Factors Affecting Early Seedling Development in Whole Pine Tree Substrates

Anthony L. Witcher[1,4], Eugene K. Blythe[2], Glenn B. Fain[3], Kenneth J. Curry[4], and Cecil T. Pounders[1]

[1]USDA-ARS Southern Horticultural Laboratory, Poplarville, MS 39470
[2]Coastal Research and Extension Center, Mississippi State University, South Mississippi Branch Experiment Station, Poplarville, MS 39470
[3]Auburn University, Department of Horticulture, Auburn, AL 36849
[4]University of Southern Mississippi, Department of Biological Sciences, Hattiesburg, MS 39406

anthony.witcher@ars.usda.gov

Index Words: seed propagation, alternative substrates, growing media, phytotoxicity

Significance to Industry: Wood-based substrates can be successfully used in nursery and greenhouse crop production, yet have not been extensively evaluated for seed or cutting propagation. Wood-based materials, such as processed whole pine trees (WPTs), contain organic compounds that can be phytotoxic to sensitive species and inhibit seedling root growth. In our study, seed germination and early root development in sensitive species were inhibited by fresh pine needles, but no such inhibition was observed in aged or fresh WPT. However, a disparity in seedling root development between WPT and a peat-lite (PL) substrate suggests factors other than phytotoxicity are involved.

Nature of Work: Wood-based materials derived from pine trees, such as WPT, can be a viable option for producers looking to offset pine bark or peatmoss usage in container substrates. Wood-based substrates have been evaluated under a variety of production environments using various crops, yet certain issues must be addressed before manufacturers will invest in the commercialization of these products. The most common issues include nitrogen immobilization associated with microbial activity (5), phytotoxicity associated with organic molecules (3, 7), and less than ideal nutrient and water retention properties (1, 4). The negative effects of such factors on plant growth are well documented and can be minimized or prevented during crop production via modified production practices. However, reduced root development of stem cuttings rooted in WPT compared with pine bark (PB) has been observed (9), but the factors involved must be identified in order to develop corrective measures.

Reduced seed germination and seedling development are commonly used as indicators of phytotoxicity, specifically due to heavy metal content, compost maturity, salinity, and growth inhibiting compounds (6, 8). Seed germination and seedling growth tests used for detecting phytotoxicity are quick, simple, and reproducible. Seed germination tests are used for evaluating responses to substrate chemical properties, while seedling

growth tests account for responses due to substrate chemical and physical properties (2, 7). Seeds have nutritional reserves that will support growth for short periods after germination. As a result, unamended substrates can be evaluated, thus minimizing the number of variables involved in plant development. The objective of our research was to identify factors affecting seed germination and seedling development in unamended WPT substrates.

Two studies were performed at the Thad Cochran Southern Horticultural Laboratory in Poplarville, MS using a Phytotoxkit™ and seedling growth test. The Phytotoxkit™ is designed for direct observation and root measurement of germinated seeds in contact with the substrate solution. The Phytotoxkit™ study included three plant species (sorghum, *Sorghum saccharatum;* cress, *Lepidium sativum*; and mustard, *Sinapis alba*). 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 2.0- to 2.5-in diameter (at 1 ft above ground) loblolly pine (*Pinus taeda*) trees harvested in Pearl River County, MS. Main stems were chipped on July 29, 2010 (WPTA) or March 14, 2011 (WPTF), 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 1/4-inch screen. On March 14, 2011, pine needles were collected directly from trees (PNF) or from the ground (PNA) surrounding the same trees and hammer-milled to pass a 3/16-inch (PNA) or 1/2-inch (PNF) screen. Saline pine bark, pine bark soaked in a sodium chloride (NaCl) solution (16 mS/cm for cress or 30 mS/cm for mustard and sorghum), was included to produce a negative effect on seed germination and initial root growth.

Substrates were passed through a 2-mm sieve, and three 95-cm^3 samples of each substrate were placed in a container (SVD-250, T.O. Plastics Inc., Clearwater, MN). Samples were bottom-saturated to the upper substrate surface with deionized water for 1 hour (SPB was saturated in NaCl for 10 hours), 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 (three per substrate) were completely randomized by species and incubated vertically in a dark growth chamber at 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. TX). Germination rate (%) and root length (mm) data were collected at the conclusion of the experiment.

In the second study, a seedling growth test was used to evaluate seedling development in unamended substrates under a simulated production environment. Test plant species included oat (*Avena sativa* 'Jerry'), lettuce (*Lactuca sativa* 'Green Ice'), and tomato (*Solanum lycopersicum* 'Brandywine'). Substrates included WPTA, WPTF, PL (3 peatmoss : 1 perlite : 1 vermiculite by vol.), and PB. Individual cells (41-cm^3) 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 ½ tsp of substrate. Trays were grouped by species

and placed in separate growth chambers at 72°F for oat and lettuce or 77°F for tomato, each receiving a 14-hour light and 10-hour dark photoperiod. At 9 days after sowing, seedlings were thinned to one per cell. At 14 (oat), 25 (tomato), or 33 (lettuce) days after sowing, roots were washed, digitally scanned, and analyzed 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, root length, and substrate physical properties (air space, container capacity, total porosity, and bulk density) data were analyzed with analysis of variance using the GLIMMIX procedure of SAS (SAS Version 9.2; SAS Institute, Inc., Cary, NC). Differences between treatment means were evaluated using the Shaffer-Simulated method.

Results and Discussion: Using the Phytotoxkit™, germination rates were lowest in PNF (cress) and SPB (mustard and sorghum) (Table 1). The highest germination rate (97%) was observed for mustard in RS, PB, and WPTA; for sorghum in PNF and WPTA; and for cress in RS. Germination rates were similar between WPTA and WPTF for all three species, whereas significant differences in germination rates between PNA and PNF occurred with cress. Cress root length was statistically similar among all substrates, yet a high level of variability of measurements within substrate could have masked differences between PNF and the other substrates. Mustard root length was significantly greater in PNA compared with PNF. Sorghum root length was greatest overall in RS, while statistically lower in SPB compared with all other substrates.

In the seedling growth test, emergence rate was similar among all substrates for lettuce and oat (Table 2), while tomato emergence rate ranged from 74% (WPTF) to 92% (WPTA). Within each species, total root length was approximately 11 (lettuce), 4.2 (tomato), and 2 (oat) times greater in PL compared to WPTF and WPTA. Aging the whole pine tree material only affected tomato emergence and oat total root length. Air space was statistically different among all substrates (Table 3), but was lowest in PL (5.5%) followed by PB (22.7%). Container capacity was greatest in PL (62.3%), while PB had the greatest bulk density (0.267 $g \cdot cm^{-3}$). Substrate pH for all substrates in both tests ranged from 4.4 (PNA and WPTA) to 5.4 (PB), while substrate soluble salt levels ranged from 76.5 (PM) to 634.5 ppm (PNF).

In both tests, root growth was a more sensitive indicator of phytotoxicity than seed germination. The Phytotoxkit™ did not reveal any significant concerns regarding phytotoxicity resulting from organic compounds present in WPT (aged or fresh), yet PNF can be phytotoxic to sensitive plant species. In the seedling growth test, greater root development occurred in PL, which had the greatest container capacity and lowest air space. All substrates were unamended and had inherently low nutrient content (based on a complete chemical analysis). Although minimal growth occurred during the seedling growth test, water and nutrient availability were undoubtedly a factor in observed differences. Modifying WPT substrate air space and container capacity to mimic that of PL would allow for a more unbiased evaluation. The goal of future research will be to evaluate the relationship among substrate physical properties, water availability, and root development in WPT.

Literature Cited:

1. 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.
2. 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.
3. 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. Europ. J. Hort. Sci. 74:73-78.
4. Jackson, B.E., R.D. Wright, and M.C. Barnes. 2008. Pine tree substrate, nitrogen rate, particle size, and peat amendment affect poinsettia growth and substrate physical properties. HortScience 43:2155-2161.
5. 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.
6. Morel, P. and G. Guillemain. 2004. Assessment of the possible phytotoxicity of a substrate using an easy and representative biotest. Acta Hort. 644:417-423.
7. 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.
8. 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.
9. Witcher, A.L., E.K. Blythe, G.B. Fain, K.J. Curry, and J.M. Spiers. 2010. Stem cutting propagation in whole pine tree substrates. Comb. Proc. Intl. Plant Prop. Soc. 59:594-598.

Table 1. Mean seed germination rate (%) and root length of three plant species after 5 (cress and sorghum) or 6 (mustard) days using a Phytotoxkit™.

	Germination rate (%)			Root length (mm)		
Substrate	Cress	Mustard	Sorghum	Cress	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 bc	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 trees (*Pinus taeda*) ground to pass a 1/4-inch screen.

Table 2. Mean seed emergence rate and total root length of three plant species after 14 (oat), 25 (tomato), or 33 (lettuce) days using a seedling growth test.

	Emergence rate (%)			Total root length (cm)		
Substrate[z]	Lettuce	Oat	Tomato	Lettuce	Oat	Tomato
Peat-lite	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	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]Peat-lite (3 peatmoss : 1 perlite : 1 vermiculite); aged and fresh processed whole pine trees (*Pinus taeda*) ground to pass a 1/4-inch screen.
[y]Means followed by different letters within columns indicate significant difference at $P < 0.05$ using the Shaffer-Simulated method.

Table 3. Physical properties[z] of processed whole pine tree (aged and fresh), pine bark, and peat-lite substrates.

	Air space	Container capacity	Total porosity	Bulk density ($g \cdot cm^{-3}$)
Substrate[y]	----------(% vol)----------			
Peat-lite	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	32.5 b	45.4 c	77.9 b	0.185 b
Fresh whole pine tree	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); aged and fresh processed whole pine trees (*Pinus taeda*) ground to pass a 1/4-inch screen.
[x]Means followed by different letters within columns indicate significant difference at $P < 0.05$ using the Shaffer-Simulated method.