

Growth and developmental responses of spinach (*Spinacia oleracea* L.) to the decomposing leaf litter of blue gum (*Eucalyptus maidenii* F. Muell.)

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ABSTRACT

We investigated the allelopathic effects of decomposing leaf litter of blue gum (*Eucalyptus maidenii* F. Muell.) on the growth and development of spinach (*Spinacia oleracea* L.). Gas chromatography–mass spectrometry (GC-MS) was used to identify and compare volatile compounds in the original leaf litter and decomposed leaf litter. No significant differences were observed in leaf number of spinach between random leaf litter treatment and the control at vegetative stage ($p > 0.05$). However in reproductive stage, the responses of two sexual types were different to leaf litter decomposition in terms of some morphological, physiological and phenological traits. The sex ratio (F/M) was increased by 60 g but reduced by 120 g leaf litter for both years. Seventy-nine volatile compounds were identified from the original leaf litter sample and after 4-months of decomposition, the volatile compounds decreased sharply in diversity and absolute peak area. Of the majority of 52 terpenes, α -pinene, aromadendrene, eucalyptol and eudesmol were optimal caused the allelopathic effects.

Key words: Allelopathic effect, blue gum, decomposition, eucalyptus, *Eucalyptus maidenii*, flowering traits, growth, leaf litter, physiological responses, spinach, *Spinacia oleracea*.

INTRODUCTION

Eucalypts (Family Myrtaceae) originates from Australia. These trees have become very popular in recent decades in India, Brazil and China, because they are not only extensively planted as an industrial raw material, but they are also introduced to farmlands as a forest policy to increase farmers' income (41,47). Intercropping using species that coexist harmoniously achieves the full and cyclic utilization of matter and energy in combined systems, and on the basis of this theory, tree-grass and tree-crop composite patterns have been increasingly practised and studied (7,21). The relatively poor understory of eucalypt plantations has been a concern for a long time (8,11,30). The allelopathic effects of eucalypts might play an important role in this phenomenon, because numerous studies have revealed that the tissue extract and volatiles of eucalypts suppresses the growth of alley agricultural and forest crops (13,14,31,35). Indoor bioassays are valuable to determine the allelopathic potential and mechanism of eucalypts; however, the

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active chemicals may potentially be concentrated, if a specific amount of tissues are extracted in water or organic solvents. In addition, during the cultivation of targeted plants, water, nutrient fluids and perlites were frequently used as a substrate. Hence, the conclusion obtained in these assays lacked ecological relevance due to the absence of soil (15).

The annual leaf litter amount of eucalypts is relatively large. Zhang and Yang (46) reported that the annual leaf litter of 6-years-old *E. grandis* Hill ex Maiden plantation reached approx. 5000 kg · ha⁻¹, and the annual leaf litter in 25-year-old *E. maidenii* F. Muell. plantation was approx. 6000 kg · ha⁻¹ as per previous investigation performed from 2010 to 2011 (5). Eucalypt leaves are rich in allelochemicals and have shown strong allelopathic effects during the decomposition. For example, Batish *et al.* (3) found that the decaying leaves of *E. citriodora* Hook contained essential oils that could inhibit the seed germination and root elongation of *Cassia occidentalis* L. and *Echinochloa crus-galli* (L.) Beauv.; besides, the decomposition of *E. camaldulensis* Dehnh. leaves also suppresses the *Phalaris minor* Retz. and several forest and agricultural crops, when the dose reached a specific level (1,34). The decomposition of *E. grandis* Hill ex Maiden leaf litter is detrimental to *Elymus sibiricus* L. and *Cichorium intybus* L. in terms of resistant and photosynthetic responses of targeted plants (6,28,43). Nevertheless, most of previous studies were limited to the vegetative growth stage of targeted plants because indoor bioassays retarded their development, or because the selected targeted plants could not enter the reproductive stage within a short time.

Spinach (*Spinacia oleracea* L.) has short lifecycle, has varied sexual types and is frequently used in flower development studies (17,32). We proposed that the decomposing leaf litter of blue gum (*Eucalyptus maidenii* F. Muell.) induced the maturation and senescence of spinach, based on several phenological and developmental indicators (5). The changes in the growth and physiology status of targeted plants exposed to allelopathic materials were due to the alterations in their developmental status. Based on our previous study, we again investigated the effect of decomposing blue gum leaf litter highlighting the sexual differences and identifying the potential active chemicals in the leaf litter, to further elucidate the allelopathic mechanism of eucalypts and to provide several theoretic and technological insights for the establishment of eucalypt-crop systems.

MATERIALS AND METHODS

Study Site: This study was done in the greenhouse, Teaching and Research Garden, Sichuan Agricultural University, Sichuan, China (29°58' 48" N, 102°59' 55" E, 600 m altitude, a. s. l.), with a subtropical humid monsoon climate with four distinct seasons, insufficient light, a small difference in day and night temperature and concentrated rains in summer. The mean annual mete parameters were : Temp: 14.1°C -17.9°C [January Temp (3-7°C), July Temp (9-29 °C)], annual rainfall : 1774.3 mm and air humidity : 79%.

Materials

(i). Leaf litter: The leaf litter of blue gum was collected in April, 2011 from a 25-year-old blue gum stand in Ya'an, China. The leaf litter was air-dried and cut into pieces [1 - 2 cm long and 1 - 2cm wide (1 - 4 cm²)] and fully mixed.

(ii). **Spinach seeds** : Spinach (*Spinacia oleracea* L. 'Chuan0165') seeds were purchased from Keda Seeds and Seedlings Service of Fucheng District, Mianyang, China. The seeds were disinfected in 0.5% potassium permanganate (KMnO₄) for 30 min, soaked in tap water for 12 h (65 °C) and covered with a piece of wet gauze at room temperature (25 °C) for 12 h.

(iii). **Pot** : The white plastic pots were made from polyvinyl with upper dia 29 cm, bottom dia 25 cm and height 26 cm. The soil was sandy loam collected from the local farmland [field capacity : 246.40 g · kg⁻¹, organic matter content : 13.527 g · kg⁻¹, total nitrogen content : 0.132 g · kg⁻¹ and hydrolyzable nitrogen content : 14.478 mg · kg⁻¹]. Gravel and grass roots were removed from the soil by sieving, which was then fully mixed and laid flat for 2 d.

Experimental design

We followed the experimental design as previously described (5), which was also used by Wu *et al.* (43), Li *et al.* (29) and Huang *et al.* (20). Briefly, we investigated the annual leaf litter quantity of blue gum stand, which was approximately 6000 kg · ha⁻¹ with an equivalence of 42 g per pot upper surface area (about 0.07 m²). Given that different stands (even different quadrates in our field investigation) differed greatly in litter quantity, we selected 60 g · pot⁻¹ as the base of leaf litter application (denoted by L60). There were 5-doses (0,30,60,90,120 g) denoted as CK, L30, L60, L90 and L120, respectively, of litter applied per pot. The pot was filled up to 20 cm depth (32) and the weight of soil per pot depended on the soil moisture content.

This study consisted of experiment done in two years.

Experiment I. The experimental treatments consisted of control (CK, 0 g leaf litter/pot), L30 (30 g leaf litter /pot), L60 (60 g leaf litter/pot), L90 (90 g leaf litter/pot) and L120 (120 g leaf litter/pot). This trial began on April 4, 2011, 12-replicates were done for Control and each leaf litter treatment and there were 60 pots. After a specified amount of leaf litter was mixed as per treatments in pre-weighed soil, 20 spinach seeds were sown on the pot surface. Thereafter 700 g soil was added per pot to cover the seeds and watered sufficiently to ensure a high germination rate. After most seeds were germinated, a portable soil moisture analyser 'HH2 (ML2x, GBR)' was used to control the soil moisture content at 18%. The growth and developmental of targeted plant were closely monitored. Thinning was done and weeding was done when required.

Experiment II. The experimental treatments were same as in Experiment I above. It began on April 4, 2012. All the operations were the same as per trial in 2011, except for the 10-replicates.

Parameters determined

(i) **Euphylla number**: The euphylla number of targeted plant was recorded at 21 and 26 days after sowing during the vegetative stage and plants could not be distinguished by sex. In 2012, the euphylla numbers of female and male individuals were counted on May 31, 2012 (57 d after sowing), when most targeted plants were in reproductive stage.

(ii) Stem length: The stem length was measured on Jun. 1, 2012 (58 d after sowing) in both sexes of spinach.

(iii) Budding and flowering dynamics: After thinning, the remaining plants in each pot were labelled and the budding and flowering of spinach were carefully observed on 34, 36, 39, 42, 46, 50, 56 and 63 d after sowing. Both sexual buds were identified by appearance of the trochiformis prominence at the stem apex. The female flower was identified by the presence of the filamentose stigma, and the male flower was identified by the exposure of pollen after anther dehiscence. The sexual type of spinach could not be distinguished at the early stage of development, hence it was done on Jun. 6, 2011. Several parameters [rate of accumulated female buds (RAFB), rate of accumulated male buds (RAMB), rate of accumulated female flowers (RAFF), and rate of accumulated male flowers (RAMF)] were determined at a specific time for each pot as under:

RAFB (%) = (No. of female and monoecism that was budding + No. of female and monoecism that was flowering + No. of female and monoecism that was seeding) \times 100 / No. of total plants in the pot

RAMB (%) = (No. of male and monoecism that was budding + No. of male and monoecism that was flowering + No. of monoecism that was seeding) \times 100 / No. of total plants in the pot

RAFF (%) = (No. of female and monoecism that was flowering + No. of female and monoecism that was seeding) \times 100 / No. of total plants in the pot

RAMF (%) = (No. of male and monoecism that was flowering + No. of monoecism that was seeding) \times 100 / No. of total plants in the pot

Besides the Logistic Equation was used to fit the budding and flowering dynamics of spinach, the expression of Logistic Equation was as under:

$$Y = a / [1 + b \cdot \exp(c \cdot X)] \quad (a, b, c \text{ are constants})$$

(iv) Sex differentiation. At the end of both trials, the gender of targeted plants was identified and the ratio of female to male (F/M) was calculated as under:

$$F/M = (\text{No. of female} + \text{No. of monoecism}) / (\text{No. of male} + \text{No. of monoecism})$$

(v) Net photosynthetic rate. The net photosynthetic rate (Pn) was determined on Jun. 4, 2012 using a portable photosynthetic apparatus called Li-6400 (Li-Cor Inc., USA). The CO₂ concentration, temperature and light intensity in the reference room were set at 400 $\mu\text{mol} \cdot \text{mol}^{-1}$, 25 °C and 1200 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, respectively. Five plants per sex per treatment were randomly selected, and the 5th euphylla on the stem counted from the bottom to the top was selected for determination of the Pn for each plant.

(vi) **Volatile compounds analysis.** Gas chromatography–mass spectrometry (GC-MS) was used to analyse the compounds in two blue gum leaf litter samples, i.e., the original blue gum leaf litter and the leaf litter decomposed for 4 months. We used the method adopted by Huang *et al.* (20). A Hewlett Packard 6890/5973 gas chromatography (GC) equipped with a quadrupole mass spectrometer and a HP-5MS column with a specification of 45 m × 0.25 mm × 0.25 µm was used, and the heating procedure was as follows: the oven temperature was held at 60 °C and programmed to rise to 240 °C at a rate of 5 °C · min⁻¹, followed by an increase to 300 °C at a rate of 5 °C · min⁻¹. The identification of compounds was based on comparisons of the mass spectra with published data (12,23,40) and records in the NIST (National Institute of Standards and Technology) 08 Databases.

Statistical analyses

SPSS 16.0 statistical analysis software (SPSS Inc., USA) was used to perform One-way ANOVA, two-factor analysis and significance tests of difference in euphylla number, stem length and net photosynthetic rate, and to perform Repeated Measures (RM-ANOVA) and nonlinear regression in the budding and flowering traits. Variance homogeneity was tested by Levene's test. Tabulation and mapping were performed using Microsoft Excel 2003.

RESULTS AND DISCUSSION

Leaf number

Plants finish their juvenile phase before reaching the flowering stage (26). In this study, when the targeted plant was in its vegetative stage, the euphylla number was not altered by the decomposing leaf litter in 2011 ($p > 0.05$). Most likely, the effect of the decomposing leaf litter was not strong due to short decomposition duration (Table 1). In 2012, the euphylla number was quantified at the reproductive stage of spinach, and the targeted plants were distinguished by sex. The leaf number response to the decomposing leaf litter varied with sex. No significant difference was observed in the euphylla number between female plants treated with different amounts of leaf litter ($p > 0.05$), while the euphylla number was significantly reduced by 90 and 120 g leaf litter for male plants ($p < 0.05$) (Table 1).

Stem length

The responses of stem length of two sexual spinach to decomposing leaf litter were similar. In both sexual types, although there were no significant differences between the L30 / L120 treatment and control (CK) ($p > 0.05$), but the L120 dose inhibited the stem length in female by 13.78% and in male by 14.94% (Fig. 1 and Table 2). At L60 there was remarkable decrease (approx. 30%) in stem length in both sexual types ($p < 0.05$) at 90 g leaf litter dose, the stem length of female was not altered ($p > 0.05$) but the stem length of male was significantly reduced (38.72%) ($p < 0.05$) (Fig. 1 and Table 2). Thus male was more sensitive to the decomposed leaf litter than female, probably due to their inherent physiological differences.

Table 1. Effects of doses of blue gum leaf litter on leaf numbers in spinach

Treatment	2011		2012	
	April 25	April 30	Female	Male
CK	5.04 ± 0.41	7.13 ± 0.53	13.00 ± 2.74	14.80 ± 2.17 a
L30	5.18 ± 0.40	6.98 ± 0.39	10.80 ± 1.64	13.20 ± 1.64 ab
L60	5.37 ± 0.40	7.19 ± 0.55	10.20 ± 1.30	12.80 ± 1.92 abc
L90	5.15 ± 0.33	7.28 ± 0.30	11.00 ± 1.26	11.17 ± 2.14 bc
L120	5.04 ± 0.36	7.05 ± 0.49	10.75 ± 2.22	9.50 ± 1.52 c
Leaf litter	—	—		0.001
Sex	—	—		0.035
Leaf litter × sex	—	—		0.129

Each value represents the mean ± SE (n=12 for 2011, and n=5 for 2012). Between-group variation in the leaf number was neither significant for both determinations in 2011, nor for the female in 2012 according to one-way ANOVA. Different lowercase letters indicate the difference reached $\alpha < 0.05$ probability level based on Tukey's test, the separate effect of leaf litter and sex, and their interaction were tested using two-factor analysis. The values obtained in 2011 were not distinguished by sex because the observation was performed at the vegetative stage of spinach.

Table 2. Effects of doses of blue gum leaf litter on stem length in spinach

Treatment	Stem length (cm)	
	Female	Male
CK (0 g leaf litter/pot)	30.34a	33.94a
L30 (30 g leaf litter/pot)	30.52a	31.37ab
L60 (60 g leaf litter/pot)	21.45b	24.07bc
L90 (90 g leaf litter/pot)	22.96ab	20.80c
L120 (120 g leaf litter/pot)	26.16ab	28.87ab
Leaf litter		< 0.001
Sex		0.341
Leaf litter × sex		0.763

Each value represents the mean ± SE (n=5). Different lowercase letters indicated the difference reached $\alpha < 0.05$ probability level based on Tukey's test, the separate effect of leaf litter and sex, and their interaction were tested using two-factor analysis.

Net photosynthetic rate

With same treatment, the net photosynthetic rate (Pn) of female was higher than male as the male entered the reproductive stage earlier than female. The Pn of male was decreased 15% ~ 36% at L60 and L90, but increased by 23.71% at L120 (Fig. 2). The L60 dose reduced the Pn in female significantly ($p < 0.05$), but not other three doses (Table 3). The L30, L60, L90 and L120 caused inhibition of 32.84%, 53.93%, 29.88% and 11.10%, respectively, in Pn of female (Fig. 2).

Buds Development

Allelochemicals affects many aspects of targeted plants from the gene level to the community level (4,27,48). However, few studies have investigated the reproductive growth of targeted plants. The genesis of male buds was earlier compared to female buds

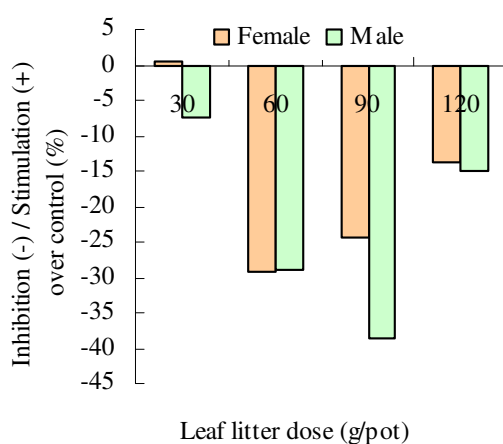


Figure 1. Effects of decomposing blue gum leaf litter on stem length of spinach

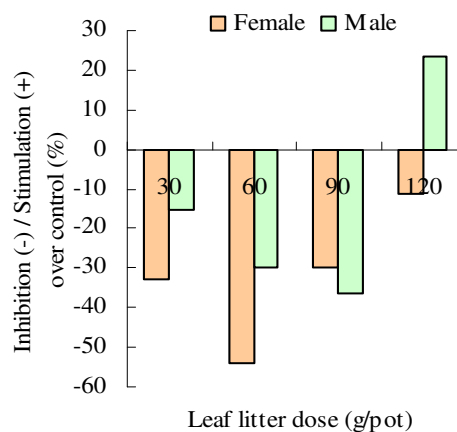


Figure 2. Effects of decomposing blue gum leaf litter on net photosynthetic rate of spinach

Table 3. Effects of doses of blue gum leaf litter on net photosynthetic rate of two sexes of spinach

Treatment	Pn ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	
	Female	Male
CK (0 g leaf litter/pot)	10.81a	4.85
L30 (30 g leaf litter/pot)	7.26ab	4.12
L60 (60 g leaf litter/pot)	4.98b	3.40
L90 (90 g leaf litter/pot)	7.58ab	3.09
L120 (120 g leaf litter/pot)	9.61ab	6.00
Leaf litter		0.060
Sex		< 0.001
Leaf litter \times sex		0.352

Each value represents the mean \pm SE (n=5). Between-group variation was not significant in the Pn for the male according to one-way ANOVA. Different lowercase letters indicate the difference reached $\alpha < 0.05$ probability level based on Tukey's test, the separate effect of leaf litter and sex, and their interaction were tested using two-factor analysis.

(Fig. 3). This corroborated the hypothesis made from Table 3, that the male entered the reproductive stage earlier and developed more senile vegetative organs than female. Prior to the 36th d after sowing, the rate of accumulated female buds (RAFB) was low (<5%) for each group. Subsequently, this rate increased rapidly and L60 > L90 \approx L30 \approx CK > L120. The L60 exceeded CK by 36.08%, while, L120 was 28.67% lower than CK ($p < 0.05$). The initiation of female bud was promoted by L60 and emerged 0.9 d earlier but L120 inhibited it, hence, emerged 2.7 d later compared to CK (Table 4). Decomposing leaf litter promoted the initiation of male buds (Table 4), the mean rate of accumulated male buds (RAMB) was 8.25%, 25.63%, 26.93% and 25.59% in L30, L60, L90 and L120, respectively. However, Repeated Measures (RM-ANOVA) showed that leaf litter effect was insignificant on RAMB of spinach ($p > 0.05$) (Fig. 3).

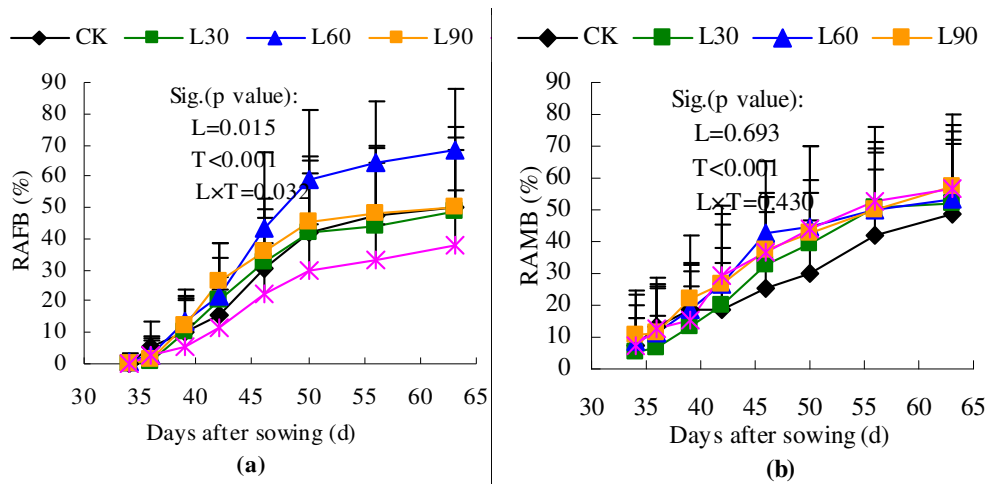


Figure 3. Effects of doses of blue gum leaf litter on rate of accumulated buds in spinach. The data are mean \pm SE (n=12). RAFB = Rate of accumulated female buds, RAMB = Rate of accumulated male buds. Repeated Measures (RM-ANOVA) was performed to exam the leaf litter effect (L), time effect (T), leaf litter and time interaction (LxT).

Table 4. Parameters obtained from the logistic equations used to fit the budding and flowering dynamics.

Treatment	Female			Male			Potential sex ratio (F/M)
	First bud day (d)	First flower day (d)	Potential maximum rate (%)	First bud day (d)	First flower day (d)	Potential maximum rate (%)	
CK	36.2	40.3	49.87	37.9	41.2	60.02	0.83
L30	35.7	42.0	46.50	34.3	42.5	53.28	0.87
L60	35.3	39.6	67.04	32.1	37.9	52.46	1.28
L90	35.9	42.1	48.67	34.0	39.8	57.96	0.84
L120	38.9	42.5	36.41	33.9	43.5	57.18	0.64

The expression of Logistic Equation is $Y = a / [1 + b \cdot \exp(c \cdot X)]$ (a, b, c are constants). For a specific treatment, potential maximum rate was obtained according to the fitted logistic equation, and first bud day and first flower day were both obtained based on the corresponding first-order derivative of the fitted logistic equation. Potential sex ratio = potential maximum rate of the female / potential maximum rate of the male.

Flowers Development

The dynamics of female and male flowering of spinach in response to decomposing leaf litter are presented in Figure 4 (a) and (b). No female flower appeared before the 42nd d after sowing for each group, but subsequently, there was gradual increase in rate of accumulated female flowers (RAFF). The responses of RAFF and RAFB were similar to decomposing leaf litter, i.e., the RAFF in L60 was highest (27.84%), which was similar to CK, L30 and L90. L120 reduced the RAFF significantly by 37.76% ($p < 0.05$). All leaf litter treatments delayed the occurrence of female flowers, except L60 (Table 4).

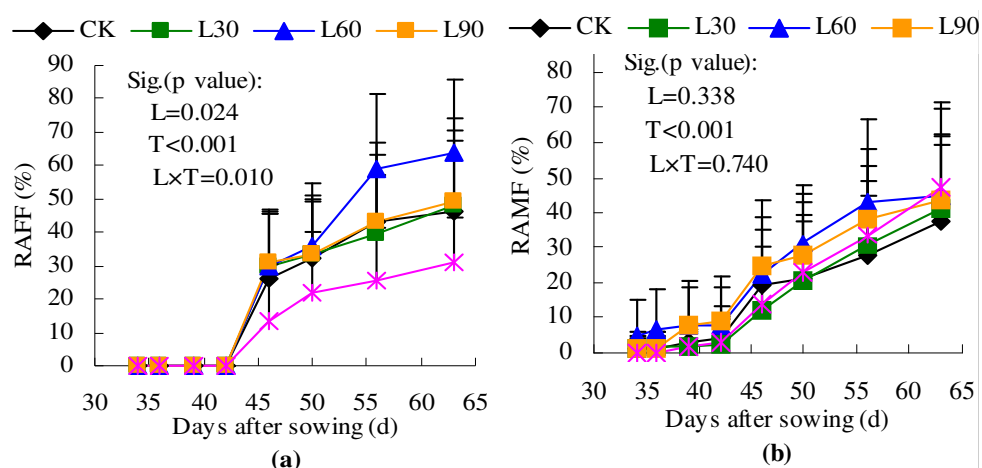


Figure 4. Effects of doses of blue gum leaf litter on rate of accumulated flowers in spinach. The data are mean \pm SE (n=12). RAFF = the rate of accumulated female flowers, RAMF = the rate of accumulated male flowers. Repeated Measures (RM-ANOVA) was performed to exam the leaf litter effect (L), time effect (T), leaf litter and time interaction (LxT).

Decomposition of 60 and 90 g leaf litter promoted the male flowers and the rate of accumulated male flowers (RAMF) in L60 and L90 was 47.17% and 33.57% higher than CK, respectively, there was no significant difference between the groups ($p>0.05$). Based on the logistic equations, the first male flower appeared 3.3 d and 1.4 d earlier in L60 and L90, respectively, than CK. The 30 and 120 g leaf litter slightly altered the RAMF but delayed the initiation of male flowers by 1.3 d and 2.3 d, respectively.

Plants at flower maturing stage are also affected by temperature (37), illumination (45) and other environmental factors, including exogenous growth regulators (26) during the flower induction and development of floral organs. The allelopathic effect is also a type of environmental stress (48). The development of both sexual floral organs of spinach were affected by decomposing leaf litter, but the mechanism underlying the alteration of allelopathic effect in budding and flowering dynamics of spinach has yet to be elucidated. Holappa and Blum (19) found that ferulic acid induced the biosynthesis of abscisic acid (ABA) in *Lycopersicon esculentum* Mill. cv. Ailsa Craig within 8 h; Chi *et al.* (9) recently reported that juglone affected the elongation of rice root via ABA, jasmonic acid (JA) and gibberellic acid (GA). Our previous study also revealed that the decomposition of blue gum leaf litter accelerated the maturation and senescence of spinach, and concluded that it was related to the imbalance of hormones and / or reactive oxygen species (ROS) (5), which was thus the likely cause of alteration in the budding and flowering dynamics of spinach in this study.

Sex differentiation

The results in 2011 and 2012 differed greatly in the percentage of three sexual types of spinach and the F/M ratio under each treatment (Table 5). However, the responses

Table 5. Effects of doses of blue gum leaf litter on sex differentiation in spinach

Treatment	2011				2012			
	Percentage (%)			F/M	Percentage (%)			F/M
	Female	Male	Monoecy		Female	Male	Monoecy	
CK (0 g leaf litter/pot)	44.19	50.00	5.81	0.90	28.07	50.88	15.79	0.66
L30 (30 g leaf litter/pot)	45.16	48.39	6.45	0.94	35.85	39.62	16.98	0.93
L60 (60 g leaf litter/pot)	42.05	36.36	21.59	1.10	33.33	43.33	10.00	0.81
L90 (90 g leaf litter/pot)	43.48	49.28	7.25	0.90	18.00	48.00	32.00	0.63
L120	36.23	56.52	7.25	0.68	20.69	56.90	15.52	0.50

Some plants remained in their vegetative phase throughout the observation period in 2012.

of sex differentiation of spinach to decomposing leaf litter were partly consistent in these two years. Treatment L90 did not influence the F/M ratio for both years. The F/M ratio was higher in L60 than CK by 0.20 and 0.15, and was lower in L120 compared to CK by 0.22 and 0.16 in 2011 and 2012, respectively. Only the plants in L30 responded differently to the F/M ratio between these two years. This was supported by the results of regression analysis, which showed that L60 had higher potential F/M ratio, while, L120 has lower potential F/M ratio than CK (Table 4).

These results indicated a positive effect of 60 g leaf litter, and contrarily a negative effect of 120 g leaf litter on the differentiation of female organs. In fact, hormones regulate the sex expression (38). Although both environmental factors (illumination, temperature, moisture, etc.) and anthropic factors (nitrogen fertiliser, wound, pruning, etc.) affects the sex differentiation of plants, but the subsequent changes in concentration of hormones is the true internal regulator (16,44). Hence, the alteration of floral development and F/M ratio caused by decomposing leaf litter (Figure 3, Figure 4 and Table 5) may be related to change in the level of endogenous hormones. As the effects of hormones depends on their concentrations e.g. IAA (26) and GA (22), we suspected that the decomposition of different amounts of leaf litter in soil may result in variable contents of hormones and thereby affected the floral development and F/M ratio of spinach. However, the identity of hormone(s) participating in regulation, and their dynamics in spinach during the leaf litter decomposition remained unknown, and further studies are needed.

Volatile compounds

In eucalypts, the leaves emit a strong smell after macerating or grinding, hence, we analysed the volatile compounds in two leaf litter samples of blue gum. There were a total of 79 compounds identified in the original blue gum leaf litter, including 28 terpanes (representing 27.26% of total compounds), 24 oxygenated terpenes (50.17%), 8 phenols (4.41%), 6 esters (4.96%), 4 carboxylic acids (1.19%), 4 alkanes or alkenes (0.96%), and 5 heterocyclic compounds (3.46%). The terpenes (terpanes and oxygenated terpenes) are major compounds among the volatile compounds of blue gum leaf litter with a total absolute peak area of 3,071,332,036. When the leaf litter was decomposed for 4-months, only 10 terpanes (25.76%) and 3 oxygenated terpenes (25.78%) remained, their total absolute peak area (707,257,444) was about only a quarter of original leaf litter (Table 6).

Table 6. Volatile compounds identified from the original blue gum leaf litter and the decomposed leaf litter. DL represents for the decomposed leaf litter

Compounds	Molecular formula	Relative content in compounds	Relative content in the DL	Compounds	Molecular formula	Relative content in compounds	Relative content in the DL
Terpenes							
α -Pinene	C ₁₀ H ₁₆	5.027	2.286	HH-Cycloprop[er]azulene, decalhyd	C ₁₅ H ₂₄	1.951	—
Camphene	C ₁₀ H ₁₆	0.043	0.284	ro-1, 1, 7-			
β -Pinene	C ₁₀ H ₁₆	0.204	—	trimethyl-4-methylene, [1 α R-(1 α , 4 β , 7 α , 7 α \beta, 7 $\beta\alpha$)]-	C ₁₅ H ₂₀	—	1.034
β -Myrcene	C ₁₀ H ₁₆	0.084	—	Agarospirol	C ₁₅ H ₂₄	0.110	1.715
				γ -Gurjunene	C ₁₅ H ₂₄	0.153	—
				Naphthalene, 1,2,3,4,4 α ,5,6,8 α -octally dro-7-			
				methyl-4-methylene-1-(1-methyl-ethyl)-, (1 α , 4 α , 8 α)-			
α -Phellandrene	C ₁₀ H ₁₆	0.226	1.197	Valencene	C ₁₅ H ₂₄	—	0.275
γ -Terpinene	C ₁₀ H ₁₆	1.519	—	1,9-Aristoladiene	C ₁₅ H ₂₂	—	0.385
(+)- δ -Cadinene	C ₁₅ H ₂₄	0.173	—	Eremophilene	C ₁₅ H ₂₄	0.389	—
(-)- α -Copaene	C ₁₅ H ₂₄	0.401	1.631	Vindiflorene	C ₁₅ H ₂₄	0.455	—
(-)- β -Elemene	C ₁₅ H ₂₄	0.086	—	Vindiflorol	C ₁₅ H ₂₀	—	7.988
γ -Elemene	C ₁₅ H ₂₄	—	2.980	Cadina-1,4-diene	C ₁₅ H ₂₄	0.141	—
α -Gurjunene	C ₁₅ H ₂₄	0.200	0.562	(-)- γ -Cadinene	C ₁₅ H ₂₄	0.173	0.535
β -Patchoulene	C ₁₅ H ₂₄	0.083	—	dehydro-Aromadendrene	C ₁₅ H ₂₂	0.087	0.714
1-Caryophyllene	C ₁₅ H ₂₄	0.406	—	Calamene	C ₁₅ H ₂₂	0.110	—
γ -Selinene	C ₁₅ H ₂₄	1.377	—	Tricyclo[6.3.0.1(2,3)]undec-7-ene, 6, 10, 11, 11-tetramethyl-	C ₁₅ H ₂₄	0.410	—
β -Selinene	C ₁₅ H ₂₄	—	4.844	Cadalol	C ₁₅ H ₁₈	—	1.179
Calarene	C ₁₅ H ₂₄	3.076	4.455	Guaiazulene	C ₁₅ H ₁₈	0.085	—
(+)-Aromadendrene	C ₁₅ H ₂₄	9.328	12.380	2, 2, 4 α , 6 α , 8 α , 9, 12 β , 14 α -Octamethyl-1, 2, 3, 4, 4 α , 5, 6, 6 α , 6 β , 7, 8, 8 α , 9, 12, 12 α , 12 β , 13, 14, 14 α , 14 β -eicosahydricene	C ₃₀ H ₅₀	0.902	—

Table 6. Contd.

Compounds	Molecular formula	Relative content in compounds	Relative content in the DL
Allicoramendrene	C ₁₅ H ₂₄	—	3.850
Ledane	C ₁₅ H ₂₆	—	0.287
Oxygenated Terpenes			
Eucalyptol	C ₁₅ H ₁₈ O	24.014	5.881
Linalool oxide	C ₁₅ H ₁₆ O ₂	0.045	—
Fenchol	C ₁₅ H ₁₆ O	0.087	—
Campholenic aldehyde	C ₁₅ H ₁₆ O	0.048	—
trans-p-2, 8-Menthadien-1-ol	C ₁₅ H ₁₆ O	0.065	—
(-)-trans-Pinocarveol	C ₁₅ H ₁₆ O	0.917	—
1, 5, 7-Octatrien-3-ol, 2, 6-dimethyl	C ₁₅ H ₁₆ O	0.103	—
Pinocarvone	C ₁₅ H ₁₄ O	0.466	—
(-)-4-Terpineol	C ₁₅ H ₁₆ O	0.906	—
Isocarveol	C ₁₅ H ₁₆ O	0.326	—
α-Terpineol	C ₁₅ H ₁₆ O	2.562	—
trans-Carveol	C ₁₅ H ₁₆ O	0.154	—
L(-)-Carvone	C ₁₅ H ₁₄ O	0.057	—
Phenols			
Thymol	C ₁₅ H ₁₄ O	0.258	—
Carvacrol	C ₁₅ H ₁₄ O	0.298	—
Fihaihone, 1-(7-hydroxy-5-methoxy-2, 2-dimethyl-2H-1-benzopyran-6-yl)-	C ₁₅ H ₁₆ O ₄	0.635	—
1, 4-Naphthoquinone, 6-acetyl-2, 5, 8-trihydroxy-Esters	C ₁₇ H ₁₄ O ₆	0.164	—
Benzoic acid, 3, 4, 5-trimethoxy-methyl ester	C ₁₇ H ₁₄ O ₇	0.461	—
Compounds	Molecular formula	Relative content in compounds	Relative content in the DL
Benzene, methyl(1-methyl-phenyl)-	C ₁₀ H ₁₂	0.062	—
p-Cymene	C ₁₀ H ₁₄	—	1.968
Geraniol	C ₁₅ H ₁₈ O	0.062	—
Elemol	C ₁₅ H ₂₆ O	0.168	—
Espatulenol	C ₁₅ H ₂₆ O	0.160	—
(-)-Globulol	C ₁₅ H ₂₆ O	4.377	9.919
Rosifolol	C ₁₅ H ₂₆ O	2.081	—
β-Endesmol	C ₁₅ H ₂₆ O	10.142	9.978
α-Endesmol	C ₁₅ H ₂₆ O	2.219	—
Perhydrofarnesyl acetone	C ₁₈ H ₃₆ O	0.136	—
Phytol	C ₂₀ H ₄₀ O	0.190	—
γ-Sitosterol	C ₂₉ H ₅₀ O	0.798	—
Stigmastan-3,5-dien *	C ₂₉ H ₅₀ O	0.089	—
Stigmast-4-en-3-one	C ₂₉ H ₄₈ O	—	0.433
1, 4-Naphthoquinone, 6-ethyl-2, 3, 5, 7-tetrahydroxy-	C ₁₇ H ₁₆ O ₆	1.064	—
γ-Tocopherol	C ₂₈ H ₄₄ O ₂	0.071	—
2H,6H-Pyranol(3, 2-b)kxanthen-6-one, 5, 9, 10-trihydroxy-2, 2-dimethyl-	C ₁₈ H ₁₁ O ₆	0.584	—
Vitamin E	C ₂₉ H ₅₀ O ₂	1.333	—
(Z)-14-Tricosenyl formate	C ₃₃ H ₆₆ O ₂	0.467	—

Table 6. Contd.

Compounds	Molecular formula	Relative content in compounds	Relative content in the DL
7, 10, 13-Hexadecatrienoic acid, methyl ester	C ₁₇ H ₃₂ O ₂	0.133	—
4, 8, 12, 16-Tetramethylheptadecan-4-olide	C ₂₁ H ₄₀ O ₂	0.049	—
3, 5-Dinitrobenzoic acid, 2- <i>t</i> -butylphenyl ester	C ₁₇ H ₁₆ N ₂ O ₆	3.562	—
Carboxylic Acids			
Tetradecanoic acid	C ₁₄ H ₂₈ O ₂	0.072	—
Palmitic acid	C ₁₆ H ₃₂ O ₂	0.308	—
Oleic acid	C ₁₈ H ₃₄ O ₂	0.062	—
Alkanes & Alkenes			
Heptadecane	C ₁₇ H ₃₆	0.052	—
Octadecane	C ₁₈ H ₃₈	0.317	—
Heicosane	C ₂₁ H ₄₄	0.537	—
Other Cyclic Compounds			
8-Quinolol	C ₉ H ₉ NO	—	1.756
Dibenz[b, f][1, 4]oxazepine	C ₁₃ H ₉ NO	0.873	—
2H-Pyran, 2, 2-(1, 6-hexanediyloxy)bis(tetrahydro-	C ₁₄ H ₁₆ O ₄	0.106	—
Episesamin	C ₂₈ H ₄₈ O ₆	0.075	—
Total		92.408	80.030

Compounds	Molecular formula	Relative content in compounds	Relative content in the DL
Olean-18-en-28-oic acid, 3-oxo-, methyl ester	C ₃₁ H ₅₀ O ₃	0.285	—
Dimethyl 2,3-bis(1,3-dimethylindol-2-yl)fumarate	C ₂₈ H ₃₈ O ₄	—	0.286
Linoleic acid	C ₁₈ H ₃₂ O ₂	—	0.276
5-Hepenoic acid, 2, 6-dimethyl-2-vinyl	C ₁₁ H ₁₈ O ₂	0.747	—
Nonacosane	C ₂₉ H ₆₀	—	0.240
17-Pentatriacontene	C ₃₅ H ₇₀	0.052	—
Benzenamine, 4-(2-phenylethyl)-	C ₁₄ H ₁₃ N	1.130	—
3, 4, 5-Trime-thoxy-N-(2-methyl-1, 3-dioxo-5-isoindolyl) benzamide	C ₁₉ H ₁₈ N ₂ O ₅	1.280	—
1H-Indene-1,3-(2H)-dione, 2-hydroxy-2-(9-methoxy-9H-fluoren-9-yl)-	C ₂₃ H ₁₈ O ₄	—	0.301
1H-Indene, 1-ethylideneoctahydro-7 α -methyl-, cis-*		—	0.411

Eucalyptus oils have insecticidal, antifungal, antimicrobial, and herbicidal activities, etc. (2) and there are numerous volatile compounds in eucalypt leaves (12,23,40). The plants produce allelopathic effects via numerous allelochemicals rather than a single one (24). Thus, determining the active chemical(s) in blue gum leaf litter is difficult. We suspected that the likely allelochemicals of blue gum leaf litter were among the 28 terpanes and 24 oxygenated terpenes (total 52 terpenes), because terpenoids is the largest family of allelochemicals (27,36) and are also potential allelochemicals of eucalypts (23,42). Further, of the 52 terpenes, α -pinene, aromadendrene, eucalyptol, and α -(β -)eudesmol were the best candidates, because their relative content was 5.027%, 9.328%, 24.014% and 12.361%, respectively, and their absolute peak area was greatly decreased after decomposition. Moreover, they are inhibitory to the growth of weeds or bacteria (18,33,39). In addition, 11 newly generated terpenes, including 10 terpanes and 1 oxygenated terpenes, were also identified from the decomposed sample. Thus the terpenes in blue gum leaf litter could also be isomerized or transformed during the decomposition process, e.g., β -selinene and alloaromadendrene found in the decomposed sample might originate from γ -selinene and aromadendrene in the original sample, respectively. This hypothesis was supported by Kong *et al.* (25) and de Albuquerque *et al.* (10).

CONCLUSIONS

Two sexual types of spinach responded differently to partially decomposed leaf litter of blue gum for morphological traits (the leaf number and stem length), net photosynthetic rate (Pn), budding and flowering dynamics. These differences might originate from the inherent genetic differences between the two sexes. The F/M ratio was not altered by application with 90 g blue gum leaf litter but was increased by 60 g leaf litter and reduced by 120 g leaf litter in both years. Seventy nine volatile compounds were identified from the blue gum leaf litter, of these 52 terpenes, with high relative content decreased greatly after the decomposition. The α -pinene, aromadendrene, eucalyptol and eudesmol were most active volatile compounds. These terpenes or their decomposing products may induce an imbalance in endogenous hormones in spinach, and thus affect its budding, flowering and sex differentiation during the leaf litter decomposition.

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