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ORIGINAL ARTICLE

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Potential allelopathic effect of *Eucalyptus grandis* across a range of plantation ages

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Abstract Allelopathy of the eucalypt has been considered as an important mechanism for the biodiversity reduction in the eucalypt plantation. To understand the allelopathic potential of the eucalypt (*Eucalyptus grandis*) roots and rhizosphere soil along a chronosequence (2, 4, 6, 8, 10 years), the germination and growth characteristics of three plant species (*Raphanus sativus*, *Phaseolus aureus*, and *Lolium perenne*) growing nearby or beneath the eucalypt plantations were measured. The results showed that aqueous extract of *E. grandis* root suppressed the germination and early seedling growth of the target plants. The younger *E. grandis* exhibited a comparatively stronger allelopathic potential. The highest dose root extracts from 4 years old *E. grandis* showed the strongest inhibitory effects on the germination rates of the target species, the inhibitory rates were about 48, 51.2, and 56.56% for *R. sativus*, *P. aureus*, and *L. perenne*, respectively. However, present biotests of rhizosphere soils from 6, 8, and 10-year-old plantations exhibited a remarkable stimulative effect on *L. perenne*, which indicated that the soil might neutralize or dilute allelopathic agents with the increase of plantation age. In addition, according to GC–MS analysis, more allelopathic potential compounds were found in the rhizosphere soil and roots of younger *E. grandis* plantation. Moreover, more allelochemicals were obtained from soil than from roots. The allelopathic compounds in roots and rhizosphere soil may play important roles in allelopathy of *E. grandis* plantation. More attention should be paid to the younger *E. grandis* plantations for the relative higher allelopathic effects.

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Introduction

Plants are known to produce secondary metabolites that affect germinations and growth of other plants, this mechanism between plant species has been defined as allelopathy (Rice 1984; Weidenhamer 2005; Fitter 2003; Inderjit and Duke 2003). Many studies have documented close evidence to the establishment of invasive species and to the decreased yields of crop and weed (Badu and Kandasamy 1997; Macias et al. 1999; Chaves et al. 2001; Ahmed et al. 2008). Allelopathy has been considered as an important mechanism for the environmental impact of commercial plantation on degradation of soil, and reduction of productivity and biodiversity (Vesterdal et al. 2002; Wall and Heiskanen 2003; Smith et al. 2000). Consequently, there is growing interest in studying the allelopathic effects in commercial plantations.

Generally, allelopathic chemicals are released into the environment by four ecological processes: volatilization, leaching, decomposition of plant residues in soil, and root exudation (Rice 1984; May and Ash 1990; Badu and Kandasamy 1997; El-Darier 2002; Ahmed et al. 2008). Many previous studies have focused on the effects of leaf aqueous extracts on the germination and early seedling growth of various target plants (Lisanework and Michelsen 1993; Badu and Kandasamy 1997; Kong et al. 2006; Ahmed et al. 2008), and have obtained a lot of volatiles and soluble allelochemical compounds from leaves that could suppress the establishment of vegetative propagules and early seedling growth of the crops and weeds (del Moral and Muller 1970; Badu and Kandasamy 1997; Ziaebrahimi et al. 2007). However, allelochemical components might be accumulated in soil by leaching, plant residues decomposition and root exudation (Lee and Monsi 1963; Chou 1993; Bonanomi et al. 2006; Ahmed et al. 2008), which could play the

primary role in allelopathic effects on other plants. Unfortunately, little was available on allelopathy of root exudation and soil in plantation. In addition, the allelopathic effect greatly relies on various environmental factors including temperature, light, soil, precipitation, nutrients and water availability, and understory vegetation (Lee and Monsi 1963; Khan et al. 1999; Ahmed et al. 2008; Catherine et al. 2006, 2008). These factors were changed with the developing of forest, and then the allelopathy could be different along the forest ages. Nevertheless, only a few studies have taken the growth stage or age of the donor plant into consideration in studying allelopathy (Rice 1984; Catherine et al. 2006, 2008). Even so, inconsistent results have been founded in the previous studies. Increased or degraded inhibitory effects with growth and development stage were observed on invasive species and crops (Finney et al. 2005; Kong et al. 2006; Catherine et al. 2006, 2008), which might be related to the donor species and target species. Therefore, it is still not clear that the allelopathic potential changed with the increase of growing age of plantations.

Eucalypt (*E. grandis*) is one of the major introduced fast-growing commercial trees in southwest China, its plantation area has exceeded 20,000 hm^2 in Sichuan (Zhang and Yang 2008). Meanwhile, the allelopathy of eucalypts has been considered as the main cause of the reduction of biodiversity and abundance of plantation forbs and graminoids, and the productivity of adjoining crops. Allelopathic extracts and allelochemicals have been obtained from various eucalyptus tissues including leaves, barks, and litters, but little was available on the allelochemicals in root and soil, much remained unknown about the changes of allelopathic potential along the plantation ages. Therefore, we hypothesized that root exudates and rhizosphere soil of eucalyptus had significant allelopathic effects on the target species, and the allelopathic responses could be different along plantation age, and aimed to (1) identify the allelopathy of roots and rhizosphere soils in *E. grandis* plantation and (2) characterize the changes of allelopathic effects in different eucalyptus plantation ages.

Materials and methods

Sampling sites

Our study was conducted in the Danling county (102°57′–103°04′E, 29°55′–29°59′N, 570–592 m a.s.l), a typical *E. grandis* plantation located in southwest China. The mean annual temperature (current decade) is 17.5°C and precipitation is 1,350–1,580 mm, relative air humidity is about 82%. The soil is classified as ferralsol with old alluvial yellow loam and granular structure according to the Chinese Soil Taxonomy (2003). In these stands, *Pinus massoniana* and *Quercus acutissima* trees are the native dominate species. There are large areas of *E. grandis* of different ages (1–10 years old) in this region afforested on agricultural lands. These plantations have not received any special management as fertilization, thinning, or weed control. The area of each age plantation is over 10 hm^2. Fifteen plantations with similar environmental characters but different plantation ages (three replicates for each age, 2, 4, 6, 8 and 10 years old) were selected as sampling sites (Table 1).

Soil properties

At each plot, soil samples at three depths (i.e., 0–10 cm, 10–20 cm, 20–30 cm) were randomly taken from five sampling points. The undisturbed soil cores were collected from the three layers using metal cylinders of 100 cm^3 volumes. Roots and other debris were removed and discarded. All samples were sieved to pass a 2-mm sieve. One part of each sample was kept field-moist in a cooler at 4°C, and another half was air-dried and stored at room temperature. The bulk density (BD) was determined by the gravimetric method. Soil pH was measured electrometrically with a glass electrode (soil:-water = 1:2.5). Dichromate oxidation–titration method and semi-microkjedahl method were used to determine SOM and N content, respectively (Lu 1999). The cation exchange capacity (CEC) was calculated as the sum of H^+ (the hydrolytic acidity) and total exchangeable bases (TEB). The hydrolytic acidity (Ha) was determined by Kappen titration method, and TEB was determined by the method described by Luo (1993). Base saturation (BS) was calculated as the ratio of TEB to CEC. The soil physico-chemical properties are given in Table 2.

Material collection

Root and rhizosphere soil samples were collected from five individuals from the field replicates. In October of 2007, roots were sampled closely to the trunk (distance <0.5 m, 3–6 mm of root diameter, 10 cm of depth) and mixed thoroughly. A part of the roots were put inside

Table 1 Characteristics of study sites and trees of *E. grandis* (2, 4, 6, 8, and 10 years old including plantation age mean diameter, and height ±SD

Sites (years)	Coordinates	Slope (°)	Altitude (m)	Mean diameter (cm)	Mean height (m)
2	29°57′N 103°31′E	NE36	557	6.8 (0.8)	6.00 (0.5)
4	29°57′N 103°31′E	NE30	556	12.0 (1.1)	13.5 (0.2)
6	29°57′N 103°31′E	NE32	556	13.7 (1.8)	14.9 (0.7)
8	29°57′N 103°31′E	NE28	556	16.6 (1.0)	18.5 (0.2)
10	29°57′N 103°31′E	NE29	556	23.1 (0.4)	25.0 (0.6)

Table 2 Soil physico-chemical properties (SD) in *E. grandis* plantations of different establishment age

Soil properties	Plantation ages (years)				
	2	4	6	8	10
Bulk density (g cm^{-3})	1.47 (0.03)	1.36 (0.02)	1.31 (0.02)	1.28 (0.05)	1.16 (0.02)
Water content (%)	0.25 (0.01)	0.27 (0.01)	0.29 (0.02)	0.32 (0.00)	0.33 (0.01)
pH	5.49 (0.15)	5.53 (0.30)	5.41 (0.30)	5.32 (0.29)	5.35 (0.32)
Base saturation	15.34 (3.01)	12.13 (1.14)	12.00 (1.81)	9.94 (0.93)	8.38 (1.50)
Organic carbon (g C kg^{-1})	3.52 (0.51)	3.12 (0.14)	4.90 (0.69)	7.44 (0.32)	8.30 (0.21)
N tot (g N kg^{-1})	0.15 (0.01)	0.13 (0.01)	0.13 (0.01)	0.14 (0.01)	0.14 (0.01)
C:N	3.50 (0.26)	3.09 (0.04)	4.90 (0.32)	7.44 (0.31)	8.30 (0.32)
CEC	34.72 (0.22)	31.96 (0.31)	35.98 (0.22)	37.84 (0.21)	39.21 (0.22)

300-ml tubes to obtain rhizosphere soils. The tubes were vigorously shaken and soil that was still held to the roots was collected and placed in plastic bags (Molina et al. 1991). All root and soil samples were stored in a cool box, and immediately transported to the laboratory. Soils collected from the nearby *Q. acutissima* secondary forest were used as the control; the roots and other debris were removed and discarded.

Bioassays

Root extracts of 2, 4, 6, 8, and 10-year-old *E. grandis* taken separately from the field replicates were prepared by soaking 100 g (fresh weight) in 1 l of distilled water (100 mg fw ml^{-1}) and stirred gently for 24 h by a shaker at room temperature. The suspension was filtered two times by Whatman filter paper no. 2 to remove the fiber and the resulting solution was passed through a sterilized 0.45-μm filter paper to avoid contamination. The solutions with 50, 25, and 12.5 mg ml^{-1} were based on this mother solution and the sterilized distiller water was used as the control. One hundred grams of rhizosphere soils taken separately from the field replicates for each age was prepared for the bioassay of rhizosphere soils.

Three target species were selected: *Raphanus sativus, Phaseolus aureus*, which were cultivated nearby the plantation and also have been frequently used for bioassays due to their sensitivity to allelopathic substances (Molina et al. 1991; Chiapusio et al. 1997; Zhang et al. 2007); *Lolium perenne* present in open environment of surveyed *E. grandis*. Seeds of target species were sterilized in 70% ethanol for 1.5 min, then in 2% sodium hypochlorite for 30 min and finally washed several times with distilled water. Trials were carried out using glass Petri dishes (diameter: 9 cm) with three layers of filter papers (Chiapusio et al. 1997; Chou et al. 1998; Erickson et al. 2001). Fifty seeds of each target plant were sown on the top of filter paper per Petri dishes. Approximately 10 ml of respective aqueous extract from one of the three field replicates was used to moisten the filter papers until the end of the experiment. The seeds watered with distilled water served as the control. All the treatments were carried out in four replicates. Petri dishes were placed in a growth cabinet at a fixed temperature (20.5 ± 1°C), and at 100% relative humidity (Catherine et al. 2006), kept moist by applying an equal volume (1 ml) of the same extract every day.

Fifty prepared *L. perenne* seeds were uniformly sown in plastic plates (diameter: 15 cm; depth: 8 cm) containing 100 g of rhizosphere soils. Seeds sown in plates containing soils collected from the nearby *Q. acutissima* secondary forest were used for control. The plates were placed in a greenhouse with 20/25°C for night/daytime and 65–90% relatively humidity maintained. Plates were watered once a day.

GC–MS analyses

Collection methods of root exudates varied with different allelochemicals, most of which are extracted from aqueous or organic solvents, but the extracted from organic solvents under ultrasonic conditions could obtain more allelochemical components compared to that from aqueous extracts (Zhang and Yang 2008).

For identification of allelochemicals in exudates of root and rhizosphere soil, the roots collected were dipped in distilled water after being air-dried and then smashed and sieved (0.2-mm mesh). The roots and soils obtained were then extracted by *n*-hexane under ultrasonic conditions for 30 min and filtrated with 0.45-μm filter paper (Zhang et al. 2007). The filtrate was then gathered and concentrated to 1 ml with rotary evaporator. All these extracts were stored at −20°C for 12 h and then transferred to a new 5-ml vial for GC–MS analysis. Three replicates were used for each combination (age, root, or rhizosphere soil).

Analyses were performed using a Hewlett-Packard (Palo Alto, CA, USA) Model G1 800A'. Samples of exudates were silylated using *N*,*O-bis*(trimethylsiy1)acetamide (BSA) and separated on a 30 cm × 0.25 mm × 0.25 mm capillary column. The column temperature was initially held at 90°C for 1 min and was then increased to 240°C at a rate of 10°C min^{-1}; the total running time was 20 min. Injection temperature was maintained at 240°C and then injection volume was 0.4 μl. Electron impact ionization energy was 1,000 V. All samples were carried out in triplicate (Zhang and Yang 2008).

Measurements and calculation

Seed germination was measured after 5 days. Germination was considered when radicle emerges from the seed coat. The length of radicles and shoots, shoot/radicle fresh mass ratio was measured 7 days after germination. Trials were carried out at root temperature (16–20°C). Germination rate was determined by seeds germinated divided by total seeds.

The similarity (C_i) between compounds in roots and soils was assessed using Jaccard's similarity coefficient based on the presence or absence of compounds:

$$C_i = a/(a+b+c)$$

where a is the number of species common in sample A and sample B; b is the number of compounds present in the root, but absent in soil; c is the number of compounds present in soil, but absent in root. The result ranged between 0 and 1 as measured by Jaccard's coefficient: 0 for no compound shared in common, and 1 for complete concurrence.

An index of allelopathic effects (RI) was used to measure the allelopathic effects of rhizosphere soils on seed germination and seedlings growth of *Raphanus sativus*.

$$\mathrm{RI} = 1 - C/T(T \geq C), \text{or } \mathrm{RI} = C/T - 1(T<C)$$

where C is the control value, T is the treatment value. The result indicates a simulative effects when RI > 0, while an inhibitory effects when RI < 0.

Statistical analyses

Statistical analysis (arithmetic mean and SD) was performed separately for each parameter. Differences in germination percentage and seedling growth of the targets according to forest age and extract dose were tested using one-way ANOVA. ANOVA was also performed to test the significant differences of the relative amounts of compounds in the soil and roots with regard to forest age. Normality and homogeneity of variances tests were examined. Data were log-transformed to satisfy the requirement of variance homogeneity if data failed equal variance tests. Homogeneity of variances was tested by the Levene test. Post-hoc means comparisons were conducted using Tukey test. The level of significance of statistical tests throughout this study is 0.05. All of the statistical analyses were performed using SPSS 11.5 software package.

Results

Allelopathic effect of root aqueous extracts

Seed germination and seedling development of the three target plants were affected by the root aqueous extracts of *E. grandis*. Germination rates of the three target species decreased with the increase of the concentrations of extracts ($P < 0.05$) (Fig. 1). At the lowest concentration, little variation was observed on germination rate among the treatments from plantations with different ages ($P > 0.05$). The highest dose roots extracts from 4-year-old *E. grandis* showed the strongest inhibitory effects on the germinations of all target specie (Fig. 2), the inhibitory rates were about 48, 51.2, 56.56% for *R. sativus*, *P. aureus*, *L. perenne*, respectively.

Seedling growth of the three target plants were reduced as the concentration of root extract increased ($P < 0.05$) (Fig. 1) and toxic effect of extract was much more pronounced for younger *E. grandis* (Fig. 2). The growth of *P. aureus* and *R. sativus* radicle increased when treated with the lower concentrations of root extracts from the younger *E. grandis*.

Rhizosphere soil bioassays

The RI value indicated that soils of 4-year-old *E. grandis* plantation exhibited the most remarkable inhibitory effect on the target plant, followed by the 2-year-old (Fig. 3). After 4 years old, the inhibitory effect was weakened and a stimulatory effect was presented with the increased forest ages on germination and growth of *L. perenne*, a remarkable stimulative effect was observed in rhizosphere soils of 10-year-old *E. grandis* (Fig. 3). Among the three parameters, radicle length was more sensitive.

Chemical analyses

Based on a range of secondary metabolites identified to be potent allelochemicals from previous studies, a total of 28 allelopathic potential compounds including alkane, arene, alkene, terpene, aldehyde, ketone, phenol, long-chain fatty acids, and aromatic ester were confirmed to be present in root extracts of *E. grandis* (Table 3). Among them, alkane, arene, phenol, long-chain fatty acids were in great abundance in younger *E. grandis* (2 and 4 years old), reaching the minimum levels at 8-year-old stages (Fig. 4). The amounts of terpene, alkenes, and ketone were lower in *E. grandis* root, but the relative contents of which also exhibited a similar trend with the components mentioned above (Fig. 5). 9,12-Octadecadienoic acid and phthalic acid phthalate were in measurable quantities in root extracts from younger *E. grandis* (2 and 4 years old) but they were hardly detected in older *E. grandis*.

A total of 38 chemical components (Table 4) including alkane, alcohol, aldehyde, ketone, phenol, long-chain fatty acids, and aromatic ester were found in *E. grandis* rhizosphere soils, the amounts of which were different with plantation ages (Fig. 6). Among which, alkanes, 1,2-Benzenedicarboxylic acid, diisooctyl ester, Mono(2-ethylhexyl)phthalate was in great abundance in

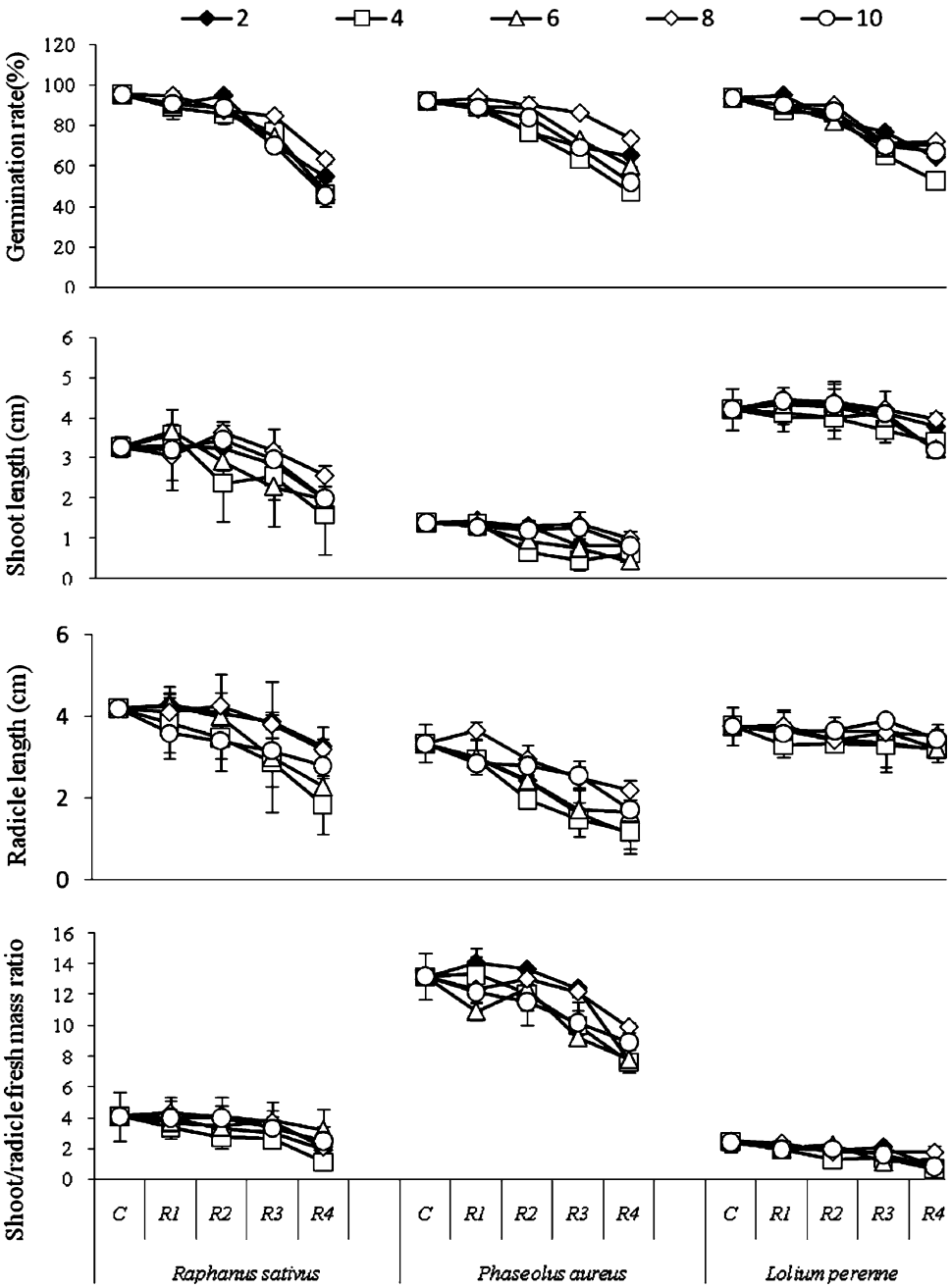

Fig. 1 Effects of varying concentration (*C* control, *R1* 12.5 mg ml^{-1}, *R2* 25 mg ml^{-1}, *R3* 50 mg ml^{-1}, *R4* 100 mg ml^{-1}) of the aqueous root extract from *E. grandis* on the germination rate and initial seedling growth of the target plants

young *E. grandis*. Twenty common components including alkane, aromatic ester, arene and phenol have been observed both in root and rhizosphere soils (C_i = 0.47).

Discussion

Allelopathic potential of *E. grandis* root and rhizosphere soils

Many studies have documented that eucalyptus leaf could produce allelochemicals and its extract suppressed the growth of other plants, but root and soil have received little attention. The present results demonstrated our hypothesis that root and rhizosphere soil exhibited significant allelopathic effects on the three target plants (*R. sativus*, *P. aureus* and *L. perenne*), and the extent of the effects was influenced by the plantation ages, which could provide efficient data to plantation mangers.

Root exudates are an important resource of allelopathic chemicals (Lee and Monsi 1963; May and Ash 1990), which could have significant allelopathic effects on other plants in the plantation. The results that seed germination and early seedling growth were inhibited by eucalyptus root extracts evidently demonstrated the theory, suggesting that the allelopathy in the eucalyptus plantation was at least partly induced by root activities. However, the responses of target species were different

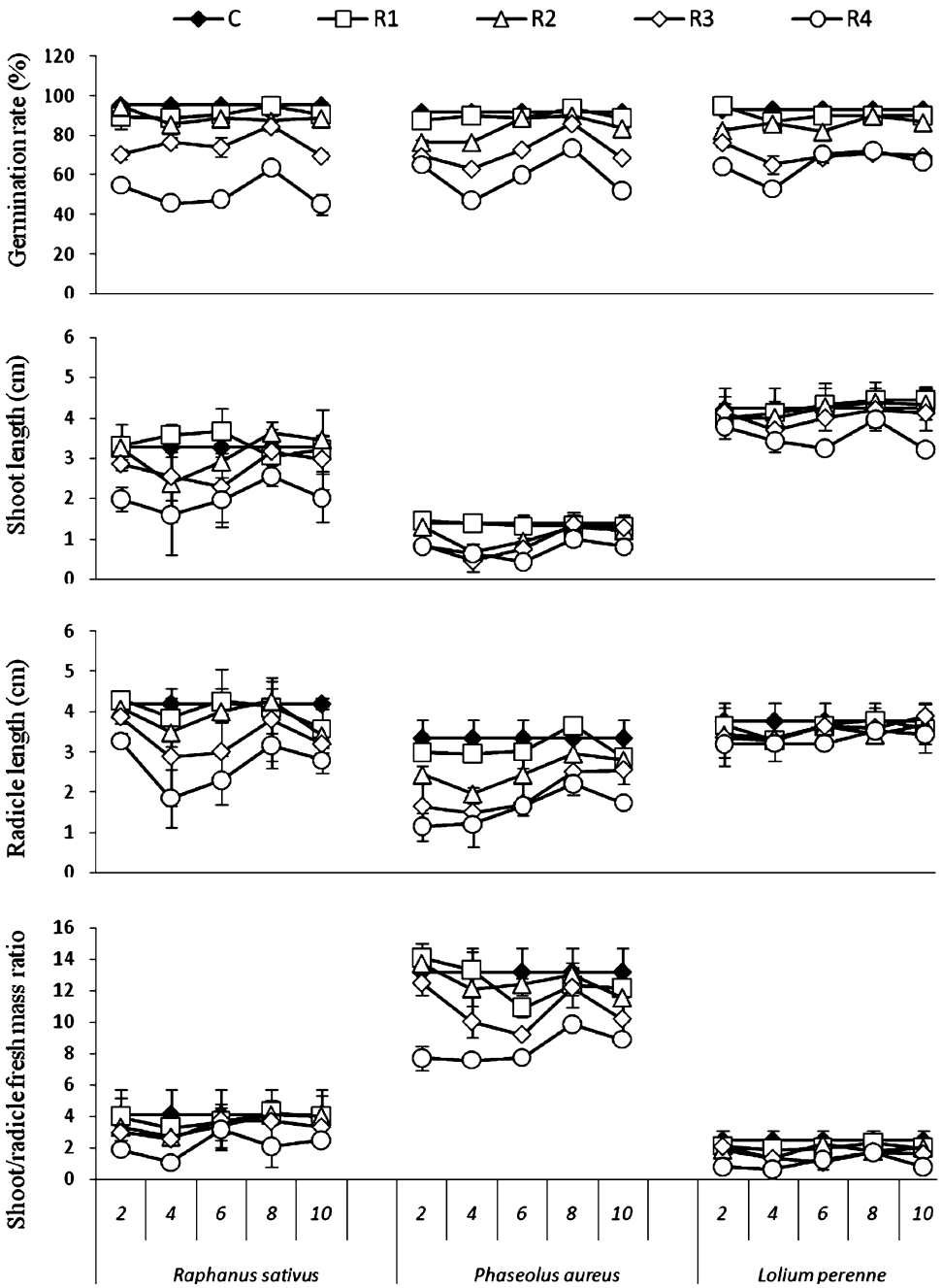

Fig. 2 Germination rate and seedlings growth of target species treated with aqueous root extract (*C* control, *R1* 12.5 mg ml^{-1}, *R2* 25 mg ml^{-1}, *R3* 50 mg ml^{-1}, *R4* 100 mg ml^{-1}) from *E. grandis* as the function of forest ages

in response to the varieties of the extract concentration. In agreement with the previous results (Lee and Monsi 1963; Ballester et al. 1982; Rafiqul Hoque et al. 2003), the allelopathic effects increased with the increase of root extract concentration, showing significantly lower length of root and radicle of target species with higher root extract concentration treatment. This suggested that the potential allelopathic effects could be more pronounced in the area with low or erratic rainfall due to relative higher content of allelochemical substances (May and Ash 1990). In addition, relatively higher seed-germination rates and early growth of target species were observed in the treatment with lower root extract concentration compared to distilled water treatment. This might be the protective mechanism that lower allelochemical concentration could stimulate the growth of other plants as declared by several studies (Reigosa et al. 2000; Hong et al. 2004; Nektarios et al. 2005). Moreover, a few studies have found that some allelochemicals could perform stimulatory effects on other plants (Chen 1999). A small amount of nutrients brought by root extracts also might partly contribute to the stimulative effect (Khan et al. 1999).

With the development of an eucalyptus plantation, internal and external environment factors would change in the community of plantation (Binkley et al. 1989;

Chou 1993; El-Darier 2002; Smal and Olszewska 2008). These changed factors might affect root growth, and then root exudates. The present results indicated that allelopathic effects of eucalyptus root extract on target species were different with the difference of plantation ages, which might be related to the composition of allelochemicals and the growth of eucalyptus. The strongest allelopathy was observed in the 4-year- old eucalyptus plantation. This could be explained by the following two reasons. On one hand, younger eucalyptus plantations often displayed a relative faster growth rate compared to older ones (Zhang and Yang 2008). The fast growth stimulated the root activities, thus increasing root allelochemicals (Nishimura et al. 1982; May and Ash 1990; Catherine et al. 2006). On the other hand, lower growth rate, more closed canopy, less understory biodiversity, and limited growing area in older eucalyptus plantations induced less stronger soil resources competition inner population, and consequently less resources were allocated to allelopathy. This might be the adaptive mechanism of fast-growing plantation as eucalyptus.

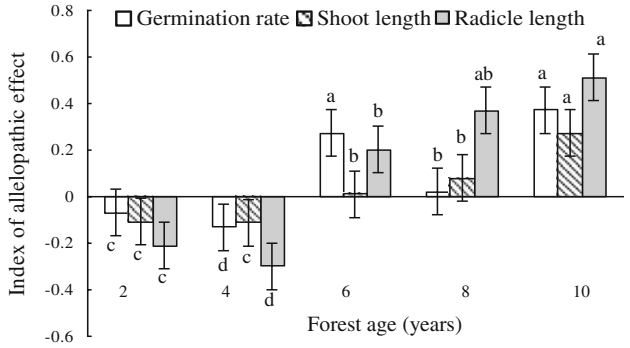

Fig. 3 The index of allelopathic effects of rhizosphere soil from different aged *E. grandis* on *R. sativus* germination and seedling growth. The *line bars* show SD. Different letters indicate significant differences according to Tukey test ($P < 0.05$)

The rhizosphere soil is the important sink that allelochemicals accumulated, which could play a key role in allelopathic effects on other plants (Inderjit and Keating 1999; Espinosa-Garcia et al. 2008). The results of this

Table 3 Chemical compounds found in roots extracts of *E. grandis* across a range of forest ages (2, 4, 6, 8, and 10-year-old *E. grandis*)

Composition	Scientific name	Relative amount (%)				
		2	4	6	8	10
Alkane						
1	2-Methyl-heptane	1.84	1.86	0.32	1.22	1.03
2	3-Methyl-heptane	1.43	1.62	1.2	0.28	0.91
3	*n*-Octane	14.01	15.2	14.58	2.5	14.13
4	*n*-Decane	2.02	2.03	2.17	0.42	1.24
5	2,2,4,4,6-Pentamethyl-heptane	3.12	3.22	0.62	0.46	2.24
6	3,7-Dimethyl-decane	1.62	0.81	0.27	0.15	0.31
7	*n*-Dodecane	1.33	1.56	1.95	0.13	1.04
8	Hexadecane	1.11	1.05	0.98	0.85	1.26
9	Tetradecane	0.99	1.03	1.94	0.12	0.89
10	Heptadecane	0.95	0.71	0.81	0.18	1.00
11	Undecane	0.32	–	0.42	–	0.13
12	Tridecane	0.42	–	0.46	0.25	0.40
13	Tetracosane	0.46	–	0.90	–	–
14	2,6,10,14-Tetramethyl-pentadecane	0.47	0.58	0.44	–	0.35
15	Eicosane	1.87	1.43	1.02	0.57	0.80
16	Octadecane	2.56	1.81	3.30	0.19	1.39
17	2,6,10,14-Tetramethyl-hexadecane	1.6	1.25	0.91	0.18	–
18	5-Methyl-tetradecane	0.39	0.98	0.31	1.06	0.72
Alkene						
19	1-Methyl-1-cyclo undecene	1.9	1.89	0.41	0.19	0.46
Arene						
20	2,6-Dimethyl-naphthalene	1.85	1.48	1.43	0.46	1.32
21	2,3-Dimethyl-naphthalene	3.01	2.77	2.78	1.08	2.41
22	1,5-Dimethyl-naphthalene	1.60	1.62	0.30	0.16	0.89
Aldehyde						
23	*n*-Nonanal	0.31	0.32	0.21	–	0.26
Long-chain fatty acids						
24	*n*-Hexadecanoic acid	25.84	13.5	3.81	2.41	7.57
25	9,12-Octadecadienoic acid	5.79	5.88	–		–
Ketone						
26	2-Heptadecanone	1.33	1.54	0.70	0.41	0.86
Phenol						
27	2,4-Di-tert-butylphenol Phenol	1.38	1.39	1.23	0.18	0.35
Aromatic ester						
28	Dimethyl ester phthalic acid	21.05	15.88	–	–	–
Others						

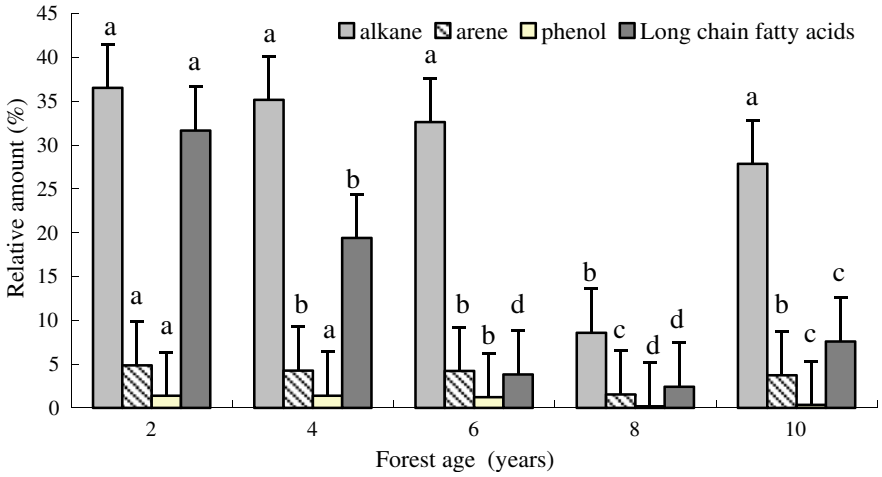

Fig. 4 The relative amounts of alkanes, aromatic esters, phenol, long-chain fatty acids in root extracts of *E. grandis* with plantation ages. The *line bars* show SD. *Different letters* indicate significant differences according to Tukey test ($P < 0.05$)

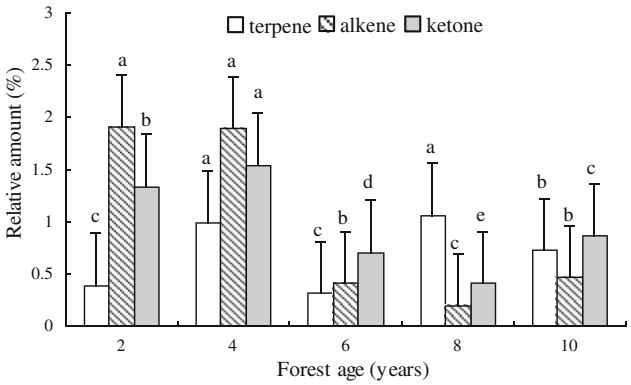

Fig. 5 The relative amounts of terpene, alkene, and ketone in root extracts of *E. grandis* with plantation ages. The *line bars* show SD. *Different letters* indicate significant differences according to Tukey test ($P < 0.05$)

study showed that inhibited effects were observed in younger plantations (2 and 4 years old) whereas remarkable stimulative effects were observed from 6 years old. The close explanation might be the synthetic effects of resources competition, growth rate, and the characteristics of allelochemicals (Lisanework and Michelsen 1993; Malik 2004). In the younger plantation, eucalyptus performed rapid growth rate and higher resource needs (Zhang and Yang 2008). In order to catch the maximum resources, eucalyptus could release allelochemicals to inhibit the growth of other plants, which was consistent with the theory that more obvious allelopathy was often observed in a resource-limited habitat (Rice 1984; El-Darier 2002; Smal and Olszewska 2008). Meanwhile, allelochemical compounds in soils might be affected by environment factors and their personality (Lee and Monsi 1963; Chou 1993; Malik 2004). Allelopathic effects in natural systems occur to a larger extent than laboratory experiments subjected to mitigation or intensification by the physicochemical characteristics of the soil and the microbial activity (Lisanework and Michelsen 1993; Malik 2004). Since inner environment and allelochemical compounds change with the growing of eucalyptus plantation, the single, synergistic and antagonistic effects of allelochemicals components and allelochemicals degradation might contribute to the changes of observed allelopathic effects in the plantations with different ages. The detailed reasons for this need to be studied further. Even so, more attention should be paid to allelopathy of rhizosphere soil in eucalyptus plantation.

Allelopathic compounds in *E. grandis* root and rhizosphere soils

According to the discussion above, allelopathic effects were different from the differences of influencing factors, suggesting that the allelopathic components might be varied. In this study, a total of 28 allelopathic potential compounds including alkane, arene, alkene, terpene, aldehyde, ketone, phenol, long-chain fatty acids, and aromatic ester were confirmed in root extracts of *E. grandis*, which are structurally similar to those that have been reported to inhibit plant germination and growth (Rice 1984; Nishimura et al. 1982; Zhang et al. 2007; Djurdjević et al. 2008). Alkanes and arene are well known to be toxic and can inhibit the K/Ca uptake of other plants. Fatty acids, ester, and ketone could inhibit seedling growth and microorganisms (Ossipov et al. 1995a, 1995b). Furthermore, several studies have declared that allelopathic effects are caused by phenolics phytotoxins that are in all plants parts (Loponen et al. 2001; Ossipov et al. 1995a, 1995b; Djurdjević et al. 2008). The presence of terpenes in eucalypt soils has also been reported by del Moral and Muller (1970) and Lovett (1985). Additionally, allelopathic potential compounds were more abundant in younger *E. grandis* (2 and 4 years old) roots, which proved the results that younger plantations displayed more significant allelopathy. More chemical components including alkane, alcohol, aldehyde, ketone, phenol, long-chain fatty acids, and aromatic ester in the present study were found

Table 4 Chemical compounds found in rhizosphere soils extracts of *E. grandis* across a range of forest ages (2, 4, 6, 8, and 10-year-old *E. grandis*)

Composition	Scientific name	Relative amount (%)				
		2	4	6	8	10
Alkane						
1	2-Methyl-heptane	1.04	0.95	0.25	1.09	0.78
2	3-Methyl-heptane	0.89	0.83	0.22	1.08	0.63
3	Octane	12.56	7.30	1.83	14.35	5.91
4	2,3,3-Trimethyl-hexane	–	–	0.19	0.14	0.54
5	2,4-Dimethyl-heptane	0.5	0.43	0.21	0.42	–
6	2,2,4,6,6-Pentamethyl-heptane	1.4	0.58	0.19	1.50	0.52
7	Decane	1.02	1	0.34	0.81	1.01
8	3,3-Dimethyl-octane	–	–	0.43	0.19	–
9	5,6-Dimethyl-undecane	0.39	–	–	0.3	0.48
10	4-Methyl-decane	0.67	0.65	–	0.65	0.65
11	3,7-Dimethyl-decane	1.67	0.52	0.39	0.2	1.83
12	Undecane	0.83	0.65	0.55	1.19	0.42
13	2,2,4,6,6-Pentamethyl-heptane	1.4	0.58	0.19	1.31	0.65
14	Dodecane	1.41	1.74	0.28	2.29	1.31
15	5-Methyl-tetradecane	1.17	1.42	0.36	1.4	1.42
16	Hexadecane	1.77	1.94	0.24	0.42	2.01
17	Nonadecane	0.56	0.6	0.69	0.64	0.57
18	Tridecane	1.19	1.93	1.05	0.89	1.64
19	Tetradecane	1.05	1.6	0.27	1.4	0.72
20	Eicosane	1.54	0.42	0.69	0.47	1.57
21	Tetracosane	1.44	0.44	0.28	0.65	0.66
22	Nonacosane	1.05	1.74	2.78	0.57	1.28
23	Heneicosane	0.88	0.99	0.52	0.81	0.92
24	Octacosane	–	1.15	0.68	0.54	1.32
25	Heptadecane	0.53	0.67	0.43	0.82	0.3
26	2,6,11-Trimethyl-dodecane	–	0.87	1.75	1.1	0.48
Alkene						
27	6,10,15,19,23-Hexamethyl-2,6,10, 14,18,22-tetracosahexaene	0.82	–	–	1.91	0.77
Alcohols						
28	Trans-9-Octadecen-1-ol	–	0.45	–	–	–
Aldehyde						
29	Octadecanal	–	0.78	–	1.48	
Long-chain fatty acids						
30	Hexadecanoic acid	1.07	1.9	0.69	–	0.77
Aromatic ester						
31	Diisooctyl ester 1,2-Benzenedicarboxylic acid	17.16	17.3	–	30.04	–
32	Mono(2-ethylhexyl) phthalate	20.19	2.19	10.97	12.73	11.46
Ketone						
33	14,16-Hentriacontanedione	–	–	–	–	4.6
34	1,4-Diphenyl-1,2,4-butanetrione	–	1.24	1.05	–	–
Phenol						
35	2,4-Di-tert-butyl-phenol	1.06	1.32	0.57	0.46	1.33
36	2,2′-Methylenebis[6-[1,1-dimethylethyl]-4-methyl-phenol	2.4	2.23	1.86	5.79	1.78
Arene						
37	2,6-Dimethyldecalin	1.11	1.7	0.39	0.83	0.96
Terpene						
38	(1S,3aR,4S,8aS)-(+)ecahydro-4,8,8-trimethyl-9-methylene-1,4-Methanoazulene	–	0.87	–	–	1.07
Others						

in soils than extracts of roots. Twenty similar components were observed both in root extracts and soils, suggesting that a considerable part of allelochemicals in the soil might be released from the root. The other components in the soil might come from other mechanisms, such as litter decomposition and leaching (Molina et al. 1991; An et al. 2001; Ahmed et al. 2008). However, the mechanism of how the specific allelochemicals in the roots and soil affect the target plants is still not well documented. Further work is urgently needed.

In conclusion, root and rhizosphere soil of eucalyptus showed strong allelopathic effects on the target species. However, a higher concentration of root extract exhibited inhibitory effects, and a lower dose displayed the opposite effect. Stronger inhibitory effects on the target species were observed in the root and soil of younger plantations, while stimulative effects were observed in rhizosphere soil of older plantations. In addition, a large amount of allelopathic potential compounds were identified in rhizosphere soil and root extract, and their components and amounts were different with the

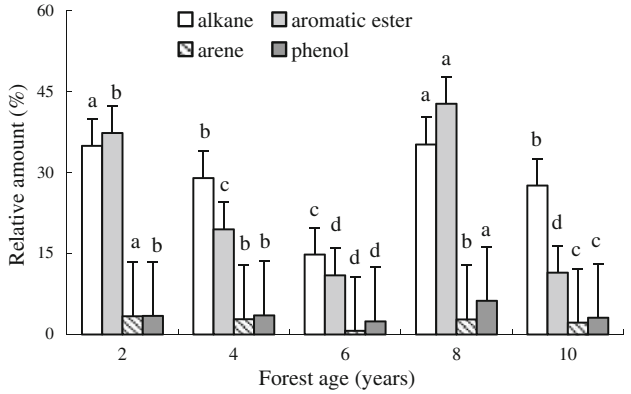

Fig. 6 The relative amounts of major compounds (alkane, aromatic ester, arene, phenol) in soil extracts of *E. grandis* with plantation ages. The *line bars* show SD. *Different letters* indicate significant differences according to Tukey test ($P < 0.05$)

differences of forest ages. The results suggest that more attention should be paid to the younger eucalyptus plantations for the relative higher allelopathic effects, which could provide important data for plantation managers.

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