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Assessing Phytotoxicity in Fresh and Aged Whole Pine Tree Substrates©

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Reduced plant growth in wood-based substrates has been attributed to a variety of factors, including phytotoxity. A detailed method for evaluating the phytotoxic potential of wood-based substrates has not been identified. Two biological assays (Phytotoxkit™ and seedling growth test) were conducted for identifying phytotoxicity in WPT, while examining the potential of such methods for testing other alternative substrates. Substrates evaluated in the Phytotoxkit included a reference soil (RS), aged (WPTA) and fresh (WPTF) whole pine tree, aged (PNA) and fresh (PNF) pine needles, pine bark (PB), peat moss (PM), and a saline pine bark (SPB) substrate. Substrates evaluated in the seedling growth test included WPTA, WPTF, PB, and a peat-lite (PL) substrate. The Phytotoxkit revealed some plant species may be sensitive to compounds present in PNF. The greatest germination/emergence rate and root length varied by species in regard to WPTA and WPTF; therefore, factors other than phytotoxicity affected seedling development in WPT. In the seedling growth test, total root length was greatest in the PL substrate for all three species, while substrate air space was lowest in the PL substrate.

INTRODUCTION

Many greenhouse and nursery crop producers have greater awareness and access to materials not traditionally used as container substrates. Materials such as composted plant debris and animal wastes, industrial by-products, and wood biomass have been successfully used for crop propagation and production. Never-

theless, many of these materials may not be ideal as the primary component of a substrate due to undesirable chemical or physical characteristics. Limited availability and lack of uniformity between sources or shipments is another concern producers must consider.

Processed whole pine tree (WPT), among other wood-based materials, are viable options as the primary component of a container substrate. Such materials can be uniformly produced over time due to consistent harvesting and processing methods. Concerns associated with wood-based substrates include availability and increased fertilizer rates required to overcome slow initial growth of some sensitive crops (Day, 2009; Gruda and Schnitzler, 1999). Questions also remain whether WPT substrates should be aged before use (Gaches, 2010). Additionally, substrate physical properties have contributed to disparity in root development of stem cuttings in WPT (Witcher et al., 2010). The most common factors associated with reduced plant growth and development in wood-based substrates include nitrogen immobilization, substrate physical properties, low buffering capacity, and phytotoxicity (Fain et al., 2008; Wright et al., 2008; Ortega et al., 1996). The detrimental effects of such factors can possibly be mitigated for crop production, but the effects may be more critical in crop propagation.

Laboratory analyses exist for determining chemical and physical properties of substrates, but the results do not account for interactions that may contribute to phytotoxicity. A method for testing the phytotoxic potential of alternative substrates would be beneficial to producers. Ideally, the method would be quick, inexpensive, and reflect the production environment. Seed germination tests are useful tools for evaluating phytotoxic effects associated with the chemical properties of a material, while seedling growth tests are used to gauge phytotoxicity due to the combined effects of substrate chemical and physical properties (Gong et al., 2001; Nassz et al., 2009). These tests have been widely adapted for evaluating compost maturity and contaminated soil, yet few protocols exist for similarly evaluating the suitability of alternative container substrate components. The objective of our research was to evaluate two biological assays for identifying phytotoxicity in WPT, while examining the potential of such methods for testing other alternative substrates.

MATERIALS AND METHODS

Two types of biological assays (Phytotoxkit™ and seedling growth test) were evaluated for determining possible phytotoxic effects of whole pine tree substrates compared with traditional substrates. The Phytotoxkit is a rapid, reproducible test designed for direct observation and root measurement of germinated seeds in contact with the substrate solution. Test plants included one monocot species (sorghum, *Sorghum saccharatum*) and two dicot species [*Lepidium sativum* (cress) and *Sinapis alba* (mustard)]. Substrates included a reference soil (RS), 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). Whole pine tree substrates were produced from 5 to 6.4 cm (2 to 2.5 in.) diameter loblolly pine (*Pinus taeda*) trees harvested in Pearl River County, MS. The main stems were chipped on 29 July 2010 (WPTA) or 14 March 2011 (WPTF) with a wood chipper (Liberty WC-6; Mesa, Arizona) and a combination of chipped stems and needles (9 : 1, w/w) was ground with a hammer mill (Model 30; C.S. Bell Co., Tiffin, Ohio) to pass a 0.63-cm (0.25-in.) screen. Pine needles were collected on 14 March 2011, directly from trees (PNF) or

from the ground (PNA) surrounding the same trees and hammer-milled to pass a 0.47 cm (0.18 in.) or 1.2 cm (0.49 in.) screen, for PNA and PNF, respectively. Saline pine bark, pine bark soaked in a sodium chloride (NaCl) solution (16 $mS \cdot cm^{-1}$ for cress or 30 $mS \cdot cm^{-1}$ for mustard and sorghum), was included to produce an inhibitory effect on seed germination and initial root growth and served as a negative control for the test procedure.

Substrates were passed through a 0.2 cm (0.08 in.) sieve to eliminate coarse particles. Three 95-ml (3.2-oz) samples (loosely filled) were collected in a coffee-filter-lined container (T.O. Plastics SVD-250) for each substrate. Samples were bottom-saturated to the upper substrate surface with deionized water for 1 h (SPB was saturated in NaCl for 10 h), drained, transferred to individual test plates, and covered with filter paper. Ten seeds of a test species were placed in a single row, a clear plastic cover was placed on each test plate, and test plates were incubated vertically in a dark growth chamber at 24 °C (75 °F) for 5 (cress and sorghum) or 6 (mustard) days. Plates were digitally scanned and analyzed using ImageTool software (ImageTool Version 3.0; UTHSA, San Antonio. Texas). Germination rate (%) and root length (mm) were collected at the conclusion of the experiment.

A seedling growth test was used to evaluate seed emergence and seedling root growth under a simulated production environment. Test plants included one monocot species (oat, *Avena sativa* 'Jerry') and two dicot species (lettuce, *Lactuca sativa* 'Green Ice' and tomato, *Solanum lycopersicum* 'Brandywine'). Substrates included WPTA, WPTF, a peat-lite (PL) substrate [peatmoss, perlite and vermiculite (3 : 1 : 1, by vol)], and pine bark (PB). Individual cells (cut from 72-cell propagation trays) were filled with substrate (36 replications), completely randomized into 72-cell propagation trays (36 cells per tray), and saturated. Two seeds of a single test species were sown on the substrate surface and covered with 2.5 ml (0.5 tsp) substrate. Trays were grouped by species and placed in separate growth chambers at 22 °C (72 °F) for oat and lettuce or 25 °C (77 °F) for tomato, each receiving a 14-h light and 10-h dark photoperiod. Seedlings were thinned to one per cell at 9 days after sowing. At 14 (oat), 25 (tomato), or 33 (lettuce) days after sowing, roots were washed and digitally scanned for analysis using WinRhizo software (WinRhizo Version 2007d; Regent Instruments Inc., Canada). Initial substrate pH and soluble salt concentration (data not shown), emergence rate (%), and total root length were collected. Germination/emergence rate and root length data were analyzed with analysis of variance using the GLIMMIX procedure of SAS (SAS Version 9.2; SAS Institute, Inc., Cary, North Carolina). Differences between treatment means were determined using the Shaffer-Simulated method.

RESULTS

Initial substrate pH ranged from 4.4 (PNA and WPTA) to 5.4 (PB). Substrate soluble salt levels ranged from 76.5 (PM) to 634.5 ppm (PNF). Using the Phytotoxkit, the lowest germination rates within a species occurred in PNF (cress) and SPB (mustard and sorghum) (Table 1). The greatest germination rate was 96.7% for mustard (RS, PB, and WPTA), 96.9% for sorghum (PNF and WPTA), and 97.0% for cress (RS). Significant differences in germination rates between PNA and PNF occurred only with cress, while germination rates were similar between WPTA and WPTF for all three species. Root length for cress was 3.8 times greater in PB compared with PNF, and 2.3 times greater in PNA compared with PNF. Although root

Table 1. Mean seed germination rate and root length of three plant species using a Phytotoxkit™.

	Germination rate (%)			Root length (mm)		
Substrate	Cress	Mustard	Sorghum		Mustard	Sorghum
Reference soil	97 a[Z]	97 a	88 a	56 a	53 bcd	87 a
Pine bark	94 a	97 a	88 a	66 a	89 a	65 ab
Peatmoss	91 a	87 a	94 a	42 a	46 cd	52 b
Saline pine bark[Y]	95 a	43 b	79 a	59 a	6e	15 c
Aged pine needles	86 a	93 a	94 a	40 a	62 cb	66 ab
Fresh pine needles	5 b	80 ab	97 a	18 a	41 d	59 ab
Aged whole pine tree[X]	93 a	97 a	97 a	51 a	52 bcd	52 b
Fresh whole pine tree	75 ab	93 a	88 a	40 a	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]Pine bark soaked in a saline solution.
[X]Processed whole pine tree.

length was statistically similar between PNA and PNF in cress, likely due to a high level of variability of measurements within a substrate, disparity between the two suggests otherwise. Mustard root length ranged from 5.8 mm (SPB) to 88.8 mm (PB), but was significantly greater in PNA compared with PNF. Sorghum root length was statistically lower in SPB compared with all other substrates and greatest overall in RS.

In the seedling growth test, emergence rate was similar among all substrates for lettuce and oat (Table 2), while tomato emergence rate was lowest for WPTF (73.6%) and greatest for WPTA (91.7%). Total root length was greatest for PL in each species, significantly different from all other substrates within species. Compared with WPTA and WPTF, total root length was approximately 11 times greater for PL with lettuce, 4.2 times greater with tomato, and 2 times greater with oat. Aging the whole pine tree material only affected tomato emergence and oat total root length. Substrate physical properties (specifically air space; data not shown) seemed to play a significant role in the greater total root length observed with PL, since the chemical analysis did not reveal any limiting factors.

DISCUSSION

We show that WPTA and WPTF can be used for seed propagation of six plant species sensitive to various phytotoxic effects. Additionally, the seed germination/emergence rate in WPTA/WPTF was similar to that obtained in traditional substrate components, specifically peatmoss and pine bark. The poor performance of cress in PNF was similar to results from a previous experiment with cress (Witcher

Table 2. Mean seed emergence rate and total root length of three plant species using a seedling growth test.

	Emergence rate (%)			Total root length (cm)		
Substrate	Lettuce	Oat	Tomato	Lettuce	Oat	Tomato
Peat-lite	86 a[Z]	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[Y]	86 a	89 a	92 a	19 c	135 d	45 c
Fresh whole pine tree	96 a	83 a	74 b	20 c	160 c	43 c

[Z]Means followed by different letters within columns indicate significant difference at $P < 0.05$ using the Shaffer-Simulated method.
[Y]Processed whole pine tree.

et al., 2011), and a separate experiment with lettuce exposed to a fresh pine needle leachate (Gaches et al., 2011). 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 radical growth. Nassz et al. (2009) suggested that air space of bark substrates (various species) was a limiting factor in seedling development, more so than the inherent nutrient and chemical composition.

The Phytotoxkit and seedling growth test could be useful tools for testing substrates in a laboratory setting when reproducible tests are required, while the seedling growth test could be conducted by producers wanting to evaluate potential substrates. In both tests, root development was a more sensitive indicator of phytotoxicity compared with germination/emergence rate. Further investigation regarding the effects of substrate physical properties on seedling growth is warranted to fully understand which factors contribute to reduced root development.

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