

SEED SIZE, COTYLEDONAL COMPENSATION, AND RESPONSE TO DEFOLIATION AND SEEDING DEPTH IN HERBACEOUS DICOTYLEDONOUS SPECIES

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Abstract

Of all series of diseases that might define survival rates, seed size was investigated intensively and is regarded as of specific importance at stages of early seedling. The larger-seeded species seedlings generally perform better than smaller-seeded species seedlings to face various hazards. However, the smaller-seeded species are to confer a growth advantage in the favorable habitats. This study studied the correlation between seed size and the compensatory capacity of cotyledon to recover from simulated herbivory, and if the capacity could be affected by seeding depth, and whether smaller seeded-species grow faster than larger ones in response to cotyledons herbivory at seedling stage. Five defoliation treatments (control, one cotyledon damage, both cotyledons damage, one cotyledon and apical meristem damage, apical meristem damage) and two seeding depths (1cm and 3cm) were used in the pot experiment. The compensation of cotyledon area, thick and mass were increased with increasing seed size, but cotyledon longevity was decreased with increasing seed size in each defoliation level, and there is a significant effect of seeding depth on them. All the factors strongly affected the seedlings biomass and significantly altered belowground and aboveground biomass ratio of seedlings. The relative growth rate of smaller-seeded species is significant higher than that of large-seeded species in response to cotyledons damage at early seedling stage. The growth advantage of smaller seeded species seedlings and the difference of cotyledonal compensation ability may account for a range of coexisting strategies.

Key words: Seed size; Cotyledon; Compensation; Defoliation; Seedling depth; Growth strategy.

Introduction

Seed size is one crucial trait that highly influence the seedlings' establishment, dispersal, growth and survival (Harper *et al.*, 1970), particularly at early seedling stages (Leishman *et al.*, 2000; Coomes & Grubb, 2003). Fenner and Thompson (2005) indicated that it was highly possible for large seeds to be established under harsh establishment conditions, whereas a good amount of tiny seeds were highly possible to have dispersal in time and space. Seed size is discovered to be one key plant attribute to understand plant strategies (Harper, 1977; Westoby, 1998; Weiher *et al.*, 1999; Leishman *et al.*, 2000) and is usually connected to a species' capacity for seedling establishment and colonisation. As an indispensable policy for plants to maintain or maximize fitness in variable abiotic and biotic environments, many ecologists have embraced the idea that the large-seeded species seedlings typically cope better than smaller-seeded species seedlings in natural conditions (Dalling & Hubbell, 2002; Moles & Westoby, 2004a) and in the face of various hazards such as competition (Parrish & Bazzaz, 1985; Rees, 1995), deep shade (Grime & Jeffrey, 1965), drought (Leishman & Westoby, 1994b), nutrient limitation (Lee, 1989; Jurado & Westoby, 1992; Leishman & Westoby, 1994a; Leishman & Westoby, 1994b) and seedling emergence depth (Gulmon, 1992; Peterson & Facelli, 1992; Vasquez-Yanes, 1992). Despite the ecological consequences of variation in seed size for seedling performance to cope with environmental hazards have been explored, comparatively little study addressed how seed size influences seedling recovery and regrowth potential, and how these relationships may vary with the hazardous environments.

The storage tissue (endosperm or cotyledons) of the seed could have two mechanisms as carbon and mineral nutrients sources (Ashcroft & Murray, 1979; Fenner, 1983; Zhang & Maun, 1991; Luan *et al.*, 2017). Seedlings are one of most venerable and sensitive stages in the plant life cycle that the initial growth and survival are totally relied on the reserves in seed storage tissue (Fenner & Thompson, 2005; Hanley & May, 2006). For species lack of endosperm, such as most dicotyledons, a relatively greater proportion of energy and nutrients were retained in the cotyledons acting as newly expanded seedling reserves (Kitajima, 1994; Poorter & Rose, 2005). Penny *et al.*, (1976) found that the cotyledons were the main organ to support seedling development for cucumber. Kircher & Schopfer (2012) showed that the cotyledons were converted into photosynthetically active leaves to support seedling development after seedling emerged for *Arabidopsis*. Given the assimilatory role of the cotyledons, any damage to them would have a negative effect on seedling growth (Bonfil, 1998; Kitajima, 2003; Boege & Marquis, 2005; Bisognin *et al.*, 2005). Although a number of factors, including disease, insects and susceptibility to abiotic stresses, often damage the cotyledons, significantly limiting seedling growth or resulting in death of seedlings (Fenner, 1987; Moles & Westoby, 2004b; Fenner & Thompson, 2005), one of the most important factors that affect seedling development is probably defoliation by herbivores (Moles & Westoby, 2004b). The damage that originated from herbivores range from the removal of a minor photosynthetic surfaces part, to complete shoot and total photosynthetic incapacity excision.

In general, larger-seeded species seedlings are more tolerant to herbivores comparing with smaller-seeded species seedlings (Armstrong & Westoby, 1993; Harms & Dalling, 1997). Westoby *et al.*, (1996) and Leishman *et al.*, (2000) have proposed the 'reserve effect' mechanistic hypotheses to better explain large-seeded species seedling performance in the face of herbivores and other hazards. For instance, large seeds are likely to have hypogeal cotyledons that play a role as seed reserves. As a result, they may have more resilience to stem grazing since they have more seed reserves, and also because they can re-sprout from hypogeal cotyledons (Forget, 1992; Harms & Dalling, 1997; Green & Juniper, 2004). In addition, some large seed species have epigeal cotyledons, which have the surplus resources interim render seedlings more resistant to herbivory hazards (Leishman *et al.*, 2000; Moles & Westoby, 2004b). However, smaller-seeded species might be less able to re-sprout because they are more likely to have epigeal cotyledons and have limited reserves, which are likely to react more negatively to intense post dispersal herbivory damage compared to larger-seeded species (Howe *et al.*, 1985; Molofsky & Fisher, 1993). Smaller-seeded species normally grow more speedy than larger-seeded species and therefore could overcome disadvantage of the less reserves compared to larger-seeded species (Pazand Martinez, 2003; Poorter, 2003; Baraloto *et al.*, 2005). However, it is unclear whether it holds true when cotyledons damaged due to defoliation at seedling stage. Two recent studies have shown that cotyledons are able to compensate the growth by area, thickness, mass, longevity and photosynthesis due to defoliation (Zheng *et al.*, 2011; Zheng *et al.*, 2012). Although the compensatory growth of cotyledons can reduce the effect of herbivore damage through the morphological and physiological characteristics, the correlation between cotyledonal compensation and seed size remains largely unresolved.

In order to understand the seed size effect on plant growth via compensation, particular attention should be paid to seedling development stage. Herbivores can have a great impact on growth by consuming seedling or juvenile plants (Huntly, 1991; Bryant & Julkunttiitto, 1995; Fritz *et al.*, 2001). Further, the seedlings often have the highest mortality in this stage (Harper, 1977; M C, 1983). However, seedling growth is a complex process that influenced by environmental factors, and which may not be the same for mature plants (Harper, 1977). For instance, experimental researches have examined effects of seed size on emergence and survival under seeding depth environment (Maun & Lapierre, 1986; Yanful & Maun, 1996; Seiwa *et al.*, 2002; Zheng *et al.*, 2005). Factually, plant communities generally include species whose seed sizes span 5~7 magnitude orders (Leishman *et al.*, 2000). Rees & Westoby (1997) proposed the multiple seed mass coexistence strategies in population and evolutionary dynamics. The small and large-seeded species coexist in the same plant community and face the same environmental hazards, such as seeding depth and foliar damage. Zheng *et al.*, (2012) did report that the compensatory growth of cotyledon and seedling can be strongly influenced by the interaction between tissue removal and seeding depth. However, the ecological

consequences of variation in seed size for compensatory growth have seldom been explored in hazardous environment, which involved the coexistence of seeding depth and seedling herbivory.

The cotyledon function is believed to have an effect on the seed size evolution (Hladik & Miquel, 1990; Garwood, 1996; Kitajima, 1996). In general, small-seeded species are usually correlated to epigeal, photosynthetic-type cotyledons, while large-seeded species often have hypogeal, storage-type cotyledons. To avoid confounders regarding correlation between seed mass and the capability of tolerating herbivores with coincident differences in seedling morphology, we limited our research to species with epigeal photosynthetic cotyledons. The aims of current investigation are to explore (1) the correlation between seed size and compensatory capacity of cotyledon recovering from simulated defoliation, (2) whether the compensatory capacity might be affected by seeding depth; and (3) whether smaller-seeded species grow more speedy than larger-seeded species with respect to cotyledon removal at early stage seedling.

Materials and Methods

Site and materials: We conducted a pot experiment in July 2010 in the Grassland Ecosystem Experimental Station of Northeast Normal University, Changling County, Jilin Province, China (E 123°31', N 44°30'; Elevation 145m), which is located at the Songnen Plain of northeast China. This place has a classical mesothermal monsoon climate with plain topography. The average rainfall per year is around 400~500 mm, of which more than 60% falls from June to September. The annual evaporation capacity is 2-3 times more than the rainfall.

Three annual, epigeal, dicotyledonous species with distinguishable seed sizes were selected for study, namely *Pharbitis purpurea* (average seed sizes 28.55 ± 0.44 mg), *Abutilon theophrasti* (9.39 ± 0.03 mg) and *Amaranthus retroflexus* (0.36 ± 0.04 mg), which are common weed those often grow in frequently disturbed environments such as roadside, cropping land and fallow field, etc. We collected seeds in autumn 2009 from the wild on the Songnen plain and stored them in cloth bags under room temperature (20°C - 25°C).

Experimental design: We filled the pots (20 cm diameter and 25 cm height) with a soil-sand mixture (1:1 by volume) and buried at ground level in the open air. Twenty seeds were sown in the pot, which were covered with either 1 or 3 cm of the soil-sand mixture. We thinned seedlings to 10 plants per pot at emergence. We irrigated pots when necessary.

There were 5 defoliation treatments as shown in the Fig. 1, namely (1) RC1: we removed one cotyledon at seedling emergence, (2) RC2: we removed both cotyledons at seedling emergence, (3) RC1M: we removed the apical meristem and one cotyledon at seedling emergence, (4) RM: we removed exclusively the apical meristem at seedling emergence and (5) CK: we did not remove any tissue (intact plants) as a control.

A randomized block design was used in the pot experiment with defoliation, seed size and seedling depth treatments with 5 replicates, thus 150 pots in total. We randomly divided pots into five groups of 30 pots. We placed the pots in five rows with 10 pots per group arranged randomly in each row. There was a 10cm distance between neighboring pots.

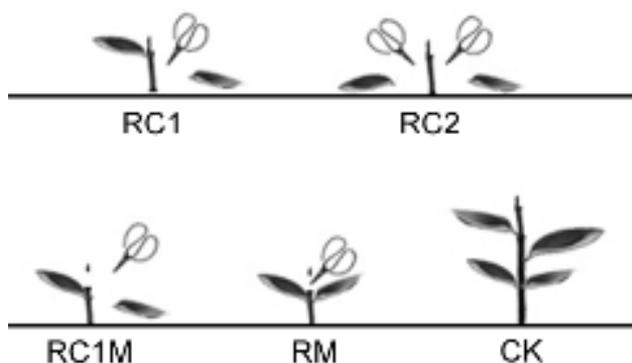


Fig. 1. Diagram to illustrate tissue defoliation treatments. RC1, we removed one cotyledon at seedling emergence; RC2, we removed both cotyledons at seedling emergence; RC1M, we removed one cotyledon and the apical meristem at seedling emergence; RM, we only removed the apical meristem at seedling emergence; and CK, no tissue was removed as control. The open symbol represents a pair of scissors.

Measurements: We randomly selected 3 out of 5 pots in each treatment for each species and harvested them for growth traits measurement after 50% of the cotyledons on control seedlings of every species that died. We used the left 2 pots to test cotyledon longevity, the cotyledon growth days from emergence to senescence.

We measured seedlings' height and root length (including length of belowground hypocotyl) with a ruler. We measured the area and thickness of remaining cotyledon with Sigmascan software program (Systat Software, Richmond, CA), and with thickness tester (Peacock, Tokyo, Japan), respectively. We weighed the cotyledon biomass, aboveground biomass (excluding cotyledon biomass), and belowground biomass after drying them at 65°C for 48 h.

We calculated belowground over aboveground biomass (including cotyledon biomass) ratio (*B/A* ratio) and relative seedling growth rate, and compensatory growth ratio (*C*) of remaining cotyledon through the following formulas:

$$B/A \text{ ratio} = \text{Belowground} / \text{Aboveground biomass} \times 100\% \quad \text{Eqn 1}$$

$$\text{RGR} = (\ln M_2 - \ln M_1) / t \quad \text{Eqn 2}$$

$$C_{(t_n)} = (S_n - S_0) / S_0 \text{ or } (T_n - T_0) / T_0 \text{ or } (M_n - M_0) / M_0 \text{ or } (L_n - L_0) / L_0 \times 100\% \quad \text{Eqn 3}$$

where in Eqn 2, M_1 represent individual seed weight, M_2 represent final plant biomass, and t represents time; and in Eqn 3, t_n represents the various treatments and S_n is final treatment t_n cotyledon area and S_0 is final control cotyledon area; M_n is final cotyledon mass of t_n and M_0 is final control cotyledon mass; T_n is final cotyledon thickness of t_n and T_0 is final control cotyledon thickness;

L_n is final cotyledon longevity of t_n and L_0 is final control cotyledon longevity.

Statistical analysis: Variance analysis was performed by a randomized block design (SPSS, Chicago, IL). We performed a three-way factorial analysis of variance (ANOVA) to detect the seed size effects, defoliation effects and seeding depth effects on relative parameters of seedling growth. Statistical differences between each trait means within each treatment were defined by the Least Significant Difference (LSD) test, where $p < 0.05$ indicated statistical significance.

Results and Discussion

Compensatory growth of cotyledons: There were significant differences in compensatory growth of the remaining cotyledon between treatments ($p < 0.05$; Table 1 and Fig. 2). Three-way factorial ANOVA showed that the compensatory growth traits of cotyledon varied significantly among different species treatments (Table 1). The compensation of cotyledonal area of *A. retroflexus* was the largest in each defoliation treatment at 1cm seedling depth, and which of *P. purpurea* was smallest. But at 3cm seeding depth, the compensation of *P. purpurea* species was the largest, and the *A. retroflexus* was the smallest. Comparing two seeding depths, the maximum compensation of cotyledonal area of *A. retroflexus* occurred at 1cm seeding depth treatment in each defoliation treatment, while which of *P. purpurea* and *A. theophrasti* occurred at 3cm seeding depth (Figs. 2a, b). The compensation of cotyledonal thickness was increased with increasing seed size in each defoliation treatment at each seeding depth treatment, and *P. purpurea* species had the greatest values for this trait. The compensation of thickness of *A. retroflexus* did not differ significantly in each defoliation treatment between two seeding depths, while *P. purpurea* and *A. theophrasti* at 3cm seeding depth had significantly greater compensation of thickness comparing with those at 1cm (Figs. 2c, d). The cotyledonal mass compensation had shown the similar consequence with thickness for all species in each defoliation treatment at each seeding depth (Figs. 2e, f). The compensation of cotyledonal longevity was decreased with increasing seed size in each defoliation treatment at each seeding depth treatment, and there was not significant difference between two seeding depths (Figs. 2g, h). All of the compensatory growth traits of cotyledon in RC1M treatment for three species were higher than which in other defoliation treatments at both seeding depth treatments (Fig. 2).

Seedlings growth: The seedling height and belowground length were significantly affected by all three factors and their interactions (Tables 1, 2). They were increased with increasing seed size in each defoliation treatment at both seeding depths. For all species, the seedling height and belowground length were reduced significantly as cotyledon defoliation at both seeding depths. The seedling heights of all species from 1cm depth were higher than those from 3cm in every defoliation treatment. But the results of belowground length were opposite response to seeding depth compare with seedling height (Table 2).

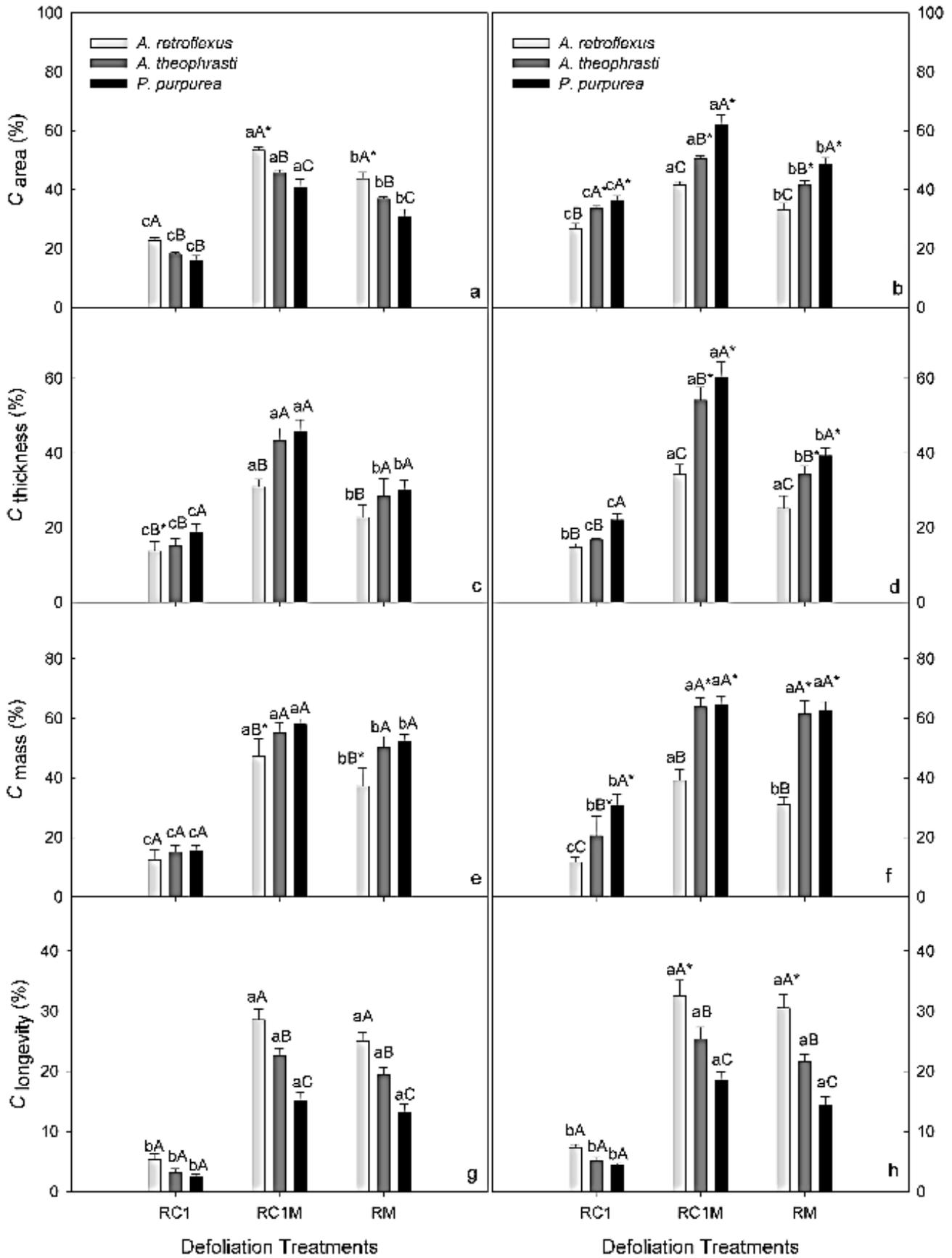


Fig. 2. The compensatory growth of cotyledonal traits of *A. retroflexus*, *A. theophrasti* and *P. purpurea* seedlings at seeding depth of 1cm (a, c, e, g) and 3cm (b, d, f, h) under different tissue defoliation treatments. Upper case letter denote significant differences between species at each defoliation treatment at $p < 0.05$; lower case letters denote significant differences among the different defoliations at each seeding depth at $p < 0.05$, and asterisks indicate a significant difference between seeding depths at the same tissue defoliation treatment.

Table 1. Three-way ANOVA for effects of seed size, defoliation, seeding depth and their interactions \on parameters of seedling growth.

| | df | C _{area} | C _{thickness} | C _{mass} | C _{longevity} | SH | BL | SB | B/A | RGR |
|-----------------------------------------|----|--------------------|------------------------|--------------------|------------------------|-----|-----|--------------------|--------------------|-----|
| Defoliation | 4 | *** | *** | *** | *** | *** | *** | *** | *** | *** |
| Seed size | 2 | *** | *** | *** | *** | *** | *** | *** | *** | *** |
| Seeding depth | 1 | *** | *** | 0.51 ^{NS} | * | *** | *** | *** | *** | ** |
| Seed size × Defoliation | 8 | *** | *** | *** | ** | *** | *** | *** | *** | *** |
| Seed size × Seeding depth | 2 | *** | *** | * | 0.71 ^{NS} | * | *** | 0.93 ^{NS} | 0.40 ^{NS} | *** |
| Defoliation × Seeding depth | 4 | 0.35 ^{NS} | ** | 0.86 ^{NS} | 0.85 ^{NS} | *** | ** | 0.86 ^{NS} | 0.45 ^{NS} | *** |
| Seed size × Defoliation × Seeding depth | 8 | 0.06 ^{NS} | 0.90 ^{NS} | 0.91 ^{NS} | 0.94 ^{NS} | *** | *** | 0.89 ^{NS} | 0.16 ^{NS} | ** |

NS, not significant ($p>0.05$); *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$

C_{area}: compensation of area; C_{mass}: compensation of mass; C_{thickness}: compensation of thickness; C_{longevity}: compensation of longevity; SH: seedling height; BL: belowground length; SB: seedling biomass; B/A: ratio of belowground over aboveground biomass; RGR: relative growth rate of seedling

Table 2. Seedling height and belowground length (means ± SE, n=15) for three species under different defoliation treatments at both seeding depths.

| Species | Seeding depth | CK | RC1 | RC2 | RC1M | RM |
|---------------------------|---------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Seedling height | | | | | | |
| <i>A. retroflexus</i> | 1cm | 8.34 ± 0.16aC* | 4.51 ± 0.11bC* | 2.9 ± 0.08cC* | - | - |
| | 3cm | 6.38 ± 0.31aC | 3.83 ± 0.25bC | 2.52 ± 0.26cC | - | - |
| <i>A. theophrasti</i> | 1cm | 10.83 ± 0.27aB* | 7.70 ± 0.19bB* | 4.25 ± 0.10cB* | - | - |
| | 3cm | 9.51 ± 0.46aB | 6.36 ± 0.20bB | 3.13 ± 0.23cB | - | - |
| <i>P. purpurea</i> | 1cm | 16.5 ± 0.30aA* | 12.25 ± 0.32bA* | 6.18 ± 0.12cA* | - | - |
| | 3cm | 13.26 ± 0.25aA | 11.17 ± 0.41bA | 5.27 ± 0.25cA | - | - |
| Belowground length | | | | | | |
| <i>A. retroflexus</i> | 1cm | 13.20 ± 1.02aC | 9.53 ± 0.97bC | 4.91 ± 0.49dC | 6.37 ± 0.80cC | 6.52 ± 0.71cC |
| | 3cm | 14.05 ± 0.69aC* | 9.45 ± 0.42bC | 4.76 ± 0.61dC | 6.75 ± 0.78cC | 6.53 ± 0.92cC |
| <i>A. theophrasti</i> | 1cm | 14.42 ± 1.17aB | 10.08 ± 0.43bB | 6.65 ± 0.85eB | 8.34 ± 0.28dB | 9.63 ± 0.30cB |
| | 3cm | 15.05 ± 0.80aB | 12.00 ± 0.66bB* | 9.85 ± 0.82cB* | 10.53 ± 0.77cB* | 10.77 ± 0.61cB* |
| <i>P. purpurea</i> | 1cm | 23.15 ± 1.22aA | 19.50 ± 1.35bA | 7.95 ± 1.10eA | 14.24 ± 1.05dA | 15.58 ± 1.01bA |
| | 3cm | 28.62 ± 1.35aA* | 22.14 ± 1.02bA* | 11.35 ± 1.21dA* | 16.45 ± 0.76cA* | 20.46 ± 1.32bA* |

Lower case letters denote significant differences between the different defoliations at each seeding depth at $p<0.05$; upper case letter denote significant differences between species at each defoliation treatment at $p<0.05$; asterisks denote a significant difference between seeding depths at the same defoliation treatment at $p<0.05$

Note: “-” stands for no measurements

Seed size, defoliation and seeding depth, as well as second-order interactions, had a clear effect on seedling biomass and B/A ratio (Fig. 3, Table 1). For all species, the seedling biomass was increased with increasing seed size in each defoliation treatment at every seeding depth. In response to defoliation treatment, the primary consequence was a reduction in seedling biomass with cotyledon defoliation in each seeding depth for all species. Compare two seeding depths treatments, all species seedlings from 1cm seeding depth had the largest biomass (Figs. 3a, b). *P. purpurea* had the highest B/A ratio in each defoliation treatment at both seeding depths. In CK and RC1 defoliation treatments, the B/A ratio of *A. retroflexus* was higher than that of *A. theophrasti*, but it showed opposite result in other defoliation treatments at both seeding depths. For every species at both seeding depths, defoliation treatments significantly changed B/A ratio. Meanwhile, all species seedlings from 3cm had a significantly higher B/A ratio comparing with those from 1cm in the same defoliation treatment (Figs. 3c, d).

Three-way factorial ANOVA showed that the RGR (relative growth rate) was significantly affected by seed size, defoliation and seeding depth, as well as their interactions (Table 1). Analysis of average RGR revealed the expected strong negative correlation between defoliation and RGR ($F=787$, $p<0.001$). Also, there was a significant correlation between seed size and defoliation ($F=152.66$, $p<0.001$), and among seed size, defoliation and seeding depth ($F=3.14$, $P=0.02$). The RGR of *A. retroflexus* was the highest in three species, and the RGR was decreased with increasing seed size in each defoliation treatment at both seeding depths. For all species, defoliation treatments significantly altered RGR of seedlings at both seeding depths. Compare two seeding depths treatments, seedlings from 1cm depth for *A. retroflexus* had a higher RGR than that from 3cm depth in each defoliation treatment. However, the higher RGR of *P. purpurea* and *A. theophrasti* seedlings were exhibited at 3cm depth in every defoliation treatment (Fig. 4).

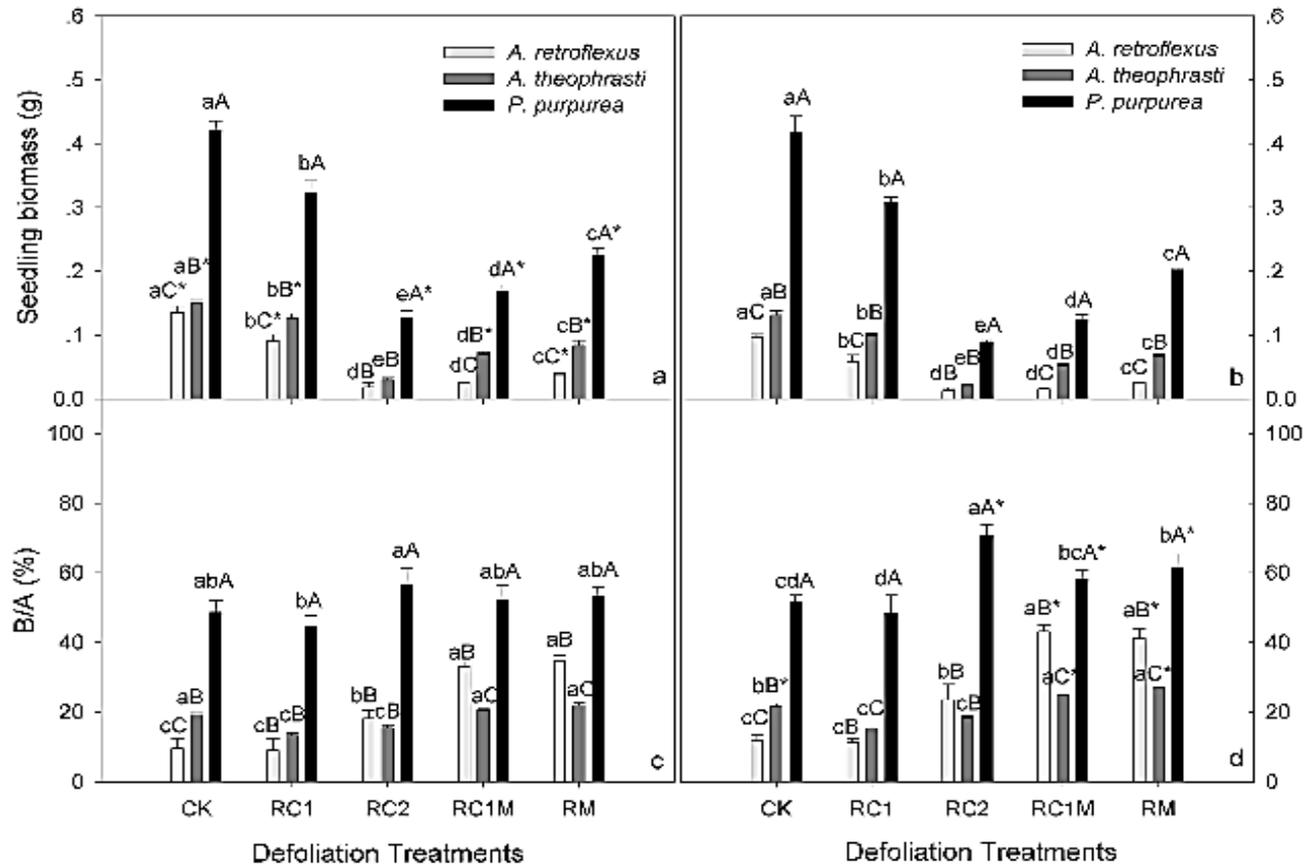


Fig. 3. Seedling biomass and *B/A* ratio of *A. retroflexus*, *A. theophrasti* and *P. purpurea* as related to defoliation and seeding depth treatments (1cm: a, c and 3cm: b, d). Upper case letter represent significant differences among species at each defoliation treatment, lower case letters represent significant differences among the different defoliations at each seeding depth, and asterisks represent a significant difference between seeding depths at the same defoliation level.

Discussion

The seed size importance has been detected from a lot of perspectives (Fenner, 1983; Armstrong & Westoby, 1993; Parker *et al.*, 2004; Munzbergova & Plackova, 2010). Few previous studies suggested that the seed size effects were predominant at early stage of species establishment under biotic and abiotic stresses conditions (Hanley & May, 2006; Moles & Westoby, 2006; Baraloto & Forget, 2007), which is the most vulnerable phase in a plant's life (Fenner & Thompson, 2005). But its relationship to ability of cotyledon compensation under defoliation or mechanical damage and seeding depth conditions does not attract much attention (Zheng *et al.*, 2011; Zheng *et al.*, 2012). The present research had illustrated, by the cotyledons experimental defoliation, seeding depth and their content analyses, that seeds size could be a major nutrient source for satisfying cotyledon compensation requirements. This function was especially pronounced under cotyledon damage conditions which compensatory growth was very sensitive to seed size, and where there was a greater increment in compensation without cotyledons (Fig. 2). Another cotyledon function is to provide photosynthetic assimilates to promote seedling growth (Ampofo *et al.*, 1976a; 1976b). The present study showed that the relative cotyledon defoliation effect on performance was a relation of cotyledon area, thickness, mass and longevity (Fig. 2), which can maximize

photosynthetic validity to minimize the effect of herbivore damaged. Tiffin (2000) did report that compensation might be an indispensable function of herbivory tolerance, allowing plants that damaged to maintain fitness levels equaling those of undamaged plants. For all species in present research, the cotyledonal traits of small seed-size species also exhibited a strong compensatory response to defoliation compare with those of large seed-size species under optimal conditions (1cm depth) (Figs. 2a, c, e, g). In contrast to the trade-off under suitable conditions, the compensation of cotyledon is considered to be the trade-off result between large seeds with a high compensation of cotyledon area, thick and mass, and small seed with a high compensation of cotyledon longevity under harsh establishment environment (3cm depth) (Figs. 2b, d, f, h). Thus the disadvantage of cotyledonal morphological index may be balanced by its longevity.

The early stage of seedling is the most vulnerable phase in a plant's life (Fenner & Thompson, 2005). There is a general consensus that partial defoliation of the cotyledon can marked reduce the seedlings size that produced (Zhang & Maun, 1991; Kitajima, 2003) and that part or entire cotyledon loss ultimately reduce the seedlings biomass (Evans, 1991; Boege & Marquis, 2005) and even cause seedlings death (Armstrong & Westoby, 1993; Moles & Westoby, 2004a). The 'reserve effect' was discovered initially in Westoby *et al.*, (1996), which is

manifested clearly in this study, that the larger seeded species seedlings perform better since they have more mobilizable reserves available for interim support while cotyledon lost by defoliation at both seeding depths (Fig. 3a, b, Table 2). However, for the smaller-seeded species, more reserves allocation to belowground development for the absorption of soil moisture and mineral nutrients to promote seedling development as the photosynthetic cotyledon loss (Fig. 3c, d, Table 2).

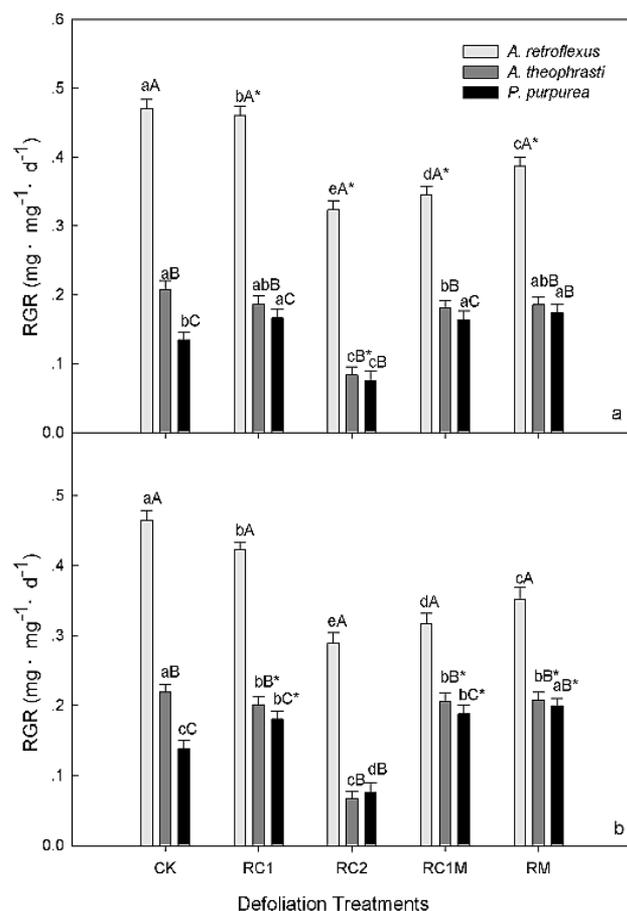


Fig. 4. The relative growth rate of *A. retroflexus*, *A. theophrasti* and *P. purpurea* seedling as related to defoliation and seeding depth treatments (1cm: a, 3cm: b). Upper case letter denote significant differences among species at each defoliation treatment, lower case letters denote significant differences among the different defoliations at each seeding depth, and asterisks denote a significant difference between seeding depths at the same defoliation level.

The large-seeded species contain a relatively high amount of uncommitted maternal reserves, to produce large and more robust seedlings that generally perform better than small-seeded species seedlings in the face of hazardous environments during early life cycle stage (Green & Juniper, 2004). However, smaller-seeded species have moderate reserves, which tend to react more negatively to intense hazardous conditions (Howe *et al.*, 1985; Molofsky & Fisher, 1993). While the higher RGR of these species might compensate somewhat for the lower support to early development provided by smaller seed reserves (Reich *et al.*, 1998; Wright & Westoby, 1999; Baraloto *et al.*, 2005). This idea also has been

bolstered that RGR response to deep shade and damage in neotropical rain forest trees by Baraloto & Forget (2007), who reported although smaller-seeded species had lower survival and faster growth, these patterns were explained better by cotyledon type rather by seed mass. Thus, it is clear from this study that the RGR of smaller-seeded species is significantly higher than that of large-seeded species in response to cotyledon loss at early seedling stage (Fig. 4, Table 1). This high growth relative rate advantage might enable seedling to compensate more effectively in an existing hazardous environment.

Seed size differs from many magnitude orders, both between and within floras (Harper, 1977; Jurado & Westoby, 1992; Moles & Westoby, 2003). Aarssen & Jordan (2001) and Henery & Westoby (2001) proposed that a negative relationship has been found that the 10-fold increase in seed biomass is correlated to a 10-fold reduce in the seed number that a plant can produce per unit canopy per year. This provides small-seeded species an initial advantage over large-seeded species. Moreover, small-seeded species have a fecundity advantage (Westoby *et al.*, 2002; Fenner & Thompson, 2005). In the study reported here, it is possible that small-seeded species may always have a compensatory advantage response to cotyledon damage in coexisted environment conditions. Meanwhile, we evaluate the advantage of smaller seeded species seedlings and the difference of cotyledonal compensation ability between larger- and smaller-seeded species response to cotyledon damage under different seeding depths, which may account for a range of coexisting strategies.

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