Torrefied wood effects on the seedling quality of Zelkova serrata and Fraxinus rhynchophylla in a containerized production system

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ABSTRACT
Biochar can be used as a kind of soil amendment in crop production, but there are few studies on containerized seedling production in forestry. The purpose of this study was to investigate the amount of torrefied wood to mix with growing medium for Zelkova serrata and Fraxinus rhynchophylla seedling production in a containerized system. We used torrefied wood made from chips of Quercus accutissima, which was mixed with growing medium by 0%, 10%, 20%, 30%, and 40% in volume. We applied two levels of 0.5 g/L (1 ×) and 1.0 g/L (2 ×) commercial fertilizer for fertilization treatment. Generally, growth parameters such as height, root collar diameter, and dry weight were comparable among 0%, 10%, and 20% mixed ratios, but those at 30% and 40% mixed ratio were lower than other mixed ratios. The 2 × fertilization significantly increased height by 29%, root collar diameter by 18%, total dry weight by 52%, and quality index by 31.4% compared to the 1 × fertilization across mixed ratios. Our results suggest that torrefied wood can be substitutable for 20% of the growth medium in total volume in containerized Zelkova serrata and Fraxinus rhynchophylla seedling production systems.

Introduction
Biochar is a kind of charcoal produced under high temperatures using crop residues, animal manure, or any type of organic waste material (Kelsi 2010). It has been used to increase soil nutrient status and soil organic carbon resulting in improved plant growth and soil properties (Glaser et al. 2002; Rondon et al. 2007), especially in agricultural fields.

However, biochar has a wide range of properties because its properties are variable as they depend on the original feedstocks (Lehmann 2007; Downie et al. 2009; Cho et al. 2017) and the temperature and heating rate of the pyrolysis process (Liang et al. 2006; Cheng et al. 2008; Downie et al. 2009; Watanabe et al. 2014). So, amending soils with biochar from various feedstocks will result in differing effects on soil properties and subsequent effects on plant growth (Amonette et al. 2009). Consequently, the effects of biochar application on soil characteristics and plant growth are not uniform. Spokas et al. (2012) reported that only 50% of the reviewed studies showed a positive growth response, 30% showed no significant differences, and 20% found negative growth.

Torrefied wood is a kind of biochar produced at low temperature under low oxygen conditions. Torrefaction has been used to upgrade fuel woody biomass by increasing heating values as well as reducing transportation costs due to evaporating internal water from the biomass (Bourgois and Guyonnet 1988). Although application of biochars has potential for renewable energy, soil amendment, and carbon sequestration (McElligott et al. 2011), there is little information about the impacts of torrefied wood application in forestry sectors. Only one report by Ogura et al. (2016) observed that torrefied wood application in African drylands improved water retention ability and mineral ability for plants. Furthermore, the combination of fertilization with torrefied wood increased initial growth of Jatropha curcas and K, P, and S mineral uptake.

In a companion study, we found woodchips of Quercus accutissima were better for feedstocks of torrefied biomass than cones of Pinus koraiensis and crab shells (Cho et al. 2017). In this study, we investigated the effect of torrefied wood on seedling growth and physiological properties of Zelkova serrata and Fraxinus rhynchophylla to verify the substitution ratio of commercial soil medium in a containerized seedling production system. We used oak chips for torrefied wood because these could be supplied from trees damaged by disease and pests, which were usually cut and abandoned on the forest floor. Zelkova serrata and F. rhynchophylla seedlings were used to verify the effects of torrefied wood in a containerized production system because of their high economic value as a commercial timber or a street tree. This study proposes an optimal mixed ratio of torrefied wood with commercial soil medium in a containerized seedling production system, which may produce a kind of forest biomass utilization with high value.

Materials and method
Study sites and species
The experiment was conducted in the greenhouse located at Chungnam National University in Daejeon city, South Korea (36°22'16"N, 127°21'08"E). The temperature and humidity were measured from May to September 2015 with HOBO (U23 Pro v2, USA). Mean temperature was 22.5 °C and mean humidity was 78.6% (Cho et al. 2017).

The Z. serrata and F. rhynchophylla seeds collected from the seed orchard were germinated in March 2015 at the Forest Practice Research Center, National Institute of Forest
mixed with 360 mL artifical soil for the 10% mixed ratio treatment. We applied up to 40% mixed ratio treatment in accordance with the findings of Cho et al. (2017). The physical properties of torrefied oak chip showed 16.4% of fixed carbon and 79.9% volatility (Table 1). The pH was 5.1, which was relatively low compared to the meta analysis of Spokas et al. 2012, but other chemical properties were in accordance with the findings of Cho et al. 2017.

**Torrefied wood production and analysis**

In May 2015, the torrefied wood was made by using a wood roaster at the College of Agriculture and Life Sciences, Chungnam National University (Cho et al. 2017). This wood roaster automatically conveys sources and carbonizes at temperatures of 200 °C to 250 °C. Oak chips (c. 2 cm × 2 cm × 0.5 cm) were used as the feedstock. More details of the torrefied wood production and analysis are described in Cho et al. (2017). The physical properties of torrefied oak chip showed 16.4% of fixed carbon and 79.9% volatility (Table 1). The pH was 5.1, which was relatively low compared to the meta analysis of Spokas et al. 2012, but other chemical properties were in accordance with the findings of Cho et al. 2017.

**Experimental treatments**

The cells for *Z. serrata* and *F. rhynchophylla* were 6.8 cm in diameter and 15 cm in depth (400 mL). Each tray was 32 cm × 40 cm and contained 20 cells. The trays were placed on a platform 60 cm above the greenhouse floor. The experimental design was 5 × 2 factorial combinations with *Z. serrata* and *F. rhynchophylla* seedlings. We applied five levels of torrefied wood chips mixed ratios and two levels of fertilization. Each treatment was applied for five replications in a completely randomized design. The artificial soil was Peat moss, Perlite, and Vermiculite at a ratio of 1:1:1 by volume, which is the recommended artificial mixture for tree seedlings in container seedling production (Landis et al. 1990). Five levels of mixed ratio were applied. Non-torrefied wood was mixed with 400 mL artificial soil for the control; 40 mL of torrefied wood was mixed with 360 mL artificial soil for the 10% mixed ratio treatment. We applied up to 40% mixed ratio treatment in this study. Ten seedlings were planted in each tray with blank cells in between seedling cells. The planted seedlings were of a similar height (c. 6 cm) (Cho et al. 2017).

Fertilizer (MultiFeed20 fertilizer [20N: 20P₂O₅: 20K₂O], Haifa Chemicals, Israel) was applied at two levels – 0.5 g/L for 1× fertilization and 1 g/L for 2× fertilization – at 110 mL per cell once a week. Fertilization started on May 28 and went on for 10 weeks. Water irrigation was applied for 20 minutes every day. The positions of the trays were rotated every 2 weeks in order to reduce unknown environmental influences such as unequal water irrigation.

**Growth measurements**

During the experiments, the height and root collar diameter (rcD) of seedlings were measured every month from late May to late September. The height was measured from the ground to apical meristem, while the rcD was measured at 1 cm above the ground. The seedlings were harvested in October and divided into stem, leaf, and root. The roots were washed with tap water in order to remove any soil particles. All components were dried to constant weight at 65 °C for 48 hours in a dry oven.

**Chlorophyll measurements**

For measuring chlorophyll content, three healthy leaves of *Z. serrata* and *F. rhynchophylla* from the 3rd to 5th order from the top for each seedling were selected and measured by SPAD-502plus (MINOLTA, Japan). The measurements were taken once in September.

**Dickson’s quality index**

To measure the quality of seedlings, Dickson’s quality index was used as follows:

\[
\text{Quality index} = \frac{\text{SD}}{\text{(HD + SR)}}
\]

where SD is seedling dry weight (g), HD is height (cm) to rcD ratio (mm), and SR is shoot to root dry weight ratio (Deans et al. 1989; Bayala et al. 2009; Park et al. 2015).

**Statistical analysis**

Analysis of variance (ANOVA) with Duncan’s multiple comparison tests was applied to test the effects of five levels of torrefied wood mixed ratio and two levels of fertilization treatment on seedling height, rcD, dry weight, quality index, and chlorophyll contents, which were measured in September. The change of height and rcD growth across months was analyzed by one-way ANOVA for each treatment. All probabilities were tested at the significance level of 0.05.

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**Table 1.** Physical and chemical properties of the growing media before mixing with torrefied wood (revised from Cho 2015).

| Bulk density (g m⁻³) | pH  | Electric conductivity (ds m⁻¹) | Organic matter (%) | Total N (%) | P₂O₅ (mg kg⁻¹) | Exchangeable cations (cmolₖg⁻¹) | CEC (cmolₖg⁻¹) |
|----------------------|-----|------------------------------|--------------------|-------------|----------------|--------------------------------|----------------|
| 0.37                 | 6.1 | 0.06                         | 4.0                | 0.08        | 3.0            | 0.3                            | 3.3            | 2.5                        | 24.3          |

**Table 2.** Physical and chemical properties of torrefied wood (revised from Cho et al. 2017). Values in parentheses represent one standard error of the mean (n = 3).

| Moisture content | Ash (%) | Fixed carbon (%) | Volatility (%) | pH | Electric conductivity (ds m⁻¹) |
|------------------|---------|------------------|----------------|----|------------------------------|
| 3.2 (0.0)        | 0.5 (0.0)| 16.4 (0.4)      | 79.9 (0.4)   | 5.1 (0.0) | 0.282 (54)                   |

| N (g kg⁻¹) | P (g kg⁻¹) | K (g kg⁻¹) | Na (g kg⁻¹) | Ca (g kg⁻¹) | Mg (g kg⁻¹) |
|------------|------------|------------|-------------|-------------|-------------|
| 0.7 (0.4)  | 0.94 (0.08)| 0.97 (0.12)| 0.64 (0.11) | 10.3 (1.2)  | 0.83 (0.09) |
Results

There were no significant interaction effects of mixed ratio and fertilization treatments on *Z. serrata*’s seedling height and rcD growth, but marginally significant effects on the growth of *F. rhynchophylla* (Table 3, Figures 1–2). However, both main factors significantly influenced the growth of both species.

In *Z. serrata*, seedling height and rcD were significantly lower in the 30% and 40% mixed ratios than in the other mixed ratios which, between them, were not significantly different (Figure 1(a) and 2(a)). The 2× fertilization significantly increased height by 29% and rcD by 18% compared to the 1× fertilization. The pattern of periodical height growth of *Z. serrata* plateaued between August and September, but rcD increased during the same period; relative growth rates between measurements were 4.1% for height and 16.4% for rcD.

In *F. rhynchophylla*, seedling height was lower in the 30% and 40% mixed ratio than in the other mixed ratios in the 1× fertilization treatment, but was not significantly different in the 2× fertilization treatment (Figure 1(b)). In other words, the differences between the average height growth of 0%, 10%, and 20% mixed ratio and the average height growth of

Table 3. ANOVA table for growth parameters and chlorophyll contents of *Zelkova serrata* and *Fraxinus rhynchophylla*. Height and root collar diameter measured in September were used in the analysis.

| Species             | Source of variable | Degree of freedom | Probability (Pr > F) |
|---------------------|--------------------|-------------------|---------------------|
|                     |                    |                   | Dry weight          |                     |
|                     |                    |                   | Height              | Root collar diameter|
|                     |                    |                   | Leaf                | Stem                |
|                     |                    |                   | Root                | Total               |
|                     |                    |                   | Quality index       | Chlorophyll contents|
| *Zelkova serrata*   | Mixed ratio        | 4                 | <0.01               | <0.01               |
|                     | Fertilization      | 1                 | <0.01               | <0.01               |
|                     | Mixed ratio × Fertilization | 4 | 0.44               | 0.20               | 0.52               | 0.28               | 0.63               | 0.30               | 0.13               | 0.48               |
| *Fraxinus rhynchophylla* | Mixed ratio | 4                 | 0.36               | <0.01               | 0.12               | 0.04               | <0.01               | <0.01               | <0.01               | 0.10               |
|                     | Fertilization      | 1                 | 0.01               | 0.16               | 0.13               | <0.01               | 0.09               | 0.03               | 0.13               | <0.01               |
|                     | Mixed ratio × Fertilization | 4 | 0.09               | 0.09               | 0.72               | 0.16               | 0.37               | 0.34               | 0.33               | 0.55               |

Figure 1. Height growth of (a) *Zelkova serrata* and (b) *Fraxinus rhynchophylla* at 1× fertilization (left) and 2× fertilization (right) torrefied wood mixed ratios. Different letters represent significant differences between mixed ratios in both fertilization treatments. Vertical bars represent one standard error of the mean (n = 5).
30% and 40% mixed ratio was 23.3% in the 1× fertilization, but only 6.2% in the 2× fertilization treatment. As with *Z. serrata*, rcD was significantly lower in the 30% and 40% mixed ratios than in the other mixed ratios which, between them, were not significantly different. The pattern of periodical height growth of *F. rhynchophylla* plateaued from the July measurement, but rcD growth was still steep until harvesting in September; relative growth rates for 8 weeks were 4.6% for height and 59.5% for rcD.

As expected, *Z. serrata*’s biomass of leaf, stem, and root showed similar patterns of height and rcD growth across biochar mixed ratio and fertilization treatment (Table 3, Figure 3(a)). Average total biomass was significantly higher, by 62.7%, for the 0%, 10%, and 20% mixed ratios (which, between them, were not significantly different) than the 30% and 40% mixed ratios. The 2× fertilization significantly increased leaf biomass by 56.6%, stem biomass by 68.8%, and root biomass by 31.2% compared to the 1× fertilization.

Like *Z. serrata*’s biomass growth pattern, there was no significant interaction between mixed ratio and fertilization treatment in *F. rhynchophylla* biomass growth (Table 3, Figure 3(b)). Total biomass at 0% and 20% mixed ratios was twice that at 30% mixed ratio and total biomass at 10% and 40% mixed ratios were not significantly different from other mixed ratios. The 2× fertilization significantly increased total biomass by 30.0% compared to the 1× fertilization.

The order of quality index, which is one of the most comprehensive to evaluate seedling quality, was 0% > 10%, 20%, and 40% > 30% mixed ratios for *Z. serrata*’s seedlings and 0% > 20%, 10% > 30%, 40% mixed ratios for *F. rhynchophylla*’s seedlings (Figure 4(a) and (b)). Fertilization significantly increased the quality index of *Z. serrata* by 31.4%, but not for *F. rhynchophylla* (Figure 4(a) and (b)).

Chlorophyll contents of both species were not statistically influenced by biochar mixed ratio, but the 2× fertilization treatment significantly increased chlorophyll contents compared to the 1× fertilization, by 14.2% for *Z. serrata* and 6.8% for *F. rhynchophylla* (Table 3, Figure 5).

**Discussion**

Because the properties of biochar depend on feedstocks and pyrolysis condition, five feedstocks were torrefied and the effects on plant growth were verified in a previous study (Cho et al. 2017). This study found that the effects of biochars made from *Pinus densiflora*, *Q. accutissima*, and rice husk...
were better than those of pine cones and crab shells in seedling production. Torrefied wood provides absorption of water and nutrient elements when incorporated into growing medium because the high temperature (c. 200 °C) during torrefaction degrades the hemicellulose components and remaining cellulose of oak chips (Ogura et al. 2016). Furthermore, torrefied wood has the capacity to increase beneficial elements such as K, P, and S and inhibit toxin absorption.

The influence of torrefied wood on soil fertility and plant growth could be negative in high application ratios - above 30% in this study. Torrefied wood in the soil can absorb, release, and transform compounds, which significantly influence plant growth and microbial activity. Torrefied wood can also release or absorb volatile organic compounds (Warnock et al. 2010; Spokas et al. 2012), which influence microbial activities resulting in positive or negative effects on plant growth (Deenik et al. 2010; Graber et al. 2010). Heavy ratios of torrefied wood could therefore have negative effects because of pH change and unbalanced nutrients in the soil medium (Sumner 2000; Major et al. 2010).

Growth of height and rcD did not respond linearly with growth period in a companion study (Cho et al. 2017). The height growth measurement can be done until August for *Zelkova serrata* and until July for *Fraxinus rhynchophylla*. However, the measurement of root collar diameter for both species should be done until September at least in the greenhouse study. Seedlings treated with less than 20% torrefied wood substitution of growing medium might improve initial survival and growth rate because the quality index (Dickson et al. 1960) was increased by this treatment by 69%.

Figure 3. Leaf, stem, and root dry weight of (a) *Zelkova serrata* and (b) *Fraxinus rhynchophylla* at 1× fertilization (left) and 2× fertilization (right) torrefied wood mixed ratio. Different letters represent significant differences between mixed ratios in both fertilization treatments. Vertical bars represent one standard error of the mean (n = 5).
Figure 4. Seedling quality index of (a) *Zelkova serrata* and (b) *Fraxinus rhynchophylla* at 1× fertilization (left) and 2× fertilization (right) torrefied wood mixed ratio. Different letters represent significant differences between mixed ratios in both fertilization treatments. Vertical bars represent one standard error of the mean (n = 5).

Figure 5. Chlorophyll contents of (a) *Zelkova serrata* and (b) *Fraxinus rhynchophylla* at 1× fertilization (left) and 2× fertilization (right) torrefied wood mixed ratio. Different letters represent significant differences between mixed ratios in both fertilization treatments. Vertical bars represent one standard error of the mean (n = 5).
In forestry biochar has the potential to curtail greenhouse gas emissions to mitigate global climate change by acting as a soil supplement that sequesters carbon in the soil and supplies nutrients to plants. According to height, rcD, and total biomass growth, 20% torrefied wood substitution of growing medium is the best ratio to apply to Z. serrata and F. rhynchophylla seedlings in a containerized production system.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This study was carried out with the support of ‘R&D Program for Forest Science Technology (project number 2014068E10-1719-AA03) provided by Korea Forest Service (Korea Forestry Promotion Institute).

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