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Herbivory-induced maternal effects on growth and defense traits in the clonal species *Alternanthera philoxeroides*



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HIGHLIGHTS

G R A P H I C A L A B S T R A C T

- Alternanthera philoxeroides is a widespread clonal invasive species in China.
- Effects from maternal and offspring herbivory on defense traits of *A. philoxeroides* were tested.
- Herbivory reduced growth of *A. philoxeroides* and modified allocation of plant metabolites.
- Herbivory effect could persist across vegetative generations.



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ABSTRACT

Plants have evolved a variety of defense traits against foliar herbivory, including the production of primary and secondary metabolites, the allocation of chemical compounds, and morphological plasticity. Using two vegetative generations of the invasive clonal species *Alternanthera philoxeroides*, we investigated the effects of maternal and offspring herbivory by *Planococcus minor* on the integrative defense strategy of plants. Herbivory severely inhibited leaf, stolon and root growth, as well as the production of primary metabolites (soluble sugars, starch, and total non-structural carbohydrates in stolons), and decreased average leaf area and specific leaf area of the second-generation *A. philoxeroides*. The changes in growth measures of the first-generation *A. philoxeroides* with herbivory were consistent with that of the second generation. By contrast, herbivory basically did not affect the concentration of non-structural carbohydrate compounds in the roots, and even increased the concentrations of N and total phenols in taproots. Furthermore, herbivory-induced maternal effects also reduced the growth of the second-generation plants. The results suggest that *A. philoxeroides* is capable of adapting to herbivory by *P. minor*, mainly via the allocation of available resources in belowground organs, and that the herbivory effect can persist across vegetative generations. These features may potentially improve the regeneration and tolerance of *A. philoxeroides* after a short-term herbivory.

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1. Introduction

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To defend against foliar herbivory, plants have evolved a variety of traits (Schaller, 2008; Schoonhoven et al., 2005), including the production of primary and secondary metabolites (Agrawal and Weber, 2015;

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Carmona et al., 2011; Johnson et al., 2009; Vergés et al., 2008), the adaptive allocation of chemical compounds among organs (Huang et al., 2011; Kaplan et al., 2008), and morphological plasticity (Ginocchio and Montenegro, 1994; Poorter et al., 2004). Of these defenses, the production of secondary metabolites (e.g., phenolic compounds and condensed tannins) is probably the most common phenomenon (Agrawal and Weber, 2015; Schoonhoven et al., 2005), which can inhibit herbivore feeding by either deterring herbivores or reducing the nutritive value of plant tissues (Forkner et al., 2004; Huang et al., 2011; Tanentzap et al., 2011). For example, neither red deer (*Cervus elaphus*) nor the ground beetle (*Badister collaris*) preferentially consumes plants with high tannin concentrations (Huang et al., 2011; Tanentzap et al., 2011).

Since the production of primary and secondary metabolites often involve same biosynthetic pathways, plants need to optimize the allocation of available resources when subject to foliar herbivory. In the framework of the growth-differentiation balance hypothesis, Herms and Mattson (1992) noted that investment in secondary metabolism could depend upon carbohydrate availability in plants. Therefore, when photosynthesis is restricted but nutrient uptake is not (e.g., the occurrence of foliar damage by herbivores), plants may allocate fewer resources to growth and more to secondary metabolism (Aspinwall et al., 2011; Hale et al., 2005; Herms and Mattson, 1992). The other alternative feedback is that plants may also translocate non-structural carbohydrates, such as water-soluble sugars and starch, or nitrogen from aboveground parts to belowground parts (i.e., roots) to alleviate herbivory-induced resource deficiency (Agrawal and Spiller, 2004; Loranger and Weisser, 2012; Poorter et al., 2004).

In some scenarios, morphological plasticity can function as a lowcost defense mechanism against herbivores (Carmona et al., 2011; Ginocchio and Montenegro, 1994; Poorter et al., 2004; Valladares et al., 2007). For example, when the shoot apices of plants have been removed by herbivores, damaged plants have been reported to produce long axillary shoots with rapidly expanding internodes to gain access to limited resources (e.g., light), and thinner leaves (i.e., high leaf area per unit mass) to improve photosynthesis and compensate for tissue loss (Dong et al., 2012; Ginocchio and Montenegro, 1994; Julien and Bourne, 1986). Apparently, simultaneously incorporating the production and allocation of primary and secondary metabolites, and morphological plasticity into plant defense models will broaden our understanding of plant-herbivore interactions and help distinguish the relative importance among plant defense mechanisms adopted by the target plant species.

Herbivory-induced defense traits have been reported to persist across generations and influence the performance of offspring (Holeski et al., 2012; Rasmann et al., 2011), by modifying plant morphology (Agrawal, 2001), or by altering the quantity and quality of primary and secondary metabolites in seeds or vegetative propagules (Ballhorn et al., 2016; Dam and Baldwin, 2001; Latzel et al., 2014; Rasmann et al., 2011). Such maternal effects are often considered adaptive in plants with sexual reproduction, especially when the herbivory experienced by material plants is similar to offspring environments (Herman and Sultan, 2011; Holeski et al., 2012). For example, plants of Impatiens capensis experiencing herbivory could produce large and vigorous progeny that, at maturity, exhibit greater size and reproductive ability than the offspring of undamaged plants (Steets and Ashman, 2010). Meanwhile, in Raphanus raphanistrum, plants subject to herbivory produce offspring with greater leaf trichome density to improve defense against herbivory (Agrawal, 2001), and in Phaseolus lunatus, plants subject to herbivory exhibited produce offspring with greater β-glucosidase activity and cyanide content than the offspring of undamaged plants, as well as lower mortality (Ballhorn et al., 2016).

The increasing body of evidence further exhibited that herbivory-induced maternal effects may also be important in clonal species that reproduce asexually and have limited opportunities to adapt to environmental changes genetically (Douhovnikoff and Dodd, 2015; Gao et al., 2010; Geng et al., 2013; Latzel and Klimesova, 2010; Schwaegerle et al., 2000; Verhoeven and Preite, 2014). For example, there was an observed greater ability for compensatory growth in plants of Trifolium repens propagated from clones subject to repeated application of jasmonic acid (González et al., 2016), or in plants of Alternanthera philoxeroides propagated from populations with a greater history of herbivory (Lu and Ding, 2012). In the present study, we conducted a greenhouse experiment to explicitly test maternal effects of herbivory across vegetative generations by a generalist insect Planococcus minor on growth and defense traits of the invasive clonal species A. philoxeroides. We tested three hypotheses: (1) clonal plants respond to foliar herbivory via a combination of defense strategies, (2) maternal effects of herbivory persist across vegetative generations, and (3) the magnitude of maternal herbivory effects are context-dependent. We predicted that (1) A. philoxeroides would adapt to P. minor herbivory by morphological plasticity, production of primary and secondary metabolite chemicals, and/or changes in resource allocation, (2) maternal herbivory would affect the performance of vegetative offspring in the form of propagule size and the internal resource availability, and (3) effects of maternal herbivory would depend on the level of herbivory experienced by vegetative offspring.

2. Material and methods

2.1. The species

Alternanthera philoxeroides (Mart.) Griseb (Amaranthaceae) is a stoloniferous, perennial herb that is native to South America. The species is listed as one of the worst invasive weeds in Asia, North America, and Australia (Holm et al., 1977; Julien et al., 1995). Populations in China possess extremely low genetic diversity (Xu et al., 2003; Ye et al., 2003) and mainly reproduce vegetatively via stolon and root fragmentation (Dong et al., 2010, 2012). This species colonizes different habitats from terrestrial to aquatic, and causes severe economic and ecological problems (Sainty et al., 1998; Wang et al., 2008, 2009).

Planococcus minor (Maskell) (Pseudococcidae: Hemiptera) is a polyphagous insect with a host range of over 250 wild and cultivated plants in nearly 80 families. The species is native to Asia and widely distributed in subtropical and tropical regions (Cox, 1989; Francis et al., 2012; Venette and Davis, 2004). The adult females are soft-bodied, wingless, covered with waxy filaments, and relatively sedentary; the males are tiny, winged, and ephemeral (Roda et al., 2013). Females are generally observed at the base of leaves and buds of host plants and feed from the phloem by piercing their mouthparts into plant tissues. Males also feed on plants, but only during the first and second stages of their development (Roda et al., 2003). Infestations by *P. minor* can cause reduced plant growth and yield, defoliation, and plant death (Cox, 1989; Venette and Davis, 2004).

The plants of *A. philoxeroides* were collected from several isolated populations in a riparian area in Zhejiang province, China (28.87° N, 121.01° E), mixed and vegetatively cultivated in a greenhouse at Forest Science Co., Ltd., Beijing Forestry University. The collected plants and their offspring ramets were very likely to belong to the same genotype because *A. philoxeroides* does not produce viable seeds and population genetic variation is very low in China based on molecular studies (Xu et al., 2003; Ye et al., 2003). Individuals of *P. minor* were collected from the same greenhouse, and only female adults were used for the experiment.

2.2. The experiment

The overall experiment involved two vegetative generations of *A. philoxeroides*. On 26 July 2015, 42 plants, each having a stolon with five nodes (ramets) and some adventitious roots, were selected from the stock plants and used for the first generation treatments. They were planted in 14 cm-diameter, 12 cm-deep pots that contained a

1:1 mixture of guartz sand (0.5–1 mm particle size) and peat (Pindstrup Seedling; Pindstrup Mosebrug A/S, Pindstrup, Denmark) and 2 g l^{-1} slow-release fertilizer (16:9:12:2 N:P:K:Mg, Osmocote Exact Standard 3-4 M; Scotts, Marysville, Ohio, USA). After 3 weeks of recovery (on 16 August 2015), 21 plants were randomly assigned to the herbivory treatment, in which seven adults of P. minor were released on the expanded young leaves of each plant (i.e., leaves of the third ramet from the stolon apex), whereas the other 21 plants were untreated (no herbivory). To avoid the escape of P. minor, each plant in each treatment (for both herbivory and no herbivory treatments) was put into a 50-cm-high cage covered with a transparent net (mesh size: $0.25 \text{ mm} \times 0.25 \text{ mm}$). The pots were place in a greenhouse, and periodically repositioned to minimize potential effects of micro-environments. Tap water was supplied on a daily basis to keep the soil moist. During the experiment, the air temperature was 19.79 \pm 0.51 °C, and relative humidity was 77.22 \pm 1.05%. On 5–6 December 2015, we harvested seven replicate plants of this first generation experiment. We counted number of ramets, number of leaves, and measured stolon length and leaf area (WinFOLIA Pro 2004a; Regent Instrument, Inc., Canada). The leaves, stolons, taproots and fine roots of each plants were separated, dried at 70 °C for 48 h, and weighed.

The other 14 replicate plants of each treatment were used as the source for the second generation experiment. A 6-cm taproot fragment was cut from each of these remaining (mother) plants and weighed to obtain fresh mass. All the root fragments were individually planted in pots with the substrate described above, and regenerated and grew for 4 months (5 December 2015 to 10 April 2016) in the same greenhouse. During this time, air temperature was 13.42 ± 0.41 °C, and relative humidity was $66.88 \pm 1.43\%$. All root fragments survived and developed into new plants (of second generation).

On 11 April 2016, of the 14 plants regenerated from root fragments of the mother plants grown in the same first-generation treatment, seven were assigned to the same herbivory treatment as in the firstgeneration experiment and other seven were untreated (no herbivory). Therefore, the second-generation experiment had two factors: maternal herbivory (i.e., the first-generation treatment; the first generation plants were subjected to herbivory or not) and offspring herbivory (i.e., the second-generation treatment; the second-generation plants were subjected to herbivory or not). The experiment lasted 16 weeks, from 11 April 2016 to 7 July 2016, and was conducted in the same greenhouse. During the experiment, the air temperature in the greenhouse was 23.79 \pm 0.33 °C, and relative humidity 54.55 \pm 1.67%. At harvest, number of ramets, number of leaves, and stolon length were measured. The leaves, stolons, taproots and fine roots of each plant were then separated, dried at 70 °C for 48 h and weighed. The remaining mass of the first-generation root fragments was excluded.

2.3. Measurements of non-structural carbohydrate compounds

After the second harvest, the non-structural carbohydrate (soluble sugars and starch) and secondary metabolite (condensed tannin and total phenols) contents of the leaves, stolons, fine roots, and taproots were measured. Before analysis, the tissues of each plant were dried and then finely ground using a Retsch MM400 Mixer Mill at a frequency of 28 Hz for 6 min (Retsch GmbH, Haan, Germany). For non-structural carbohydrate analysis, dry mass subsamples (approx. 50 mg) were analyzed using the perchloric acid/anthrone method (Luo et al., 2014; Morris, 1948). Briefly, the soluble sugars were extracted in 80% ethanol at 80 °C for 30 min, the extracts were centrifuged at $4000 \times$ for 10 min, and then the supernatants of three successive extractions were pooled and thoroughly mixed. Finally, the concentration of soluble sugars was determined by measuring the absorbance at 620 nm in a spectrophotometer. Meanwhile, the starch in the pellet was reacted with perchloric acid, extracted, and analyzed using the anthrone reaction with the method previously described for soluble sugars. The concentration of total non-structural carbohydrates was estimated as the sum of the concentrations of the soluble sugars and starch.

2.4. Measurements of secondary plant metabolites

Dry mass subsamples (approx. 50 mg) were extracted in a 1:1 mixture of methanol and distilled water (5 ml), incubated for 30 min, and then centrifuged at $4000 \times$ for 10 min. The supernatants were collected, diluted to 10 ml using the methanol:water mixture, and analyzed for total phenols and condensed tannin contents. The concentration of total phenols was measured using Folin-Ciocalteu reagent, as in Mcdonald et al. (2001). Briefly, 0.5 ml aliquots of the diluted extracts were mixed with 2.5 ml Folin-Ciocalteu reagent (0.25 M) and 2 ml Na₂CO₃ (7.5%, w/v), heated to 45 °C for 15 min, and then subject to absorbance analysis at 765 nm, using gallic acid monohydrate as a standard. The concentration of condensed tannin was measured using butanol-HCl-iron reagent, as in Gessner and Steiner (2005). Briefly, 0.5 ml aliquots of the diluted extracts were mixed with 7 ml butanol-HCl-iron reagent (cf. Gessner and Steiner, 2005) incubated at 95 °C for 50 min, and then subject to absorbance analysis at 550 nm, using cyanidin chloride as a standard. All concentrations of chemicals were calculated as % dry weight.

2.5. Measurements of nitrogen concentration

The N concentration is commonly used as an indicator of ecophysiological state (activity) of damaged plants after herbivory (Schoonhoven et al., 2005). The concentration of total N in each plant organ was measured at the Analytical and Testing Center, Beijing Institute of Botany, Chinese Academy of Sciences, by using an elemental analyzer (Vario EL III; Elementar, Hanau, Germany). Besides, the amount of N in each plant organ was then calculated by multiplying the N concentration of each plant organ by its corresponding organ mass.

2.6. Data analysis

For the first-generation *A. philoxeroides*, independent *t*-tests were used to examine the effects of herbivory on growth (total biomass, leaf mass, stolon mass, fine root mass, taproot mass, number of ramets, number of leaves, and stolon length), and morphological measures (average leaf area, specific leaf area (SLA), and internode length) of plants.

For the second-generation A. philoxeroides, we used traditional twoway ANCOVAs with block to test the effects of maternal and offspring herbivory on the growth (total biomass, leaf mass, stolon mass, fine root mass, taproot mass, number of ramets, number of leaves, and total stolon length), morphological (average leaf area, specific leaf area, and average internode length) and chemical measures (concentrations of tannins, phenols and N, and the amount of N) of the plants. The initial fresh mass of the second-generation root fragments was used as a covariate. Because data on the concentrations of soluble sugars, starch, and nonstructural carbohydrates violated the assumption of homoscedasticity and normality, we employed two-way ANCOVAs with permutation tests using the R package "ImPerm" (Wheeler, 2010). Leaf mass of plants in the offspring herbivory treatment were insufficient for further chemical analyses, so that only independent *t*-tests were employed to test the effects of maternal herbivory on the leaf chemistry of undamaged offspring. All data were analyzed using R version 3.3.2 (R Development Core Team, 2016).

3. Results

3.1. Growth and morphology of the first-generation plants

Herbivory had negative effects on the accumulation of total biomass and the growth of leaves and roots, but not stolon mass, number of ramets, or stolon length (Appendix Fig. 1). More specifically, the plants subjected to herbivory had approx. 25% less total biomass, 30–41% less root growth (fine root and taproot mass), and 38–50% less leaf number and mass than undamaged plants (Appendix Fig. 1). However, herbivory significantly affected none of the morphological measures (Appendix Fig. 1i–k).

3.2. Growth and morphology of the second-generation plants

Offspring herbivory significantly affected all growth measures of the second-generation plants, expect fine root mass (Fig. 1, Appendix Table 1). Offspring herbivory reduced total biomass, number of ramets, stolon length, stolon mass and taproot mass by 43–53%, and leaf number and leaf mass by approx. 98% compared to the corresponding untreated plants (Fig. 1). Maternal herbivory also significantly reduced total biomass, number of leaves, stolon length, leaf mass, and taproot mass by 12–15% (Fig. 1). There was no significant interaction effect of maternal herbivory \times offspring herbivory on any of the growth measures (Fig. 1, Appendix Table 1).

Offspring herbivory significantly reduced average leaf area and specific leaf area (SLA), but not stolon internode length (Fig. 2, Appendix Table 1). Average leaf area and SLA of plants subject to offspring herbivory were lower than those in undamaged plants (Fig. 2a and b). No effect of maternal herbivory was detected on average leaf area or SLA (Fig. 2a and b, Appendix Table1), but there was a significant interactive effect of maternal and offspring herbivory on internode length (Fig. 2c, Appendix Table1). Offspring taken from the damaged first-generation plants had a longer internode than those taken from the undamaged first-generation plants, but this effect only occurred when offspring herbivory was present rather than when it was absent (Fig. 2c).

3.3. Chemistry of the second-generation plants

Both maternal and offspring herbivory significantly affected the concentrations of non-structural carbohydrates in stolons and fine roots, but not in taproots (Fig. 3, Appendix Table 2). Offspring herbivory significantly reduced the concentrations of soluble sugars, starch, and nonstructural carbohydrates in stolons (Fig. 3a, d and g), and the concentration of soluble sugars in fine roots (Fig. 3b). Maternal herbivory significantly reduced the concentration of soluble sugars in stolons (Fig. 3a). There were also (marginally) significant interactive effects of maternal and offspring herbivory on the concentrations of starch and non-structural carbohydrates in fine roots (Fig. 3e and h, Appendix Table 2). Offspring taken from the damaged first-generation plants had greater concentrations of starch and non-structural carbohydrates in fine roots than those taken from the undamaged first-generation plants, but this is true only when offspring herbivory was absent (Fig. 3e and h).

Offspring herbivory significantly increased the concentration of total phenols in taproots and the concentration of condensed tannin in fine roots (Fig. 4c and e, Appendix Table 2). Offspring herbivory increased the concentration of N in stolons, fine roots, and taproots, but did not affect the total amount of N (Fig. 5a, b and c, Appendix Table 2). By contrast, there were no any maternal effects as detected in the corresponding chemical measures (Appendix Table 2). For the leaves of undamaged plants, maternal herbivory only decreased the concentration of total phenols (Appendix Fig. 2g).

4. Discussion

In the present study, herbivory by the insect *P. minor* caused continuous defoliation of *A. philoxeroides* (approx. 90% reduction in leaf mass and number at the second harvest), thereby severely limiting its mass production, stolon expansion, and ramet reproduction. These results are consistent with those of previous studies that have investigated responses of *A. philoxeroides* to herbivory by the native generalist insects *Atractomorpha sinensis* (Fan et al., 2013) and *Cassida piperata* (Wei et al., 2016) and the introduced specialist beetle *Agasicles hygrophila* (Lu and Ding, 2012), and to defoliation (simulated herbivory) (Schooler et al., 2006, 2007). Besides, herbivory also exerted strong negative effects on the production of non-structural carbohydrate compounds. For example, herbivory significantly reduced the concentrations of soluble sugars, starch, and non-structural carbohydrates in stolons and soluble sugars in fine roots of *A. philoxeroides*. The reason is possible that the reduced photosynthetic capacity of *A. philoxeroides* (including leaf



Fig. 1. Effects of maternal herbivory (Mat) and offspring herbivory (Off) on the growth of second-generation *Alternanthera philoxeroides*. Values and error bars represent means \pm SE. Open and hatched bars represent maternal environments without and with herbivory, respectively. Symbols indicate the level of statistical significance (*P*): ns > 0.1; *0.05–0.1; *0.01–0.05; **0.001–0.01; ***<0.001.

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Fig. 2. Effects of maternal herbivory (Mat) and offspring herbivory (Off) on the morphology of second-generation *Alternanthera philoxeroides*. Values and error bars represent means \pm SE. Open and hatched bars represent maternal environments without and with herbivory, respectively. Symbols indicate the level of statistical significance (*P*): ns > 0.1; #0.05–0.1; *0.01–0.05; ***<0.001.

production and quality) caused by foliar defoliation inhibited the production of carbon-based primary compounds and their translocation from leaves to stolons and fine roots (Fan et al., 2013).

We also found that offspring herbivory had no significant effects on the non-structural carbohydrates in taproots, suggesting that *A. philoxeroides* still maintained a relatively high concentration of nonstructural carbohydrates to ensure the investment of available resources to the development of belowground storage organs, even when they was attacked by herbivores (Mao et al., 2011). This seems to be important for the compensatory growth of *A. philoxeroides* after damage, since roots can function as potential pools of dormant buds, and the high nutrient quality of taproots can improve aboveground regrowth and vegetative reproduction (Jia et al., 2009; Wilson et al., 2007). A previous study of shoot removal reported that damaged *A. philoxeroides* plants were capable of returning to a normal growth trajectory within a short period (e.g., four weeks after shoot removal), owing to rapid regrowth that was supported by the carbohydrate reserves of taproots (Wilson et al., 2007).

More strikingly, the N concentration of the stolons and taproots in *A. philoxeroides* subject to herbivory could be about twice as those in undamaged plants. This is likely because foliar herbivory to some degree enhanced the distribution of N contents in the storage organs within plants (Glass, 1989). Excessive defoliation could inhibit the translocation of N ions from roots to leaves or shoot tips, so that some active N ions are stored in undamaged underground organs (Newingham et al., 2007). Meanwhile, based on the fact that the high foliar N concentration



Fig. 3. Effects of maternal herbivory (Mat) and offspring herbivory (Off) on the concentrations of soluble sugar, starch, and non-structural carbohydrate in different organs of second-generation *Alternanthera philoxeroides*. Values and error bars represent means \pm SE. Open and hatched bars represent maternal environments without and with herbivory, respectively. Symbols indicate the level of statistical significance (*P*): ns > 0.1; *0.05–0.1; *0.01–0.05; **0.001–0.01.



Fig. 4. Effects of maternal herbivory (Mat) and offspring herbivory (Off) on the concentrations of total phenols and condensed tannin in different organs of second-generation *Alternanthera philoxeroides*. Values and error bars represent means \pm SE. Open and hatched bars represent maternal environments without and with herbivory, respectively. Symbols indicate the level of statistical significance (*P*): ns > 0.1; *0.05–0.1; *0.01–0.05.

could elevate the foliar consumption rate of herbivores (Agrawal and Spiller, 2004; Agrawal and Weber, 2015; Fan et al., 2016), herbivory might also induce damaged plants to transport existing foliar N back to undamaged tissues, to obtain an equilibrium state of N among different plant organs, i.e., no significant changes in N amount in stolons, fine roots, or taproots (Newingham et al., 2007). Such modifications of N might also serve for the compensatory growth of plants (Newingham et al., 2007; Roiloa et al., 2014; Wilson et al., 2007). This is consistent with the responses of shrub and tree species to disturbance (Millard et al., 2001; Millard and Grelet, 2010).

To prevent herbivory damage, many plant species can produce carbon-based secondary chemicals of high concentration, like condensed tannins and other polyphenol compounds, to reduce food digestibility and nutritive quality in attacked organs (Agrawal and Weber, 2015; Schoonhoven et al., 2005). However, the increase in defense chemicals in damaged *A. philoxeroides* only occurred in root organs (fine roots or taproots), suggesting that the modification of plants secondary metabolites may be organ-specific, with roots being more sensitive to herbivory damage (Larbat et al., 2012). If roots of A. philoxeroides served as a potential source of vegetative propagule, the secondary chemicals may to some degree improve the resistance ability of vegetative offspring. The other plausible explanation is that the regulation of secondary metabolites depends upon the life-history traits (Herms and Mattson, 1992). The life-history traits of A. philoxeroides (including high intrinsic growth rate, rapid resource uptake ability, and strong phenotypic plasticity), for example, may require the greater investment of resources in storage organs for compensatory growth after damage (Geng et al., 2007; Luo et al., 2009, 2011). Because the concentrations of phenols (0.097-0.252% dry weight) and condensed tannins (0.018-0.034% dry weight) in roots of A. philoxeroides were lower than the normal range of other natural species (reviewed by Hattenschwiler and Vitousek, 2000), we presumed that the increased secondary chemicals in roots



Fig. 5. Effects of maternal herbivory (Mat) and offspring herbivory (Off) on the concentration and amount of N in different organs of second-generation *Alternanthera philoxeroides*. Values and error bars represent means \pm SE. Open and hatched bars represent maternal environments without and with herbivory, respectively. Symbols indicate the level of statistical significance (*P*): ns > 0.1; #0.05–0.1; ***<0.001.

may be a byproduct of resource allocation between plant organs (i.e., the concentration of secondary chemicals in roots increased due to the increase in root to shoot ratio, which increased from 0.59 in plants without herbivory to 0.81 in plants with herbivory; unpublished data). Overall, the results partly supported the first hypothesis, implying that *A. philoxeroides* in the introduced range may adapt to foliar herbivory by generalists using the tolerance traits related to the allocation of non-structural carbohydrates and nitrogen in belowground organs.

Our results supported the second hypothesis that maternal herbivory effects can span vegetative generations of A. philoxeroides, especially for growth traits. Meanwhile, the effect of maternal herbivory across vegetative generations was basically independent of offspring environments, which is inconsistent with the prediction from the third hypothesis. Maternal herbivory could independently and remarkably reduce biomass accumulation, ramet production, and stolon expansion, as well as the concentration of soluble sugars in stolons; maternal herbivory also induced the internode elongation in A. philoxeroides when plants were attacked by *P. minor*. The change in internode length may reflect a morphological plasticity of A. philoxeroides in response to foliar herbivory, by increasing the feeding distance to alleviate negative effects of slowly-moving insects such as P. minor (Ginocchio and Montenegro, 1994). Such a morphological response has also been previously described as a strategy of clonal species to escape from stressful conditions such as flooding (Luo et al., 2009), metal pollutions (Roiloa and Retuerto, 2012), and interspecific competition from neighbor species (Evans and Cain, 1995). In the present experiment, only damaged parent plants of A. philoxeroides may activate the escape strategy in their offspring subject to the same foliar herbivory. Therefore, morphological plasticity of internodes mediated by maternal herbivory may be selected, which may potentially contribute to the invasiveness of A. philoxeroides in habitats with frequent herbivory by native generalists.

Including initial mass as a covariate in the statistical analyses allowed us to distinguish whether the herbivory-induced maternal effect depended upon the initial size of offspring (Cendán et al., 2013; Hereford and Moriuchi, 2004; Zas et al., 2013). The results suggest

Appendix A

Appendix Table 1

ANCOVA results for effects of maternal and offspring herbivory on the growth and morphology of second-generation Alternanthera philoxeroides.

that the maternal herbivory effects as observed in A. philoxeroides cannot be simply explained by the variation in the initial size of the firstgeneration root fragments (i.e., no significant initial mass effect on the majority of growth, morphology and chemical measures in Appendix Tables 1 and 2). Similarly, Cendán et al. (2013) previously reported that the differences in seed weight between maternal environments could not completely account for the germination phenology of Pinus pinaster seeds (Cendán et al., 2013). It is possible that the provisions of internal resources (non-structural carbohydrates and N) in offspring, rather than the absolute mass, may really influence the subsequent performance of offspring (Newingham et al., 2007; Wilson et al., 2007). Besides, it is also possible that herbivory-induced maternal effects may be driven by epigenetic changes, such as DNA methylation and histone modification, that is transmitted through vegetative propagates and alters offspring gene activity and phenotype (Douhovnikoff and Dodd, 2015; Latzel et al., 2016).

In conclusion, our work provides evidence that *A. philoxeroides* can cope with foliar herbivory by *P. minor* through both morphological plasticity such as internode elongation, and physiological flexibility such as the allocation of resources related to non-structural carbohydrates and nitrogen. These responses may potentially improve the regrowth ability and herbivory-tolerance of vegetative offspring in *A. philoxeroides*, thereby contributing to plant fitness in habitats with herbivores. Notably, herbivory-induced maternal effects could also play a detrimental role in the growth traits of *A. philoxeroides*, but could not provide important cues for the choice of the resistance strategy of vegetative offspring against future herbivory, at least by *P. minor*.

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	Block	Initial mass	Maternal (Mat)	Offspring (Off)	$Mat \times Off$
Growth					
Total biomass	1.6	0.4	4.6 *	35.2***	1.0
No. of ramets	2.1	0.2	0.9	16.2 ****	1.0
No. of leaves	1.5	0.2	6.0 *	109.5***	2.5
Stolon length	1.1	0.8	6.5 *	49.9 ***	1.2
Leaf mass	2.3#	0.7	6.8 *	217.3***	4.3#
Stolon mass	1.2	<0.1	4.3#	32.7***	1.1
Fine root mass	1.5	1.9	0.4	3.0	0.01
Taproot mass	1.6	2.3	5.0 *	15.7**	0.6
Morphology					
Average leaf area	1	6.6 *	2.2	174.5***	2.3
SLA	$2.4^{\#}$	4.9 *	0.5	58.8 ***	3.4#
Internode length	1.1	0.5	0.3	1.7	4.8 *

Symbols indicate the level of statistical significance, as follows: no symbol P > 0.1; *P = 0.05 - 0.1; *P = 0.01 - 0.05; *P = 0.001 - 0.01; *P < 0.001. *F* values for which P < 0.05 are shown in bold and for which *P* is between 0.05 and 0.1 are in italic. Degrees of freedom (*df*) are (6, 17) for the block effect and (1, 17) for the other effects. Initial mass is a covariate.

Appendix Table 2

ANCOVA results for effects of maternal and offspring herbivory on the chemistry of second-generation Alternanthera philoxeroides.

	Block	Initial mass	Maternal (Mat)	Offspring (Off)	$Mat \times Off$
Stolon					
Sugar ^a	2.7#	0.48	7.0 *	3.7#	1.8
Starch ^a	1.3	0.08	0.1	9.5 **	1.1
NSC ^a	1.6	0.02	0.5	11.9**	0.6
Phenol	2.7*	0.02	0.001	0.2	2.1
Tannin	0.9	0.55	0.6	0.6	3.4#

Appendix Table 2 (continued)

	Block	Initial mass	Maternal (Mat)	Offspring (Off)	$Mat \times Off$
N conc.	0.7	1.46	0.002	21.7***	0.4
N amount	1.1	1.9	2.6	1.9	1.2
Fine root					
Sugar ^a	0.1	0.33	0.4	10.7**	0.02
Starch ^a	0.6	1.46	0.8	0.2	4.9 *
NSC ^a	0.6	1.71	1.1	0.3	4.1#
Phenol	0.8	2.56	0.1	1.1	0.4
Tannin	0.6	1.98	0.6	6.3 *	2.0
N conc.	0.8	3.95#	0.2	3.5#	0.7
N amount	1.8	1.07	0.6	1.9	0.2
Taproot					
Sugar ^a	0.5	0.01	0.7	0.8	0.3
Starch ^a	0.9	1.8	0.1	1.4	2.3
NSC ^a	1.0	1.9	0.04	1.6	2.3
Phenol	0.7	3.1#	0.004	5.4 *	0.9
Tannin	0.6	0.001	0.7	0.4	0.1
N conc.	1.4	10.2**	0.5	71.1***	0.7
N amount	2.1	0.04	2.5	3.1#	0.7

Symbols indicate the level of statistical significance, as follows: no symbol P > 0.1; *P = 0.05 - 0.1; *P = 0.001 - 0.05; **P = 0.001 - 0.01; ***P < 0.001. *F* values for which P < 0.05 are shown in bold and for which *P* is between 0.05 and 0.1 are in italic. Degrees of freedom (*df*) are (6, 17) for the block effect and (1, 17) for the other effects. Initial mass is a covariate.

^a These chemical measures were analyzed by permutation ANCOVAs to avoid the biased estimation from violation of assumptions of heterogeneity of variance and normality.



Appendix Fig. 1. Effects of herbivory on the growth and morphology of first vegetative generation of *Alternanthera philoxeroides*. Degrees of freedom (*df*) is 12, and both *t*- and *P*-values are shown. Values and error bars represent means ± SE.



Appendix Fig. 2. Effects of maternal herbivory on the leaf chemistry of second-generation *Alternanthera philoxeroides* under the offspring treatment without herbivory. Values and error bars represent means ± SE. Degrees of freedom (*df*) is 12, and both *t*- and *P*-values are shown. Open and hatched bars represent maternal environments without and with herbivory, respectively.

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