causes of poor germination.

Conclusions and Future Directions

Overall, this study suggests that media and container type can influence growth and development of *Pinus occidentalis* seedlings from germination to pre-outplanting. None of the media types alone, chosen for this study, proved to be viable options for use in Haitian nurseries. Further investigation into media types that are both locally available and sustainable is required to promote successful seedling development in a nursery. Due to the overall low germination percentage of *Pinus occidentalis* seeds, despite container type, further studies on how to improve germination in this species should be conducted. Additionally, further study as to why germination differed between container types could prove beneficial for nurseries in many areas.

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Table 1.1 Morphological growth characteristics of *Pinus occidentalis* seedlings following nursery culture. Statistical means for measured values and the associated standard error; height, root-collar diameter (RCD), root volume (RV), root dry mass (RDM), shoot dry mass (SDM), root-to-shoot ratio (R:S) were measured.

Media	**Height (cm)**		**RCD (mm)**		**RV (cm^3)**		**RDM (g)**		**SDM (g)**		**R:S**	
P	3.78 (0.07)	ab	0.93 (0.02)	a	1.03 (0.04)	a	0.09 (0.00)	a	0.09 (0.00)	a	1.51 (0.18)	ab
T	3.68 (0.07)	a	0.91 (0.02)	a	0.72 (0.03)	a	0.11 (0.00)	ab	0.08 (0.00)	a	1.86 (0.14)	b
CT	4.04 (0.10)	ac	0.94 (0.03)	a	0.72 (0.04)	a	0.11 (0.01)	ab	0.14 (0.01)	b	1.07 (0.10)	a
CG	4.92 (0.08)	d	1.40 (0.03)	c	1.56 (0.05)	a	0.27 (0.01)	c	0.33 (0.01)	c	0.91 (0.05)	a
CTG	4.24 (0.11)	c	1.02 (0.03)	b	0.62 (0.04)	a	0.12 (0.01)	b	0.15 (0.01)	b	1.13 (0.11)	a

Container	**Height (cm)**		**RCD (mm)**		**RV (cm^3)**		**RDM (g)**		**SDM (g)**		**R:S**	
D-40	3.94 (0.05)	a	0.99 (0.01)	a	0.91 (0.03)	a	0.13 (0.00)	a	0.13 (0.01)	a	1.47 (0.09)	b
Poly	4.32 (0.08)	b	1.09 (0.02)	b	0.96 (0.04)	a	0.14 (0.01)	a	0.18 (0.01)	b	1.13 (0.08)	a

*different letters indicate significant differences (α=0.05); n=91-132 for media treatments, n=313 & 211 for container treatments. Peat-based media (P), Topsoil (T), Compost:Topsoil (CT), Compost:Grit (CG), Compost:Topsoil:Grit (CTG)

Table 1.2 Plant tissue nutrient concentration following nursery culture. Statistical means for measured values and associated standard error and significant difference indicator.

Nutrients	Adequate	P		T		CT		CG		CTG	
Sulfur (%)	**.10-.20**	0.19 (0.01)	b	0.15 (0.01)	a	0.23 (0.01)	d	0.21 (0.01)	c	0.24 (0.01)	d
Phosphorus (%)	**.20-.60**	0.45 (0.03)	b	0.43 (0.04)	a	0.77 (0.05)	d	0.40 (0.02)	a	0.65 (0.06)	c
Zinc (ppm)	**30-150**	69.00 (2.90)	c	64.01 (3.16)	b	80.90 (4.13)	d	61.88 (3.24)	ab	68.85 (5.08)	bc
Iron (ppm)	**60-200**	210.00 (18.77)	d	241.84 (17.20)	e	144.06 (6.54)	c	91.26 (4.61)	a	122.77 (8.40)	b
Boron (ppm)	**20-100**	32.50 (1.85)	a	65.47 (2.26)	b	114.41 (6.37)	c	110.28 (5.95)	c	131.57 (19.30)	c
Copper (ppm)	**4-20**	9.30 (0.34)	c	7.63 (0.67)	b	7.33 (0.39)	b	5.28 (0.45)	a	5.55 (0.39)	a
Manganese (ppm)	**100-250**	1138.30 (70.48)	d	383.98 (14.70)	c	84.68 (5.93)	b	54.02 (3.24)	a	73.01 (6.46)	b
Magnesium (%)	**.10-.30**	0.25 (0.02)	c	0.27 (0.02)	c	0.17 (0.01)	b	0.12 (0.01)	a	0.17 (0.01)	b
Calcium (%)	**.30-1.0**	0.45 (0.02)	d	0.69 (0.03)	e	0.40 (0.02)	b	0.30 (0.01)	a	0.41 (0.02)	c
Potassium (%)	**.70-2.50**	1.72 (0.02)	a	2.20 (0.07)	c	2.32 (0.08)	d	2.06 (0.08)	b	2.15 (0.10)	b
Nitrogen (%)	**1.3-3.5**	1.37 (0.07)	b	1.18 (0.07)	a	1.78 (0.09)	e	1.65 (0.09)	c	1.60 (0.20)	c

*different letters indicate significant differences (α=0.05); n=10 for measurements. Peat-based media (P), Topsoil (T), Compost:Topsoil (CT), Compost:Grit (CG), Compost:Topsoil:Grit (CTG)

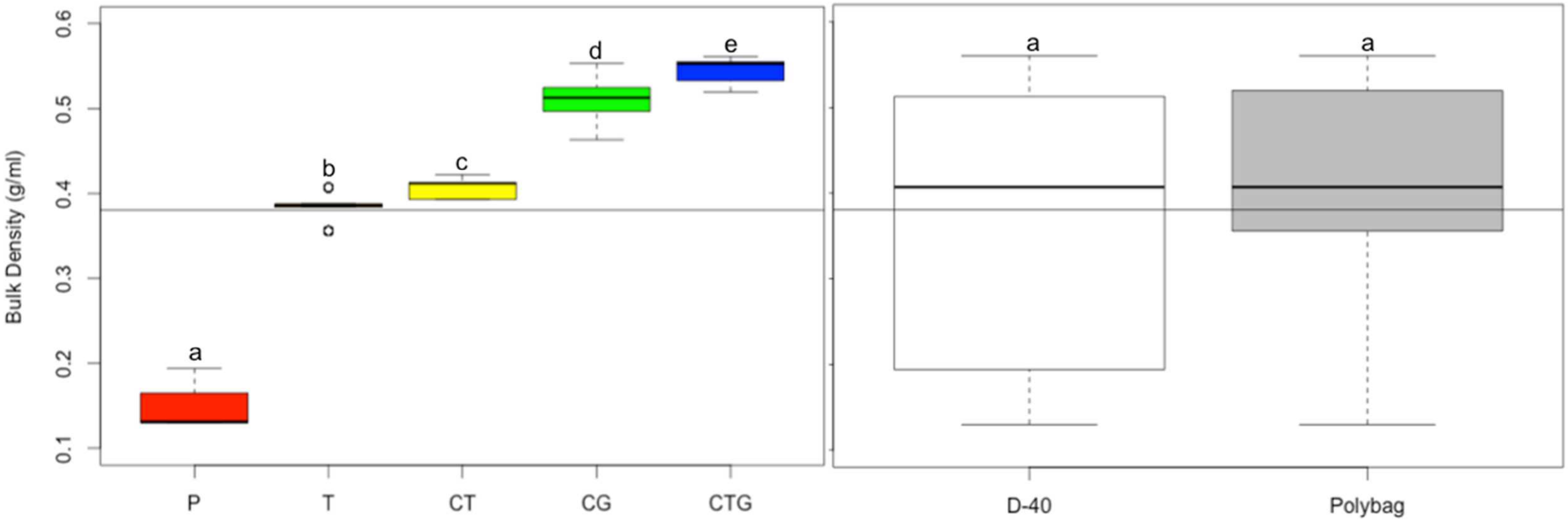

Figure 1.1 Mean bulk density across media and container types with associated significant difference indicators. Peat-based media (P), Topsoil (T), Compost:Topsoil (CT), Compost:Grit (CG), Compost:Topsoil:Grit (CTG).

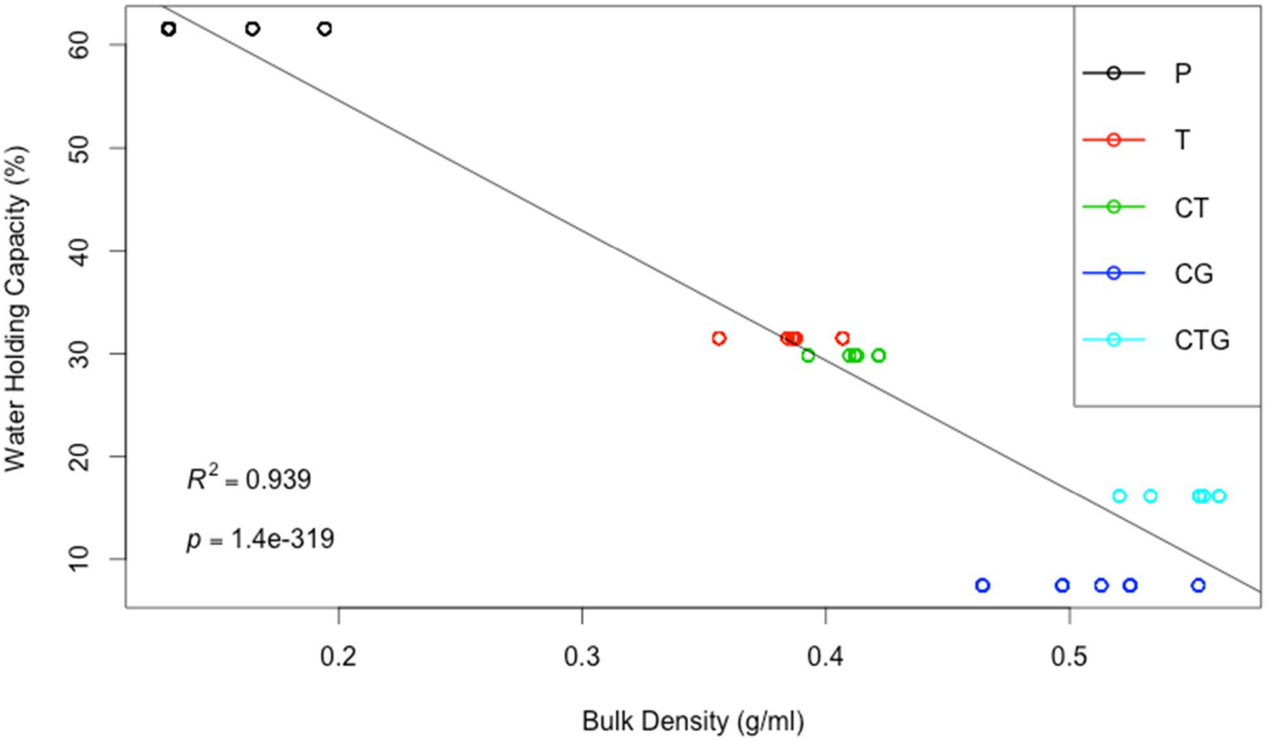

Figure 1.2 Linear regression of soil bulk density (g/ml) and water holding capacity (%). CG=compost:gravel, CT=compost:topsoil, CTG=compost:topsoil:gravel, P=peat-based, T=topsoil

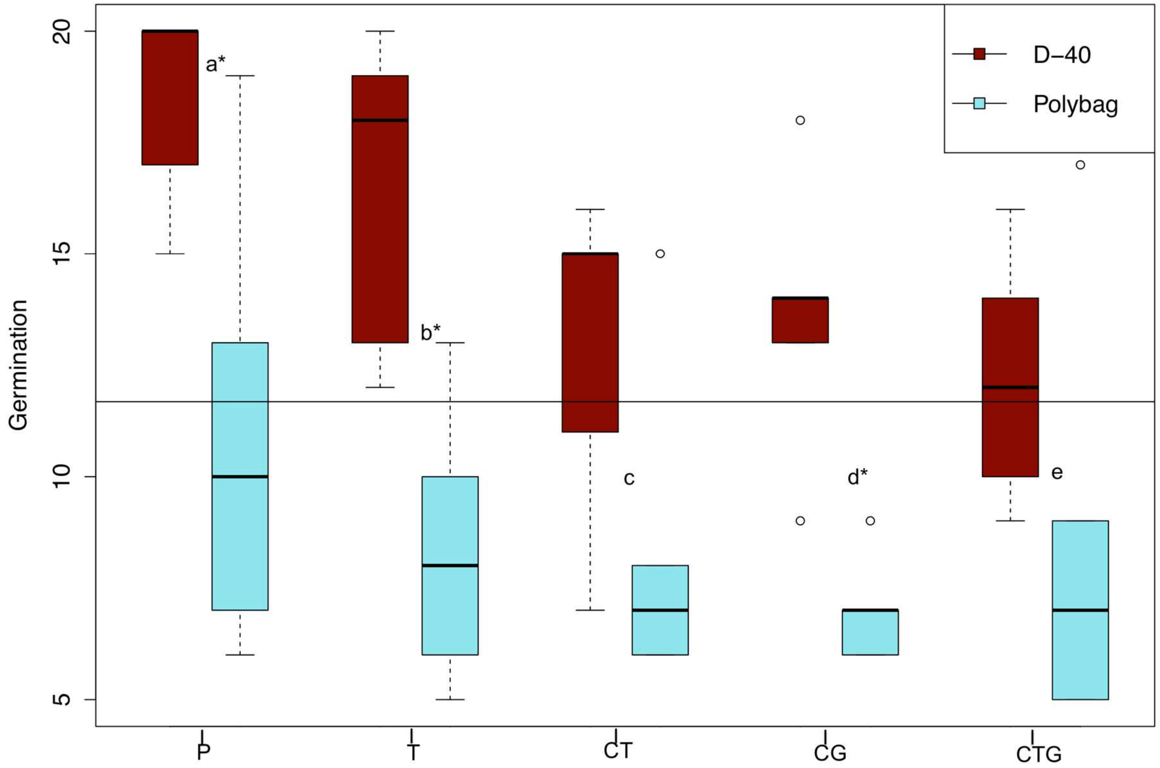

Figure 1.3 Mean germination across media and container types with associated significant difference indicators. Letters indicate differences across media types and * indicate differences between container types. Peat-based media (P), Topsoil (T), Compost:Topsoil (CT), Compost:Grit (CG), Compost:Topsoil:Grit (CTG).

Chapter 2

Effects of plant moisture stress on growth and photosynthesis of *Pinus occidentalis*

Abstract

Seasonal monsoons were at one time associated with abundant rainfall in Haiti. However, with increased deforestation, these large precipitation events have become less reliable and are yielding lower amounts of rainfall in conjunction with higher temperatures. During the dry season, plants must be able to withstand drought conditions or suffer decreased growth or mortality. Thus, seedling performance under drought conditions becomes important for nursery and reforestation practitioners, as these variations must be considered in order to determine the appropriate outplanting window for each species. The objective of this study was to determine the relative contribution of nursery cultural practices on the subsequent growth and photosynthetic capabilities of Pinus occidentalis seedlings under two irrigation treatments: drought and non-drought.

Pinus occidentalis seedlings were grown in five different media types during nursery cultivation in 2014. In May 2015, a subset of these seedlings were transplanted into 5.5-liter long pots for s a simulated outplanting. After transplantation seedlings were divided into two groups: drought and non-drought. Relative seedling growth was not affected by previous media types but was highly affected by irrigation treatments. Photosynthesis and plant moisture stress also differed only between irrigation treatments. Seedlings in the drought treatment, over time, eventually had significantly lower photosynthesis measurements as well as increased plant moisture stress. Alternatively, seedlings grown in the non-drought treatment maintained even photosynthesis and decreased plant moisture stress.

Introduction

Rainfall in tropical forests can be highly variable and may fluctuate from 800 to more than 10,000mm annually (Richards 1998). Fortunately, plants in dry tropical forests and savannahs have evolved traits that help them survive these variable conditions even in times periodic drought. However, the importance of drought in wet and moist tropical forests for plant growth and survival has been somewhat under-studied; particularly for those tropical forests where rainfall is seasonal with one or two dry seasons per year (Engelbrecht *et al.* 2007). This is especially true for Haiti, with its decreased forest cover (Gorte RW and Sheikh 2010), where dry seasons are becoming increasingly pronounced and are associated with higher temperatures (Malhi and Wright 2004) as well as reduced rainfall (Makarieva *et al.* 2014).

During the dry season, plants can be exposed to considerable drought stress that may result in decreased plant vigor and increased mortality (Haase *et al*. 2014); this is likely exacerbated for seedlings as they do not yet have fully developed root systems (Grossnickle 2005). Therefore, the extent to which a seedling may be able to resist drought stress becomes important for nursery and reforestation practitioners, as these fluctuations in moisture must be used to determine the appropriate outplanting window for each species. In areas where wet and dry seasons are prevalent, the outplanting window needs to be timed with the beginning of reliable rains and increased soil moisture (Haase *et al.* 2014). This is to ensure that plant roots have sufficient time to develop and establish before the next dry season begins. When wet seasons are interrupted by drought, however, knowledge pertaining to species drought tolerance is essential. Additionally, available soil moisture relative to seedling drought tolerance can help to determine ideal edaphic conditions at outplanting sites (Engelbrecht *et al.* 2007).

Mechanisms of drought resistance can be divided into two categories: desiccation delay and desiccation tolerance. Characteristics of desiccation delay include deep roots, early stomatal closure, low cuticular conductance, water-storage in plant organs, osmotic adjustments, and leaf shedding. All of these traits are aimed at increased access to soil water and decreased plant water loss (Tyree *et al.* 2014). Desiccation tolerance, however, is aimed at promoting physiological traits that permit continued water transport, gas exchange, cell survival at lower water content (ω) and low water potentials (Ψ), increased resistance to xylem embolism, and ability of cells to remain alive at low ω or Ψ (Tyree *et al.* 2014). Therefore, desiccation tolerance is the ability to survive drought while minimizing reductions in growth, and ultimately, fitness.

Drought resistance of conifer species varies widely (Engelbrecht *et al.* 2005). These variations occur both within and outside of specific forest types, i.e. temperate, tropical, etc. But, research pertaining to drought resistance of conifer species within the tropics is particularly lacking. Engelbrecht *et al.* (2003) posits that this may be due to the assumption that plants in these areas suffer little from drought stress, but this may not always be true.

Pinus occidentalis, also known as Hispaniolan pine, is a tropical pine endemic to the island of Hispaniola. This species has been known to researchers for over 200 years but has received little scientific attention (Darrow and Zanoni 1990). While much is to be learned about this species, the rate of deforestation in Hispaniola exceeds that of reforestation (Brothers 1997; McClintock 2010). Thus, it is imperative to start in the nursery with this species and improve its outplanting success via nursery cultural practices in conjunction with proper outplanting procedures, while also including community engagement programs to address issues related to deforestation. Some preliminary work has given insight to some methods by which to improve seedling quality in a nursery setting, indicating the importance of media as well as container type selection (Hubbel 2015).

This study will examine drought resistance of *Pinus occidentalis*, which will help to determine the appropriate outplanting window as well as ideal edaphic conditions for seedling establishment. Drought susceptibility for *P. occidentalis* seedlings was characterized under dry and moist conditions in a controlled setting, with the objective of identifying critical timing for post-outplanting irrigation. Seedling gas exchange and moisture stress were measured to identify changes in physiological status, and morphological factors (height, root-collar diameter, root length, root and shoot dry mass) were recorded to determine the effects on more readily evaluated parameters. All of these measurements can help us to better understand the overall well-being of each seedling, and thus the likelihood of seedling establishment (Grossnickle and Folk 1993). Furthermore, this method allowed us to isolate the effects of drought from other ecological factors that determine survival and growth. We chose young seedlings because, due to their limited root systems, they are likely to be the life stage most sensitive to drought (Engelbrecht *et al.* 2005). Additionally, seedlings grown in a nursery environment must be outplanted at this life stage, and information pertaining to species drought tolerance is essential.

Methods

Nursery Culture

This study was performed as the second part of the Hubbel (2015) study that examined the relative contribution of media and container type on the growth of *P. occidentalis* seedlings. After 105 days of growth, a randomly selected subset of 50 *P. occidentalis* seedlings was chosen from Hubbel (2015). These seedlings were grown in D-40 containers (Stuewe & Sons, Tangent, OR), and in one of the five media types utilized in the original study (100% peat-based [P], 100% topsoil [T], 80:20 compost and topsoil [CT], 80:20 compost and grit [CG], 70:20:10 compost, topsoil and grit, [CTG]), for a total of 10 seedlings per media type. Due to differences in nutritional access of seedlings across media treatments there were variations in seedling size at the beginning of this study. P seedlings had the smallest height and root-collar diameter followed by T, CT, CTG and CG seedlings.

After selection, seedlings continued growth for 6 months in 2 growth chambers (Model E-30BH0, Percival Scientific, Inc., Perry, IA). The growth chambers were programmed to maintain an environment similar to that of a typical growing season in Kenscoff, Haiti, where *P. occidentalis* seedlings are currently propagated (400ppm CO_2, 11 hour day length and 30° C during the day, 27°C at night, 60% humidity) (Fiondella 2010). While in the growth chambers seedlings were fertigated twice weekly, at a rate of 150ppm nitrogen (ammonium-n 25.5 ppm, nitrate-n 43.5 ppm, urea-n 82.5 ppm) phosphorus 22.5 ppm, potassium 118.5 ppm, calcium 0 ppm, magnesium 5.25 ppm, boron 0.15 ppm, copper 0.375 ppm, iron 0.75 ppm, manganese 0.375 ppm, molybdenum 0.00375 ppm, zinc 0.375 ppm fertilizer (WIL-SOL Pro Grower 20-7-19, Wilbur-Ellis, Spokane, WA).

Experimental Design and Treatments

A complete randomized design (CRD) was established with a factorial structure of 5 media types × 2 irrigation treatments (drought [D] and a no-drought control [ND]) × 5 replications. There were 7-10 seedlings per treatment combination for a total of 48 seedlings (2 seedlings died during the growth chamber portion of this study).

Seedlings were sampled and evaluated (see next section) prior to being bareroot transplanted into 5.5 liter "Long pots" (Stuewe & Sons, Tangent, OR) filled with 100% perlite (Wilbur-Ellis, Spokane, WA). Care was taken to ensure proper root alignment as well as adequate media compaction during the transplant process. Once all seedlings had been transplanted, each was irrigated to full saturation. Approximately one hour after saturation, weights of each seedling in the control group were taken to determine field capacity. Seedlings in the control group were irrigated once they reached 80% of their weight at field capacity according to the operational methods described by Dumroese *et al.* (2015). Seedlings in the drought group received no additional irrigation for the duration of this study.

Sampling

Initial as well as periodic photosynthesis and plant moisture stress measurements were taken on each seedling throughout this study (n=48). Leaf water potential measurements of both predawn and midday were taken on eight separate events (Days 1,9,14,19,22,26,33, and 34) after transplanting. Leaf water potential measurements of both predawn (ψ_{pd}) and midday (ψ_{md}) were taken in the nursery using a pressure chamber (PMS Instrument Company, Albany, OR). Predawn measurements were conducted between 0200 and 0400 h, and midday measurements between 1100 and 1400 h (midday,

ψ_{md}) on the same day. On day 34 of the study, ψ_{pd} was measured via the stem of each seedling or a primary branch.

Photosynthesis measurements were taken on ten separate events (Days 1, 5, 11, 16, 21, 23, 26, 28, 31, and 35). Day 1 measurements were taken prior to transplanting, all other measurements were taken after seedlings were transplanted. Measurements were conducted using the LI-6400XT portable photosynthesis system (Li-COR, Inc., Lincoln, Nebraska) equipped with 6400-22L lighted conifer chamber. Three light-response curves were averaged to determine photosynthetic active radiation (PAR) settings for the study. Settings on the LI-6400 for all sampling periods were as follows: PAR of 1200 μmol m^{-2} s^{-1}, reference CO_2 concentration of 400 μmol m^{-2} s^{-1}, flow rate at 500 μmol m^{-2} s^{-1}, block temperature at 24°C, and relative humidity of 50-75%.

Due to the frequency of photosynthesis measurements and small size of the seedlings, needle fascicles were marked for repeated measures: the same section of fascicles was used for all seedlings on each photosynthesis measurement event. Needle surface area was determined non-destructively via the adapted methods of Pinto *et al.* (2012). Fascicle diameters were measured at the center of each fascicle predetermined for photosynthesis measurements. Operating under the assumption that five needles of a fascicle form a cylinder, the abaxial leaf area was calculated by multiplying the circumference (of the cylinder) by the length of the predetermined needle segments. Total adaxial leaf area was calculated by multiplying the radius of the fascicle by the length of the predetermined needles, this number was then multiplied by ten (ten is the total number of adaxial surface areas of one needle fascicle). The following equation was used to calculate leaf area for one fascicle:

$$\mathrm{LA}=\pi dl + 10\frac{d}{2}l$$

where *d* is the diameter of the five-needle fascicle and *l* is the length of the predetermined needles in the chamber.

Morphological measurements for height (HT), root-collar diameter (RCD), root dry mass (RDM), shoot dry mass (SDM) and root length (RL) were recorded on all seedlings post harvest, 27 May 2015. Seedling HT was measured from the root-collar to the terminal end of the longest branch. RCD measurements were obtained at the root-collar. Shoots and roots were bagged separately and dried for approximately 48 hours at 71°C. Following drying RDM and SDM were measured and subsequently used to assess seedling root-to-shoot ratio (R:S). RL was measured from the root-collar to the end of the longest root.

Given seedlings selected for this study varied in size due to treatment differences from the first study, relative growth was calculated across treatment types for HT and RCD. The equation used for this calculation is as follows:

$$\mathrm{RG}=(\mathrm{F} - \mathrm{I}) / \mathrm{I}$$

where F and I are plant HT or RCD morphological measurements (F = final measurement; I = initial measurement). Relative growth parameters are now RHT and RRCD for HT and RCD, respectively.

Statistical Analysis

Statistical analyses were completed using R (Version 3.1.1, The R Foundation for Statistical Computing, 2013). An ANOVA was used to test media and irrigation treatment main effects as well as their interactions for seedling height (RHT), root-collar diameter (RRCD), root dry mass (RDM), shoot dry mass (SDM) root length (RL), water potential (ψ_{pd}; ψ_{md}), and photosynthesis (*A*). When ANOVA indicated significant differences ($\alpha = 0.05$) among media treatments, Tukey's HSD was used to identify significant mean differences at $\alpha = 0.05$. Repeated measures ANOVA was performed on *A*, ψ_{pd}, and ψ_{md}.

Results

Photosynthesis and Leaf Water Potential

The repeated measures ANOVA performed for Ψ_{pd} revealed significant differences in irrigation treatments ($P < 0.01$) and dates ($P < 0.001$) as well as the interaction of irrigation treatment and date ($P < 0.001$). During the weeks where drought stress was induced, Ψ_{pd} revealed significant differences (Figure 2.1[A]) on two separate occasions: day 26, where Ψ_{pd} was 164% more stressed in D than ND and day 34 where Ψ_{pd} was 53% more stressed in D than ND (Figure 2.1[A]). The data show that day 22 is when time begins to play a significant role in affecting Ψ_{pd}.

As a main effect, Ψ_{md} was not significant between irrigation treatments ($P = 0.1647$). Conversely, date main effects were significant ($P < 0.001$) as well as the irrigation × date interaction ($P < 0.001$)(Figure 2.1[B]). Differences in irrigation Ψ_{md} occurred on days 14, 22, and 33. On these days, Ψ_{md} was 85, 175, and 150% more stressed in D than ND, respectively. For Ψ_{md} significant differences in date, as an interaction with irrigation treatment, were somewhat erratic. Days 9 and 19 were significantly lower than immediately adjacent dates. Alternatively, days 14, 22, and 33 were higher than the dates in between. Due to the variable nature of the Ψ_{md} data it is difficult to describe any trending behavior.

A Repeated Measures ANOVA revealed significant main effects differences for *A* in the (media [P = 0.006], irrigation [P = 0.04], and date [P <0.001]) (Figure 2.2 and 2.3) as well as their interactions (media × date [P = 0.009] and irrigation × date [P = 0.001]) (Figure 2.3). No significant differences were detected for the three-way interaction (media × irrigation × date [P = 0.59]). Media × date interaction showed an overall gradual decrease in photosynthesis as number of days after transplanting increased. A similar trend was seen in the interaction between irrigation × date. As days after transplant increased overall photosynthesis between both groups began decreasing, however after day 11 seedlings in the ND group maintained higher photosynthesis rates than those in the D group.

Significant differences across media types (Figure 2.2[A]) were detected between T and CT, CTG, and P. Overall, T averaged 44% greater *A* than the overall mean for all media types.

On day 35, average A measurements (Figure 2.3[A]) for the ND treatment were significantly higher than those in D treatment. For ND, the average net *A* rate was 1.95 μmol m^{-2} s^{-1} (± 0.44) while the average net *A* for D was -0.01 μmol m^{-2} s^{-1} (± 0.33) (Figure 2.2[B]). Time becomes a factor very early in this study as significant differences in *A* were seen between days 1 and 5 across media and irrigation treatment types. However, after day 5 *A* is not significantly affected again by time until day 23 between irrigation types and day 35 between media types.

Morphological Measurements

RRCD was significantly (P = 0.0103) influenced by irrigation; specifically, there was a 12% mean increase in ND and 6% decrease in D-treated seedlings. No significant differences were detected across media types (P = 0.769) and no interaction was present (P = 0.667). Relative growth differences across media (P = 0.729) and irrigation treatment types concerning HT were insignificant (P = 0.147). Additionally, there were no significant interactions between media and irrigation treatment type (P = 0.741) (Table 2.1).

An analysis of seedling RDM across treatment types revealed significant differences with both media (P < 0.001) and irrigation (P = 0.025) treatments but no interaction was present (P = 0.285). Seedlings grown in P media had a mean RDM that was 46% greater than the overall average across media treatments. T seedlings exhibited the overall lowest RDM with a mean 60% lower than the average across treatments (Table 2.1). ND seedlings exhibited 50% larger RDM overall, than those in D.

Seedling SDM differed significantly across media types (P < 0.0001) but not between irrigation treatments (P > 0.05) (Table 2.1). Mean P was 70% greater than the overall average across media types and CG measured 18% above this average. CT, CTG and T all measured below the average with T measuring the lowest at 65% below average.

No significant differences were detected for R:S media or irrigation treatments (P > 0.05) or their interaction (P = 0.369) (Table 2.1). As well, RL analysis revealed no significant differences across media or irrigation treatments (P > 0.103) and showed no significant interaction (P = 0.869).

Discussion

Plant Moisture Stress and Photosynthesis

Average predawn plant moisture stress values for the entire 34 day study period varied by irrigation type but did not vary significantly by media type. This indicates that despite differences in seedling size, media was drying down at similar rates within irrigation treatments. While research exists that suggests seedling quality can be somewhat equal across a range of nursery cultural practices (Duryea 1984), this was not found in *P. occidentalis* seedlings in a previous study (Hubbel 2015). Therefore, this finding was somewhat unexpected. A study performed by Lamhamedi *et al.* (1996) compared the effect of drought stress across small, medium, and large black spruce seedlings. The findings showed that small and medium seedlings did not differ physiologically when drought stressed. Large seedlings, however, were significantly more stressed than both the small and medium seedlings after the same amount of time. Large seedlings, thus, supported the study hypothesis that there would be a direct link between increased foliar mass and increased susceptibility to water stress (Lamhamedi *et al.* 1996). Perhaps, in this study, if seedlings had been grown in differently sized containers, allowing for greater RDM differences, moisture absorption would have differed by seedling size. Additionally, multiple studies have been performed concerning the relationship between drought and seedling establishment (ultimate measure of seedling quality) in various plant species (Engelbrecht and Kursar 2003; Engelbrecht *et al.* 2005; Moser *et al.* 2014; Tyree *et al.* 2014). These studies show high variability when it comes to exact amounts of drought that may be tolerated before reduced establishment occurs. Generally speaking, as drought increases seedling growth is reduced thus establishment becomes less likely (Engelbrecht and Kursar 2003).

Furthermore, when under drought stress, in an effort to conserve moisture, plants tend to close their stomata. However, stomatal closure inhibits gas exchange that is necessary to perform photosynthesis (Lopushinsky 1969). Therefore, as drought stress increases, photosynthesis typically decreases (Brix 1978; Pinto *et al.* 2012). Results concerning photosynthesis in *P. occidentalis* are no exception to this concept.

In this study, a significant decrease in photosynthesis was observed after seedlings were transplanted (Figure 2.3). This decrease could be compared to the type of transplant shock often experienced in bare-root seedling stock, as all seedling root systems in this study were washed (similar to "wrenching") and transplanted into a dry medium. Multiple studies have shown that containerized seedlings exhibit higher overall seedling quality than their bare-root counterparts on dry sites (Barnett and McGilvray 1993; Barnett 1984), including higher photosynthesis rates.

Post transplant, however, seedlings in the ND group were able to increase their photosynthetic capacity while those in the D group maintained lower photosynthesis rates. By day 36, seedlings in the D group exhibited significantly lower rates of photosynthesis than those in the ND group (Figure 2.3[A]). The ability of seedlings in the ND group to increase photosynthesis rates is supported by similar findings in a study by Barnett and McGilvray (1993). This study compared relative performance of containerized and bare-root loblolly pine seedlings on high quality sites with adequate available soil moisture. Results of this study showed that if seedlings of either stock type were planted at the appropriate time of year when conditions were ideal than seedling establishment and performance were equal.

Morphological Measurements

Two important morphological measurements in this study were RHT and RRCD (Thompson 1985; Mattsson 1997). Results of comparisons concerning RHT and RRCD revealed that there were no differences across media types when these seedlings were subjected to drought conditions. Therefore, reduced photosynthesis due to transplant shock, as mentioned above, most likely inhibited any substantial growth differences that may have surfaced between media types.

Alternatively, differences in growth were detected between irrigation treatments. In this study, when drought was induced, it was not found to affect RHT, a measure sometimes associated with seedling establishment. This may also have been due to transplant shock, as studies have shown that photosynthesis, together with cell growth are among the primary processes to be impacted by drought (Chaves *et al.* 2009; Brix 1978). Furthermore, shoot growth tends to be more sensitive to drought than root growth as roots are better equipped to rapidly adjust to changing osmotic conditions (Franco *et al.* 2011).

RRCD, however, is a factor more commonly associated with seedling establishment (Gardiner *et al.* 2009). This is due to a high positive correlation between RCD and root growth (Dey and Parker 1997). Roots have a high capacity to sense the physicochemical parameters of the soil and to adjust their development and performance accordingly. Therefore, seedlings exhibiting greater root growth after transplant are better equipped to seek out necessary moisture and nutrients allowing for greater establishment success

(Franco *et al.* 2011; Grossnickle 2005). In this study, RRCD was significantly and negatively impacted by drought (Table 2.1).

SDM, another measure of above ground plant tissue, resulted in significant differences across media as well as irrigation treatments (Table 2.1). However, the subset of seedlings selected for this study were of varying initial size. Thus, significant differences in SDM across media treatments cannot be definitively attributed to this specific study, as relative values are unavailable. Differences between irrigation treatments are more informative in this case and the induction of drought was reflected in reduced SDM found in seedlings in the D group (Table 2.1).

RDM reductions in drought stressed seedlings were somewhat analogous to those reported for SDM, showing 50% less RDM in D when compared with ND (Table 2.1). According to research performed by Pearson (1974), increasing moisture stress usually results in relatively less shoot than root growth. Despite the strain imposed on root systems during root washing and transplantation before imposing drought stress, results of this study parallel those of Pearson.

Another variable commonly used in the nursery industry to determine seedling quality and thus outplanting success is R:S. Research concerning outplanting success of seedlings in harsh environments suggests that seedlings with higher R:Ss tend to have increased survival rates (Elliot *et al.* 2008). This can be favorable in harsh outplanting situations, as the root surface area will be more adept at obtaining adequate moisture and nutrients for the connected shoot area (Mokany *et al.* 2006). An evaluation of R:S ratio in this study shows that seedlings grown in the CG mixture had the greatest R:S ratio while those grown in P media exhibited the lowest overall ratio (Table 2.1).

RL was not significantly impacted by drought stress in this study. However, in a similar study performed over a longer period of time, drought stressed *Pinus resinosa* seedlings had significantly shorter root lengths illustrating that long-term moisture stress can reduce root elongation (Becker *et al.* 1987). The same study also found that roots exposed to drought stress had fewer active root tips, lending to reduced potential for establishment at an outplanting site. While differences between drought treatments in this study were not significant, given a longer period of time, results from this study may have mirrored those of Becker as seedlings in the ND treatment averaged longer roots than those in the D treatment.

Conclusions

It was clearly observed that reductions in media water, i.e. drought, caused a decline in photosynthesis as well as in RRCD in this study. Therefore, *P. occidentalis* nursery growth and development should be well-timed so that seedlings are planted when there is adequate soil moisture; in Haiti, this period may often be associated with monsoon seasons. As those seedlings that received continued irrigation developed more robust root systems, it is likely that a seedling's ability to withstand drought increases with increased time from planting while there is still adequate soil moisture. As the seedlings used in this study showed an appreciable decline in physiological function approximately 26 days after irrigation ceased, one can assume that seedling response to drought would follow a similar pattern in dry post-planting conditions; thus those involved in planting should be able to identify the key point to irrigate should sufficient rainfall not occur.

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Table 2.1. Morphological characteristics of *Pinus occidentalis* seedlings. Values are statistical means for measured values and the associated standard error; media type and irrigation treatments were evaluated by measures of relative height (RHT), relative root-collar diameter (RRCD), shoot dry mass (SDM), root dry mass (RDM), root-to-shoot ratio (R:S), and root length (RL).

Media	**RHT (cm)**		**RRCD (mm)**		**SDM (g)**		**RDM (g)**		**R:S**		**RL (cm)**	
CG	0.01 (0.01)	a	0.01 (0.01)	a	5.80 (0.26)	ac	4.33 (0.16)	a	0.78 (0.02)	a	30.24 (0.44)	a
CT	0.00 (0.01)	a	0.06 (0.01)	a	4.48 (0.19)	bc	2.58 (0.09)	b	0.61 (0.01)	a	32.52 (0.79)	a
CTG	-0.02 (0.01)	a	0.02 (0.02)	a	4.13 (0.12)	bc	2.45 (0.08)	c	0.60 (0.01)	a	33.45 (0.89)	a
P	0.01 (0.03)	a	0.14 (0.05)	a	8.32 (0.25)	a	4.38 (0.11)	a	0.55 (0.01)	a	33.33 (0.62)	a
T	-0.09 (0.02)	a	0.03 (0.02)	a	1.68 (0.05)	b	1.19 (0.03)	d	0.75 (0.03)	a	37.97 (0.75)	a
Irrigation	**HT (cm)**		**RCD (mm)**		**SDM (g)**		**RDM (g)**		**R:S**		**RL (cm)**	
ND	0.02 (0.01)	a	0.15 (0.01)	a	5.68 (0.13)	a	3.45 (0.07)	a	0.63 (0.01)	a	35.08 (0.30)	a
D	-0.05 (0.00)	a	-0.04 (0.01)	b	4.23 (0.10)	a	2.53 (0.04)	b	0.66 (0.01)	a	32.10 (0.30)	a

*different letters indicate significance differences (α=0.05); n~10 for media type and n=24 for irrigation treatments

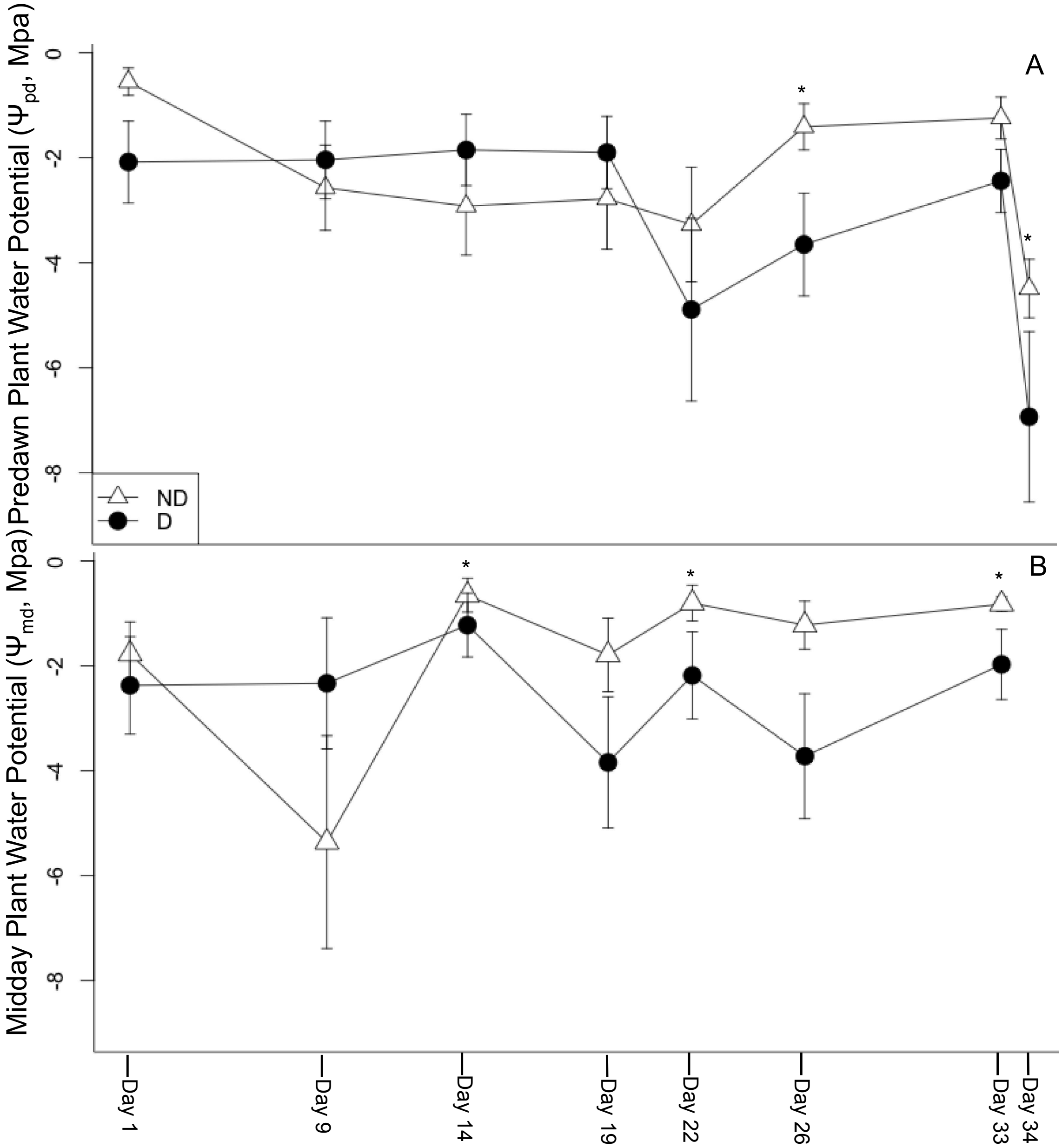

Figure 2.1. Mean predawn (A) and midday (B) plant water potentials between irrigation types ND (No-Drought) and D (Drought) as they interact with date. *Indicates significant differences (α=0.05).

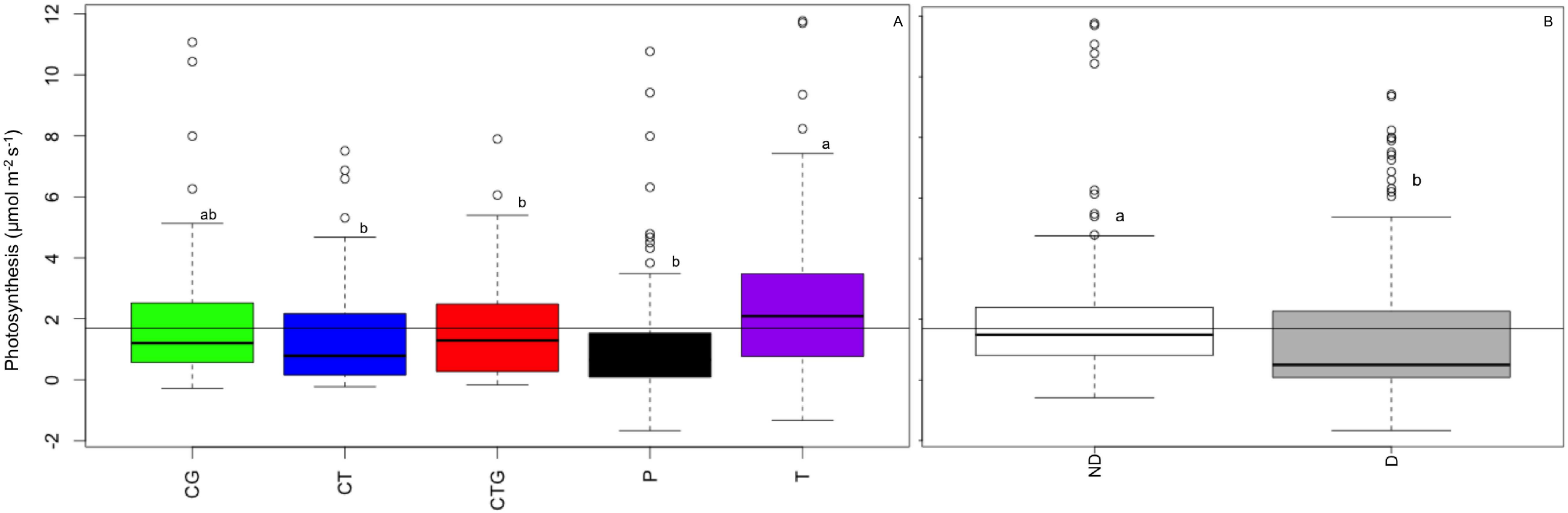

Figure 2.2. Mean photosynthesis (*A*) across media types (A) and irrigation treatments (B). Includes associated significant difference indicators (α=0.05). Solid line indicates overall mean. CG=compost:gravel, CT=compost:topsoil, CTG=compost:topsoil:gravel, P=peat-based, T=topsoil, ND=no drought, D=drought.

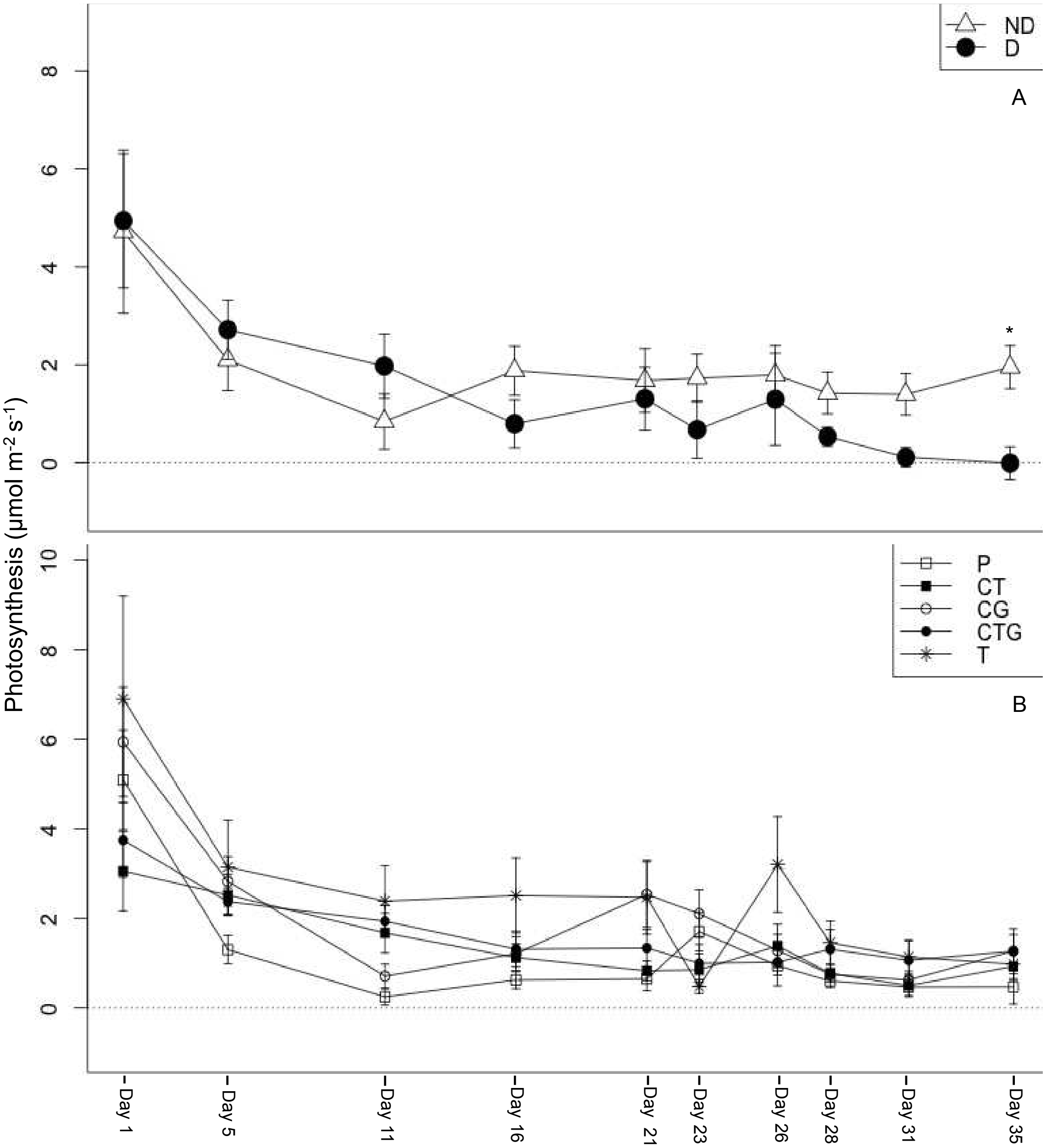

Figure 2.3. Mean photosynthesis (A) across irrigation (A) and media (B) types as they interact with date. *Indicates significant differences (α=0.05). Dotted line indicates absence of photosynthesis. ND=no drought, D=drought CG=compost:gravel, CT=compost:topsoil, CTG=compost:topsoil:gravel, P=peat-based, T=topsoil.