

PART OF A HIGHLIGHT ON CLONAL PLANT ECOLOGY

## Light limitation and litter of an invasive clonal plant, *Wedelia trilobata*, inhibit its seedling recruitment

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• **Background and Aims** Invasive clonal plants have two reproduction patterns, namely sexual and vegetative propagation. However, seedling recruitment of invasive clonal plants can decline as the invasion process proceeds. For example, although the invasive clonal *Wedelia trilobata* (Asteraceae) produces numerous seeds, few seedlings emerge under its dense population canopy in the field. In this study it is hypothesized that light limitation and the presence of a thick layer of its own litter may be the primary factors causing the failure of seedling recruitment for this invasive weed in the field.

• **Methods** A field survey was conducted to determine the allocation of resources to sexual reproduction and seedling recruitment in *W. trilobata*. Seed germination was also determined in the field. Effects of light and *W. trilobata* leaf extracts on seed germination and seedling growth were tested in the laboratory.

• **Key Results** *Wedelia trilobata* blooms profusely and produces copious viable seeds in the field. However, seedlings of *W. trilobata* were not detected under mother ramets and few emerged seedlings were found in the bare ground near to populations. In laboratory experiments, low light significantly inhibited seed germination. Leaf extracts also decreased seed germination and inhibited seedling growth, and significant interactions were found between low light and leaf extracts on seed germination. However, seeds were found to germinate in an invaded field after removal of the *W. trilobata* plant canopy.

• **Conclusions** The results indicate that lack of light and the presence of its own litter might be two major factors responsible for the low numbers of *W. trilobata* seedlings found in the field. New populations will establish from seeds once the limiting factors are eliminated, and seeds can be the agents of long-distance dispersal; therefore, prevention of seed production remains an important component in controlling the spread of this invasive clonal plant.

**Key words:** Clonal plant ecology, leaf litter, biological invasion, invasive plant, light limitation, reproductive strategy, seedling recruitment inhibition, *Wedelia trilobata*, Asteraceae.

### INTRODUCTION

Many plants reproduce in two ways, namely sexual reproduction through seeds and clonal regeneration through vegetative propagation (Koivunen *et al.*, 2004). Sexual reproduction occurs via seeds that are dispersed over long distances by wind, birds or mammals, whereas clonal propagation occurs via bulbils, corms or rhizomes for rapid colonization (Rautiainen *et al.*, 2004; Liu *et al.*, 2010). However, environmental resources are often limited to a certain range. More resources are allocated to sexual reproduction, while fewer resources might be allocated to rapid clonal propagation (Thompson and Eckert, 2004; Chu *et al.*, 2011). The balance between these two reproduction modes is highly significant in the population expansion of a plant.

Various studies on the biological invasion mechanisms of the reproduction strategy of invasive plants have been reported.

Invasive plants spread or recruit their population via sexual (e.g. *Centaurea solstitialis*) (Swope and Parker, 2010) or clonal reproduction (e.g. *Oxalis pes-caprae*) (Castro *et al.*, 2007). Clonal growth is common and important for alien plant invasion (Otinowski and Kenkel, 2008; Wang *et al.*, 2008b); it facilitates population establishment and expansion in a new range (Yu *et al.*, 2009; Roiloa *et al.*, 2010). Clonal species often invest fewer resources in seedling recruitment than non-clonal species (Dorken and Eckert, 2001). Since many populations grow predominantly via vegetative reproduction, seedling recruitment has a minor significance for these populations, especially for invasive clonal plants, and may decline as the invasion process continues (Harald and Roldan, 1997). What are the reasons for the restriction of seedling recruitment of invasive clonal plants?

Plant regeneration is affected by numerous environmental parameters, such as light, temperature, water availability, nutrients

and litter, among which light and litter may be two of the pivotal factors influencing seed germination and seedling establishment (Fuchs *et al.*, 2000; Olson and Wallander, 2002; Sonohat *et al.*, 2004; Wei *et al.*, 2009). Although previous studies have reported that seeds of many Asteraceae plants are photosensitive (Schutz *et al.*, 2002; Luo and Cardina, 2012), the light requirement still varies among different Asteraceae plants (Galindez *et al.*, 2009; Sun *et al.*, 2009; Li *et al.*, 2012). The response of seed germination to light is significantly associated with seed characteristics such as seed weight and size (Schutz *et al.*, 2002). Plant litter is another factor that affects seed germination and seedling establishment (Cavieres *et al.*, 2007; Hata *et al.*, 2010; Valera-Burgos *et al.*, 2012). Wang *et al.* (2012) found that the litter of the invasive weed *Wedelia trilobata* inhibited seedling growth of radish (*Raphanus sativus* L.) and turnip (*Brassica campestris* L.).

*Wedelia trilobata* (Asteraceae), native to the tropics of South America (Weber *et al.*, 2008), is a perennial evergreen creeping clonal herb (Supplementary Data Fig. S1). It has been listed as one of the 100 world's worst invasive alien species (IUCN, 2001). This noxious weed was introduced to South China on a large scale as a common groundcover plant in the 1970s, but it rapidly spread to the field (Li and Xie, 2003). Fast dispersal through vegetative propagation (clonal growth) is one of the pivotal factors for the successful invasion of *W. trilobata* (Wu *et al.*, 2005; Song *et al.*, 2010b). Once established in plantations, *W. trilobata* can overgrow into a dense groundcover and prevent the regeneration of other species (Song *et al.*, 2010a). Abundant flowers of *W. trilobata* were found in the field (Supplementary Data Fig. S2A). *Wedelia trilobata* also produces seeds (Thaman, 1999). Most studies have focused on its vigorous clonal reproduction, whereas sexual reproduction has not received much attention. Also, seedling recruitment of *W. trilobata* has been poorly studied. In a previous investigation of vegetation in the field across the tropical Hainan Island in China (unpublished results), we found that invasion of a community of flora by a dense population of *W. trilobata* resulted in a microhabitat with low light and a thick layer of *W. trilobata* litter, and hardly any detectable *W. trilobata* seedlings.

In the field, *W. trilobata* produces numerous seeds; however, few seedlings were found under its population canopy in the field. We hypothesized that both the dense mother ramets and the thick litter layer caused light limitation for the seeds under *W. trilobata* populations. Darkness and litter might inhibit seed germination and seedling growth of *W. trilobata*. Therefore, lack of light and the presence of a layer of its own litter under ramets might be the primary reasons causing the failure of seedling recruitment for this invasive weed in the field. To test these hypotheses, we quantified both the seed production and seedling establishment of *W. trilobata* in the field, and assessed the effects of light and its own litter on seed germination and seedling growth in the greenhouse and in the field.

## MATERIALS AND METHODS

### Field survey and sampling

A field survey was conducted in Hainan Province, China in June 2012. In order to examine biomass allocation in sexual reproduction of *Wedelia trilobata* (L.) Hitchc., 48 individuals were collected randomly from eight sites across eight cities in Hainan

Province (six individuals from each site). Roots, stems, leaves and flowers from each individual were separated, washed, dried at 80 °C for 72 h, and weighed to calculate their biomass allocation ratios.

Many species of Asteraceae produce dimorphic achenes in a single capitulum (Brandel, 2004); this is the case for *W. trilobata*, which produces Type I and Type II achenes (Supplementary Data Fig. S2C). Type I seed is plump and fertilized, whereas Type II seed is shrivelled and sterile. A total of 267 mature capitulum were randomly selected to count the number of Type I and II seeds in one capitulum. Also, Type I seeds were collected from each sample site, mixed evenly and stored in a gauze bag at room temperature for seed germination experiments.

We also randomly established 117 quadrats (1 × 1 m) in the sites that were seriously invaded by *W. trilobata* both in Haikou and in Sanya city. Each quadrat was separated from the others by a distance of about 1 km. The number of *W. trilobata* seedlings (Supplementary Data Fig. S1) was counted in each quadrat. We also calculated light transmittance under each population canopy. Light intensity under the *W. trilobata* population canopy and that of the bare ground nearby for each quadrat were recorded using a CL-200 illumination photometer (Konica Minolta, Japan). Light transmittance (LT) of the *W. trilobata* population was calculated as the ratio of light intensity of the substrate under the *W. trilobata* population canopy ( $L_p$ ) to the light intensity of the bare ground nearby ( $L_b$ ), i.e.  $LT = L_p/L_b$ . The plant cover was estimated using a sub-divided quadrat method (Kolb *et al.*, 2002; Karalius and Alpert, 2010).

### Experiment 1: effects of light on seed germination

For all germination experiments, seeds of *W. trilobata* were surface-sterilized with 5 % sodium hypochloride solution for 10 min and washed thoroughly five times with sterilized distilled water. Seed germination was conducted in 9 cm Petri dishes with two layers of sterilized Whatman qualitative filter papers. To test the effects of light on seed germination of *W. trilobata*, five light intensity levels were created: full light (the light intensity of the incubator,  $450 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), 1/2, 1/4 and 1/8 of full light, and darkness. These five treatments were chosen to simulate light regimes in the field. The Petri dishes were wrapped in perforated aluminium foil with a different hole area for the 1/2, 1/4 and 1/8 light intensity treatments, intact aluminium foil without perforation for dark treatment, and no aluminium foil for full light treatment (Experiment 1 in Supplementary Data Fig. S3). Each light treatment was repeated five times (a total of 25 Petri dishes) and contained 50 surface-sterilized seeds (a total of 1250 seeds). All Petri dishes were placed in an incubator with a constant temperature of 24 °C and humidity of 70 % under light ( $450 \mu\text{mol m}^{-2} \text{s}^{-1}$ ; photoperiod: 16 h day and 8 h night). About 7 d after the study was set up, germinated seeds (radicle visible) were counted daily for about 7 weeks until no more germinated seeds were registered.

### Experiment 2: effects of leaf extracts and light on seed germination

A factorial block design (Experiment 2 in Supplementary Data Fig. S3) was applied to study the effects of leaf extracts and light intensity. Due to the potential difference in chemical leaf constituents between fresh and dry leaves during decomposition (Gessner, 1991), we used both fresh and dry leaf extracts to test

the effects on seed germination. The leaf extracts were obtained from fresh or dry leaves of *W. trilobata*. Fresh leaves were collected randomly from individuals in the field. Half of the samples were soaked in sterilized distilled water (0.2 g f. wt mL<sup>-1</sup>) for 72 h at room temperature, and then filtered. The other half of the fresh leaves were dried to constant mass at 80 °C. Fresh leaves (1 kg) were dried to yield 86 g of dry material. The dry leaves were soaked in sterilized distilled water (0.05 g d. wt mL<sup>-1</sup>) for 72 h at room temperature. The extracts were filtered and stored at 4 °C. The fresh leaf extracts were diluted to the final working concentrations of 0.05, 0.1 and 0.2 g f. wt mL<sup>-1</sup> following Dornig and Cipollini (2006). The dry leaf extracts were diluted to 0.005, 0.025 and 0.05 g d. wt mL<sup>-1</sup> (Hou et al., 2011), and sterilized distilled water was used as control treatment. Thus, a total of seven concentrations of leaf extract were used. Light intensity included high light (450  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and darkness (by covering with aluminium foil). All 14 treatments were arranged as one block and repeated five times.

Fifty seeds were placed on each Petri dish with filter paper. Each Petri dish was treated with 5 mL of prepared leaf extracts every 2 d, or treated with 5 mL of sterilized distilled water as control. Germination conditions were the same as those of expt 1. Germinated seeds (radicle visible) were counted daily for 7 weeks until germination stopped.

#### Experiment 3: effects of leaf extracts and light on seedling growth

To assess the effects of its own litter and light on seedling growth of *W. trilobata*, seven concentrations of leaf extract and two levels of light treatments were examined (Experiment 3 in Supplementary Data Fig. S3). The seven concentrations of leaf extract were consistent with those in expt 2. The two light levels were high light (450  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and shade treatment (approx. 22.5  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , by covering with kraft paper).

Seedlings that were 2 d old after germination were transferred from the Petri dishes into pots (7 × 7 × 8 cm, one individual seedling per pot) with a 1:1:1 mixture of sterilized soil (total nitrogen, 0.88 ± 0.23 %; total phosphorus, 0.85 ± 0.33 %), perlite and vermiculite. Vigorous seedlings were chosen for further experiment after 7 d of culture. For each leaf extract concentration, 14–20 individual seedlings were planted under high light treatment (123 seedlings in total), and 15 individual seedlings were planted for shade treatment (105 seedlings in total). The seedlings were watered every 3 d with 10 mL of leaf extracts of each concentration or 10 mL of sterilized distilled water (control treatment) until harvest. All the pots of this experiment were randomly placed in an incubator with a diurnal rhythm of 16 h day and 8 h night. We harvested the seedlings after 6 weeks and weighed above- and below-ground mass. Their shoot and root lengths were also measured.

#### Experiment 4: seed germination in the field

The field seed germination experiment was conducted at a location in Haikou, China (110°20'24.2"E, 20°00'16.4"N) where the cover of the *W. trilobata* population was homogeneous and >90 %. Light transmission under the plant canopy was about 5 % of full sunlight. Twelve 1 × 1 m plots were randomly selected. The plant canopy was removed and litter was cleaned to

make the ground bare in six plots for the full sunlight treatment, whereas that of the other six plots was left for shade treatment. In half of the plots of these two light treatments, we added 30 seeds of *W. trilobata* per plot, whereas no seeds were added to the remaining plots. Thus, four treatments were created (two light levels and two seed addition treatments) with three replications (Experiment 4 in Supplementary Data Fig. S3). Germinated seeds were counted every day for 2 months.

#### Data analysis

Data were analysed using SAS statistical software (version 9.1) (SAS Institute Inc., 2004). Experiments 1 and 3 were analysed using one-way analysis of variance (ANOVA) with light or leaf extract treatment as fixed factors. Duncan's multiple range test was used to compare pairwise least squares means of seed germination and seedling growth (shoot and root length, above- and below-ground biomass) of *W. trilobata*. Experiment 2 was analysed using two-way ANOVA with both light and leaf extract treatment as factors. Tukey's multiple comparison test was used to compare pairwise least squares means of seed germination of *W. trilobata*. Data are presented as the mean ± s.e. Statistical significance was set at  $P \leq 0.05$ , and the results were adjusted by Bonferroni test. A quadratic non-linear regression model was used to detect the relationships between the canopy cover of *W. trilobata* and relative LT under the population canopy. The results of the regression analysis were visualized using SigmaPlot Version 11.

## RESULTS

#### Sexual reproduction and seedling recruitment of *W. trilobata* in the field

Although biomass allocation to flowers was about  $6 \pm 1.9\%$  of the whole individual biomass ( $n = 48$ ), *W. trilobata* produced flowers profusely year-around and a number of ramets with numerous stem nodes where a capitulum could possibly develop (Supplementary Data Fig. S2A, B). An average of 48 seeds was found in one capitulum based on 267 capitulum, and 12 (25.4 %) Type I seeds were counted in one capitulum. In the field, the stem length of *W. trilobata* was not constant, and the number of individuals per square metre varied. Thus, we used two scenarios to estimate seed production based on the number of stem nodes, capitulum and total seed number in a 1 × 1 m plot with 90 % cover of *W. trilobata*. In the first scenario, almost 1240 stem nodes (310 ramets × 4 nodes per ramet on average) were noted, and 1240 capitulum could exist in 1 m<sup>2</sup>. In the second scenario, there were approx. 30 individuals with about 30 nodes, i.e. 900 capitulum (30 individuals × 30 nodes × 1 capitulum per node) could exist in 1 m<sup>2</sup>. According to our survey in the field and the two scenarios of estimated capitulum number, approx. 10 800 (900 capitulum × 12 Type I seeds per capitulum on average) to 14 880 fertilized seeds (1240 capitulum × 12 Type I seeds) were present in 1 m<sup>2</sup>.

In the communities invaded by *W. trilobata*, light transmittance decreased to <20 % when the plant canopy cover was >50 %, and it was almost murky when the cover was >75 % (Fig. 1A). Thick litter layers were also frequently found under the ramets of *W. trilobata*. The frequency of seedling numbers

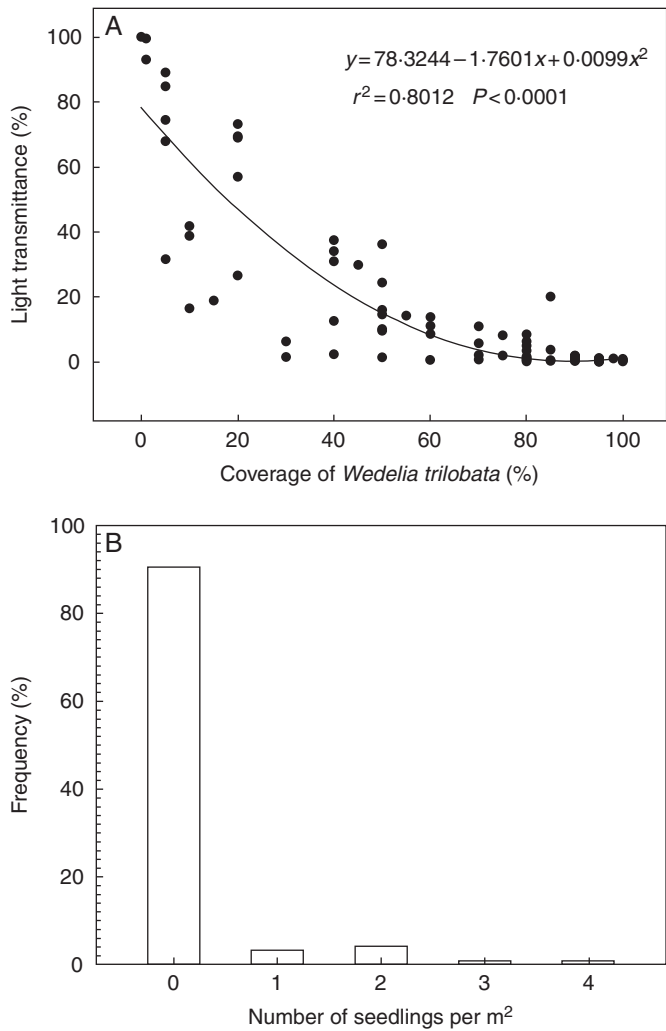


FIG. 1. Relationship between cover of the *W. trilobata* population and light transmittance (A) and the frequency of the number of seedlings of *W. trilobata* per m<sup>2</sup> which emerged in the surveyed plots (B).

in the sampled plots is shown in Fig. 1B. For the total of 117 plots, 91 % of the plots had no detectable seedlings (Fig. 1B) under the mother ramets with low LT and thick leaf litter layer. Only 21 seedlings, which were all distributed in the bare ground around the adjacent mother ramets (Supplementary Data Fig. S4), were found in 11 plots.

*Effects of light intensity on seed germination in the laboratory (expt 1)*

Darkness significantly limited seed germination ( $P < 0.001$ , Table 1; Fig. 2A). The seed germination rates were between 70 and 80 % under the four levels of light intensity, but the germination rate was only 9.6 % under the dark condition (Fig. 2A).

*Effects of leaf extracts and light on seed germination in the laboratory (expt 2)*

Seed germination was greatly influenced by both leaf extracts and light ( $P < 0.001$ , Table 2; Fig. 2B). The interaction effects

TABLE 1. One-way ANOVA for the effects of light on seed germination of *W. trilobata* (expt 1)

Factor	d.f.	Mean square	F	P
Light	4	4363.04	63.64	< 0.001
Error	20	68.56		

between leaf extracts and light on seed germination were also significant ( $P < 0.001$ , Table 2).

In high light treatment, seed germination was significantly inhibited by the presence of both fresh and dry leaf extracts. Moreover, the aqueous leaf extract presented concentration-dependent effects on seed germination (Fig. 2B).

The seed germination rate without leaf extract (control treatment) declined from approx. 71 % in high light treatment to approx. 10 % in dark treatment. Germination of the seeds that were simultaneously exposed to leaf extracts (especially for high concentrations) and dark conditions was drastically decreased; more extremely, seeds treated with the highest concentration of dry leaf extracts (0.05 g d. wt mL<sup>-1</sup>) did not germinate under dark conditions (Fig. 2B).

*Effects of leaf extracts and light on seedling growth (expt 3)*

In the high light treatment, the presence of fresh or dry leaf extracts (especially moderate and high concentrations) significantly suppressed *W. trilobata* seedling growth (shoot and root length, above- and below-ground biomass) (Table 3; Fig. 3), but did not cause any seedling death. More notably, under the shade treatment, almost all the seedlings treated with dry leaf extracts died, and the surviving seedlings hardly grew at all and eventually died. There were insufficient data for the shade treatment to conduct statistical analysis; instead, the high mortality is shown in Fig. 4.

*Seed germination in the field (expt 4)*

Seeds did not germinate well in the field germination experiment. Seedlings were only detected in the plots where the plant canopy was removed and seeds were added (the germination ratio was about  $12.22 \pm 3.9$  %). No seedlings were found in any of the plots where seeds were not added, or in the plots where the plant canopy was not removed but seeds were added. Therefore, no statistical analysis was possible for the field germination.

DISCUSSION

In this study, we showed that the resources of the invasive clonal weed *W. trilobata* were also allocated to its sexual reproduction. In the field, *W. trilobata* produced fecund vigorous seeds every season, but few seedlings were found and population recruitment via seedlings was obstructed. The simulated experiments in the laboratory and the field germination experiment showed that seed germination of *W. trilobata* was constrained by shade and its own aqueous leaf extracts. Thus, we considered light

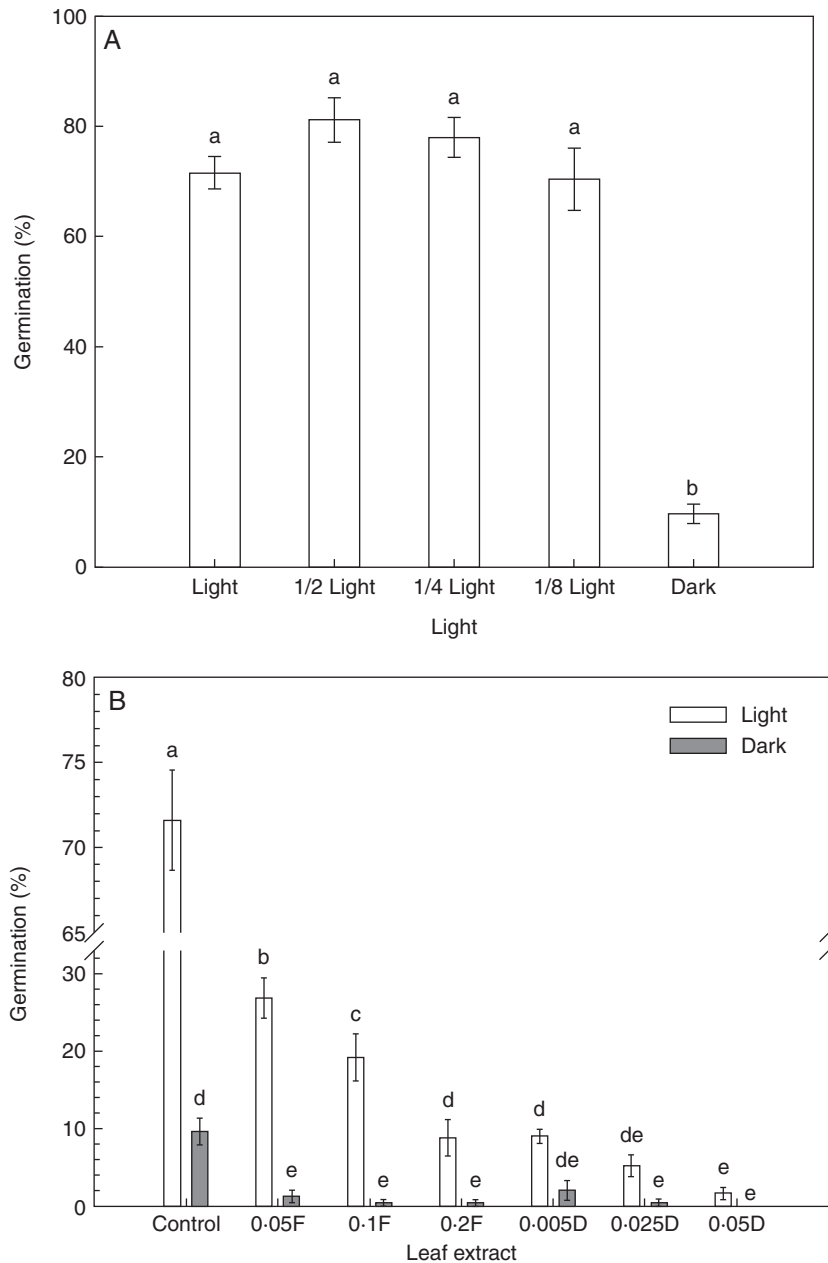


FIG. 2. (A) Effects of different light intensity on seed germination of *W. trilobata*. (B) Germination (%) of *W. trilobata* seeds treated with different concentrations ( $\text{g mL}^{-1}$ ) of fresh (F) or dry (D) leaf extract under high light ( $450 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) or dark conditions (as indicated in the key). Error bars are the s.e. ( $n = 5$ ). Different letters indicate a significant difference at  $P < 0.05$ .

TABLE 2. Two-way ANOVA for the effects of both light and leaf extract on seed germination of *W. trilobata* (expt 2)

Factor	d.f.	Mean square	F	P
Light (L)	1	5870.18	424.45	< 0.001
Leaf extract (E)	6	1881.48	136.04	< 0.001
L $\times$ E	6	1104.15	79.84	< 0.001
Error	56	13.83		

limitation and its own leaf litter to be two of the crucial factors causing scarcely detectable seedling recruitment for this invasive clonal weed.

#### Sexual reproduction of *W. trilobata*

A review of seedling dynamics and life histories found that >40% of clonal plants establish populations by seedling recruitment, especially for plants growing in grasslands (Eriksson, 1989). *Wedelia trilobata* also spreads rapidly through very vigorous clonal ramets (Wu *et al.*, 2005; Song *et al.*, 2010b) and a greater ratio of resources was allocated to its clonal reproduction. In the field survey, *W. trilobata* flowered profusely in almost all seasons. Thus, the number of seeds was considerable because at least one capitulum which possessed 10–20 fertilized seeds could be produced in every stem node. However, few seedlings of *W. trilobata* were found beneath the canopy of mother

TABLE 3. One-way ANOVA for the effects of leaf extracts on seedling growth of *W. trilobata* (expt 3)

Traits	Source	d.f.	Mean square	F	P
Shoot length	Leaf extracts	6	2.44	3.16	0.007
	Error	116	0.77		
Root length	Leaf extracts	6	63.31	24.15	<0.001
	Error	116	2.62		
Above-ground biomass	Leaf extracts	6	0.019	9.91	<0.001
	Error	116	0.0019		
Below-ground biomass	Leaf extracts	6	0.0011	14.48	<0.001
	Error	116	0.0008		

ramets. Also seedlings seldom emerged in the bare ground near the populations. Our findings were similar to those reported by Singh and Singh (2002) in the invasive plant *Centella asiatica*, i.e. the population is mainly maintained by ramets in spite of many emerging seeds and seedlings, which experience high mortality.

The inhibition of seedling recruitment will save more resources (e.g. light, water and nutrients) to promote the spread of clonal reproduction in a short period, because sexual and clonal reproduction may directly compete for resources (Thompson and Eckert, 2004). Thus, a balance between these two reproduction modes could contribute to the rapid population expansion and colonization of *W. trilobata* by inhibiting its seedling recruitment.

#### Potential mechanisms regulating seedling recruitment of *W. trilobata*

Critical effects of light on seed germination have been found in various species (Milberg *et al.*, 2000). Only very few seedlings of *W. trilobata* in the field were found on the bare ground near to the population, and no seedlings were detected beneath *W. trilobata* adult ramets. The light intensity under the *W. trilobata* population in the field was frequently poor because of its dense and reticular branches. Moreover, seeds that fell onto the substrate of the population were often covered by the thick litter layer. Moreover, our controlled experiments showed that seeds of *W. trilobata* germinated better in light than in darkness, and seeds could germinate after removing the canopy in the field. Thus, lack of light might be one of the main factors causing few seeds of *W. trilobata* to germinate in the field. However, the response of seeds to light varies in different plant species (Ahmed and Khan, 2010). Exposure to light reduces the germination of pyrethrum (*Tanacetum cinerariifolium*) seed and inhibits its seedling growth (Li *et al.*, 2011). The germination rate of larger seeds is higher in the dark than in the light, whereas smaller seeds require more light (Bell, 1999; Milberg *et al.*, 2000). Light may act as a depth-sensing cue to small seeds that are buried too deep in the soil or for seeds to germinate on the soil surface with decreasing humidity (Schutz *et al.*, 2002).

The presence of *W. trilobata* litter might be another important factor that explains the low number of seedlings detected in the field. In our chamber experiments, the application of leaf extracts of *W. trilobata* significantly decreased seed germination and prevented seedling growth, and seedlings even died in the presence

of leaf extract and absence of light. Allelopathy of the chemicals produced from leaf litter may explain this phenomenon (Wu *et al.*, 2009; Inderjit *et al.*, 2011). However, numerous mechanisms may also explain the effects of litter on seedling recruitment. For instance, accumulation of a massive amount of litter may reduce the emergence of seedlings by mechanical damage (Hovstad and Ohlson, 2008), or by the osmotic potential of chemicals released from the litter (Wardle *et al.*, 1992). Also, plant litter may affect seedling growth by altering the microbial community (Elgersma *et al.*, 2012), or changing the light conditions of the seedling habitat (Koyama and Tsuyuzaki, 2012). After all, litter layers can affect seed germination and seedling development (Holmgren *et al.*, 1997). In addition, thick litter layers (including fresh leaves and dry leaves) were frequently found under the mother ramets of *W. trilobata* in the field. Therefore, the changes in the microhabitat of dropped seeds caused by thick litter might affect seed recruitment of *W. trilobata*.

The results of our field investigation and laboratory experiments show that both light limitation and a layer of its own litter resulting in an unfavourable microhabitat might be the factors preventing seedling recruitment of the invasive clonal weed *W. trilobata*. These factors are considered to be the potential mechanisms regulating seedling recruitment of *W. trilobata*, and they are thought to be the potential mechanisms that regulate the balance of clonal and sexual reproduction (seedling recruitment) of clonal invasive plants. However, clonal reproduction is also affected by light conditions (Yu and Dong, 2003; Wang *et al.*, 2008a), and the effects of litter feedback on plant growth differ among plant species (Dorrepal *et al.*, 2007). Further, more research on the effects of light and litter on the clonal growth of *W. trilobata* should be conducted in the future.

#### Implications for biological invasion control

For the prevention and control of alien invasive plants which rapidly expand by clonal reproduction with maintenance of sexual reproduction, such as *W. trilobata*, their seeds must be taken into consideration. Although seedling recruitment is limited by the presence of litter and the absence of light, *W. trilobata* still allocates resources for sexual reproduction and produces fertilized seeds. This is significant for population dynamics of invasive plants in new sites by long-distance seed dispersal or in the very bare ground where there is no possibility for their vegetative propagation. Seeds will establish new populations once the limiting factors are eliminated. Seeds and seedlings might be the pioneer in new habitats. Therefore, to prevent seedling recruitment, invasive plants should be eradicated before they yield seeds.

#### SUPPLEMENTARY DATA

Supplementary data are available online at [www.aob.oxfordjournals.org](http://www.aob.oxfordjournals.org) and consist of the following. Figure S1: illustration of a ramet and seedling of *W. trilobata*. Figure S2: images of flowers in the field, flowers on a branch of a greenhouse-grown plant, and the two types of seed in one capitulum. Figure S3: layout of experiments on the effects of light and/or leaf extract on seed germination and seedling growth. Figure S4: images of a seedling emerging in bare ground near to mother ramets.

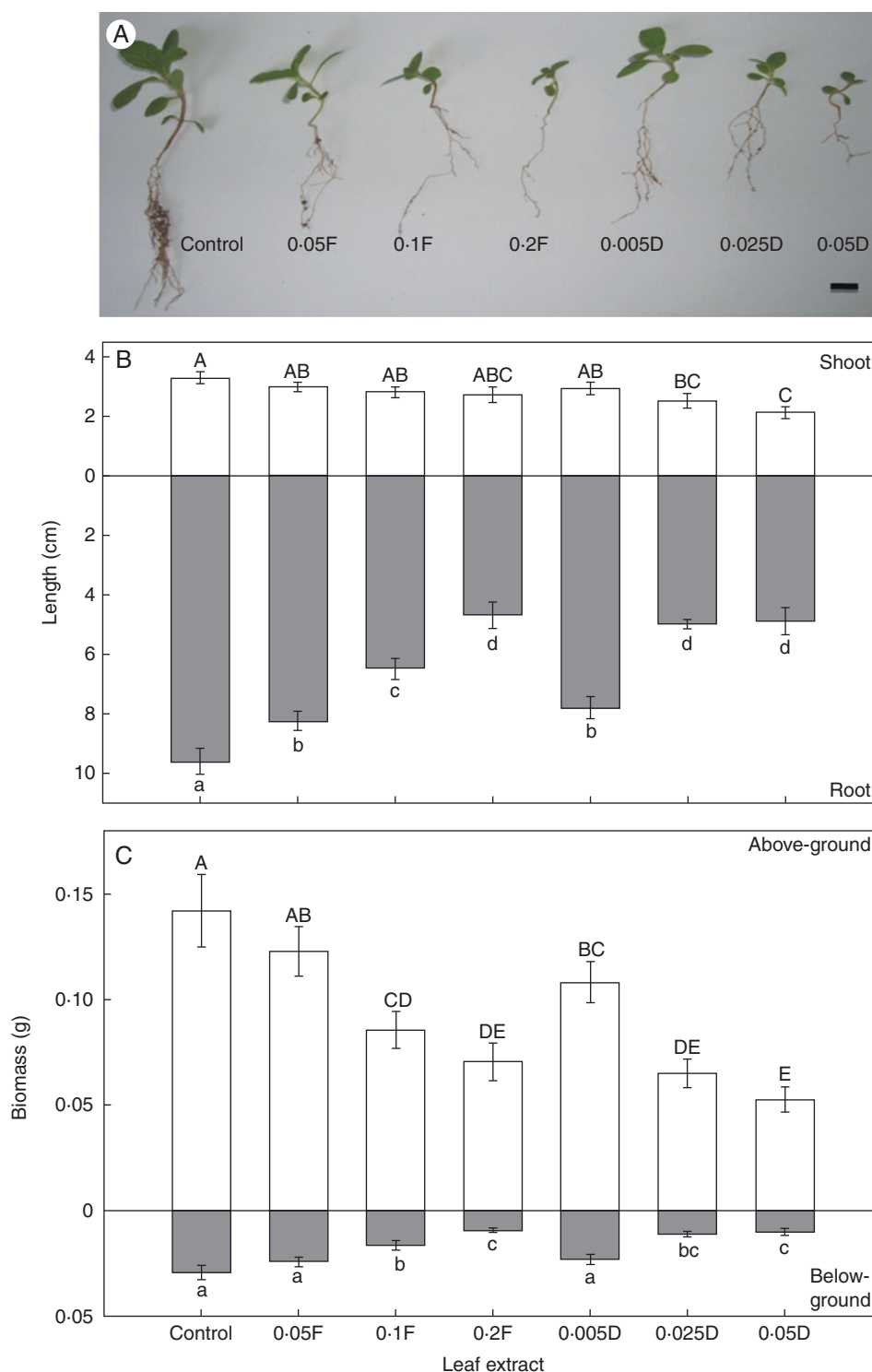


FIG. 3. (A) Seedling growth (scale bar = 1 cm), (B) seedling shoot and root length, and (C) above- and below-ground seedling biomass of *W. trilobata* treated with different concentrations ( $\text{g mL}^{-1}$ ) of fresh (F) or dry (D) leaf extracts under high light ( $450 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) for 6 weeks. Error bars are the s.e. Different letters indicate a significant difference at  $P < 0.05$ .

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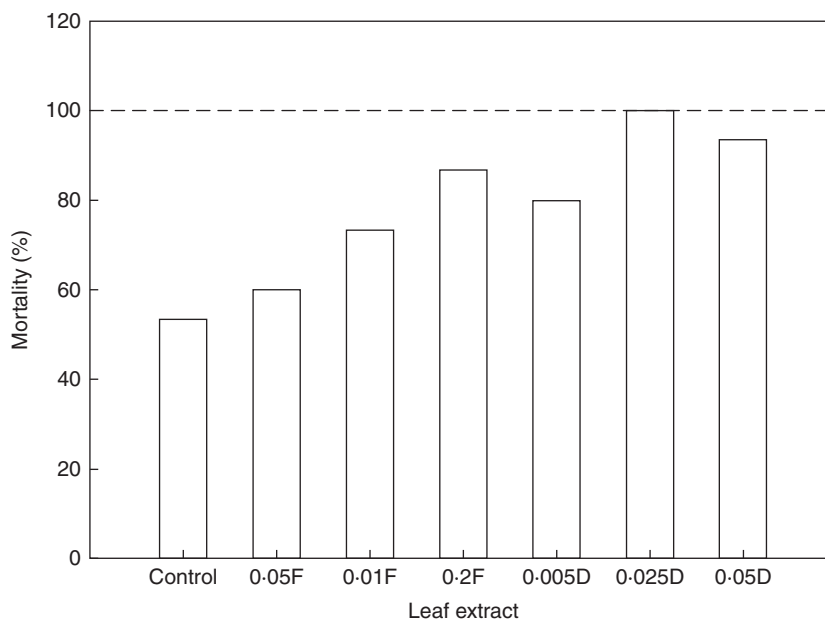


FIG. 4. Mortality of *W. trilobata* seedlings treated with different concentrations ( $\text{g mL}^{-1}$ ) of fresh (F) or dry (D) leaf extracts under shade treatment ( $22.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) for 6 weeks. There were 15 seedlings for each treatment.

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