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Characterization of Whole Pine Tree Substrates for Adventitious Rooting of Cuttings and Initial Growth of Seedlings

Anthony Lynn Witcher
University of Southern Mississippi

The University of Southern Mississippi

CHARACTERIZATION OF WHOLE PINE TREE SUBSTRATES FOR ADVENTITIOUS ROOTING OF CUTTINGS AND INITIAL GROWTH OF SEEDLINGS

by

Anthony Lynn Witcher

Abstract of a Dissertation
Submitted to the Graduate School
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ABSTRACT

CHARACTERIZATION OF WHOLE PINE TREE SUBSTRATES FOR ADVENTITIOUS ROOTING OF CUTTINGS AND INITIAL GROWTH OF SEEDLINGS

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Processed whole pine (*Pinus taeda* L.) trees have been extensively evaluated to supplement peatmoss and pine bark usage in container substrates for greenhouse and nursery crop production. The suitability of whole pine tree (WPT) substrates for crop propagation has not been investigated. Demonstrating the versatility of WPT substrates is essential to expanding their commercial availability and use.

The objective of this work was to evaluate WPT substrates for stem cutting and seed propagation of ornamental crops, and to identify factors affecting root development. Stem cutting and seedling root development was evaluated in WPT and traditional (peatmoss and pine bark) substrates. In the first study, stem cuttings of *Chrysanthemum*, *Cupressocyparis*, *Euonymus*, *Evolvulus*, *Ligustrum*, *Persicaria*, *Rosa*, and *Salvia* were set in whole pine tree and pine bark substrates. Rooting percentage was similar among substrates for each species, but root growth increased with the addition of peatmoss. In the second study, a phytotoxicity assessment of aged and fresh WPT substrates was conducted using a Phytotoxkit and a seedling growth test. Using the Phytotoxkit, seed germination rate and seedling root growth was similar for aged WPT and peatmoss. Fresh pine needles had an inhibitory effect on seed germination and seedling growth. Using the seedling growth test, lettuce, oat, and tomato seed emergence rate was similar for aged

WPT and a peatmoss substrate. Root development was greatest in a peatmoss substrate compared with pine bark and aged and fresh WPT. In the final study, the effect of WPT particle size on seedling and stem cutting root development was evaluated. Processing WPT into finer particle sizes resulted in decreased air space and increased container capacity, but did not affect stem cutting or seedling root growth. Overall, root development was greater in peatmoss substrates compared with WPT substrates.

Whole pine tree substrates can be used for germinating seeds and rooting stem cuttings. Differences in seed germination/emergence rate and seedling root length could not be attributed to phytotoxic compounds in WPT substrates. Nutrient availability and retention properties of WPT substrates during propagation should be further evaluated.

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Approved:

_Kenneth Curry_____
Director

_Eugene Blythe_____

_Glenn Fain_____

_Brian Kreiser_____

_Mac Alford_____

_Cecil Pounders_____

_Susan A. Siltanen_____
Dean of the Graduate School

May 2013

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TABLE OF CONTENTS

LIST OF TABLES

Table

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Introduction

The environmental horticulture industry, also known as the ornamental horticulture industry or green industry, refers to businesses (public and private) involved in the production, sale, installation, and maintenance of ornamental crops (Hall et al., 2005). Financial, environmental, and social benefits of the environmental horticulture industry impact everyone in some manner. Residential energy costs can be reduced by proper tree selection and placement, while landscaped homes are valued higher than non-landscaped homes (Project Evergreen, 2005a). Reduced energy consumption means lower demand for fossil fuels, while air contaminants are absorbed by trees, shrubs, and turfgrass. Additionally, groundwater contamination and surface erosion are minimized by reduced runoff from landscaped areas (Project Evergreen, 2005b). Well-planned landscapes can provide privacy and soften the loud sounds of congested areas. In urban areas, communities with green spaces are more appealing to potential residents and are linked to fewer incidents of crime (Project Evergreen, 2005c). An improved quality of life is the greatest contribution of the environmental horticulture industry.

Major sectors of the environmental horticulture industry include wholesale and retail nurseries, landscaping services, florists, golf courses, and urban forestry. Over the past 20 years, the environmental horticulture industry has been one of the fastest growing segments of agriculture in the United States, largely due to the strong economy and real estate market of the 1990s. Expansion of the environmental horticulture industry has resulted in job creation and enhanced revenues for state and local governments where

these businesses are located. Allied companies, which provide supplies and support for the various sectors, have also benefitted from industry growth. (Hall et al., 2005; Shields and Willits, 2003).

Wholesale nurseries are a key component of the environmental horticulture industry because their products are used throughout all sectors of the industry. Wholesale nurseries vary greatly in respect to the production method used, the production environment, and the type of crop produced. The most common crop production methods are field-grown, container-grown, and pot-in-pot. Field-grown crops are planted directly into the soil and grown to an ideal size. Field-grown crops can be harvested as bare-root plants, or can be dug with the root ball wrapped for storage and transport. Container-grown crops are planted and grown to a finished size in plastic containers filled with a soilless substrate (also known as growing medium, potting soil, or potting mix) for plant support and root development. At harvest, the plants are transported in the containers, which are removed prior to planting in the landscape. The pot-in-pot method requires two containers and combines field and container production. The first container is permanently buried in the soil as a socket for the second container. The crop is planted into a substrate-filled second container and inserted into the socket container. At harvest, the containerized plants are lifted from the socket container and treated as a typical container crop (Diver and Greer, 2001).

Container-grown crops can be produced outdoors or inside a greenhouse. The production environment for container-grown plants will vary depending on the crop type or species. Hardy species of trees, shrubs, ground covers, and perennials can be grown outdoors. Tender species such as annuals, tropical foliage, and certain seasonal crops

must be grown inside a greenhouse. Greenhouses are used for the propagation and initial plant growth of most crops and for off-season production of hardy species.

Most wholesale nurseries produce a variety of plant species, regardless of production method or environment. The United States Department of Agriculture (USDA) conducts surveys of horticulture crops every 10 years. The survey collects information from operations that grew and sold $10,000 or more of horticultural crops during the census year in order to accurately estimate national and state ornamental horticulture crop production. The two most valuable crop types for the 2009 survey were floriculture and nursery crops (USDA, 2010). The terms, *floriculture crop* and *nursery crop*, are used extensively in the fields of research and cooperative extension, although the terms are not universal and some overlap may occur because each category is broad in respect to plant type.

Nursery crops are hardy species grown outdoors in full sun or under shade cloth and can be field-grown, container-grown, or grown pot-in-pot. Nursery crops are typically plants with woody stems and can be classified as broadleaf evergreens, coniferous evergreens, deciduous flowering trees, deciduous shade trees, deciduous shrubs, fruit and nut plants, ornamental grasses, ground covers and vines, palms, or propagative material (Jerardo, 2007). The wholesale value of all nursery crop categories totaled $3.9 billion for 2009 (USDA, 2010).

Most floriculture crops are tender species which are container-grown inside a greenhouse, although some hardy species can be produced outdoors as field-grown or container-grown crops. Floriculture crops are typically herbaceous plants and can be classified as annual bedding plants, herbaceous perennial plants, seasonal flowering

plants, foliage plants, cut flowers, and propagative material. The total wholesale value of all floriculture crop categories totaled $5.0 billion in 2009. (USDA, 2010).

Nursery and floriculture crops are started from propagative material, plant material produced for further growing. Examples of propagative material include rooted and unrooted cuttings, seedlings (bare root, liners, or plugs), and tissue cultured plantlets. Typically, the propagative material is transplanted into larger containers for final growth and sale. Most growers purchase propagative material, although some nurseries have the resources and facilities to propagate and produce certain crops. Propagative material accounted for $600 million (3%) of the total wholesale value of horticultural crops in 2009 (USDA, 2010).

Substrate Chemical and Physical Properties

The propagation and production of container-grown crops requires a substrate that is uniform, free of weed seed and pathogens, easily re-wetted, physically stable in storage and during production, cost effective, light-weight, and readily available (Davidson et al., 1994; Reid, 2001). Substrate is a universal term that refers to the contents of a container used to support the plant roots (Yeager et al., 1997). A universally accepted substrate does not exist, so a substrate selected for propagation or production is largely based on grower preference and availability. Before a substrate is selected, the grower should consider the chemical and physical properties of the material or combination of materials used to formulate the substrate.

Substrate chemical properties such as pH, electrical conductivity, and cation exchange capacity are important to plant production. A pH range of 5.5 to 6.5 is desirable for most plant species, although some species may require or tolerate a higher or lower

pH. Electrical conductivity, typically measured in milliSiemens per centimeter (equivalent to deciSiemens per meter), is used to quantify the capacity of a solution to carry an electrical current. The ion concentration, or soluble salt concentration, of a solution will affect the conductance, pure water having a very low electrical conductivity due to the lack of ions. Periodic electrical conductivity analysis of substrate solution samples is a useful tool for monitoring the nutritional status of a substrate. The pour-through method is a simple, non-destructive process for extracting substrate solution from container grown plants (Ingram et al., 2003). Synthetic fertilizers are composed of a variety of salts, thus electrical conductivity can be linked to the nutritional status of a substrate and be used as a guideline to determine if supplemental fertilizer is required. Cation exchange capacity is a measure of a substrate's ability to retain positively charged ions against leaching. Negatively charged binding sites on substrate particles act as a reservoir for positively charged nutrients. Substrates with a high cation exchange capacity are buffered against drastic changes in pH and nutrient supply. Cation exchange capacity is typically measured in cmol/kg substrate, but is converted to a volumetric unit ($cmol \cdot L^{-1}$) for comparison of soilless substrates with varying bulk density (Argo and Biernbaum, 1997).

The substrate physical properties important for healthy plant growth include air space, container capacity, total porosity, bulk density, and particle size distribution. The North Carolina State University porometer method (Fonteno et al., 1995) is used to calculate total porosity, container capacity, air space, and bulk density. Air space is the percent volume of a substrate filled with air after the substrate has been saturated and allowed to drain. Container capacity, or water-holding capacity, is the percent volume of

a substrate filled with water after the substrate has been saturated and allowed to drain. Total porosity is the percent volume of pore space containing air and water in a substrate. Bulk density refers to the dry weight of a substrate per unit volume and is represented as weight per volume ($g \cdot cm^{-3}$). Sufficiency ranges have been developed for physical properties of substrates used for nursery crop production (Yeager et al., 1997), but no such universally accepted values are available for substrates used in greenhouse crop production and propagation. Sufficiency ranges for nursery substrate physical properties include air space (10% to 30%), container capacity (45% to 65%), total porosity (50% to 85%), and bulk density (0.19 to 0.70 g/cc).

Particle size distribution is typically reported as the proportion (percentage) of a substrate sample composed of specific particle sizes. Particle size distribution is obtained by separating an air-dried substrate sample through a series of sieves, each sieve representing a separate range of particle size. After separation, the particles from each sieve are weighed and the proportion of particles for each sieve size is calculated using the following equation: [(particle weight / total sample weight) x 100]. Particle size distribution has a direct effect on the values of the other physical properties. Handreck (1983) reported particle sizes less than 0.5 mm had a significant effect on air space and container capacity for pine bark substrates, while it has been reported that particle sizes less than 1 mm contribute to reduced air space and increased container in peatmoss (Raviv and Lieth, 2008). In container production, a substrate must be able to retain adequate water for plant use between irrigation events, while providing sufficient air space for drainage (Argo, 1998b).

Container height and environmental conditions of the production site must be considered when selecting a substrate. Container height will affect the air space and container capacity of a substrate inside the container. As container height increases, the force of gravity on the upper portion of the substrate results in more water draining from the container compared to a shorter container. The substrate in a taller container will have more air space in the top portion and higher water content in the lower portion. The substrate in a shorter container has less air space in relation to water content, thus a higher container capacity (Fonteno et al., 1995; Owen and Altland, 2008).

The production environment for containerized crops can range from a climate-controlled greenhouse to an area exposed to the natural elements. Substrates with a high container capacity are acceptable in a greenhouse environment, but not recommended for outdoor production environments. Greenhouse-grown crops receive water strictly from mechanical irrigation, so growers can monitor the moisture content of the substrate and determine if irrigation is necessary. Crops grown outdoors are also mechanically irrigated, although excess water can be a problem during extended periods of rain (Ingram et al., 1993; Altland, 2006).

A propagation environment usually involves frequent mist applications to plant material in short containers. A substrate with a higher percentage of air space is desirable for adequate drainage, a characteristic useful in the prevention of plant damage due to pathogens or physiological stress from overwatering. An ideal substrate will contain an optimum balance of air and water, which may be difficult to achieve if the substrate is used for the propagation and production of numerous plant species.

Substrate Components

Traditional substrate components for container-grown crops include peat, pine bark, perlite, and vermiculite. Peat is a generic term that refers to a variety of partially decomposed plants in a bog habitat, including mosses, sedges, reeds, and grasses. The low oxygen environment present in bogs results in the slow decomposition and accumulation of dead plant material and other organisms. The vegetation type (plant species) and degree of decomposition affect the physical and chemical properties of peat. The high degree of decomposition for sedge and reed peats results in a high container capacity and low air space, typically at undesirable levels for a container substrate. Sphagnum peat (peatmoss), derived from slightly decomposed *Sphagnum* moss, is the predominant component of container substrates used for floriculture crops (Reid, 2001; Jaenicke, 1999). The physical and chemical characteristics of peatmoss make it an ideal component for substrates used in small containers. Peatmoss quality may vary as will values for container capacity (42% to 83%), air space (14% to 55%), and pH (3.0 to 4.0). The addition of agriculture grade limestone to peatmoss substrates can increase the pH to a desirable range for crop production. Characteristics such as a light weight, absence of weed seed and pathogens, and a slow rate of decomposition contribute to the popularity of peatmoss as the predominate component of horticultural substrates around the world (Ingram et al., 1993; Schmilewski, 2008; Yeager et al., 1997).

Pine bark has been used in the eastern United States as a container substrate since the 1960s. Pine bark is a byproduct of the forestry industry and is obtained by stripping the bark from pine logs harvested to make lumber, paper, or other wood products. The physical and chemical characteristics of pine bark will vary due to the type of debarking

equipment used, processing technique, and the age of processed pine bark. Although variations exist in the pine bark supply, most sources of pine bark are suitable as the sole substrate component for container production of nursery crops (Ingram et al., 1993; Lu et al., 2006). Pine bark may have a pH 3.5 to 6.0 and values may also vary for container capacity (40% to 70%) and air space (10% to 40%). Pine bark substrate pH can be increased to a desirable range for crop production by the addition of agriculture grade limestone (Argo, 1998a; Bilderback and Lorscheider, 1995; Yeager et al., 1997). Although pine bark is typically used for nursery crop production, screened pine bark is commonly used in smaller containers for propagation.

The physical and chemical characteristics of a substrate can be modified by the addition of inorganic components such as perlite and vermiculite. Inorganic components are not commonly used as the sole component of a container substrate due to increased expense or unacceptable chemical or physical characteristics. The air space and drainage of a container substrate can be increased with the addition of perlite. Perlite is a lightweight, structurally stable, sterile, chemically inert material with a neutral pH. Perlite is derived from a volcanic alumino-silicate mineral that has been crushed and heated at 1100 to 1800°F. Depending on the grade, which is based on particle size, perlite can have an air space of 14% to 46% and a container capacity of 22% to 54% (Davidson et al., 1994; Fonteno et al., 1995; Landis, 1990; Robbins and Evans, 2005). Vermiculite is used to increase the container capacity and nutrient retention of container substrates, due to its physical structure and high cation exchange capacity, respectively. Vermiculite is the product of heating an aluminum-iron-magnesium silicate mineral to over 1400°F. Vermiculite has a pH of 6.0 to 8.9, container capacity of 59% to 70%, and a cation

exchange capacity of 2.0 to 4.9. (Ingram et al., 1993; Robbins and Evans, 2005; Yeager et al., 1997).

Cutting Propagation

Cutting propagation is the most widely used method for cloning nursery and floriculture crops. Various vegetative portions of a plant can be used for cutting propagation, although stem cuttings are preferred for most crops due to the simple technique and abundance of cutting material. A cutting propagation substrate should physically support the cutting, have adequate aeration around the base of cutting, have adequate moisture retention, and create a darkened environment around the cutting base. Cuttings obtain water directly from the substrate, although the lack of roots prevents adequate uptake of water to replenish water loss from transpiration. Therefore, cutting propagation is conducted in a modified environment where water is applied intermittently as a mist or fog. The smaller containers used for propagation are filled with substrate and grouped into flats so a number of containers can be transported at once (Hartmann et al., 2002).

A proper balance of air space and container capacity is critical for healthy root system development from a cutting, so the combined effects of frequent mist application and small container size must be well understood when selecting a propagation substrate (Threadgill et al., 1985). For example, an environment that uses frequent mist to maintain adequate humidity would need a substrate with a high ratio of air space to container capacity for adequate aeration, compared to an environment in which high humidity is maintained by fogging and less water is applied to the substrate. The suggested range of chemical and physical properties for a propagation substrate include a 5.5 to 6.5 pH, 15%

to 40% air space, 20% to 60% container capacity , and 0.3 to 0.8 $g \cdot cm^{-3}$ bulk density (Hartmann et al., 2002). The recommended physical properties for production substrates can be used as a guide for propagation substrate selection, yet the desired proportion of air space to container capacity will vary among nurseries due to container preference and propagation environment (Bilderback and Lorscheider, 1995; Regulski, 1984).

Economic and Environmental Factors for Peatmoss and Pine Bark

Economic and environmental factors affect the cost and availability of peatmoss and pine bark. Reed-sedge peat and peatmoss account for 82.1% and 8.5%, respectively, of total U.S. peat production. In 2008, Canadian peatmoss represented 97% of the total peatmoss imported into the U.S. The reliance on Canadian peatmoss can negatively affect the U.S. horticulture industry. For example, abnormal weather conditions during the harvesting period, coupled with rising transportation costs, can contribute to a shortage in supply and increased prices of sphagnum peat (United States Geological Survey, 2008; Canadian Sphagnum Peat Moss Association, 2011). In Europe, a large portion of peatlands have been destroyed or damaged due to centuries of harvesting for fuel and clearing for agricultural purposes. As a result, strict environmental policies have been adopted to decrease the use of peat to protect intact peatlands and to reclaim and restore areas that have been previously harvested. Only 0.02% of Canadian peatlands are currently being harvested, none for fuel, so Canada has adopted less stringent policies for peatland management and restoration (Barkham, 1993; Daigle and Daigle, 2001). Future environmental policies may limit the amount of peatmoss available for U.S. consumption.

Pine bark availability is linked to timber production, the economic status of the environmental horticulture industry, and fuel costs. Pine bark has been used increasingly

as an energy source since the 1970s and accounted for 50% of the energy consumed by the forest products industry in 2001. Domestic timber production has remained stable since 1986, but the recent closing and potential relocation of timber processing facilities to other countries will result in a higher cost and reduced availability of pine bark (Lu et al., 2006). In addition, the forestry industry has adopted in-field processing methods involving bark removal in the field. Therefore, bark is left in the field and not readily available to the environmental horticulture industry. The need for alternative components for container substrates will increase as traditional components become more expensive and more difficult to obtain.

Alternative Substrate Components

Increased demand for alternative substrate components is evident in the United Kingdom, where the proportion of container substrates composed of peat decreased by 19% between 1999 and 2009 (Department for Environmental Food and Rural Affairs, 2010). Various composted and raw organic materials have been evaluated as alternative components of container substrates. The addition of composted materials as a component of container substrates can improve substrate chemical and physical properties, be a source of essential plant nutrients, and reportedly suppress soil-borne pathogens (Hadar and Mandelbaum, 1992; Wilson et al., 2003). Spent mushroom compost is the substrate left from commercial edible mushroom production. Nursery and greenhouse crops have been successfully grown in substrates composed of up to 100% spent mushroom compost, although precautions must be taken if a grower plans on using a substrate containing greater than 50% spent mushroom compost. High proportion spent mushroom compost substrates may contain salt levels detrimental to plant growth, have reduced

water holding capacity, and may exhibit shrinking over time (Chong, 2005; Moore, 2005). Composted cotton gin trash is composed of residual plant material from the ginning process. A variety of ornamental crops have been successfully grown in substrates that contained various proportions of composted cotton gin trash. Although benefits from the addition of composted cotton gin trash to a substrate include increased water holding capacity compared to a 100% pine bark substrate (Cole et al., 2005; Jackson et al., 2005), the steady decline in cotton acreage since 2005 has led to reduced composted cotton gin trash availability (USDA, 2008).

Composted green wastes, composed of lawn and garden waste, have become more accessible due to the recycling efforts of proactive municipalities. Quality herbaceous and woody crops have been produced in composted green waste substrates as part of experimental evaluations, although the greatest benefit was achieved with composted green waste concentrations of 50% or less. Although substrates composed of composted green waste may have increased nutrient content and similar plant growth compared to a traditional substrate, substrates with greater than 50% composted green waste may exhibit increased water holding capacity and an undesirable soluble salt concentration (Burger et al., 1997; Hartz et al., 1996; Moore, 1999). Common problems associated with composted materials include availability limited to localized area, inconsistent quality (physical and chemical properties) among batches, and uncertainty about a long-term supply (Chong, 2005).

Coconut coir, the residual dust and short fibers of the husk after the desirable long fibers have been processed for commercial use, is an organic material that can be used without any composting. Coconut coir is currently available as a component of certain

commercial greenhouse substrates, although the cost of such substrates is similar to peat-based substrates. Conflicting results have been observed from experimental evaluations of coir as a container substrate component. Variability among the experiments can be linked to differences in the physical structure of the coir and differences in sodium and chlorine content, most likely due to the various procedures used to process coconut husks worldwide (Abad et al., 2005; Evans et al., 1996; Meerow, 1994). Although various materials can be used to produce acceptable container-grown crops, such substrates may not be suitable for propagation. Few of the alternative materials mentioned possess all of the critical characteristics of a propagation substrate, which include being highly uniform, low in soluble salts, and having a consistent balance of air space and container capacity.

Wood-based Substrates

Non-composted wood-based materials can have various compositions of wood, bark, leaves, and reproductive structures depending on the source, although all have wood as the major component. Wood-based materials have been increasingly used as peat replacements for container substrates in Europe. In 2009, wood-based materials accounted for 6% of all materials used for container substrates and 16% of non-peatmoss materials used for container substrates in the United Kingdom (Department for Environmental Food and Rural Affairs, 2010). The European wood-base materials are primarily composed of coniferous species and are obtained from forestry operations or as waste from wood product manufacturing. Wood-based materials typically comprise up to 30% of a container substrate and are rarely used as the sole substrate component (Gruda and Schnitzler, 2004; Schmilewski, 2008). In the United States, wood shavings and sawdust of coniferous species have been used as components container substrates.

Substrates composed of up to 50% pine wood sawdust and shavings are used at various nurseries in the southeastern United States, whereas redwood and Douglas fir sawdust and shavings are used as components of substrates in the Western U.S (Jackson et al., 2008; Schaefer, 2009). The same issues associated with composted materials have led to limited use of sawdust and wood shavings.

The residual pine tree material created by in-field processing equipment has been identified as a component for container substrates. Clean chip residual is composed of, on average, 50% wood, 40% bark, and 10% needles, although the actual composition will vary depending on tree age and production site. Substrates composed entirely of clean chip residual have been deemed acceptable, compared to pine bark substrates, for the production of various annual and perennial herbaceous crops. In order to maximize plant growth, clean chip residual may require processing into smaller particles to achieve a greater water holding capacity desirable for such crops. Clean chip residual may be a more suitable substrate for nursery crop production, although further research on such crops is required (Boyer et al., 2008a, 2008b).

The importance of high (>50%) wood content substrates was recognized by Laiche and Nash (1986), who evaluated whole pine tree chips and a wood/bark substrate as alternatives to pine bark. More recent studies have been conducted to determine the effectiveness of high wood content substrates. Readily available raw materials composed of greater than 50% wood include chipped pine logs and chipped whole pine trees. Processed pine logs may contain up to 90% wood, while processed debarked pine logs contain 100% wood. Plant growth of marigold and holly comparable to that obtained in a pine bark substrate has been reported in a pine log substrate composed of 90% wood

(Wright and Browder, 2005). Jackson et al. (2008) later discovered that additional fertilizer was required in a pine log substrate to attain azalea and holly plant growth comparable to that in a pine bark substrate. Wright et al. (2008) determined additional fertilizer was required for increased chrysanthemum growth in a pine log substrate.

Whole pine tree substrate is composed of the entire above ground portion of a pine tree and contains about 80% wood. The raw material, widely sold as industrial fuel, is readily available throughout the southeastern United States during pine plantation thinning. Fain et al. (2008b) demonstrated that Vinca produced in whole pine tree substrates derived from three species had similar growth compared to plants in a pine bark substrate. Additional starter fertilizer was required to produce petunia in a 100% whole pine tree substrate compared to a peat-based substrate, although marigold had similar growth in all the substrates evaluated (Fain et al., 2008a).

According to Jackson et al. (2008), Wright et al. (2008), and Fain et al. (2008a), the higher fertilizer requirement of plants grown in high wood content substrates is most likely due to a combination of nitrogen immobilization, particle size distribution, and reduced cation exchange capacity. Proposed solutions for increasing the overall nutrient availability in high wood content substrates include increasing container capacity and modifying fertilizer practices. Substrate container capacity may be increased by further mechanical processing to obtain a substrate with finer particles or by the addition of peatmoss. Nitrogen impregnation during processing, the use of starter fertilizers, or higher fertilizer rates during production could be used to offset nitrogen immobilization (Fain et al., 2008a; Gruda and Schnitzler, 1999; Wright et al., 2008).

Phytotoxicity

Phytotoxicity may be a function of certain organic or inorganic compounds found in soil, compost, or other substrates used for growing plants. In substrates composed of various tree components, phytotoxicity may occur due to the presence of organic phenolic and terpenoid compounds, or from inorganic metal compounds (Harkin and Rowe, 1971; Sjöström, 1993). Seed germination bioassays and seedling growth tests are universally accepted procedures for determining whether a solid substrate has any phytotoxicity properties. Such tests are simple to conduct, relatively inexpensive (compared to laboratory chemical analysis), and reproducible. Chemical reactions detrimental to plant development may be observed in a bioassay, whereas such a response would not be obvious just by reviewing a chemical analysis. Although a single standard has not been universally accepted for the germination bioassay, the most common procedures involve exposing seeds to a liquid extract of a substrate or placing seeds in direct contact with a substrate (Archambault et al., 2004; Kapanen and Itävaara, 2001; Macias et al., 2000; Ortega et al., 1996;). The direct contact method accounts for any phytotoxic compounds bound to the solid particles, in addition to those dissolved in water (Naasz et al., 2009).

Although much information is available on using bioassays to test compost maturity and quality (Emino and Warman, 2004; Hartz and Giannini, 1998; Kapanen and Itävaara, 2001; Murillo et al., 1995), little information exists on such tests for the phytotoxic effects of non-composted tree components such as wood, bark, and leaves. Rau et al. (2006) evaluated tomato seedling growth after 30 days in wood substrates derived from five tree species and found that plant dry weight decreased as the

polyphenolic concentration of the wood increased. Ortega et al. (1996) demonstrated that higher phenolic levels in an oak bark resulted in significantly reduced seedling growth of six vegetable species. In the same study, two seed germination bioassay experiments were conducted to determine the effects of possible oak bark phytotoxicity on germination percentage and radicle growth. Two types of germination bioassays, liquid extract and direct contact, were conducted to determine the applicability for determining potential phytotoxicity. They concluded the direct contact method was the optimum method due to its similarity to actual production procedures. In both methods, seed germination was negatively affected in the presence of greater phenolic compound concentrations.

Naasz et al. (2009) conducted lettuce seed germination and tomato seedling growth bioassays using the bark of seven tree species. The degree of phytotoxicity varied among the barks, although there was a correlation between plant growth and substrate air space. They concluded low air space, rather than select chemical and biochemical properties, had the greatest effect on plant growth. The allelopathic effects of pine needles were investigated in seedling growth and seed germination bioassays conducted by Nektarios et al. (2005). They concluded pine needles contained compounds that inhibited plant development. The phytotoxic effect was more pronounced for fresh pine needles compared with senesced and decaying pine needles.

Dissertation Research

Stem cutting propagation has not been evaluated in whole pine tree substrates. Root development in whole pine tree and traditional substrates were evaluated through a series of experiments designed with modifications to substrate chemical and physical

properties. Seed germination and seedling growth experiments were conducted to detect potential phytotoxicity associated with whole pine tree and traditional substrates. Results from these experiments were used to identify chemical and physical properties suitable for propagation in a whole pine tree substrate.

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CHAPTER II

STEM CUTTING PROPAGATION IN WHOLE PINE TREE SUBSTRATES

Abstract

Wood-based substrates have been extensively evaluated for greenhouse and nursery crop production, yet these substrates have not been evaluated for crop propagation. The objective of this study was to evaluate processed whole pine trees as a rooting substrate for stem cutting propagation of ornamental crops. Substrates included processed whole pine tree (WPT), pine bark (PB), and each mixed with equal parts peatmoss (WPT:PM and PB:PM). Substrate physical (air space, container capacity, total porosity, bulk density, and particle size distribution) and chemical (pH and electrical conductivity) properties were determined for all substrates. Rooting percentage, total root length, total root volume, and total shoot length were evaluated for four species in 2008 and five species in 2009. Substrate air space was similar between PB and WPT in the 2008 experiment, and likewise between PB:PM and WPT:PM. In the 2009 experiment, PB and WPT had similar substrate air space. The addition of peatmoss to PB and WPT resulted in reduced air space and increased container capacity in both experiments. The proportion of fine particles doubled for PB:PM and WPT:PM compared with PB and WPT, respectively. Substrate pH for all substrates ranged 6.0 to 6.9 at 7 DAS and 6.9 to 7.1 at 79 DAS. Rooting percentage was similar among substrates within each species in both experiments. The addition of peatmoss resulted in significantly greater total root length for PB:PM and WPT:PM compared with PB and WPT, respectively, for five of the eight species. Shoot growth was most vigorous for PB:PM compared with the other substrates for all species.

Introduction

Cutting propagation is the most widely used method for cloning nursery and floriculture crops. Factors that affect successful cutting propagation include stock plant quality, timing of cuttings, propagation environment, container size, and rooting substrate. A proper balance of air space and container capacity are critical for healthy root development, so the combined effects of propagation environment (mist application volume/frequency) and container size must be well understood when selecting a propagation substrate (Threadgill et al., 1985).

Sphagnum peatmoss, pine bark, perlite, and vermiculite are commonly used as substrates for propagation, either individually or in combination at various proportions. The high transportation costs and variable annual harvest of Canadian peatmoss have negatively impacted greenhouse crop producers in the United States (Canadian Sphagnum Peat Moss Association, 2011; Fain et al., 2008). Likewise, nursery crop producers have experienced a decline in pine bark supplies and a rise in cost due to pine bark's use as boiler fuel and a decline in the timber market (Lu et al., 2006). Although many alternative substrates have been used to produce quality container-grown crops, such substrates may not be suitable for propagation. Ideally, an alternative substrate component should be cost effective, sustainable, and regionally available.

Alternative substrates should be evaluated within a propagation environment prior to extensive use. Offord et al. (1998) demonstrated coconut coir was a suitable alternative to peatmoss for propagation of *Pultenaea parviflora* Sieber ex DC. Shah et al. (2006) reported silt and sawdust as acceptable substrates for *Ficus binnendijkii* (Miq.) Miq. 'Amstel Queen' cutting propagation, yet a traditional substrate was not included for

comparison. Composts derived from a variety of materials have also been used for cutting propagation. Cuttings of three foliage plant species had similar root development in composts mixed with peatmoss or pine bark and in a standard substrate (Chen et al., 2003). Chong (1999) noted composted municipal waste blended with perlite was a satisfactory substrate for cutting propagation of several woody plant species.

Wood-based substrates have been identified as acceptable supplements or replacements for peatmoss and pine bark in crop production. Wood-based materials derived from pine trees are readily available throughout the southeastern United States and include clean chip residual (bark, limbs, and needles), processed whole pine trees (wood, bark, limbs, and needles), and chipped pine logs (wood and bark). These substrates have been extensively evaluated for greenhouse and nursery crop production (Boyer et al., 2008; Fain et al., 2008; Jackson et al., 2009; Wright and Browder, 2005). Although crops grown in these substrates commonly required additional fertilizer when compared to those grown in traditional substrates (Fain et al., 2008; Jackson et al., 2008; Wright et al., 2008), nutrient and water availability issues can be readily managed during crop production.

The suitability of pine wood-based substrates for cutting propagation has not been investigated. Demonstrating the versatility of these substrates is essential to expanding their commercial availability and use. The objective of the current experiments was to evaluate WPT as a rooting substrate for stem cutting propagation of ornamental crops.

Materials and Methods

Root development of stem cuttings in four substrates was evaluated in two experiments conducted in 2008 and 2009 at the USDA-ARS Thad Cochran Southern

Horticultural Laboratory in Poplarville, MS. The substrates included processed whole pine tree (WPT), pine bark (PB), and each mixed with equal parts peatmoss (PM) by volume to produce two additional substrates (WPT:PM and PB:PM). In the 2008 experiment, WPT was produced from 12-year-old loblolly pine (*Pinus taeda* L.) trees fed through a portable heavy-duty horizontal grinder with 10.19-cm screens (Peterson 4700B; Peterson Pacific Corp. Eugene, OR) in Jan. 2007 and the resulting material was stored outside in full sun. In April 2007, the material was further processed through a hammer mill (C.S. Bell No. 30, Tiffin, OH) fitted with a 0.47-cm screen and stored in 1.8-m^3 polypropylene bulk bags placed under a canopy. In the 2009 experiment, WPT was produced from 20- to 25-cm diameter loblolly pine (*Pinus taeda* L.) trees harvested in Macon County, AL and chipped with a Woodsman Model 334 Biomass Chipper (Woodsman, LLC Farwell, MI) on 19 Jan. 2009. Chips were ground with a Williams Crusher hammer mill (Meteor Mill #40, Williams Patent Crusher and Pulverizer Co. Inc., St. Louis, MO) to pass a 0.95-cm screen.

In both experiments, each substrate was amended with 3.37 $kg \cdot m^{-3}$ 16N–2.6P–10K (5-month formulation; Harrell's, Sylacauga, AL) and 2.97 $kg \cdot m^{-3}$ dolomitic limestone. Individual 6.6-cm square (232-mL) plastic containers (SVD-250; T.O. Plastics Inc., Clearwater, MN) were filled with substrate, completely randomized in 6 carry trays (SPT-250-32-PF; T.O. Plastics Inc.), and placed under a greenhouse mist system to saturate substrates before use.

The plant species used in the two experiments were chosen due to accessibility and to represent a range of plant types and rooting difficulty. In the 2008 experiment, species used were *Chrysanthemum* ×*morifolium* Ramat. 'Dazzling Stacy',

×*Cupressocyparis leylandii* (A.B. Jacks. & Dallim.) Dallim. 'Murray', *Ligustrum japonicum* Thunb. 'Texanum', and *Salvia leucantha* Cav. In the 2009 experiment, species used were *Euonymus fortunei* (Turcz.) Hand.-Mazz., *Evolvulus glomeratus* Nees & Mart. 'Blue Daze', *Persicaria microcephala* (D. Don) H. Gross 'Red Dragon', *Rosa* 'Red Cascade', and *Salvia leucantha*.

Stem cuttings from individual plant species were prepared (Table 1), all species (except *Persicaria*) received a 1-sec basal quick-dip in a 1000 ppm indole-3-butyric acid solution (Dip'N Grow Lite, Dip'N Grow Inc., Clackamas, OR), and a single cutting was inserted into each container for a total of 192 experimental units per species. Intermittent mist was maintained for all species at 8 sec every 15 min from 8:00 AM to 6:00 PM (2008 experiment) and at 5 sec every 15 min from 7:00 AM to 6:00 PM (2009 experiment). Pin-Perfect nozzles (Dramm Corp., Manitowoc, WI) were used in the 2008 experiment and mister nozzles (809 Series; Ein-Dor Co., Israel) were used in the 2009 experiment. In the 2008 experiment, average monthly greenhouse temperature was 20°C (Feb.), 20°C (Mar.), 20°C (Apr.), 22°C (May), and 25°C (June). In the 2009 experiment, average monthly greenhouse temperature was 22°C (Apr.), 22°C (May), 24°C (June), and 27°C (July). Day length ranged from 11 to 13.75 hours in 2008 and 13 to 14.1 hours in 2009.

Rooting periods varied by species, but all cuttings within a species were harvested at the same time (Table 1). Upon harvest, roots (if present) were washed and digitally scanned for analysis (total root length and total root volume) using WinRhizo software (WinRhizo Version 2007d; Regent Instruments Inc., Canada). New shoot growth (if present) was recorded as total shoot length. In the 2009 experiment, substrate solution

Table 1

Plant Type, Cutting Data, Rooting Period, Cutting Description, Auxin Treatment, and Stock Plant Type/Location for Eight Plant Species Used in Two Rooting Experiments.

Species	Plant type	Cutting date	Rooting period	Cutting description	Auxin Treatment[z]	Stock plant type/location
Chrysanthemum ×morifolium 'Dazzling Stacy'	Herbaceous perennial	22 Jan. 2008	52 days	Terminal	1000 ppm IBA	Purchased from Yoder Brothers Inc.
Ligustrum japonicum 'Texanum'	Large shrub	11 Feb. 2008	90 days	Subterminal; semi-hardwood; 2.25-3.5 in	1000 ppm IBA	Landscape planting; MSU[y], Poplarville, MS
Salvia leucantha	Herbaceous perennial	11 Mar. 2008	49 days	Subterminal	1000 ppm IBA	Landscape planting in Rancho Cucamonga, CA
×Cupressocyparis leylandii 'Murray'	Large shrub	14 Feb. 2008	138 days	Subterminal; 4.25 in; brown wood of previous year's growth	1000 ppm IBA	Avery Christmas Tree Farm, Purvis, MS
Euonymous fortunei	Evergreen ground cover	24 Apr. 2009	81 days	Subterminal; 2 in	1000 ppm IBA	Container plants; MSU greenhouse, Poplarville, MS
Evolvulus glomeratus 'Blue Daze'	Herbaceous perennial	24 Apr. 2009	66 days	Subterminal; three node	1000 ppm IBA	Container plants; MSU greenhouse, Poplarville, MS

Table 1 (continued).

Species	Plant type	Cutting date	Rooting period	Cutting description	Auxin Treatment[z]	Stock plant type/location
Persicaria microcephala 'Red Dragon'	Herbaceous perennial	1 May 2009	33 days	Subterminal; single node	none	Container plants; MSU greenhouse, Poplarville, MS
Rosa 'Red Cascade'	Ground cover/ climbing rose	17 Apr. 2009	60 days	Subterminal; single node	1000 ppm IBA	Container plants; MSU shade house, Poplarville, MS
Salvia leucantha	Herbaceous perennial	14 May 2009	40 days	Subterminal	1000 ppm IBA	Container plants; USDA greenhouse, Poplarville, MS

[z]1-sec basal quick-dip; IBA = indole-3-butyric acid (Dip'N Grow Lite)
[y]MSU = Mississippi State University South Mississippi Branch Experiment Station.

was extracted from fallow containers (n = 4) at 7, 29, 52, and 79 days after setting (DAS) the cuttings via the pour-through method (Wright, 1986). Substrate solution pH and electrical conductivity (EC) were analyzed using an Accumet Excel XL50 multiparameter meter (Fisher Scientific, Pittsburgh, PA). Substrate air space, container capacity, total porosity, and bulk density were determined (n = 3) using the North Carolina State University porometer method (Fonteno et al., 1995). Substrate particle size distribution (PSD) was determined by passing 500-mL air-dried substrate samples (n = 3) through 11 sieves (9.5- to 0.05-mm). Sieves were shaken for 3 min with a Ro-Tap (Ro-Tap RX-29; W.S. Tyler, Mentor, OH) sieve shaker (278 oscillations/min, 159 taps/min). Particles collected on each sieve and in the pan (<0.05-mm) were weighed and grouped into three texture classes [coarse (>2.0-mm), medium (<2.0 to >0.5-mm), and fine (<0.5-mm)].

Assumptions of normality and common variance were tested (except for rooting percentage) using the GLM and UNIVARIATE procedures of SAS (Version 9.3; SAS Institute, Inc., Cary, NC). Rooting percentage data were analyzed using the MULTTEST procedure of SAS, with differences between treatment means determined using Fisher's exact test with a permutation adjustment for multiple comparisons ($P < 0.05$). Total root length, total root volume, total shoot length, porometer data, PSD data, pH, and EC were analyzed with linear models using the GLIMMIX procedure of SAS. Differences between treatment means were determined using the Shaffer-Simulated method ($P < 0.05$). Linear contrasts were used to test differences between means for peatmoss-amended substrates (included PB:PM and WPT:PM) and the non-peatmoss-amended substrates (included PB and WPT), and differences between means for whole pine tree

substrates (included WPT and WPT:PM) and pine bark substrates (included PB and PB:PM).

Results and Discussion

Substrate air space ranged from 16.3% (PB:PM) to 35.5% (PB) in the 2008 experiment (Table 2), and from 17.7% (PB:PM) to 31.7% (WPT) in the 2009 experiment (Table 3). Substrate air space was similar between PB and WPT in the 2008 experiment, and likewise between PB:PM and WPT:PM. In the 2009 experiment, PB and WPT had similar substrate air space. Substrate container capacity ranged from 51.8% to 66.6% (2008 experiment) and 53.9% to 60.5% (2009 experiment).

The addition of peatmoss to PB and WPT resulted in reduced air space and increased container capacity in both experiments. Substrate air space was significantly lower in PB:PM and WPT:PM compared with PB and WPT, respectively, in both experiments. In the 2008 experiment, substrate container capacity was significantly greater in PB:PM and WPT:PM compared with PB and WPT, respectively. Total porosity was greatest in PB compared with the other substrates in the 2008 experiment, but similar between PB and PB:PM and between WPT and WPT:PM in the 2009 experiment. Bulk density decreased with the addition of peatmoss to PB, but increased with the addition of peatmoss to WPT in both experiments. Peatmoss has high water retention properties and is routinely used to enhance the container capacity of substrates used for crop production (Robbins and Evans, 2005).

Substrate air space and container capacity are critical factors in propagation substrate selection. Cuttings require sufficient aeration and moisture content for root initiation and

Table 2

Physical Properties[z] *of Pine Bark and Whole Pine Tree Substrates in a 2008 Cutting Propagation Experiment*.

	Air space	Container capacity	Total porosity	Bulk density ($g \cdot cm^{-3}$)
Substrate	----------(% vol)----------			
Pine bark	35.5 a[y]	52.0 b	87.3 a	0.292 a
1 Pine bark : 1 peatmoss	16.3 b	66.6 a	82.9 b	0.264 b
Whole pine tree[x]	31.0 a	51.7 b	82.7 b	0.184 c
1 Whole pine tree : 1 peatmoss	16.5 b	64.8 a	81.3 b	0.219 d
Bark v. Tree[w]	0.1067	0.214	0.0168	<.0001
Peat v. None[v]	<.0001	<.0001	0.0238	0.6703

[z]Data presented as means (n = 3) and obtained using the North Carolina State University porometer method.
[y]Means followed by different letters within columns indicate significant difference at $P < 0.05$ using the Shaffer-Simulated method.
[x]12-year-old whole pine (*Pinus taeda*) trees harvested, chipped, and hammermilled to pass a 0.47-cm screen.
[w]Tested differences between substrates containing pine bark (Bark) and substrates containing whole pine tree substrates (Tree); $P < 0.05$.
[v]Tested differences between substrates amended with peatmoss (Peat) and substrates with no peatmoss (None); $P < 0.05$.

Table 3

Physical Properties[z] *of Pine Bark and Whole Pine Tree Substrates in a 2009 Cutting Propagation Experiment*.

	Air space	Container capacity	Total porosity	Bulk density ($g \cdot cm^{-3}$)
Substrate	----------(% vol)----------			
Pine bark	24.0 b[y]	53.9 b	77.9 b	0.312 a
1 Pine bark : 1 peatmoss	17.7 c	58.0 ab	75.7 b	0.248 b
Whole pine tree[x]	31.7 a	55.5 b	87.1 a	0.163 c
1 Whole pine tree : 1 peatmoss	22.6 b	60.5 a	83.1 a	0.190 d
Bark v. Tree[w]	<.0001	0.1437	<.0001	<.0001

Table 3 (continued).

	Air space	Container capacity	Total porosity	Bulk density ($g \cdot cm^{-3}$)
Substrate	----------(% vol)----------			
Peat v. None[v]	<.0001	0.006	0.0182	0.0235

[z]Data presented as means (n = 3) and obtained using the North Carolina State University porometer method.
[y]Means followed by different letters within columns indicate significant difference at $P < 0.05$ using the Shaffer-Simulated method.
[x]20- to 25-cm diameter whole pine (*Pinus taeda*) trees harvested, chipped, and hammermilled to pass a 0.95-cm screen.
[w]Tested differences between substrates containing pine bark (Bark) and substrates containing whole pine tree substrates (Tree); $P < 0.05$.
[v]Tested differences between substrates amended with peatmoss (Peat) and substrates with no peatmoss (None); $P < 0.05$.

subsequent growth. A continuous film of water surrounding the cutting base would restrict cellular respiration and prevent root development, and waterlogged substrates may provide an ideal environment for pathogens to persist. Substrate air space between 15% and 40% is recommended for adequate aeration during propagation, while substrate container capacity between 20% and 60% is recommended for adequate water retention (Hartmann et al., 1990; Threadgill et al., 1985). Substrate air space was within the recommended range for substrates used in the 2008 and 2009 experiments, while substrate container capacity was slightly greater than the recommended range for PB:PM and WPT:PM in the 2008 experiment.

Pine bark substrate had the lowest proportion of fine particles followed by WPT, PB:PM, and WPT:PM in both experiments (Tables 4 and 5). The proportion of fine particles doubled for PB:PM and WPT:PM compared with PB and WPT, respectively. It has been reported that substrate particles less than 0.5 mm can have a significant effect on substrate air space and container capacity (Jackson et al., 2010; Owen and Altland, 2008).

The greater proportion of fine particles most likely resulted in the greater substrate container capacity and lower substrate air space of the substrates amended with peatmoss.

Rooting percentage was similar among substrates within each species in both experiments (Table 6). Rooting percentage was 90% or greater for all species except *Ligustrum*. The high rooting success is an indication that substrate did not have a significant effect on root initiation or rooting percentage in either experiment. Proper selection of propagation material (stock plant age, cutting type, seasonal timing, etc.) is critical in order to reduce variability during root initiation. Additionally, stem cuttings require adequate endogenous nutrients for root initiation and emergence so the relative health of the stock plants is also an important factor to consider when selecting cuttings (Hartmann et al., 1990).

Table 4

Particle Size Distribution[z] *of Pine Bark and Whole Pine Tree Substrates in a 2008 Cutting Propagation Experiment*.

	Substrate			
Sieve opening (mm)	Pine bark	1 Pine bark : 1 peatmoss (v:v)	Whole pine tree[y]	1 Whole pine tree : 1 peatmoss (v:v)
6.3	10.1	6.1	0.1	0.0
3.4	29.7	15.4	8.6	5.4
2.4	17.3	9.4	21.4	13.0
2.0	6.6	3.9	10.2	6.0
1.4	11.3	10.0	16.3	10.7
1.0	6.1	9.1	10.3	8.0
0.5	7.3	18.3	13.3	16.0
0.25	7.3	17.1	9.9	16.0
0.106	3.0	7.9	7.5	17.2

Table 4 (continued).

Sieve opening (mm)	Substrate: Pine bark	1 Pine bark : 1 peatmoss (v:v)	Whole pine tree[y]	1 Whole pine tree : 1 peatmoss (v:v)
0.053	0.6	1.9	1.8	5.9
Pan	0.6	0.9	0.6	1.7
Texture class[x]				
Coarse	63.8 a[w]	34.9 c	40.2 b	24.4 d
Medium	24.8 d	37.4 b	40.0 a	34.8 c
Fine	11.5 d	27.8 b	19.8 c	40.9 a

[z]Data presented as means (n = 3) of percent of particles collected on sieves and in pan.
[y]12-year-old whole pine (*Pinus taeda*) trees harvested, chipped, and hammermilled to pass a 0.47-cm screen.
[x]Texture classes: coarse (>2.0-mm), medium (<2.0 to >0.5-mm), and fine (<0.5-mm).
[w]Means followed by different letters within rows indicate significant difference at $P < 0.05$ using the Shaffer-Simulated method.

Table 5

Particle Size Distribution[z] of Pine Bark and Whole Pine Tree Substrates in a 2009 Cutting Propagation Experiment.

Sieve opening (mm)	Substrate: Pine bark	1 Pine bark : 1 peatmoss (v:v)	Whole pine tree[y]	1 Whole pine tree : 1 peatmoss (v:v)
6.3	20.4	16.7	0.0	2.2
3.4	17.9	15.1	1.7	5.2
2.4	10.1	7.7	9.1	7.0
2.0	3.8	2.7	8.8	5.1
1.4	9.9	7.6	24.4	15.3
1.0	8.1	6.6	17.7	11.5
0.5	14.3	14.5	21.1	18.5
0.25	8.6	12.9	11.3	15.9

Table 5 (continued).

Sieve opening (mm)	Substrate: Pine bark	1 Pine bark : 1 peatmoss (v:v)	Whole pine tree[y]	1 Whole pine tree : 1 peatmoss (v:v)
0.106	4.6	11.3	4.9	14.0
0.053	1.4	3.5	0.8	4.1
Pan	0.9	1.5	0.2	1.3
Texture class[x]				
Coarse	52.2 a[w]	42.1 b	19.6 c	19.5 c
Medium	32.2 c	28.7 d	63.2 a	45.3 b
Fine	15.5 d	29.2 b	17.2 c	35.2 a

[z]Data presented as means (n = 3) of percent of particles collected on sieves and in pan.
[y]20- to 25-cm diameter whole pine (*Pinus taeda*) trees harvested, chipped, and hammermilled to pass a 0.95-cm screen.
[x]Texture classes: coarse (>2.0-mm), medium (<2.0 to >0.5-mm), and fine (<0.5-mm).
[w]Means followed by different letters within rows indicate significant difference at $P < 0.05$ using the Shaffer-Simulated method.

Table 6

Mean Rooting Percentage of Cuttings from Eight Species Rooted in Pine Bark and Whole Pine Tree Substrates.

Species	Substrate: Pine bark	1 Pine bark : 1 peatmoss (v:v)	Whole pine tree[z]	1 Whole pine tree : 1 peatmoss (v:v)
2008 experiment				
Chrysanthemum ×*morifolium* 'Dazzling Stacy'	100 a[y]	100 a	100 a	100 a
×*Cupressocyparis leylandii* 'Murray'	94 a	96 a	90 a	96 a
Ligustrum japonicum 'Texanum'	88 a	83 a	75 a	75 a

Table 6 (continued).

Species	Substrate: Pine bark	1 Pine bark : 1 peatmoss (v:v)	Whole pine tree[z]	1 Whole pine tree : 1 peatmoss (v:v)
Salvia leucantha	100 a	100 a	100 a	100 a
2009 experiment				
Euonymus fortunei	100 a	100 a	100 a	100 a
Evolvulus glomeratus 'Blue Daze'	100 a	100 a	94 a	94 a
Persicaria microcephala 'Red Dragon'	100 a	100 a	100 a	100 a
Rosa 'Red Cascade'	94 a	98 a	94 a	100 a
Salvia leucantha	100 a	100 a	100 a	100 a

[z]12-year-old whole pine (*Pinus taeda*) trees harvested, chipped, and hammermilled to pass a 0.47-cm screen (2008 experiment); 20- to 25-cm diameter whole pine (*P. taeda*) trees harvested, chipped, and hammermilled to pass a 0.95-cm screen (2009 experiment).
[y]Means followed by different letters within rows indicate significant difference at $P < 0.05$ using Fisher's exact test with a permutation adjustment for multiple comparisons.

Root development response to substrate varied by species in both experiments. Root development in WPT was less vigorous compared with the other substrates, yet the differences were not always significant. Total root length (Table 7) and total root volume (Table 8) were similar in WPT and PB for *Persicaria*, while total root length was similar among all substrates for *Salvia* in the 2009 experiment. Total root length and total root volume was similar between PB and WPT:PM for *Euonymus* and *Evolvulus*. Maximum total root length was observed in PB:PM for all species except *Cupressocyparis* and *Salvia* (2009 experiment). In most cases, results for total root volume mirrored the results for total root volume within a species.

The addition of peatmoss resulted in significantly greater total root length for PB:PM compared with PB for *Chrysanthemum*, *Euonymus*, *Evolvulus*, *Persicaria*, and *Rosa*. The increased total root length between PB and PB:PM ranged from 9% (*Chrysanthemum*) to 174% (*Rosa*). Similarly, significantly greater total root length in WPT:PM compared with WPT occurred for *Salvia* (2008 experiment), *Euonymus*, *Evolvulus*, *Persicaria*, and *Rosa*. The increased total root length between WPT and WPT:PM ranged from 26% (*Salvia* - 2008 experiment) to 337% (*Rosa*).

Shoot growth was most vigorous for PB:PM compared with the other substrates for all species (Table 9). A positive response for total shoot length was observed in PB:PM and WPT:PM, compared with PB and WPT, respectively.

Peatmoss has a greater water holding capacity and lower aeration compared with pine bark and wood-based substrates (Raviv and Leith, 2008). Therefore, greater substrate container capacity and lower substrate air space was expected for PB:PM and WPT:PM. High rooting percentages and subsequent root development was an indication that sufficient water content and adequate aeration was present in all substrates and maintained within the propagation system used for these experiments.

Disparities in root development among substrates are rarely attributed to differences in physical properties, unless extreme values are observed. Typically, low substrate air space (<10%) and high substrate container capacity (>60%) are considered undesirable for cutting propagation due to low oxygen content (Chen et al., 2003). Substrate air space above the recommended values, or a high proportion of coarse particles, may provide inadequate moisture or hinder contact between roots and substrate particles, but such conditions have not been widely reported for cutting propagation.

Table 7

Mean Total Root Length (cm) of Cuttings from Eight Species Rooted in Pine Bark and Whole Pine Tree Substrates.

	Substrate					
Species	Pine bark	1 Pine bark : 1 peatmoss (v:v)	Whole pine tree[z]	1 Whole pine tree : 1 peatmoss (v:v)	Bark v. Tree[y]	Peat v. None[x]
2008 experiment						
Chrysanthemum ×morifolium 'Dazzling Stacy'	1353 b[w]	1481 a	1046 c	1051 c	<.0001	0.0635
×Cupressocyparis leylandii 'Murray'	570 a	379 b	249 c	295 c	<.0001	0.0144
Ligustrum japonicum 'Texanum'	474 a	485 a	277 b	330 b	<.0001	0.3616
Salvia leucantha	990 a	1036 a	623 c	790 b	<.0001	0.0011
2009 experiment						
Euonymus fortunei	165 b	226 a	111 c	165 b	<.0001	<.0001
Evolvulus glomeratus 'Blue Daze'	752 b	1173 a	462 c	907 b	<.0001	<.0001
Persicaria microcephala 'Red Dragon'	1055 b	1469 a	916 b	1431 a	0.0608	<.0001
Rosa 'Red Cascade'	236 c	647 a	91 d	398 b	<.0001	<.0001
Salvia leucantha	801 a	793 a	632 a	738 a	0.0231	0.3179

[z]12-year-old whole pine (*Pinus taeda*) trees harvested, chipped, and hammermilled to pass a 0.47-cm screen (2008 experiment); 20- to 25-cm diameter whole pine (*P. taeda*) trees harvested, chipped, and hammermilled to pass a 0.95-cm screen (2009 experiment).

[y]Tested differences between substrates containing pine bark (Bark) and substrates containing whole pine tree substrates (Tree); $P < 0.05$.

[x]Tested differences between substrates amended with peatmoss (Peat) and substrates with no peatmoss (None); $P < 0.05$.

[w]Means followed by different letters within rows indicate significant difference at $P < 0.05$ using the Shaffer-Simulated method.

Table 8

Mean Total Root Volume (cm^3) of Cuttings from Eight Species Rooted in Pine Bark and Whole Pine Tree Substrates.

	Substrate					
Species	Pine bark	1 Pine bark : 1 peatmoss (v:v)	Whole pine tree[z]	1 Whole pine tree : 1 peatmoss (v:v)	Bark v. Tree[y]	Peat v. None[x]
2008 experiment						
Chrysanthemum ×morifolium 'Dazzling Stacy'	2.16 a[w]	2.36 a	1.81 b	1.9 b	<.0001	0.0411
×Cupressocyparis leylandii 'Murray'	1.75 a	1.20 b	0.79 c	0.94 c	<.0001	0.025
Ligustrum japonicum 'Texanum'	3.41 a	3.24 a	2.18 b	2.31 b	<.0001	0.947
Salvia leucantha	2.00 a	2.01 a	1.38 b	1.66 ab	<.0001	0.2115
2009 experiment						
Euonymus fortunei	0.21 b	0.28 a	0.15 c	0.22 b	<.0001	<.0001
Evolvulus glomeratus 'Blue Daze'	1.12 b	1.80 a	0.69 c	1.35 b	0.0003	<.0001
Persicaria microcephala 'Red Dragon'	0.56 b	0.85 a	0.53 b	0.96 a	0.3499	<.0001
Rosa 'Red Cascade'	0.31 c	0.84 a	0.15 d	0.54 b	<.0001	<.0001
Salvia leucantha	1.28 a	1.33 a	0.87 b	1.16 ab	0.0059	0.0988

[z]12-year-old whole pine (*Pinus taeda*) trees harvested, chipped, and hammermilled to pass a 0.47-cm screen (2008 experiment); 20- to 25-cm diameter whole pine (*P. taeda*) trees harvested, chipped, and hammermilled to pass a 0.95-cm screen (2009 experiment).

[y]Tested differences between substrates containing pine bark (Bark) and substrates containing whole pine tree substrates (Tree); $P < 0.05$.

[x]Tested differences between substrates amended with peatmoss (Peat) and substrates with no peatmoss (None); $P < 0.05$.

[w]Means followed by different letters within rows indicate significant difference at $P < 0.05$ using the Shaffer-Simulated method.

In the 2008 experiment, root development was superior in PB:PM compared with WPT:PM, despite similar substrate air space. In the 2009 experiment, root development was similar (for most species) in PB and WPT:PM corresponding to similarities in substrate air space. As a result, differences in root development cannot be attributed solely to substrate air space. Although substrate nutrient content is not a critical factor during root initiation, newly developed roots require an external source of nutrients for continued growth. Substrate cation exchange capacity refers to how effectively mineral nutrients (cations specifically) are bound to the substrate particles. Peatmoss and aged pine bark have a greater cation exchange capacity compared with wood-based substrates (Jackson et al., 2010; Raviv and Leith, 2008). Nitrogen immobilization is another issue associated with wood-based substrates. Less nitrogen is available for plant absorption due to high microbial activity (Boyer et al., 2012).

In the 2009 experiment, substrate pH for all substrates ranged 6.0 to 6.9 at 7 DAS and 6.9 to 7.1 at 79 DAS (Table 10). Substrate pH was above the recommended range (5.5 to 6.5) for all substrates at 29 DAS and thereafter. An increase in substrate pH was observed between 7 and 29 DAS for all substrates, yet remained relatively stable within substrates from 29 to 79 DAS. Substrate EC was in an acceptable range for all substrates at 7 DAS, but was in the low range for all substrates at 29 DAS and thereafter. Substrate EC was similar among all substrates throughout the experiment. Changes in substrate pH and EC within the first 29 days are likely due to the nutrient release rate of the controlled-release fertilizer (Merhaut et al., 2006).

During propagation, nutrients are more readily leached from the substrate due to high substrate porosity and excessive mist application rates (Santos et al., 2011).

Table 9

Mean Total Shoot Length (cm) of Cuttings from Eight Species Rooted in Pine Bark and Whole Pine Tree Substrates.

	Substrate					
Species	Pine bark	1 Pine bark : 1 peatmoss (v:v)	Whole pine tree[z]	1 Whole pine tree : 1 peatmoss (v:v)	Bark v. Tree[y]	Peat v. None[x]
2008 experiment						
Chrysanthemum ×morifolium 'Dazzling Stacy'	NA	NA	NA	NA	NA	NA
×Cupressocyparis leylandii 'Murray'	NA	NA	NA	NA	NA	NA
Ligustrum japonicum 'Texanum'	NA	NA	NA	NA	NA	NA
Salvia leucantha	30.9 b[w]	37.1 a	23.7 c	31.6 b	<.0001	<.0001
2009 experiment						
Euonymus fortunei	NA	NA	NA	NA	NA	NA
Evolvulus glomeratus 'Blue Daze'	18.3 b	30.0 a	10.5 c	26.6 a	0.0016	<.0001
Persicaria microcephala 'Red Dragon'	16.9 b	31.0 a	13.2 b	26.3 a	0.01	<.0001
Rosa 'Red Cascade'	6.0 b	11.7 a	3.3 b	7.0 b	0.0024	0.0001
Salvia leucantha	15.3 a	15.5 a	11.6 b	14.8 a	0.0036	0.0223

[z]12-year-old whole pine (*Pinus taeda*) trees harvested, chipped, and hammermilled to pass a 0.47-cm screen (2008 experiment); 20- to 25-cm diameter whole pine (*P. taeda*) trees harvested, chipped, and hammermilled to pass a 0.95-cm screen (2009 experiment).

[y]Tested differences between substrates containing pine bark (Bark) and substrates containing whole pine tree substrates (Tree); $P < 0.05$.

[x]Tested differences between substrates amended with peatmoss (Peat) and substrates with no peatmoss (None); $P < 0.05$.

[w]Means followed by different letters within rows indicate significant difference at $P < 0.05$ using the Shaffer-Simulated method.

Table 10

Substrate pH and Electrical Conductivity (EC) of Pine Bark and Whole Pine Tree Substrates in Fallow Containers at 7, 29, 52, and 79 Days After Setting (DAS) Cuttings in a 2009 Cutting Propagation Experiment.

	7 DAS		29 DAS		52 DAS		79 DAS	
Substrate	pH	EC ($dS \cdot m^{-1}$)	pH	EC ($dS \cdot m^{-1}$)	pH	EC ($dS \cdot m^{-1}$)	pH	EC ($dS \cdot m^{-1}$)
Pine bark	6.9 a[z]	0.47 a	7.2 a	0.19 a	7.1 a	0.21 a	7.1 a	0.15 ab
1 Pine bark : 1 peatmoss	6.1 c	0.81 a	7.1 b	0.20 a	6.9 ab	0.18 a	7.1 a	0.12 b
Whole pine tree[y]	6.4 b	0.91 a	7.2 a	0.18 a	7.1 a	0.23 a	7.0 a	0.17 a
1 Whole pine tree : 1 peatmoss	6.0 c	0.84 a	7.0 b	0.16 a	6.7 b	0.18 a	6.9 a	0.13 b

[z]Means followed by different letters within columns indicate significant difference at $P < 0.05$ (n = 4) using the Shaffer-Simulated method.

[y]20- to 25-cm diameter whole pine (*Pinus taeda*) trees harvested, chipped, and hammermilled to pass a 0.95-cm screen.

Although water and nutrient availability can be readily managed in wood-based substrates used for crop production, such issues are more difficult in a propagation environment. The combined effects of leaching, low cation exchange capacity, and reduced nitrogen availability most likely contributed to less vigorous root and shoot growth in WPT.

I demonstrated a range of plant species can be propagated from stem cuttings in WPT substrates. Combinations of WPT and peatmoss or other organic component with a high cation exchange capacity may be required for optimum root development in WPT substrates. A wealth of information is available regarding crop production in wood-based substrates, but stem cutting propagation in such substrates has not been evaluated. A single, universal propagation substrate has not been developed due to the unique set of factors associated with species variation and individual cultural practices. Rooting success is ultimately determined by the combined effects of container size, mist application rate and frequency, and substrate. Development of guidelines for propagation in WPT substrates would benefit manufacturers and growers interested in alternatives to traditional substrates.

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CHAPTER III

PHYTOTOXICITY ASSESSMENT OF WHOLE PINE TREE SUBSTRATES

Abstract

Reduced plant growth in wood-based substrates has been attributed to a variety of factors, including phytotoxity, yet a detailed method for evaluating the phytotoxic potential of wood-based substrates has not been identified. The objective of this study was to evaluate the Phytotoxkit and seedling growth test for identifying potential phytotoxicity in horticultural substrates and to identify factors affecting seed germination and seedling development in nonamended whole pine tree substrates. Substrates evaluated using the Phytotoxkit included a reference soil, aged (WPTA) and fresh (WPTF) whole pine tree, aged (PNA) and fresh (PNF) pine needles, pine bark (PB), peatmoss (PM), and saline pine bark (SPB). Substrates evaluated using the seedling growth test included WPTA, WPTF, PB, and a peat-lite (PL) substrate. Substrate physical (air space, container capacity, total porosity, and bulk density) and chemical properties (pH and soluble salt concentration), along with a complete mineral analysis, were determined for all substrates. Seed germination rate (%) and total root length (mm) were evaluated for 3 biosensor species (cress, mustard, and sorghum) in the Phytotoxkit experiments (2010 and 2011). Seed germination rate was similar among all substrates, except for cress in PNF. Total root length was inhibited by PNF for cress, but varied among the substrates for mustard and sorghum. Total root length was similar or greater in WPTA compared with PM for all species. The only observed statistical differences between WPTA and WPTF were for sorghum total root length in 2010. Inhibitory effects associated with phytotoxic compounds were only observed with PNF. Seedling

emergence rate (%) and total root length (cm) were evaluated for 3 biosensor species (lettuce, tomato, and oat) in the seedling growth experiments (2010 and 2011). Seedling emergence rate varied among substrates, but was substantially greater in PL and WPTA compared with PB and WPTF in the 2010 experiment. Seedling emergence rate was similar among all substrates for lettuce and oat in the 2011 experiment. Total root length was greatest in PL compared to the other substrates for all species. Peat-lite substrate had significantly lower air space and greater container capacity compared with the other substrates. Differences in seed germination/emergence rate and seedling root length could not be attributed to phytotoxic compounds in the whole pine tree substrates. A combination of nutrient and water availability is likely responsible for reduced root development in PB, WPTA, and WPTF in the seedling growth test.

Introduction

Wood-based materials have been evaluated extensively as alternative substrate components for nursery and greenhouse crop production. A wood-based material is predominately composed of wood (secondary xylem), yet may contain various proportions of other plant parts including bark and leaves. Pine trees have been the prominent subject matter for such scientific evaluations in the United States, particularly in the southeastern United States where pine plantations are widespread. Ongoing interest in alternative substrates has sparked similar research efforts for evaluating a wide range of plant species.

Nursery and/or greenhouse crop production has been demonstrated in wood-based substrates composed of loblolly pine (Fain et al., 2008; Wright et al., 2008), spruce (Gruda and Schnitzler, 2004), melaleuca (Brown and Duke, 2000; Ingram and Johnson,

1983), and various other tree species (Murphy et al., 2011; Rau et al., 2006). Nevertheless, reduced plant performance in high wood content substrates (compared with pine bark and/or peat-based substrates) has been observed and linked to various factors. Nitrogen immobilization has been reported in wood-based substrates, due to high levels of microbial activity (Gruda et al., 2000; Jackson et al., 2009). In order to offset reduced nitrogen availability in wood-based substrates, supplemental nitrogen applications can be used to provide sufficient concentrations for both microbial and plant requirements (Fain et al., 2008; Jackson et al., 2008). Less than ideal water and nutrient retention properties have also been reported in wood-based substrates, although these issues can be minimized by processing materials into a finer particle size or blending with peatmoss (Fain et al., 2008; Jackson et al., 2010). Although nutrient and water availability can be readily managed in wood-based substrates, concerns persist about potential phytotoxicity due to compounds present in wood.

Phytotoxicity may be a function of certain organic or inorganic compounds found in soil, compost, or other substrate used for growing plants. In substrates composed of various tree components, phytotoxicity may occur due to the presence of organic phenolic and terpenoid compounds or inorganic metal compounds (Harkin and Rowe, 1971; Sjöström, 1993). Seed germination tests and seedling growth tests are universally accepted procedures for determining the phytotoxic potential of a material. Such tests are simple to conduct, relatively inexpensive (compared to laboratory chemical analysis), and reproducible. Chemical reactions detrimental to plant development may be observed with these tests, whereas such a response would not be obvious simply by reviewing a chemical analysis. Although a single standard has not been identified for the germination

test, the most common procedures involve seeds exposed to a liquid extract of a substrate or seeds placed in direct contact with a substrate or substrate solution (Kapanen and Itävaara, 2001; Ortega et al., 1996; Archambault et al., 2004; Macias et al., 2000). The direct contact method accounts for any phytotoxic compounds bound to the solid particles, in addition to those dissolved in water (Naasz et al., 2009).

A wealth of knowledge is available on using seed germination and seedling growth tests for evaluating compost maturity and quality (Emino and Warman, 2004; Hartz and Giannini, 1998; Kapanen and Itävaara, 2001; Murillo et al., 1995), yet little information exists on such tests for the phytotoxic effects of non-composted tree components such as wood, bark, and leaves. Rau et al. (2006) evaluated tomato seedling growth after 30 days in wood substrates derived from five tree species and concluded plant dry weight decreased as the polyphenolic concentration of the wood increased. Ortega et al. (1996) demonstrated that higher phenolic levels in oak bark resulted in the significantly reduced seedling growth of six vegetable species. In the same study, two types of germination bioassays, liquid extract and direct contact, were conducted to determine their applicability for determining potential phytotoxicity. In both methods, seed germination was negatively affected in the presence of greater phenolic compound concentrations. The investigators concluded direct contact was the optimum method due to its similarity to actual production procedures.

Gruda et al. (2009) treated tomato and lettuce seeds with leachate extracted from a pine tree substrate and found that washing the substrate reduced the phytotoxic effects, indicated by germination rate and radicle growth. Nektarios et al. (2005) investigated the allelopathic effects of pine needles in seed germination and seedling growth tests. In this

study, the phytotoxic effect was more pronounced for fresh pine needles compared with senesced and decaying pine needles. Similar results were reported by Gaches et al. (2011a), wherein lettuce seedlings exhibited reduced growth when exposed to fresh pine needle leachate compared with exposure to aged pine needle leachate. In all three studies, the investigators posited that phytotoxic compounds within the wood/needles were responsible for the reduced germination and growth rates.

Factors other than substrate chemical properties may also be responsible for reduced seed germination and seedling growth. Naasz et al. (2009) conducted lettuce seed germination and tomato seedling growth tests using the bark of seven tree species. The degree of phytotoxicity varied among the barks, but the investigators concluded that air space in the bark substrate, rather than select chemical and biochemical properties, had the greatest effect on plant growth.

Reduced plant growth in pine tree-based substrates has been attributed to a variety of factors, but here I focus on the phytotoxic potential of these materials. Seed germination and seedling growth tests can be readily adapted for screening horticultural substrates. Seed germination tests are used for detecting phytotoxicity associated with substrate chemical properties, whereas seedling growth tests account for phytotoxicity associated with the individual or combined effects of substrate chemical and physical properties (Gong et al., 2001; Naasz et al., 2009). Seeds have nutritional reserves that will support growth for short periods after germination. As a result, nonamended substrates can be evaluated, minimizing the number of variables involved in plant development.

A commercially available seed germination test, the Phytotoxkit (MicroBioTests Inc., Belgium), is a standardized, sensitive, rapid, reproducible, and cost-effective

procedure for determining the potential phytotoxicity of a solid substrate. The Phytotoxkit includes all the hardware required to perform a phytotoxicity test. It also includes a sterile reference soil (control) and seeds of three test species, specifically selected for rapid germination and sensitivity to a variety of factors. The Phytotoxkit is designed for contact between the seed and substrate solution, and for direct observation and measurement of germinated seeds and root/shoot growth. The Phytotoxkit test may be a useful laboratory procedure for scientists evaluating alternative horticultural substrates.

The objectives of this study were to (1) evaluate the Phytotoxkit and seedling growth test for identifying potential phytotoxicity in horticultural substrates; and (2) identify factors affecting seed germination and seedling development in non-amended whole pine tree substrates.

Materials and Methods

Two biological tests (Phytotoxkit and seedling growth) were used to assess potential phytotoxicity in whole pine tree substrates compared with traditional substrate components. Each test was conducted as an individual experiment in 2010 and in 2011 (four experiments total) at the USDA-ARS Thad Cochran Southern Horticultural Laboratory in Poplarville, MS.

Phytotoxkit test - 2010

The Phytotoxkit was supplied with a reference soil (RS) and seeds of three test plant species, one monocot species [sorghum, *Sorghum saccharatum* (L.) Moench] and two dicot species (cress, *Lepidium sativum* L. and mustard, *Sinapis alba* L.). Seed germination rates of the selected test species were determined prior to the experiment

[cress (82%); mustard (90%); sorghum (78%)]. Substrates evaluated with the Phytotoxkit included aged (WPTA) and fresh (WPTF) whole pine tree, aged (PNA) and fresh (PNF) pine needles, saline pine bark (SPB), and RS. Whole pine tree substrates were produced from 20- to 25-cm diameter loblolly pine (*Pinus taeda* L.) trees harvested and chipped on 29 Sept. 2009 (WPTA) and 26 May 2010 (WPTF) in Macon County, AL, then ground with a Williams Crusher hammer mill (Meteor Mill #40; Williams Patent Crusher and Pulverizer Co. Inc., St. Louis, MO) to pass a 0.95-cm screen. Pine needles were collected from a 12-year-old loblolly pine plantation in Stone County, MS, either directly from trees (PNF) or from the ground (PNA) surrounding the same trees. Pine needles were hammer-milled (model 30; C.S. Bell Co., Tiffin, OH) to pass a 0.47-cm (PNA) or 0.95-cm (PNF) screen. Saline pine bark [pine bark soaked in a sodium chloride (NaCl) solution (16 $dS \cdot m^{-1}$ for cress and sorghum; 30 $dS \cdot m^{-1}$ for mustard) overnight] was included to produce a negative effect on seed germination and initial root growth for verification of the procedure.

All substrates were passed through a 2-mm sieve to eliminate coarse particles. Three 95-mL samples (loosely filled) of each substrate were collected in coffee-filter-lined containers (T.O. Plastics SVD-250), bottom-saturated to the upper substrate surface with deionized water (NaCl solution used for SPB) for 1 hour, and drained. Samples were transferred to individual test plates (3 plates per substrate) and covered with filter paper onto which 10 seeds of a test species were placed in a single row. A clear plastic cover was placed on each test plate, then test plates were incubated vertically in a dark growth chamber at 25 °C for 4 (cress) or 5 (mustard and sorghum) days. Plates were digitally scanned and analyzed using ImageTool software (ImageTool Version 3.0; UTHSA, San

Antonio, TX). Data collected included seed germination rate (%) and total root length (mm). A complete laboratory soil test analysis was conducted on all substrates to determine pH, soluble salt concentration, and mineral nutrient content.

Germination data were analyzed with generalized linear models using the binary distribution and a logit link function using the GLIMMIX procedure of SAS (Version 9.3; SAS Institute, Inc., Cary, NC). Total root length data were analyzed with linear models using the GLIMMIX procedure of SAS. The ten seeds in each plate were analyzed as subsamples. Differences between treatment means were determined using the Shaffer-Simulated method ($P < 0.05$). Data from SPB was not included in the overall statistical analyses, but separate statistical analyses were conducted to test the sensitivity of the Phytotoxkit by comparing seed germination rate and total root length between RS and SPB.

Phytotoxkit test - 2011

A separate Phytotoxkit experiment was conducted in 2011, with design and procedural differences described below. Seed germination rates of the selected test species were determined prior to the experiment [cress (90%); mustard (94%); sorghum (96%)]. Substrates included WPTA, WPTF, PNA, PNF, pine bark (PB), peatmoss [(PM); Fertilome Pure Canadian Peat Moss; Cheek Garden Products, Austin, TX], SPB, and RS. Whole pine tree substrates were produced from 5.0- to 6.4-cm diameter *P. taeda* trees harvested in Pearl River County, MS. The main stems were chipped on 29 July 2010 (WPTA) and 14 Mar. 2011 (WPTF) with a wood chipper (Liberty WC-6; Mesa, AZ) and a combination of 9 chipped stems : 1 needles (by weight) was ground with a hammer mill (Model 30; C.S. Bell Co., Tiffin, OH) to pass a 0.63-cm screen. Pine needles were

collected on 14 Mar. 2011 directly from trees (PNF) or from the ground (PNA) surrounding the same trees and hammer-milled to pass a 0.47-cm or 1.2-cm screen, for PNA and PNF, respectively. Saline pine bark was prepared using a NaCl concentration of 16 $dS \cdot m^{-1}$ for cress and 30 $dS \cdot m^{-1}$ for mustard and sorghum. Test plates were incubated at 25 °C for 5 (cress and sorghum) or 6 (mustard) days.

Seedling Growth Test - 2010

Substrates included WPTA, WPTF, PB, and a peat-lite (PL) mix [3 peatmoss : 1 perlite (Coarse grade; SunGro Horticulture, Bellevue, WA) : 1 vermiculite (Medium grade; SunGro Horticulture, Bellevue, WA); by volume]. Pine bark was passed through a 5-mm screen, while WPTA and WPTF were prepared as described in the 2010 Phytotoxkit test. Individual cells were cut from 72-cell sheets (PROP-72-RD; T.O. Plastics Inc., Clearwater, MN) and filled with substrate (36 replications per substrate), randomized in 72-cell trays (36 cells/tray), and saturated under mist. Two seeds of a single test plant species (lettuce, *Lactuca sativa* L. 'Buttercrunch' and tomato, *Solanum lycopersicum* L. 'Better Boy') were sown in each cell. Plant species were chosen based on standards developed for conducting phytotoxicity tests using plants as the test species (Kapanen and Itävaara, 2001; U.S. E.P.A., 1996). Seed germination rates of the selected test species were determined prior to the experiment [lettuce (87%) and tomato (95%)].Trays were grouped by species and placed in separate growth chambers (25 °C day/21 °C night) with no light until germination occurred, thereafter receiving a 14-h light (375 – 415 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and 10-h dark photoperiod. All trays were hand-watered as needed and all 4 trays of individual test species were watered equally.

At 11 (tomato) and 12 (lettuce) days after sowing (DAS), seedling emergence rate was recorded and seedlings were thinned to 1 per cell. At 35 (tomato) and 39 (lettuce) DAS, roots were washed and digitally scanned for analysis (total root length) using WinRhizo software (WinRhizo Version 2007d; Regent Instruments Inc., Canada). Substrate air space, container capacity, total porosity, and bulk density were determined using the North Carolina State University porometer method (Fonteno et al., 1995). A complete laboratory soil test analysis was conducted on all substrates to determine pH, soluble salt concentration, and mineral nutrient content.

Seed emergence rate was analyzed with generalized linear models using the binary distribution and a logit link function using the GLIMMIX procedure of SAS. Total root length and porometer data were analyzed with linear models using the GLIMMIX procedure of SAS. Differences between treatment means were determined using the Shaffer-Simulated method ($P < 0.05$).

Seedling Growth Test - 2011

A separate seedling growth experiment was conducted in 2011, with design and procedural differences described below. Substrates included WPTA and WPTF (prepared as described in the 2011 Phytotoxkit test), PB (passed through a 5-mm screen), and PL [3 peatmoss (Fertilome Natural Organic Pure Canadian Sphagnum Peat Moss) : 1 perlite (Coarse grade; SunGro Horticulture, Bellevue, WA) : 1 vermiculite (Medium grade; SunGro Horticulture, Bellevue, WA)]. Test plant species were lettuce (*Lactuca sativa* L.'Green Ice'), oat (*Avena sativa* L. 'Jerry'), and tomato (*Solanum lycopersicum* L. 'Brandywine'). Seed germination rates of the selected test species were determined prior to the experiment [lettuce (100%), oat (74%), and tomato (100%)]. Seeds were covered

with 2.5 mL of substrate, flats were placed in growth chambers (22 °C day/18 °C night for oat and lettuce; 25 °C day/21 °C night for tomato) and subjected to a 14-h light (349 – 387 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and 10-h dark photoperiod. Seedling emergence rate was recorded at 8 (oat) or 9 (lettuce and tomato) DAS and seedlings were thinned to 1 per cell. The experiment was terminated at 14 (oat), 25 (tomato), or 33 (lettuce) DAS and roots were washed and digitally scanned for analysis.

Seed emergence rate was analyzed with generalized linear models using the binary distribution and a logit link function using the GLIMMIX procedure of SAS. Total root length and porometer data were analyzed with linear models using the GLIMMIX procedure of SAS. Differences between treatment means were determined using the Shaffer-Simulated method ($P < 0.05$).

Results and Discussion

Separate statistical analyses were conducted to compare the sensitivity of the Phytotoxkit, comparing seed germination rate and total root length between RS and SPB. Significant inhibition of germination rate was observed for cress in 2010 and for mustard in both experiments (Table 11). Total root length was inhibited for mustard and sorghum in both experiments. These results verify salinity may not be an issue in the substrates evaluated in the experiments discussed within, although Phytotoxkit could be used to identify other sources of phytotoxicity. The Phytotoxkit has been used in previous studies for evaluating the phytotoxic potential of trace and heavy metals in sewage sludge (Oleszczuk, 2010) and herbicide contaminated soil (Sekutowski and Sadowski, 2009).

Table 11

Mean Seed Germination Rate and Total Root Length of Three Biosensor Species Using a Phytotoxkit.

	Germination rate (%)			Total root length (mm)		
Substrate	Cress	Mustard	Sorghum	Cress	Mustard	Sorghum
			2010 Experiment			
Reference soil	97 a[z]	100 a	93 a	44 a	50 a	94 a
Saline pine bark[y]	20 b	40 b	83 a	32 a	2 b	58 b
			2011 Experiment			
Reference soil	97 a	97 a	87 a	56 a	53 a	87 a
Saline pine bark[x]	93 a	43 b	77 a	59 a	6 b	15 b

[z]Means followed by different letters within columns of each experiment indicate significant difference at $P < 0.05$ using the Shaffer-Simulated method.
[y]Pine bark soaked in a sodium chloride (NaCl) solution overnight (16 $dS \cdot m^{-1}$ for cress and sorghum; 30 $dS \cdot m^{-1}$ for mustard).
[x]Pine bark soaked in a NaCl solution overnight (16 $dS \cdot m^{-1}$ for cress; 30 $dS \cdot m^{-1}$ for mustard and sorghum)

Phytotoxkit tests

In the 2010 experiment, cress seed germination rate was lowest in PNF (10%), but germination rate was similar among all other substrates ranging from 90% to 97% (Table 12). Mustard seed germination rate was 100% in all substrates, while sorghum seed germination rate was similar among all substrates, ranging from 77% to 93%. Cress total root length was greatest in WPTA (57 mm) and lowest in PNF (12 mm), yet each was statistically similar to the remaining substrates. Total root length for mustard was similar among all substrates. Sorghum total root length was greatest in RS (94 mm) and WPTA (98 mm), but total root length was similar among the remaining substrates.

In the 2011 experiment, PM and PB were included so direct comparisons could be made with commercially available substrate components. Such comparisons allow investigators to determine how the results may relate to current horticultural production

practices. In this experiment, cress germination rate was lowest in PNF (7%), but similar among the remaining substrates (Table 13). Seed germination rate was similar among all

Table 12

Mean Seed Germination Rate and Total Root Length of Three Biosensor Species Evaluated in 2010 Using a Phytotoxkit.

	Germination rate (%)			Total root length (mm)		
Substrate	Cress	Mustard	Sorghum	Cress	Mustard	Sorghum
Reference soil	97 a[z]	100 a	93 a	44 ab	50 a	94 a
Aged pine needles	93 a	100 a	90 a	41 ab	30 a	60 b
Fresh pine needles	10 b	100 a	77 a	12 b	39 a	65 b
Aged whole pine tree[y]	97 a	100 a	93 a	57 a	42 a	98 a
Fresh whole pine tree[x]	90 a	100 a	83 a	47 ab	60 a	62 b

[z]Means followed by different letters within columns indicate significant difference at $P < 0.05$ using the Shaffer-Simulated method.
[y]Processed whole pine (*Pinus taeda*) trees harvested and chipped on 29 Sept. 2009.
[x]Processed whole pine (*P. taeda*) trees harvested and chipped on 26 May 2010.

Table 13

Mean Seed Germination Rate and Total Root Length of Three Biosensor Species Evaluated in 2011 Using a Phytotoxkit.

	Germination rate (%)			Total root length (mm)		
Substrate	Cress	Mustard	Sorghum	Cress	Mustard	Sorghum
Reference soil	97 a[z]	97 a	87 a	56 ab	53 bcd	87 a
Peatmoss	90 a	87 a	93 a	42 b	46 cd	52 b
Pine bark	93 a	97 a	87 a	66 a	89 a	65 ab
Aged pine needles	83 a	93 a	93 a	40 b	62 bc	66 ab
Fresh pine needles	7 b	80 a	97 a	18 b	41 d	59 ab

Table 13 (continued).

Substrate	Germination rate (%)			Total root length (mm)		
	Cress	Mustard	Sorghum	Cress	Mustard	Sorghum
Aged whole pine tree[y]	93 a	97 a	97 a	51 ab	52 bcd	52 b
Fresh whole pine tree[x]	70 ab	93 a	87 a	40 b	67 b	73 ab

[z]Means followed by different letters within columns indicate significant difference at $P < 0.05$ using the Shaffer-Simulated method.
[y]Processed whole pine (*Pinus taeda*) trees harvested and chipped on 29 July 2010.
[x]Processed whole pine (*P. taeda*) trees harvested and chipped on 14 Mar. 2011.

substrates for mustard (ranging from 80% to 97%) and sorghum (ranging from 87% to 97%). Cress total root length ranged from 18 (PNF) to 66 mm (PB), but was greatest for PB, RS, and WPTA. Mustard total root length was greatest in PB (89 mm) and lowest in PNF (41 mm). Sorghum total root length was significantly greater in RS compared with WPTA and PM, but similar to the remaining substrates.

Substrate pH ranged from 4.8 (PNA) to 6.1 (WPTA) in 2010 and 4.1 (PNA) to 5.4 (PB) in 2011 (Tables 14 and 15). It has been reported that seed germination rates may vary when seeds are subjected to a range of pH values (Koger et al., 2004; Shoemaker and Carlson, 1990). Nevertheless, substrate pH likely not significantly affect seed germination rate in either experiment due to the high germination rates exhibited for all substrates except PNF. Substrate soluble salt concentration ranged from 19 (RS) to 192 ppm (PNA) in 2010 and from 79 (PM) to 568 ppm (PNF) in 2011. These values are within acceptable ranges for plug production (Cavins et al., 2000) and should not adversely affect seed germination rate or early seedling root growth.

Unsatisfactory germination rates were observed in PNF in both experiments. Compounds (phenols, terpenoids, and organic acids) found in needles of certain *Pinus* spp. can have an inhibitory effect on seed germination (Alvarez et al., 2005). Nektarios et al. (2005)

reported pine needles had an inhibitory effect on initial radicle growth and seedling development of two turfgrass species and two biosensor species. In their experiments, the inhibitory effects were more pronounced in fresh pine needles compared with decaying pine needles. Gaches et al. (2011a) evaluated seed germination and early radicle growth for lettuce seeds subjected to leachates of fresh and aged pine needles. In their study, seed germination was not affected but radicle growth was reduced in the fresh pine needle leachate compared with the aged leachate. In both studies, the authors posited that compounds within fresh pine needles are responsible for the observed phytotoxicity.

In my experiments, PNF had a substantially greater concentration of potassium compared with the other substrates in both experiments. The PNF potassium concentration is considered high for greenhouse substrates (Bailey et al., nd), but no published data were found indicating a high potassium concentration would inhibit seed germination. High concentrations of other minerals (phosphorus, iron, manganese, and aluminum) were observed in PNF, but could not be considered inhibitory to seed germination or initial root growth due to their presence in PNA and other substrates in the experiments. Inhibitory effects observed for seed germination and initial root growth are likely caused by phytotoxic compounds present in PNF, but these compounds break down over time.

Overall, germination rate in WPTA and WPTF was similar to germination rate in RS in both experiments, and similar to PM and PB in the 2011 experiment. The whole pine tree material used in the 2011 experiment was composed of 10% by weight pine needles, yet did not exhibit any inhibitory properties. Gruda et al. (2009) treated lettuce

Table 14

pH, Soluble Salt Concentration, and Mineral Nutrient Content of Substrates in 2010 Phytotoxkit and Seedling Growth Tests.

Substrate	pH	Soluble Salts	NO_3 -N	NH_4 -N	P	Ca	Mg	K	Na	B	Fe	Mn	Cu	Zn	Al	Mo
		ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
Reference soil	5.4	19	0.5	3.6	4.3	24.8	2.5	7.1	24.2	0.12	0.22	0.10	0.03	0.04	1.86	< 0.05
Aged pine needles	4.8	192	< 0.5	1.0	15.4	30.1	19.0	47.7	6.3	0.37	0.92	5.41	0.05	0.51	5.77	< 0.05
Fresh pine needles	5.5	70	1.0	6.2	26.8	15.7	26.3	343.3	8.8	0.48	3.46	7.31	0.04	1.97	10.56	< 0.05
Aged whole pine tree[z]	6.1	51	< 0.5	< 0.5	3.3	2.3	0.6	22.2	2.2	0.15	0.27	0.05	0.01	0.03	0.76	< 0.05
Fresh whole pine tree[y]	5.7	141	< 0.5	< 0.5	2.1	6.5	3.1	58.7	3.3	0.19	0.76	0.57	0.02	0.07	0.99	< 0.05
Peat-lite[x]	4.7	70	< 0.5	< 0.5	0.2	3.7	2.7	7.6	12.8	0.18	0.70	0.06	0.03	0.04	0.61	< 0.05
Pine bark	4.9	128	< 0.5	< 0.5	6.4	11.8	4.7	48.8	8.7	0.29	9.90	0.45	0.06	0.12	22.69	< 0.05

[z]Processed whole pine (*Pinus taeda*) trees harvested and chipped on 29 Sept. 2009.
[y]Processed whole pine (*P. taeda*) trees harvested and chipped on 26 May 2010.
[x]Peat-lite (3 peatmoss : 1 perlite : 1 vermiculite).

Table 15

pH, Soluble Salt Concentration, and Mineral Nutrient Content of Substrates in 2011 Phytotoxkit and Seedling Growth Tests.

Substrate	pH	Soluble Salts	NO_3 - N	NH_4 - N	P	Ca	Mg	K	Na	B	Fe	Mn	Cu	Zn	Al	Mo
		ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
Reference soil	5.1	165	< 0.5	< 0.5	2.5	29.5	3.2	7.3	26.0	0.13	0.22	0.09	0.02	0.05	1.21	< 0.05
Peatmoss	5.2	79	< 0.5	< 0.5	0.3	4.3	2.7	2.2	12.9	0.18	0.27	0.07	0.02	0.06	0.37	< 0.05
Pine bark	5.4	116	< 0.5	< 0.5	4.8	4.1	1.2	22.2	15.8	0.49	0.68	0.03	0.01	0.04	1.75	< 0.05
Aged pine needles	4.1	211	0.6	< 0.5	6.6	28.5	28.2	42.6	12.8	0.38	0.79	10.77	0.05	0.62	25.76	< 0.05
Fresh pine needles	4.8	568	1.3	< 0.5	20.2	43.8	53.8	328.6	7.8	0.50	3.89	10.68	0.03	2.62	20.83	< 0.05
Aged whole pine tree[z]	4.4	349	< 0.5	< 0.5	7.3	22.5	11.7	122.1	6.2	0.35	2.62	2.49	0.04	0.36	3.49	< 0.05
Fresh whole pine tree[y]	4.7	236	< 0.5	< 0.5	3.1	13.2	6.6	67.4	4.9	0.26	5.70	1.50	0.04	0.18	5.45	< 0.05
Peat-lite[x]	4.9	134	< 0.5	< 0.5	2.5	4.9	4.1	9.6	15.6	0.18	0.92	0.21	0.04	0.06	0.73	< 0.05

[z]Processed whole pine (*Pinus taeda*) trees harvested and chipped on 29 Sept. 2009.
[y]Processed whole pine (*P. taeda*) trees harvested and chipped on 26 May 2010.
[x]Peat-lite (3 peatmoss : 1 perlite : 1 vermiculite).

and tomato seeds with aqueous extracts of a pine tree substrate (containing no needles) and found that seed germination rate and radicle length was lower in a cold water extract compared with distilled water. They also noted that washing the pine tree substrate before performing the extracts improved seed germination rate and radicle length. In our study, cress and mustard seed germination rate and total root length were similar for RS, WPTA, and WPTF in both experiments. The direct contact method used in our experiment was chosen to more closely simulate typical production conditions. The aqueous extract method is commonly used in phytotoxicity evaluations, but results may not accurately represent the conditions encountered during production.

Although seed germination rate and total root length tended to increase after the whole pine tree material was aged, there were exceptions. Mustard total root length was actually greater for WPTF in both experiments and for sorghum in the 2011 experiment. The only observed statistical differences between WPTA and WPTF were for sorghum total root length in 2010. Gaches et al. (2011b) reported greater plant growth for annuals grown in aged whole pine tree substrate compared with a fresh whole pine tree substrate. Taylor et al. (2012) also noted that marigold growth was greater in a peat-lite substrate compared with fresh pine tree substrate and a substrate composed of equal parts fresh pine tree substrate and peatmoss. The investigators both posited that several factors, including phytotoxic compounds in the wood-based materials, may be responsible for reduced plant growth. In my experiments, whole pine tree substrates did not exhibit any effects that could be definitively interpreted as phytotoxic, especially when compared with PM and RS. Nevertheless, the disparity in plant growth of crops produced in aged and fresh wood-base substrates should be more thoroughly investigated.

In my experiments, the Phytotoxkit was used to identify potential phytotoxicity associated with compounds present in whole pine tree substrates. All substrates were sieved to pass a 2-mm screen to minimize the effect of substrate physical properties. Differences in nutrient availability among substrates could potentially affect initial seedling development, yet such factors could not be identified in the Phytotoxkit experiments.

Seedling Growth Test

Substrate pH ranged from 4.7 (PL) to 6.1 (WPTA) in 2010 and 4.4 (WPTA) to 5.4 (PB) in 2011 (Tables 14 and 15). Substrate soluble salt concentration ranged from 45 (WPTA) to 128 ppm (PB) in 2010 and from 116 (PB) to 349 ppm (WPTA) in 2011. In the 2010 experiment, lettuce seed emergence rate ranged from 58% (PB) to 85% (WPTA) (Table 16). Tomato seedling emergence rate was similar for PL and WPTA, but both were significantly greater than PB and WPTF. Total root length was greatest for PL in both test species, 2.3 to 4.5 times greater than the other substrates. In the 2011 experiment, seedling emergence rate was similar in all substrates for lettuce (ranging from 86% to 96 %) and oat (ranging from 83% to 89%) (Table 17). Tomato seedling emergence rate was greatest in WPTA (92%) and lowest in WPTF (74%). Total root length was greatest in PL for all test species, 2.2 to 11.1 times greater than the other substrates.

Table 16

Mean Seedling Emergence Rate and Total Root Length of Three Biosensor Species Evaluated in a 2010 Seedling Growth Test.

	Emergence rate (%)		Total root length (cm)	
Substrate	Lettuce	Tomato	Lettuce	Tomato
Peat-lite[z]	82 a[y]	99 a	197 a	183 a
Pine bark	58 b	81 b	48 b	81 b
Aged whole pine tree[x]	85 a	96 a	44 b	72 b
Fresh whole pine tree[w]	71 ab	76 b	52 b	81 b

[z]Peat-lite (3 peatmoss : 1 perlite : 1 vermiculite).
[y]Means followed by different letters within columns indicate significant difference at $P < 0.05$ using the Shaffer-Simulated method.
[x]Processed whole pine (*Pinus taeda*) trees harvested and chipped on 29 Sept. 2009.
[w]Processed whole pine (*P. taeda*) trees harvested and chipped on 26 May 2010.

Table 17

Mean Seedling Emergence Rate and Total Root Length of Three Biosensor Species Evaluated in a 2011 Seedling Growth Test.

	Emergence rate (%)			Total root length (cm)		
Substrate	Lettuce	Oat	Tomato	Lettuce	Oat	Tomato
Peat-lite[z]	86 a[y]	88 a	81 ab	208 a	294 a	186 a
Pine bark	92 a	88 a	85 ab	35 b	258 b	67 b
Aged whole pine tree[x]	86 a	89 a	92 a	19 c	135 d	45 c
Fresh whole pine tree[w]	96 a	83 a	74 b	20 c	160 c	43 c

[z]Peat-lite (3 peatmoss : 1 perlite : 1 vermiculite).
[y]Means followed by different letters within columns indicate significant difference at $P < 0.05$ using the Shaffer-Simulated method.
[x]Processed whole pine (*Pinus taeda*) trees harvested and chipped on 29 July 2010.
[w]Processed whole pine (*P. taeda*) trees harvested and chipped on 14 Mar. 2011.

Substrate physical properties (air space, container capacity, total porosity, and bulk density) were analyzed for both seedling growth experiments (Tables 18 and 19). Peat-lite had the lowest air space and greatest container capacity in both experiments.

Aged and fresh whole pine tree had the greatest air space in both experiments when compared with PL and PB. Mineral concentrations for WPTA and WPTF were within the acceptable ranges in both experiments, except for WPTF in 2011 which had high iron and aluminum concentrations (Tables 14 and 15).

Seedling emergence rate varied among substrates, but was substantially greater in PL and WPTA compared with PB and WPTF in the 2010 experiment. In contrast, seedling emergence rate was similar among all substrates for lettuce and oat in the 2011 experiment. Seedling emergence rate tended to be greater in WPTA compared with WPTF in both experiments. The opposite was observed for total root length, which tended to be greater in WPTF compared with WPTA. Differences in seedling emergence rate did not necessarily have an impact on total root length.

Table 18

Physical Properties[z] *of Processed Whole Pine Tree (Aged and Fresh), Pine Bark, and Peat-lite Substrates in a 2010 Seedling Growth Test*.

	Air space	Container capacity	Total porosity	Bulk density ($g \cdot cm^{-3}$)
Substrate	----------(% vol)----------			
Peat-lite[y]	10.9 c[x]	62.1 a	73.0 d	0.190 b
Pine bark	28.4 b	50.1 b	78.6 c	0.213 a
Aged whole pine tree[w]	36.1 a	55.3 b	91.4 a	0.141 c
Fresh whole pine tree[v]	34.9 a	50.8 b	85.7 b	0.148 c

[z]Data presented as means (n = 3) and obtained using the North Carolina State University porometer method.
[y] Peat-lite (3 peatmoss : 1 perlite : 1 vermiculite).
[x]Means followed by different letters within columns indicate significant difference at $P < 0.05$ using the Shaffer-Simulated method.
[w]Processed whole pine (*Pinus taeda*) trees harvested and chipped on 29 Sept. 2009.
[v]Processed whole pine (*P. taeda*) trees harvested and chipped on 26 May 2010.

Table 19

Physical Properties[z] *of Processed Whole Pine Tree (Aged and Fresh), Pine Bark, and Peat-lite Substrates in a 2011 Seedling Growth Test.*

	Air space	Container capacity	Total porosity	Bulk density ($g \cdot cm^{-3}$)
Substrate	----------(% vol)----------			
Peat-lite[y]	5.5 d[x]	62.3 a	67.7 c	0.209 b
Pine bark	22.7 c	53.5 b	76.3 b	0.267 a
Aged whole pine tree[w]	32.5 b	45.4 c	77.9 b	0.185 b
Fresh whole pine tree[v]	37.6 a	49.9 b	87.6 a	0.196 b

[z]Data presented as means (n = 3) and obtained using the North Carolina State University porometer method.
[y]Peat-lite (3 peatmoss : 1 perlite : 1 vermiculite).
[x]Means followed by different letters within columns indicate significant difference at $P < 0.05$ using the Shaffer-Simulated method.
[w]Processed whole pine (*Pinus taeda*) trees harvested and chipped on 29 July 2010.
[v]Processed whole pine (*P. taeda*) trees harvested and chipped on 14 Mar. 2011.

Minimal shoot growth was observed in either experiment and no more than one set of true leaves was produced by any of the test plant species. Shoot growth was not measured in either experiment, but seedlings in PL were visually larger compared with seedlings in the remaining substrates, corresponding to the total root length data. Seedling growth tests conducted to detect phytotoxicity typically involve sowing seeds in the test substrates, then watering and fertilizing the seedlings until the experiment is terminated (Gruda et al., 2009; Hartz and Giannini, 1998; Nektarios et al., 2005; Ortega et al, 1996). Fertilizer was not applied to seedlings in our experiments in order to reduce the number of factors affecting seedling development. Thus, seedling development resulted from nutrients obtained from seed reserves, the nonamended substrates, and the irrigation water.

Gruda et al. (2009) reported marigold seedling dry mass was lower in a pine tree substrate compared with a pine tree substrate that was leached or soaked with water prior to use. The investigators suggest a lower concentration of phytotoxins was present in the pretreated substrates. Ortega et al. (1996) reported that leaching an oak bark substrate resulted in greater shoot dry mass for seedlings, compared with those grown in nontreated bark. In the same study, phenolic acid compounds tended to be less concentrated in the leached bark substrate. Naasz et al. (2009) evaluated the phytotoxic properties of washed and nonwashed bark from seven tree species. The investigators evaluated several factors including substrate physical, chemical, and biochemical properties. They determined substrate air porosity as the predominant factor contributing to reduced germination index in lettuce seeds and reduced dry weight of tomato seedlings. Moreover, they noted low air porosity led to increased competition for oxygen among microorganisms and plant roots.

In my seedling growth experiments, substrate air space was significantly lower in PL compared with the other substrates. Total root length was substantially greater in PL, thus seedlings could have responded more positively to a lower air space. Additionally, PL had significantly greater container capacity compared with the other substrates, thus water availability could have also affected seedling growth. Throughout both experiments, seedlings were watered evenly at each irrigation event until all substrates reached saturation. Substrates with greater air space and lower container capacity would drain faster and could possibly limit water availability between irrigations and be a limiting factor in seedling growth.

Jackson et al. (2009) reported high levels of nitrogen immobilization in a pine tree substrate compared with pine bark and peatmoss substrates, while pine bark had intermediate levels of nitrogen immobilization compared with pine tree substrate and peatmoss. Wood-based substrates also have a low cation exchange capacity compared with peatmoss and pine bark (Jackson et al., 2010; Raviv and Leith, 2008). Although nitrogen immobilization and low cation exchange capacity could be responsible for reduced root development in WPTA and WPTF, it would not fully account for the significantly lower total root length in PB compared with PL. A combination of nutrient and water availability is likely responsible for reduced root development in PB, WPTA, and WPTF.

I demonstrated seeds of six biosensor plant species could be germinated and seedlings could be established in aged and fresh whole pine tree substrates. Differences in seed germination/emergence rate and seedling root length could not be attributed to phytotoxic compounds in the whole pine tree substrates. An abundance of information has been published regarding producing crops in wood-based substrates, but little emphasis has been placed on seed or cutting propagation in wood-based substrates. I determined whole pine tree substrates could be used to germinate and establish young seedlings, yet further research is required to enhance seedling development in these substrates.

The Phytotoxkit was sensitive to high soluble salt concentrations in pine bark, but further investigations are needed to determine its sensitivity for other potential phytotoxic properties in horticultural substrates. Including traditional substrates as “controls” in a Phytotoxkit evaluation would allow investigators to establish a baseline for inhibitory

effects observed in the test. The seedling growth test was successfully used to detect differences in shoot and root growth between whole pine tree and peat-lite substrates. The Phytotoxkit and seedling growth tests could be useful tools for researchers evaluating alternative horticultural substrates.

Seed germination and early seedling development in pine tree-based substrates has not been extensively evaluated. Substrates composed of processed whole pine trees or other wood-based materials have recently become commercially available in the United States, but many growers are reluctant to switch from peatmoss substrates due to their proven performance within various production methods. Demonstrating the versatility of whole pine tree substrates, from seed/cutting propagation to crop production, will positively influence growers' perceptions of these substrates.

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CHAPTER IV

PARTICLE SIZE INFLUENCE ON INITIAL SEEDLING GROWTH AND STEM CUTTING ROOT DEVELOPMENT IN WHOLE PINE TREE SUBSTRATES

Abstract

High wood content substrates derived from pine trees have been extensively evaluated for crop production, and commercially available substrate blends composed of wood-based materials are becoming more commonplace. Processing wood-based materials into finer particle sizes can result in increased container capacity and reduced air space, but how this may affect seedling and cutting root development is unknown. The objective of this study was to evaluate the effects of whole pine tree substrate particle size on initial seedling growth and stem cutting root development. Substrates evaluated in an experiment examining seedling growth included processed whole pine tree (WPT), WPT further processed through a smaller screen to produce a fine WPT, and two substrates composed of peatmoss and perlite at two proportions (1:3 peatmoss:perlite and 9:1 peatmoss:perlite). In an experiment examining rooting of stem cuttings, WPT and WPT processed through a smaller screen to produce a fine WPT (Fine WPT) were used alone and blended at three proportions. Peatmoss and perlite were also used alone and blended at three proportions for a total of ten substrates. Substrate physical properties (air space, container capacity, total porosity, bulk density, and particle size distribution) and chemical properties (pH, electrical conductivity, and cation exchange capacity) were determined for all substrates. Seedling emergence (seedling experiment), rooting percentage (stem cutting experiment), total root length, average root diameter, and number of root tips were evaluated. Processing whole pine tree substrate into finer

particle sizes resulted in reduced substrate air space and increased container capacity for both experiments. Nevertheless, whole pine tree substrates with finer particle sizes did not significantly affect seedling emergence (seedling experiment), rooting percentage (stem cutting experiment), or root development for either experiment. In the stem cutting experiment, total root length and number of root tips was superior in substrates composed of 50% or more peatmoss compared with the substrates composed of WPT and/or Fine WPT. In the stem cutting experiment, overall root development increased with an increasing proportion of peatmoss. Whole pine tree substrates can be used for germinating seeds and rooting stem cuttings. Further improvement of nutrient availability and retention properties in these substrates will likely be necessary for optimal root development during seed and stem cutting propagation.

Introduction

Sphagnum peatmoss, perlite, vermiculite, and pine bark are the most common components of substrates used for seed and stem cutting propagation of ornamental crops. These materials can be used alone or combined at various proportions resulting in countless substrate blends. Seeds and stem cuttings require substrates with high container capacity to provide ample moisture, yet adequate air space is necessary for drainage and to prevent oxygen deficiency. Peatmoss, aged pine bark, and vermiculite are used to increase water retention, while perlite is added to increase air space.

Wood-based materials produced from loblolly pine trees are viable alternatives to offset peatmoss and pine bark usage in crop production (Murphy et al., 2010; Taylor et al., 2012). Pine wood-based materials include chipped whole pine trees, chipped pine logs, and clean chip residual. Although these materials are readily available throughout

the southeastern United States, they must be further processed for use as a container substrate (Boyer et al., 2008; Jackson et al., 2010).

Pine wood-based substrates have been successfully used for crop production, but these substrates have not been evaluated for crop propagation. Wood-based substrates have a lower cation exchange capacity compared with peatmoss and aged pine bark (Jackson et al., 2010; Raviv and Leith, 2008). Nitrogen immobilization is another issue associated with wood-based substrates, whereby less nitrogen is available for plant uptake due to high microbial activity. Nevertheless, water and nutrient availability can be readily managed in wood-based substrates used for crop production. For example, such substrates can be amended with peatmoss for increased water retention (Boyer et al., 2008; Jackson et al., 2009b), while higher fertilizer rates can be used to offset nitrogen immobilization (Jackson et al., 2009a). Saunders et al. (2006) processed pine chips through different hammermill screen sizes to produce substrates with a range of particle sizes. They demonstrated pine chip substrate air space and container capacity could be modified due to differences in particle size. Pine wood-based substrate particle size may also vary due to differences in processing equipment (Altland and Krause, 2012).

Water and air content have long been considered the most important factors in selecting a substrate for cutting propagation (Bilderback and Lorscheider, 1995; Threadgill et al., 1985). Once roots are formed, an external source of nutrients is required for continued plant development. The small volume of containers used for seed and cutting propagation limits the amount of nutrients available for plant uptake, thus the added effects of nutrient leaching and reduced nitrogen availability could be detrimental to seedling and rooted cutting growth in wood-based substrates.

Water and nutrient management practices for propagation in pine wood-based substrates have not been investigated. In a previous study, this author demonstrated whole pine tree (WPT) substrates could be used to root stem cuttings of several plant species. In that study, the addition of peatmoss to WPT and pine bark substrates resulted in greater root development. It was indicated that several factors possibly contributed to less vigorous root development in WPT, including low cation exchange capacity, reduced nitrogen availability, and excessive leaching.

Wood-based substrates typically have high air space compared with peatmoss and aged pine bark substrates, thus oxygen deficiency would not be a problem associated with wood-based substrates. High substrate air space can contribute to nutrient leaching and may limit moisture content due to reduced contact between roots and substrate particles. Although processing wood-based materials into finer particle sizes can result in increased container capacity and reduced air space, how this affects seedling and cutting root development is unknown. The objective of these experiments was to evaluate the effects of WPT particle size on initial seedling development and stem cutting root development.

Materials and Methods

Two experiments were conducted to evaluate the effect of WPT substrate particle size on early seedling development (seedling experiment) and stem cutting root development (stem cutting experiment). The experiments were conducted at the USDA-ARS Thad Cochran Southern Horticultural Laboratory in Poplarville, MS.

Seedling Experiment

Whole pine tree (WPT) substrate was produced from 20- to 25-cm diameter loblolly pine (*Pinus taeda* L.) trees harvested in Macon County, AL and chipped with a

Woodsman Model 334 Biomass Chipper (Woodsman, LLC Farwell, MI) on 19 Jan. 2009. Chips were ground with a Williams Crusher hammer mill (Meteor Mill #40; Williams Patent Crusher and Pulverizer Co., Inc St. Louis, MO) to pass a 0.95-cm screen, and ground WPT was stored in 1.73 m^3 polypropylene bulk bags. On 5 Jan. 2012, WPT was further processed through a hammer mill (C.S. Bell No. 30, Tiffin, OH) fitted with a 0.3-cm screen to produce a substrate with smaller particle sizes (Fine WPT). Peatmoss (Fertilome Pure Canadian Peat Moss; Cheek Garden Products, Austin, TX) and perlite (Coarse grade; SunGro Horticulture, Bellevue, WA) were combined at two proportions by volume to produce substrates (1:3 peatmoss:perlite and 9:1 peatmoss:perlite) with physical properties similar to WPT and Fine WPT, respectively.

Individually cut cells (PROP-72-RD, T.O. Plastics Inc., Clearwater, MN) were filled with substrate (36 replications per substrate), randomized in 72-cell trays (36 cells/tray), and saturated under mist. Two seeds of a single test plant species (lettuce, *Lactuca sativa* L.'Optima'; oat, *Avena sativa* L. 'Jerry'; tomato, *Solanum lycopersicum* L. 'Arkansas Traveler') were sown in each cell. Seed germination rates of the selected test species were determined prior to the experiment [lettuce (100%), oat (95%), and tomato (98%)]. Seeds were covered with 2.5 mL of substrate, flats were placed in growth chambers (22 °C day/18 °C night, oat and lettuce; 24 °C day/21 °C night, tomato) and subjected to a 14-h light (349 – 387 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and 10-h dark photoperiod. All trays were hand-watered as needed, all 4 trays of individual test species watered equally.

At 7 and 13 days after sowing (DAS), seedling emergence rate (%) was recorded and seedlings were thinned to 1 per cell at 13 DAS. At 17 (oat), 31 (lettuce), and 39 (tomato) DAS, roots were washed and digitally scanned for analysis (total root length,

average root diameter, and number of root tips) using WinRhizo software (WinRhizo Version 2007d; Regent Instruments Inc., Canada). Substrate air space, container capacity, total porosity, and bulk density were determined (n = 3) using the North Carolina State University porometer method (Fonteno et al., 1995). Substrate particle size distribution (PSD) was determined by passing 500-mL air-dried substrate samples (n = 3) through 11 sieves (9.5- to 0.05-mm). Sieves were shaken for 3 min with a Ro-Tap (Ro-Tap RX-29; W.S. Tyler, Mentor, OH) sieve shaker (278 oscillations/min, 159 taps/min). Particles collected on each sieve and in the pan (<0.05-mm) were weighed and grouped into three texture classes [coarse (>2.0-mm), medium (<2.0 to >0.5-mm), and fine (<0.5-mm)].

Initial (0 DAS) and final (each species at termination) substrate solution pH were determined using an Accumet Excel XL50 multiparameter meter (Fisher Scientific, Pittsburgh, PA). Substrate solution was extracted using the 1:2 dilution method. Individual 45-mL substrate samples (n = 9) were saturated in 90-mL deionized water for 30 min, and the mixture was filtered through a non-bleached coffee filter (#4 Cone Style; Supervalu Inc., Eden Prairie, MN).

Seedling emergence rate data were analyzed with generalized linear models using the binary distribution and a logit link function using the GLIMMIX procedure of SAS (Version 9.3; SAS Institute, Inc., Cary, NC). Total root length, average root diameter, number of root tips, porometer data, PSD data, and pH were analyzed with generalized linear models using the GLIMMIX procedure of SAS. Differences between treatment means were determined using the Shaffer-Simulated method ($P < 0.05$).

Stem Cutting Experiment

Root development of stem cuttings from four species (*Chrysanthemum ×morifolium* Ramat. 'Dark Splendid Reagan', *Ficus benjamina* L., *Ligustrum japonicum* Thunb. 'Texanum', and *Tagetes lucida* Cav.) was evaluated in twelve substrates. Whole pine tree substrate (WPT) was produced from 20- to 25-cm diameter loblolly pine (*Pinus taeda* L.) trees harvested in Macon County, AL and chipped with a Woodsman Model 334 Biomass Chipper (Woodsman, LLC Farwell, MI) in April 2012. Chips were ground with a Williams Crusher hammer mill (Meteor Mill #40; Williams Patent Crusher and Pulverizer Co., Inc St. Louis, MO) to pass a 0.95-cm screen. On 17 Sept. 2012, WPT was further processed through a hammer mill (C.S. Bell No. 30, Tiffin, OH) fitted with a 0.3-cm screen to produce a substrate with smaller particle sizes (Fine WPT).

Whole pine tree substrate and Fine WPT were used alone and combined at three proportions (3:1 WPT:Fine WPT, 1:1 WPT:Fine WPT, and 1:3 WPT:Fine WPT) by volume to produce five substrates with varying physical properties. Peatmoss (Blonde Golden Sphagnum Peat Moss; Berger Peat Moss Inc., Quebec) and perlite (Coarse grade; SunGro Horticulture, Bellevue, WA) were used alone (Peatmoss 100% and Perlite 100%, respectively) and combined at three proportions (3:1 peatmoss:perlite, 1:1 peatmoss:perlite, and 1:3 peatmoss:perlite) by volume to produce five substrates with varying physical properties. Two commercially available substrates [Fafard 3B (Conrad Fafard, Agawam, MA) and Sunshine Rediearth PS (SunGro Horticulture, Bellevue, WA)] were also include for observational purposes. Each substrate (except Fafard and Sunshine Rediearth) was amended with 2.37 $kg \cdot m^{-3}$ 16N–2.6P–10K (5-month formulation; Harrell's; Sylacauga, AL). Dolomitic limestone was added to Peatmoss 100% (3.3

$kg \cdot m^{-3}$), 3:1 peatmoss:perlite (2.5 $kg \cdot m^{-3}$), and 1:1 peatmoss:perlite (1.7 $kg \cdot m^{-3}$) for substrate pH adjustment.

Individual cells were cut from 72-cell sheets (PROP-72-RD; T.O. Plastics Inc., Clearwater, MN) and filled with substrate (36 replications per substrate). Cells were randomized in 72-cell trays and placed under a greenhouse mist system to saturate substrates before use. Stem cuttings from individual plant species were prepared (Table 20), all species received a 1-sec basal quick-dip in a 1000 ppm indole-3-butyric acid + 500 ppm 1-naphthaleneacetic acid solution (Dip'N Grow; Dip'N Grow Inc., Clackamas, OR), and a single cutting was inserted into each container for a total of 432 experimental units per species. Intermittent mist was applied with mister nozzles (809 Series; Ein-Dor Co., Israel) for 8 to 12 sec (varied by species) every 15 min from 7:00 AM to 7:00 PM. Average monthly greenhouse temperature was calculated for September (23 °C, +/-1 degrees), October (21 °C, +2/-3 degrees), and November (19 °C, +/-3 degrees). Natural day length ranged from 10.3 to 12 hours.

Rooting periods varied by species, but all cuttings within a species were harvested at the same time (Table 20). At this time, roots (if present) were washed and digitally scanned for analysis (total root length, average root diameter, and number of root tips) using WinRhizo software. Substrate air space, container capacity, total porosity, and bulk density were determined (n = 3) using the North Carolina State University porometer method (Fonteno et al., 1995). Substrate particle size distribution (PSD) was determined by passing 500-mL air-dried substrate samples (n = 3) through 11 sieves (9.5- to 0.05-mm). Sieves were shaken for 3 min with a Ro-Tap sieve shaker (278 oscillations/min, 159 taps/min). Particles collected on each sieve and in the pan (<0.05-mm) were weighed

and grouped into three texture classes [coarse (>2.0-mm), medium (<2.0 to >0.5-mm), and fine (<0.5-mm)].

Initial (0 DAS) and final (each species at termination) substrate pH and electrical conductivity were analyzed using an Accumet Excel XL50 multiparameter meter. Substrate solution was extracted using the 1:2 dilution method. Individual 45-mL substrate samples (n = 4) were saturated in 90-mL deionized water for 30 min, and the mixture was filtered through a nonbleached coffee filter. A complete laboratory soil test analysis was conducted on the four substrate components (WPT, Fine WPT, peatmoss, and perlite) to determine mineral nutrient content. Cation exchange capacity was analyzed for all substrates, and converted from meq/100 g substrate to $cmol \cdot L^{-1}$ substrate using bulk density values ($g \cdot cm^{-3}$).

An *F*-test was used to test differences among means for substrates with WPT and/or FWPT as a component (WPT, 3:1 WPT:Fine WPT, 1:1 WPT:Fine WPT, 1:3 WPT:Fine WPT and Fine WPT) using the GLIMMIX procedure of SAS. An *F*-test was also used to test differences between means for substrates with peatmoss and/or perlite as a component (Peatmoss 100%, 3:1 peatmoss:perlite, 1:1 peatmoss:perlite, 1:3 peatmoss:perlite, and Perlite 100%). Rooting percentage data were analyzed with generalized linear models using the binary distribution and a logit link function using the GLIMMIX procedure of SAS. Total root length, average root diameter, number of root tips, porometer data, PSD data, pH, and EC were analyzed with generalized linear models using the GLIMMIX procedure of SAS. Differences between treatment means were determined using the Shaffer-Simulated method ($P < 0.05$).

Table 20

Plant Type, Cutting Data, Rooting Period, Cutting Description, Auxin Treatment, and Stock Plant Type/Location for Four Plant Species Rooted in Peat-lite and Whole Pine Tree Substrates.

Species	Plant type	Cutting date	Rooting period	Cutting description	Auxin Treatment[z]	Stock plant type/location
Chrysanthemum ×morifolium 'Dark Splendid Reagan'	Herbaceous perennial	4 Oct. 2012	25 days	Terminal	1000 ppm IBA + 500 ppm NAA	Provided by GroLink Plant Company
Ficus benjamina	Tropical tree	2 Oct. 2012	34 days	Terminal; 3-node; 1.75-3.25 in;	1000 ppm IBA + 500 ppm NAA	Container plants; MSU[y] greenhouse, Poplarville, MS
Ligustrum japonicum 'Texanum'	Large shrub	24 Sept. 2012	63 days	Subterminal; 2-node; 1.75-2.25 in	1000 ppm IBA + 500 ppm NAA	Landscape planting; MSU, Poplarville, MS
Tagetes lucida	Herbaceous perennial	27 Sept. 2012	46 days	Subterminal; 3-node; 1.25-2.5 in	1000 ppm IBA + 500 ppm NAA	Field planting; MSU, Poplarville, MS

[z]1-sec basal quick-dip; IBA = indole-3-butyric acid and NAA = 1-naphthaleneacetic acid solution (Dip'N Grow).
[y]MSU = Mississippi State University South Mississippi Branch Experiment Station.

Results and Discussion

Seedling Experiment

Seedling emergence rate was similar among all substrates for lettuce and oat at 13 DAS (Table 21). Tomato seedling emergence rate at 13 DAS was significantly lower in 9:1 peatmoss:perlite compared with the other substrates. The reduced seedling emergence rate in 9:1 peatmoss:perlite for lettuce (7 DAS) and tomato (7 and 13 DAS) is unusual considering similar substrates are used for commercial seedling production. Nevertheless, processing WPT into finer particle sizes did not affect seedling emergence rate.

Table 21

Mean Seedling Emergence Rate (%) for Three Species at 7 and 13 Days After Sowing (DAS) Seeds in Peat-lite and Whole Pine Tree Substrates.

	Lettuce		Oat		Tomato	
Substrate	7 DAS	13 DAS	7 DAS	13 DAS	7 DAS	13 DAS
1:3 peatmoss:perlite[z]	80 a[y]	99 a	85 a	86 a	54 b	90 a
9:1 peatmoss:perlite[x]	41 b	83 a	92 a	92 a	11 c	75 b
WPT[w]	84 a	94 a	88 a	90 a	81 a	93 a
Fine WPT[v]	75 a	96 a	90 a	90 a	71 ab	94 a

[z]Composed of 1 peatmoss : 3 perlite (v:v).
[y]Means followed by different letters within columns indicate significant difference at $P < 0.05$ using the Shaffer-Simulated method.
[x]Composed of 9 peatmoss : 1 perlite (v:v).
[w]20- to 25-cm diameter whole pine (*Pinus taeda*) trees harvested, chipped, and hammermilled to pass a 0.95-cm screen.
[v]WPT hammermilled to pass a 0.3-cm screen.

Initial substrate pH ranged 5.3 (9:1 peatmoss:perlite) to 6.0 (1:3 peatmoss:perlite) (Table 22). Substrate pH was within or slightly below the recommended range (5.5 to 6.5) for all substrates with each species at project termination. Any differences observed for seed emergence rate or root development would not be attributed to differences in substrate pH.

Substrate air space ranged from 19% (9:1 peatmoss:perlite) to 39% (WPT) (Table 23). Substrate air space was lower for Fine WPT compared with WPT. Similar substrate air space was observed for Fine WPT and 1:3 peatmoss:perlite. Substrate container capacity was similar between WPT and 1:3 peatmoss:perlite, and likewise between Fine WPT and 9:1 peatmoss:perlite. Substrate container capacity was greater in Fine WPT compared with WPT. Substrate total porosity was significantly lower in 1:3 peatmoss:perlite compared with the other substrates. Bulk density ranged from 0.10 (1:3 peatmoss:perlite) to 0.15 $g \cdot cm^{-3}$ (Fine WPT). Recommended ranges for substrate physical properties are not available for seedling production, but substrates composed of 75% to 90% peatmoss and have 10% to 20% substrate air space are commonly used for commercial seed propagation (personal observation).

Table 22

Initial (Fallow Containers) and Final (Three Species) Substrate pH of Peat-lite and Whole Pine Tree Substrates for a Seedling Growth Test.

Substrate	Initial (0 DAS[z])	Lettuce (31 DAS)	Oat (17 DAS)	Tomato (39 DAS)
1:3 peatmoss:perlite[y]	6.0 a[x]	5.9 a	6.0 a	6.3 a
9:1 peatmoss:perlite[w]	5.3 c	5.8 a	5.3 b	5.9 b
WPT[v]	5.6 b	5.3 c	5.2 b	5.6 c
Fine WPT[u]	5.6 b	5.5 b	5.3 b	5.7 c

[z]DAS = days after sowing.
[y]Composed of 1 peatmoss : 3 perlite (v:v).
[x]Means followed by different letters within columns indicate significant difference at $P < 0.05$ using the Shaffer-Simulated method.
[w]Composed of 9 peatmoss : 1 perlite (v:v).
[v]20- to 25-cm diameter whole pine (*Pinus taeda*) trees harvested, chipped, and hammermilled to pass a 0.95-cm screen.
[u]WPT hammermilled to pass a 0.3-cm screen.

Table 23

Physical Properties[z] *and Particle Size Distribution*[y] *of Peat-lite and Whole Pine Tree Substrates in a Seedling Growth Experiment.*

	Air space	Container capacity	Total porosity	Bulk density ($g \cdot cm^{-3}$)	Texture class[x]		
Substrate	----------(% vol)----------				Coarse	Medium	Fine
1:3 peatmoss:perlite[w]	28.5 b[v]	50.4 b	78.9 b	0.104 b	35.6 a	34.3 d	30.1 b
9:1 peatmoss:perlite[u]	19.3 c	66.9 a	86.1 a	0.117 ab	21.0 c	38.3 c	40.8 a
WPT[t]	39.3 a	48.3 b	87.6 a	0.134 ab	26.0 b	53.4 b	20.6 c
Fine WPT[s]	26.9 b	60.9 a	87.7 a	0.146 a	1.8 d	58.3 a	39.9 a

[z]Data presented as means (n = 3) and obtained using the North Carolina State University porometer method.
[y]Air-dried samples passed through 11 sieves (9.5- to 0.05-mm). Data presented as means (n = 3) of percent of particles collected on sieves and in pan.
[x]Texture classes: coarse (>2.0-mm), medium (<2.0 to >0.5-mm), and fine (<0.5-mm).
[w]Composed of 1 peatmoss : 3 perlite (v:v).
[v]Means followed by different letters within columns indicate significant difference at $P < 0.05$ using the Shaffer-Simulated method.
[u]Composed of 9 peatmoss : 1 perlite (v:v).
[t]20- to 25-cm diameter whole pine (*Pinus taeda*) trees harvested, chipped, and hammermilled to pass a 0.95-cm screen.
[s]WPT hammermilled to pass a 0.3-cm screen.

The proportion of fine particles ranged from 20% (WPT) to 41% (9:1 peatmoss:perlite), and was similar between 9:1 peatmoss:perlite and Fine WPT (Table 23). The proportion of fine particles increased nearly two fold by processing WPT through the smaller screen size. A larger proportion of medium particles were also found in Fine WPT compared with WPT. This likely accounted for the differences in substrate air space and container capacity since particle sizes smaller than 0.5 mm affect substrate air and water content (Jackson et al., 2010; Owen and Altland, 2008).

Overall, root development was less in 9:1 peatmoss:perlite than in the other substrates. The delayed emergence rate likely negatively affected root development of lettuce and tomato, but oat also had inferior root development in 9:1 peatmoss:perlite. Total root length and number of root tips was lowest in 9:1 peatmoss:perlite for all species, while average root diameter was greatest in 9:1 peatmoss:perlite for all species (Table 24). Among the remaining substrates, the maximum root development response varied by species. Maximum total root length was observed in 1:3 peatmoss:perlite for tomato, likewise for the number of root tips for lettuce and tomato. Total root length, average root diameter, and number of root tips were similar between WPT and Fine WPT for lettuce and tomato, whereas average root diameter of oat was greater in Fine WPT.

Processing WPT into smaller particle sizes did not result in greater seedling root development for the species evaluated. In a previous study conducted by the author, total root length for lettuce, oat, and tomato was superior in a peat-lite (3:1:1 peatmoss:perlite:vermiculite) substrate compared with fresh and aged WPT substrates. The author posited water and nutrient availability was limited in WPT substrates. Increased substrate container capacity and reduced substrate air space was achieved for

Fine WPT in the current study, yet improved root growth did not occur. Thus, limited nutrient availability may be the major limiting factor for seedling root development. All of the substrates in this experiment had inherently low nutrient content (data not shown) and no limestone or fertilizer was added.

Although I demonstrated WPT substrates could be used for seed propagation, seedling nutrient requirements have not been determined for these substrates. Further research is necessary to determine the effectiveness of WPT substrates during a complete seedling production cycle.

Stem Cutting Experiment

Substrate air space, container capacity, and total porosity were each similar among the substrates composed of WPT and/or Fine WPT (Table 25). Substrate air space ranged from 18% (Peatmoss 100%) to 34% (Perlite 100%) among the substrates composed of peatmoss and/or perlite. Substrate container capacity was greatest for Peatmoss 100% (72%) and lowest for Perlite 100% (41%). Substrates with WPT and/or Fine WPT as a component had similar container capacity compared with 1:3 peatmoss:perlite. Substrate bulk density ranged 0.08 $g \cdot cm^{-3}$ (Perlite 100%) to 0.12 $g \cdot cm^{-3}$ (WPT).

Processing WPT into finer particles led to a reduction in substrate air space and an increase in substrate container capacity. Nevertheless, substrate air space for Fine WPT was greater compared with substrates composed of peatmoss. Substrate air space between 15% and 40% is sufficient for adequate aeration during propagation, while substrate container capacity between 20% and 60% is sufficient for adequate water retention (Hartmann et al., 1990; Threadgill et al., 1985). All substrates composed of WPT and/or

Table 24

Mean Total Root Length (cm), Average Root Diameter (mm), and Number of Root Tips for Seedlings Grown in Peat-lite and Whole Pine Tree Substrates.

	Total root length			Average root diameter			Number of root tips		
Substrate	Lettuce	Oat	Tomato	Lettuce	Oat	Tomato	Lettuce	Oat	Tomato
1:3 peatmoss:perlite[z]	28 b[y]	141 b	120 a	0.31 b	0.42 b	0.33 b	34 a	240 b	122 a
9:1 peatmoss:perlite[x]	2 c	63 c	13 c	0.42 a	0.54 a	0.41 a	11 c	146 c	29 c
WPT[w]	40 a	191 a	62 b	0.24 c	0.36 c	0.29 c	30 ab	232 b	54 b
Fine WPT[v]	41 a	204 a	65 b	0.24 c	0.35 c	0.30 c	28 b	288 a	58 b

[z]Composed of 1 peatmoss : 3 perlite (v:v).
[y]Means followed by different letters within columns indicate significant difference at $P < 0.05$ using the Shaffer-Simulated method.
[x]Composed of 9 peatmoss : 1 perlite (v:v).
[w]20- to 25-cm diameter whole pine (*Pinus taeda*) trees harvested, chipped, and hammermilled to pass a 0.95-cm screen.
[v]WPT hammermilled to pass a 0.3-cm screen.

Table 25

Physical Properties[z], Cation Exchange Capacity (CEC), and Particle Size Distribution[y] of Peat-lite[x] and Whole Pine Tree[w] Substrates in a Rooting Experiment.

	Air space	Container capacity	Total porosity	Bulk density ($g \cdot cm^{-3}$)	CEC ($cmol \cdot L^{-1}$)	Texture class[v]		
Substrate	----------(% vol)----------					Coarse	Medium	Fine
Perlite 100%	33.8 bc[u]	40.5 c	74.2 e	0.075 f	0.2	58.3 a	28.3 g	13.4 g
1:3 peatmoss:perlite	27.0 cd	52.4 b	79.3 d	0.088 d	3.9	45.1 b	28.4 g	26.6 d
1:1 peatmoss:perlite	20.9 d	62.6 a	83.5 c	0.083 e	5.7	36.8 c	29.3 fg	33.9 c
3:1 peatmoss:perlite	19.6 d	67.7 a	87.2 b	0.082 e	7.2	29.2 de	31.0 f	39.8 a
Peatmoss 100%	18.0 d	72.1 a	90.2 a	0.082 e	10	23.0 f	36.0 e	41.1 a
WPT[t]	48.0 a	42.8 bc	90.8 a	0.119 a	2.2	37.1 c	49.8 d	13.1 g

Table 25 (continued).

	Air space	Container capacity	Total porosity	Bulk density ($g \cdot cm^{-3}$)	CEC ($cmol \cdot L^{-1}$)	Texture class[v]		
Substrate	----------(% vol)----------					Coarse	Medium	Fine
3:1 WPT:Fine WPT	45.9 a	45.9 bc	91.7 a	0.116 ab	2.6	30.3 d	53.4 c	16.3 f
1:1 WPT:Fine WPT	47.3 a	44.4 bc	91.7 a	0.113 bc	2.3	26.7 e	36.3 e	37.0 b
1:3 WPT:Fine WPT	42.9 ab	48.6 bc	91.5 a	0.114 bc	2.4	15.5 g	61.6 b	22.9 e
Fine WPT[s]	40.4 ab	52.4 b	92.8 a	0.111 c	2.3	9.4 h	63.7 a	26.8 d

[z]Data presented as means (n = 3) and obtained using the North Carolina State University porometer method.
[y]Air-dried samples passed through 11 sieves (9.5- to 0.05-mm). Data presented as means (n = 3) of percent of particles collected on sieves and in pan.
[x]Peatmoss and perlite used alone (Peatmoss 100% and Perlite 100%, respectively) and in combination at three volumetric proportions (v:v).
[w]WPT and Fine WPT used alone and in combination at various three proportions (v:v).
[v]Texture classes: coarse (>2.0-mm), medium (<2.0 to >0.5-mm), and fine (<0.5-mm).
[u]Means followed by different letters within columns indicate significant difference at $P < 0.05$ using the Shaffer-Simulated method.
[t]WPT produced from 20- to 25-cm diameter whole pine (*Pinus taeda*) trees harvested, chipped, and hammermilled to pass a 0.95-cm screen.
[s]Fine WPT produced from WPT hammermilled to pass a 0.3-cm screen.

Fine WPT were at or above 40% substrate air space, but within the sufficiency range for substrate container capacity.

The proportion of fine particles ranged from 13% (WPT) to 41% (Peatmoss 100%) (Table 25). Proportion of fine particles was similar between WPT and Perlite 100%, and likewise between Fine WPT and 1:3 peatmoss:perlite. Although processing WPT through a smaller screen size resulted in a greater proportion of fine particles, Peatmoss 100% had 1.5 times more fine particles compared with Fine WPT. Handreck (1983) reported particle sizes less than 0.5 mm had a significant effect on air space and container capacity for pine bark substrates, while it has been reported that particle sizes less than 1 mm contribute to reduced air space and increased container capacity in peatmoss (Raviv and Lieth, 2008).

In the seedling experiment, substrate air space was lower in WPT and Fine WPT, compared with WPT and Fine WPT in the cutting experiment. The WPT substrate used in the seedling experiment had been stored for several months and likely decomposed over time, resulting in a material with a greater proportion of fine particles. High substrate air space would contribute to nutrient leaching and could limit the contact between roots and substrate particles, both having a negative effect on nutrient uptake. The importance of maintaining a high percentage of fine particles in substrates is well documented, and is critical for producing a WPT substrate for propagation.

Rooting percentage was high among all substrates (97% or greater) for all species except *Ligustrum* (Table 26). *Ligustrum* rooting percentage ranged from 58% (Fine WPT) to 97% (1:1 peatmoss:perlite). Rooting percentage for *Ligustrum* tended to decline as air space decreased for the substrates composed of WPT and/or Fine WPT, while no

such trend was observed for rooting percentage with the substrates composed of peatmoss and/or perlite. Overall, *Ligustrum* rooting percentage was greater in the substrates composed of peatmoss and/or perlite. Stem cuttings require adequate endogenous nutrients for root initiation and emergence (Hartmann et al., 1990). The high rooting success of all species (except *Ligustrum*) would be an indication that substrate likely did not have a significant effect on rooting percentage, but more species need to be evaluated.

Root development responses (total root length, average root diameter, and number of root tips) were similar overall among means for substrates with WPT and/or Fine WPT as a component, except for *Chrysanthemum* average root diameter (Table 27). Contrastingly, root development responses were different overall among means for substrates composed of peatmoss and/or perlite, except for *Ficus* average root diameter. Total root length was lower for all substrates with WPT and/or Fine WPT as a component compared with Peatmoss 100%, 3:1 peatmoss:perlite, and 1:1 peatmoss:perlite, except for *Ficus* (Table 26). A similar trend was observed for number of root tips among the same substrates in all species (Table 28). Differences in average root diameter among substrates were more difficult to explain. Maximum average root diameter varied by species in regard to substrate (Table 28), thus various factors including plant species may contribute to differences in average root diameter.

Table 26

Mean Rooting Percentage (%) and Total Root Length (cm) of Cuttings from Four Species Rooted in Peat-lite[z] and Whole Pine Tree[y] Substrates.

Substrate	*Ficus benjamina*	*Ligustrum japonicum* 'Texanum'	*Chrysanthemum ×morifolium* 'Dark Splendid Reagan'	*Tagetes lucida*
		Rooting percentage		
Perlite 100%	97 a[x]	94 a	100 a	97 a
1:3 peatmoss:perlite	100 a	92 a	100 a	100 a
1:1 peatmoss:perlite	100 a	97 a	100 a	100 a
3:1 peatmoss:perlite	100 a	92 a	100 a	100 a
Peatmoss 100%	100 a	92 a	100 a	100 a
WPT[w]	100 a	69 a	100 a	97 a
3:1 WPT:Fine WPT	100 a	78 a	100 a	100 a
1:1 WPT:Fine WPT	100 a	67 a	100 a	100 a
1:3 WPT:Fine WPT	100 a	64 a	100 a	100 a
Fine WPT[v]	100 a	58 a	100 a	100 a
		Total root length		
Perlite 100%	111 c	224 b	157 d	177 c
1:3 peatmoss:perlite	160 abc	118 c	100 e	59 d
1:1 peatmoss:perlite	213 a	362 a	272 b	308 a
3:1 peatmoss:perlite	198 a	341 a	316 a	276 ab
Peatmoss 100%	197 a	375 a	311 a	270 b
WPT	166 abc	114 c	206 c	58 d
3:1 WPT:Fine WPT	162 abc	109 c	202 c	72 d
1:1 WPT:Fine WPT	159 abc	104 c	198 c	70 d
1:3 WPT:Fine WPT	171 ab	134 c	198 c	59 d
Fine WPT	134 bc	102 c	202 c	60 d

[z]Peatmoss and perlite used alone (Peatmoss 100% and Perlite 100%, respectively) and in combination at three volumetric proportions (v:v).
[y]WPT and Fine WPT used alone and in combination at three volumetric proportions (v:v).

Table 26 (continued).

[x]Means followed by different letters within columns indicate significant difference at $P < 0.05$ using the Shaffer-Simulated method.
[w]WPT produced from 20- to 25-cm diameter whole pine (*Pinus taeda*) trees harvested, chipped, and hammermilled to pass a 0.95-cm screen.
[v]Fine WPT produced from WPT hammermilled to pass a 0.3-cm screen.

Table 27

Results from F-tests Used to Test Differences in Root Development Among Means for Peat-lite[z] Substrates and Also Test Differences Among Means for Whole Pine Tree[y] Substrates.

Substrate	*Ficus benjamina*	*Ligustrum japonicum* 'Texanum'	*Chrysanthemum ×morifolium* 'Dark Splendid Reagan'	*Tagetes lucida*
		Total root length		
Peat-lite	≤0.0001	≤0.0001	≤0.0001	≤0.0001
Whole pine tree	0.2815	0.8127	0.9378	0.7053
		Average root diameter		
Peat-lite	0.3731	0.0011	≤0.0001	≤0.0001
Whole pine tree	0.9753	0.1121	0.0428	0.0643
		Number of root tips		
Peat-lite	≤0.0001	≤0.0001	≤0.0001	≤0.0001
Whole pine tree	0.8233	0.8881	0.9875	0.8416

[z]Five substrates composed of peatmoss and perlite used alone and in combination at three volumetric proportions.
[y]Five substrates composed of WPT (hammermilled to pass a 0.95-cm screen) and Fine WPT (WPT hammermilled to pass a 0.3-cm screen) used alone and in combination at three volumetric proportions.

Table 28

Average Root Diameter (mm) and Number of Root Tips of Cuttings from Four Species Rooted in Peat-lite[z] and Whole Pine Tree[y] Substrates.

Substrate	*Ficus benjamina*	*Ligustrum japonicum* 'Texanum'	*Chrysanthemum ×morifolium* 'Dark Splendid Reagan'	*Tagetes lucida*
		Average root diameter		
Perlite 100%	0.89 a[x]	0.96 a	0.86 a	0.59 b
1:3 peatmoss:perlite	0.86 a	0.97 a	0.76 b	0.71 a
1:1 peatmoss:perlite	0.90 a	0.86 a	0.67 cd	0.53 b
3:1 peatmoss:perlite	0.86 a	0.89 a	0.65 d	0.54 b
Peatmoss 100%	0.91 a	0.87 a	0.66 cd	0.55 b
WPT[w]	0.70 b	0.91 a	0.70 c	0.73 a
3:1 WPT:Fine WPT	0.70 b	0.94 a	0.69 c	0.70 a
1:1 WPT:Fine WPT	0.70 b	0.86 a	0.67 cd	0.76 a
1:3 WPT:Fine WPT	0.69 b	0.93 a	0.66 cd	0.72 a
Fine WPT[v]	0.70 b	0.86 a	0.67 cd	0.74 a
		Number of root tips		
Perlite 100%	208 b	178 bc	175 b	221 b
1:3 peatmoss:perlite	376 a	132 cd	177 b	167 b
1:1 peatmoss:perlite	497 a	293 a	392 a	389 a
3:1 peatmoss:perlite	468 a	269 ab	425 a	350 a
Peatmoss 100%	430 a	325 a	396 a	384 a
WPT	263 b	93 d	188 b	69 c

Table 28 (continued).

Substrate	*Ficus benjamina*	*Ligustrum japonicum* 'Texanum'	*Chrysanthemum ×morifolium* 'Dark Splendid Reagan'	*Tagetes lucida*
		Number of root tips		
3:1 WPT:Fine WPT	261 b	89 d	186 b	83 c
1:1 WPT:Fine WPT	264 b	80 d	183 b	86 c
1:3 WPT:Fine WPT	272 b	102 d	191 b	70 c
Fine WPT	235 b	83 d	188 b	72 c

[z]Peatmoss and perlite used alone (Peatmoss 100% and Perlite 100%, respectively) and in combination at three volumetric proportions (v:v).
[y]WPT and Fine WPT used alone and in combination at three volumetric proportions (v:v).
[x]Means followed by different letters within columns indicate significant difference at $P < 0.05$ using the Shaffer-Simulated method.
[w]WPT produced from 20- to 25-cm diameter whole pine (*Pinus taeda*) trees harvested, chipped, and hammermilled to pass a 0.95-cm screen.
[v]Fine WPT produced from WPT hammermilled to pass a 0.3-cm screen.

Significant differences in total root length and number of root tips was observed among substrates composed of peatmoss and/or perlite for all species. The least total root length was observed for 1:3 peatmoss:perlite or Perlite 100%, while the greatest total root length varied among Peatmoss 100%, 3:1 peatmoss:perlite, and 1:1 peatmoss:perlite. Increased total root length (between the least and greatest mean total root length within each species) ranged 92% (*Ficus*), 216% (*Chrysanthemum*), 217% (*Ligustrum*), and 422% (*Tagetes*). Such disparity was not observed among substrates composed of WPT and/or Fine WPT.

Although root development was less vigorous for the substrates composed of WPT and/or Fine WPT, total root length and number of root tips were comparable or superior to those observed for 1:3 peatmoss:perlite and Perlite 100% for *Chrysanthemum* and *Ficus*. Although these substrates had similar substrate air space (except 1:3

peatmoss:perlite) and container capacity, physical properties are not likely the main factor affecting root development.

Perlite is an inert material with an extremely low cation exchange capacity compared with peatmoss (Ingram, 1993), while wood-based substrates also have a low cation exchange capacity compared with peatmoss and pine bark (Jackson et al., 2010; Raviv and Leith, 2008). Cation exchange capacity was analyzed for all substrates in this experiment (Table 25). Cation exchange capacity ranged from 0.2 (Perlite 100%) to 10.0 meq/L (Peatmoss 100%). Cation exchange capacity increased with increasing proportion of peatmoss, yet processing WPT into fine particles did not result in increased cation exchange capacity.

Initial substrate pH ranged from 5.8 to 6.3 among all substrates (Table 29). However, final substrate pH (for all species) remained within or slightly above the recommended range (5.5 to 6.5). Substrate electrical conductivity was below the recommended range throughout the experiment.

All substrate components had inherently low nutrient content (Table 30). All substrates were amended with controlled-release fertilizer, thus substrates with greater cation exchange capacity had enhanced nutrient retention properties and could have contributed to differences in root development. Nitrogen immobilization has been associated with wood-based substrates, due to high levels of microbial activity and competition for nitrogen (Gruda et al., 2000; Jackson et al., 2009a). Supplemental nitrogen applications are required during crop production to offset reduced nitrogen availability in wood-based substrates (Fain et al., 2008; Jackson et al., 2008). Similar

strategies may be required during propagation if high proportions of WPT are used in the substrate.

Table 29

Initial (Fallow Containers) and Final (Four Species) Substrate pH and Electrical Conductivity (EC) of Peat-lite[z] and Whole Pine Tree[y] Substrates for a Rooting Experiment.

Substrate	Initial	*Ficus benjamina*	*Ligustrum japonicum* 'Texanum'	*Chrysanthemum ×morifolium* 'Dark Splendid Reagan'	*Tagetes lucida*
			Substrate pH		
Perlite 100%	6.3 a[x]	6.6 a	6.4 b	6.5 a	6.7 a
1:3 peatmoss:perlite	5.9 cde	6.4 ab	6.4 ab	6.4 a	6.4 a
1:1 peatmoss:perlite	6.1 b	6.5 a	6.4 ab	6.2 a	6.5 a
3:1 peatmoss:perlite	6.0 bcd	6.4 ab	6.3 b	6.4 a	6.5 a
Peatmoss 100%	6.0 bc	6.5 ab	6.4 ab	6.4 a	6.4 a
WPT[w]	5.8 f	6.5 ab	6.7 a	6.1 a	6.5 a
3:1 WPT:Fine WPT	5.8 ef	6.1 b	6.4 ab	6.2 a	6.6 a
1:1 WPT:Fine WPT	5.8 ef	6.3 ab	6.3 b	6.2 a	6.5 a
1:3 WPT:Fine WPT	5.8 f	6.5 a	6.5 ab	6.4 a	6.5 a
Fine WPT[v]	5.9 def	6.4 ab	6.6 ab	6.3 a	6.5 a
			Substrate EC ($dS \cdot m^{-1}$)		
Perlite 100%	0.11 b	0.13 a	0.09 a	0.05 a	0.05 a
1:3 peatmoss:perlite	0.13 ab	0.08 ab	0.04 a	0.08 a	0.10 a
1:1 peatmoss:perlite	0.17 ab	0.08 ab	0.07 a	0.12 a	0.05 a
3:1 peatmoss:perlite	0.20 a	0.1 ab	0.05 a	0.05 a	0.06 a

Table 29 (continued).

Substrate	Initial	*Ficus benjamina*	*Ligustrum japonicum* 'Texanum'	*Chrysanthemum ×morifolium* 'Dark Splendid Reagan'	*Tagetes lucida*
			Substrate EC ($dS \cdot m^{-1}$)		
Peatmoss 100%	0.19 a	0.08 ab	0.04 a	0.08 a	0.08 a
WPT	0.17 ab	0.04 b	0.06 a	0.06 a	0.06 a
3:1 WPT:Fine WPT	0.16 ab	0.06 ab	0.06 a	0.07 a	0.06 a
1:1 WPT:Fine WPT	0.14 ab	0.05 b	0.06 a	0.07 a	0.05 a
1:3 WPT:Fine WPT	0.16 ab	0.05 b	0.05 a	0.05 a	0.08 a
Fine WPT	0.14 ab	0.05 b	0.04 a	0.06 a	0.04 a

[z]Peatmoss and perlite used alone (Peatmoss 100% and Perlite 100%, respectively) and in combination at three volumetric proportions (v:v).
[y]WPT and Fine WPT used alone and in combination at three volumetric proportions (v:v).
[x]Means followed by different letters within columns indicate significant difference at $P < 0.05$ using the Shaffer-Simulated method.
[w]WPT produced from 20- to 25-cm diameter whole pine (*Pinus taeda*) trees harvested, chipped, and hammermilled to pass a 0.95-cm screen.
[v]Fine WPT produced from WPT hammermilled to pass a 0.3-cm screen.

Industrial hammermills are the preferred equipment for processing substrates from wood chips. The resulting substrates have adequate physical properties for greenhouse crop production, but substrate physical properties may vary due to differences in processing equipment and methods (Altland and Krause, 2012). In the current study, processing WPT into finer particle sizes led to improved substrate air space and container capacity, yet did not result in significant differences in root development. Current practices for producing WPT substrates need not be modified, but methods for improving nutrient availability in WPT substrates during propagation should be further evaluated.

In a previous study, the author demonstrated amending WPT with peatmoss led to improved root development of stem cuttings. Peatmoss is commonly blended with perlite

for improved aeration and drainage for propagation applications. Perlite is a dusty material that can irritate eyes and lungs, and it also requires a significant amount of energy to produce (Evans and Gachukia, 2004; Ingram et al., 1993). Perlite and WPT have similar chemical and physical properties, thus WPT may be a viable substitute for perlite in substrates used for propagation. A thorough evaluation of nutrient inputs (starter and controlled-release fertilizers) and alternative amendments for seed and cutting propagation in wood-based substrates would be a valuable resource for producers. Commercially available substrate blends composed of wood-based materials are becoming more commonplace, thus producers would benefit from the development of best management practices for optimizing nutrient and irrigation in these products.

Table 30

pH, Conductivity, and Mineral Nutrient Content (ppm) of Substrate Components Used in a Rooting Experiment.

Substrate	pH	Conductivity ($dS \cdot m^{-1}$)	NH_4 -N	NO_3 -N	P	K	Ca	Mg	Fe	Mn	Zn	Cu	B	S	Na	Al	Mo
Perlite	7.7	0.30	0.00	0.00	0.8	6.5	21	1	1.4	0.3	0.3	0.1	0.1	1	23	9.0	0
Peatmoss	3.7	0.50	1.00	0.00	0.7	3.7	8	12	25.2	0.3	1.3	0.1	0.2	10	21	3.1	0
WPT[z]	5.5	0.27	0.00	0.00	8.0	55.8	37	4	3.6	11.1	1.8	0.1	0.3	1	7	0.7	0
FWPT[y]	5.4	0.34	0.00	0.00	7.1	72.1	21	7	6.9	14.0	2.7	0.1	0.2	2	7	1.2	0

[z]WPT produced from 20- to 25-cm diameter whole pine (*Pinus taeda*) trees harvested, chipped, and hammermilled to pass a 0.95-cm screen.
[y]Fine WPT produced from WPT hammermilled to pass a 0.3-cm screen.

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CONCLUSIONS

These studies were conducted to determine the suitability of whole pine tree (WPT) substrates for propagating ornamental crops. Chipped whole pine trees are readily available throughout the southeastern United States due to their use as an alternative energy source. Whole pine tree chips are processed through a hammermill to produce a material with suitable water holding capacity for use as a container substrate. Whole pine tree substrates have been extensively evaluated for greenhouse and nursery crop production, and have been identified as acceptable supplements or replacements for peatmoss and pine bark. Demonstrating the versatility of WPT substrates, from propagation to production, is essential to expanding their commercial availability and use.

In the first study, stem cuttings of *Chrysanthemum*, *Cupressocyparis*, *Euonymus*, *Evolvulus*, *Ligustrum*, *Persicaria*, *Rosa*, and *Salvia* were set in WPT and pine bark substrates used alone or combined with equal parts peatmoss. Cuttings were maintained under intermittent mist until project termination. Rooting percentage was similar among substrates for each species. Root growth increased with the addition of peatmoss to WPT and pine bark for five of the eight species. Shoot growth was greatest for pine bark amended with peatmoss compared with the other substrates for all species. I demonstrated a variety of plant species could be rooted in WPT substrates, yet the addition of peatmoss or other organic component with greater nutrient retention properties may be required for optimum root development in WPT substrates.

Reduced plant growth in wood-based substrates has been attributed to a variety of factors, including phytotoxity. In the second study, a phytotoxicity assessment of aged

and fresh WPT substrates was conducted using a Phytotoxkit and a seedling growth test. The Phytotoxkit is a standardized, sensitive, rapid, reproducible, and cost-effective procedure for determining the potential phytotoxicity of a solid substrate. Aged and fresh WPT, aged and fresh pine needles, peatmoss, and pine bark were evaluated using the Phytotoxkit. Overall, seed germination rate in fresh and aged WPT was similar to germination rate in peatmoss and pine bark. Seedling root growth was similar for aged WPT and peatmoss. Fresh pine needles had an inhibitory effect on seed germination and seedling growth. A seedling growth test was used to evaluate potential phytotoxicity of aged and fresh WPT, pine bark, and a peatmoss substrate under typical production conditions. Lettuce, oat, and tomato seed emergence rate was similar for aged WPT and the peatmoss substrate. Root development was greatest in the peatmoss substrate compared with pine bark and aged and fresh WPT. I demonstrated seeds of six biosensor plant species could be germinated and seedlings could be established in aged and fresh whole pine tree substrates. Differences in seed germination/emergence rate and seedling root length could not be attributed to phytotoxic compounds in the WPT substrates.

Processing wood-based materials into finer particle sizes can result in improved substrate moisture retention, but how this may affect seedling and cutting root development is unknown. In the final study, the effect of WPT particle size on seedling and stem cutting root development was evaluated. Lettuce, oat, and tomato seedling development was evaluated in WPT, fine WPT, and two peatmoss substrates. Stem cuttings of *Chrysanthemum*, *Ficus*, *Ligustrum*, and *Tagetes* were evaluated in five WPT substrates with a range of particle sizes, along with peatmoss and perlite used alone or in combination at various proportions. Processing WPT into finer particle sizes resulted in

decreased air space and increased container capacity, but did not affect stem cutting or seedling root growth. In the stem cutting experiment, total root length and number of root tips was superior in substrates composed of 50% or more peatmoss compared with the substrates composed of WPT. Overall, root development increased with an increasing proportion of PM. I demonstrated WPT substrates can be used for germinating seeds and rooting stem cuttings, yet nutrient availability and retention properties in these substrates during propagation should be evaluated.

Supplemental nitrogen applications are required during crop production to offset reduced nitrogen availability in wood-based substrates compared with peatmoss-based substrates, thus similar strategies may be required during propagation if high proportions of WPT are used. Perlite and WPT have similar chemical and physical properties, thus WPT may be a viable substitute for perlite in substrates used for propagation. Current practices for producing WPT substrates are acceptable for propagation, but methods for improving nutrient availability in WPT substrates during propagation should be further evaluated. A thorough evaluation of nutrient inputs (starter and controlled-release fertilizers) and alternative amendments for seed and cutting propagation in wood-based substrates would be a valuable resource for producers.