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Low-level expression of induced defences and increased compensation in *Eupatorium adenophorum* relative to its native congeneric plant to mechanical wounding and insect herbivory

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Abstract

Native herbivory may be detrimental to plant fitness and thus can contribute a biotic resistance to exotic plant invasion. *Eupatorium adenophorum*, a non-native species, has formed dense monocultures in many southern Provinces of China. Because several native generalist insects feed on *E. adenophorum*, we evaluated whether the lower level of induced defences and greater compensatory growth contributed to successful invasion of *E. adenophorum* by comparison with a native congener *E. heterophyllum*. Early events in the jasmonic acid cascade and chlorophyll fluorescence, non-target metabolomic profiles and recovery in biomass compensation of *E. adenophorum* and its native congener *E. heterophyllum* were measured during mechanical wounding and herbivory by the generalist insect *Helicoverpa armigera*. Mechanical and simulated chewing damage immediately initiated defences in the two species. However, *E. heterophyllum* was more sensitive to tissue loss, and it produced more metabolic defensive compounds than invasive *E. adenophorum*. Although *H. armigera* feeding amplified the effects of mechanical wounding on defence induction, the biomass of *E. adenophorum* was only weakly affected, as its higher compensatory growth could offset the biomass loss and metabolic cost. Overall, our results suggest that *H. armigera* herbivory and mechanical wounding could induce different physiological responses, and are often specific to invasive *E. adenophorum* and native *E. heterophyllum*. Moreover, lower metabolic defences and higher compensation growth may contribute to the invasion success of *E. adenophorum*.

Keywords herbivory, chlorophyll fluorescence, metabolic, defence, compensation, *Eupatorium adenophorum*

机械损伤和昆虫取食对紫茎泽兰与本地同属植物防御表达和补偿作用的差异

摘要：入侵地群落中的本地草食昆虫取食可以影响植物生长的适合度，从而有助于抵抗外来植物入侵。紫茎泽兰(*Eupatorium adenophorum*)是一种入侵性很强的外来物种，在中国南方许多省份已形成密集的单优群落。很多学者认为天敌逃逸是外来植物入侵的一个重要原因，但前期研究发现当地的几种广谱天

敌能够取食紫茎泽兰。因此，本研究通过与本地同属植物异叶泽兰(*E. heterophyllum*)比较，来评估是否低水平的防御表达和高的生物量补偿促进了紫茎泽兰的成功入侵。本文分别比较了在广谱天敌棉铃虫(*Helicoverpa armigera*)取食和机械损伤情况下紫茎泽兰和异叶泽兰早期防御信号茉莉酸(JA)和茉莉酸–异亮氨酸(JA-Ile)、叶绿素荧光、非靶代谢组学特征以及补偿生长等方面的差异。研究结果表明，机械损伤和模拟取食能立即启动入侵植物紫茎泽兰和本地同属植物异叶泽兰的天敌防御，诱导两种植物特异性的生理反馈。异叶泽兰对组织损伤更为敏感，相比于入侵植物紫茎泽兰产生了更丰富的防御化合物。棉铃虫取食比机械损伤对防御诱导的影响更大；与异叶泽兰相比，紫茎泽兰具有较高的补偿生长能力，可以抵消生物量损失和防御成本的投入，受到的影响相对较小。较低的代谢防御和较高的补偿生长可能有助于紫茎泽兰的入侵成功。

关键词：食草动物取食，荧光，代谢，防御，补偿，紫茎泽兰

INTRODUCTION

According to the natural enemy release hypothesis (Keane and Crawley 2002), exotic plants may have advantages over native plants owing to herbivory reduction in the introduced region. However, invasive plants may encounter novel generalist herbivores in introduced regions (Agrawal and Kotanen 2003). Generalists may have similar attack rates (Stutz *et al.* 2016) or prefer palatable exotic plants over native plants (Verhoeven *et al.* 2009; Yu *et al.* 2011), particularly generalist enemies from congeneric plants (Agrawal and Kotanen 2003), because the short co-evolutionary duration of exotic plants with local herbivores may lead to its ineffective defences (Morrison and Hay 2011; Parker *et al.* 2006). Herbivory can trigger physiological processes, including photosynthesis inhibition and defensive metabolic shifts, thereby affecting the fitness and growth of invasive plants (Liu *et al.* 2022a; Yu and Fan 2018). To some extent, native generalist herbivores may play roles in biological resistance, reshaping the invasion outcomes of exotic plants (Elton 1958; Levine *et al.* 2004; Parker *et al.* 2006). However, little is known about how exotic plants cope with native generalist herbivores over time.

Herbivory-induced changes in photosynthesis have been documented in many plants; however, the results were inconsistent. For example, chewing herbivory has been shown to decrease plant photosynthesis by physically disrupting the supply of water and nutrients via direct reductions in leaf area (Aldea *et al.* 2005; Peschiutta *et al.* 2018). Additionally, herbivory can downregulate the activity of photosynthesis-related genes (Wu and Baldwin 2010). Therefore, short-term feeding by chewing herbivory can immediately reduce the efficiency of photosystem II (PSII) in remaining tissue (Aldea *et al.* 2006; Halitschke *et al.* 2011; Nabity *et al.* 2013; Zangerl *et al.* 2002). However, increased carbon fixation following partial defoliation was also reported, which may arise from increasing flow of nitrogen (N) to remaining leaves (Turnbull *et al.* 2007), or which may reduce the feedback inhibition of photosynthetic end-products, such as sugars and starch (Schultz *et al.* 2013). Therefore, photosynthetic responses to herbivore attack may be highly species specific and could be manipulated by the initial signal perception induced by the attacker (Halitschke *et al.* 2011; Nabity *et al.* 2013).

During early signalling responses, jasmonic acid (JA) accumulates when plants perceive attacker-derived physical and chemical cues, which play pivotal roles in triggering specific defensive metabolites (Babst *et al.* 2005; Howe and Jander 2008; Hummel *et al.* 2010; Machado *et al.* 2017). Caterpillar (*Spodoptera exigua*), wounding and aphid treatments all upregulate genes associated with JA signalling, cell wall thickening for *Arabidopsis* plants and other defence-related compounds for invasive plant *Eupatorium adenophorum* (Appel *et al.* 2014; Chen *et al.* 2019; Liu *et al.* 2021). Feeding by the generalist *Helicoverpa zea* can induce the production of simple phenolics and precursor amino acids involved in simple phenylpropanoids synthesis within 24 h for tomato plants (*Solanum lycopersicum*) (Steinbrenner *et al.* 2011). Because growth and defence share some limited metabolic resources, allocating resources to defence may result in a reduction in plant growth (Züst and Agrawal 2017).

Plant compensation is the ability of a plant to buffer the negative effects of herbivores (Strauss and Agrawal 1999). For example, some plants have compensatory mechanisms for increasing the photosynthetic rate (Thomson *et al.* 2003), facilitating resources remobilization (Babst *et al.* 2005; Gómez *et al.* 2010; Peschiutta *et al.* 2018), and increasing the branch number and relative growth rate (Li *et al.* 2012) when suffering enemy attack. Additionally, plants may fully compensate or overcompensate for tissue loss by herbivores (Babst *et al.* 2005; Peschiutta *et al.* 2018). Thus, herbivory may have no detectable or even positive effects on growth in some cases (Wang and Feng 2016).

Belsky *et al.* (1993) proposed that compensatory effects may represent a feedback mechanism of plants in response to threats, including fire, tissue damage, freezing damage and pathogen infection. In studies of compensation, plants were manually defoliated to mimic insect feeding in some experiments (Lieurance and Cipollini 2013). However, the intensity and degree are different between artificial simulation and natural herbivore damage, which may trigger different responses (Howe and Jander 2008; Wu and Baldwin 2010). For example, Machado *et al.* (2017) found that mechanical wounding reduced glucose and fructose concentrations in *Nicotiana attenuata* leaves, and these effects were further amplified by applying oral secretions (OSs) of *Manduca sexta*. Mechanical wounding and caterpillar (*S. exigua*) damage induced only 8%–12% overlap in upregulated genes of *Arabidopsis thaliana* (Appel *et al.* 2014). However, continuous mechanical wounding using a computer-controlled mechanical device (completely devoid of salivary secretions), can also induce herbivory-related volatile emissions in *Phaseolus lunatus* plants (Mithöfer *et al.* 2005). These findings highlighted the need to explore the difference of inducing mechanisms between mechanical wounding and real herbivory.

Eupatorium adenophorum Sprengel (also known as *Ageratina adenophora*), native to Central America, is a perennial herb or semi-shrubby plant i.e. considered a noxious invasive plant in many regions of the world (Poudel *et al.* 2019). *Eupatorium adenophorum* can be attacked by a few generalist enemies (Zheng *et al.* 2012). However, little is known about how *E. adenophorum* responds to the damage induced by manual (mechanical) wounding and generalist herbivores. Accordingly, in this study, we investigated the effects of herbivory attack on phytohormone levels and chlorophyll fluorescence, as well as changes in non-target metabolomes in leaves of *E. adenophorum* and native congener (*E. heterophyllum*) under mechanical wounding and generalist herbivory. Meanwhile, we also measured the recovery of above parameters after damage. We evaluated the differences between the invader and its native congener in terms of physiological responses to manual wounding and generalist herbivory, and the different impacts of mechanical wounding and herbivory on plants. We predicted that: (i) both mechanical wounding and generalist chewing can induce early defence response for *Eupatorium* plants in this study, and (ii) lower metabolic defences and higher compensation of *E. adenophorum* compared with its native congener, may contribute to its successful invasion.

MATERIALS AND METHODS

Plant material

E. heterophyllum is a perennial native forb distributed extensively in China, sharing distribution areas with *E. adenophorum* in multiple habitats (Feng 2008). Seeds of *E. adenophorum* and *E. heterophyllum* were collected in Kunming (24°52′ N, 102°49′ E), Yunnan Province, southwest China. The seeds were air dried for 1 week at room temperature and then stored in paper bags at room temperature until the experiment started.

This study was conducted at Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Menglun (21°56′ N, 101°15′ E), Mengla, Yunnan Province. Seeds were germinated in planting trays filled with a 1:1 mixture of sand and peat (Pindstrup substrate, Denmark), and similar-sized seedlings were then transplanted into 12-L pots filled with mixed soil (topsoil from a nearby forest and river sand, 3:1). All plants were grown in a shade house (with approximately 40% irradiance) for 2 weeks to reduce transplanting shock, and were then transferred to full light. All of the seedlings were fertilized once a month (0.01 g diluted 500 times; $N:P_2N_5:K_2O$:micronutrient element, 20:20:20:3; Sanning, Hubei). All plants were watered daily with an automated drip irrigation system. Herbivory treatments were applied after 2 weeks of growth under full light. Five plants (replicates) for each treatment were harvested and dried at 70 °C for at least 72 h to estimate initial biomass.

Generalist insect

Helicoverpa armigera Hubner is a chewing generalist insect, sharing geographic areas with *E. adenophorum* and *E. heterophyllum* (Chen *et al.* 2019). Larvae at one to two instars were purchased and reared on an artificial diet (Keyun Biopesticide Technology Research and Development Center of Institute of Zoology, CAS and Henan Jiyuan Baiyun Industrial Co., Ltd), and three or four instar larvae (when insects eat the most) were used for herbivory treatments.

Experiment 1: chlorophyll fluorescence responses of *Eupatorium* plants to mechanical and insect-induced damages

To test the immediate effects of mechanical wounding and insect herbivory on photosynthetic capacity, the first fully expanded leaves of five individual plants were wounded by punching along the mid-vein and gently rubbing with 20 μL deionized water (WW: wounding plus water, defined as mechanical wounding) or by feeding of *H. armigera* larvae (WH: wounding herbivory). The larvae were starved for 24 h, and then one larva was put on the selected leaf, and both the leaf and one larva were caged using a clip cage for 4 h. In order to keep environment homogeneity, leaves in the control (Con) and WW groups were caged with empty clip cages. After measuring fluorescence, the leaves were traced along the feeding edge to determine the final feeding area. Approximately 25% of the leaf area of plants from the WW treatment had been removed, whereas approximately 20% and 30% of the leaf area had been removed for *E. adenophorum* and *E. heterophyllum* plants from WH treatment, respectively.

Chlorophyll fluorescence was measured using an imaging chlorophyll fluorometer and camera (AVT Manta 033B [MAXI]; ImagingWin 2.47; Walz GmbH, Effeltrich, Germany). After removal of the insects from the target leaf, the chlorophyll fluorescence measurements were performed on one leaf immediately, all measurements were carried out from 9:30 to 11:30 AM. There were eight measurements for each leaf in the WW and WH groups, respectively; four were performed at the damaged edge, and another four were performed in undamaged parts in the same leaf. Four measurements were also performed for each leaf in Con group. The leaves were then exposed to actinic light (230 μmol photons m^{-2} s^{-1}, intensity of 9, instrument setting) for approximately 5 min, and a saturation pulse was applied. The light-adapted maximum (F'_m) and steady state fluorescence output (F_s) were recorded, and PSII efficiency was calculated: $\Phi_{PSII} = (F'_m F_s)/F'_m$ (Genty *et al*. 1989). All measurements were conducted on attached leaves.

Experiment 2: early signalling and induced metabolites of *Eupatorium* plants in response to mechanical and insect-induced damage

Induced signals and metabolites are sensitive to leaf damage and can change greatly over time (Howe and Jander 2008). Previous studies have shown that OSs from *H. armigera* larvae can induce numerous primary metabolic shifts in plants (Chen *et al.* 2019; Peiffer and Felton 2005). Therefore, we used insect OSs from *H. armigera* larvae to treat leaves owing to the difficulty in controlling the chewing behaviours. *Helicoverpa armigera* larvae were starved for 24 h, and the larvae were then fed *E. adenophorum* and *E. heterophyllum* plants for 12 h. OSs were then collected from approximately 200 larvae, and divided into small amounts (Chen *et al.* 2019). The entire process was performed on ice, and the OSs were subsequently stored at −80 °C in Eppendorf tubes until use.

In order to quantify metabolite expression after simulated leaf damage, the leaves above the first fully expanded leaf of eight individual plants were chosen for non-targeted metabolomic analysis. The leaf was generated one strip wound by rolling a metal wheel along the mid-vein. Then the leaf was immediately and gently rubbed with 20 μL deionized water (WW; wounding plus water) or *H. armigera* OSs (WH_S; wounding plus OSs) on the leaf wounds. OS from *H. armigera* was re-applied every day after repeating the damage with the roll. All treatments were performed from 9:00 to 10:00 AM to minimize environmental and growth effects on signalling. At the first day treatment, after 1 h of treatment, together with untreated controls (Con), leaves obtained manually from three individual plants were sampled, frozen in liquid nitrogen, and were stored at −80 °C until use. The leaf samples were then freeze-dried and ground into powder. Each sample was weighed (±0.0001 g) and transferred to an Eppendorf tube. JA and JA–isoleucine conjugate (JA-Ile) determinations were performed using ultra-high-performance liquid chromatography/tandem mass spectrometry (SCIEX), as previously described (Simura *et al.* 2018).

Leaves in the WW and WH_S groups of another five plants were continued to be wounded, at the same time every day with a final three rolls on each side of one leaflet during the remaining 5-day period. Treated leaves obtained from individual plant were then harvested at 10 days after the initial damage, immediately frozen in liquid nitrogen and stored at −80 °C until use for metabolite analysis to quantify changes in induced metabolites after continuous leaf damage.

The freeze-dried samples were crushed with a mixer mill for 30 s at 60 Hz. Then, a 10-mg aliquot of each individual sample was transferred to an Eppendorf tube. After addition of 1 mL extract solution (methanol/water = 3:1, precooled at −40 °C, containing internal standard), samples were vortexed for 30 s, homogenized at 35 Hz for 4 min and treated with sonicate for 15 min in ice-water bath. The samples were then extracted overnight at 4 °C on a shaker and centrifuged at 12 000 rpm for 15 min at 4 °C. The supernatants were carefully filtered through a 0.22-microporous membrane, and the resulting supernatants were diluted 20 times with the methanol/water mixture (v:v = 3:1, containing internal standard), vortexed for 30 s and transferred to 2-mL glass vials. Then 20 µL from each sample was taken and evaluated as the pooled quality control sample. Samples were stored at −80 °C until the liquid chromatography/mass spectrometry (LC–MS) analysis according to Shi *et al.* (2019).

Experiment 3: compensatory response of *Eupatorium* plants to mechanical and insect-induced damage

To determine the compensation capacity of plants after damage, the third instar larvae of *H. armigera* were released (10–12 larvae/plant) at 3, 5 and 8 weeks after transplanting to simulate the feeding habits of *H. armigera* in nature. The larvae of *H. armigera* fed on plants for 3–4 days and then were removed (WH treatment). During the same period, at WW treatment, we cut the leaves along the main vein of plants and sprayed each plant with 15 mL deionized water immediately after cutting. Approximately 45%–55% leaf area (visually estimated) of each plant was removed in WH treatment, and approximately 50% leaf area of each plant was removed in WW treatment. During the herbivory period, each plant was enclosed with a white nylon cage (length × width: 130 cm × 45 cm, mesh size 1 mm^2). The larvae and cages were removed immediately after herbivory. At 88th day after the initial herbivory, the first fully expanded leaf was harvested manually from the main stem for detecting N and ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) contents. Leaf N concentrations were measured with an elemental analyser (EA 3000; EuroVector, Italy) after drying in an oven at 60 °C for 48 h. The leaves for RuBisCO contents were frozen quickly with liquid nitrogen and stored at −80 °C. Then RuBisCO contents were determined with a RubisCO enzyme-linked immunosorbent assay quantification kit (CUSABIO, Wuhan, China), which employed a competitive inhibition enzyme immunoassay technique. Finally, the above- and below-ground biomasses of whole individual plant were then harvested at 21 days (H1), 46 days (H2) and 88 days (H3) after herbivory and carefully washed, and dried at 60 °C for 72 h to a constant weight and then weighed.

Statistical analysis

Principle component analysis and orthogonal partial least squares discrimination analysis of the identified metabolites from LC–MS data were performed using SIMCA software (v16.0.2; Sartorius Stedim Data Analytics AB, Umea, Sweden) to analyse the metabolic differences between mechanical/herbivory and Con treatments. Metabolite peak areas from gas chromatography–MS were log transformed. Based on a variable importance in the projection value greater than or equal to 1.0 and *P* value less than or equal to 0.05 (two-tailed Student's *t*-test), we define metabolites as significantly differential metabolites (DMs). Furthermore, fold change greater than or equal to 2 or fold change less than 0.5 was performed to identify the most representative differential metabolites (RDMs) that contributed significantly to the grouping and defence-related representative differential metabolites (D-RDMs) in alkaloids, phenols, flavonoids, phenylpropanoids and coumarins.

The effects of herbivory treatment, species and their interaction on phytohormones, chlorophyll fluorescence and compensatory variables were analysed in linear models (Supplementary Table S1). *Post hoc* tests were used for multiple comparisons of the variables across different levels of each factor. Data were checked for normal distribution and homogeneity of variance before the analyses. All linear models were carried out using the *lm* function in R 4.0.2 (R Core Team 2020).

Relative Φ_{PSII}, JA and JA-Ile, and relative biomass were calculated as the ratio of the value in damaged plant (WW and WH) to the value in the control treatment, the former three were used to measure early defence response, the relative biomass was used to evaluate the capacity of plants for compensatory growth.

RESULTS

Chlorophyll fluorescence responses of *Eupatorium* plants to mechanical and insect-induced damages

For both *E. adenophorum* and *E. heterophyllum*, relative Φ_{PSII} was significantly lower at WH (herbivory) than

WW (mechanical wounding) treatment (Fig. 1). There were no significant differences between leaves of the two species in relative Φ_{PSII} at same treatment.

Early signalling and induced metabolites in *Eupatorium* plants in response to mechanical and insect-induced damage

Insect-damage induced higher increment in JA and JA-Ile concentrations for both *Eupatorium* species than mechanical wounding. *E. heterophyllum* leaves exhibited higher relative JA/JA-Ile than *E. adenophorum* at both mechanical wounding and simulated herbivory (Fig. 2a and b).

Compared with Con leaves, there were 37 DMs (16 upregulated and 21 downregulated) for the simulated herbivory group (WH_S) and 39 DMs (24 upregulated and 15 downregulated) for the mechanical wounding group (WW) in leaves of *E. adenophorum* (Fig. 3). Additionally, there were 57 DMs (42 upregulated and 15 downregulated) in the WH_S group and 49 DMs (36 upregulated and 13 downregulated) in the WW group in leaves of *E. heterophyllum* (Fig. 3). Among the DMs, 8 (four upregulated and four downregulated) and 11 (eight upregulated and three downregulated) were the same for leaves in the WW/Con and WH_S/Con comparisons of *E. adenophorum* and *E. heterophyllum*, respectively (Fig. 3; Supplementary Table S2). OSs induced fewer uniquely upregulated DMs (n = 12) than downregulated DMs (n = 17) for *E. adenophorum*. However, a strikingly higher number of upregulated DMs was found for *E. heterophyllum* leaves under both damage treatments than for *E. adenophorum* (Fig. 3).

RDMs in mechanical wounding and simulated herbivory are different between the two *Eupatorium* species. The concentrations of Demethoxycurcumin (phenols, 144-fold), Harmol hydrochloride monohydrate (alkaloids, 84-fold), Androstanediol (steroids, and steroid derivatives, 12-fold) increased in *E. adenophorum* WH_S/Con (Fig. 4) and Robustine (alkaloids, 31-fold), Genipin (iridoids, 17-fold), DL-Benzylsuccinic acid (phenylpropanoids, 11-fold) and Wedelolactone (coumarins, 11-fold) also increased in *E. heterophyllum* WH_S/Con (Fig. 4). However, the concentrations of 4-Aminobutyric acid (amino acids, 98-fold) and Ganoderic acid (triterpenoids, 13-fold) decreased in *E. heterophyllum* in WH_S/Con. The concentration of fewer compounds changed more than 10-fold in WW/Con. For example, only the concentration of Delphinidin-3-*O*-glucoside (flavonoids) decreased 11-fold in *E. adenophorum*, Psychosine (sphingolipids) increased 10-fold in *E. heterophyllum*, and the concentration change of other compounds was less than 10-fold (Fig. 4; Supplementary Table S2) (but Psychosine, sphingolipids, 10-fold).

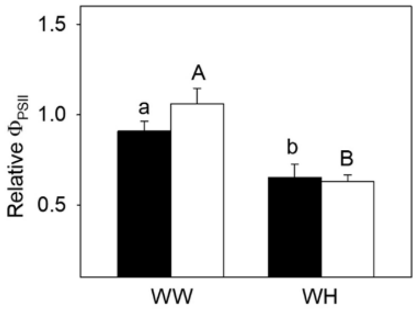

Figure 1: Relative photochemical efficiency of PSII (Φ_{PSII}) in the leaves of *Eupatorium adenophorum* (black bars) and *E. heterophyllum* (white bars) after mechanical wounding (WW) or *Helicoverpa armigera* larvae feeding (WH) treatment. Values are means ± standard errors. n = 5 for each plant species. Different lowercase letters above bars indicate significant differences between treatments for *E. adenophorum*, and different capital letters above bars indicate significant differences between treatments for *E. heterophyllum*.

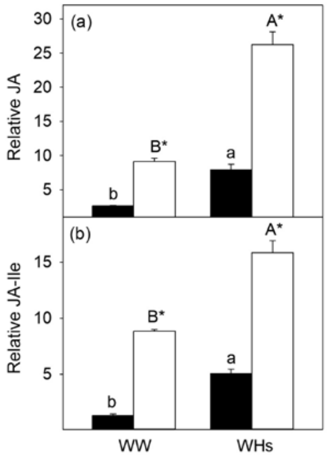

Figure 2: Relative Jasmonate (JA, **a**) and JA-Ile (JA–isoleucine conjugate, **b**) concentrations in the leaves of *E. adenophorum* (black bars) and *E. heterophyllum* (white bars) within 1 h after mechanical wounding (WW) or *H. armigera* OS elicitation (WH_S). Values are means ± standard errors, and three replicates for each species. Different lowercase letters above bars indicate significant differences between treatments for *E. adenophorum*, and different capital letters above bars indicate significant differences between treatments for *E. heterophyllum*. Asterisk indicates significant difference between *E. adenophorum* and *E. heterophyllum* at the same treatment.

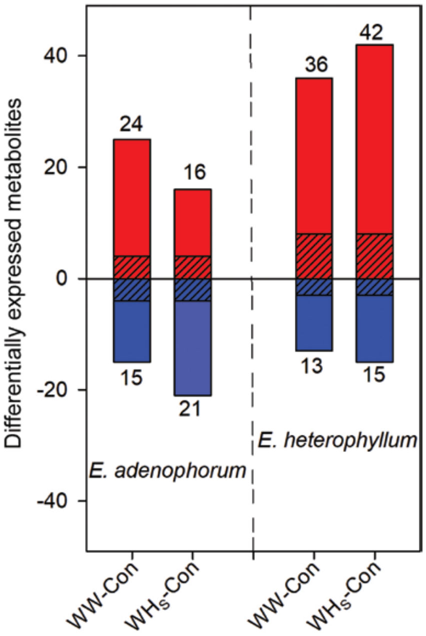

Figure 3: Number of DMs in pairwise comparisons of *E. adenophorum* and *E. heterophyllum*. Red and blue bars indicate the numbers of metabolites showing increases and decreases in concentrations, respectively. Dashed line bars indicate the numbers of overlapping DMs between WW/Con and WH$_S$/Con comparisons, respectively.

E. heterophyllum had more D-RDMs in alkaloids, phenols, flavonoids, phenylpropanoids and coumarins, but *E. adenophorum* only had alkaloids, phenols, flavonoids metabolites. Although *E. heterophyllum* had more D-RDMs than *E. adenophorum*, D-RDMs change much greatly for *E. adenophorum* in WH$_S$/Con comparisons (Fig. 5; Supplementary Table S2).

Compensatory response of *Eupatorium* plants to mechanical and insect-induced damage

On average, *E. adenophorum* had lower leaf N content but significantly higher RuBisCO content than *E. heterophyllum* (Fig. 6). Compared with Con treatment, both WW and WH damage increased RubisCO but not leaf nitrogen content of *E. adenophorum* (Fig. 6). However, for *E. heterophyllum*, both WW and WH damage had no significant effect on RubisCO content, and only WW damage increased its leaf N content (Fig. 6).

Both *Eupatorium* species could recover after induction of damage. However, *E. heterophyllum* compensated the biomass loss quickly at the early growth stage (relative biomass >1, WH in H1), and *E. adenophorum* gradually compensated and had significantly higher biomass than *E. heterophyllum* at the last harvest (relative biomass >1, H3) (Fig. 7).

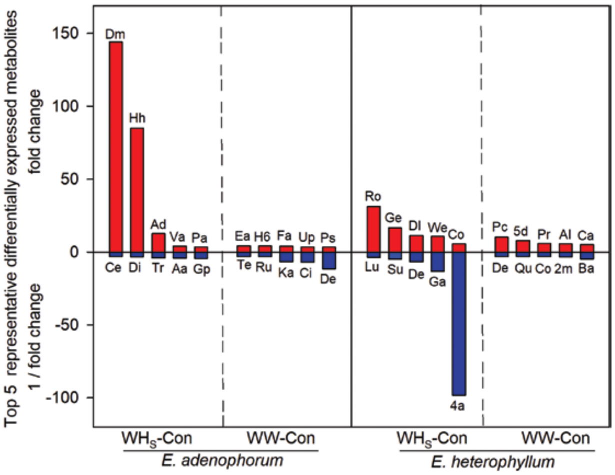

Figure 4: The top 5 representative DMs (fold change) between WW/Con and WH$_S$/Con comparisons, respectively. Red and blue bars indicate the metabolites increase and decrease in concentrations, respectively. Three replicates for each treatment. (In WH$_S$/Con comparison of *E. adenophorum*, Dm: Demethoxycurcumin, Hh: Harmol hydrochloride monohydrate, Ad: Androstanediol, Va: Vanillic acid, Pa: Physalin D, Ce: Cedrelone, Di: Isomangiferin 3-(2,3-Dihydroxyphenyl)propanoate, Tr: 1,5,6-Trihydroxyxanthone, Aa: Arachidonic acid, Gp: Geniposid. In WW/Con comparison of *E. adenophorum*, Ea: Eicosadienoic acid, H6: 2-Hydroxy-6-oxo-6-(2-carboxyphenyl)-hexa-2,4-dienoate, Fa: Farrerol, Up: Uplandicine, Ps: Pseudouridine, Te: Tenulin, Ru: Rutin, Ka: Kaempferol-3-*O*-rutinoside, Ci: Cimifugin, De: Delphinidin-3-*O*-glucoside. In WH$_S$/Con comparison of *E. heterophyllum*, Ro: Robustine, Ge: Genipin, Dl: DL-Benzylsuccinic acid, We: Wedelolactone, Co: Cordycepin, Lu: Luteolin-6-C-glucoside, Su: Sugiol, De: Deoxyvasicinone, Ga: Ganoderic acid, 4a: 4-Aminobutyric acid. In WW/Con comparison of *E. heterophyllum*, Pc: Psychosine, 5d: 5′-Deoxyadenosine, Pr: Prunetin, Al: alpha-Sinensal, Ca: Cardanol, De: Delphinidin-3-*O*-glucoside, Qu: Quercetin-7-*O*-beta-D-glucopyranoside, Co: Corticosterone, 2m: 2′-*O*-Methyladenosine, Ba: Bakkenolide.).

Both *Eupatorium* plants compensated more slowly after mechanical wounding (WW) than after WH (herbivory) at H1. However, *E. heterophyllum* exhibited similar biomass compensation between WW and WH at last harvests (H3), and *E. adenophorum* showed higher compensation ability in WW than in WH treatment (Fig. 7).

DISCUSSION

In this study, we found that *Eupatorium* plants initiated defence after mechanical and simulated herbivory damages. For both *E. adenophorum* and *E. heterophyllum*, several defence-related DMs (D-RDMs) increased more strongly in plants subjected to WH treatment than

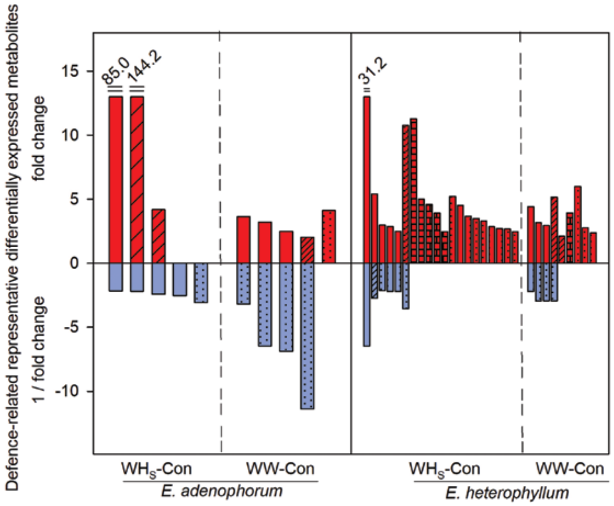

Figure 5: Defence-related representative DMs between WW/Con and WH_S/Con comparisons, respectively. Each comparison of *E. adenophorum* and *E. heterophyllum* shows representative DMs (fold change >2) of alkaloids (blank bars), phenols (slash bars), flavonoids (spot bars), phenylpropanoids (brick bars) and coumarins (curve bars). Red and blue bars indicate the metabolites increase and decrease in concentrations, respectively. Three replicates for each treatment. In order to show the change of each kind metabolites clearly, three bars with large fold changes are represented by broken lines, and the numbers above each bar represent the actual data.

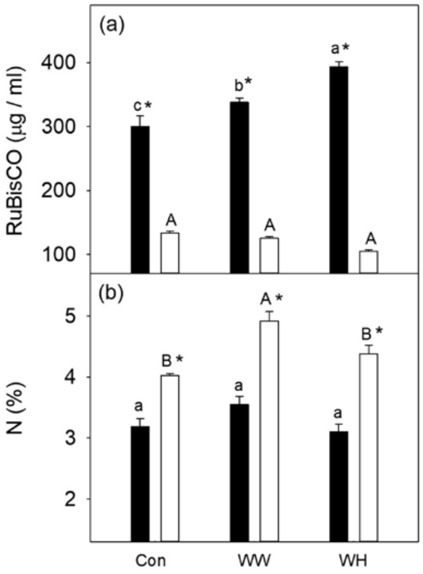

Figure 6: RuBisCO content (**a**) and leaf nitrogen content (**b**) of *E. adenophorum* (black bars) and *E. heterophyllum* (white bars) under mechanical wounding (WW) or *H. armigera* larvae feeding (WH) and in control (Con) plants. Values are means ± standard errors. n = 4–5 for each plant species. Different lowercase letters above bars indicate significant differences between treatments for *E. adenophorum*, and different capital letters above bars indicate significant differences between treatments for *E. heterophyllum*, respectively. The asterisk indicates a significant difference between *E. adenophorum* and *E. heterophyllum* at the same treatment.

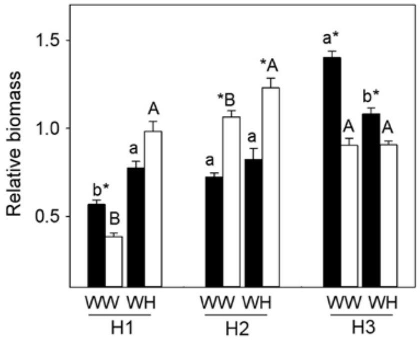

Figure 7: Relative biomass of *E. adenophorum* (black bars) and *E. heterophyllum* (white bars) under mechanical wounding (WW) or *H. armigera* larvae feeding (WH) at 21 days (H1), 46 days (H2) and 88 days (H3). Values are means ± standard errors. n = 4–5 for each plant species. Different lowercase letters above bars indicate significant differences between treatments for *E. adenophorum*, and different capital letters above bars indicate significant differences between treatments for *E. heterophyllum*, respectively. The asterisk indicates a significant difference between *E. adenophorum* and *E. heterophyllum* at the same treatment.

WW treatment. However, different damages induced a different set of DMs for both *Eupatorium* species, and a higher number of induced DMs were detected for *E. heterophyllum* than for *E. adenophorum* (Fig. 3); additionally, invasive *E. adenophorum* had significantly lower leaf N contents but higher RuBisCO contents than *E. heterophyllum* at both WW and WH treatments (Fig. 6), which indicated the invader allocated more leaf nitrogen to photosynthetic apparatus, and might contribute to its higher compensation capacity (Feng 2008; Lei *et al.* 2012; Turnbull *et al.* 2007). Liu *et al.* (2022b) also found significant differences in warming-induced changes in leaf chemistry between invasive *Alternanthera philoxeroides* and native congener.

The efficiency of PSII is a key factor affecting photosynthetic activity. Therefore, reduction in PSII efficiency (Aldea *et al.* 2006; Nabity *et al.* 2013; Zangerl *et al.* 2002) can indicate the suppression of photosynthetic activity in damaged leaves (Halitschke *et al.* 2011; Yu and Fan 2018; Zheng *et al.* 2012). In this study, we observed a significant reduction in Φ_{PSII} for both *Eupatorium* species in the remaining area of the damaged leaf with herbivory (Fig. 1), which may result from interruption of electron transport by physically disrupting tissue integrity (Halitschke *et al.*

2011; Nabity *et al.* 2013). This effect may be amplified by high transpiration water loss from the cut edges, which can lead to tissue desiccation (Aldea *et al.* 2005). However, both *Eupatorium* species maintain similar Φ_{PSII} in mechanically wounded leaves relative to undamaged leaves (relative $\Phi_{PSII} = 0.96$ and 1.06 for *E. adenophorum* and *E. heterophyllum*, respectively). The different PSII responses to herbivory and mechanical wounding may be due to specific damage inducing specific recognition and metabolic procedure in plants (Maffei *et al.* 2004; Mithöfer *et al.* 2005).

According to specific stimuli within 1 h, the suppression of PSII was accompanied with rapid biosynthesis of JA and the downstream active compound JA-Ile (Fig. 2), which suggested that the JA cascade may mediate plant responses to the specific initial cues, such as mechanical wounding, herbivory and pathogen infection (Howe and Jander 2008; Hummel *et al.* 2010; Yates-Stewart *et al.* 2020). It could result in transient suppression of photosynthesis by reducing expression of light-harvesting complex- and stomatal closure-related genes (Wu and Baldwin 2010). For example, in genetically edited *N. attenuata* plants, JA defence signalling was suppressed, and showed minimal suppression of photosynthesis (Nabity *et al.* 2013). Activation of the JA cascade can induce the defence response to protect plants from attack (Howe and Jander 2008). However, these metabolic changes caused by specific tissue injury signals are poorly understood (Schwachtje and Baldwin 2008; Steinbrenner *et al.* 2011). Construction of a defence system may require a long duration of interactions between plants and herbivores (Agrawal and Kotanen 2003; Morrison and Hay 2011; Parker *et al.* 2006). Therefore, the short co-evolutionary history of *E. adenophorum* with native herbivores (approximately 80 years) may limit the production of effective defence metabolites against herbivores (Fig. 3).

Different damage cues are able to trigger dramatically different metabolic procedures in the attacked leaves (Halitschke *et al.* 2011; Maffei *et al.* 2004; Peiffer and Felton 2005; Steinbrenner *et al.* 2011), which was also proved in our results that only 8 DMs were the same for leaves in the WW/Con and WH_S/Con for *E. adenophorum* and 11 DM_S for *E. heterophyllum*, respectively (Fig. 3), RDM_S and D-RDM_S upregulated greatly were all in WH_S/Con (Figs 4 and 5; Supplementary Table S2). It is likely that elicitors in *H. armigera* OSs constitute the main source of information through which plants recognize insect attack (Nabity *et al.* 2013). Indeed, induced defences are energy/resource costly, and tissue disruption is not always a reliable indicator of insect attack, such as that caused by hail or wind in the natural setting. Therefore, discerning real herbivory from mechanical wounding and then quickly deploying economical defences are critical skills in the battle between plants and herbivores, which can avoid wasting of energy allocated to defence-related compounds (Howe and Jander 2008).

Allocating resources to defence may lead to a reduction in plant growth (Peschiutta *et al.* 2018; Züst and Agrawal 2017). Although leaf N content in *E. adenophorum* was not higher than in *E. heterophyllum*, the higher proportion of N allocation to photosynthetic machinery as reported in earlier *E. adenophorum* studies (Feng 2008; Lei *et al.* 2012) may have resulted in increasing content of the photosynthetic enzyme RuBisCO in this study (Fig. 6) and promoted the high carbon income efficiency of *E. adenophorum* (Feng 2008; Lei *et al.* 2012). Compared with native *E. heterophyllum*, lower levels of induced defensive metabolites of *E. adenophorum* may relate to its lower compensation in biomass at H1 (WH) and H2 stages (Fig. 7). Besides, the difference of biomass allocation pattern between *E. adenophorum* and *E. heterophyllum* may also relate to their compensation difference. In previous studies, we found *E. heterophyllum* had lower leaf mass fraction (LMF) and life span but higher root/crown (R/C) ratio than *E. adenophorum* (Feng 2008; Wang and Feng 2016). Higher R/C could facilitate the remobilization of resources sequestrated in the site away from injury (Babst *et al.* 2005; Schwachtje and Baldwin 2008; Wang *et al.* 2013), which contributed higher compensation growth to *E. heterophyllum* in the early stage (Fig. 7). However, higher LMF, life span and nitrogen allocation to photosynthetic machinery could contribute high carbon accumulation to *E. adenophorum* in long-term duration, which may relate with its higher compensation in biomass at later stage (H3).

At H3 stage, compared with mechanical treatment, the lower compensation rate of *E. adenophorum* in *H. armigera* herbivory treatment (Fig. 7) might due to a much longer-lasting energy-consuming process of herbivory and the substantial increase in defence investments in D-RDM_S, which may hinder the compensatory construction of new photosynthetic organs. For example, feeding by another native generalist insect, *S. exigua*, exacerbated the effects of mechanical damage for *E. adenophorum* by inducing more cadinene sesquiterpenes, and herbivory could induce a larger quantity of antifeedant chemical (compound 17) than mechanical damage (Liu *et al.* 2021). After 24 h of leaf damage, the generalist *H. zea* altered many more defence-related metabolites than mechanical damage in leaves, which suggests an enhanced plant response to *H. zea* herbivory (Steinbrenner *et al.* 2011).

In summary, the recognition and differentiation of specific attackers by invasive *E. adenophorum* and native *E. heterophyllum* were clearly different. The low-level expression of induced defences and increased compensation relative to its native congeneric plant *E. heterophyllum* (in long-term damage) could explain the successful invasion of *E. adenophorum*. Future findings on the effects of specific metabolites combined with molecular biology will increase our understanding of the mechanisms through which exotic plants respond to biological threats in new introduced locations.

Supplementary Material

Supplementary material is available at *Journal of Plant Ecology* online.

Table S1: Results from linear effect models of the effects of herbivory treatment, species and their interaction on phytohormones, chlorophyll fluorescence and compensatory variables.

Table S2: Total differentially expressed metabolites of *E. adenophorum* and *E. heterophyllum* after WW and WH_s treatments.

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