Suitability of Whole Pine Tree Substrates for Seed Propagation[1]

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Abstract

Wood-based substrates are a viable option for producing crops in containers, but seed propagation in such substrates has not been sufficiently examined. Seed germination and seedling development in processed whole pine tree (*Pinus taeda* L.) substrates were evaluated using the Phytotoxkit and seedling growth tests. Substrates compared 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), peat moss (PM), and saline pine bark (SPB). Substrates evaluated using the seedling growth test included WPTA, WPTF, PB, and a peat-lite (PL) substrate. Seed germination percentage and total root length were evaluated for garden cress (*Lepidium sativum* L.), white mustard (*Sinapis alba* L.), and sorghum [*Sorghum bicolor* (L.) Moench] in repeated Phytotoxkit experiments (2010 and 2011). Seed germination percentage was lowest for garden cress in PNF, but similar among all substrates for white mustard and sorghum. Total root length was similar or greater in WPTA compared with PM for all species. Seedling emergence percentage and total root length were evaluated for lettuce (*Lactuca sativa* L.), tomato (*Solanum lycopersicum* L.), and oat (*Avena sativa* L.) in repeated seedling growth experiments (2010 and 2011). Seedling emergence percentage varied among substrates and was substantially greater in PL and WPTA compared with PB and WPTF in 2010. Total root length was greatest in PL compared to the other substrates for all species in both years. In addition, PL had significantly lower air space and greater container capacity compared with the other substrates.

Index words: growing media, pine bark, peat moss, alternative substrate, seed germination, seedling development.

Species used in this study: loblolly pine (*Pinus taeda* L.), garden cress (*Lepidium sativum* L.), white mustard (*Sinapis alba* L.), sorghum [*Sorghum bicolor* (L.) Moench], lettuce (*Lactuca sativa* L.), tomato (*Solanum lycopersicum* L.), oat (*Avena sativa* L.).

Significance to the Horticulture Industry

Wood-based substrates can be used for container production and stem cutting propagation, yet these substrates have not been thoroughly evaluated for seed propagation. There are concerns that pine tree-based substrates may have an inhibitory effect on seed germination due to compounds present in the wood and needles. Seed germination and initial seedling growth were evaluated in traditional (peat moss and pine bark) and processed whole pine tree (aged and fresh) substrates. Seed germination percentages were similar among traditional and whole pine tree substrates, whereas seed germination was inhibited in fresh pine needles. In a second study, seedling root development was greater in a peat-lite substrate compared with pine bark and whole pine tree substrates (aged and fresh). Whole pine tree substrates (aged and fresh) can be used for seed germination and initial seedling establishment, but further research is required to examine cultural methods (irrigation, fertility, etc.) for enhancing seedling development in these substrates.

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 material 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 as a source of substrate components.

Nursery and greenhouse crop production has been demonstrated in wood-based substrates composed of loblolly pine (*Pinus taeda* L.) (Fain et al. 2008, Wright et al. 2008), spruce (*Picea spp.*) (Gruda and Schnitzler 2004), melaleuca [*Melaleuca quinquenervia* (Cav.) S. T. Blake] (Brown and Duke 2000, Ingram and Johnson 1983), and various other tree species (Murphy et al. 2011, Rau et al. 2006). Additionally, stem-cutting propagation has been evaluated in whole pine tree substrates (Witcher et al. 2014). 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 growth (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 peat moss (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.

Certain organic or inorganic compounds found in soil, compost, or other substrates used for growing plants can be phytotoxic. In substrates composed of various tree compo-

[1]Received for publication on September 16, 2014; in revised form February 27, 2015. This article was prepared from a chapter of a dissertation submitted by Anthony L. Witcher for the degree of Ph.D. in Environmental Biology at the University of Southern Mississippi.

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nents, 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. Compounds detrimental to plant development may be identified 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 (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).

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 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 higher concentrations of phenolic compounds. 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 increased germination percentage and radicle growth in the washed substrates. 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.

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., Mariakerke (Ghent), 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 materials required to perform a phytotoxicity test: a sterile reference soil (control) and seeds of three biosensor plant 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 objective of this study was to evaluate seed germination and seedling development in nonamended whole pine tree substrates using the Phytotoxkit and seedling growth tests.

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 contained a reference soil (RS) and seeds of three biosensor plant species: one monocot species (sorghum) and two dicot species (garden cress and white mustard). Seed germination percentages of the selected test species were determined prior to the experiment using 50 seeds per species [garden cress (82%); white 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 (7.9 to 9.8 in) diameter loblolly pine (*Pinus taeda* L.) trees harvested and chipped on September 29, 2009 (WPTA) and May 26, 2010 (WPTF) in Macon County, AL. The chips were then ground (within 1 to 2 days of the respective harvest date) 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 (0.38 in) screen. Processed materials were stored in covered plastic tubs until use. Pine needles were collected from a 12-year-old loblolly pine plantation in Stone County, MS, either fresh needles (PNF) collected directly from trees or aged needles (PNA) collected from the ground surrounding the same trees. Pine needles were hammer-milled (model 30; C.S. Bell Co., Tiffin, OH) to pass a 0.47-cm (0.19 in) (PNA) or 0.95-cm (0.38 in) (PNF) screen. Saline pine bark [pine bark soaked overnight in a sodium chloride (NaCl) solution (16 $dS \cdot m^{-1}$ for garden cress and sorghum; 30 $dS \cdot m^{-1}$

for white mustard)] 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 (0.01 in) sieve to eliminate coarse particles. Three 95-mL (3.2 oz) samples (loosely filled) of each substrate were collected in coffee-filter-lined containers (SVD-250; T.O. Plastics, Clearwater, MN), bottom-saturated to the upper substrate surface with deionized water (NaCl solution used for SPB) for 1 hour, and then 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 (77 F) for 4 (garden cress) or 5 (white 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 percentage (percentage) and total root length (mm). A laboratory analysis was conducted on all substrates to determine pH, soluble salts, nitrate (NO_3-N), ammonium (NH_4-N), P, Ca, Mg, K, Na, B, Fe, Mn, Cu, Zn, Al, and Mo using the Saturated Media Extract method (Warncke 1998). Inductively coupled plasma-emission spectrometry was used to analyze all elements except N. Nitrate (NO_3-N cadmium reduction) and NH_4-N were determined by spectrophotometric flow injection analysis.

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 were not included in the overall statistical analyses, but separate statistical analyses were conducted to test the sensitivity of the Phytotoxkit by comparing seed germination percentage and total root length between RS and SPB.

Phytotoxkit test — 2011. A second Phytotoxkit experiment was conducted in 2011, with design and procedural differences described below. Seed germination percentages of the selected test species were determined prior to the experiment [garden cress (90%); white mustard (94%); sorghum (96%)]. Substrates included WPTA, WPTF, PNA, PNF, pine bark (PB), peat moss [(PM); Fertilome Pure Canadian Peat Moss; Cheek Garden Products, Austin, TX], SPB, and RS. The methods for processing the whole pine tree substrates were altered for 2011 in order to produce a substrate with 10% pine needles by weight, considered a high proportion for a typical whole pine tree harvest. Whole pine tree substrates were produced from 5.0- to 6.4-cm (2.0 to 2.5 in) diameter *P. taeda* trees harvested in Pearl River County, MS. The main stems were chipped on July 29, 2010 (WPTA) and March 14, 2011 (WPTF) with a wood chipper (Liberty WC-6; Mesa, AZ) and a combination of chipped stems:needles (9:1, by weight) was ground (the following day) with a hammer mill (Model 30; C.S. Bell Co., Tiffin, OH) to pass a 0.63-cm (0.25 in) screen. Pine needles were collected on March 14, 2011, directly from trees (PNF) or from the ground (PNA) surrounding the same trees and hammer-milled to pass a 0.47-cm (0.19 in) or 1.2-cm (0.5 in) screen, for PNA and PNF, respectively. Saline pine bark was prepared using a NaCl concentration of 16 $dS \cdot m^{-1}$ for garden cress and 30 $dS \cdot m^{-1}$ for white mustard and sorghum. Test plates were incubated at 25 C (77 F) for 5 (garden cress and sorghum) or 6 (white mustard) days.

Seedling growth test — 2010. Substrates included WPTA, WPTF, PB, and a peat-lite (PL) mix [peat moss (Fertilome Pure Canadian Peat Moss):perlite (Coarse grade; SunGro Horticulture, Bellevue, WA):vermiculite (Medium grade; SunGro Horticulture, Bellevue, WA) 3:1:1, by vol]. Pine bark was passed through a 5-mm (0.2 in) 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), substrates were randomized in 72-cell trays (36 cells per tray), and thoroughly wetted 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. Environmental Protection Agency 1996). Seed germination percentages of the selected test species were determined prior to the experiment using 50 seeds per species [lettuce (87%) and tomato (95%)]. Trays were grouped by species and placed in separate growth chambers [25 C (77 F) day/21 C (70 F) 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 percentage 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 of total root length using WinRhizo software (WinRhizo Version 2007d; Regent Instruments Inc., Quebec, 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 laboratory analysis was conducted on all substrates to determine pH, soluble salts, nitrate (NO_3-N), ammonium (NH_4-N), P, Ca, Mg, K, Na, B, Fe, Mn, Cu, Zn, Al, and Mo using the Saturated Media Extract method (Warncke 1998). Inductively coupled plasma-emission spectrometry was used to analyze all elements except N. Nitrate (NO_3-N cadmium reduction) and NH_4-N were determined by spectrophotometric flow injection analysis.

Seed emergence percentage 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 second 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 (0.2 in) screen], and PL. Test plant species were 'Green Ice' lettuce, 'Jerry' oat, and

Table 1. Mean seed germination percentage and total root length of three biosensor species to compare the sensitivity of the Phytotoxkit in experiments conducted in 2010 and 2011.

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

[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·m^{-1} for garden cress and sorghum; 30 dS·m^{-1} for white mustard).

[x]Pine bark soaked in a NaCl solution overnight (16 dS·m^{-1} for garden cress; 30 dS·m^{-1} for white mustard and sorghum).

'Brandywine' tomato. Seed germination percentages 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 (0.5 tsp) of substrate and the flats were placed in growth chambers [22 C (72 F) day/18 C (64 F) night for oat and lettuce; 25 C (77 F) day/21 C (70 F) night for tomato] and subjected to a 14-h light (349-387 µmol·m^{-2}·s^{-1}) and 10-h dark photoperiod. Seedling emergence percentage 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.

Results and Discussion

Phytotoxkit tests. Preliminary statistical analyses were conducted to assess the sensitivity of the Phytotoxkit, comparing seed germination percentage and total root length between RS and SPB. Garden cress (2010) and white mustard (2010 and 2011) germination percentage was significantly lower in SPB compared with RS (Table 1). Total root length was reduced for white mustard and sorghum in both years. These results suggest the Phytotoxkit could be used for assessing salinity, but the Phytotoxkit may also be a useful tool for identifying 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).

In the 2010 experiment, garden cress seed germination percentage was lowest in PNF (10%), but germination percentage was similar among all other substrates, ranging from 90 to 97% (Table 2). White mustard seed germination percentage was 100% in all substrates, while sorghum seed germination percentage was similar among all substrates, ranging from 77 to 93%. Garden cress total root length was numerically greatest in WPTA [57 mm (2.2 in)] and lowest in PNF [12 mm (0.5 in)], yet each was statistically similar to the remaining substrates. Total root length for white mustard was similar among all substrates. Sorghum total root length was greatest in RS [94 mm (3.7 in)] and WPTA [98 mm (3.9 in)], while total root length was similar among the remaining substrates.

In the 2011 experiment, PM and PB were included so that 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, garden cress germination percentage was lowest in PNF (7%), but garden cress germination percentage was similar among the remaining substrates (Table 3). Seed germination percentage was similar among all substrates for white mustard (ranging from 80 to 97%) and sorghum (ranging from 87 to 97%).

Table 2. Mean seed germination percentage and total root length of three biosensor species evaluated in 2010 using a Phytotoxkit.

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

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

[y]Pine needles (*Pinus taeda*) collected from the ground surrounding the trees. Hammer-milled to pass a 0.47-cm (0.19 in) screen.

[x]Pine needles (*P. taeda*) collected directly from trees. Hammer-milled to pass a 0.95-cm (0.38 in) screen.

[w]Processed whole pine (*P. taeda*) trees harvested and chipped on September 29, 2009.

[v]Processed whole pine (*P. taeda*) trees harvested and chipped on May 26, 2010.

Table 3. Mean seed germination percentage and total root length of three biosensor species evaluated in 2011 using a Phytotoxkit.

Substrate	Germination percentage (%)			Total root length (mm)		
	Garden cress	White mustard	Sorghum	Garden cress	White mustard	Sorghum
Reference soil	97a[z]	97a	87a	56ab	53bcd	87a
Peat moss	90a	87a	93a	42b	46cd	52b
Pine bark	93a	97a	87a	66a	89a	65ab
Aged pine needles[y]	83a	93a	93a	40b	62bc	66ab
Fresh pine needles[x]	7b	80a	97a	18b	41d	59ab
Aged whole pine tree[w]	93a	97a	97a	51ab	52bcd	52b
Fresh whole pine tree[v]	70ab	93a	87a	40b	67b	73ab

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

[y]Pine needles (*Pinus taeda*) collected from the ground surrounding the trees. Hammer-milled to pass a 0.47-cm (0.19 in) screen.

[x]Pine needles (*P. taeda*) collected directly from trees. Hammer-milled to pass a 1.2-cm (0.5 in) screen.

[w]Processed whole pine (*P. taeda*) trees harvested and chipped on July 29, 2010.

[v]Processed whole pine (*P. taeda*) trees harvested and chipped on March 14, 2011.

Garden cress total root length ranged from 18 (PNF) to 66 mm (PB), but was similar for PB, RS, and WPTA. White mustard total root length was greatest in PB [89 mm (3.5 in)] and lowest in PNF [41 mm (1.6 in)]. 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 4 and 5). Seed germination may be inhibited when seeds (various species) are subjected to a pH below 3 or above 7 (Koger et al. 2004, Shoemaker and Carlson 1990). Nevertheless, substrate pH likely did not significantly affect seed germination percentage in either year due to the high germination percentages exhibited in 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 percentage or early seedling root growth.

Unsatisfactory germination percentages were observed in PNF in both years. 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 (*P. halepensis* L.) had an inhibitory effect on initial radicle growth and seedling development of two turfgrass species (*Festuca arundinacea* Schreb. and *Cynodon dactylon* [L.] Pers.) and two biosensor species (*Avena sativa* L. and *Lemna minor* L.). 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 pine needle leachate. In both studies, the authors posited that compounds within fresh pine needles are responsible for the observed phytotoxicity.

In our experiments, PNF had a greater concentration of potassium compared with the other substrates in both years. The PNF potassium concentration is considered high for greenhouse substrates (Bailey et al. 2002), 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 compounds present in PNF, but these compounds probably break down over time resulting in less inhibitory effects in aged pine needles.

Overall, germination percentage in WPTA and WPTF were similar to germination percentage in RS in both years, and similar to PM and PB in 2011. The whole pine tree material used in 2011 was composed of 10% (by weight) fresh pine needles, yet did not exhibit any inhibitory properties. Gruda et al. (2009) treated lettuce and tomato seeds with aqueous extracts of a pine tree substrate (containing no needles) and found that seed germination percentage and radicle length were lower in a cold water extract compared with distilled water. They also noted that washing the pine tree substrate before collecting the extracts improved seed germination percentage and radicle length. In our study, garden cress and white mustard seed germination percentage and total root length were similar for RS, WPTA, and WPTF in both years.

Although seed germination percentage and total root length tended to be numerically greater using aged whole pine tree material compared with fresh material, there were exceptions. White mustard total root length was actually numerically greater for WPTF in both years and for sorghum in 2011 compared with WPTA. The only statistically significant differences between WPTA and WPTF were for sorghum total root length in 2010, where total root length was greater for WPTA. Gaches et al. (2011b) reported greater plant growth for annuals (*Petunia ×hybrida* Vilm. and *Tagetes patula* L.) grown in aged whole pine tree substrate compared with a fresh whole pine tree substrate. Taylor et al. (2012) also noted that *T. patula* 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 peat moss. These investigators believed that several factors, including phytotoxic compounds in the wood-based materials, may be responsible for reduced plant growth. In our 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,

Table 4. Chemical properties[z] of substrates prior to use in assessing seed germination of biosensor plant species using a Phytotoxkit and seedling growth test in 2010.

Substrate	pH	Soluble salts	NO_3-N	NH_4-N	P	Ca	Mg	K	Na	B	Fe	Mn	Cu	Zn	Al	Mo
								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[y]	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[x]	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[w]	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[v]	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[u]	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]Extractions using the Saturated Media Extract method (Warncke 1998).

[y]Pine needles (*Pinus taeda*) collected from the ground surrounding the trees. Hammer-milled to pass a 0.47-cm (0.19 in) screen.

[x]Pine needles (*P. taeda*) collected directly from trees. Hammer-milled to pass a 0.95-cm (0.38 in) screen.

[w]Processed whole pine (*Pinus taeda*) trees harvested and chipped on September 29, 2009.

[v]Processed whole pine (*P. taeda*) trees harvested and chipped on May 26, 2010.

[u]Peat-lite composed of peat moss:perlite:vermiculite (3:1:1, by vol).

Table 5. Chemical properties[z] of substrates prior to use in assessing seed germination of biosensor plant species using a Phytotoxkit and seedling growth test in 2011.

Substrate	pH	Soluble salts	NO_3-N	NH_4-N	P	Ca	Mg	K	Na	B	Fe	Mn	Cu	Zn	Al	Mo
								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
Peat moss	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[y]	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[x]	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[w]	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[v]	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[u]	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]Extractions using the Saturated Media Extract method (Warncke 1998).

[y]Pine needles (*Pinus taeda*) collected from the ground surrounding the trees. Hammer-milled to pass a 0.47-cm (0.19 in) screen.

[x]Pine needles (*P. taeda*) collected directly from trees. Hammer-milled to pass a 1.2-cm (0.5 in) screen.

[w]Processed whole pine (*Pinus taeda*) trees harvested and chipped on September 29, 2009.

[v]Processed whole pine (*P. taeda*) trees harvested and chipped on May 26, 2010.

[u]Peat-lite composed of peat moss:perlite:vermiculite (3:1:1, by vol).

Table 6. Mean seedling emergence percentage and total root length of three biosensor species evaluated in 2010 using a seedling growth test.

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

[z]Peat-lite composed of peat moss:perlite:vermiculite (3:1:1, by vol).

[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 September 29, 2009.

[w]Processed whole pine (*P. taeda*) trees harvested and chipped on May 26, 2010.

the disparity in plant growth of crops produced in aged and fresh wood-base substrates should be investigated more thoroughly.

Seedling growth tests. Substrate pH ranged from 4.7 (PL) to 6.1 (WPTA) in 2010 and 4.4 (WPTA) to 5.4 (PB) in 2011 (Tables 4 and 5). 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 2010, lettuce seed emergence percentage ranged from 58% (PB) to 85% (WPTA) (Table 6). Tomato seedling emergence percentage was similar for PL and WPTA and both were significantly greater than PB and WPTF. Total root length of both test species was greatest with PL in both test species and was 2.3 to 4.5 times greater than with other substrates. In 2011, seedling emergence percentage was similar in all substrates for lettuce (ranging from 86 to 96 %) and oat (ranging from 83 to 89%) (Table 7). Tomato seedling emergence percentage 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 in the other substrates.

Substrate physical properties (air space, container capacity, total porosity, and bulk density) were analyzed for both seedling growth experiments (Tables 8 and 9). Peat-lite had the lowest air space and greatest container capacity in both years. Aged and fresh whole pine tree had the greatest air space in both years when compared with PL and PB. Mineral concentrations for WPTA and WPTF were within acceptable ranges in both years, except for WPTF in 2011 which had high iron and aluminum concentrations (Tables 4 and 5).

Seedling emergence percentage varied among substrates, but was substantially greater in PL and WPTA compared with PB and WPTF for tomato in 2010. In contrast, seedling emergence percentage was similar among all substrates for lettuce and oat in 2011. Seedling emergence percentage tended to be greater in WPTA compared with WPTF in both years. The opposite was observed for total root length, which tended to be greater in WPTF compared with WPTA.

Minimal shoot growth was observed in either year and no more than one set of true leaves was produced by any of the test plant species (data not shown). Shoot growth was not measured in either year, 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 [*Picea glauca* Moench Voss, *Picea mariana* Mill. B.S.P., *Pinus banksiana* Lamb., *Populus tremuloides* Michx., *Abies balsamea* (L.) Mill., *Betula papyrifera* Marsh., and *Thuja orientalis* L.]. The investigators evaluated several factors including substrate physical, chemical, and biochemical properties. They determined substrate air-filled porosity (reported range of 0.13 to 0.40) as the predominant factor contributing to reduced germination index in lettuce seeds and reduced dry weight of tomato seedlings. Moreover, they

Table 7. Mean seedling emergence percentage and total root length of three biosensor species evaluated in 2011 using a seedling growth test.

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

[z]Peat-lite composed of peat moss:perlite:vermiculite (3:1:1, by vol).

[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 July 29, 2010.

[w]Processed whole pine (*P. taeda*) trees harvested and chipped on March 14, 2011.

Table 8. Physical properties[z] of processed whole pine tree (aged and fresh), pine bark, and peat-lite substrates in 2010 using a seedling growth test.

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

[z]Data presented as means (n = 3) and obtained using the North Carolina State University porometer method.

[y]Peat-lite composed of peat moss:perlite:vermiculite (3:1:1, by vol).

[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 September 29, 2009.

[v]Processed whole pine (*P. taeda*) trees harvested and chipped on May 26, 2010.

Table 9. Physical properties[z] of processed whole pine tree (aged and fresh), pine bark, and peat-lite substrates in 2011 using a seedling growth test.

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

[z]Data presented as means (n = 3) and obtained using the North Carolina State University porometer method.

[y]Peat-lite composed of peat moss:perlite:vermiculite (3:1:1, by vol).

[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 July 29, 2010.

[v]Processed whole pine (*P. taeda*) trees harvested and chipped on March 14, 2011.

noted low air porosity led to increased competition for oxygen among microorganisms and plant roots.

In our seedling growth experiments, substrate air space was significantly lower in PL compared with the other substrates. Total root length was substantially greater in PL, which had significantly greater container capacity compared with the other substrates. Thus, seedlings could have responded more favorably to increased water availability in PL. In both years, 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 peat moss substrates, whereas pine bark had intermediate levels of nitrogen immobilization compared with pine tree substrate and peat moss. Wood-based substrates also have a low cation exchange capacity compared with peat moss and pine bark (Jackson et al. 2010, Raviv and Lieth 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.

We 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 percentage and seedling root length could not be solely attributed to 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 propagation in wood-based substrates. We determined whole pine tree substrates could be used to germinate and establish young seedlings, yet further research is required to enhance and sustain 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 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.

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 peat moss substrates due to their proven performance within various production methods. Demonstrating the versatility of whole pine tree substrates, from seed and cutting propagation to crop production, will positively influence growers' perceptions of these substrates.

Literature Cited

Alvarez, R., L. Valbuena, and L. Calvo. 2005. Influence of tree age on seed germination response to environmental factors and inhibitory substances in *Pinus pinaster*. Intl. J. Wildland Fire 14:277–284.

Archambault, D.J., J.J. Slaski, X. Li, and K. Winterhalder. 2004. A rapid, sensitive, seedling-based bioassay for the determination of toxicity of solid and liquid substrates and plant tolerance. Soil Sediment Contam. 13:53–63.

Bailey, D.A., W.C. Fonteno, and P.V. Nelson. 2002. Greenhouse substrates and fertilization. http://www.ces.ncsu.edu/depts/hort/floriculture/plugs/ghsubfert.pdf. Accessed January 11, 2013.

Brown, S.H. and E.R. Duke. 2000. Melaleuca as an alternative to pine bark in the potting medium. Proc. Fla. State Hort. Soc. 113:180–182.

Cavins, T.J., B.E. Whipker, W.C. Fonteno, B. Harden, I. McCall, and J.L. Gibson. 2000. Monitoring and managing pH and EC using the pourthru extraction method. Hort. Inf. Lfl. 590. N.C. Coop. Ext. Serv., Raleigh, NC. 17 pp.

Emino, E.R. and P.R. Warman. 2004. Biological assay for compost quality. Compost Sci. Util. 12:342–348.

Fain, G.B., C.H. Gilliam, J.L. Sibley, C.R. Boyer, and A.L. Witcher. 2008. *WholeTree* substrate and fertilizer rate in production of greenhouse-grown petunia (*Petunia ×hybrida* Vilm.) and marigold (*Tagetes patula* L.). HortScience 43:700–705.

Fonteno, W.C., C.T. Harden, and J.P. Brewster. 1995. Procedures for determining physical properties of horticultural substrates using the NCSU porometer. Hort. Substr. Lab., N.C. State Univ., Raleigh, NC. 27 pp.

Gaches, W.G., G.B. Fain, D.J. Eakes, C.H. Gilliam, and J.L. Sibley. 2011a. Allelopathic influences of fresh and aged pine needle leachate on germination of *Lactuca sativa*. Proc. South. Nur. Assn. Res. Conf. 56:250–253.

Gaches, W.G., G.B. Fain, D.J. Eakes, C.H. Gilliam, and J.L. Sibley. 2011b. Comparison of aged and fresh *WholeTree* as a substrate component for production of greenhouse-grown annuals. J. Environ. Hort. 29:39–44.

Gong, P., B.M. Wilke, E. Strozzi, and S. Fleischmann. 2001. Evaluation and refinement of a continuous seed germination and early seedling growth test for the use in the ecotoxicological assessment of soils. Chemosphere 44:491–500.

Gruda, N., B.J. Rau, and R.D. Wright. 2009. Laboratory bioassay and greenhouse evaluation of a pine tree substrate used as a container substrate. Eur. J. Hort. Sci. 74:73–78.

Gruda, N. and W.H. Schnitzler. 2004. Suitability of wood fiber substrates for production of vegetable transplants II. The effect of wood fiber substrates and their volume weights on the growth of tomato transplants. Scientia Hort. 100:333–340.

Gruda, N., S.V. Tucher, and W.H. Schnitzler. 2000. N-immobilization of wood fiber substrates in the production of tomato transplants (*Lycopersicon lycopersicum* (L.) Karst. ex Farw.). J. Appl. Bot. 74:32–37.

Harkin, J.M. and J.W. Rowe. 1971. Bark and its possible uses. U.S. Dept. Agr. For. Serv. Res. Note FPL-091. Madison, WI. 60 pp.

Hartz, T.K. and C. Giannini. 1998. Duration of composting of yard wastes affects both physical and chemical characteristics of compost and plant growth. HortScience 33:1192–1196.

Ingram, D.L. and C.R. Johnson. 1983. Melaleuca: An alternative container media component for woody ornamentals. Proc. Fla. State Hort. Soc. 96:254–256.

Jackson, B.E, R.D. Wright, J.F. Browder, J.R. Harris, and A.X. Niemiera. 2008. Effect of fertilizer rate on growth of azalea and holly in pine bark and pine tree substrates. HortScience 43:1561–1568.

Jackson, B.E., R.D. Wright, and M.M. Alley. 2009. Comparison of fertilizer nitrogen availability, nitrogen immobilization, substrate carbon dioxide efflux, and nutrient leaching in peat-lite, pine bark, and pine tree substrates. HortScience 44:781–790.

Jackson, B.E., R.D. Wright, and M.C. Barnes. 2010. Methods of constructing a pine tree substrate from various wood particle sizes, organic amendments, and sand for desired physical properties and plant growth. HortScience 45:103–112.

Kapanen, A. and M. Itävaara. 2001. Ecotoxicity tests for compost applications. Ecotoxicol. Environ. Saf. 49:1–16.

Koger, C.H., K.N. Reddy, and D.H. Poston. 2004. Factors affecting seed germination, seedling emergence, and survival of texasweed (*Caperonia palustris*). Weed Sci. 52:989–995.

Macias, F.A., D. Castellano, and J.M.G. Molinillo. 2000. Search for a standard phytotoxic bioassay for allelochemicals: Selection of standard target species. J. Agr. Food Chem. 48:2512–2521.

Murillo, J.M., F. Cabrera, R. Lopez and P. Martin-Olmedo. 1995. Testing low-quality urban composts for agriculture: Germination and seedling performance of plants. Agr. Ecosyst. Environ. 54:127–135.

Murphy, A., C.H. Gilliam, G.B. Fain, H.A. Torbert, T.V. Gallagher, J.L. Sibley, and C.R. Boyer. 2011. Low-value trees as alternative substrates in greenhouse production of three annual species. J. Environ. Hort. 29:152–161.

Naasz, R., J. Caron, J. Legault, and A. Pichette. 2009. Efficiency factors for bark substrates: Biostability, aeration, or phytotoxicity. Soil Sci. Soc. Am. J. 73:780–791.

Nektarios, P.A., G. Economou, and C. Avgouolas. 2005. Allelopathic effects of *Pinus halepensis* needles on turfgrass and biosensor plants. HortScience 40:246–250.

Oleszczuk, P. 2010. Testing of different plants to determine influence of physico-chemical properties and contaminants content on municipal sewage sludges phytotoxicity. Environ. Toxicol. 25:38–47.

Ortega, M.C., M.T. Moreno, J. Ordovas, and M.T. Aguadol. 1996. Behaviour of different horticultural species in phytotoxicity bioassays of bark substrates. Scientia. Hort. 66:125–132.

Rau, B.J., J.F. Browder, B.E. Jackson, and R.D. Wright. 2006. Wood substrates derived from a variety of tree species affect plant growth. Proc. South. Nur. Assn. Res. Conf. 51:43–45.

Raviv, M. and H. Lieth. 2007. Soilless Culture: Theory and Practice. 1st ed. Elsevier. Oxford, UK. 608 pp.

Sekutowski, T. and J. Sadowski. 2009. Phytotoxkit™ microbiotest used in detecting herbicide residue in soil. Environ. Protection Eng. 35:105–110.

Shoemaker, C.A. and W.H. Carlson. 1990. pH affects seed germination of eight bedding plant species. HortScience 25:762–764.

Sjöström, E. 1993. Wood chemistry: Fundamentals and applications. 2nd ed. Academic Press, Inc. San Diego, CA. 293 pp.

Taylor, L.L., A.X. Niemiera, R.D. Wright, and J.R. Harris. 2012. Storage time and amendments affect pine tree substrate properties and marigold growth. HortScience 47:1782–1788.

U.S. Environmental Protection Agency. 1996. Ecological effects test guidelines: Seed germination/root elongation toxicity test. OPPTS 850.4200. 8 pp.

Warncke, D. 1998. Recommended test procedure for greenhouse growth media, p. 34–37. *In*: Dahnke, W.C. (ed.). Recommended chemical soil test procedures for the North Central Region. North Central Reg. Res. Pub. No.221. Miss. Agr. Expt. Stat. SB 1001.

Witcher, A.L., E.K. Blythe, G.B. Fain, and K.J. Curry. 2014. Stem cutting propagation in whole pine tree substrates. HortTechnology 24:30–37.

Wright, R.D., B.E. Jackson, J.F. Browder, and J.G. Latimer. 2008. Growth of chrysanthemum in a pine tree substrate requires additional fertilizer. HortTechnology 18:111–115.