# Rhizometrics: A Review of Three In Situ Techniques for Observation and Measurement of Plant Root Systems in Containers

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**Abstract**

**Rhizometrics is a term derived from *rhizo-* (rhizosphere) and *-metrics* (series of parameters or measures of quantitative assessment used for measuring, comparisons or tracking performance or production), to describe several methods either developed or examined by North Carolina State University to observe and quantify root growth of plants in containers. Three new techniques have been developed and/or investigated as potential new methods of quantifying root growth; 1) Mini-Horhizotron; 2) Rhizometer; and 3) Hydraulic Conductance Flow Meter (HCFM). First, the mini-Horhizotrons have a clear, three-arm configuration suitable for observing root growth of small container plant material. The clear arms allow for visible access and measurements of plant roots. Potential measurements include root length, quantity of root hairs, and root architecture. Second, the Rhizometer is made from a clear cylinder that is 7.6 cm tall x 7.6 cm inside diameter, which allows for visible observations of root systems and they can be fitted in the North Carolina State University Porometer for in situ measurements of the influence of root growth on physical properties in containers during crop production. Thirdly, the HCFM is an apparatus that measures root and shoot conductance based on pressure and water flow through the roots, in the opposite direction of normal transpiration under quasi-steady-state conditions. Conductance values are directly indicative (and correlated) with root mass. These Rhizometric techniques are novel methods of observing and quantifying root growth and potentially identifying ways of improving root growth productivity and efficiency to maximize crop growth. These techniques have also been used to quantify root growth differences between/among various substrates. A summary of the initial experiments testing the usefulness of these three techniques for quantifying undisturbed root growth have yielded promising results.**

## INTRODUCTION

With a large portion of the horticultural industry producing plants in containers, it is important to understand the factors that influence root growth in order to achieve optimal benefits from container production. To understand these factors and quantify the effects, root system development must be measured accurately. The study of root growth began in the field over nine decades ago with agronomists who studied root growth in various soils (McDougall, 1916; Weaver et al., 1922). Over these decades, techniques of root growth measurements have ranged from arduous hand-drawn pictures to using visible materials to view root growth, such as rhizotrons and minirhizotrons (Taylor et al., 1990). Rhizotrons are permanently installed underground buildings that have glass walls placed against the soil to make nondestructive, repeated observations and measurements of root systems (Klepper and Kaspar, 1994). Most of these techniques are more suited for field-grown plants, and with the large amount of woody nursery crops, annual bedding plants, potted foliage plants and greenhouse vegetable crops produced in containers, there is a need for methods that more accurately and precisely measure root growth in containers.

Currently, there are two methods of quantifying root growth that are used commonly in scientific literature: subjective ratings and root washing. Subjective root ratings can be a method to quantify root systems; however this method is completely dependent on the rater (Walters and Wehner, 1994). The person rating roots might have difficulty creating a well-defined rating system, and depending on the rating system and how broad/general it is, differences in amount of root hairs or root diameter would not be accounted for. Root washing is a destructive method involving removing all the substrate from the root ball in order to view the roots unobstructed. Washing roots will reveal the root system to be viewed; however this removes the roots from their natural position/ architecture and 20-40% of the fine roots (including root hairs) are lost in the washing process (Oliveira et al., 2000). This creates a need for new non-destructive methods that can measure the whole root system in situ and root growth over time.

In an effort to overcome some of the disadvantages on the mentioned techniques, new root measuring methods have been created. The Horhizotron™, a non-destructive technique, measures horizontal root growth from the root ball of a container-grown nursery crop, allowing for post-transplant assessment (Wright and Wright, 2004). Advancements have been done to the rhizotrons as well, and an above-ground rhizotron was developed to observe root growth in an environment closer to natural soil conditions (Silva and Beeson, 2011). However, both these techniques are intended for woody plants with large root balls, to imitate post-transplant conditions. This creates a need for accurate and effective techniques to measure root growth of smaller plants during production.

At North Carolina State University, the Horticultural Substrates Lab (HSL) has been investigating different methods to observe and quantify root growth, in a project termed Rhizometrics. Three new techniques to measure root growth of plants during production have been developed and/or explored as potential new methods of quantifying root growth; 1) Mini-Horhizotron, 2) Rhizometer, and 3) Hydraulic Conductance Flow Meter (HCFM). These techniques are used as a tool to better understand Rhizometrics and the influences of root growth by having the ability to change the root environment (substrates) both chemically and physically (Judd, 2013). The HSL has also been investigating using the effects of replacing perlite with different wood components in greenhouse substrates (peat).

**Mini-Horhizotron**

A new technique was developed to study root growth of seeds, liners and plugs during production, termed the mini-Horhizotron. The mini-Horhizotron is a root box designed with a three-arm configuration suitable for observing and measuring root growth of small container plant material. Arms are clear sided which allows visible measurements to be taken from a plant/seed planted in the center, and these measurements include: root length, speed of root growth, presence and quantity of root hairs, and root architecture/ branching. This design allows for the measurement of roots from a plug/liner/seed as they would fill out a standard greenhouse container and aids in better understanding of root growth patterns, problems and potential. The mini-Horhizotrons have a substrate volume approximately three times larger than a standard greenhouse container (such as a 16.5 cm dia container). The mini-Horhizotron was constructed to be permanent, with no loose pieces except for the shade panels. Shade panels were constructed to fit snuggly against the plexiglass arms and completely block sunlight from the rhizosphere. The shade panels do not cover the substrate, allowing for water to directly contact the substrate surface which is the same as traditional surface applied irrigation for container-grown plants. The objectives of the mini-Horhizotron study were to 1) measure root growth in the mini-Horhizotron of *Chrysanthemum* planted in one of three substrates: a traditional peat: perlite substrate or peat amended with either pine-wood-chips or shredded-pine wood, and 2) compare final root mass of *Chrysanthemums* grown in either the mini-Horhizotrons or standard greenhouse containers to determine if the increased surface area of the mini-Horhizotrons effected root growth.

## Rhizometer

For container-grown plants, the stability of the substrate's physical properties are of primary concern because changes in these properties may adversely affect plant growth (Allaire-Leung et al., 1999). The influence of root growth on the physical properties of substrates is poorly documented with unconvincing and contradictory results (Cannavo et al., 2011). As plant roots grow into the container substrate, there can be modification of total porosity, pore size distribution and pore connectivity. Total porosity, container capacity and air space can be measured with the North Carolina State University (NCSU) Porometer method (Fonteno et al., 1995).

Rhizometers are an apparatus that allow for both viewing a growing root system and in situ measurements of substrate physical properties (Judd, 2013). The meaning of the term Rhizometer is derived from *rhizo-* meaning rhizosphere, and *-meter*, stemming from porometer (a device that measures physical properties of substrates). The Rhizometer is made from a clear cylinder that is 7.6 cm tall x 7.6 cm inside diameter, which allows for visible observations for root growth measurements. The Rhizometer cores can be fitted in the NCSU Porometer method. The Rhizometers have a 3.8 cm tall collar attached to the top to aid in packing the Rhizometer with substrate and having extra space to plant a plug or seed. The rationale of this apparatus was to measure both the physical properties of substrates and the effects of growing roots on substrates, while also having the ability to observe and measure roots in situ (Judd, 2013). The objectives of the Rhizometer study were: 1) measure changes in physical properties over time 2) measure the root mass in the Rhizometers to observe substrate effects and compare with changes in substrate physical properties.

## Hydraulic Conductance Flow Meter

The HCFM (Dynamax, Inc., Houston, TX) is an apparatus that can measure both root and shoot hydraulic conductance. Shoots are excised from the root system a few cm above substrate level and the rootstock or shoot stem is fitted with water filled tubing of the HCFM. The HCFM uses constantly increasing pressure to cause water to flow into the root or shoot system. The pressure measurement versus the flow measurement is used to estimate root/shoot conductance from the slope. The objectives of the HCFM study were to 1) test the capability of measuring root conductance on herbaceous plant material and 2) observe substrate effect on root conductance of herbaceous plants.

These three techniques of Rhizometrics are novel methods of observing and quantifying root growth which can aid in the modification of container substrates and the substrate environment to maximize plant growth in containers. These techniques have also been used to quantify root growth differences between/among various substrates. The initial experiments testing the usefulness of these three techniques for quantifying undisturbed root systems have yielded promising results.

# MATERIALS AND METHODS

## Substrate Preparation and Plant Care

Eight-year-old loblolly pine trees (*Pinus taeda* L.) were harvested on 19 Dec. 2011 at ground level, and stored under shelter for protection from the weather. On 2 Jan. 2012 the delimbed pine logs were either chipped or shredded in different wood processing machines to produce either blockular wood chips or fiberous shredded wood. Both the chipped and shredded wood was then processed in a hammermill through a 6.35 mm screen to produce two end products, pine-wood-chips (PWC) and shredded-pine-wood (SH). The SH component was selected for trial because the particles have properties similar to peat and the PWC component was selected for trial because it has properties similar to perlite. Both wood components have been researched as respective alternatives to peat and perlite (PL) in greenhouse substrates (Judd, 2013).

All experiments were completely randomized on a greenhouse bench, in Raleigh, NC. Plants were over-head watered as needed depending on weather conditions, and never showed symptoms of water stress. Plants were fertilized at each watering with 200 ppm Nitrogen.

**Rhizometrics**

**1. Mini-Horhizotron.** Three substrates were used in this study, 70% peat (by volume) amended with 30% of either PL, PWC, or SH. On 1 June 2012, substrates were mixed and amended with dolomitic limestone at 3.86 $kg \cdot m^{-3}$ to achieve a desired pH of 5.8. On 2 June 2012, three mini-Horhizotrons were filled with each individual substrate. One plug of *Chrysanthemum* 'Garden Alcala Red' was planted into the center of each mini-Horhizotron. Three substrates x three replications of each substrate made a total of 9 mini-Horhizotrons. Plants were also grown in the same substrates in greenhouse containers to compare root dry weights at the end of the study to show any effects the shape of the mini-Horhizotron may have contributed, compared to a container of similar substrate volume. Six greenhouse containers (16.5 cm dia) were filled with each substrate and filled to the top of the container and tapped three times to settle the substrate. One plug of *Chrysanthemum* was planted into the center of the containers, three substrates x six replications of each substrate made a total of 18 containers.

Root length measurements (cm) were taken on the three longest roots appearing on the face of each arm on 11, 18, 25, and 32 days after planting (DAP). Measurements were taken by attaching a transparent sheet with a printed $cm^2$ grid on each arm face, and roots were measured from the start of the gridlines, which was at the center of the mini-Horhizotron (where the plant was planted), to the end of the gridlines which reached the end of the arms. Measuring three roots per arm face x six arm faces per mini-Horhizotron x three replications per substrate equals 54 data points collected. Data were subjected to the general linear model procedures and regression analysis (SAS Institute version 9.2, Cary, NC). Means were separated by least significant differences at $P \leq 0.05$. Both the mini-Horhizotrons and the container-grown plants were harvested on the last measurement date; shoots were removed at the substrate surface and the root balls were washed to remove substrate. Both the shoots and washed root systems were oven dried at 70°C for 48 hours. The comparison of root dry weights between the mini-Horhizotrons and the greenhouse containers were subjected to least square means analysis (SAS Institute version 9.2, Cary, NC). Means were separated by Tukey's studentized range (HSD) at $P \leq 0.05$.

**2. Rhizometer.** Two substrates were used in this study, 75% peat (by volume) amended with 25% of either PWC or PL. The substrates were mixed on 3 Sept. 2012 and both substrates were amended with dolomitic limestone at 3.85 $kg \cdot m^{-3}$ to bring the pH value to 5.8. On 4 Sept. 2012, 40 Rhizometers were filled with the PWC substrate and 40 Rhizometers were filled with the PL substrate, and both substrates had a marigold (*Tagetes erecta* 'Inca Orange') plug planted into each one. All Rhizometers were then wrapped with the dark foil to restrict light from the rhizosphere and completely randomized on a greenhouse bench.

Every week after the installation of the experiment, 10 Rhizometers were chosen randomly and removed from the greenhouse, for four weeks. Five planted Rhizometers were prepared for testing in the NCSU Porometer method. For the remaining five planted Rhizometers, the marigolds were harvested at the base of the substrate and all substrate was washed from the root systems, to determine root biomass. This was conducted so that data of root growth mass over time was known and correlated with the changes in substrate physical properties. To prepare the Rhizometer for the porometer method, shoots were severed at the base of the substrate and the collar extension was removed, revealing 1-2 cm of substrate above the 7.6 cm core. This substrate and any roots above the main core were removed such that the substrate within the core was level with the top of the core. The bottom screen was removed, exposing the bottom of the Rhizometer for insertion into the base plate of the porometer method. Rhizometers were then processed through the NCSU Porometer procedure described by Fonteno et al. (1995) to determine physical properties, including total porosity (TP), air space (AS) and container capacity (CC).

Means separation using least significant difference (P≤0.05) was used to compare means of substrate physical properties and root dry mass (SAS Institute version 9.2, Cary NC).
**3. Hydraulic Conductance Flow Meter.** Three substrates were used in this study; peat amended with 20% (by volume) PL; 20%, 30% (by volume) SH. The substrates were mixed on 4 June 2012; all substrates were tested for initial pH and then amended with dolomitic limestone. On 5 June 2012, five greenhouse containers (15.2 cm dia) were filled with each individual substrate to the top of the container and tapped three times, by lifting the containers 10 cm from a hard surface and dropping, to settle the substrate. *Chrysanthemum* 'Garden Alcala Red' plugs were planted into the center of each container. Three substrates x five replications made a total of 15 containers.

On 6 July 2012, all container-grown *Chrysanthemums* were removed from the greenhouse into a controlled temperature room (21°C). The container-grown plants were placed in a vessel of tap water in order to saturate the substrate and pores around the roots so that air bubbles were easier to remove from the substrate and from the xylem. While saturated, plants were then singly and randomly severed 3 cm above the substrate level to leave enough stem for later manipulations. The HCFM connection was immediately placed on the root system and the HCFM was turned on to begin measuring root hydraulic conductance. Transient root conductance was computed from the linear slope of the pressure versus flow data points given by the HCFM. After the conductance measurements were taken, the HCFM connection was removed and substrate was removed from the root system in order to place the washed roots in the oven to be dried (70°C for 48 hours) for biomass measurements. Each plant was plotted on a graph in order to find correlations between root conductance and root dry mass and linear regression was run to define the relationship (SAS Institute version 9.2, Cary NC).

## RESULTS AND DISCUSSION

### Rhizometrics

**1. Mini-Horhizotron.** Beginning at 18 DAP and continuing to 25 DAP, *Chrysanthemums* grown in PWC had more root growth than plants grown in PL and SH (Fig. 1). Observed for all species, the PWC or SH components either enhanced root growth at certain time periods or were not significantly different from root growth observed in the PL substrate.

There were no differences between the growth of the shoots and roots of *Chrysanthemum* in all three substrates, when grown in either the mini-Horhizotron or containers (Table 1). Since *Chrysanthemum* species show no significant root or shoot growth differences between the two growing methods, the experimental design of the mini-Horhizotron does not influence root growth (treatment effects) of these species.
**2. Rhizometers.** Container capacity and TP for both PL and PWC substrates were not significantly different over the four weeks (data not shown). Air space measurements were not significantly different between PL and PWC substrates over the four weeks (Fig. 2). Marigold root growth between the PL and PWC substrate was not significantly different until 28 DAP when the marigold roots in PWC substrate had a larger dry mass than the roots in PL substrate (Fig. 2). Although AS was not significantly different, AS of PWC substrate was 2.1% less than PL substrate at 28 DAP when marigold root growth was greater in the PWC substrate.
**3. Hydraulic Conductance Flow Meter.** There was a linear response observed in root conductance plotted against root dry weight of *Chrysanthemums* grown in 30% SH substrate (Fig. 3). For *Chrysanthemums* grown in 20% SH substrate, root conductance increased with increasing root dry mass in a quadratic model (Fig. 3). *Chrysanthemums* grown in 20% PL substrate have an observed increase in root conductance with increasing root dry mass; however there is not a significant linear or quadratic relationship. Further work needs to be done to investigate why the relationship changed from linear to quadratic between 30% SH and 20% SH, respectively. This observation could possibly be explained by observing the root system architecture/branching and the effects of substrate type on these root characteristics.

## CONCLUSION

The study of Rhizometrics at NCSU is investigating several new methods of accurately and efficiently measuring root growth of container-grown plants. The mini-Horhizotron has been determined to be effective in measuring root growth of small herbaceous plants and these data shown here convey that root growth of plants grown in the mini-Horhizotrons are representative of the same plants grown in containers, to allow for accurate measurements of root systems. The Rhizometer is an effective tool in measuring physical properties of substrates, as well as the effect of root growth on the substrate physical properties over time. The HCFM has shown potential for quantifying root growth with root conductance values due to the correlations seen with this data, however the differences observed between substrates needs to be further studied. Observations on root characteristics could be measured by growing the plants in the mini-Horhizotron to view the differences in the root systems before using the HCFM to measure root conductance. These techniques of Rhizometrics have shown promising results in quantifying undisturbed root systems. The ability to visualize, observe and measure the growth of roots in a non-destructive way will further expand root growth research and understanding.

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## Tables

Table 1. Comparison of shoot and root dry weight of *Chrysanthemum* species between plants grown in containers and plants grown in the mini-Horhizotrons.

| Plant | Substrate | Shoot[z] (g) | | Root[y] (g) | |
|---|---|---|---|---|---|
| | | CT[x] | MH[w] | CT | MH |
| *Chrysanthemum* | PL[v] | 7.1 a[s] | 7.0 a | 4.4 a | 4.6 a |
| | PWC[u] | 5.6 a | 6.5 a | 4.3 a | 4.5 a |
| | SH[t] | 6.4 a | 5.6 a | 5.0 a | 6.9 a |

[z] Shoot dry weight, severed plant at substrate surface and oven dried.
[y] Root dry weight, washed root system to remove all substrate and oven dried.
[x] CT is plant dry weights from plants grown in containers.
[w] MH is plant dry weights from plants grown in mini-Horhizotrons.
[v] PL substrate is 70:30 peat:perlite (v/v).
[u] PWC substrate is 70:30 peat:pine-wood-chips (v/v).
[t] SH substrate is 70:30 peat:shredded-pine-wood (v/v).
[s] Means separated within row separated for shoot and root by Tukey-Kramer significant difference, $P \leq 0.05$. Means followed by the same letter are not significantly different.

## Figures

Fig. 1. A planted mini-Horhizotron, seen here with two arm faces visible (without the shade panel) and the root branching/architecture can be observed. Shade panels are in place against the other arm sections.

Fig. 2. Planted Rhizometers, without foil covers so that the clear sides and the attached collar are visible. The bottom ring is holding a screen to allow for drainage.

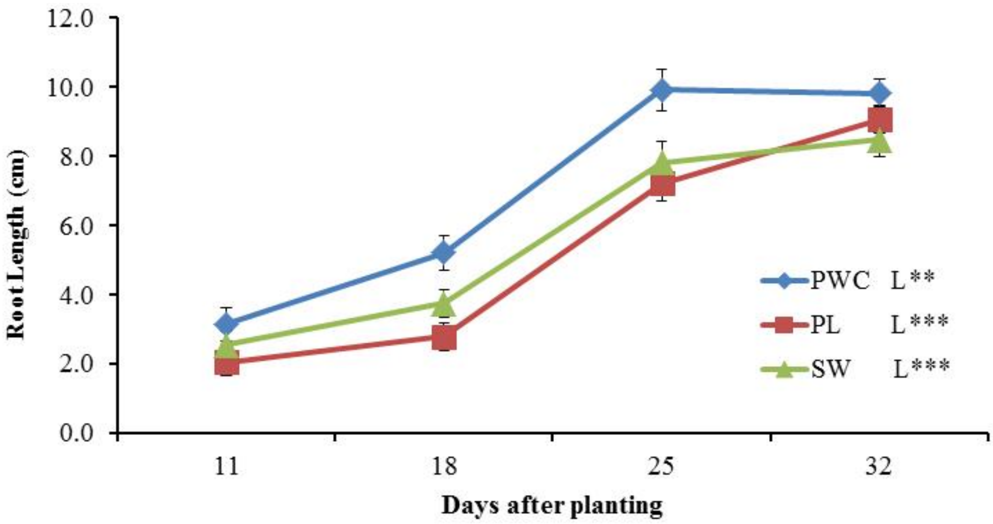


Fig. 3. Root length measurements of *Chrysanthemum* from 11 to 32 days after planting (DAP) for 70% (by volume) peat moss amended with either 30% perlite (PL), pine wood chips (PWC), or shredded-pine-wood (SH).

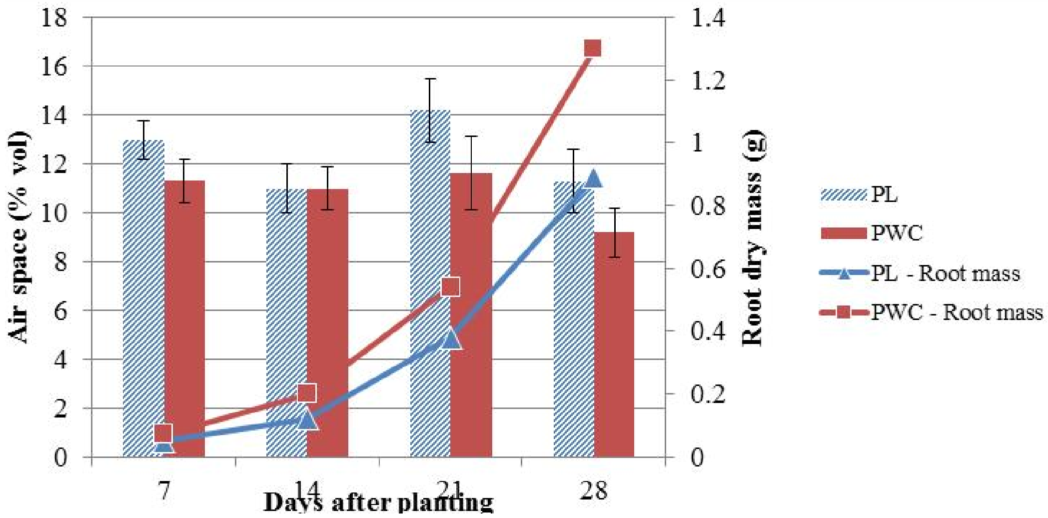


Fig. 4. Air space measured with NCSU Porometer method for 75% (by volume) peat amended with either perlite (PL) or pine-wood-chip (PWC) substrates over four weeks. Root dry mass of marigolds planted in both substrates over four weeks.

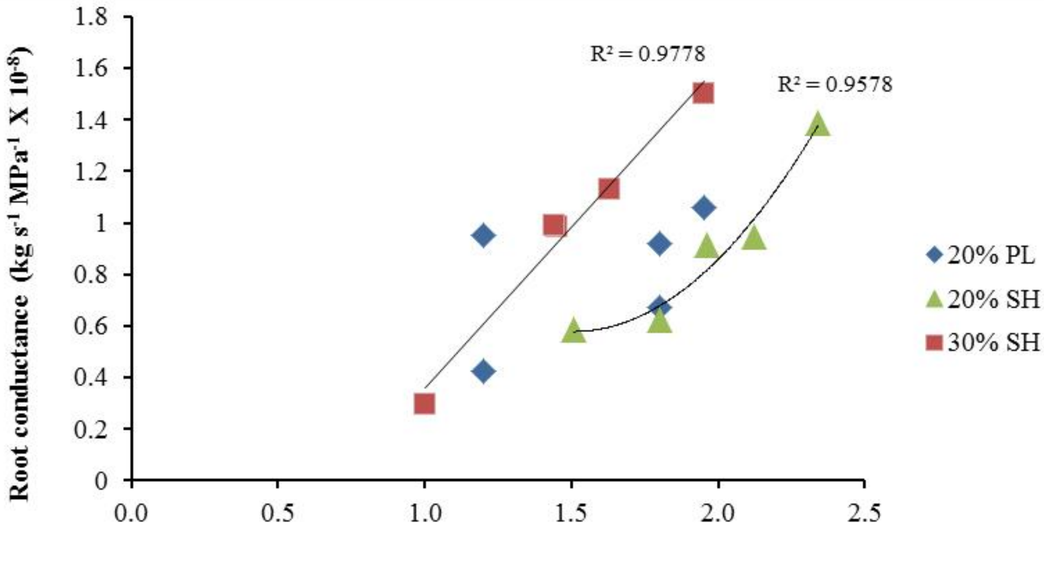


Fig. 5. Root conductance plotted against root dry mass of all *Chrysanthemum* plants in peat amended with either 20% (by volume) perlite (PL), 20% shredded-pine-wood (SH), or 30% SH. 20% PL substrate was non-significant with both linear and quadratic regression. 20% SH had a significant quadratic relationship ($R^2$=0.957, y = -0.086 + 0.253$x^2$). 30% SH had a significant linear relationship ($R^2$=0.978, y = -0.895 + 1.254x).