Pine wood chip aggregates for greenhouse substrates: effect of age on plant growth

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Abstract

Recent work has shown the potential for pine wood (*Pinus taeda* L.) to be a suitable and cost-effective organic alternative to perlite in horticultural substrates in the United States. The objective of this research was to determine the effect of pine wood chip (PWC) aggregate age on young plant growth. Pine trees were harvested and chipped in a wood chipper which created coarse wood chips (1 L × .2 W × .9 H – cm). Wood chips were then hammer-milled through a 6.35 mm screen to produce PWC (0.11 L × 0.4 W × 0.2 H – cm). Phytotoxicity germination bioassays were used to determine the potential presence and effect of phytotoxins released from substrates amended with fresh or aged PWC and the effect on growth of cucumber (*Cucumis sativus* L. 'Muncer'), tomato (*Solanum lycopersicum* L. 'Ace 55'), and radish (*Raphanus sativus* L. 'Easter Egg'). Germination count and seedling dry mass were similar among peat-based substrates formulated with 20, 30, or 40% fresh or aged PWC. Growth trials of three popular bedding plants including: celosia (*Celosia plumose* L. 'Fresh Look Mix'), impatiens (*Impatiens walleriana* Hook f. 'Super Elfin Bright Orange'), and African marigold (*Tagetes erecta* L. 'Moonsong Deep Orange' were conducted in substrates amended with 20% perlite (v/v) or 20, 30, or 40% fresh, or 4-monthed aged PWC aggregates. Results from germination bioassays conclude no visual or detrimental effects on seedling emergence, growth, or substrate chemical properties. In plant growth trials, the plant response was varied (by species and by aggregate type and percent) but the overall trends indicated in most cases as the percent PWC increases, pH increases and EC decreases. Plant shoot growth was often as large in fresh PWC-grown plants compared to aged.

Keywords: potting media, horticultural substrate, loblolly pine, *Pinus taeda*

INTRODUCTION

The chemical composition of peat-alternative substrates and components may influence plant growth. Problems may occur when organic biomass is used in container substrates, promoted by secondary metabolites or alleochemicals, respectively phytochemicals and phytotoxins (Maher and Thomson, 1991; Rathinasabapathi et al., 2005). Moreover, the concentration of secondary metabolites differs among plant species, but also varies from tree to tree, from season to season, and during the growth season (Kanerva et al., 2008). For example, phenolic compounds, are a wide spread group of secondary metabolites which have been identified and located primarily in the bark and cambial tissues of softwood trees, but are more prevalent in hardwood tree species (Farmer, 1998). These allelopathic compounds can inhibit plant growth. Substrates formulated with fresh coniferous bark or sawdust may, in certain conditions, reduce plant growth due to high concentrations of phenolic compounds (Parvez et al., 2004), terpenes (Aaron, 1982), or organic acids.

In response to phytotoxicity correlated to substrates and substrate components, investigators have developed multiple evaluation procedures to predict plant behavior. Complex and analytic laboratory techniques can detect and quantify phytotoxic molecules, while rapid, low technical bioassays or aqueous substrate extracts can test the germination rate of plant species sensitive to toxic elements. Allison (1965) determined the phytotoxicity of 28 species of tree wood and bark by evaluating pea seedlings in a germination bioassay.

Similarly, Ortega et al. (1996) conducted germination bioassays of eight different vegetables and reported germination rate and radicle growth of tomato (*Solanum lycopersicum* L.) and lettuce (*Lactuca sativa* L.) seedlings to be the most sensitive to phenolic compounds. Whilst previous work evaluated the phytotoxicity of barks and sawdust derived from numerous tree species, Rau et al. (2006) determined if differential growth could be related to the relative amounts of polyphenolics in substrates containing loblolly pine, white pine (*Pinus strobus* L.), sycamore (*Platanus occidentalis* L.), red maple (*Acer rubrum* L.), and white oak (*Quercus alba* L.). Based upon the Rau et al. (2006) work, Gruda et al. (2009) explored the possibility of determining, avoiding or mitigating the effects of phytotoxins in pine tree substrates (PTS). Gruda et al. (2009) evaluated growth of marigold (*Tagetes erecta* 'Inca Gold') plants grown in 100% untreated, leached, or soaked PTS and in a peat-lite control mix. Results indicate pretreatments (leached or soaked) of PTS improved plant growth and similar to plants grown in the PL control. Witcher et al. (2011) determined phytotoxicity of a reference soil, ground whole pine trees, and pine needles, peat moss, and pine bark substrates using a Phytotoxkit™ (rapid, reproducible test design directly for direct observation and root measurement of germinated seeds).

To use pine wood substrates fresh or delay use for some period of time prior to the production of greenhouse annuals (Gaches et al., 2011) is a concern for many growers. Jackson et al. (2010) indicated wood fiber substrates can be used fresh, however Gaches et al. (2011) investigated a comparison between aged and fresh WholeTree substrates. In this study, substrates were formulated to contain 1:1 (v/v) aged (94 day after processing) or fresh (2 days after processing) WholeTree with the remainder being peat moss. It was reported that substrate solution pH in general increased while EC decreased through the duration of the study for both petunia (*Petunia* × *hybrida* 'Dreams White') and French marigold (*Tagetes patula* L. 'Little Hero Yellow'). Growth response of marigold was reported to be more evident than petunia, however both species demonstrated higher bloom count, greater growth indices and dry weights of those grown in aged WT compared to fresh WT. Gaches et al. (2011) suggested differences in plant growth may be attributed, at least partly, to physical properties, N-immobilization, or from an allelopathic relationship between fresh WT and the plant, which diminished during the aging process.

No information is available regarding pine wood chip (PWC) aggregates phytotoxic effects on plant growth and aging requirements. Therefore, the objectives of this study were to: 1) determine phytotoxicity effects on seed germination in peat-based substrates amended with various ratios of either perlite, fresh PWC, or aged PWC; 2) Evaluate herbaceous plant growth in peat-based substrates amended with either perlite, fresh PWC, or aged PWC aggregates.

MATERIALS AND METHODS

Pine wood chip processing

On 19 Dec. 2011, eight-year-old loblolly pine trees were harvested (Chatham County, NC) at ground level, de-limbed, and subsequently stored under shelter for protected from the weather. On Jan. 3, 2012, pine logs were chipped in a DR Chipper (18 HP DR Power Equipment, model 356447; Vergennes, VT) resulting in small wood chips (1 L × .2 W × .9 H – cm). On Jan. 5, 2012 wood chips were then hammer-milled through a 6.35 mm screen (Meadows Mills, North Wilkesboro, NC) to produce pine wood chips ((PWC); 0.11 L × 0.4 W × 0.2 H – cm). Pine wood chips were stored in bulk tote bags (top of the bags were not sealed and open to the air) under shelter. On April 20, 2012, eight-year-old loblolly pine trees from the same site were harvested again in the same manner as previously described. On 24 April, the pine logs were chipped on May 5, 2012 the coarse wood chips were then hammer-milled and stored similarly as the previous harvest (January 2012). At this point the first harvested and processed PWC were four months old (aged in bag while stored). These two PWC ages (four month and zero month) make up the treatments for the following studies.

Expt. 1: phytotoxicity assessment of aged and fresh PWC-amended substrates

On May 6, moistened (50%) sphagnum peat moss (Pro-Moss Sphagnum Peat, Quakertown, PA) was amended with 10, 20, 30 or 40% (v/v) perlite, aged PWC (processed on Jan. 5, 2012) or fresh PWC (processed on May 5, 2012), to produce a total of 12 substrate treatments. Initial substrate pH was determined on all treatments and dolomitic limestone was amended to each substrate at the rate of 4.5 kg m^{-3} to adjust pH to 5.4. Substrates were incubated for 2 d in sealed plastic bags to allow for lime activation and pH equilibration before potting. Substrates did not contain a pre-plant starter-charge fertilizer. On June 6, 2012, 11.5-cm diameter plastic containers filled with each substrate. For three replications of each of the substrate treatments, five seeds of cucumber (*Cucumis sativus* L. 'Muncer'), tomato (*Solanum lycopersicum* L. 'Ace 55'), and radish (*Raphanus sativus* L. 'Easter Egg') were evenly spaced on the substrate surface with a circular plastic stencil and direct sown to a depth of 1-cm. Pots were randomly placed in a glasshouse in Raleigh, NC and watered with a mist nozzle using tap water containing no fertilizer solution. The experimental design was a randomized complete block design with three replications of each plant species × twelve substrates. Fourteen days after planting, substrate solution was extracted 1 h after the last irrigation, collected using the pour-through method (Wright, 1986) and was analyzed for EC and pH. Fourteen days after planting, germination count and visual phytotoxicity symptoms were recorded. Seedlings were then severed at the substrate surface, dried at 70°C for one week, and weighed. The mean germination count from five seeds sown represents one replication per pot (three pots per treatment). Data were subjected to analysis of variance by the general linear model procedures and means were separated by least significant differences with Duncan's means separation at $P \leq 0.05$ (SAS Institute, Cary, NC).

Expt. 2: plant growth evaluation of aged and fresh PWC-amended substrates

On May 8, 2012 moistened (50%) sphagnum peat moss was amended with 20% perlite (v/v), 20, 30, or 40% aged PWC (processed on Jan. 5, 2012), or fresh PWC (processed on May 5, 2012), to produce a total of seven substrate treatments. Substrates were amended with 4.5 kg m^{-3} dolomitic limestone and subsequently incubated for 2 d in sealed plastic bags. On May 10, five-week-old plugs of celosia (*Celosia plumosa* L. 'Fresh Look Mix'), impatiens (*Impatiens walleriana* Hook f. 'Super Elfin Bright Orange') and *Tagetes erecta* 'Moonsong Deep Orange' were transplanted in 12.7-cm, diameter plastic containers filled with each individual substrate. Pots were placed at bench level in a glasshouse in Raleigh, N.C. and were watered at the same time as needed depending upon weather conditions, and were never allowed to showed symptoms of water stress. While all plants were irrigated at the same time, similar leaching fractions (percent of applied irrigation water that drains from the container) were targeted for the different substrates in an attempt to minimalize the different water holding capacities of the different substrates. Plants were fertilized at each irrigation with 200 mg L^{-1} nitrogen (N) formulated from Peters Professional 20N-10P-20K Peat-Lite Special (Israeli Chemicals Ltd, Israel) containing 8.1% ammonical- (NH_4-N) and 11.9% nitrate-Nitrogen (NO_3-N) and injected by a Dosatron injector ((D14MZ2); Dosatron International, Inc., Clearwater, FL). Substrate solution was extracted and collected weekly for four weeks and analyzed for EC and pH. For celosia and marigold, a final growth index (GI) ((height + widest width + perpendicular width) ÷ 3) of each plant was recorded 28 days after transplanting. For all species, stems were severed at the substrate surface, dried at 70°C for one week, and weighed. The experimental design was a randomized complete block design (by species) with 6 single-plant replications × 7 substrates. Data were subjected to analysis of variance by the general linear model procedures and regression analysis (SAS Institute, Cary, NC). Means were separated by least significant differences with Duncan's means separation at $P \leq 0.05$.

RESULTS AND DISCUSSION

Expt. 1: phytotoxicity assessment of aged and fresh PWC-amended substrates

For all species, substrate pH increased with increasing percent of perlite or PWC aggregates (Table 1). Substrate solution EC levels were similar between aggregate type (perlite or PWC) and age for all species in all but a couple isolated cases. Germination rates of cucumber seedlings were similar among all perlite-amended substrates, substrates amended with 20 and 40% fresh PWC aggregates, and 20-40% aged PWC aggregates. Germination rate was lowest in substrates amended with 20% aged PWC aggregates. Radish and tomato germination rates were similar between among all substrates. Dry mass of cucumber seedlings was similar in all perlite substrate rates, and was generally higher than dry weights of plants grown in most rates of fresh or aged PWC (except 20% fresh PWC and 10% aged PWC). Radish seedling mass was highest in 40% perlite than all other treatments, but all other treatments were similar. Tomato dry mass was similar among all perlite rates/treatments and mostly similar with the 10-20% rates of fresh PWC and all rates of aged PWC. Visually, seedlings of all species in all substrates did not exhibit toxicity symptoms.

Expt. 2: plant growth evaluation of aged and fresh PWC-amended substrates

Substrate solution pH for celosia and impatiens grown in 40% fresh PWC-amended substrates were highest but were within the recommended pH range of 6.0-6.6 (Nau, 2011; (Table 2)); compared to other ratios of fresh or aged PWC- and perlite-amended substrates. Although substrates amended with 20% perlite or >30% aged PWC were not within recommended pH range, Nelson (2012) suggests a pH range of 5.4-6.6 to be desirable for most greenhouse crops. From 7 to 28 DAT, substrate pH of celosia were similar in substrates amended with 20-30% fresh PWC, 30-40% aged PWC, and in the 20% perlite-amended substrate. The substrate pH of impatiens followed a similar trend to 14 DAT. Substrate pH of marigolds were similar in substrates amended with 20% perlite, >30% fresh or aged PWC, and in substrates amended with 20% fresh and 20% aged PWC (Table 2). Among all substrates, pH values were not within recommended pH range of 6.0-6.6 (Nau, 2011) for marigold culture, however no visual indication of iron or manganese toxicities were observed. In contrast to high substrate solution pH, EC values of substrates amended with >20% fresh PWC-amended substrates were lower than recommended (1.0-2.6 mS cm^{-1}; Whipker et al., 2001) and compared to all other EC solutions reported. For impatiens, there is a significant difference between EC values of substrates amended with fresh or aged PWC and the 20% perlite-amended substrate. From 7 to 21 DAT, marigold EC values of substrates amended with >20% aged PWC were similar to the 20% perlite-amended substrate and thereafter, EC values were similar among all substrates at 28 DAT. Overall, plant growth and shoot dry weight of celosia and impatiens grown in either fresh or aged PWC and in each ratio were similar to those plants grown in the 20% perlite-amended substrate. However, the greatest shoot dry mass was determined in peat-based substrates amended with 30% aged PWC. Final growth indices of celosia and marigolds indicate similarities in some treatments and variability among other treatments. No clear trend can be seen in the plant growth response for any species. Marigold shoot dry weight of plants were similar in 20% perlite and most of the fresh or aged PWC treatments.

Table 1. Phytotoxicity test was used to determine the germination rate and seedling dry weight of cucumber/cuke (*Cucumis sativus* L. 'Muncer'), radish (*Raphanus sativus* L. 'Easter Egg'), and tomato (*Solanum lycopersicum* L. 'Ace 55') grown in peat-based amended substrates containing 20, 30, 40, or 50% perlite, fresh pine wood chips (PWC), or aged PWC.

Substrates[3]	Pour-through[1]						Germination rate[2]			Shoot dry weight (g)		
	pH			EC								
	Cuke	Radish	Tomato	Cuke	Radish	Tomato	Cuke	Radish	Tomato	Cuke	Radish	Tomato
Perlite												
10	5.6 de[4]	5.6 f	5.6 e	0.27 b	0.40 ab	0.46 a	5.0 a	4.7 a	5.0 a	0.36 abc	0.12 b	0.03 ab
20	6.1 c	6.3 c	6.3 c	0.22 c	0.39 ab	0.46 a	5.0 a	4.3 a	4.0 a	0.45 a	0.12 b	0.03 ab
30	6.6 b	6.6 b	6.6 b	0.28 b	0.38 ab	0.47 a	5.0 a	5.0 a	5.0 a	0.41 ab	0.12 b	0.04 a
40	7.0 a	6.9 a	7.1 a	0.28 b	0.41 ab	0.46 a	5.0 a	5.0 a	4.3 a	0.41 abc	0.52 a	0.03 ab
Fresh PWC[5]												
10	5.7 d	5.6 f	5.4 e	0.29 b	0.40 ab	0.45 ab	4.3 bc	4.3 a	5.0 a	0.26 de	0.05 b	0.02 bc
20	6.1 c	5.9 e	6.1 cd	0.29 b	0.31 c	0.40 c	5.0 a	4.7 a	4.7 a	0.33 bcd	0.07 b	0.02 bc
30	6.0 c	6.1 d	6.1 d	0.28 b	0.37 ab	0.40 c	4.3 bc	5.0 a	5.0 a	0.25 de	0.08 b	0.01 c
40	6.7 b	6.9 a	6.9 a	0.35 a	0.46 a	0.44 ab	5.0 a	4.7 a	4.0 a	0.29 de	0.07 b	0.01 c
Aged PWC[6]												
10	5.5 e	5.4 f	5.5 e	0.27 b	0.36 bc	0.43 ab	4.0 c	5.0 a	4.7 a	0.40 abc	0.12 b	0.04 a
20	6.2 c	6.2 cd	6.2 cd	0.31 ab	0.41 ab	0.44 ab	4.7 ab	5.0 a	4.7 a	0.28 de	0.07 b	0.02 bc
30	6.1 c	6.2 cd	6.2 cd	0.30 b	0.38 ab	0.41 bc	5.0 a	5.0 a	4.7 a	0.30 dce	0.07 b	0.02 bc
40	6.6 b	6.6 b	6.7 b	0.31 ab	0.37 ab	0.43 ab	5.0 a	5.0 a	5.0 a	0.22 e	0.07 b	0.02 bc

[1]pH and electrical conductivity (mS cm^{-1}) of substrate solution determined on pour-through extracts (n=4; Wright, 1986).

[2]Germination rate was the mean number of seedlings germinated at 14 days after planting.

[3]Substrates were formulated on a volume basis to contain 10, 20, 30, or 40% perlite (v/v) or PWC aggregates.

[4]Means separated within column by Duncan's multiple range test ($P \leq 0.05$).

[5]*Pinus taeda* trees were harvested, de-limbed, chipped, and hammer milled through a 6.35-mm screen to produce PWC in Jan. 2012.

[6]*Pinus taeda* trees were harvested, de-limbed, chipped, and hammer milled through a 6.35-mm screen to produce PWC in May 2012.

Table 2. Growth data of herbaceous annuals and chemical properties of peat-based substrates amended with perlite, fresh pine wood chips (PWC) or aged PWC.

Substrates[2]	Pour-through data[1]								GI[4]	Shoot dry weight (g)
	Days after transplanting									
	7		14		21		28			
	pH	EC[4]	pH	EC	pH	EC	pH	EC		
	Celosia plumose 'Fresh Look Mix'									
Perlite										
20	5.4 b[5]	1.61 a	5.5 b	1.65 a	5.3 b	1.76 ab	5.3 b	1.38ab	18.4 abc	1.48 cd
Fresh PWC[6]										
20	5.2 bc	1.31 c	5.4 bc	1.31 bc	5.3 b	1.50 bc	5.3 b	1.35 ab	16.7 c	1.00 d
30	5.6 b	1.07 d	5.8 b	0.90 c	5.6 b	1.10 d	5.6 b	0.93 c	20.6 a	2.22 b
40	6.4 a	1.13 d	6.6 a	0.90 c	6.4 a	1.05 d	6.3 a	0.91 c	19.1 ab	1.82 bc
Aged PWC[7]										
20	4.9 c	1.53 ab	5.0 c	1.63 a	4.9 c	1.94 a	4.9 c	1.63 a	17.7 bc	1.48 cd
30	5.3 b	1.42 bc	5.4 bc	1.40 b	5.3 b	1.33 cd	5.4 b	1.15 bc	20.5 a	2.75 a
40	5.6 b	1.48 ab	5.7 b	1.34 b	5.7 b	1.48 c	5.6 b	1.05 c	19.4 ab	1.82 bc
	Impatiens Walleriana 'Super Elfin Bright Orange'[9]									
Perlite										
20	5.5 cb	1.59 a	5.6 cb	1.68 a	5.4 cd	1.72 ab	5.3 c	1.82 ab	---	1.19 a
Fresh PWC										
20	5.1 cd	1.37 b	5.3 cd	1.44 b	5.3 d	1.51 b	5.2 c	1.37 bcd	---	1.07 a
30	5.7 b	1.09 c	5.9 b	1.06 c	5.9 ab	1.10 c	5.7 b	1.02 d	---	1.27 a
40	6.2 a	1.00 c	6.5 a	0.98 c	6.2 a	1.05 c	6.1 a	1.10 cd	---	1.08 a
Aged PWC										
20	5.0 d	1.65 a	5.1 d	1.68 a	4.9 e	1.94 a	4.8 d	1.89 a	---	1.38 a
30	5.4 ab	1.48 ab	5.4 cd	1.66 a	5.3 de	1.75 ab	5.2 c	1.56 abc	---	1.05 a
40	5.7 b	1.57 ab	5.8 b	1.58 ab	5.7 bc	1.68 ab	5.5 bc	1.88 a	---	1.27 a
	Tagetes erecta 'Moonsong Deep Orange'[10]									
Perlite										
20	5.7 ab[5]	1.45 a	5.9 a	1.71 a	5.9 a	1.50 ab	5.7 a	1.42 a	20.8 ab	2.26 a
Fresh PWC										
20	5.1 cd	1.20 ab	5.2 b	1.45 b	5.3 b	1.30 bc	5.3 b	1.28 a	19.8 abc	2.05 ab
30	5.9 a	1.02 bc	6.0 a	1.35 bc	5.9 a	1.40 ab	5.8 a	1.40 a	18.92 c	1.77 b
40	5.8 ab	0.81 c	6.0 a	1.18 c	6.1 a	1.18 c	5.9 a	1.27 a	19.0 bc	1.98 ab
Aged PWC										
20	4.9 d	1.54 a	5.2 b	1.73 a	5.3 b	1.56 a	5.3 b	1.44 a	19.0 bc	1.68 b
30	5.4 bc	1.44 a	5.6 a	1.52 ab	5.8 a	1.36 ab	5.8 a	1.24 a	21.1 a	2.27 a
40	5.5 abc	1.37 ab	5.8 a	1.46 b	5.9 a	1.42 ab	5.8 a	1.33 a	20.5 abc	2.23 a

[1] pH and EC (mS cm^{-1}) of substrate solution determined on pour-through extracts (Wright, 1986).

[2] Substrates were formulated on a volume basis to contain 20% perlite or 20, 30, or 40% PWC aggregates.

[3] EC = electrical conductivity.

[4] GI = growth index – ((plant height+plant width+perpendicular width)/3).

[5] Means separated within column, within species, using Duncan's multiple range test ($P \leq 0.05$).

[6] *Pinus tadea* (loblolly pine) trees were harvested, de-limbed, chipped, and hammer milled through a 6.35-mm screen to produce PWC in Jan. 2012.

[7] *Pinus taeda* trees were harvested, de-limbed, chipped, and hammer milled through a 6.35-mm screen to produce PWC in May 2012.

CONCLUSION

Evaluating fresh and aged PWC aggregates for general phytotoxicity issues were determined to address questions of potential toxicity during crop production. Results from germination bioassays conclude no visual or detrimental effects on seedling emergence, growth, or substrate chemical properties. In plant growth trials, the plant response was varied (by species and by aggregate type and percent) but the overall trends indicated in most cases as the percent PWC increases, pH increases and EC decreases. These observations of higher pH and lower EC have been seen and reported previously in the literature (Jackson et al., 2009, 2010). While plant growth response was varied, no severe plant loss or severe toxicity, regardless of age could be seen in these experiments. This information assessing the biological and chemical characteristics of PWC aggregates will help growers and investigators alike, to potentially make PWC a suitable aggregate in greenhouse substrates.

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