ABSTRACT

JUDD, LESLEY ANN. Rhizometrics: Novel Techniques to Observe and Measure Root Growth of Container-Grown Crops. (Under the direction of Dr. Brian E. Jackson).

Approximately 90% of the $16 billion greenhouse, nursery and floriculture industries are generated from plants produced in containers, excluding food crops grown under cover. Root growth of crops grown in containers is a central element in overall plant performance and one of the main factors effecting root growth in containers is the substrate in which the plant is growing. Considering the large portion of the horticulture industry involved with growing plants in containers and the importance of understanding the physiology and morphology of roots, the factors that influence root growth in container production need to be investigated. However, root growth and root architecture are frequently excluded in horticultural and the study of natural root development is a challenge due to the difficulty of observation in containers. The most common root system evaluations of plants grown in containers that are reported in scientific literature today include: subjective root ratings and root dry weight measurements. Due to the disadvantages of these root measuring methods, there is a need for developing new techniques in order to study and measure root growth, especially root growth of small plant material to evaluate root growth during production.

In the first study, an apparatus (mini-Horhizotron) was developed and constructed that could measure root growth of small plant material. The mini-Horhizotron was tested with several different types of plants grown in peat amended with either perlite (PL), pine-wood-chips (PWC) or shredded-pine-wood (SW). Experiments conducted with the mini-Horhizotron reports that several herbaceous plants can be grown in the mini-Horhizotron with no effect on root development compared to a regular container. Several experiments

were conducted with the mini-Horhizotron, in comparing substrates and experimental design. Data from these studies show that root length for floriculture and nursery plants are similar among PL, PWC and SW substrates, and the experimental designs did not affect root growth of plants in PL and SW substrates until close to the end of the study. Another experiment compared root growth of plants grown in substrates with increasing rates of PWC; and at the end of the study, plants grown in the higher rates of PWC substrates had significantly longer root lengths.

In the second study, a device was designed (Rhizometer) to allow for both viewing a growing root system and in situ measurement of substrate physical properties. In the first experiment, substrate physical properties and root dry weights were measured over four weeks and these data showed that the Rhizometer could successfully measure substrate physical properties and the effect of root growth. There were several other experiments conducted with the Rhizometers that reported an effect of plant root type on substrate physical properties and the ease of root measurements of different seedlings in the Rhizometers.

The third study investigated the use of the Hydraulic Conductance Flow Meter (HCFM) on container-grown small herbaceous and woody plant material to find the effects of substrate on root conductivities. The HCFM was used to measure root conductivities of plants in peat with PL, PWC or SW, and pine bark (PB) with PTS. Dry root mass was measured as well to find the effects of these substrates on root development. The studies showed that there is not any effect of using PTS and compared to PL or PB on root growth of plants. One experiment showed an observed effect of root mass on root conductance and substrate influenced weighted root conductance.

Rhizometrics: Novel Techniques to Observe and Measure Root Growth of Container-Grown Crops

by
Lesley Ann Judd

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APPROVED BY:

_____	_____
Brian E. Jackson Committee Chair	William C. Fonteno

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J. – C. Domec

DEDICATION

"You have brains in your head

You have feet in your shoes

You can steer yourself any direction you choose

You're on your own

And you know what you know

And YOU are the one who'll decide where to go…"

(Dr. Seuss, from '*Oh the Places You Will Go'*)

BIOGRAPHY

Lesley Ann Judd grew up in Mason, Michigan and graduated from Mason High School in June 2006. Lesley's family had always been Spartan fans, and in August 2006, Lesley moved on to campus and joined her brother at Michigan State University. Lesley started as a freshman majoring in Mathematics with plans to be a high school math teacher. However, Lesley worked at a local garden center during high school and college and decided her main passion was for horticulture and switched her degree to Horticulture. Lesley graduated from Michigan State University in December 2010, receiving a Bachelor of Science degree in Horticulture with a specialization in Landscape Design. While completing her B.S. degree, Lesley worked several jobs that included greenhouse research technician, student worker on campus healing garden and landscape designer at a local garden center. After these experiences, Lesley realized she liked the science aspect of Horticulture more than designing landscapes. With this in mind, Lesley looked into graduate school at several universities and when she found out she was accepted to North Carolina State University, she made the 14 hour journey south from Mason to Raleigh, North Carolina. While Lesley was completing her Master of Science degree in Horticultural Science, she had enjoyed teaching so much that she decided to continue her education with the ultimate goal of teaching at a university. In May 2013, Lesley graduated with a M.S. from North Carolina State University, and shortly before graduation, found out she was accepted to stay at NC State and continue her education in the Ph.D. program.

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My graduate program would not have been successful without the contribution of several people. My advisor, Dr. Brian Jackson, made every day a learning experience and showed me how to be a better, more independent thinker. Dr. Jackson has made substrates his life's work and the intelligence and dedication he brings to the substrate group makes it one of a kind. I appreciate how much Dr. Jackson worked with me to help develop my writing skills, so that I can sit down with a blank sheet of paper and write an exceptional piece of work. Dr. Bill Fonteno also deserves recognition, because of his input and help through my work with substrate physical properties. Dr. Fonteno was in the lab or greenhouse beside me during my work and I cannot thank him enough for that. Both Dr. Jackson and Dr. Fonteno started me on the path to a great researcher and a professional, and they have my everlasting gratitude. I owe thanks to Dr. J.-C. Domec for taking the time to sit down with me to evaluate all my data, and for always being patient with me as I learned from him.

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Introduction

Approximately 90% of the ornamental plants grown in the $16 billion greenhouse, nursery and floriculture industries are produced in containers (U.S. Department of Agriculture, 2009). These industries include bedding plants, foliage plants, potted flowering plants, potted nursery stock and other floriculture/nursery crops, all grown in a wide variety of container sizes. Container production has increased substantially over the last several decades, due to several advantages container production has over traditional field production. With developments in water quality, fertilizers and pesticides, container production became an easier enterprise, especially because it requires less land, ease of harvesting, shipping can occur almost any time of the year, and greater returns per acre than field production (Halcomb et al., 2009). However, the disadvantages of container production include; the requirement for large quantities of quality water and higher costs of operation (irrigation, containers, substrates, fertilizer, potting machine, poly covered houses, shade cloth, etc.). Container production also has advantages for the plants grown in containers. Plants grown in plastic containers have been shown to have a greater fine root mass compared to field-grown plants (Gilman and Beeson, 1996; Harris and Gilman, 1993). After transplanting, these plants were better able to meet transpiration needs with no roots loss compared to harvested field-grown plants. Also, container-grown plants have more fine roots along the outside of the root ball, which come into contact with the backfill soil when transplanted (Harris and Gilman, 1993). However, trees grown in the field or in fabric containers had greater trunk diameter and height than trees grown in plastic containers (Gilman and Beeson, 1996). Different production methods have a direct effect on tree growth, root growth/extension, and

distribution of biomass (Gilman and Beeson, 1996; Harris and Gilman, 1993). Woody plants in containers are generally above ground and are therefore subject to extremes in temperature, moisture and nutrient levels (Mertens and Wright, 1978).

Until the 1960's, almost all the greenhouse substrates were soil-based. Typical mixes included equal volumes of loam soil, sphagnum peat moss, and concrete grade sand (Nelson, 2011). Soil-less substrates arose in the 1960's due to three factors: 1) soil-less substrates do not need to be pasteurized as soil-based substrates do, 2) soil-less substrates have much lower bulk densities which reduced shipping and handling costs, and 3) field soil can vary greatly from batch to batch, unlike soil-less substrate components that tend to be more consistent over time (Nelson, 2011).

Root Growth of Containerized Horticultural Crops

Plants grown in containers are limited by the volume of substrate in which water, gas and solute availability can fluctuate over short periods of time (Polak and Wallach, 2001). The two primary functions of roots are acquisition of water with dissolved ions and anchorage of the plant (Fitter, 1996). Secondary functions include carbohydrate storage, synthesis of some growth regulators and propagation by adventitious roots (Fitter, 1996). Fitter (1996) believed that most of the first land plants had poorly developed root systems, since these plants were small and lived in very wet environments where neither anchorage nor acquisition of water was a problem. Therefore, it is possible to believe the most difficult function of early root systems was the procurement of poorly mobile resources, such as phosphate. Roots can alter their surroundings by releasing root exudates (e.g. organic acids, amino acids and hydrogen ions) to alter pH to affect ion uptake. Some exudates have been

shown to be used to enhance the symbiotic relationship with nitrogen fixing bacteria (Marschner and Romheld, 1996).

The first step into the science of how roots influence the surrounding soil was by Lorenz Hiltner (1904), when he introduced the term “rhizosphere” to describe a zone of soil surrounding roots in relation to nitrogen fixing bacteria (Hartmann et al., 2008; Rovira, 1991). Although the diversity of root systems in modern plants is quite dramatic, the external features of root morphology are extremely similar, unlike leaves and flowers that can show distinguishing features that allow for identification. This lack of variation is presumably related to the limited range of variation of the root environment, and therefore any variations that occur, such as root diameter, color and surface texture, may be interpreted in terms of some root environment variation (Fitter, 1996). Of course, some variances are governed by hereditary growth characteristics (Weaver, 1926).

It is important to understand the factors that influence root growth in containers in order to achieve optimal benefits from container production. Plants grown in containers are generally limited by the volume of substrate in which water, gas, and solute availability can fluctuate over a short period of time (Polak and Wallach, 2001). Substrates for container production must meet several criteria including; low soluble salts, high cation exchange capacity (CEC), suitable physical and chemical properties, no pests, consistent, available and cost effective (Mathers et al., 2007). Common substrate components used in the industry today are peat moss, pine bark, coir, perlite, vermiculite, rice hulls and compost. Physical properties of substrates known to affect roots include aeration, water holding capacity, total porosity, particle size, and bulk density (Baligar and Nash, 1978; Mathers et al., 2007). These

physical properties are not only important to root growth, but also to container size and irrigation strategy. According to Cannavo et al. (2011), the air-filled porosity, the water retention capacity and water availability have a considerable impact on plant growth. Air-filled pores allow for drainage and gas exchange between the root environment and the outside atmosphere (Bunt, 1988). Chemical properties of substrates, such as pH, CEC, soluble salts, and pesticides can also have immense impact on root growth and function of containers (Mathers et al., 2007). Understanding how chemical properties influence roots is important for selecting substrates, amendments (e.g. lime), and fertilizer additions. The air and water holding capacities of a substrate are dependent upon container depth and width as well as on the type/mix of substrate (Bilderback and Fonteno, 1987). Also, as plant roots grow into the container, there can be modification of the substrate's total porosity, pore size distribution and pore connectivity (Cannavo et al., 2011).

Importance of Root Hairs

As early as the seventeenth century, researchers began to realize that plants absorbed water rather than soil and that nutrient substances were absorbed by the roots at the level of the root hairs (Hofer, 1996; Landmeyer, 2011; Rosene, 1943). Root hairs constitute, along with the adjoining cells, the rhizodermis (Hofer, 1996). Typical root hairs have a cylindrical, straight form with a dome-shaped tip, that often form a right angle with the main root surface. They appear a short distance behind the root tip where the cells are dividing, and persist for a relatively short time depending on the plant species (Whitaker, 1923). As the hairs die off, new ones are formed closer to the root tip, a process termed the migration of root hair zones (Whitaker, 1923). Whitaker (1923) writes that root hairs are the chief means

through which water and mineral salts are taken from the soil into the plant. Dittmer (1937) reported that root hairs were present in many root systems of different plants and were important enough to attempt to measure. Dittmer (1937) reports that living root hairs were scattered over the entire surface of all the roots of a winter rye plant (*Secale cereal* L.) and that the surface area of these root hairs was nearly twice that of the main, larger roots. In 1949, Dittmer investigated root hair variations between different families of angiosperms and noted that considerable variation was found in the diameters, lengths, shape, and color between the different species, but within any one species the size and color was relatively constant. In 1962, researchers could only hypothesize the mechanism of root hair development based on the studies completed in earlier years (Cormack, 1962). More recent studies have expanded the functions of root hairs, including; nutrient and water uptake, organic acid secretion (exudation), anchorage, interactions with soil microorganisms, and a particularly important role in the uptake of immobile nutrients, like phosphate (Datta et al., 2011; Jones and Dolan, 2012).

Root Types, Diameter and Architecture

Another important root characteristic that has been linked with mechanical support and acquisition of water and nutrients is root architecture/types and diameter. Three classes of roots usually described are: the taproot, lateral roots, and adventitious roots. Cannon (1949) was the first to attempt to classify root systems, by striving to recognize characteristics that were constant or similar between genetically similar plants growing in varied habitats. Other authors have reported that there is a fourth root class, termed basal roots (Nicola, 1998; Zobel, 2005). The taproot is the first root to emerge from the seed, and

is considered the primary root while the basal and lateral roots that develop from the taproot are considered to be secondary roots (Zobel, 2005). These secondary roots then in turn produce tertiary, quaternary and further roots (Fitter, 1996; Malamy, 2005). When plants develop secondary shoots, such as tillers, and the secondary shoots develop roots, these roots are commonly called adventitious or shootborne roots (Zobel, 2005). Fitter (1996) writes that adventitious roots are believed to aid in stability and found in many plants of water-logged soils. The types of roots in root systems can be used to characterize the plant and environment; for example if the root system consists of a taproot with little basal or lateral roots, it is considered an early root system on a young plant for most species (Zobel, 2005).

The structure of the root organ itself is very consistent between different species; but the number, placement and direction of growth of each root in the system is highly variable, even among genetically similar plants (Malamy, 2005). The apical regions of roots allow plants to adapt their morphology and organ development to the encountered environmental conditions (Hodge et al., 2009). The number and length of secondary roots varies greatly depending on the plant species, soil composition and water and nutrient availability (Malamy, 2005).

Root architecture refers to the spatial configuration of the entire root system; however studies of root architecture usually do not include fine structural details, such as root hairs (Lynch, 1995). Root architecture is generally quite complex, and is different from morphology, root topology (branching) and distribution (amount/presence of roots in a positional gradient) (Lynch, 1995). Root architecture can include topography and distribution, and these descriptions are usually easier to measure. The architecture of a root

system can determine its exploration of spatial domains in the soil, as well as its ability to respond to possibly localized available nutrients in the soil/media (Fitter, 1996). However, little is known about root architecture and its roles for the plant because it is difficult to observe, quantify, and interpret without destroying the native architecture (Lynch, 1995).

Root diameter varies both within and between species and can sometimes be used to describe what the root and plant experience in the surrounding environment. Root diameter determines the length of root that the plant can produce for unit input of resources to the system (Fitter, 1996). The diameter of fine roots in forests have a strong role in determining the fine root turnover; as diameter increases, the root turnover decreased (Gill and Jackson, 2000; Kucbel et al., 2011).

Peat and Fitter (1993) report that it is often found that the roots of species that form mycorrhizal associations, especially obligate mycorrhizal species, have much coarser roots (larger diameter) and no, or very few, root hairs compared to species with no mycorrhizal associations that have very fine roots and copious amounts of root hairs. Some plant species produce fine roots when grown at a low nutrient supply (Fitter, 1996). The diameter of roots also seems to be a good predictor of the effect of mechanical impedance and substrate pore size, because data obtained by Wiersum (1957) demonstrates in a greenhouse study that a root is only able to penetrate a pore which has a diameter exceeding that of a young root and Goss (1977) reported results that mechanical impedance caused the plant to grow superficial and densely branched root system where the roots did not grow past eight centimeters of depth. In the field, it was demonstrated that roots can force their way through pores smaller in diameter (Gill and Miller, 1956).

Methods of Root Growth Measurements

The study of root growth began in the field over nine decades ago with agronomists who studied root growth in various soils. According to Weaver et al. (1922), an exact knowledge of root development of crop plants, their position, extent and activity, is of paramount importance to a scientific understanding of plant production. Several techniques used to measure root growth in the field included trenches, photography and excavation (Weaver et al., 1922). McDougall (1916) used the horizontal glass-plate method, where a square foot of glass was buried two inches below the surface of the soil and covered with felt roofing so it could be removed to count the number of roots growing against the glass. McDougall (1916) also used the vertical glass-plate method, where holes were dug into the earth two and a half feet wide by five feet long and two feet deep, and a glass plate was placed against one side of the hole and the entire hole was covered with a board cover to block out light. Other techniques described by Schuurman and Goedewaagen (1971) include monoliths, soil cores, and profile walls. More recent work has been done with rhizotrons, minirhizotrons or transparent walls/windows (Smit et al., 2000).

Drawings, Pinboards, Rhizotrons and Minirhizotrons

Methods of measuring roots has varied over the years, and many past methods that were thought of as illustrative during that time are now limited and no longer used. Weaver et al. (1922) used the same excavating method that was commonly used in 1922, digging a trench along the side of the plant at a depth of five feet and of a convenient width. In some cases, it was possible to view the entire root system and the usual practice was completed. The usual practice of this time consisted of writing a working description, noting variation

and hand-drawing a replication of the root system on a large drawing-sheet to the exact measurements (Weaver et al., 1922). In other cases, the roots were destroyed by digging and had to be reconstructed. Photographs were often found to be blurry, not allowing the viewer to perceive the finer roots; therefore hand-drawn pictures were the best representative of the root system (Weaver et al., 1922). Monoliths, or pinboards, can be used both with field-grown and with container-grown plants. The pinboard method is thought to give a fairly complete picture of the structure and shape of the root system (Schuurman and Goedewaagen, 1971). The pinboards can be constructed from 1-1.5 cm thick plywood with holes drilled 5 cm apart both horizontally and vertically that hold pins (made from cut-off knitting needles or stainless steel wire with a length of 9.5 – 15 cm long; Schuurman and Goedewaagen, 1971). In the field, a pit is dug against the plant, the dug-out wall smoothed and the pinboard may be placed and pressed against this wall and then a steel cable may be passed down each side of the pinboard in a sawing movement so that the soil surrounding the pinboard is cut away and the pinboard is free to pull out with the soil and roots still held by the pins (Schuurman and Goedewaagen, 1971). The specimen can now be transported to a laboratory where the soil will be washed off, leaving the roots arranged around the pins in a similar architecture found in nature (Schuurman and Goedewaagen, 1971). Kono et al. (1987) and Kano-Nakata et al. (2011) used a “root box-pinboard” method to quantitatively and qualitatively measure root system morphology. The root box was made of transparent solid vinyl chloride with dimensions of 25 cm length, 2 cm width and 40 cm depth, making a relatively small box due to all the handling required to place the pinboard on it at day of harvest (Kono et al., 1987). Both authors found this method was an easy and effective way to

view the natural morphology of the root systems, only requiring around 15 minutes per sample per person to harvest (Kano-Nakata et al., 2011; Kono et al., 1987). However, there were several disadvantages; Kono et al. (1987) reported the size of the box was limiting for growth, and three different types of dyes had to be used to get an optimum contrast among the root system members. Kano-Nakata et al. (2011) used digital photography and put the image in a computer program to measure root length, and they found this program underestimated root length because of overlapping roots, especially fine lateral roots.

Another common technique currently used today is rhizotrons. The first rhizotron, designed by Rogers (1969) in 1933, was constructed in East Malling, Kent, England from 1960-1961 at the East Malling Research Station, which is famous for its development of dwarfing rootstocks of fruit trees. The word rhizotron is coined from Greek words *rhizos* for root and *tron* for instrument and can be defined as a facility or building designed underground for viewing and measuring plant roots and underground structures through transparent surfaces that may be in contact with the natural soil (Klepper and Kaspar, 1994). It is a tool for making nondestructive, repeated measurements of root systems at a large, field scale. Rhizotrons are one of the earliest non-destructive techniques for observing root growth in soil, and they have several advantages and limitations (Taylor et al., 1990). Advantages include the ability to take successive measurements on the same individual root and to rapidly see the length increases (Taylor et al., 1990). Sensors and cameras can be installed to measure soil conditions and record time-lapse photography. Roots growing along the transparent wall can be traced as the roots grow, to provide information on speed of root growth and root density (Glinski et al., 1993). However, the primary disadvantage of the

rhizotron is its expense of construction and operation (Taylor et al., 1990). A rhizotron constructed at Auburn, Alabama in 1969 cost about $40,000 and during the 13 years of operation added to this cost by $50-100,000, spent on instruments, control systems, and updated computer systems (Huck and Taylor, 1982). Current costs of constructing a rhizotron would be substantially greater (Taylor et al., 1990). Huck and Taylor (1982) discuss several disadvantages of rhizotrons; the finite number of repetitions, the immobility of the structure and changing of the soil environment when the rhizotron is installed. Also, the viewing surface of the rhizotron may not be representative of the roots in the bulk soil at depth and after research is conducted, the soil might need to be replaced, in which case the replacement soil may have altered populations of worms, fungi, bacteria and insects compared to the native profile (Klepper and Kaspar, 1994).

A similar technique to the rhizotrons is the minirhiztron, originally proposed by Bates (1937) using a mirror and a battery-operated lamp mounted on the end of a stick to see roots intersecting a glass tube in the ground. Throughout later years, this minrhizotron was improved by others to create the modern minirhizotron that uses a color video camera with a right-angle viewing attachment that can be lowered into the underground tube, and images can be recorded on video or photographs taken, both of which have improved quality images due to the modern technology (Taylor et al., 1990). One of the greatest limitations of the minirhizotron is the number of tubes required to accurately estimate rooting (Taylor et al., 1990). It is suggested to use a minimum of eight tubes in a single plot and it requires 30 to 45 minutes to install each tube (Taylor et al., 1990). Another disadvantage of the minirhizotron is the amount of labor/time required to collect the pictures from every tube and

analyze them. In 1985, James et al. suggested a new nondestructive root measurement technique similar to the rhizotron, which eliminated the issues of expense and requirement for specialized equipment. James et al. (1985) still called their apparatus a rhizotron or mini-rhizotron, and it was constructed of two 20 x 20 x 0.5 cm transparent plexiglas plates held one centimeter apart by plastic tubing. This rhizotron could be used in or out of a greenhouse, shaded by panels or aluminum foil, inexpensive and created a small box to view growing roots in. Neufeld et al. (1989) created a root box similar to James et al. (1985) that was slightly larger and grew plant roots between a plexiglass sheet and a nylon sheet with soil medium on the other side of the nylon so complete view of the roots could be had. Pan et al. (1998) developed a new portable rhizotron system called mesorhizotron, to observe root growth in different cropping systems, soil conditions and environments. The mesorhizotron has a transparent face on a box that is buried in the soil, and a portable hand scanner can be placed into the box to scan the transparent wall view (Pan et al., 1998). Supporting hardware for the scanner and software for storing and analyzing the images were also required for the mesorhizotron, and each box required five vertical scans (Pan et al., 1998). Silva and Beeson (2011) developed a large-volume rhizotron for aboveground observation of undisturbed, natural root growth of woody plants. The large-volume rhizotron was to mimic in-ground conditions, including enhanced drainage for evaluating effects of soil moisture deficits on root growth (Silva and Beeson, 2011).

Digital Imaging

Rhizotrons, minirhizotrons and other transparent wall/container designs commonly use digital imaging to measure root systems. Digital imaging includes photographs or videos

of the transparent walls, scanned images of roots floating in water, or scanned drawings of root tracings. These photographs or scanned images can be used by computer programs to evaluate several root measurements. There are numerous computer programs, both commercially and freely available, and there are 19 commonly used and known computer programs (Lobet, 2011). Several of these programs include RootLM, RootReader 2D, EZ-Rhizo, WinRHIZO and WinRHIZO Tron. One software program that has been used in scientific literature is the WinRHIZO (Regents Instruments, Quebec City, Canada) program. WinRHIZO is based on an optical scanner instead of a video camera, because scanners produce high-quality images (Arsenault et al., 1995). Possible measurements for the WinRHIZO system are; total length, projected area, surface area, root tips, branching points, and root length for different width intervals chosen by the user (Arsenault et al., 1995). According to Fang et al. (2012), WinRHIZO is relatively inexpensive and suitable for both large and small-scale experiments. Villordon et al. (2012) used WinRHIZO to classify lateral root growth of sweetpotato (*Ipomoea batatas* 'Beauregard'). Sweetpotato roots were washed, placed in water and scanned to be analyzed by WinRHIZO (Villordon et al., 2012). Debris, such as sand particles and broken root segements, in the washed roots were removed manually in the WinRHIZO program (Villordon et al., 2012). Root type classification was based on predetermined diameter intervals designed by the researchers, and used to show different root stages (Villordon et al., 2012). While the scanner allows for clearer images, the roots must be washed and the substrate removed, and measurements of the root system over time cannot be observed with this program. Washing roots can cause a loss of fine roots and disturb the natural architecture of the root system. Unless the roots are floated in water,

as done by Villordon et al. (2012), the natural architecture is gone. Another program WinRHIZO Tron, developed by the same company, is used to analyze images from rhizotrons, minirhizotrons or other transparent wall techniques. Root tracings can also be scanned into the WinRHIZO Tron program, and root length can be converted to root mass (Metcalfe et al., 2007). However, the images to be used with WinRHIZO Tron are often unfocused and blurry, so the user has to manually select the roots on the image and trace the length for the program to know what to measure.

The smaller, above-ground rhizotrons, minirhizotrons and root boxes are still currently used, and adaptions to these techniques have created new designs, such as the Horhizotron™. The Horhizotron™ is a non-destructive technique used to measure lateral root growth from an original root ball of a container-grown plant, allowing for post-transplant assessment (Wright and Wright, 2004). The center of the Horhizotron™ fits a range of 1-3 gallon size root balls. Plants are placed in the center with eight panes of glass that extend away from the root ball in a 4-pointed star shape (Wright and Wright, 2004). The substrate in each quadrant can be modified in various ways in order to examine the effects of different rhizosphere conditions (Wright and Wright, 2004). Each quadrant can be filled with a different substrate (Jackson et al., 2005b), or the quadrants can be divided with one type of substrate on the lower half and a different substrate on the upper half (Price et al., 2009). The Horhizotron™ can easily be used in a greenhouse or in the field, due to the lightweight materials used and ease of assembly. The disadvantages of the materials used are; the glass panels are not permanently placed and can move and crack, the shade box does not restrict all

light from the root system, and the limitation of only being able to use large container plants to observe root growth.

Dry Weights and Subjective Root Rating

The importance of root system development in relation to plant growth is often overlooked due to the roots being below ground and not directly observed. In viewing the whole plant, the shoots and roots are in constant competition for energy and nutrients for their development. A measure of the resultant pattern of differential growth, expressed as the shoot:root ratio, provides an index for the performance of each organ in a certain growth environment (Aung, 1971). These shoot:root ratios may help ascertain how environmental and chemical factors affect and modify the growth of the shoot and root (Aung, 1971). Shoot:root ratios are often measured with the destructive method of comparing dry weights of roots and shoots. Media must be washed away from the roots and then the roots are placed in an oven at 70° C for several days, until all water has evaporated. Using this method, much of the fine roots and root hairs are lost in the process, as well as the natural positions and arrangements of the roots. In standard methods of washing and storage of root samples, losses of dry weight from 20% to 40% may occur (Oliveira et al., 2000; van Noordwijk and Floris, 1979). Very fine roots are difficult to wash and even by using a sieve with a mesh size of 0.2 mm^2, these roots still may be lost (Bohm, 1979).

Root rating can be a simple and easy way to qualitatively describe root balls, washed roots and propagative rooted cuttings. Ratings can evaluate root density, appearance, branching and distribution (Cid et al., 1993; Jackson et al., 2005b). Subjective root ratings can be done on root balls, where the roots are observed growing on the outside of the

substrate or by destructively washing the roots and rating the uncovered root system (Jackson et al, 2005a; Walters and Wehner, 1994). Root ratings can also be measured with the rhizotrons, minirhizotrons and Horhizotron™ by estimating the root density observed through the transparent walls (Jackson et al., 2005b). Walters and Wehner (1994) found root rating was a simple and accurate method for determining cucumber root growth in the greenhouse. The authors note that that rating is a subjective measurement, and the person rating must first understand how to accurately rate the size of the root system (Walters and Wehner, 1994). The rater must determine beforehand the categories of the rating scale, and can even lay out some root systems that refer to one of the categories, to be consulted to when a judgment is made (Walters and Wehner, 1994).

Hydraulic Conductance Flow Meter

One of the essential functions of roots is to supply the plant with water from the surrounding environment (soil or substrate). It is well known that the hydraulic properties of roots vary with species and environmental conditions (Miyamoto et al., 2001). These environmental factors can strongly influence root morphology and anatomy (Steudle and Peterson, 1998). When the plant is experiencing high transpiration, the force driving water across the root will be the hydrostatic pressure difference between the root medium (substrate) and the root xylem (Steudle, 2000). When transpiration stops, the cell-to-cell passage, or the symplastic and apoplastic pathways, is left to transport water and has a high hydraulic resistance (Steudle, 2000). Water movement from the soil solution into the root xylem and then up into the shoot can be treated as fluid flow through a complex structure with variable hydraulic resistances, such as the different tissues in the root cylinder or the

different cellular pathways for water (Steudle and Peterson, 1998). Hydraulic resistances of the root will also be great when the surrounding environment is dry and may help to limit water stress (Miyamoto et al., 2001). Research completed by Ramos and Kauffmann (1979) observed an increase in hydraulic root resistance with the seedlings that did not receive enough water (water stressed). The observed increase in hydraulic resistance did not result from decreased soil-to-root contact, but may have resulted from suberization of the cortical cell walls in the root or from a change in membrane permeability (Ramos and Kauffmann, 1979). Also, an analysis of the variance of root length showed no significant effect due to water stress. Reiger and Litvin (1999) noticed in their experiment that root system hydraulic conductivity decreased with increasing root diameter and cortex width, resulting in the belief that more dense tissue resists water movement.

Roots impose the greatest resistance to water flow in the soil-plant-atmosphere continuum (SPAC), thus their hydraulic conductivity has been the subject of numerous studies (Rieger and Litvin, 1999). Hydraulic resistance is the reciprocal of hydraulic conductance (Tsuda and Tyree, 2000). Hydraulic conductivity can be measured for both roots and shoots, and are important parameters to the model of the SPAC, that will help predict the rate of water flow through whole plants and to predict the water potential of various plant organs (Tyree et al., 1994). The usual method to estimate root and whole plant hydraulic conductance, known as the evaporative flux method, required a measurement of soil water potential from predawn leaf water potential; a midday leaf water potential; and midday evaporative flux density, which is usually estimated from stem water flow measurements divided by leaf area (Tsuda and Tyree, 2000; Tyree et al., 1994). These measurements

required a number of devices, such as a pressure chamber to measure leaf water potential. Measurements of root and whole plant hydraulic conductance are usually subject to a number of sources of error, especially with the stem flow measurements that usually have a range of accuracy of 10 to 20% (Tyree et al., 1994).

With advancing technology, new apparatuses have been developed to measure hydraulic conductivity/resistance with rapid water-flow measurements. The two devices are; the high-pressure flow meter and the hydraulic conductance flow meter (Dynamax, Inc., Houston, TX). The hydraulic conductance flow meter (HCFM), preforms the same measurements as the high pressure flow meter (HPFM), but the HCFM is generally used on smaller stock while the HPFM is used on large caliber plants. Both the HCFM and the HPFM measure the hydraulic conductance of shoot and root systems. Shoots are excised from the root system a few cm above substrate level and the rootstock or shoot stem is fitted with water filled tubing of the HCFM. The HCFM uses constantly increasing pressure to cause water to flow into the root or shoot system (whichever the HCFM is attached to) and this water flows in the opposite direction of normal transpiration (Tyree et al., 1995). The pressure measurement versus the flow measurement is used to estimate root/shoot conductance from the slope. Tsuda and Tyree (2000) experimented with the HPFM, showing that HPFM method and the conventional evaporative flux method yield consistent values of plant hydraulic conductance under quasi-steady-state conditions. Tsuda and Tyree (2000) also note that the HPFM method is much faster and permits the determination of whole-shoot conductance. These hydraulic conductance values, which are reflective of root mass and

development, are believed to be correlated with dry weights as an assessment of overall growth.

Greenhouse Substrates

Growing media, or substrates, are the medium in which container plants are grown in the greenhouse or nursery. The substrates are considered soilless, because the container plants are not grown in field soil and the substrate is composed of a mixture of several organic and inorganic materials (Schmilewski, 2008). Such materials include peat, composted bark, perlite vermiculite and others. Substrates usually require additional ingredients of fertilizers, lime, wetting agents, pesticides and other substances (Schmilewski, 2008). The substrates must be at an optimal pH for the uptake of nutrients and must have the ability to be maintained at a certain pH. Horticultural crops grown in these mixes demand a stable rooting environment (Verhagen, 2007). The physical aspects of a good root environment are structural stability, water capacity, air capacity, bulk density and wettability. It is important to know the physical and chemical properties of the substrates used in horticultural crop production to ensure an optimal environment for the root system.

A combination of peat and perlite, with other possible components such as vermiculite and bark, make up the industry's common substrate mixtures. The horticultural industry has been using peat moss as a substrate component for several decades. Peat is essentially incomplete decomposition of plant matter (organic matter) formed under anaerobic conditions in bogs and fens, from where peat is harvested. Peat moss is known to be an effective growing medium because of its chemical and physical properties create an ideal growing environment for horticultural crops, such as its high cation exchange capacity

(nutrient availability), high porosity and water-holding capacity (Maher et al., 2008). In general, peat substrates have a pore volume of 85-95% depending on particle size and particle density (Michiels et al., 1993). After fertilizing and liming, peat can be the sole constituent of substrates because it provides roots with a satisfactory environment to promote positive root growth (Schmilewski, 2008). Perlite, the most common aggregate component of substrates, is created from naturally occurring siliceous volcanic rock mined from the earth (Bolen, 2003). This mined rock is heated to its softening range so it will expand, four to twenty times its original volume, and become the well-known white/grey, lightweight, porous particle used in substrates. Perlite is most commonly used to create air-filled pore space in substrates (Bunt, 1988). However, perlite is inert and does not add any chemical properties, such as nutrient availability, when mixed into a substrate (Ghehsareh, 2011).

In recent years, the use of peat moss has become a topic of concern for both growers and consumers of horticultural crops because of the cost of the material due to transport and availability (Pruett, 2011). For many years, peat moss has been readily available in Canada and other parts of the United States. However, the price for peat has been rising over the last five years due to transport costs and reduced availability (Griffith, 2007). It must be dry in order to harvest peat, but during seasons when there is too much rain and cool weather with a lack of drying during the harvesting time creates a reduction in the amount of peat available for the industry. The quality of peat moss available has been declining, especially in Denmark and this has created interest to develop a more stable alternative (Hansen et al., 1993). Perlite has also come under the scrutiny of sustainability concerns. The largest producers of perlite in the world are Greece, U.S. and Japan (Bolen, 2003). Perlite is a

relatively expensive substrate component due to the costs associated with mining, transportation, and heating (Evans and Gachukia, 2007). In addition its cost, when perlite is in its dry state, it produces a siliceous dust that is classified as an eye and lung irritant (Evans and Gachukia, 2007; Schundler Co., 2002). Due to these sustainability concerns of peat and perlite, some potential alternative components, such as chipped wood and parboiled rice hulls, have been researched for their physical and chemical properties.

Chipped Pine Logs as an Alternative Component

Currently, there is a quest for a substrate component that is renewable, of consistent quality, economically priced and of unlimited supply. The use of wood as an aggregate can be traced back to Viljoen and Fred (1924). Viljoen and Fred (1924) report the use of hardwood pulp and sawdust in soil and the effects on plant growth. The authors' results show using wood does not cause any toxicity to the plant, and there was an increase of microorganisms using nitrates that depleted nitrogen for the plant (Viljoen and Fred, 1924). However, any injury caused by loss of nitrogen soon passed off and the following season showed no injury (Viljoen and Fred, 1924). Work done by Maas and Adamson (1975) further showed that plants could be grown in softwood bark and sawdust. This has led to the belief that whole tree logs processed into a container substrate may be a suitable and economical alternative. Trees are renewable, reasonably priced, and widespread geographically, as well as small chipped wood particles are often byproducts of the forestry industry (Wright and Browder, 2005). In the southeast, loblolly pine trees (*Pinus taeda* L.) are abundant and reasonably priced; making loblolly pine trees the ideal tree to test the appropriateness of as an alternative aggregate/substrate.

Work with wood fibers or wood chips, as an aggregate and/or substrate, has been done both internationally and nationally. Gruda and Schnitzler (2004a; 2004b) worked with spruce (*Picea* sp.) wood fiber substrates (WFS) supplied by INTERTORESA (Switzerland). Gruda and Schnitzler (2004a) examined the physical properties of both a coarser and finer spruce WFS. Both wood fiber substrates resembled peat substrates with the amount of total pore space, the finer WFS had a high air volume compared to peat, and mixing both WFS and peat substantially improved the air volume (Gruda and Schnitzler, 2004a). Gruda and Schnitzler (2004b) saw no difference in plant growth between WFS and white peat, and mention that they saw a particularly well developed root system in the wood fiber substrates. Gruda and Schnitzler (2004) conclude that wood fiber can be an alternative component for greenhouse and nursery substrates.

Other work in the United States with chipped wood as encompassed the species of loblolly pine trees. Work done by Wright and Browder (2005) also showed loblolly pine chips to have a high air space, well above the acceptable range, and there was no apparent shrinkage due to decomposition during the course of their experiment. Generally, the wood is processed by chipping logs and hammer-milling the chips to a certain particle size, usually the majority of the particles in the 2-4 mm size range (Wright and Browder, 2005). Jackson et al. (2008) researched the effect of fertilizer rate on nursery crops grown in pine tree substrate (PTS) made of delimbed, chipped and hammer-milled loblolly pine. The plants achieved shoot growth in PTS comparable to shoot growth in traditional pine bark with 2.4 $kg \cdot m^{-3}$ additional fertilizer for PTS (Jackson et al., 2008), showing that woody plants grown in PTS required more fertilizer and an increase in nitrogen immobilization that was also

concluded by Fain et al. (2008b), Gruda and Schnitzler (2004b), and Wright et al. (2008) as well. Several authors have mentioned that substrate particles less than 0.5 mm (fines) are needed to provide adequate water-holding properties (Gruda and Schnitzler, 2004a; Jackson et al., 2010). Jackson et al. (2010) reported results showing that amending coarsely ground PTS with finer particle PTS or with other materials, such as peat moss, aged pine bark, or sand, can result in a substrate with similar physical properties and plant growth compared with 100% peat-lite or pine bark. The additions of peat moss or aged pine bark would also increase the cation exchange capacity of PTS, which is low (Wright et al., 2008). Fain et al. (2006) also reported that whole tree substrates, especially amended with peat moss, are a potential alternative to conventional greenhouse substrates.

Other species or sources of chipped wood have been investigated as well. Boyer et al. (2008a; 2008b; 2009) evaluated loblolly pine trees as a wood alternative made from a forestry residue generated from an in-field clean chipping operation that processes pine logs into chips for paper and the by-products are bark, limbs and needles, which can be chipped and hammer-milled to specified particle sizes. Fain et al. (2008a) looked into difference between three tree species; loblolly pine, longleaf pine (*Pinus palustris*), and slash pine (*Pinus elliottii*). The entire shoot portion of the trees were harvested and chipped in a tree chipper, termed as *WholeTree* substrates (Fain et al., 2008a). Initial substrate pH indicated that *WholeTree* made from either slash or longleaf pine had an average pH of 4.5 and loblolly pine had a pH of 5.3 (Fain et al., 2008a). All three *WholeTree* substrates have the potential as an alternative sustainable source for producing short-term horticultural crops (Fain et al., 2008a).

The use of chipped pine logs and trees have been widely researched in the past few years and have led to the discovery that pine tree substrates are an acceptable and sustainable alternative greenhouse and nursery substrate, as well as a suitable substrate component with peat moss and pine bark. A few authors mention the observation of better, well developed root systems in the pine tree substrates (Gruda and Schnitzler, 2004b; Jackson et al., 2010; Wright and Browder, 2005) (Table 1). However, these were observations were quantified with root dry weight or subjective ratings, which both measurements have flaws and this phenomenon needs to be correctly and accurately quantified.

Table 1. Comparison of results in scientific literature showing improved root growth in wood substrates.

Author	Species Used	Evaluation Method	Results
Gruda and Schnitzler (2004b)	Spruce sp. (*Picea* sp.)	Root rating	Highest rating in both coarse and fine wood fiber substrates compared to white peat and rockwool.
Wright and Browder (2005)	Loblolly pine (*Pinus taeda* L.)	Root dry weight	Highest dry weight for 75% pine chips: 25% pine bark compared to 100% pine bark.
Jackson et al. (2010)	Loblolly pine	Root rating	Highest rating for all pine tree substrates compared to peatlite and pine bark.

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Chapter 1

Mini-Horhizotron: A Small-Scale Horhizotron™ for Observing and Measuring Seedling and Small Plant Root Growth In Situ

(In the format appropriate for submission to HortScience)

Mini-Horhizotron: A Small-Scale Horhizotron™ for Observing and Measuring Seedling and Small Plant Root Growth In Situ[1]

Lesley A. Judd[2,6], Brian E. Jackson[3], Ted C. Yap[4] and William C. Fonteno[5]

North Carolina State University, Department of Horticultural Science, 227 Kilgore Hall, Campus Box 7609, Raleigh, NC 27695

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[2]Graduate Research Assistant.
[3]Assistant professor.
[4]Undergraduate student worker.
[5]Professor.
[6]To whom reprint requests should be addressed; e-mail lajudd@ncsu.edu

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Abstract. An apparatus that allows for a range of measurements of whole system root growth in containers (pot culture) was developed. The mini-Horhizotron was designed to measure root growth of small plant material, such as seedlings, herbaceous plugs or woody plant liners. Potential measurements include root length, speed of root growth, presence and quantity of root hairs, and root architecture/branching. The design of the mini-Horhizotron has three quadrants extending away from the center that could be filled separately with different substrates/treatments to observe root growth response. This design allows for the measurement of roots from a plug or liner as they would fill out a standard greenhouse container and aids in better understanding of root growth patterns, problems and potential. This knowledge can be used to better improve the rooting environment (substrate), study patterns and root response to disease, water stress or various other stresses that occur during the plant culture that previously we have been unable to observe and quantify in situ. The objectives of this work were: 1) to design mini-Horhizotron, 2) test the suitability of the design for small plant material, 3) use the mini-Horhizotrons to compare root growth in different container substrates, and 4) test different experimental designs on the mini-Horhizotrons for research purposes.

Introduction

A large portion of the U.S. green industry is involved with growing plants in containers, including bedding plants, vegetable plants, foliage plants, potted flowering plants, potted nursery stock and other assorted floriculture crops. Root growth of crops grown in containers is a central element in overall plant performance, whether it is during propagation, production or post-production (e.g. transplant success). Container production of horticulture crops has increased substantially over the past several decades, due to advantages container production has over traditional field production and the gradual shift from using soil in containers to soilless substrates. Container production is an easier enterprise, because it requires less land, ease of harvesting/handling and greater returns per acre than field production (Halcomb et al., 2009). Soilless substrates were adopted for many reasons, including; 1) soilless substrates do not need to be pasteurized as soil-based substrates do; 2) soilless substrates are lighter in weight, and therefore reduce shipping and handling costs; and 3) soil can vary greatly from batch to batch (Nelson, 2011). Also, woody plants grown in containers have been shown to have a greater fine root mass and better post-transplant success compared to field-grown plants (Gilman and Beeson, 1996; Harris and Gilman, 1993). Considering the large portion of the industry involved with growing plants in containers and the importance of understanding the physiology and morphology of roots, the factors that influence root growth in container production need to be investigated. However, root growth and root architecture are frequently excluded in horticultural research (Wright and Wright, 2004) and the study of natural root development is a challenge due to the difficulty of root observations in containers during crop production (Silva and Beeson, 2011).

Strategies and techniques for observing, studying, and quantifying root growth have been reported for over nine decades. The earliest records of observing root growth of field-grown plants were by McDougall (1916) and Weaver et al. (1922). The techniques of the early twentieth century were to dig a trench and either draw/trace the roots or take photographs. The photographs taken were often blurry and did not allow the viewer to observe the finer roots. The inadequate photographs resulted in researchers hand-drawing root systems, which was extremely time-consuming (Weaver et al., 1922). Fortunately, many advances have been made over the decades in the study of root measurements; including techniques that can be easier, faster and more descriptive/clarifying observations of root growth.

Advances in techniques of quantifying root growth for field-grown plants include the rhizotron. A rhizotron is an underground chamber designed for viewing and measuring plant roots through transparent (glass) surfaces (walls) in contact with the soil (Klepper and Kaspar, 1994). Rhizotrons are one of the earliest non-destructive techniques for observing root growth in soil, and the ability to make successive measurements on the same individual root is a major advantage of the rhizotron (Taylor et al., 1990). However, rhizotron construction and upkeep is expensive, there are a finite number of repetitions, and the soil environment may be changed during construction (Huck and Taylor, 1982). A similar technique to the rhizotron is the minirhizotron, originally proposed by Bates (1937). The modern minirhizotron uses a color video camera with a right-angle viewing attachment that can be lowered into a transparent tube buried in the ground, images can be recorded and analyzed, and field-grown plant root systems can then be quantified (Taylor et al., 1990). A

major disadvantage of the minirhizotron is the number of underground tubes required to accurately estimate rooting (Taylor et al., 1990) and the amount of time required to collect the video or images from every tube and analyze them. Another common technique of measuring root growth of field-grown plants is the pinboard method. Pinboards are constructed with a sturdy, hard surface (such as a wood board or a metal board) with several pins, or nails, attached to the surface. These pinboards can be pressed into a trenched side of the soil column, a section of soil can be cut and removed such that the soil can be rinsed from the pinboard leaving the root system arranged around the pins in what is thought to be the natural positions of the root system (Schuurman and Goedewaagen, 1971).

With the increasing shift to container production and soilless substrates, it is important to understand the factors that influence root growth in containers in order to achieve optimal benefits/efficiency from container production. The importance of root hairs/fine roots and their function has increased with the study of root growth. Whitaker (1923) hypothesized that root hairs were the chief means through which water and mineral salts are taken from the soil into the plant. In 1937, author H.J. Dittmer commented in his paper that several of his peers had measured the general picture of plant roots and the roots distribution through the soil, however none of them had made a count of root hairs for the entire root development. Dittmer (1937) reported that root hairs were present on many root systems of different plants and were important enough to attempt to measure. Another important root characteristic that has been linked with mechanical support and acquisition of water and nutrients is root architecture/types and diameter. Three classes of roots usually described are: the taproot, lateral roots, and adventitious roots. The number and length of

secondary roots (e.g. lateral roots) varies greatly depending on the plant species, soil composition and water and nutrient availability (Malamy, 2005). The architecture of a root system can determine its exploration of spatial domains in the soil, as well as its ability to respond to possibly localized available nutrients in the soil/media (Fitter, 1996).

The shift to container production and the advances in understanding root growth led to new approaches to measuring root growth of container-grown plants. The pinboard method is a method that can also be used with container-grown plants, by pressing the pinboard against a root ball and rinsing the substrate away to reveal roots arranged around the pins in what is thought to be the natural positions of the root system (Schuurman and Goedewaagen, 1971). Currently, root system evaluations of plants grown in containers are subjective root ratings and/or root dry weight measurements. Subjective root ratings can be a simple and easy way to qualitatively describe root balls, washed roots and propagative rooted cuttings. Ratings can evaluate root density, appearance, branching and distribution. However, the person rating the root system must first understand how to accurately rate the quality of the root system (Walters and Wehner, 1994) and root rating is subjective and will vary between different examiners. Shoot:root ratio is a measurement of the performance of each organ in a certain environment (Aung, 1971). Shoot:root ratios is a destructive method which involves comparing dry weights of roots and shoots. Media must be washed away from the roots, and much of the fine roots and root hairs are lost in this process, as well as the natural positions and arrangements of the roots. In standard methods of washing and storage of root samples, losses of dry weight from 20-40% may occur (Oliveira et al., 2000; van Noordwijk and Floris, 1979).

The Horhizotron™ was developed at Auburn University and Virginia Tech as a non-destructive technique to measure horizontal root growth from root balls of plants grown in nursery containers, allowing for post-transplant assessment (Wright and Wright, 2004). The Horhizotron™ is constructed out of eight panels of glass attached to an aluminum base to form four wedge-shaped quadrants, and is suitable for greenhouse or field use and fits a range of nursery stock root balls. The substrate in each quadrant can be modified in various ways in order to examine the effects of different rhizosphere conditions (Wright and Wright, 2004). However, the size of the Horhizotron™ is restricted to large sized root balls (3.8 -11.4 L), the glass panels are not permanent and can move and crack and the shade box does not restrict all light from the root system. Silva and Beeson (2011) developed a large-volume rhizotron, to observe root growth in an environment closer to natural soil conditions, and still have the apparatus aboveground and therefore relatively easier to collect measurements. However, this design is even larger and intended for woody plants, with large root balls, to imitate post-transplant/or field growing conditions.

Currently, rhizotrons, minirhizotrons and other transparent wall/container designs commonly use digital imaging to measure root systems. Digital imaging includes photographs or videos, scanned images of exposed roots, or scanned root tracings. These images can be used by computer programs to evaluate root systems. There are numerous computer programs, both commercially and freely available that can be used, and there are 19 commonly used and known computer programs (Lobet, 2011). Some of these programs include RootLM, RootReader 2D, EZ-Rhizo, WinRHIZO and WinRHIZO Tron. Possible measurements for WinRHIZO (Regents Instruments, Quebec City, Canada) include; total

length, projected area, surface area, root tips, and branching points (Arsenault et al., 1995). The WinRHIZO is relatively inexpensive and suitable for both large and small-scale experiments (Fang et al., 2012). However, WinRHIZO only analyzes scanned images of washed roots, and this is a destructive method that loses fine roots. WinRHIZO Tron is used with rhizotrons and other techniques where a photograph or video can be analyzed by this software. However, the images are often unfocused, blurry and the substrate might not contrast with the root enough to be completely visible. This causes the user of WinRHIZO to have to manually pick out each root and trace the length of it themselves, and depending on the number of roots per image, could be time consuming.

The use of chipped pine logs and trees have been widely researched in the past few years and have led to the discovery that pine tree substrates are an acceptable and sustainable alternative greenhouse and nursery substrate, as well as a suitable substrate component with peat moss and pine bark. Several researchers have observed better, well-developed root systems of herbaceous and woody plants grown in wood substrates (Gruda and Schnitzler, 2004b; Jackson et al., 2010; Wright and Browder, 2005). Gruda and Schnitzler (2004b) examined spruce (Picea sp.) wood fiber substrates (WFS) and noted particularly well developed root systems of plants grown in the WFS compared to plants grown in both peat and rockwool substrate. The physical properties of both a coarser and finer WFS resembled peat substrates with the amount of total pore space; the finer WFS had a high air volume compared to peat, and mixing both WFS and peat substantially improved the air volume (Gruda and Schnitzler, 2004a). Wright and Browder (2005) also noted increase in root growth with plants grown in 75% pine bark: 25% pine chips compared to 100% pine bark.

Wright and Browder (2005) used loblolly (*Pinus taeda* L.) pine chips as the substrate component, and there was no apparent shrinkage due to decomposition during the course of their experiment. Jackson et al. (2010) also used loblolly pine chips as the substrate component, and observed the highest root rating for plants grown in all pine tree substrates compared to plants grown in peatlite or pine bark. However, these observations were quantified with root dry weights or subjective ratings, so this enhanced rooting phenomenon remains to be accurately quantified and explained.

Understanding root growth in containers and the factors that affect it are critical during plant production and ways to improve root growth of container-grown plants would be valuable to the horticulture industry. There is a need for developing new techniques in order to study and measure root growth of seeds, and small sized plant material during production (vegetable transplants, plugs for floricultural crops and nursery liners). The objectives of this work were: 1) to design the mini-Horhizotron, 2) test the suitability of the design for small plant material, 3) use the mini-Horhizotrons to compare root growth of plants in different container substrates, and 4) test different experimental designs on potential usefulness/influences when using the mini-Horhizotron for research purposes.

Materials and Methods

Design and construction of mini-Horhizotron. The mini-Horhizotron was designed to have three quadrants with concave curves forming a geometric deltoid (Fig. 1) and to have a substrate volume (1573 cm^3) similar to the volumes of common containers (16.5 cm dia, 1720 cm^3) used to grow greenhouse crops. The base of the mini-Horhizotron area is a

triangle with an area of 907.2 cm^2 (44.3 cm base length with corners removed, 40.95 cm height length) and was cut from polyvinylchloride (PVC) board (Plasticlad®; Franklin, VA). Three end caps (PVC trimboard, Plasticlad®; Franklin, VA) were placed at each corner of the triangle base and permanently fastened with 4.45 cm wood screws (Power Pro; Lowe's, Smithfield, NC; Fig. 2A) drilled from the bottom through the base and the corners of the base were trimmed/beveled to remove sharp corners. Each end cap has a trapezoid shape 12.7 cm tall (Fig. 2) with two 6.35 mm notches (2.54 cm apart) to hold the ends of two quadrant walls (Fig. 1). Transparent acrylic sheets (6.35 mm dia; Lucite International, UK) were cut to make the three quadrants, 10.2 cm tall and 41.9 cm in length (Fig. 1). The concave curves of both the acrylic sheets were created by placing the acrylic sheets into an industrial oven heated to 150° C for 3 minutes and when removed, placed on a form-fitting, curved mold which was constructed to have the desired angle of the quadrants. On the mini-Horhizotron, the acrylic sheets are held in place by screws (#5 x 1.3 cm, The Hillman Group; Lowe's, Smithfield, NC) which were drilled into the end cap groves and through the acrylic sheets to hold them in place. To minimize excess drainage by leaks, the acrylic sheets were attached to the base with adhesive caulk (Loctite® Polyseamseal®; Lowe's, Smithfield, NC). To facilitate drainage, three 0.07 cm holes were drilled into the base of each quadrant 5.5 cm apart; starting 3 cm from the end caps (Fig. 2C). Shade panels (6.35mm dia, PVC panel, Plasticlad®; Franklin, VA) were constructed from the same PVC material used to make the base, and the shade panels fit tightly against the concave clear acrylic sheets in order to block sunlight to the rhizosphere, and a flange strip was attached with a staple gun to create a "lip" over the acrylic sides to aid in the ability to remove the shade panel and to block sunlight (40

cm length, 11.4 cm tall, lip 1.3 cm; Fig. 2B). Shade panels were constructed in the same procedure as the acrylic sides, by being placed in an oven for several minutes and then pressed against a mold.

Testing the design: Experiment 1. Three substrates were used in the initial testing of the mini-Horhizotron, 70% peat amended with either 30% perlite (PL), pine wood chips (PWC), or shredded-pine-wood (SW; v/v). Eight-year-old loblolly pine trees were harvested on 19 Dec. 2011 at ground level and delimbed in Chatham County, NC and stored under shelter from weather. On 2 Jan. 2012 the delimbed pine logs were chipped in a DR Chipper (18 HP DR Power Equipment, model 356447; Vergennes, VT) to produce small wood chips. The pine logs destined for shredding were processed in a Wood Hog shredder (Morbark® model 3800; Winn, MI). Both the chipped and shredded wood was then processed in a hammermill through a 6.35 mm screen (Meadows Mills, North Wilkesboro, NC) to produce two end products, PWC and SW. The SW component was selected for trial because the particles have properties similar to peat and the PWC component was selected for trial because it has properties similar to perlite. Both wood components have been researched as respective alternatives to peat and perlite. Substrates were mixed on 1 June 2012, tested for initial pH and then amended with dolomitic limestone (#200; Mississippi Lime Company, Vicksburg, MS) at 3.86 $kg \cdot m^3$ to achieve a desired pH of 5.8. On 2 June 2012, three mini-Horhizotrons were filled with each individual substrate. To account for substrate settling which occurs after initial irrigation events, the mini-Horhizotrons were tapped three times, by lifting the mini-Horhizotron 10 cm from a hard surface and dropping, to settle the substrate and then filled to the top with substrate again. Three species, *Echinacea purpurea* ‘Prairie

Splendor'(162-tray; C. Raker & Sons, Inc., Litchfield, MI) , *Chrysanthemum* 'Garden Alcala Red' (51-tray; C. Raker & Sons, Inc, Litchfield, MI), and *Ilex crenata* 'Steeds'(10 cm, 16-liners; Casey Nursery, Inc., Goldsboro, NC) were used in this experiment. One plug or liner (*Ilex*) was planted into the center of each mini-Horhizotron, and one mini-Horhizotron is considered a replication since all three quadrants contain the same substrate. Three substrates x three replications of each substrate x three species made a total of 27 mini-Horhizotrons. Plants were also grown in the same substrates in greenhouse containers to compare root dry weights at the end of the study to show any effects the mini-Horhizotron's shape may have contributed, compared to a container of similar substrate volume. Six greenhouse containers (16.5 cm dia; Dillen Products, Middlefield, OH) were filled with each substrate on 2 June 2012, and filled to the top of the container and tapped three times to settle the substrate. One plug/liner of each species was planted into the center of the containers, three substrates x six replications of each substrate x three species made a total of 54 containers. Mini-Horhizotrons and containers were completely randomized separately by species on a greenhouse bench, in Raleigh, NC. Plants were over-head watered as needed depending on weather conditions, and never showed symptoms of water stress. The design of the mini-Horhizotron was purposely similar to the design of a container, so the shade panels are to remain in place when plants were watered, because the substrate surface of all quadrants are exposed to air/light, like a plant grown in a container. Plants were fertilized at each watering with 200 ppm nitrogen (N) injected at 1:100 ratio by Dosatron injector [(D14MZ2); Dosatron International, Inc., Clearwater, FL] with Peters Professional 20-10-20 Peat-Lite Special (The Scotts Co., Marysville, OH) containing 8.1% ammonium (NH_4-N) and 11.9% nitrate (NO_3-

N). Root length measurements (cm) were taken on the three longest roots appearing on the face of each quadrant on 11, 18, 25, 32, 39, and 46 days after planting (DAP). Each quadrant has two measureable faces giving a sum of six quadrant faces per mini-Horhizotron. Measurements were taken by attaching a transparent sheet (20.5 cm x 10.5 cm transparency film; 3M Visual Systems Division, Austin, TX) with a printed cm^2 grid on each quadrant face, and roots were measured from the start of the gridlines, which was at the center of the mini-Horhizotron (where the plant was planted), to the end of the gridlines, which reached the end of the quadrants (at the end cap). Measuring three roots per quadrant face x six quadrant faces per mini-Horhizotron x three replications per substrate equals 54 data points collected per plant species tested. Data were subjected to the general linear model procedures and regression analysis (SAS Institute version 9.2, Cary, NC). Means were separated by least significant differences at $P \leq 0.05$. Both the mini-Horhizotrons and the container-grown plants were harvested on 54 DAP; shoots were removed at the substrate surface and the root balls were washed to remove substrate. Both the shoots and washed root systems were oven dried at 70°C for 48 hours. The comparison of root dry weights between the mini-Horhizotrons and the greenhouse containers were subjected to general linear model procedures and least square means analysis (SAS Institute version 9.2, Cary, NC). Means were separated by Tukey's studentized range (HSD) at $P \leq 0.05$.

Comparing experimental designs: Experiment 2. Previous studies with the Horhizotron™ have shown the design allows for each quadrant to be modified in various ways in order to examine the effects of different rhizosphere conditions (Wright and Wright, 2004). Jackson et al. (2005) conducted an experiment with the Horhizotron™, in which each

quadrant was filled with a different substrate; Price et al. (2009) divided the quadrants of the Horhizotron™ with one type of substrate on the lower half and a different substrate on the upper half. Since the mini-Horhizotron was designed to be a smaller Horhizotron™, an experiment was conducted to test the functionality of the mini-Horhizotron when the quadrants were divided and filled with a separate substrate. Three substrates were used in the comparison of experimental designs of the mini-Horhizotrons, 70% (v/v) peat moss amended with either 30% perlite (PL), pine wood chips (PWC), or shredded-pine-wood (SW). Eight-year-old loblolly pine trees were harvested on 4 July, 2012 at ground level and delimbed in Chatham County, NC and subsequently stored under shelter for protected from the weather. The delimbed pine trees were then either chipped or shredded and hammermilled in the same process described in Expt. 1. The substrates were mixed on 28 July 2012, all substrates were tested for initial pH and then amended with dolomitic limestone (#200; Mississippi Lime Company, Vicksburg, MS) at 4.45 $kg \cdot m^{-3}$ to achieve a desired pH of 5.8. On 30 July 2012 three mini-Horhizotrons were filled and tapped in the same manner as described in Expt. 1 with each individual substrate. Six mini-Horhizotrons were divided in the center with a cardboard divider and each quadrant was filled with one of the three substrates in random order and the same tapping and refilling procedure occurred. One plug of *Rudbeckia hirta* 'Becky Yellow' (288-tray; C. Raker & Sons, Inc., Litchfield, MI) was planted into the center of each mini-Horhizotron. Each of the mini-Horhizotrons that contained the same substrate in all quadrants was considered a single replication. The six mini-Horhizotrons with a different substrate in each quadrant was considered a block design with six replications. A total of 15 mini-Horhizotrons were used in this experiment. Mini-Horhizotrons were completely

randomized on a greenhouse bench, grown in Raleigh, NC. Plants were watered and fertilizer in the same manner as previously described in Expt. 1. Root length measurements (cm) were taken on the three longest roots appearing on the face of each quadrant on 15, 19, 23, 27, 31, 35, 39, 43, 47, 51, 55, 59, 63, and 67 DAP. Measurements were taken as previously described in Expt. 1. Data were subjected to the general linear model procedures and regression analysis (SAS Institute version 9.2, Cary, NC). Means were separated by Tukey's HSD at $P \leq 0.05$.

Testing substrates: Experiment 3. Three substrates were tested in the mini-Horhizotron; 60, 70 and 80% peat moss amended with either 40, 30 or 20% (v/v) PWC, respectively. The substrates were mixed on 28 July 2012, initial pH of the substrates were tested, and all the substrates were amended with dolomitic limestone (#200; Mississippi Lime Company, Vicksburg, MS) at 4.45 $kg \cdot m^3$ to achieve desired pH of 5.8. On 30 July 2012, three mini-Horhizotrons were filled and tapped with each individual substrate in the same manner described in Expt. 1. One plug of *Rudbeckia hirta* 'Becky Yellow' (288-tray; C. Raker & Sons, Inc., Litchfield, MI) was planted into the center of each mini-Horhizotron. Only one plug was planted into each mini-Horhizotron, and one mini-Horhizotron is considered a replication since all three quadrants contain the same substrate. Three substrates x three replications of each substrate x one species made a total of 9 mini-Horhizotrons. Mini-Horhizotrons were completely randomized on a greenhouse bench, in Raleigh, NC. Plants were watered and fertilizer in the same manner as previously described in Expt. 1. Root length measurements (cm) were taken on the three longest roots appearing on the face of each quadrant on 15, 19, 23, 27, 31, 35, 39, 43, 47, 51, 55, 59, 63, and 67 DAP. Data

were subjected to the general linear model procedures and regression analysis (SAS Institute version 9.2, Cary, NC). Means were separated by Tukey's HSD at $P \leq 0.05$.

Results and Discussion

Testing the design: Experiment 1. All species exhibited linear rates of root growth over the course of the experiment in all three substrates (Fig. 3). At 11 DAP, all three species had similar root growth in all three substrates. Beginning at 18 DAP and continuing to 25 DAP, *Chrysanthemum* grown in PWC had more root growth than plants grown in PL and SW (Fig. 3). At 39 DAP, PL had more root growth than PWC (Fig. 3). However, at the end of the study (46 DAP), root growth of *Chrysanthemum* in all three substrates were not significantly different (Fig. 3). At 18 and 25 DAP, *Echinacea* had more root growth in the PWC and SW substrates compared to plants grown in PL substrate (Fig. 3). The fourth and fifth measurements dates had faster root growth for *Echinacea* in grown in SW substrate compared to plants grown in PL substrate, and plants grown in PWC substrate were not significantly different from either. The final measurement date had more significant root growth in PWC substrate compared to plants grown in PL substrate, and plants grown in SW substrate were not significantly different from either (Fig. 3). *Ilex* had faster root growth in PWC beginning at 25 DAP, until 39 DAP when plants grown in all three substrates had no significant difference in root growth (Fig. 3). Observed for all species, the PWC or SW components either enhanced root growth at certain time periods or were not significantly different from root growth observed in the PL substrate.

There were no differences between the growth of the shoots and roots of both *Echinacea* and *Chrysanthemum* in all three substrates, when grown in either the mini-

Horhizotron or containers (Table 1). The only differences observed are with *Ilex* grown in the PWC and SW substrates (Table 1). *Ilex* showed more shoot and root growth in the mini-Horhizotron compared to container-grown plants in the PWC substrate, and only more shoot growth with the plants grown in the SW substrate within the mini-Horhizotrons. Since *Echinacea* and *Chrysanthemum* species show no significant root or shoot growth differences between the two growing methods, the experimental design of the mini-Horhizotron does not influence root growth (treatment effects) of these species. The differences observed in *Ilex* root and shoot growth show that the woody plant species was influenced differently than the herbaceous plant species and more work needs to be done to determine the cause of this. Based on this experiment, data can be easily collected on root growth of small herbaceous and woody plants in the same manner the large Horhizotron™ is used to observe and measure root growth from root balls. This experiment provides evidence that the mini-Horhizotron can be used to show treatment/substrate effects on root growth. The mini-Horhizotron as an apparatus for scientific study and quantification of root growth can be used to generate results that are indicative of plant growth in traditional containers (actual production situations).

Comparing experimental designs: Experiment 2. In comparing the two experimental designs (mini-Horhizotron as a block versus a replication) by substrate, root growth in the PL substrate was similar from 15 DAP through 55 DAP, after which root growth was greater in the block experimental design until the end of the experiment (Table 2). Root growth in the PWC substrate was different between the experimental designs from 15 DAP through 27 DAP, it was observed that plants grown in PWC substrates in the replication design had

greater root growth. After 27 DAP, root growth was similar between the experimental designs with PWC substrate until 51 DAP (Table 2). After 51 DAP, plants grown in PWC substrate had more root growth in the replication design through the end of the study (Table 2). Root growth in the SW substrate was similar from 15 to 51 DAP, except at 19 DAP when the replication design had more root growth than the block design (Table 2). After 51 DAP, root growth in the SW substrate was greater in the replication design (Table 2). Differences between the two experimental designs for PL and SW substrate started after 51/55 DAP, while differences in the PWC substrates used in the two experimental designs started at the beginning of the study through 27 DAP, and then differences were observed again after 51 DAP. Both the experimental design and the substrate had a significant effect on root growth (Table 2).

Testing substrates: Experiment 3. All three substrates had similar root growth from the beginning through 35 DAP (Fig. 4). Beginning at 39 DAP, plants grown in 40% PWC substrate had more root growth compared to plants grown in the other substrates (Fig. 4). At 51 DAP, root growth was similar between the 40% PWC and 30% PWC substrates, but plants grown in 40% PWC substrate were significantly longer than plant roots in 20% PWC substrate. At 55 DAP through the end of the study, plants grown in 40% PWC and 30% PWC substrates had significantly longer plant roots compared to plants grown in 20% PWC substrate. These data show a general increase in *Rudbeckia* root growth in 40% PWC substrate starting at 39 DAP through 51 DAP (Fig. 4). The mini-Horhizotron can be used to quantify root growth and the effects of different substrates on root growth.

Conclusion

The mini-Horhizotron was designed to be a small-scale Horhizotron to measure and observe root growth of small plant material, such as seedling, floriculture plugs and woody liners. The design succeeded with this objective, due to the ability to easily observe and measure root growth of plants grown in the mini-Horhizotron. These experiments provide results showing the ability to easily manipulate the root environment (rhizosphere) in the mini-Horhizotron, and to also have the capacity to detect and quantify the influence of the rhizosphere on root growth of plants. These experiments also show the mini-Horhizotron has the ability to manipulate the experimental design, with different rhizosphere conditions (e.g. substrate), similar to research done with the Horhizotron™.

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Table 1: Comparison of shoot and root dry weight of *Echinacea*, *Ilex* and *Chrysanthemum* species between plants grown in containers and plants grown in the mini-Horhizotrons.

		Shoot[z] (g)		Root[y] (g)	
Plant	Substrate	CT[x]	MH[w]	CT	MH
Echinacea	PL[v]	5.7 a[s]	6.3 a	3.0 a	2.5 a
	PWC[u]	6.1 a	6.5 a	4.4 a	3.4 a
	SW[t]	6.8 a	8.4 a	3.3 a	3.5 a
Ilex	PL	4.4 a	5.1 a	1.0 a	1.1 a
	PWC	4.1 b	7.1 a	1.1 b	1.8 a
	SW	4.0 b	5.6 a	1.0 a	1.3 a
Chrysanthemum	PL	7.1 a	7.0 a	4.4 a	4.6 a
	PWC	5.6 a	6.5 a	4.3 a	4.5 a
	SW	6.4 a	5.6 a	5.0 a	6.9 a

[z]Shoot dry weight, severed plant at substrate surface and oven dried.
[y]Root dry weight, washed root system to remove all substrate and oven dried.
[x]CT is plant dry weights from plants grown in containers.
[w]MH is plant dry weights from plants grown in mini-Horhizotrons.
[v]PL substrate is 70:30 peat:perlite (v/v).
[u]PWC substrate is 70:30 peat:pine-wood-chips (v/v).
[t]SW substrate is 70:30 peat:shredded-pine-wood (v/v).
[s]Means separated within row separated for shoot and root by Tukey-Kramer significant difference, $P \leq 0.05$. Means followed by the same letter are not significantly different.

Table 2: Comparison of *Rudbeckia* root growth in different experimental designs using the mini-Horhizotrons with three different substrates.

		Days after planting															
Substrate	Exp. Design[z]	15	19	23	27	31	35	39	43	47	51	55	59	63	67		
PL[w]	Block[y]	1.7 a[t]	2.9 a	5.2 a	6.7 a	8.5 a	10.9 a	14.3 a	16.7 a	17.6 a	18.0 a	18.7 a	18.7 b	18.7 b	18.7 b	L***[s]	Q***
	Rep[x]	1.4 a	2.7 a	5.7 a	7.4 a	8.9 a	10.0 a	14.0 a	16.1 a	17.1 a	18.3 a	19.6 a	20.1 a	20.4 a	20.5 a	L***	Q***
PWC[v]	Block	1.4 b	2.6 b	5.3 b	6.9 b	9.7 a	11.2 a	14.6 a	16.6 a	17.2 a	17.8 a	18.4 b	18.5 b	18.6 b	18.6 b	L***	Q***
	Rep	2.9 a	4.1 a	6.7 a	8.3 a	10.3 a	12.3 a	14.9 a	17.2 a	18.4 a	19.0 a	19.8 a	20.1 a	20.3 a	20.4 a	L***	Q***
SW[u]	Block	2.6 a	3.6 a	7.0 a	8.6 a	11.9 a	13.0 a	15.4 a	16.3 b	17.6 a	18.3 a	18.5 b	18.5 b	18.5 b	18.5 b	L***	Q***
	Rep	2.8 a	4.4 a	7.5 a	9.4 a	12.6 a	14.1 a	17.1 a	18.3 a	18.9 a	19.6 a	20.1 a	20.2 a	20.3 a	20.3 a	L***	Q***

Significance	Experimental design	Substrate	Interaction
	***	***	NS

[z]Experimental design was either block or replication of mini-Horhizotrons.
[y]Block was the experimental design with each quadrant of mini-Horhizotrons filled with an individual substrate.
[x]Rep was the experimental design with each quadrant of mini-Horhizotrons filled with the same substrate.
[w]PL substrate is 70:30 peat:perlite (v/v).
[v]PWC substrate is 70:30 peat:pine-wood-chips (v/v).
[u]SW substrate is 70:30 peat:shredded-pine-wood (v/v).
[t]Means separated by substrate within column by Duncan's multiple range test, $P \leq 0.05$. Means followed by the same letter are not significantly different.
[s]NS, L, and Q represent no significant response, linear, and quadratic response, respectively, over time of individual substrates and experimental design type, *, **, *** represent significant effects when $P \leq 0.05$, 0.01, and 0.001, respectively.

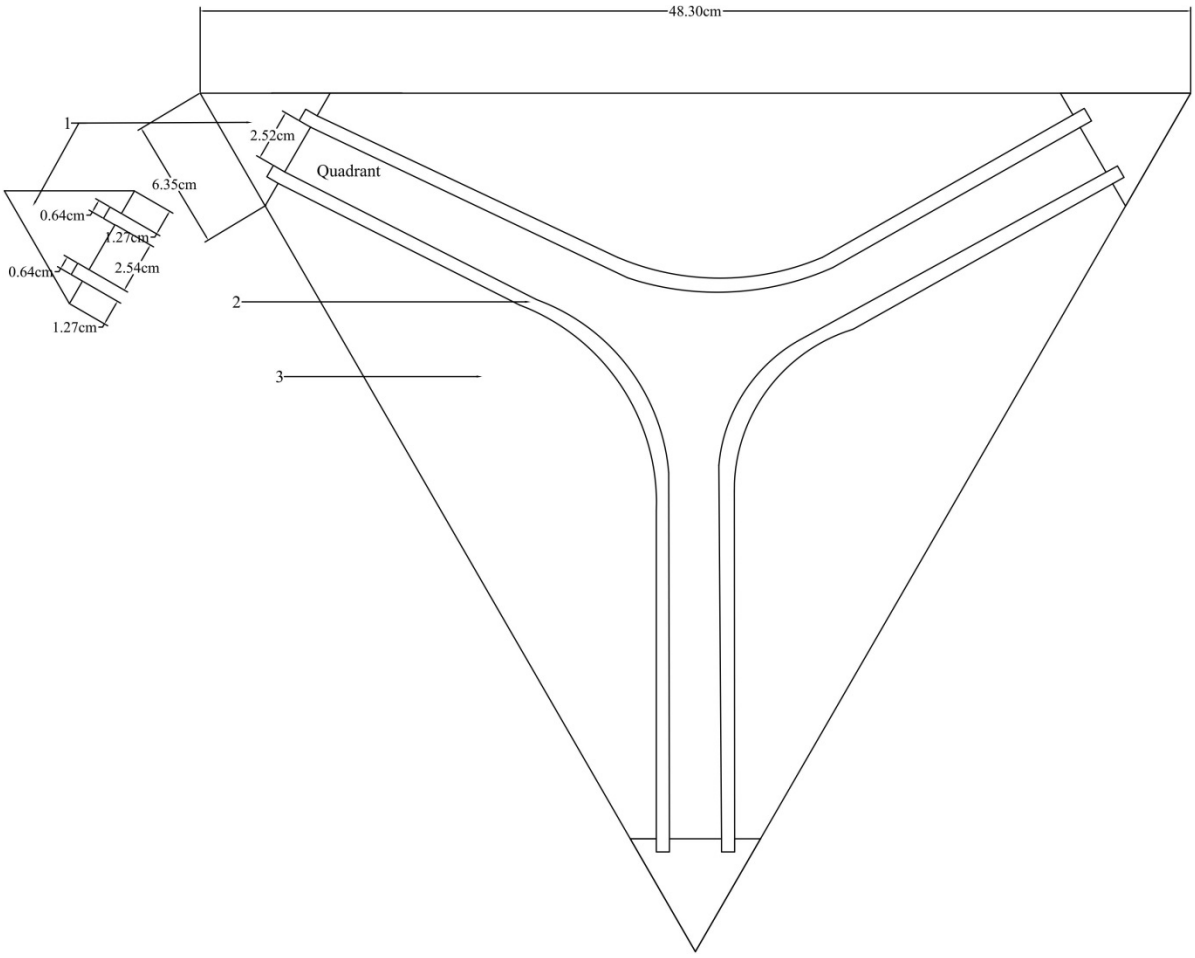

Figure 1. Schematic drawing of the mini-Horhizotron. Top view of the mini-Horhizotron with (1) the end cap, (2) the acrylic sides and quadrants, and (3) the base, before the corners of the end caps and base were trimmed.

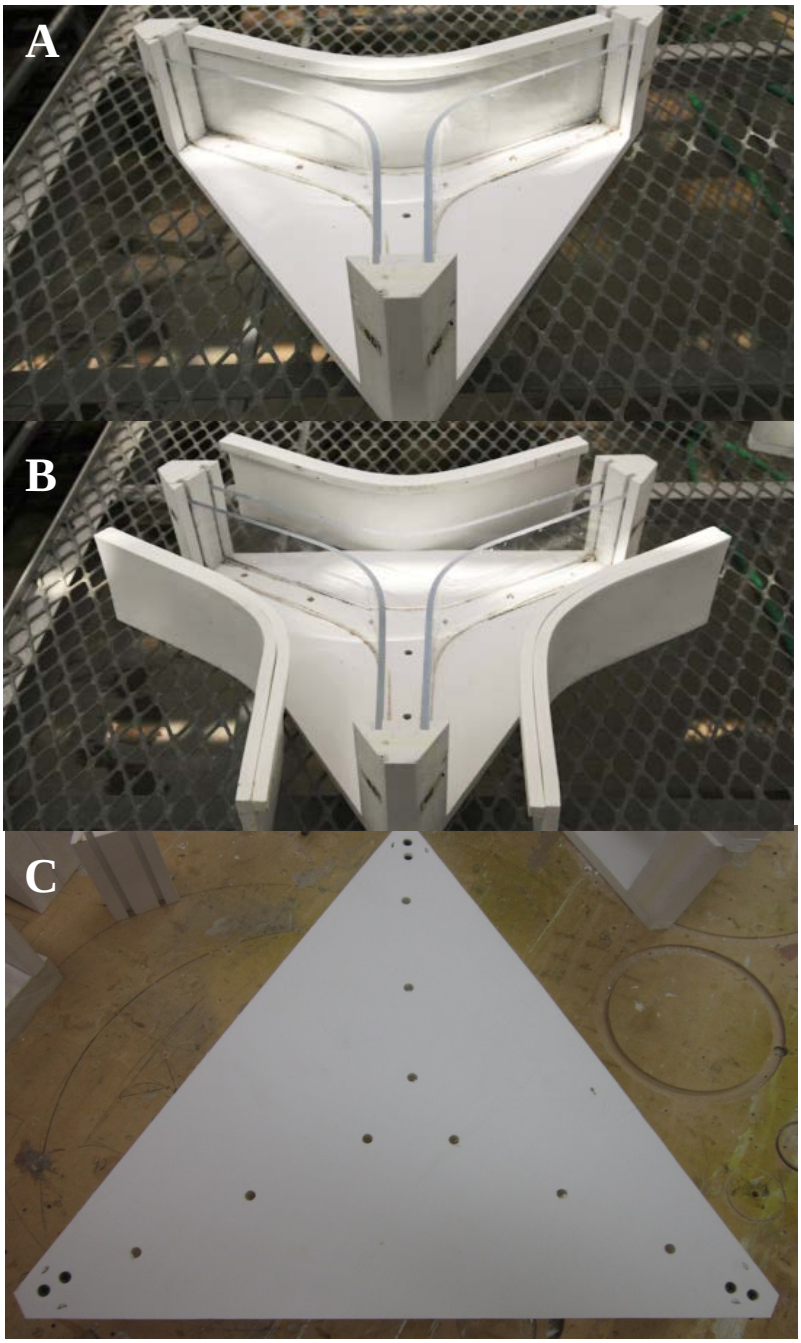

Figure 2. (A) Design of the mini-Horhizotron illustrating the three quadrant configuration, and (B) the removable shade panels which fit directly against the acrylic quadrants to restrict all light from the rhizosphere. (C) the bottom view of the mini-Horhizotron, with drainage holes drilled into the base.

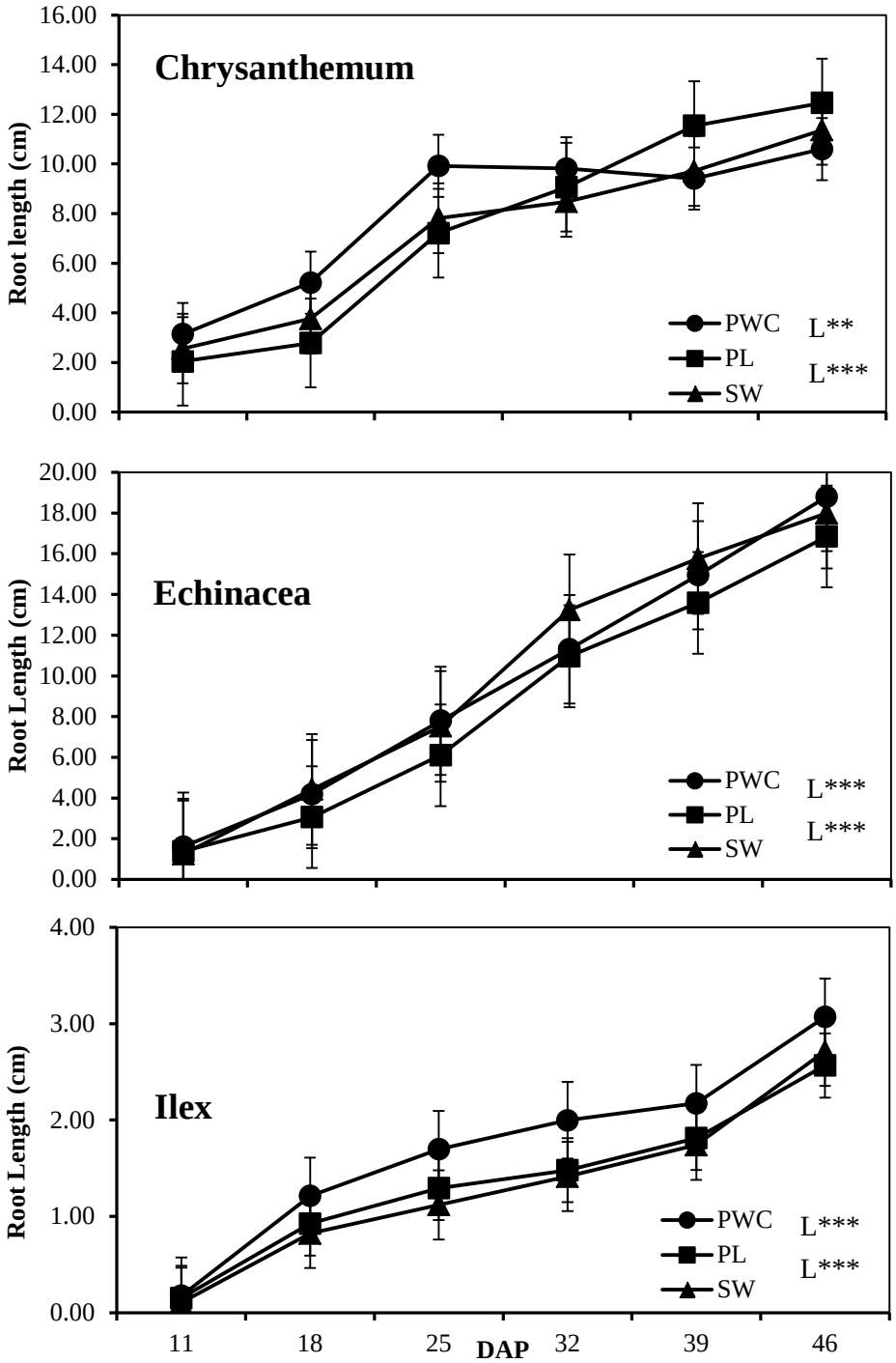

Figure 3. Root length measurements of *Chrysanthemum*, *Echinacea* and *Ilex* from 11 to 46 days after planting (DAP) for 70% (v/v) peat moss amended with either 30% perlite (PL), pine wood chips (PWC), or shredded-pine-wood (SW).

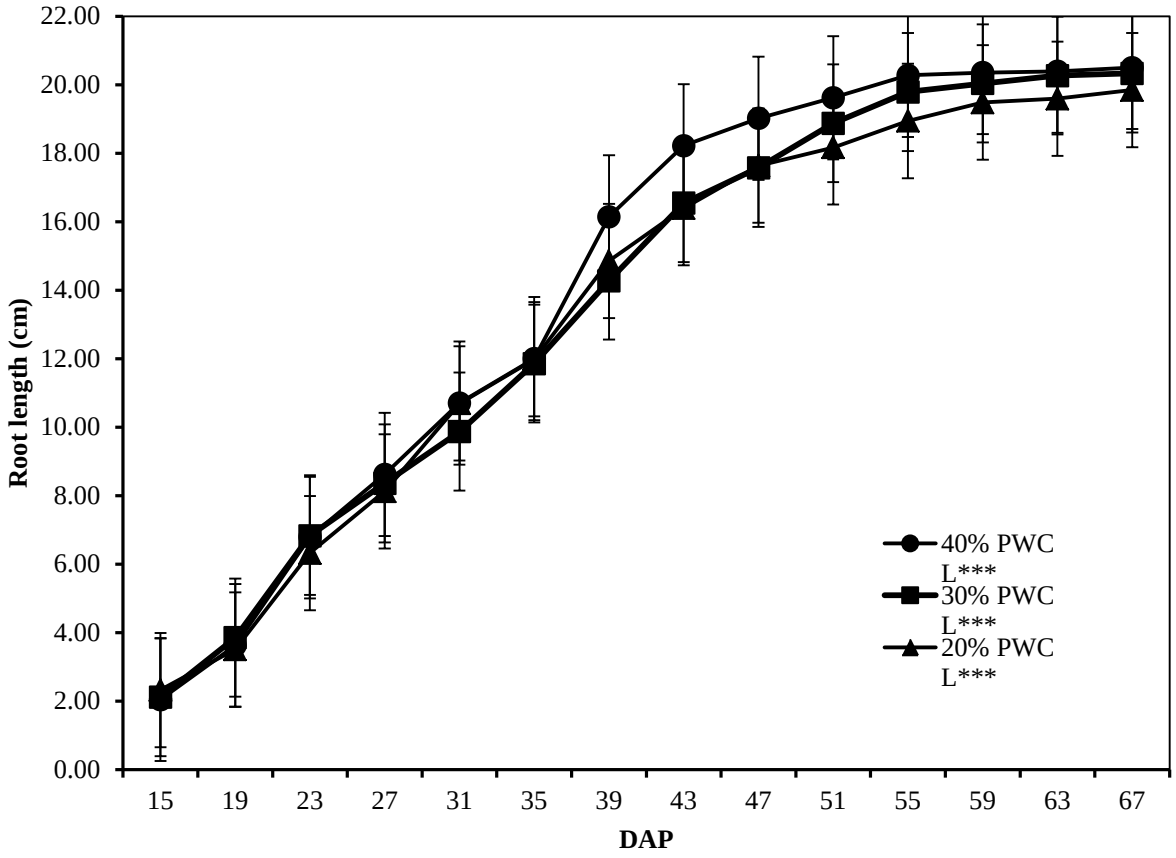

Figure 4. Root length measurements of *Rudbeckia* from 15 to 67 days after planting (DAP) for substrates of 60, 70 and 80% (v/v) peat moss amended with either 40, 30 or 20% PWC, respectively.

Chapter 2

Rhizometer: A New Technique to Observe and Measure Root Growth and Their Effects on Substrate Physical Properties Over Time

(In the format appropriate for submission to HortScience)

Rhizometer: A New Technique to Observe and Measure Root Growth and Their Effects on Substrate Physical Properties Over Time[1]

Lesley A. Judd[2,5], Brian E. Jackson[3] and William C. Fonteno[4]

North Carolina State University, Department of Horticultural Science, 227 Kilgore Hall, Campus Box 7609, Raleigh, NC 27695

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[2]Graduate Research Assistant.
[3]Assistant Professor.
[4]Professor.
[5]To whom reprint requests should be addressed; e-mail lajudd@ncsu.edu

Title: Rhizometer: A New Technique to Observe and Measure Root Growth and Their Effects on Substrate Physical Properties Over Time

Additional index words: air space, container capacity, rhizosphere, pine tree substrate

Abstract. An apparatus (Rhizometer) was developed that allows for the effects of plant roots on physical properties of substrates to be measured over time during crop production in containers. The design of the Rhizometer includes a clear core which allows for measuring a range of root system characteristics in situ. Physical properties of different substrates can be tested with the Rhizometer, and the effect of different root systems (seedlings or plugs) on substrate physical properties can also be observed. The objectives of this study were 1) to design and construct the Rhizometer, 2) test the functionality of the Rhizometer, 3) compare the effects of different plant root types on substrates physical properties over time, 4) compare the effects of different substrates on plant roots and physical properties, and 5) test the ability/ease of measuring roots systems via observation in Rhizometers.

Introduction

Approximately 90% of the $16 billion greenhouse, nursery and floriculture industries are generated from plants produced in containers, excluding food crops grown under cover (U.S. Department of Agriculture, 2009). This industry includes bedding plants, foliage plants, potted flowering plants, potted nursery stock and other floriculture/nursery crops, all grown in a wide variety of container types and sizes, as well as different substrate mixes. Container production has increased dramatically over the last several decades, due to several advantages container production has over traditional field production. With developments in water quality, fertilizers and pesticides, container production became an easier enterprise, especially because it requires less land, ease of handling and harvesting, shipping can occur almost any time of the year, and greater returns per acre than field production (Halcomb et al., 2009). Container production also has advantages to the plants grown in containers. Plants grown in plastic containers have been shown to have a greater fine root mass and less water loss due to not having water capillary movement in soil (Gilman and Beeson, 1996; Harris and Gilman, 1993). With such a large portion of the industry involved with growing plants in containers, it is important to understand the physiology of roots and the factors that influence root growth to attain optimal benefits from container production.

Until the 1960's, almost all greenhouse substrates were soil-based. The most typical mix was equal volumes of loam soil, sphagnum peat moss, and concrete grade sand (Nelson, 2011). Soilless substrates were adopted for many reasons, including; 1) soilless substrates do not need to be pasteurized as soil-based substrates do, 2) soilless substrates are lighter in weight, and therefore reduce shipping and handling costs, and 3) soil can vary greatly from

batch to batch (Nelson, 2011). A substrate must serve four functions in order to support optimal plant growth; 1) serve as a reservoir for plant nutrients; 2) it must hold a reasonable amount of plant available water; 3) must provide for the exchange of gases between roots and the atmosphere outside the substrate, and; 4) it must provide anchorage/support for the plant (Nelson, 2011). Common substrate components used in the industry today are peat moss, pine bark, rice hulls, coir, perlite, and vermiculite.

Plants grown in containers are generally limited by the volume of substrate in which water, gas, and solute availability can fluctuate over a short period of time (Polak and Wallach, 2001). Proper substrates for container production must meet several selection criteria including; salt free, high cation exchange capacity (CEC), suitable physical and chemical properties, supportive, pest free, uniform, available and inexpensive (Mathers et al., 2007). Physical properties of substrates known to affect roots include air space (AS), container capacity (CC), total porosity (TP), percentage of fine particles, and bulk density (BD) (Baligar and Nash, 1978; Mathers et al., 2007). These physical properties are not only important to root growth, but also to cultural practices like decisions on container type/size and irrigation strategy. According to Cannavo et al. (2011), AS, CC and water availability have a considerable impact on plant growth. The pores of a substrate allow for drainage and pores devoid of water allow for gas exchange between the root environment and the outside atmosphere (Bunt, 1988). Chemical properties of substrates, such as pH, CEC, soluble salts, and pesticides can have an immense impact on root growth and function of containers (Mathers et al., 2007). Understanding how chemical properties can influence roots is

important for selecting substrates as well as cultural practices like substrate amendments (e.g. lime) and fertilizer additions.

Chemical properties of substrates may change because roots can influence their surroundings, including pH and nutrient availability, especially micronutrients, by releasing root exudates (e.g. organic acids, amino acids and hydrogen ions) for cation/anion uptake and some exudates form the symbiotic relationship with nitrogen fixing bacteria (Marschner and Romheld, 1996). The first step into the science of how roots influence the surrounding soil was achieved by Lorenz Hiltner (1904), when he gave a lecture and introduced the term "rhizosphere" to describe the zone of soil surrounding roots in relation to nitrogen fixing bacteria (Hartmann et al., 2008; Rovira, 1991).

For container-grown plants, the stability of the substrate's physical properties are of primary concern because changes in these properties may adversely affect plant growth (Allaire-Leung et al., 1999). The influence of root growth on the physical properties of substrates is poorly documented with unconvincing and contradictory results (Cannavo et al., 2011). As plant roots grow into the container substrate, there can be modification of TP, pore size distribution and pore connectivity (Cannavo et al., 2011). According to Allaire-Leung (1999), root growth leads to a decrease in porosity as the roots grow in the gaseous phase of the porosity, i.e., the macroporosity. The diameter of roots also seems to be a good predictor of the effect of mechanical impedance and substrate pore size, as data obtained by Baligar and Nash (1978) and Wiersum (1957) demonstrates that a root is only able to penetrate a pore which has a diameter exceeding that of a young root. Goss (1977) reported results that mechanical impedance caused plants to grow superficial and densely branched

root systems where the roots did not grow past eight centimeters of depth. Other factors may include the size of the container and temperature of the substrate while the roots are growing in it. The AS and CC of a substrate are dependent upon the container depth and width and not completely dependent on the type/mix of substrate (Bilderback and Fonteno, 1987).

Air-filled pores are an important physical property of substrates, because the pores allow for drainage and gas exchange between the root and the outside atmosphere (Bunt, 1988). Various materials/aggregates are used to provide, at least in part, air-filled pore space, with one of the most common being perlite (Bunt, 1988; Evans and Gachukia, 2007). The largest producers of perlite in the world are Greece, U.S. and Japan (Bolen, 2003). Perlite is a relatively expensive substrate component due to the costs associated with mining, transportation, and heating/popping (Evans and Gachukia, 2007). In addition its cost, when perlite is in its dry state, it produces a siliceous dust that is classified as an eye and lung irritant (Evans and Gachukia, 2007; Schundler Co., 2002). Some potential alternative components, such as processed wood (e.g. wood chips), have been researched for their physical and chemical properties. Wood substrates in particular have received a good amount of attention in the recent years, due to their acceptable chemical and physical properties, as well as a noted increase in root growth with plants grown in these substrates. Gruda and Schnitzler (2004) examined spruce (Picea sp.) wood fiber substrates (WFS) and noted particularly well developed root systems of plants grown in the wood fiber substrates compared to plants grown in both peat and rockwool substrate. The physical properties of both a coarser and finer WFS resembled peat substrates with the amount of total pore space; the finer WFS had a high air volume compared to peat, and mixing both WFS and peat

substantially improved the air volume (Gruda and Schnitzler, 2004). Wright and Browder (2005) also noted increase in root growth with plants grown in 75% pine bark: 25% pine chips compared to 100% pine bark. Wright and Browder (2005) used loblolly (*Pinus taeda* L.) pine chips as the substrate component, and there was no apparent shrinkage due to decomposition during the course of their experiment. Jackson et al. (2010) also used loblolly pine chips as the substrate component, and observed the highest root rating for plants grown in all pine tree substrates compared to plants grown in peatlite or pine bark.

There are several ways to measure physical properties of substrates mentioned in scientific literature. Total porosity, CC and AS can be measured with the North Carolina State University (NCSU) Porometer method (Fonteno et al., 1995). The NCSU Porometer method uses aluminum 7.6 cm cores to measure physical properties. Similarly, Altland et al. (2011) reported using 15.2 cm aluminum cores to grow nursery crops in pumice to test the changes in AS, TP and substrate shrinkage over time. In general, Altland et al. (2011) observed over all treatments a decrease in AS, with an increase in CC and TP while BD remained constant over time. Altland et al. (2011) also noted the presence of the plant in the core tended to exacerbate the decrease in AS and the increase in CC, and shrinkage was decreased minimally by the presence of a plant. Nelson et al. (2004) described using greenhouse containers, plugging the holes in the bottom of the container and saturating the substrate in order to measure AS and CC. Results from their study showed AS decreasing and CC increasing during crop time, and no consistent change in BD (Nelson et al., 2004), similar to the observations by Altland et al. (2011).

Based on the work of Altland et al. (2011) and Fonteno et al. (1995), an apparatus was designed (Rhizometer) to allow for both viewing a growing root system and in situ measurements of substrate physical properties. The meaning of the term Rhizometer stems from *rhizo-* meaning rhizosphere, and *–ometer* or *-meter*, from the term porometer and an instrument used in scientific measuring. The rationale of this apparatus was to measure both the physical properties of substrates and the effects of growing roots on substrates, while also having the ability to observe and measure roots in situ. The objectives of this study were 1) to design and construct the Rhizometer, 2) test the functionality of the Rhizometer, 3) compare the effects of different plant root types on substrates physical properties over time, 4) compare the effects of different substrates on plant roots and physical properties, and 5) test the ability/ease of measuring roots systems via observation in Rhizometers.

Materials and Methods

Design of Rhizometer. Clear cylindrical plexiglass tubes [7.6 cm inside diameter (i.d.) x 1.8 m; Plastics and Fiberglass Products, Raleigh, NC] were cut into 7.6 cm tall x 7.6 cm i.d. pieces to make a core the same dimensions as the aluminum NCSU Porometer cores, so that these Rhizometer cores would fit into the base plates and containers used in the NCSU Porometer method. The porometer procedure requires a level core, however watering over time would cause the substrate to settle and/or shrink. Another thought of the design process included the impact of the plug or seed planted in the Rhizometer; in order to not affect the substrate physical properties with pressing a seed/plug in the substrate or having the plug substrate in the part of the Rhizometer used in the porometer method, the

Rhizometer needed to be taller for extra substrate space. The solution to these issues was to include an attachment (collar) which would be affixed on top of the main core (Fig. 1). The collars were removable, and in order to ready the Rhizometers for the porometer method, the collars were removed and the excess substrate removed (including any remaining plug/seed part) and leveled to the top of the 7.6 cm core. These collars were also cut from the plexiglass tubes, 3.8 cm tall x 7.6 i.d., and were then attached to the top of the core with Parafilm M® (American Can Company, Greenwich, CT) when the Rhizometers were assembled. Mesh screen (18 x 16-square mesh, model # 13507; New York Wire, York, PA) was cut in 11.5 cm x 11.5 cm squares in order to fit the bottom of the core and held in place with an adjustable metal hose clamp (MintCraft™ #56, 78-101 mm with 1.3 cm band; Burke Brothers Hardware, Raleigh, NC; Fig. 1). The screen on the bottom of the Rhizometers allowed for irrigation drainage, held the substrate in the Rhizometers, and aided in air pruning of plant roots. The screen was held in place with a detachable metal clamp so it could be removed on the harvest date and the NCSU Porometer base plate could replace it in order to measure the physical properties. Dark colored, decorative pot covers (foil) were placed over the Rhizometers and held in place with rubber bands to restrict light from the root system. The bottom of the foil covers were removed so Rhizometer base was exposed to the air.

Testing the Rhizometer: Experiment 1. On 14 March 2012, marigolds (*Tagetes erecta* 'Inca Orange') seeds (Wyatt-Quarles Seed Co., Garner, NC) were sown into 288 plug trays (1.5 L x 1.5 W x 3.5 H -cm) containing Fafard Germination mix (Fafard, Anderson, South Carolina) in a greenhouse in Raleigh, North Carolina. On 18 May 2012, 60 Rhizometers were filled with a moistened 60:20:20 peat:perlite:vermiculite substrate, which had an initial

pH value of 5.8. The weight of each Rhizometer was measured on a scale and 105 g of substrate was added to each Rhizometer. At time of planting, the substrate had a mass wetness of 1.5 (% wt.), which assures similar packing and substrate volume filled in the Rhizometers, and also prevents potential hydrophobicity or swelling when watered. Each Rhizometer was tapped five times by dropping the filled Rhizometers from a height of 10 cm on a flat surface to achieve similar bulk density in every core, mimicking the porometer packing process. On 18 May 2012, marigold plugs were planted into packed Rhizometers by creating a 2 cm hole in the center of 40 Rhizometers and placing the plug into the hole, thereby barely disturbing the packed nature of the Rhizometer core. The remaining 20 Rhizometers were not planted (fallow) to allow for measurements of undisturbed (i.e. no root growth) physical properties. All Rhizometers were then wrapped with the dark foil to restrict light from the rhizosphere and completely randomized on a greenhouse bench, in Raleigh, NC. All Rhizometers were over-head watered as needed depending on weather conditions, and never showed symptoms of water stress. The fallow Rhizometers were watered as well, so the effects of irrigation and any substrate settling would not influence any differences in physical properties between the planted and fallow Rhizometers. Rhizometers (fallow and planted) were fertilized at each watering with 200 ppm nitrogen (N) injected at 1:100 ratio by Dosatron injector [(D14MZ2); Dosatron International, Inc., Clearwater, FL] with Peters Professional 20-10-20 Peat-Lite Special (The Scotts Co., Marysville, OH) containing 8.1% ammonium (NH_4-N) and 11.9% nitrate (NO_3-N).

Every week after the installation of the experiment, 15 Rhizometers were chosen randomly and removed from the greenhouse, for four weeks. Five planted Rhizometers and

five fallow Rhizometers were prepared for testing in the NCSU Porometer method, as described by Fonteno et al. (1995). For the remaining five planted Rhizometers, the marigolds were harvested at the base of the substrate and all substrate was washed from the root systems, to determine root biomass. This was conducted so that data of root growth mass over time was known and correlated with the changes in substrate physical properties. To prepare the Rhizometer for the porometer method, shoots were severed at the base of the substrate and the collar extension was removed, revealing 1-2 cm of substrate above the 7.6 cm core. This substrate and any roots above the main core were removed such that the substrate within the core was level with the top of the core. The bottom screen was removed, exposing the bottom of the Rhizometer for insertion into the base plate used in the porometer method. Rhizometers were then processed through the NCSU Porometer procedure described by Fonteno et al. (1995) to determine physical properties, including TP, AS and CC. Means separation using least significant difference ($P \leq 0.05$) was used to compare means of fallow versus planted physical properties and root dry mass (SAS Institute version 9.2, Cary NC).

Comparing different root types: Experiment 2. Except where indicated, procedures for Expt. 2 were as described for Expt. 1. On 19 July 2012, 100 Rhizometers were filled with a 60:20:20 peat:perlite:vermiculite substrate, which had an initial pH value of 5.8. For this study, two different plant species were used to examine the change of physical properties with different root types. For fine roots, *Rudbeckia hirta* 'Becky Yellow' (288-tray; C. Raker & Sons, Inc., Litchfield, MI) plugs were selected, and planted in 40 Rhizometers. For larger/tuberous roots, *Begonia* x *hybrida* 'Dragon Wing Red' (128-tray; C. Raker & Sons, Inc., Litchfield, MI) plugs were used, and planted in 40 Rhizometers. The remaining 20

Rhizometers were fallow, to be used in the same manner as Expt. 1. Rhizometers were randomly arranged by species on a greenhouse bench in Raleigh, NC and watered as described in Expt. 1.

Comparing different substrates: Experiment 3. Except where indicated, procedures for Expt. 3 were as described for Expt. 1. Two substrates were used in this study, 75% peat amended with 25% of either pine-wood-chips (PWC) or perlite (PL; v/v). Eight year old loblolly pine trees (*Pinus taeda* L.) were harvested on 4 July, 2011 at ground level and delimbed in Chatham County, NC and subsequently stored under shelter for protected from the weather. These delimbed pine trees were then chipped in a DR Chipper (18 HP DR Power Equipment, model 356447; Vergennes, VT) to produce wood chips. These wood chips were then processed in a hammermill through a 6.35 mm screen [Meadows Mills, North Wilkesboro, NC] to produce a smaller aggregate. The substrates were mixed on 3 Sept. 2012 and both substrates had initial pH tested to determine lime requirements. Both substrates were amended with dolomitic limestone (#200; Mississippi Lime Company, Vicksburg, MS) at 3.85 $kg \cdot m^{-3}$ to bring the pH value to 5.8. On 2 Aug. 2012, marigolds (*Tagetes erecta* 'Inca Orange') seeds (Wyatt-Quarles Seed Co., Garner, NC) were sown into 288 plug trays (1.5 L x 1.5 W x 3.5 H -cm) containing Fafard Germination mix (Fafard, Anderson, South Carolina) in a greenhouse in Raleigh, North Carolina. On 4 Sept. 2012, 60 Rhizometers were filled with the PWC substrate and 60 Rhizometers were filled with the PL substrate. Both substrates had marigold plugs planted into 40 Rhizometers, and the remaining 20 Rhizometers of each substrate were left fallow. All Rhizometers were randomly arranged on a greenhouse bench in Raleigh, NC and watered as described in Expt. 1.

Feasibility of measuring roots: Experiment 4. The clear cylinder design of the Rhizometer allows for visible observations of the substrate sides so that root data collection, including root count, root branching/architecture, quantifying root hairs, etc. can be quantified without disturbance. The purpose of this study was to test the effectiveness of the Rhizometer design on root data collection and provide evidence of the ability to measure several root system parameters in the Rhizometer. Three substrates were compared in this study, 80% (v/v) peat amended with 20% of either PWC, PL or shredded-pine-wood (SW; v/v). The process of PWC is the same as listed in Expt. 3. Delimbed loblolly pine trees were shredded through a Wood Hog shredder (Morbark® model 3800; Winn, MI) and on 18 Jan. 2013, the shredded-pine-wood was processed in a hammermill through a 6.35 mm screen (Meadows Mills, North Wilkesboro, NC) yield the fiberous SW end product. On 19 Jan. 2013, the substrates were blended and initial pH values were taken to determine lime requirements. All three substrates were amended with dolomitic limestone (#200; Mississippi Lime Company, Vicksburg, MS) at 5.04 $kg \cdot m^{-3}$ in order to reach a pH of 5.8. All substrates were moistened to a moisture content of 2.5 (% wt.) for consistency in packing the Rhizometer. On 20 Jan. 2013, a total of 60 Rhizometers were filled; 20 with each individual substrate. Four species of seeds were planted directly into the Rhizometers; bean (*Phaseolus vugaris* 'Gold Rush') and corn (*Zea mays* 'Jubilee') seeds (Livingston Seed Co., Columbus, OH) were planted at a depth of 2 cm; and tomato (*Solanum lycopersicum* L. 'Better Boy') and marigolds (*Tagetes erecta* 'Inca Orange') seeds (Wyatt-Quarles Seed Co., Garner, NC) were planted at a depth of 1 cm. These species were chosen for their fast germination rate and variability in root types. Planted Rhizometers were randomly placed by species on a

greenhouse bench in Raleigh, NC. Rhizometers were watered as needed without fertilizer until germination occurred, then the Rhizometers were fertigated as described in Expt. 1.

From date of emergence for each seedling species, three root measurements were taken every four days for one and a half weeks. Visible root measurements taken include; 1) number of root tips (RT), 2) number of roots with visible root hairs (RH) and 3) accumulative root length (RL) of Rhizometer surface (root coverage). Number of root tips and roots with hairs were measured by counting the visible roots against the clear cylinder of the Rhizometers. A transparency (27.9 cm x 7.6 cm transparency film; 3M Visual Systems Division, Austin, TX) was cut to the dimensions of the Rhizometer, wrapped around and held in place with a rubber band. Cumulative root length could then be measured on every Rhizometer by tracing the roots on the transparency sheet, taking a digital photograph, and uploading the image to root reading software (RootReader 2D version 4.3.1; Cornell University, USDA-ARS, Ithaca, NY). The RootReader 2D software selected the traced roots and measured total root length of the entire picture, providing this data. The tracing on the transparency sheet was then erased and the sheet was used for the next tracing. Data were analyzed using least significant difference ($P \leq 0.05$) (SAS Institute version 9.2, Cary, NC).

Results and Discussion

Testing the Rhizometer: Experiment 1. In comparing the physical properties between planted and fallow Rhizometers, CC was not significantly different over time (Table 1). A linear decrease was observed with AS over time for both the planted and fallow Rhizometers. At the start of the study, planted Rhizometers had a lower AS than the fallow Rhizometers,

and this trend was observed to the end of the study, except at 21 DAP when AS was not significant between planted and fallow Rhizometers (Table 1). A linear decrease was also observed in TP for both planted and fallow Rhizometers (Table 1). Planted Rhizometers had a lower TP, compared to the fallow Rhizometers, from the beginning of the study, continuing to the end of the study, which can be attributed to the decrease in AS. Nelson et al. (2004) reported a similar pattern in a study with peat and coir substrates where settling caused a decrease in AS and an increase in CC was observed, and this was thought to be the conversion of large pores (i.e. AS) into smaller pores for holding water (i.e. CC). Marigold root growth in the planted Rhizometers linearly increased over time; and the change in AS, and therefore TP, may be explained with the presence of the roots in the Rhizometers. Several other authors have reported decrease in AS over time with peat-based substrates with the presence of a plant (Aendekerk, 1997; Allaire-Leung et al., 1999; Nelson et al., 2004). As these authors noted, there is a decrease in AS due to decomposition of the peat-based substrates (Aendekerk, 1997; Allaire-Leung et al., 1999; Nelson et al., 2004), however the lower AS observed in this study shows that the presence of roots further decreases AS compared to the decrease in AS of the fallow Rhizometers, which Altland et al. (2011) also reported. As the roots grew, it is possible that they filled the pore space therefore causing a decrease in substrate AS. Altland et al. (2011) reasoned that as roots explore the substrate and displace some of the pore spaces, AS is expected to decrease over time.

Testing differences in roots: Experiment 2. The fallow Rhizometers show a linear increase in CC over time (Table 2). However, both AS and TP had no significant response over time (Table 2). The Rhizometers planted with ‘Dragon Wing Red’ *Begonia* had both

decreasing CC and AS over time, therefore affecting a decrease in TP over time (Table 2). Container capacity was not significantly different across the *Begonia* Rhizometers, *Rudbeckia* Rhizometers and fallow Rhizometers at every harvest date except the first harvest, when fallow Rhizometers had a lower CC than the *Begonia* Rhizometers (Table 2). *Rudbeckia* Rhizometers had no change in CC, AS and TP, leading to the conclusion that different plant species will effect substrate physical properties in different ways. Increasing root growth of *Begonia* lead to a decrease in both CC and AS, whereas increasing root growth of *Rudbeckia* did not change the physical properties (Table 2). The root dry mass of *Begonia* was larger at every harvest date compared the *Rudbeckia* root dry mass (Table 2).

Testing substrates: Experiment 3. The planted Rhizometers in the PL substrate had an increase in CC over time, with a significant quadratic response (Table 3). Air space of the PL substrate in the planted Rhizometers had no significant linear/quadratic response, but at the end of the study AS was lower compared to the beginning (11.3 % compared to 13%, respectively) (Table 3). The fallow Rhizometers had no significant response in CC and TP over time (Table 3). A linear response (decrease) was observed in AS over time in the PL substrate in the fallow Rhizometers (Table 3). The planted Rhizometers in PL substrate showed no change in TP (Table 3).

There was no change observed over time in CC, AS and TP for planted Rhizometers with PWC substrates (Table 3). The fallow Rhizometers with PWC substrate show no change over time in CC and AS, with an increasing linear response in TP (Table 3). Comparing 7 DAP to 28 DAP between the substrates; planted Rhizometers in the PL substrate had an increased change of 3.5% in CC and a decreased change of 1.7% in AS;

compared to the planted Rhizometers in the PWC susbtrate that had an increased change of 3.1% in CC and a decreased change of 2.1% in AS. The fallow Rhizometers in the PL substrate had an increased change of 2.8% in CC and a decreased change of 4.4% in AS; and the fallow Rhizometers in the PWC substrate had an increased change of 2.6% in CC and a decreased change of 0.7% in AS. Similar to Expt. 1, this could be explained by settling causing the large pores to become smaller, water-holding pores and rearrangement of substrate particles as roots grow into the substrate. Root growth between the PL and PWC substrate was not significantly different until 28 DAP when the marigold roots in PWC substrate had a larger dry mass than the roots in PL substrate (Table 3).

Root measurements of Rhizometer: Experiment 4. The marigold seeds were the first to emerge, however there was no visible root growth against the clear Rhizometer for any of the three substrates until 12 days after emergence (DE). Corn and bean seeds were emerged next, and root growth was visible against the Rhizometer after 4 DE. The tomato seeds were the last to emerge, and root growth was visible against the Rhizometer starting at 8 DE. The three root measurements discussed above were immediately taken on both the corn and bean Rhizometers, starting at 4 DE until 16 DE. At 16 DE, most of the visible area of the Rhizometers were covered with roots and it was decided that further measurements were not needed.

The corn seedlings grown in the Rhizometers had significant differences among the substrates for both the RT and RH measurements at 12 DE. There were more corn root tips and roots with root hairs when grown in the SW substrate than the corn grown in the PWC substrate (Table 4). There were no significant differences observed across the three substrates

for the other species (Table 4). Number of root tips, RH and RL measurements had linear responses over time for all four species (Table 4). At 12 DE, corn had the longest total root length in the SW substrate, whereas on the same day bean had the lowest total root length in the SW substrate (Table 4). Tomato had a higher total root length in PWC substrate on 12 DE, whereas marigold had the lowest total root length in PWC substrate. The rest of the measurement dates showed no difference in RL between the substrates (Table 4).

Conclusion

The above studies provide evidence that the Rhizometer has the ability to measure physical properties of substrate either with or without a plant growing in the substrate over time. The Rhizometer can also measure the effects of different plant root types on substrate physical properties, and showed that different plant roots affect the substrate differently. The Rhizometers can easily be filled with different substrates, enabling a wide variety of physical properties to be tested. The clear cylinder of the Rhizometer aids in the range of functionality, and allows for many different root observations and measurements to be made.

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Table 1. Physical properties over four weeks with or without the presence of 'Inca Orange' marigold plants growing in 60:20:20 peat:perlite:vermiculite substrate and root dry weight data for marigold grown in 2012.

		Physical Properties[z]			
		Container capacity[y]	Air space[x]	Total porosity[w]	
Plant	DAP[v]	(% vol)			Root dry mass[u] (g)
Marigold	7	74.1 a[t]	16.6 b	90.7 b	0.10 b[r]
	14	77.2 a	14.6 b	91.8 b	0.22 b
	21	74.8 a	15.1 a	89.9 b	0.69 a
	28	75.6 a	13.2 b	88.8 b	0.95 a
		NS[s]	L**, Q**	L*, Q**	L***, Q***
None	7	73.7 a	20.0 a	93.7 a	
	14	74.6 a	18.4 a	93.0 a	
	21	75.4 a	16.6 a	92.0 a	
	28	75.0 a	16.8 a	91.8 a	
		NS	L**, Q**	L**, Q**	
Plant		NS	***	***	
Root dry mass		NS	*	**	
Interaction		NS	*	**	

[z]Physical properties data were collected from five samples represented as means. Analysis performed using the North Carolina State University Porometer method (Fonteno et al., 1995).
[y]Container capacity is (wet weight – oven dry weight) ÷ volume of the sample.
[x]Air space is the volume of water drained from the sample ÷ volume of the sample.
[w]Total porosity = container capacity + air space.
[v]DAP is days after planting.
[u]Root dry mass from washed root systems and oven dried.
[t]Means separated between planted and fallow by DAP using Tukey's significant difference, $P \leq 0.05$. Means followed by the same letter are not significantly different.
[s]NS, L, and Q represent no significant response, linear, and quadratic response, respectively, of individual physical properties over time, *, **,*** represent significant effects when $P \leq 0.05$, 0.01, and 0.001, respectively.
[r]Means separated within column using Tukey's significant difference, $P \leq 0.05$. Means followed by the same letter are not significantly different.

Table 2. Physical properties of 60:20:20 peat:perlite:vermiculite substrate with or without either 'Dragon Wing Red' begonia or 'Becky Yellow' *Rudbeckia* growing in the Rhizometer and root dry weight data over four weeks.

		Physical Properties[z]			
		Container capacity[y]	Air space[x]	Total porosity[w]	
Plant	DAP[v]	(% vol)			Root dry mass[u] (g)
Begonia	7	82.1 a[t]	8.0 b	90.1 a	0.51 a[r]
	14	80.0 a	9.9 a	89.9 a	0.49 a
	21	78.0 a	5.4 b	83.4 b	1.10 a
	28	76.9 a	5.9 b	82.8 b	1.20 a
		L***, Q***[s]	L*, Q*	L***, Q***	L***, Q***
Rudbeckia	7	79.9 ab	11.2 ab	91.1 a	0.11 b
	14	79.3 a	12.9 a	92.2 a	0.08 b
	21	79.6 a	8.7 ab	88.3 ab	0.39 b
	28	79.2 a	11.2 a	90.4 a	0.58 b
		NS	NS	NS	L***, Q***
None	7	76.6 b	14.3 a	90.9 a	
	14	79.0 a	12.0 a	91.0 a	
	21	79.1 a	11.0 a	90.1 a	
	28	79.0 a	15.1 a	94.1 a	
		L*	NS	NS	
Plant interaction		NS	***	***	
Root dry mass-*Rudbeckia*		NS	NS	*	
Root dry mass-*Begonia*		**	**	***	

[z]Physical properties data were collected from five samples represented as means. Analysis performed using the North Carolina State University Porometer method (Fonteno et al., 1995).

[y]Container capacity is (wet weight – oven dry weight) ÷ volume of the sample.

[x]Air space is the volume of water drained from the sample ÷ volume of the sample.

[w]Total porosity = container capacity + air space.

[v]DAP is days after planting.

[u]Root dry mass from washed root systems and oven dried.

[t]Means separated among planted and fallow by DAP using Tukey's significant difference, $P \leq 0.05$. Means followed by the same letter are not significantly different.

[s]NS, L, and Q represent no significant response, linear, and quadratic response, respectively, of individual physical properties over time, *, **,*** represent significant effects when $P \leq 0.05$, 0.01, and 0.001, respectively.

[r]Means separated within column using Tukey's significant difference, $P \leq 0.05$. Means followed by the same letter are not significantly different.

Table 3. Expt. 3 physical properties of perlite (PL) and pine-wood-chip (PWC) substrates with or without 'Inca Orange' marigold growing in the core and root growth data for marigold grown in 2012.

			Physical Properties[z]			
			Container capacity[y]	Air space[x]	Total porosity[w]	
Substrate	Plant	DAP[v]	(% vol)			Root dry mass[u] (g)
80:20 peat:PL[t]	Marigold	7	68.6 a[r]	13.0 b	81.6 a	0.05 a[q]
		14	67.6 a	16.0 a	83.6 a	0.12 a
		21	69.9 a	14.2 a	84.0 a	0.38 a
		28	72.1 a	11.3 a	83.3 a	0.89 b
			L*, Q**[p]	NS	NS	L***, Q***
	None	7	65.4 b	18.4 a	83.9 a	
		14	66.1 a	16.0 a	82.1 a	
		21	69.1 a	15.8 a	83.9 a	
		28	68.2 a	14.0 a	82.2 a	
			NS	L*, Q*	NS	
80:20 peat:PWC[s]	Marigold	7	73.9 a	11.3 b	85.2 a	0.07 a
		14	77.4 a	11.0 a	88.4 a	0.20 a
		21	77.4 a	11.6 a	89.0 a	0.54 a
		28	77.0 a	9.2 b	86.2 b	1.30 a
			NS	NS	NS	L***, Q***
	None	7	72.6 a	14.3 a	86.9 a	
		14	74.3 b	12.5 a	86.8 a	
		21	74.6 a	14.1 a	88.7 a	
		28	75.2 a	13.6 a	88.8 a	
			NS	NS	L**, Q**	
Plant			***	***	NS	
Substrate			***	***	***	
Interaction			NS	NS	NS	

[z]Physical properties data were collected from five samples represented as means. Analysis performed using the NCSU Porometer method (Fonteno et al., 1995).
[y]Container capacity is (wet weight – oven dry weight) ÷ volume of the sample.
[x]Air space is the volume of water drained from the sample ÷ volume of the sample.
[w]Total porosity = container capacity + air space.
[v]DAP is days after planting.
[u]Root dry mass from washed root systems and oven dried.
[t]Substrate 80:20 peat:perlite (v/v).
[s]Substrate 80:20 peat:pine-wood-chips (v/v).
[r]Means separated by substrate and DAP using Tukey's significant difference, $P \leq 0.05$. Means followed by the same letter are not significantly different.
[q]Means separated by DAP using Tukey's significant difference, $P \leq 0.05$. Means followed by the same letter are not significantly different.
[p]NS, L, and Q represent no significant response, linear, and quadratic response, respectively, of individual physical properties over time, *, **,*** represent significant effects when $P \leq 0.05$, 0.01, and 0.001, respectively.

Table 4. Expt. 4 root system measurements of Rhizometers planted with four plant species in three different substrates with measurements taken every four days after date of emergence (DE) of the seedlings.

		Days after emergence												Significance		
		4			8			12			16					
Plant[r]	Substrate	RT[z]	RH[y]	RL[x]	RT	RH	RL	RT	RH	RL	RT	RH	RL	RT	RH	RL
corn	PL[w]	6.0 a[t]	3.2 a	2.1 a	29.2 a	21.6 a	47.7 a	116.0 ab	104.0 ab	126.5 b	174.6 a	136.6 a	257.4 a	L***[s]	L***	L***
	PWC[v]	2.2 a	1.2 a	9.1 a	20.4 a	15.6 a	36.5 a	97.0 b	88.4 b	140.3 b	161.4 a	128.2 a	292.4 a	L***	L***	L***
	SW[u]	7.0 a	5.2 a	13.3 a	35.2 a	27.4 a	78.8 a	145.0 a	133.4 a	213.7 a	179.0 a	153.4 a	335.8 a	L***	L***	L***
bean	PL	7.2 a	5.6 a	18.0 a	48.8 a	19.6 a	101.3 a	160.4 a	134.8 a	280.3 a	270.2 a	238.0 a	369.6 a	L***	L***	L***
	PWC	8.2 a	5.6 a	13.9 a	76.6 a	25.0 a	158.7 a	176.2 a	149.6 a	249.7 a	284.8 a	220.8 a	320.2 a	L***	L***	L***
	SW	5.0 a	3.5 b	6.5 a	64.3 a	21.0 a	119.4 a	166.0 a	145.5 a	243.4 a	278.5 a	242.3 a	296.9 b	L***	L***	L***
		Days after emergence														
		8			12			16			20					
tomato	PL	0.3 a	0.3 a	0.3 a	6.5 a	6.0 a	6.6 b	9.0 a	7.25 a	12.2 a	58.0 a	47.0 a	75.3 a	L**	L**	L***
	PWC	1.6 a	1.4 a	3.7 a	10.0 a	10.0 a	19.0 a	15.2 a	13.6 a	21.5 a	88.2 a	79.0 a	71.3 a	L***	L***	L***
	SW	0.6 a	0.6 a	1.5 a	4.6 a	4.4 a	6.0 b	8.8 a	6.8 a	9.8 a	58.8 a	47.6 a	46.3 a	L***	L***	L***
		Days after emergence														
		12			16			20			24					
marigold	PL	4.0 a	3.6 a	12.0 a	12.6 a	11.0 a	26.0 a	27.2 a	22.2 a	49.1 a	48.2 a	33.6 a	100.1 a	L***	L***	L***
	PWC	2.0 a	1.4 a	2.8 b	12.2 a	12.0 a	19.6 a	28.8 a	22.8 a	40.7 a	52.8 a	39.8 a	93.3 a	L***	L***	L***
	SW	3.0 a	0.6 a	6.9 a	14.3 a	13.8 a	26.8 a	35.8 a	29.8 a	49.1 a	60.0 a	46.3 a	114.0 a	L***	L***	L***

[z]RT is number of root tips visible inside the Rhizometer.
[y]RH is number of roots with roots hairs visible on any point along the root inside the Rhizometer.
[x]RL is total root length (cm) = the sum of the lengths of all visible roots, calculated by RootReader 2D (Cornell).
[w]PL substrate is 80:20 peat:perlite (v/v).
[v]PWC substrate is 80:20 peat:pine-wood-chip (v/v).
[u]SW substrate is 80:20 peat:shredded-pine-wood (v/v).
[t]Means separated across substrates for each plant species using Tukey's significant difference, $P \leq 0.10$. Means followed by the same letter are not significantly different.
[s]NS, L, and Q represent no significant response, linear, and quadratic response, respectively, *, **,*** represent significant effects when $P \leq 0.05$, 0.01, and 0.001, respectively.
[r]Plant species includes: corn (*Zea mays* 'Jubilee'), bean (*Phaseolus vugaris* 'Gold Rush'), tomato (*Solanum lycopersicum* L. 'Better Boy') and marigold (*Tagetes erecta* 'Inca Orange')

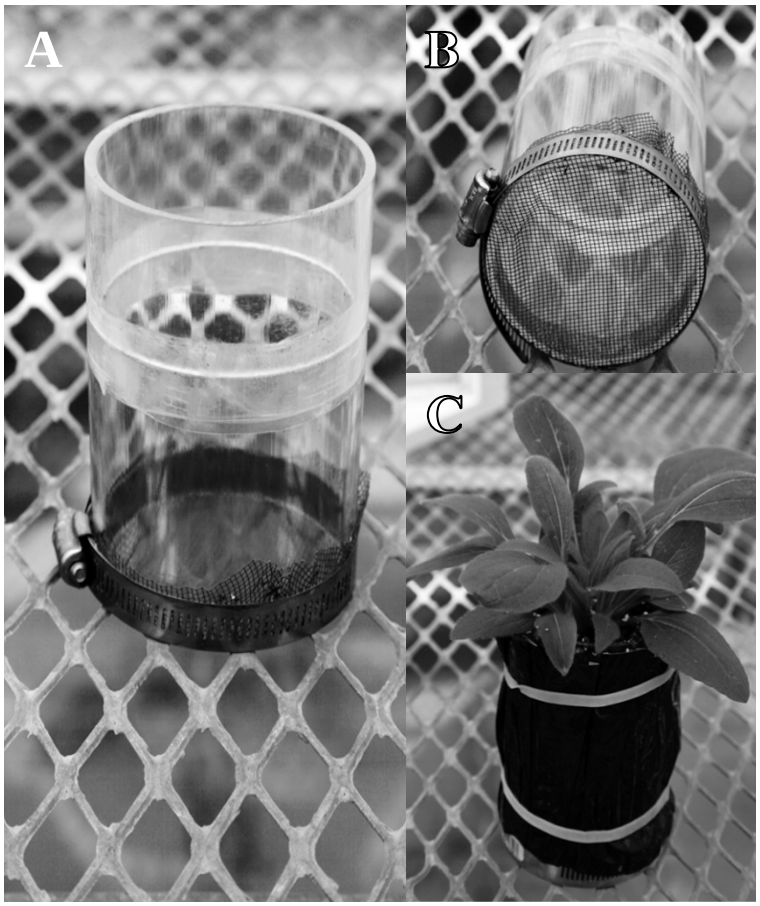

Figure 1. A) Design of Rhizometer illustrating the clear sided plexiglass allowing for root observations and measurements, B) the screened bottom and C) a complete planted Rhizometer.

Chapter 3

Substrate Influence on Root Hydraulic Conductance of Herbaceous and Woody Plants

(In the format appropriate for submission to HortScience)

Substrate Influence on Root Hydraulic Conductance of Herbaceous and Woody Plants [10]

Lesley A. Judd[11,6], Brian E. Jackson[12], William C. Fonteno[13]

North Carolina State University, Department of Horticultural Science, 227 Kilgore Hall, Campus Box 7609, Raleigh, NC 27695

J.-C. Domec[14]

North Carolina State University, Department of Forestry and Environmental Resources, Biltmore Hall, Campus Box 8001, Raleigh, NC 27695

[10]Received for publication _____. From a thesis submitted by L.A.J. in partial fulfillment of the requirements for the M.S. degree.
[11]Graduate Research Assistant.
[12]Assistant Professor.
[13]Professor.
[5]Research Associate Professor.
[6]To whom reprint requests should be addressed; e-mail brian_jackson@ncsu.edu

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Abstract. A Hydraulic Conductance Flow Meter (HCFM) was used to measure root hydraulic conductivities of herbaceous and woody plant roots. The herbaceous plants were grown in peat amended with different pine tree substrates. The woody plants were grown in pine bark amended with pine tree substrate. The objectives of this study were; 1) To test the ability of the HCFM to measure root conductivities on small, herbaceous and woody crops in containers, 2) measure root conductivities of plants in peat and pine tree substrates and compare to dry root mass to find the effects of these substrates on root conductance, and 3) measure root conductivities of plants in pine bark and pine tree substrates and compare to dry root mass to find the effects of these substrates on root conductance.

Introduction

A large portion of the U.S. green industry is involved with growing plants in containers, including bedding plants, vegetable plants, foliage plants, potted flowering plants, potted nursery stock and other assorted floriculture crops. Root growth of crops grown in containers is a central element in overall plant performance, whether it is during production (e.g. propagation) or post-production (e.g. transplant success). Container production has increased substantially over the last several decades, due to advantages container production has over traditional field production and the gradual shift from using soil in containers to soilless substrates. Container production is an easier enterprise, because it requires less land, ease of harvesting/handling and greater returns per acre than field production (Halcomb et al., 2009). Soilless substrates were adopted due to several factors; 1) soilless substrates do not need to be pasteurized as soil-based substrates do; 2) soilless substrates are lighter in weight, and therefore reduce shipping and handling costs; and 3) soil can vary greatly from batch to batch (Nelson, 2011). Also, plants grown in containers have been shown to have a greater fine root mass and better post-transplant success compared to field-grown plants (Gilman and Beeson, 1996; Harris and Gilman, 1993). Considering the large portion of the horticulture industry involved with growing plants in containers and the importance of understanding the physiology and morphology of roots, the factors that influence root growth in container production need to be investigated.

Substrates can have a significant influence on root growth and development. Substrates include materials such as; peat, pine bark, perlite vermiculite and others. Horticultural crops grown in these mixes demand a stable rooting environment (Verhagen,

2007). The physical aspects of a good root environment are structural stability, water capacity, air capacity, bulk density and wettability. Various materials/aggregates are used to provide, at least in part, air-filled pore space, with one of the most common aggregates for greenhouse substrates being perlite (Bunt, 1988; Evans and Gachukia, 2007). However, perlite is a relatively expensive substrate component due to the costs associated with mining, transportation, and heating/popping (Evans and Gachukia, 2007). In addition to its cost, when perlite is in its dry state, it produces a siliceous dust that is classified as an eye and lung irritant (Evans and Gachukia, 2007; Schundler Co., 2002). In the recent years, peat has also increased in transportation costs and in some areas, its availability is threatened due to weather during the harvest. When costs of peat started to rise in the late 1970's, growers sought less costly substitutes for greenhouse and nursery substrates (Pokorny, 1979). This is when bark was evaluated for its physical and chemical properties as a substitute. Now, the primary components of nursery substrates, especially in the southern states of the U.S., are hardwood and softwood bark. The advantages of using bark are; it is a renewable resource, can be processed by hammer-milling and screening to provide a standardized product, and has a resistance to excessive or rapid decay (Pokorny, 1979). Unfortunately, supplies of pine bark in many areas across the southeastern states have been erratic (Jackson et al., 2009). The increasing use of pine bark as a fuel source and the decrease in harvesting pine trees for the paper and timber industry have reduced the availability and raised the costs of pine bark. Recently, alternative for peat, perlite and pine bark are being sought and evaluated.

Some potential alternative components to peat, perlite and pine bark, such as processed wood (e.g. wood chips), have been researched for their physical and chemical

properties. Wood substrates have received a good amount of attention in the recent years, due to their chemical and physical properties, as well as a noted increase in root growth with plants grown in the pine tree substrates. Gruda and Schnitzler (2004) examined spruce (Picea sp.) wood fiber substrates (WFS) and noted particularly well developed root systems of plants grown in the wood fiber substrates compared to plants grown in both peat and rockwool substrate. The physical properties of both a coarser and finer WFS resembled peat substrates with the amount of total pore space; the finer WFS had a high air volume compared to peat, and mixing both WFS and peat substantially improved the air volume (Gruda and Schnitzler, 2004). Jackson et al. (2010) also used loblolly pine chips as the substrate component, and observed the highest root rating for plants grown in pine tree substrates compared to plants grown in peatlite (80:20 peat:perlite) or pine bark. For nursery/woody substrate, Wright and Browder (2005) noted an increase in root growth with plants grown in 75% pine bark: 25% pine chips compared to 100% pine bark, using loblolly (*Pinus taeda* L.) pine chips as the substrate component. Wright et al. (2006) reported that pine-wood-chips, with adjustments to fertility, are a suitable substrate for container production of woody ornamental plants. Jackson et al. (2009) described decomposition in pine tree substrate (PTS); however the decomposition was similar between both the PTS and pine bark substrate with plants grown in the containers, and fallow containers with PTS had higher shrinkage than container-grown plants in PTS. Substrate air space was highest in PTS and container capacity was equal in pine bark and PTS at potting, and over time both air space and container capacity remained in acceptable ranges for container substrates (Jackson et al., 2009). Jackson et al. (2009) provided data showing PTS had substrate shrinkage

similar to pine bark substrate, therefore PTS had similar performance as pine bark substrate. With data supporting that an acceptable alternative to peat, perlite and pine bark, is pine-wood-chips; and the increased root growth noticed in pine tree substrates in several of these studies, more work needs to be done to determine the impact of these substrates on root growth.

One of the essential functions of roots is to supply the plant with water from the surrounding environment (soil or substrate). It is well known that the hydraulic properties of roots vary with species and environmental conditions (Miyamoto et al., 2001). These environmental factors can strongly influence root morphology and anatomy (Steudle and Peterson, 1998). When the plant is experiencing high transpiration, the force driving water across the root will be the hydrostatic pressure difference between the root medium (substrate) and the root xylem (Steudle, 2000). When transpiration stops, the cell-to-cell passage, or the symplastic and apoplastic pathways, is left to transport water and has a high hydraulic resistance (Steudle, 2000). Water movement from the soil solution into the root xylem and then up into the shoot can be treated as fluid flow through a complex structure with variable hydraulic resistances, such as the different tissues in the root cylinder or the different cellular pathways for water (Steudle and Peterson, 1998). Hydraulic resistances of the root will also be great when the surrounding environment is dry and may help to limit water stress (Miyamoto et al., 2001). Research completed by Ramos and Kauffmann (1979) observed an increase in hydraulic root resistance with the seedlings that did not receive enough water (water stressed). The observed increase in hydraulic resistance did not result from decreased soil-to-root contact, but may have resulted from suberization of the cortical

cell walls in the root or from a change in membrane permeability (Ramos and Kauffmann, 1979). Also, an analysis of the variance of root length showed no significant effect due to water stress. Reiger and Litvin (1999) noticed in their experiment that root system hydraulic conductivity decreased with increasing root diameter and cortex width, resulting in the belief that more dense tissue resists water movement.

Roots impose the greatest resistance to water flow in the soil-plant-atmosphere continuum (SPAC), thus their hydraulic conductivity has been the subject of numerous studies (Rieger and Litvin, 1999). Hydraulic resistance is the reciprocal of hydraulic conductance (Tsuda and Tyree, 2000). Hydraulic conductivity can be measured for both roots and shoots, and are important parameters to the model of the SPAC, that will help predict the rate of water flow through whole plants and to predict the water potential of various plant organs (Tyree et al., 1994). The usual method to estimate root and whole plant hydraulic conductance, known as the evaporative flux method, required a measurement of soil water potential from predawn leaf water potential; a midday leaf water potential; and midday evaporative flux density, which is usually estimated from stem water flow measurements divided by leaf area (Tsuda and Tyree, 2000; Tyree et al., 1994). These measurements required a number of devices, such as a pressure chamber to measure leaf water potential. Measurements of root and whole plant hydraulic conductance are usually subject to a number of sources of error, especially with the stem flow measurements that usually have a range of accuracy of 10 to 20% (Tyree et al., 1994).

With advancing technology, new apparatuses have been developed to measure hydraulic conductivity/resistance with rapid water-flow measurements. Two such devices

are; the high-pressure flow meter and the hydraulic conductance flow meter (Dynamax, Inc., Houston, TX). The hydraulic conductance flow meter (HCFM), preforms the same measurements as the high pressure flow meter (HPFM), but the HCFM is generally used on smaller stock while the HPFM is used on large caliber plants. Both the HCFM and the HPFM measure the hydraulic conductance of shoot and root systems. Shoots are excised from the root system a few cm above substrate level and the rootstock or shoot stem is fitted with water filled tubing of the HCFM. The HCFM uses constantly increasing pressure to cause water to flow into the root or shoot system (whichever the HCFM is attached to) and this water flows in the opposite direction of normal transpiration (Tyree et al., 1995). The pressure measurement versus the flow measurement is used to estimate root/shoot conductance from the slope. Tsuda and Tyree (2000) experimented with the HPFM, showing that HPFM method and the conventional evaporative flux method yield consistent values of plant hydraulic conductance under quasi-steady-state conditions. Tsuda and Tyree (2000) also note that the HPFM method is much faster and permits the determination of whole-shoot conductance. These hydraulic conductance values, which are reflective of root development, are believed to show differences/effects of substrate on the root system. The objectives of this study were; 1) To test the ability of the HCFM to measure root conductivities on small, herbaceous and woody crops in containers, 2) measure root conductivities of plants in peat and pine tree substrates and compare to dry root mass to find the effects of these substrates on root conductance, and 3) measure root conductivities of plants in pine bark and pine tree substrates and compare to dry root mass to find the effects of these substrates on root conductance.

Materials and Methods

Hydraulic conductance flow meter (HCFM) operation. The HCFM is an apparatus designed to inject water into the base of either a root system, shoot system or leaf stem while rapidly changing the delivery pressure and simultaneously measuring the flow of water. The rapid change in water pressure was achieved using the pressure regulator (R), a needle valve (NV), and a captive air tank (CAT; Fig. 1). A pressure regulator delivered compressed air at a pressure of 4-5 MPa through a needle valve. A rubber diaphragm separated the air from water in the CAT. A pressure release valve (PR) prevented accidental over-pressurization, due to the PR limit set to vent air when the pressure exceeds about 0.6 MPa. Since the pressure in the CAT never exceeds 0.6 MPa, and the air supply is 4.2 MPa, the pressure drop across the NV is approximately constant. This allows the rate of pressurization in the water to be approximately linear with time. Pressurized water was delivered from the CAT to an 8-way manifold by way of 9 mm inside diameter (ID) nylon-reinforced Tygon tubing. At a distance of 0.3 m from the CAT the water passes through a 0.1 micron water filter, then the diameter of the tubing is reduced to 3 mm outside diameter (OD) plastic tube; the plastic tubing is connected to the input side of the 8-way manifold (8WI; Fig. 1). The 8WI is an valve of octagonal geometry with 8-tubes emerging from a common point in the center and each tube terminated by a valve. On the inlet side, the tube from the CAT is connected to one of the 8 valves. A pressure transducer (PT1) is connected to another valve. On the outlet side another pressure transducer (PT2) is connected to 8WI as well as the outlet tube. During a measurement, one valve of the 8-way manifold on the outlet side (8WO) is selected by opening the outlet valve and water can then flow through the selected valve, and the

differential pressure between the 8WI and the 8WO is measured with PT1 and PT2, respectively. The compression fitting (CF) placed on the stem of a root or shoot is a 1 mm ID HPLC tube to be used on smaller sized roots and shoots with 1-20 mm diameter. The HCFM is supplied with Windows software for controlling the A/D circuits, logging data, and for preliminary data analysis.

Pine-wood-chips substrate: Experiment 1. Six substrates were used in this study; peat amended with either 10, 20 or 30%, respectively, perlite (PL) or pine-wood-chips (PWC; v/v). Eight-year-old loblolly pine trees were harvested on 19 Dec. 2011 at ground level and delimbed in Chatham County, NC and stored under shelter from weather. On 2 Jan. 2012 the delimbed pine logs were chipped in a DR Chipper (18 HP DR Power Equipment, model 356447; Vergennes, VT) to produce small wood chips. The wood chips were then processed in a hammermill through a 6.35 mm screen (Meadows Mills, North Wilkesboro, NC) to produce PWC. Pine-wood-chips were selected for this study because it has properties similar to perlite and has been shown to have potential as an alternative to perlite. The substrates were mixed on 2 July 2012; all substrates were tested for initial pH and then amended with dolomitic limestone (#200; Mississippi Lime Company, Vicksburg, MS) at 3.86 $kg \cdot m^3$ to achieve a desired pH of 5.8. On 4 July 2012, five greenhouse containers (12.7 cm dia; Dillen Products, Middlefield, OH) were filled with each individual substrate to the top of the container and tapped three times, by lifting the containers 10 cm from a hard surface and dropping, to settle the substrate. On 28 May 2012, Coleus seeds (*Solenostemon* 'Giant Exhibition'; Fred C. Gloeckner Co., Harrison, NY) were sown into a 288 plug tray (1.5 L x 1.5 W x 3.5 H -cm) containing Fafard Germination mix (Fafard,

Anderson, South Carolina) in a greenhouse in Raleigh, North Carolina. On 4 July 2012, the coleus plugs were planted into the center of each container. Six substrates x five replications of each substrate made a total of 30 containers. Plants in containers were completely randomized on a greenhouse bench, grown in Raleigh, NC. Plants in each substrate were over-head watered as needed depending on weather conditions, and never showed symptoms of water stress. Plants were fertilized at each watering with 200 ppm nitrogen (N) injected at 1:100 ratio by Dosatron injector [(D14MZ2); Dosatron International, Inc., Clearwater, FL] with Peters Professional 20-10-20 Peat-Lite Special (The Scotts Co., Marysville, OH) containing 8.1% ammonium (NH_4-N) and 11.9% nitrate (NO_3-N). On 1 Aug. 2012, all coleus plants were removed from the greenhouse into a controlled temperature room (21°C) and were readied to have the root conductance measured by the HCFM. The container-grown coleus were placed in a tub of tap water in order to saturate the substrate and pores around the roots so that air bubbles were easier to remove from the substrate and from the xylem. While saturated, plants were then singly and randomly severed 3 cm above the substrate level to leave enough stem for later manipulations. The HCFM connection was immediately placed on the root system and the HCFM was turned on to begin measuring root hydraulic conductance. Transient root conductance, K_{rt}, was computed from the slope of the linear region by linear regression of the data. After the measurements of K_{rt} were taken, the HCFM connection was removed and substrate was removed from the root system in order to place the washed roots in the oven to be dried (70°C for 48 hours) for biomass measurements. Weighted root conductance (K_{rdm}) was calculated by dividing K_{rt} by the dry root mass. Means separation using Tukey's studentized range (HSD) ($P \leq 0.05$) was used to

compare means of root conductance and root dry mass across all six substrates, and the interaction between root conductance or root dry mass and substrate (SAS Institute version 9.2, Cary NC).

Physical properties, including air space (AS), container capacity (CC), total porosity (TP), and bulk density (BD), were determined on three replicate samples of each substrate using the NCSU Porometer method as described by Fonteno et al. (1995). Samples of all substrates were collected on 4 July 2012 when plants were potted. These substrates were taken from the same source used to pot this experiment and therefore amended similarly. Means separation using Tukey's HSD ($P \leq 0.05$) was used to compare means of substrate physical properties (AS, CC, TP and BD).

Shredded-pine-wood substrate: Experiment 2. Except where indicated, procedures for Expt. 2 were as described for Expt. 1. Four substrates were used in this study; peat amended with 20% (v/v) PL; 20, 30 or 40% (v/v) shredded-pine-wood (SW). On 2 Jan. 2012 the delimbed pine logs were shredded through a Wood Hog shredder (Morbark® model 3800; Winn, MI) and the product of this procedure was then processed in a hammermill through a 6.35 mm screen (Meadows Mills, North Wilkesboro, NC) yield the fiberous SW end product. Shredded-pine-wood was selected for this study because it has properties similar to peat and has been shown to have potential as an alternative substrate component for peat. The substrates were mixed on 4 June 2012; all substrates were tested for initial pH and then amended with dolomitic limestone. On 5 June 2012, six greenhouse containers (15.2 cm dia; Dillen Products, Middlefield, OH) were filled with each individual substrate as described in Expt. 1. Mum plugs (*Chrysanthemum* 'Garden Alcala Red', 51-tray; C. Raker & Sons, Inc,

Litchfield, MI) were planted into the center of each container. Four substrates x six replications made a total of 24 containers. Plants were randomized on a greenhouse bench in Raleigh, NC and fertigated as described in Expt. 1. On 6 July 2012, all container-grown mums were removed from the greenhouse to the same temperature-controlled room as Expt. 1. The mums were then readied for root conductance measurements and root dry mass measurements in the same manner as described in Expt. 1. Physical properties of these substrates were measured as described in Expt. 1. Means separation using Tukey's HSD ($P \leq 0.05$) was used to compare means of root conductance and root dry mass across all four substrates, and the interaction between root conductance or root dry mass and substrate, as well as compare the means of the substrate physical properties (SAS Institute version 9.2, Cary NC).

Pine bark substrates: Experiment 3. Except where indicated, procedures for Expt. 3 were as described in Expt. 1 and 2. Three substrates were used in this study; pine bark (PB) amended with 0, 25 and 50% SW, respectively. On 4 June 2012, substrates were mixed and tested for initial pH. The substrates were then limed with dolomitic limestone at 3.6 $kg \cdot m^{-3}$ (#100; Rockydale Quarries Corporation, Roanoke, VA). On 5 June 2012, 12 nursery pots (3.8 L, 16.2 cm dia., C300S; Nursery Supplies, Inc., Kissimmee, FL) were filled with each individual substrate. Two species, 'Pink Delight' *Buddleja davidii* and 'Luna Rose' *Hibiscus moscheutos* (36-tray plugs; C. Raker & Sons, Inc, Litchfield, MI) were used in this experiment, and one plug was planted into the center of each container. Three substrates x six replications x two species made a total of 36 containers. Plants were randomized on a greenhouse bench in Raleigh, NC and fertigated as described in Expt. 1. On 10 July 2012, all

container-grown *Buddleja* and *Hibiscus* were removed from the greenhouse to a temperature-controlled room. The *Buddleja* and *Hibiscus* were then readied for root conductance measurements and root dry mass measurements in the same manner as described in Expt. 1. Physical properties were measured as described in Expt. 1. Means separation using Tukey's HSD ($P \leq 0.05$) was used to compare means of root conductance and root dry mass across all four substrates, and the interaction between root conductance or root dry mass and substrate, as well as compare the means of the substrate physical properties (SAS Institute version 9.2, Cary NC).

This study was repeated on 19 June 2012, with the same three substrates, five replications of both 'Pink Delight' *Buddleja davidii* and 'Luna Rose' *Hibiscus moscheutos*. Containers were packed with the substrates and planted as described above. Three substrates x five replications x two species made a total of 30 plants in containers. Plants were randomly placed on a greenhouse bench in Raleigh, NC and fertigated as described in Expt. 1. Plants were harvested on 31 July 2012 and root conductance, dry mass, and substrate physical properties measurements were taken as described above and means were compared as described above.

Results and Discussion

Pine-wood-chips substrate: Experiment 1. There was no difference in root growth of coleus grown in the six substrates, 10, 20 or 30% - PL or PWC (Table 1). There was also no difference observed in coleus root conductivities, both K_{rt} and K_{rdm}, across substrates (Table 1). There was no linear response for both K_{rt} and K_{rdm} across all substrates, when plotted

against root dry mass (Table 1). For this study using coleus plants, there was no difference in root growth in plants grown in the six substrates and the HCFM did not measure differences in root conductance. There was no effect of substrate or root dry mass on K_{rt} and K_{rdm} (Table 1). There were no significant differences for CC and AS across all substrates (Table 2). 10% PWC had significantly higher TP compared to 20% PL and 30% PWC substrates, but not significantly different from the other substrates (Table 2).

Shredded-pine-wood substrate: Experiment 2. Mums grown in 20% SW substrate had more root dry mass than plants grown in 30 or 40% SW substrates, and was not significantly different from plants grown in 20% PL substrate (Table 3). There were no differences between the means of K_{rt} and K_{rdm} between the plants grown in all four substrates. There was a linear response observed in K_{rt} plotted against root dry weight of plants grown in 70% peat with 30% SW substrate (Table 3). There was no effect of substrate or root dry mass on K_{rt} and K_{rdm} (Table 3). Physical properties of these substrates show that 20 and 40% SW substrates had higher AS than 20% PL substrate, but these substrates were not significantly different in AS from 30% SW substrate (Table 4).

Pine bark substrates: Experiment 3. Buddleja grown in the three PB substrates had no significant differences in root dry mass, even when the experiment was repeated (Table 5). There was also no significant difference between the means of K_{rt} and K_{rdm} (Table 5). There was a linear response observed in the repeated study, with both K_{rt} and K_{rdm} plotted against root dry mass in plants grown in 100% PB (Table 5). In the repeated study, there was a significant effect of root dry mass on K_{rt} and K_{rdm}; and there was an effect of substrate on K_{rdm} (Table 5). *Hibiscus* grown in the three pine bark substrates had significant differences in

root growth the first time the study was conducted, with plants grown in 75% PB with 25% SW and 50% PB with 50% SW had more root dry mass than plants grown in 100% PB (Table 6). The first study also showed an effect of substrate type on root dry mass (Table 6). There were no significant differences in root growth of the plants grown in all three substrates when the study was repeated (Table 6). There were no linear responses observed in both studies and no effect of substrate and root dry mass on K_{rt} and K_{rdm} (Table 6).

Measuring physical properties showed 100% PB substrate had the highest BD compared to other substrates (Table 7). Total porosity was not different among the substrates (Table 7). 100% PB substrate had low AS and high CC compared to 75:25% PB:SW substrate, and 100% PB substrate was not significantly different from 50:50% PB:SW substrate in AS and CC (Table 7). All physical properties for the three substrates were in the acceptable range of properties (Yeager et al., 2007). The higher AS in the 75:25% PB:SW and 50:50% PB:SW could explain why there was more *Hibiscus* root growth in these substrates compared to plants grown in 100% PB substrate.

Conclusion

The above studies show that there is not any effect of using pine tree substrates compared to perlite or PB on root growth of plants. Observed in several of the experiments, when there were differences in root growth, there were not differences in root conductance measurements. This could be due to unseen characteristics of the root on a cellular scale. Experiment 3 showed there was an observed effect of root mass on root conductance, and substrate influenced the weighted root conductance. More work needs to be done to show if

the HCFM measures influences of substrates on root growth, and if the root conductance measurements are reflective of root development.

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Table 1: Hydraulic conductance, weighted conductance and root dry mass measurements of coleus (*Solenstemon* 'Giant Exhibition') grown in 90, 80 or 70% (v/v) peat amended with either perlite (PL) or pine-wood-chips (PWC), respectively.

	Root system measurements			Linear	
	K_{rt}[z]	K_{rdm}[y]	Root dry mass[x]		
Substrate	(kg/s/MPa)	(kg/s/MPa/g root)	(g)	K_{rt}	K_{rdm}
10% PL[w]	1.6E-6 a[v]	1.0E-6 a	1.2 a	NS[u]	NS
20% PL[t]	1.6E-6 a	1.2E-6 a	1.3 a	NS	NS
30% PL[s]	8.5E-7 a	1.0E-6 a	1.0 a	NS	NS
10% PWC[r]	6.1E-7 a	4.5E-7 a	1.4 a	NS	NS
20% PWC[q]	1.3E-6 a	1.1E-6 a	1.4 a	NS	NS
30% PWC[p]	5.6E-7 a	3.7E-7 a	1.7 a	NS	NS
Significance					
Substrate	NS[o]	NS	NS		
Root dry mass	NS	NS	-		
Interaction	NS	NS	-		

[z]K_{rt} (kg/s/MPa) is the hydraulic root conductance.

[y]K_{rdm} (kg/s/MPa/g root) is the weighted root conductance (K_{rt} divided by root dry mass).

[x]Root dry mass is the mean of all three replications, after removing substrate and oven drying.

[w]Substrate composed of 90% peat/ 10% perlite (v/v).

[v]Means separated within column across all substrates by Tukey's studentized range HSD, $P \leq 0.05$. Means followed by the same letter are not significantly different.

[u]NS, L, and Q represent no significant response, linear, and quadratic response, respectively, between K_{rt} or K_{rdm} and root dry mass, *, **,*** represent significant effects when $P \leq 0.05$, 0.01, and 0.001, respectively.

[t]Substrate composed of 80% peat / 20% perlite (v/v).

[s]Substrate composed of 70% peat / 30% perlite (v/v).

[r] Substrate composed of 90% peat / 10% pine-wood-chips (v/v).

[q]Substrate composed of 80% peat / 20% pine-wood-chips (v/v).

[p]Substrate composed of 70% peat / 30% pine-wood-chips (v/v).

[o]NS, *, **, *** represent no significant response, and significant effects when $P \leq 0.05$, 0.01, and 0.001, respectively.

Table 2. Physical properties of perlite and pine-wood-chip substrates.[z]

Substrate	Container capacity[y] (% vol)	Air space[x] (% vol)	Total porosity[w] (% vol)	Bulk density[v] (g/cc)
10% PL[u]	74.4 a[o]	15.7 a	90.1 abc	0.11 d
20% PL[t]	73.6 a	14.0 a	87.6 bc	0.13 b
30% PL[s]	73.5 a	14.5 a	88.0 abc	0.11 cd
10% PWC[r]	75.1 a	16.5 a	91.6 a	0.12 c
20% PWC[q]	73.4 a	17.5 a	91.0 ab	0.13 ab
30% PWC[p]	71.1 a	15.6 a	86.6 c	0.14 a

[z]Physical properties data were collected from three samples per substrate and represented as means. Analysis performed using the North Carolina State University Porometer method (Fonteno et al., 1995).
[y]Container capacity is (wet weight – oven dry weight) ÷ volume of the sample.
[x]Air space is the volume of water drained from the sample ÷ volume of the sample.
[w]Total porosity is container capacity + air space.
[v]Bulk density after forced-air drying at 105 °C for 48 h.
[u]Substrate is composed of 90% peat/ 10% perlite (v/v).
[t]Substrate is composed of 80% peat/ 20% perlite (v/v).
[s]Substrate is composed of 70% peat/ 30% perlite (v/v).
[r]Substrate is composed of 90% peat/ 10% pine-wood-chips (v/v).
[q]Substrate is composed of 80% peat/ 20% pine-wood-chips (v/v).
[p]Substrate is composed of 90% peat/ 10% pine-wood-chips (v/v).
[o]Means separated within column across all substrates by Tukey's HSD, $P \leq 0.05$. Means followed by the same letter are not significantly different.

Table 3: Hydraulic conductance, weighted conductance and root dry mass measurements of mum (*Chrysanthemum* 'Garden Alcala Red') grown in 80% peat amended with 20% perlite or 80, 70 and 60% peat amended with 20, 30 or 40% (v/v) shredded-pine-wood (SW), respectively.

	Root system measurements				
	K_{rt}[z]	K_{rdm}[y]	Root dry mass[x]	Linear	
Substrate	(kg/s/MPa)	(kg/s/MPa/g root)	(g)	K_{rt}	K_{rdm}
20% PL[w]	1.0E-8 a[v]	6.0E-9 a	1.7 ab	NS[u]	NS
20% SW[t]	8.7E-9 a	4.6E-9 a	2.0 a	NS	NS
30% SW[s]	9.0E-9 a	5.7E-9 a	1.5 b	L*	NS
40% SW[r]	9.0E-9 a	6.3E-9 a	1.5 b	NS	NS
Significance					
Substrate	NS[q]	NS	*		
Root dry mass	NS	NS	-		
Interaction	NS	NS	-		

[z]K_{rt} (kg/s/MPa) is the hydraulic root conductance.

[y]K_{rdm} (kg/s/MPa/g root) is the weighted root conductance (K_{rt} divided by root dry mass).

[x]Root dry mass is the mean of all three replications, after removing substrate and oven drying.

[w]Substrate composed of 80% peat/ 20% perlite (v/v).

[v]Means separated within column across all substrates by Tukey's studentized range HSD, $P \leq 0.05$ (n = 3). Means followed by the same letter are not significantly different.

[u]NS, L, and Q represent no significant response, linear, and quadratic response, respectively, between K_{rt} or K_{rdm} and root dry mass, *, **,*** represent significant effects when $P \leq 0.05$, 0.01, and 0.001, respectively.

[t]Substrate composed of 80% peat / 20% shredded-pine-wood (v/v).

[s]Substrate composed of 70% peat / 30% shredded-pine-wood (v/v).

[r]Substrate composed of 60% peat/ 40% shredded-pine-wood (v/v).

[q]NS, *, **, *** represent no significant response, and significant effects when $P \leq 0.05$, 0.01, and 0.001, respectively.

Table 4. Physical properties of perlite and shredded-pine-wood substrates.[z]

Substrate	Container capacity[y] (% vol)	Air space[x] (% vol)	Total porosity[w] (% vol)	Bulk density[v] (g/cc)
20% PL[u]	73.6 a[q]	14.0 b	87.6 a	0.13 a
20% SW[t]	68.0 b	22.0 a	90.0 a	0.13 a
30% SW[s]	66.0 b	18.0 ab	84.0 b	0.13 a
40% SW[r]	66.1 b	22.5 a	88.6 a	0.13 a

[z]Physical properties data were collected from three samples per substrate and represented as means. Analysis performed using the North Carolina State University Porometer method (Fonteno et al., 1995).
[y]Container capacity is (wet weight – oven dry weight) ÷ volume of the sample.
[x]Air space is the volume of water drained from the sample ÷ volume of the sample.
[w]Total porosity is container capacity + air space.
[v]Bulk density after forced-air drying at 105 °C for 48 h.
[u]Substrate composed of 80% peat/ 20% perlite (v/v).
[t]Substrate composed of 80% peat/ 20% pine-wood-chips (v/v).
[s]Substrate composed of 70% peat/ 30% pine-wood-chips (v/v).
[r]Substrate composed of 60% peat/ 40% pine-wood-chips (v/v).
[q]Means separated within column across all substrates using Tukey's HSD, $P \leq 0.05$. Means followed by the same letter are not significantly different.

Table 5: Hydraulic conductance, weighted conductance and root dry mass measurements of 'Pink Delight' *Buddleja davidii* grown in 100, 75 and 50% (v/v) pine bark (PB) amended with 0, 25 and 50% (v/v) shredded-pine-wood (SW).

		Root system measurements				
		K_{rt}[z]	K_{rdm}[y]	Root dry mass[x]	Linear	
Substrate	Harvest date[w]	(kg/s/MPa)	(kg/s/MPa/g root)	(g)	K_{rt}	K_{rdm}
100:0 PB:SW[t]	7/10/12	1.5E-8 a[v]	3.3E-9 a	4.6 a	NS[u]	NS
75:25 PB:SW[s]		2.0E-8 a	4.6E-9 a	4.3 a	NS	NS
50:50 PB:SW[r]		6.7E-9 a	1.7E-9 a	4.0 a	NS	NS
Significance						
Substrate		NS[q]	NS	NS		
Root dry mass		NS	NS	-		
Interaction		NS	NS	-		
100:0 PB:SW	7/31/12	2.4E-8 a	4.8E-9 a	5.2 a	L*	L*
75:25 PB:SW		1.7E-8 a	3.4E-9 a	5.3 a	NS	NS
50:50 PB:SW		8.4E-9 a	1.6E-9 a	5.2 a	NS	NS
Significance						
Substrate		NS	*	NS		
Root dry mass		**	**	-		
Interaction		NS	*	-		

[z]K_{rt} (kg/s/MPa) is the hydraulic root conductance.

[y]K_{rdm} (kg/s/MPa/g root) is the weighted root conductance (K_{rt} divided by root dry mass).

[x]Root dry mass is the mean of all three replications, after removing substrate and oven drying.

[w]Harvest date corresponds to the first harvest date of the study and the repeated harvest date, 10 July 2012 and 31 July 2012, respectively.

[v]Means separated within column across all substrates by Tukey's studentized range HSD, $P \leq 0.05$ (n = 3). Means followed by the same letter are not significantly different.

[u]NS and L represent no significant response and linear response, respectively, between K_{rt} or K_{rdm} and root dry mass, *, **,*** represent significant effects when $P \leq 0.05$, 0.01, and 0.001, respectively.

[t]Substrate composed of 100% pine bark (v/v).

[s]Substrate composed of 75% pine bark/ 25% shredded-pine-wood (v/v).

[r]Substrate composed of 50% pine bark/ 50% shredded-pine-wood (v/v).

[q]NS, *, **, *** represent no significant response, and significant effects when $P \leq 0.05$, 0.01, and 0.001, respectively.

Table 6. Hydraulic conductance, weighted conductance and root dry mass measurements of 'Luna Rose' *Hibiscus moscheutos* grown in 100, 75 and 50% (v/v) pine bark (PB) amended with 0, 25 and 50% (v/v) shredded-pine-wood (SW).

		Root system measurements				
		K_{rt}[z]	K_{rdm}[y]	Root dry mass[x]	Linear	
Substrate	Harvest date[w]	(kg/s/MPa)	(kg/s/MPa/g root)	(g)	K_{rt}	K_{rdm}
100:0 PB:SW[t]	7/10/12	9.1E-7 a[v]	1.4E-7 a	6.2 b	NS[u]	NS
75:25 PB:SW[s]		6.5E-7 a	9.0E-8 a	7.0 a	NS	NS
50:50 PB:SW[r]		4.7E-7 a	7.0E-8 a	6.8 a	NS	NS
Significance						
Substrate		NS[q]	NS	**		
Root dry mass		NS	NS	-		
Interaction		NS	NS	-		
100:0 PB:SW	7/31/12	9.9E-8 a	8.2E-9 a	5.2 a	NS	NS
75:25 PB:SW		6.2E-8 a	6.0E-9 a	5.3 a	NS	NS
50:50 PB:SW		9.2E-8 a	8.5E-9 a	5.2 a	NS	NS
Significance						
Substrate		NS	NS	NS		
Root dry mass		NS	NS	-		
Interaction		NS	NS	-		

[z]K_{rt} (kg/s/MPa) is the hydraulic root conductance.

[y]K_{rdm} (kg/s/MPa/g root) is the weighted root conductance (K_{rt} divided by root dry mass).

[x]Root dry mass is the mean of all three replications, after removing substrate and oven drying.

[w]Harvest date corresponds to the first harvest date of the study and the repeated harvest date, 10 July 2012 and 31 July 2012, respectively.

[v]Means separated within column across all substrates by Tukey's studentized range HSD, $P \leq 0.05$ (n = 3). Means followed by the same letter are not significantly different.

[u]NS and L represent no significant response and linear response, respectively, between K_{rt} or K_{rdm} and root dry mass, *, **,*** represent significant effects when $P \leq 0.05$, 0.01, and 0.001, respectively.

[t]Substrate composed of 100% pine bark (v/v).

[s]Substrate composed of 75% pine bark/ 25% shredded-pine-wood (v/v).

[r]Substrate composed of 50% pine bark/ 50% shredded-pine-wood (v/v).

[q]NS, *, **, *** represent no significant response, and significant effects when $P \leq 0.05$, 0.01, and 0.001, respectively.

Table 7. Physical properties of pine bark and shredded-pine-wood substrates.[z]

Substrate	Container capacity[y] (% vol)	Air space[x] (% vol)	Total porosity[w] (% vol)	Bulk density[v] (g/cc)
100:0 PB:SW[u]	43.4 a[r]	32.4 b	75.8 a	0.20 a
75:25 PB:SW[t]	33.2 b	42.7 a	75.9 a	0.18 b
50:50 PB:SW[s]	40.0 a	39.3 ab	79.3 a	0.18 b

[z]Physical properties data were collected from three samples per substrate and represented as means. Analysis performed using the North Carolina State University Porometer method (Fonteno et al., 1995).
[y]Container capacity is (wet weight – oven dry weight) ÷ volume of the sample.
[x]Air space is the volume of water drained from the sample ÷ volume of the sample.
[w]Total porosity is container capacity + air space.
[v]Bulk density after forced-air drying at 105 °C for 48 h.
[u]Substrate composed of 100% pine bark (v/v).
[t]Substrate composed of 75% pine bark/ 25% shredded-pine wood (v/v).
[s]Substrate composed of 50% pine bark/ 50% shredded-pine-wood (v/v).
[r]Means separated within column across substrates by Tukey's HSD $P \leq 0.05$. Means followed by the same letter are not significantly different.

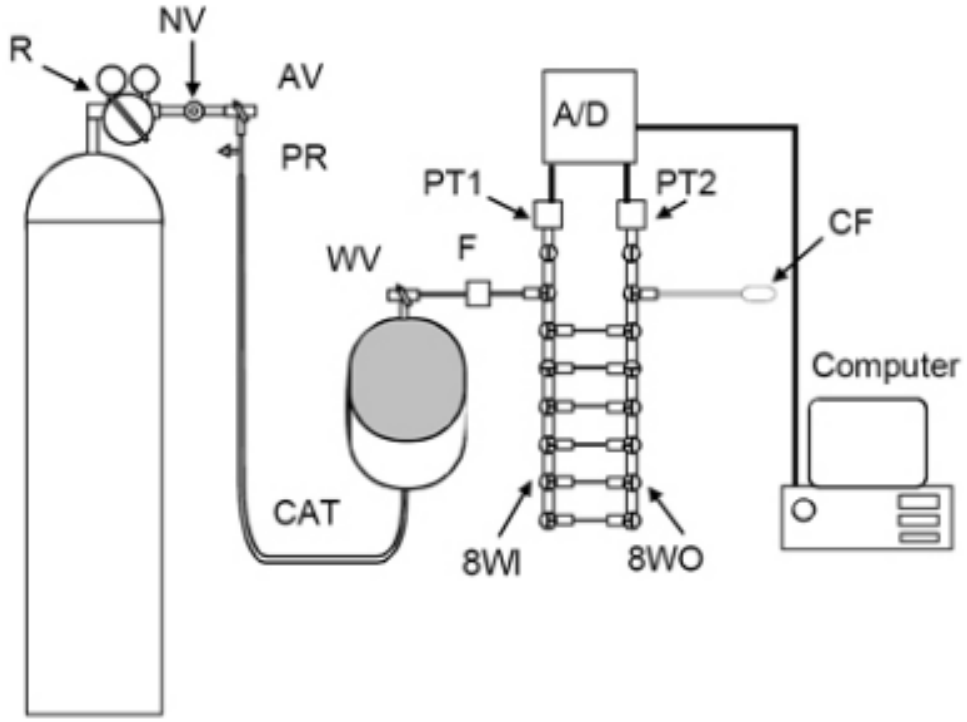

Figure 1. Diagrammatic representation of the hydraulic conductance flow meter (HCFM) for rapid measurements of hydraulic conductance of roots or shoots. Abbreviations are as follows: 8WI – 8-way valve on inlet side, 8WO – 8-way valve on outlet side, A/D – location of A/D circuit, AV – compressed air valve, CAT – captive air tank, CF – compression fitting to connect HCFM to root or shoot, NV – needle valve, PR – pressure release valve, PT1 – pressure transducer #1 on inlet side, PT2 – pressure transducer #2 on outlet side, R – pressure regulator, WV – water valve.