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Foliar spray of growth regulators significantly increases *trans*-1,4-polyisoprene production from *Eucommia ulmoides* Oliver short-rotation coppice

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ABSTRACT

Eucommia ulmoides Oliver produces *trans*-1,4-polyisoprene (TPI or Eucommia rubber), with leaves and bark as the main sources. Raw materials from short rotation coppice (SRC) cultures for TPI industrialization are a valuable option. Currently, there is a shortage of field yield data beginning from early establishment of Eucommia SRC to support TPI manufacturers. We investigated an Eucommia SRC plantation established in 2015 with application of four plant growth regulators in Mengzhou Forest Farm, China, during its first two annual rotations. By using an orthogonal experimental design, productivity related data were derived after each growing season. We observed significant variations in biomass yield and TPI productivity among the 12 dosages of 4 regulator types and control, with plants treated with brassinolide at 5 mg L^{-1}, DCPTA at 500 mg L^{-1} and DTA-6 at 20 mg L^{-1} reaching the highest TPI productivity among treatments of 406 kg ha^{-1}, 391 kg ha^{-1} and 382 kg ha^{-1} in the second rotation, respectively, all of which were more than 1.4 times that of the control. Foliage spray of regulators led to more and larger leaves, more bark yield, higher tree height, and higher leaf TPI content, which reflected the enhancing effects of regulators on leaf and bark biomass as well as TPI productivities in both the first and second rotations. Meanwhile, the harvest of Eucommia leaf and bark from the coppice plantation was less labor intensive than that from tall mature trees. Our study suggested that the annual rotation period and increase of TPI from Eucommia SRC by application of regulators could reduce the payback period. Therefore, we recommend the application of growth regulators to coppice Eucommia plantations beginning from the first rotation as an economic means to increase TPI production.

1. Introduction

Eucommia ulmoides Oliver (Eucommia, Eucommiaceae), a living-fossil tree species native to central and southern China, is one of the few woody plants that produces *trans*-1,4-polyisoprene (TPI) in its leaves, bark, root bark, and fruit coatings (Bamba et al., 2002; Nakazawa et al., 2009; Yan, 1995). TPI is a thermoplastic crystalline polymer with properties similar to plastic that can be used as an industrial raw material substitute for natural rubber (*cis-1*, 4-polyisoprene CPI) and petroleum (Du et al., 2008; Du et al., 2009; Yan, 2010). In recent years, significant progress has been made in application of Eucommia TPI to production of tires, plastic modifications, medicinal materials (Ren et al., 2013), and other industrial uses (Jin et al., 2013). As a result, the demand for Eucommia TPI has increased significantly and there is an urgent need to increase Eucommia TPI production.

However, large-scale Eucommia TPI production is currently hindered by Eucommia shortages. The total area of Eucommia plantation is less than 400,000 ha worldwide, of which 95% are in China. For the past few decades, Eucommia TPI has mainly been extracted from Eucommia leaves (Zhang, 1996; Bamba et al., 2002; Du et al., 2003; Zhang et al., 2008). A well-managed mature Eucommia plantation (> 6 years old) at a density of 2000 to 5000 stems per hectare can yield 4200–4900 kg of dry leaves annually, equivalent to 60 kg to 100 kg per hectare of Eucommia TPI (Du, 2010; Sun et al., 2013), which is much lower than the amount of rubber produced from Brazilian natural rubber (*Hevea brasiliensis*) plantations with a rubber yield ranging from 500 kg per hectare per year in small plots to more than 1500 kg per hectare per year in large plantations (Balsiger et al., 2000). Another

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issue is the high labor cost of leaf harvest. Adult Eucommia trees are usually 7–15 m in height, which makes leaf collection quite labor-intensive. Therefore, the low biomass and high labor cost of Eucommia leaf harvest has resulted in relatively low profitability.

Theoretically, there are two approaches to overcome the aforementioned problems. The first is to develop high-density short-rotation coppice (SRC) plantations of Eucommia to produce large amounts of leaf and bark biomass annually. This approach is also advantageous because the harvest of leaves and bark from SRC is less labor intensive. Eucommia trees usually have a life cycle of 40–60 years (Sun et al., 2013). This species is highly resistant to frost, adaptable to variable soil conditions, has strong re-sprouting capacity from its stump base and grows fast when cultivated under suitable climatic and soil conditions (Wang and Xiong, 2003; Du et al., 2012). These characteristics make this deciduous tree suitable for harvest of leaves and barks for TPI production in annual rotation cycles. Recent studies have confirmed the commercial potential of SRC plantations to supply large amounts of high quality lignocellulosic biomass in a managed, sustained and cost-effective manner (Field et al., 2008; Hinchee et al., 2011; Liu et al., 2012; Nissim et al., 2013; Testa et al., 2014). Another advantage is that periodic harvesting of SRC may last for approximately 20 years without the need to replant (Nissim et al., 2013). To date, there have not been any systematic studies of the above-ground biomass production from Eucommia SRC plantations.

Nevertheless, some recent reports claimed that the annual production of leaves, bark and branches or stems can reach about 10.0–15.0 tons ha^{-1}, 5.0–7.5 tons ha^{-1} and 18.0–22.5 tons ha^{-1} from a Eucommia SRC at three years after planting, respectively (Zhu et al., 2016; Zhu et al., 2017). However, the authors did not mention whether these values were in dry weight or fresh weight. Moreover, we were unable to find similar values in the literature they cited (see references in Ji and Su (2006)). Therefore, the production they reported was in doubt, necessitating that the aboveground biomass of Eucommia SRC needs to be measured in a systematic way.

The second method is to apply plant growth regulator to increase leaf or bark biomass or TPI content in Eucommia leaves or bark. The positive effects of exogenous hormones have been widely demonstrated in stimulation of shoot elongation, improvement of fruit set or crop yield, or enhancement of secondary metabolites (Rademacher, 2015). For example, foliage spray of 2-(3, 4-dichlorophenoxy)-triethyl amine (DCPTA) increased the rubber (CPI) accumulated in the treated guayule (*Parthenium argentatum* A. Gray) plants (Yokoyama et al., 1977), as well as the TPI content in greenhouse-grown Eucommia leaves (Hayman et al., 1994). Studies in China also reported that applications of exogenous hormones, such as gibberellin (GA_3), 2, 4-dichlorophenoxyacetic acid (2, 4-D), 6-benzyl adenine (6-BA), and naphthylacetic acid (NAA) increased TPI concentration in Eucommia leaves, as well was growth of leaves and shoots (Xu, 2007; Zhang et al., 2008; Zhang et al., 2010). However, no field studies conducted to date have investigated the effects of application of growth regulators on leaf and bark biomass to evaluate TPI productivity by Eucommia SRC.

In 2015, a Eucommia SRC plantation for TPI purposes was established. In this study, we measured the annual leaf and bark production and evaluated the efficacy of four different plant growth regulators on Eucommia leaf and bark biomass and TPI content and productivities during the first annual rotation and the second annual rotation after coppice (2015 and 2016). The objectives of this study were: (i) to quantify the efficacy of regulators on the leaf and bark biomass production of the plantation during both rotations; (ii) to determine the impact of regulators on TPI accumulation in Eucommia leaf and bark and to evaluate the total TPI productivities; and (iii) to test the enhancement of plant growth characteristics by regulators during the two rotations and select the optimal regulator and its concentrations for practical uses.

2. Materials and methods

2.1. The study site

The study site was located at Mengzhou State Forest Farm (112°42′58″E, 34°51′38″N), Henan Province, China. The elevation is 112 m above sea level and the climate is temperate, with an annual rainfall of 549.9 mm, a mean annual temperature of 14.3 °C, and 224 frost-free days per year on average. The soil is a typical ancient Yellow River coarse sandy loam. The chemical characteristics of the top 40 cm soil are as follows: pH (H_2O) 8.67, organic matter 4.07 $g\,kg^{-1}$, total N 0.033 $g\,kg^{-1}$, total P_2O_5 0.05 $g\,kg^{-1}$, total K_2O 0.1 $g\,kg^{-1}$, rapidly available K_2O 156.6 $mg\,kg^{-1}$and rapidly available P_2O_5 4.8 $mg\,kg^{-1}$.

2.2. Experimental design

An area of 3000 m^2 that was 30 m wide from south to north and 100 m long from east to west was selected for the experiment. Trenches of 0.5 wide and 0.5 deep were dug along the north-south direction at the beginning of March 2015. Two adjacent trenches were 1 m apart from each other. After adding farmyard manure at 50,000 $kg\,ha^{-1}$ to the bottom, the trenches were backfilled. One-year-old *E. ulmoides* seedlings with a mean height of 1.2 m were then planted in a double-row planting scheme with alternating inter-row distances of 0.5 m and 1.0 m and a distance of 0.2 m between trees within the rows, corresponding to a tree density of about 66,666 ha^{-1}. All the seedlings were immediately watered and then cut, a stump with an average height of 5 cm above the soil was left for each seedling. Seedlings were then watered once every 10–15 days during the first 60 days, after which they were watered once every 20–30 days. During the first 30 days of growth, all lateral buds were removed and only one main bud per tree was allowed to grow. Moreover, manual weed control was applied to decrease competition for light and nutrients during the first months. No seedlings were fertilized during the study period.

An orthogonal experimental design consisting of the following four regulators was used: 1% brassinolide (BR) powder, 2-(3, 4-dichlorophenoxy)-triethyl amine (DCPTA), 2-(diethylamino ethyl hexanoate) (DTA-6), and gibberellin (GA) (Zhengzhou Zhengshi Chemical Products Co., Ltd.). Three treatments (dosages) were designed for each regulator with three replicates for each treatment (see Table 1). Ten seedlings were treated in each replicate. Spray water was used as the control treatment. Seedlings with similar height were selected and numbered in each treatment, then sprayed at 8:00–10:00 am on June 30, 2015, and re-sprayed one week later. To avoid mis-spraying regulator on nearby seedlings, a protected box was used to cover the treated seedlings when spraying. Overall, 390 seedlings were used for rotation 1. After one growing season from March to October, *i.e.* at the end of rotation 1 (R1), the treated plantlets were harvested for the first time on 20 October 2015.

Trees continued to grow as a coppice culture with one main bud per stool in the following annual rotation, *i.e.*, rotation 2 (R2). Regulators were applied on June 25, 2016 exactly as described above. The second harvest occurred manually on 15 October 2016.

Table 1
Type and concentration of regulators.

Regulator type	Regulator concentration ($mg\,L^{-1}$)		
Brassinolide	1 (BR1)	5 (BR2)	10 (BR3)
2-(3, 4-dichlorophenoxy)-triethyl amine	100 (DC1)	300 (DC2)	500 (DC3)
Diethyl aminoethyl hexanoate	20 (DA1)	60 (DA2)	100 (DA3)
Gibberellin	100 (GA1)	300 (GA2)	500 (GA3)
Control	H_2O (Control)		