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Allelopathic interactions between rubber tree and *Chromolaena odorata* as measured by seed germination and/or seedling growth of the two species

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Chromolaena odorata (L.) R.M. King & H. E. Robinson (Asteraceae) is one of the most important invasive alien plants in Hainan, China. To understand the allelopathic effects between rubber tree, *Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg. (Euphorbiaceae), and *C. odorata*, an experiment was conducted to evaluate the effects of extracts of three types of rubber tree leaves on seed germination and seedling growth of *C. odorata*. Extracts of *C. odorata* stems plus leaves and roots were also evaluated on the effect on seedling growth of rubber trees. The studies showed that 0.25g/ml extracts of all types of rubber tree leaves (mature, light-green and bronze), significantly inhibited the seed germination rate of *C. odorata* and resulted in a significantly lower germination index. Seedling growth of *C. odorata* was only significantly inhibited by extracts of light-green rubber leaves at 0.125g/ml and bronze leaves at 0.25g/ml. However, seedling growth of *C. odorata* was greater with exposure to most extracts of rubber tree leaves than those seedlings in the control treatments. This indicates that rubber tree leaves could inhibit seed germination of *C. odorata* but does not inhibit seedling growth of *C. odorata*. Extracts of *C. odorata* stems plus leaves at 0.25g/ml significantly promoted rubber seedling growth. This suggests that the leaves and stems of *C. odorata* are good green manure that should promote rubber tree growth. This may also be a way to control *C. odorata*.

KEYWORDS: allelopathy; green manure; invasive alien plant; water extracts

INTRODUCTION

Chromolaena odorata (L.) R.M. King & H. E. Robinson (Asteraceae) is native to Central and South America and is now widely distributed in most tropical and subtropical regions of Africa, Asia, Australia and the West Pacific islands (Muniappan et al. 2005). It is one of the world's 100 worst invasive alien species (Lowe et al. 2000) and was listed in the first (i.e. top) group of invasive alien species of China in 2003 (SEPA and CAS 2003). *Chromolaena odorata* was first recorded in southern China in 1930 (Wu 1982) and is now distributed in Hainan, Guangdong, Guangxi, Guizhou, Taiwan, Hong Kong and Macao (SEPA and CAS 2003).

Chromolaena odorata mainly influences pasture and young rubber trees in Hainan and Yunnan. It is strongly allelopathic (Ambika and Jayachandra 1980), which may assist its establishment. However, rubber tree, *Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg. (Euphorbiaceae), also has allelopathic effects (Yang and Chen 2005; Yan et al. 2006). No previous studies on allelopathic effects between rubber tree and *C. odorata* have been reported. This paper reports on the results of such a study.

MATERIALS AND METHODS

Water extracts of rubber tree leaves and *Chromolaena odorata* stems, leaves and roots

Mature, light-green and bronze rubber tree leaves and *C. odorata* stems-plus-leaves and roots were collected from the respective plant species and dried at room temperature. The five samples were pounded and sieved separately. A

500g portion of each sample was soaked in 1,000ml of water for 24h, then subjected to ultrasonic extraction for 1h and the liquid was filtered through gauze. Each residue was again soaked in 1,000ml of water for 12h. The two extracts from each sample were combined and filtered through filter paper. The five extracts at 0.25g/ml were stored at 4°C prior to the experiments.

Effect of extracts of rubber leaves on seed germination and seedling growth of *C. odorata*

Thirty seeds of *C. odorata* were placed in each of 13 Petri dishes with two-layer filter paper, after surface sterilization of seeds with 0.3% $KMnO_4$ for 10min. Subsamples of each of the three extracts of rubber tree leaves (mature, light-green and bronze) were diluted to 0.125, 0.062 and 0.031g/ml, and 5ml of each of the undiluted (0.25) and the three diluted extracts was added individually to the Petri dishes. A control with 0/ml was also set up and all Petri dishes were kept in a plant growth chamber at 25°C, 70% relative humidity and a 12h/12h day/ night cycle.

The number of seeds germinating each day was counted for 10d. Germination was scored as having occurred when sprout length was equal to half of the seed length. The germination rate and germination index was calculated as follows:

Germination rate (%) = (number of germinated seeds/total number of seeds) x 100

Germination index = $\sum$ (Gt/Dt) where Gt = number of germinations at day t, Dt = day t

After their fresh mass and height were measured, seedlings of *C. odorata* were planted in plastic cups (15cm tall and 10cm diameter), with 200g of fine sand at room temperature. 10ml of each of the three extracts of rubber tree leaves at 0.25, 0.125, 0.062, 0.031 and 0g/ml irrigated the seedlings every day. The seedling height and fresh mass were determined after 30d, after the seedlings had been uprooted and their roots washed. The increase in height and weight was determined by subtracting the initial values from the final values.

Table 1. The effect of different rubber tree leaf extracts on the seed germination of *Chromolaena odorata*.

Extracts of rubber leaves	Concentration (g/ml)	Seed germination of *C. odorata*[1]			
		Rate (%)	Average	Index	Average
Control	0	97.81 ab/A	97.81	25.44ab/A	25.44
Mature leaves	0.25	66.67 c/C	82.90	9.28 c/C	18.50
	0.125	96.62 b/A		24.33 a/A	
	0.062	84.78 b/B		20.25b/AB	
	0.031	83.55 b/B		20.14b/AB	
Light-green leaves	0.25	46.67 d/D	79.35	5.67 d/D	18.48
	0.125	98.35 a/A		26.35 a/A	
	0.062	90.00 b/A		22.36 ab/A	
	0.031	82.36 b/B		19.54b/AB	
Bronze leaves	0.25	43.33 d/D	78.31	5.08 d/D	17.38
	0.125	97.26 ab/A		25.15 ab/A	
	0.062	90.00 ab/B		20.02b/AB	
	0.031	82.66 b/B		19.25b/AB	

[1]Different letters within a column indicate significant difference. Lower case letters indicate significance, while upper case letters indicate high significance.

Table 2. *Chromolaena odorata* seedling growth after treatment with extracts of rubber leaves.

Extracts of rubber leaves	Concentration (g/ml)	Seedling growth of *C. odorata*[1]			
		Increased height (cm)	Average	Increased fresh weight (g)	Average
Control	0	1.3 c/C	1.3	1.4 c/C	1.4
Mature leaves	0.25	3.6 a/A	2.5	3.7 a/A	2.6
	0.125	2.7 ab/A		2.8 ab/B	
	0.062	2.1 b/AB		2.3 b/B	
	0.031	1.5 c/C		1.7 bc/BC	
Light-green leaves	0.25	1.0 c/CD	1.1	1.1 c/CD	1.1
	0.125	0.2 d/D		0.6 d/D	
	0.062	1.7 bc/BC		1.6 c/C	
	0.031	1.3 c/C		1.2 c/CD	
Bronze leaves	0.25	0.6 d/D	2.3	0.8 d/D	2.2
	0.125	2.6 b/B		2.3 b/B	
	0.062	3.5 a/A		3.1 ab/A	
	0.031	2.5 b/B		2.4 b/B	

[1]Different letters within a column indicate significant difference. Lower case letters indicate significance, while upper case letters indicate high significance.

Effects of extracts of *C. odorata* stems-plus-leaves and roots on the growth of rubber tree seedlings

Rubber tree seedling buddings (height: 41-57cm, girth: 0.5cm) were planted in pots (height 35cm height and 10cm diameter) with fine sand. 20ml of extracts of *C. odorata* stems-plus-leaves and roots at 0.25, 0.125, 0.062, 0.031 and 0g/ml irrigated the buddings every day from 7d after initial planting. The height and girth of each budding were measured at the beginning of the trial (i.e. 7d after initial planting) and after 30d. The increase in height and girth was determined by subtracting the initial values from the final values.

Experiment design and analysis

Experiments were conducted using a completely randomized block design with three repeats. An ANOVA was conducted with DPS 7.0. Means were compared using Duncan's multiple range test.

RESULTS

Extracts of rubber tree leaves on seed germination and seedling growth of *C. odorata*

Seed germination rate of *C. odorata* was significantly inhibited by all three extracts of rubber tree leaves (mature, light-green and bronze leaves) at 0.25g/ml. At 0.25g/ml, extracts of light-green and bronze rubber tree leaves caused significantly greater inhibition than mature leaves. The germination index of *C. odorata* was also significantly less for all extracts at 0.25 g/ml. There was no significant difference in germination rates or the germination index for all other extract concentrations. The order of inhibition was bronze leaves > light-green leaves > mature leaves (Table 1).

Compared to the control, *C. odorata* seedling height and fresh weight were significantly reduced by extracts of only light-green rubber

Table 3. Rubber seedling budding growth after treatment with extracts of *Chromolaena odorata*.

Extracts of *C. odorata*	Concentration (g/ml)	Growth of rubber seedling budding[1]			
		Increased girth (cm)	Average	Increased height (cm)	Average
Control	0	0.01 b/B	0.01	1.53 b/B	1.53
Stems + leaves	0.25	0.03 a/A	0.02	3.62 a/A	2.05
	0.125	0.01 b/B		1.52 b/B	
	0.062	0.01 b/B		1.53 b/B	
	0.031	0.01 b/B		1.52 b/B	
Roots	0.25	0.01 b/B	0.01	1.44 b/B	1.43
	0.125	0.01 b/B		1.43 b/B	
	0.062	0.01 b/B		1.44 b/B	
	0.031	0.01 b/B		1.39 b/B	

[1]Different letters within a column indicate significant difference. Lower case letters indicate significance, while upper case letters indicate high significance.

tree leaves at 0.125g/ml and of bronze rubber tree leaves at 0.25g/ml. Most other extracts either had no effect on height or weight or significantly promoted seedling growth of *C. odorata*, compared to the control (Table 2).

Extracts of *C. odorata* stems plus leaves and roots on the growth of rubber seedling buddings

The increases in girth and height of rubber seedling buddings treated with extracts of *C. odorata* stems-plus-leaves at 0.25g/ml were significantly larger than those of the control. No other treatments were significantly different to the control (Table 3).

DISCUSSION

Extracts of rubber tree leaves inhibited seed germination of *C. odorata* but mostly promoted seedling growth of *C. odorata*. This suggests that rubber tree seedling buddings alone, with limited mature leaves, were unable to inhibit growth of *C. odorata* under field conditions.

Extracts of *C. odorata* stems-plus-leaves at 0.25g/ml promoted growth of rubber tree seedling buddings, suggesting that *C. odorata* stems and leaves had potential for use as a good green manure for rubber tree growth.

These results were obtained after only 30 days, using potted plants. This period may not be enough to display allelopathic effects between rubber tree and *C. odorata*, which are complex and which depend on factors such as the populations of each species, litter layers and canopy height.

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