

Plant genotypes affect aboveground and belowground herbivore interactions by changing chemical defense

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Abstract Spatially separated aboveground (AG) and belowground (BG) herbivores are closely linked through shared host plants, and both patterns of AG-BG interactions and plant responses may vary among plant genotypes. We subjected invasive (USA) and native (China) genotypes of tallow tree (*Triadica sebifera*) to herbivory by the AG specialist leaf-rolling weevil *Heterapoderopsis bicallosicollis* and/or the root-feeding larvae of flea beetle *Bika-sha collaris*. We measured leaf damage and leaves rolled by weevils, quantified beetle survival, and analyzed flavonoid and tannin concentrations in leaves and roots. AG and BG herbivores formed negative feedbacks on both native and invasive genotypes. Leaf damage by weevils and the number of beetle larvae emerging as adults were higher on invasive genotypes. Beetles reduced weevil damage and

weevils reduced beetle larval emergence more strongly for invasive genotypes. Invasive genotypes had lower leaf and root tannins than native genotypes. BG beetles decreased leaf tannins of native genotypes but increased root tannins of invasive genotypes. AG herbivory increased root flavonoids of invasive genotypes while BG herbivory decreased leaf flavonoids. Invasive genotypes had lower AG and BG herbivore resistance, and negative AG-BG herbivore feedbacks were much stronger for invasive genotypes. Lower tannin concentrations explained overall better AG and BG herbivore performances on invasive genotypes. However, changes in tannins and flavonoids affected AG and BG herbivores differently. These results suggest that divergent selection on chemical production in invasive plants may be critical in regulating herbivore performances and novel AG and BG herbivore communities in new environments.

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Introduction

Plant-mediated linkages between aboveground (AG) and belowground (BG) herbivore systems are increasingly recognized as important in shaping herbivore population dynamics and community structure (Johnson et al. 2013; Rasmann and Turlings 2007; Wang et al. 2014). AG and BG herbivore interactions may be mediated by changes in quantities and spatial distributions of primary and secondary metabolites (Huang et al. 2014; Maestre et al. 2003; Masters et al. 1993; van Geem et al. 2013), resulting in negative, positive, or neutral effects on plant fitness (Fournier et al. 2006; Maron 1998; Morris et al. 2007). However, variable responses in plants and herbivores make

it difficult to predict plant-mediated AG-BG interactions (Bezemer and van Dam 2005).

Plant genotypes can differ in their responses to AG or BG herbivores (Harvey et al. 2011; Wurst et al. 2008), reciprocally affecting the preference and performance of AG and BG herbivores (Johnson 2008) and higher trophic levels (Harvey et al. 2011). Wurst et al. (2008) found that a root-feeding *Agriotes* wireworm induced different responses in *Plantago lanceolata* genotypes that varied with iridoid glycosides, thus affecting AG herbivore damage. Plant responses to herbivores can be highly species-specific (Singh et al. 2014), and plant genotypes may also vary in responses to herbivory (Miller et al. 2014). However, few studies have compared induced responses of different plant genotypes to AG-BG herbivore interactions, or how these induced responses mediate AG and BG herbivore interactions (Huang et al. 2014).

Invasive plants are ideal subjects to study plant genotype mediated AG-BG herbivore interactions. Invasive plants are often released from AG and BG specialists of their native ranges (enemy release hypothesis) (Keane and Crawley 2002), however, they may suffer damage from AG and/or BG generalist herbivores in their introduced ranges. In addition, the guilds of herbivores in the introduced range may only be a subset of those in the native range (DeWalt et al. 2004; Joshi and Vrieling 2005). The altered herbivore interactions in new ranges can potentially impose selection on secondary defenses of invasive plants in terms of types, concentrations and/or distributions of chemical defenses (Agrawal et al. 2005; Engelkes et al. 2008; Van der Putten 2012). In particular, such evolved defense responses of invasive plants may modify negative or positive feedbacks on AG and BG herbivores, which potentially underpin community structure in new environments (Harvey et al. 2010; Van der Putten 2012). Therefore, comparison of the defense responses of invasive and native genotypes to AG and BG herbivores may yield new insights into the mechanisms of plant genotype mediated AG-BG herbivore interactions (van Dam and Heil 2011; Van der Putten 2012). Furthermore, to increase competitive ability in introduced ranges, especially when BG herbivores may be lacking, invasive plants may adopt an “aboveground first strategy” of allocating more resources aboveground (Huang et al. 2012). These asymmetric resource allocations for growth and defense between shoots and roots may alter the interactions between above and belowground herbivores in invasive plants. To date, tests on these predictions are rare (He et al. 2014; Huang et al. 2012; van Geem et al. 2013).

In this study, we employed *Triadica sebifera* (L.) Small (tallow tree, Euphorbiaceae) (*Triadica* hereafter) as a model species to test whether introduced and native populations of invasive plants differ in mediating AG and BG herbivore interactions. *Triadica* is a woody species native

to China that is aggressively invasive in many parts of the southeastern United States (Bruce et al. 1997). *Triadica* supports a diversity of AG and BG herbivores in China (Zheng et al. 2004), but populations in the introduced range experience only low levels of foliar herbivory by generalists and no belowground herbivore attack (Bruce et al. 1997; Siemann and Rogers 2003a, b). Recent studies found that *Triadica* invasive genotypes have lower resistance but higher tolerance to leaf-chewing herbivores compared to those from the native range (Wang et al. 2011, 2012; Zou et al. 2008). Chemical analyses found that *Triadica* plants from the invasive range have higher flavonoids but lower tannins than plants from native populations (Wang et al. 2012). In the context of AG-BG interactions, Huang et al. (2013) showed that adults of *Bikasha collaris* (Chrysomelidae: Coleoptera), a specialist flea beetle on *Triadica*, facilitated the survival of its conspecific larvae, while the larvae inhibited adult survival (Huang et al. 2013), and these feedbacks were stronger for U.S genotypes (Huang et al. 2012). As plant defense may be species-specific and differ among herbivore types (Bezemer and van Dam 2005; Huang et al. 2014), the outcomes of AG-BG interactions may differ between conspecifics and heterospecifics (Clark et al. 2011; Huang et al. 2014; Wurst and van der Putten 2007). Moreover, native and invasive genotypes may differ in their responses to damage by heterospecific herbivores to shoots and roots but this has never been tested for *Triadica* or any other invasive plants. Therefore, investigation of plant genotype responses to heterospecific AG and BG herbivore species can lead to a better understanding of how plant genotypes mediate AG-BG interactions.

Here, we subjected invasive (USA) and native (China) genotypes of *Triadica* trees to herbivory by adults of the foliar specialist leaf-rolling weevil *Heterapoderopsis bicallosicollis* (Attelabidae: Coleoptera) and the root-feeding larvae of *B. collaris*, to compare patterns of AG-BG herbivore interactions on different plant genotypes. We also examined induced defense responses of genetically differentiated plants to these AG-BG heterospecific herbivores. *Heterapoderopsis bicallosicollis* and *B. collaris* adults feed on leaves while *B. collaris* larvae feed on roots. Given these heterospecific AG and BG herbivore occurrences often overlap and our previous study suggested that *Triadica* introduced and native genotypes differed in resource allocation between roots and shoots (Huang et al. 2012), we hypothesized that patterns of AG-BG heterospecific herbivore interactions and plant defenses responses may be different between native and invasive genotypes. We specifically ask the following questions: (1) How do the presence of AG or BG herbivores affect the performances of heterospecific herbivores from the opposite group? (2) Do the interactions between AG and BG herbivores vary between plant invasive and native genotypes? (3) Do secondary

chemical defense responses (tannins and flavonoids) of different plant genotypes differ between AG and BG herbivores? Results will elucidate the effects of evolved chemical defense in invasive plants on AG and BG herbivore communities in new environments.

Materials and methods

Herbivore system

The leaf-rolling weevil *H. bicallosicollis* and the flea beetle *B. collaris* are two major specialist insects of *Triadica* in China (Huang et al. 2011; Wang et al. 2009). Adults of *H. bicallosicollis* feed on leaves and lay eggs inside cradles constructed by the female weevils (Wang et al. 2009). Adults of *B. collaris* feed on leaves and oviposit in the soil and larvae feed on roots. In the laboratory, *B. collaris* egg, larval and pupal life stages last 9, 18 and 14 days, respectively (Huang et al. 2011). There is an extensive overlap in the times when they are found on *Triadica* in the field and each can be very abundant (Huang et al. 2014).

Seeds and seedlings

We hand-collected *Triadica* seeds from at least five trees in each of ten native populations across southern China and nine invasive populations across the southeastern United States in late November 2011 (Table S1). After removing the waxy coats, we buried seeds in moist sand and stored them at 4 °C to break dormancy. In May 2012, we planted 100 seeds per population in topsoil mixed with sphagnum peat moss (1:1) in a greenhouse. After 1 month of growth, we transplanted similar-sized seedlings individually into pots (25 cm diameter), and randomly arranged them in a common garden at the Wuhan Botanical Garden (30°32' N, 114°24' E), Hubei, China. We watered seedlings every 2 days to keep the soil moist. We enclosed each seedling in a nylon mesh cage (100 cm height, 28 cm diameter) with the cage fastened tightly to the stem and then to pot rim with an elastic band to exclude other herbivores.

Common garden experiment

To examine AG and BG herbivore interactions on native and invasive *Triadica* populations, we conducted a full factorial experiment starting in July 2012. We assigned each seedling to an AG weevil and BG beetle treatment in a factorial design [0 vs. 2 pairs of *H. bicallosicollis* adults/seedling (AG) × 0 vs. 10 *B. collaris* larvae/seedling (BG)]. In total, there were 228 seedlings (19 × 2 × 2 × three replicates).

For AG herbivory, we collected adults of *H. bicallosicollis* from Luotain county, Hubei province, in June 2012, and raised them in large nylon cages with *Triadica* trees at Wuhan Botanical Garden. For BG herbivory, we used eggs of *B. collaris* instead of larvae for experimental manipulations. We collected approximately 300 pairs of naturally mating adults of *B. collaris* from *Triadica* trees growing at Wuhan Botanical Garden, and transferred each pair of adults to a 10 cm diameter petri dish with moist filter paper. We supplied each dish with a fresh *Triadica* leaf as food for adults. We kept all the dishes at 28 °C with 50 ~ 70 % relative humidity and a 14 h photoperiod in the laboratory.

One week after transplanting, we randomly assigned similar-sized seedlings to AG and BG herbivory treatments. To establish BG herbivory, we transferred ten newly laid eggs directly to a ~3.5 cm hole dug in the soil at the base of each plant, and covered them with moist soil. Nine days later, which is approximately the developmental duration from egg to larva, we released two pairs of naturally mating adults of *H. bicallosicollis* into the cages of seedlings in the AG herbivory treatment. Because we sealed all the nylon cages including the control group to the seedling stem, AG and BG insects did not contact each other.

Beginning 3 days after adding AG herbivores, we recorded the numbers of cradles and the percentage of leaf area damaged by *H. bicallosicollis* adults every 2 days to assess the performance of AG herbivores. We determined damage percentage (%) by visual estimates for each leaf and then averaging the visual estimates for all damaged leaves. We recorded the number of *B. collaris* adults emerging from soil every 2 days.

Chemical analyses

We analyzed total tannins and total flavonoids for plants with different herbivory treatments. We ground dried leaves and roots of each seedling to a fine powder. We assessed four tannins (gallic acid, catechin, tannic acid and ellagic acid) and five flavonoids (quercetin, isoquercetin, quercetin glycoside, kaempferitrin and kaempferol) by high-performance liquid chromatography (HPLC) according to the methods in Wang et al. (2012). We calculated the total concentrations of tannins and flavonoids in leaves, roots as the sum of the above chemical concentrations, and their ratios in roots to leaves.

Statistical analyses

We tested the dependence of AG weevil damage (binomial model) and leaf rolls (poisson model) on origin, population nested in origin, BG beetle presence, and their interactions using generalized linear mixed models (GLMMs,

Proc Glimmix, SAS 9.4). We examined the dependence of BG larval survival (binomial model) on origin, population nested in origin, AG weevil presence, and their interactions in a GLMM.

We conducted four-way ANOVAs to examine origin, population nested in origin, AG weevil presence, BG beetle presence, and their interactions on tannin and flavonoid concentrations in leaves and roots, and the ratio of their concentrations in roots to those in leaves (Proc Mixed, SAS 9.4). We used plant population (ten from China and nine from US) nested in origin to test for significance of origin in these and other models. We used the interactions of population nested in origin with beetle and/or weevil presence to test for the significance of the interactions of origin with these factors. We used adjusted means partial difference tests to determine whether treatment levels differed for significant terms with more than two levels.

Results

Leaf damage from weevils was greater on invasive plants than native plants when beetle larvae were absent, but beetles reduced weevil damage more strongly for invasive plants so they experienced comparable amounts of damage when beetles were present (Table 1; Fig. 1). The amounts of weevil damage varied among populations (Table 1, Fig S1a). The number of leaf rolls made by weevils was not affected by population origin ($F_{1,17} = 0.4$, $P = 0.54$), beetle presence ($F_{1,76} = 2.0$, $P = 0.16$), or their interaction ($F_{1,17} = 2.9$, $P = 0.10$).

The number of beetle larvae emerging as adults was higher for invasive plants than for native plants, especially when weevils were absent because they reduced emergence more strongly for invasive plants (Table 1; Fig. 2).

Tannin concentrations in leaves varied with population origin, weevil presence, origin \times beetle presence, and weevil presence \times beetle presence (Table 2). Plants with

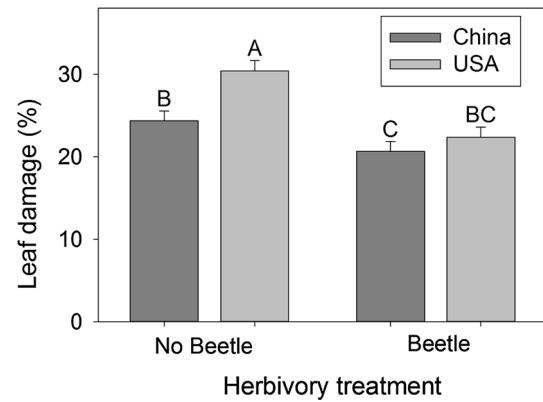


Fig. 1 Foliar chewing damage from AG feeding *Heterapoderopsis bicallosicollis* weevils for invasive (USA) or native (China) tallow populations with BG feeding *Bikasha collaris* beetle larvae absent or present. Values are means + 1 SE ($n = 114$). Means with the same letters were not significantly different in post hoc tests

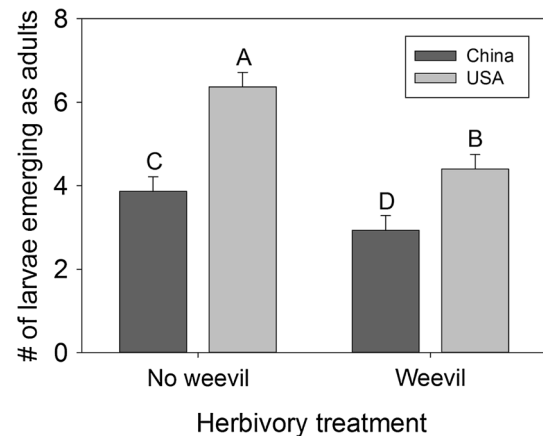


Fig. 2 The number of BG feeding *Bikasha collaris* beetle larvae emerging as adults for invasive (USA) or native (China) tallow populations with AG feeding *Heterapoderopsis bicallosicollis* weevils absent or present. Values are means + 1 SE ($n = 114$). Means with the same letters were not significantly different in post hoc tests

Table 1 The effects of plant origin (USA vs. China), the presence or absence of the other herbivore, population nested in origin, and their interactions on leaf chewing damage from weevils or number of beetle larvae emerging as adults in GLMMs (Proc Glimmix)

AG weevil performance				BG beetle performance			
Effect	Leaf damage			Effect	Larval emergence		
	df	F	P		df	F	P
Origin	1,17	7.4	0.0146	Origin	1,17	139.6	<0.0001
Beetle	1,76	48.7	<0.0001	Weevil	1,76	24.5	<0.0001
Origin \times beetle	1,17	3.6	0.0749	Origin \times weevil	1,17	6.5	0.0208
Population (origin)	17,76	2.8	0.0012	Population (origin)	17,76	0.3	0.9940
Population \times beetle (origin)	17,76	1.6	0.0794	Population \times weevil (origin)	17,76	0.4	0.9848

F tests for origin terms use the corresponding population nested in origin term for error. Significant results with P value <0.05 are shown in bold. N = 114 for each model

Table 2 The effects of plant origin (USA vs. China), the presence or absence of BG beetle herbivory, the presence or absence of AG weevil herbivory, population nested in origin, and their interactions on total tannin and flavonoid concentrations in leaves and roots, and the ratio of their concentrations in roots to those in shoots (R:S)

Effect	df	Leaf tannins		Root tannins		Tannin R:S		Leaf flavs		Root flavs		Flav R:S	
		F	P	F	P	F	P	F	P	F	P	F	P
Origin	1,17	32.6	<0.0001	0.1	0.7969	9.6	0.0065	0.8	0.3713	5.1	0.0380	3.2	0.0911
Beetle	1,152	0.3	0.5592	9.3	0.0027	9.7	0.0022	13.3	0.0004	0.6	0.4534	0.8	0.3731
Weevil	1,152	27.5	0.0001	3.4	0.0672	0.8	0.3752	9.3	0.0028	27.8	0.0001	21.3	0.0001
Origin × beetle	1,17	8.5	0.0098	30.7	<0.0001	12.2	0.0028	7.9	0.0121	0.1	0.7849	1.3	0.2791
Origin × weevil	1,17	0.1	0.7488	1.6	0.2258	2.0	0.1777	3.6	0.0735	3.0	0.1003	4.8	0.0428
Beetle × weevil	1,152	6.5	0.0119	0.8	0.3876	<0.1	0.8344	3.1	0.0824	0.7	0.4056	0.2	0.6821
Origin × B × W	1,17	1.6	0.2256	0.9	0.3481	2.0	0.1786	1.2	0.2897	<0.1	0.8428	0.1	0.8181
Population (origin)	17,152	2.0	0.0168	1.2	0.2939	1.3	0.2291	2.0	0.0120	3.4	<0.0001	2.6	0.0010
Population × B (origin)	17,152	0.7	0.7679	0.4	0.9802	0.4	0.9878	0.7	0.8357	0.7	0.8506	0.6	0.9171
Population × W (origin)	17,152	1.5	0.0973	0.9	0.5998	1.3	0.1892	0.6	0.8997	3.3	<0.0001	2.0	0.0150
Population × B × W(origin)	17,152	0.9	0.6181	0.6	0.8994	0.6	0.8660	1.4	0.1255	0.7	0.7545	1.1	0.3543

F tests for origin terms use the corresponding population nested in origin term for error. Significant results with P value <0.05 are shown in bold. N = 228

only weevils had the highest foliar tannin concentrations and those with no herbivores had the lowest concentrations (Fig. 3a). Beetles reduced foliar tannin concentrations in native plants, but foliar tannin concentrations in native plants were always higher than those in invasive plants (Fig. 3b). The concentration of tannins in leaves varied among populations (Table 2, Fig S1b). Tannin concentrations in roots were highest in invasive plants with beetles and lowest in invasive plants without beetles and intermediate for native plants (Table 2; Fig. 3c). The ratio of tannin concentrations in roots to those in leaves was higher for invasive populations with beetles (0.311 ± 0.018) than for invasive plants without beetles (0.219 ± 0.018) or native plants with (0.213 ± 0.018) or without beetles (0.196 ± 0.018) which were all similar to each other.

Beetle or weevil presence each affected flavonoid concentrations in leaves (Table 2). Plants with no herbivores had higher foliar flavonoid concentrations than those with only weevils, only beetles, or weevils and beetles simultaneously (Fig. 4a). The interaction of plant origin and beetle presence affected foliar flavonoid concentrations (Table 2) with reductions by beetle herbivory only significant for invasive plants (Fig. 4b). Flavonoids in roots varied with weevil presence and plant origin × weevil presence (Table 2) with increases with weevil herbivory only significant for invasive plants (Fig. 4c). The ratio of flavonoid concentrations in roots to those in leaves was higher for invasive populations with weevils (0.369 ± 0.022) than for invasive plants without weevils (0.207 ± 0.022), or native plants with (0.244 ± 0.022) or without weevils (0.211 ± 0.022) which were all similar to each other. Effects of weevil herbivory on both root flavonoids and the

ratio of flavonoid concentrations in roots to those in leaves varied among plant populations (Table 2; Fig S2).

Discussion

Patterns of AG-BG herbivore interactions and induced defense responses in plants can vary among plant genotypes and herbivore identities (Huang et al. 2012; Singh et al. 2014; Wurst et al. 2008). Previous studies found that *B. collaris* adults facilitated their conspecific larval survival, and these feedbacks were stronger for US plants. Our study with *Triadica* populations and interactions between AG *H. bicallosicollis* adults and BG *B. collaris* larvae found that heterospecific weevils and beetles formed negative feedbacks, which were stronger for invasive genotypes. Lower tannins explained overall better AG and BG herbivore performances on invasive genotypes. However, changes in tannin and flavonoid concentrations in leaves and/or shoots differently affected AG and BG herbivores: tannins were likely transported from shoots to roots with BG herbivory, while AG herbivory might have limited transportation of flavonoids from shoots to roots. These results may suggest that differences among plant genotypes can underpin AG and BG herbivore interactions because of their varying defense metabolite responses.

The interactive effects between AG and BG herbivores are frequently mediated by host plants (Wardle et al. 2004) and herbivore types (such as chewing herbivores and sucking herbivores) (Hooper et al. 2000; Johnson et al. 2012; Soler et al. 2013). In our study, both the feeding of weevils and survival of the beetles were inhibited by the presence

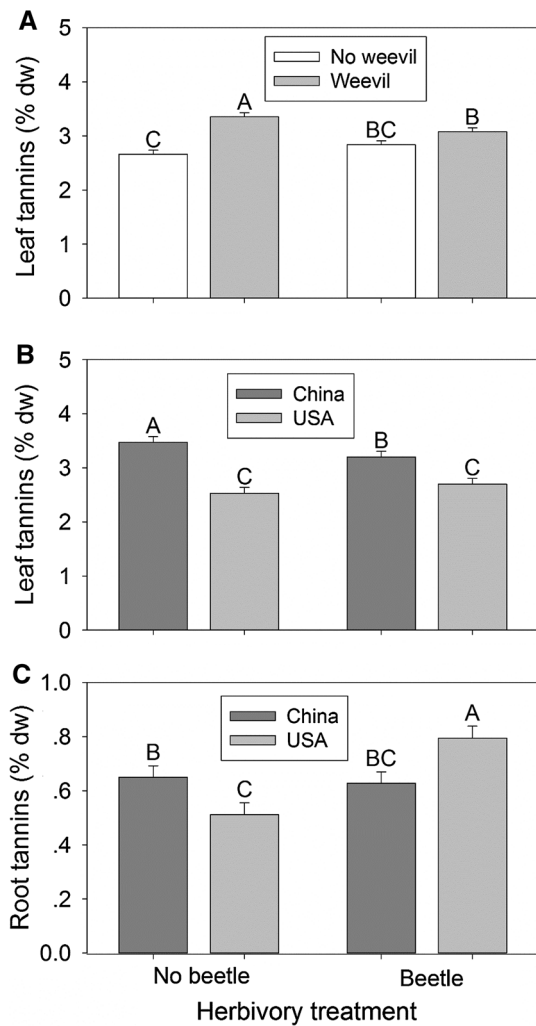


Fig. 3 **a** Effects of BG feeding *Bikasha collaris* beetle herbivory and/or AG feeding *Heterapoderopsis bicallosicollis* weevil herbivory on total tannin concentrations in leaves. Effects of BG feeding *B. collaris* beetle herbivory on total tannin concentrations in **b** leaves and **c** roots of invasive (USA) and native (China) tallow genotypes. Values are means + 1 SE (n = 228). Means with the same letters were not significantly different in post hoc tests

of herbivory from the opposite space (Figs. 2, 3), indicating AG-BG heterospecific herbivores formed a negative feedback. In fact, a number of studies have found that AG herbivores could negatively affect the performance of other herbivores in soil (Huang et al. 2014; Masters et al. 1993; Soler et al. 2005, 2007). However, Huang et al. (2012) found that the survival of *B. collaris* larvae was significantly higher with the presence of its AG conspecific adults. So patterns of AG-BG interactions may differ in conspecific and heterospecific herbivores.

The patterns of AG-BG herbivore interactions may also differ among plant genotypes (van Dam and Heil 2011; van Geem et al. 2013). In our study, although leaf damage and the number of beetle larvae emerging as adults were greater

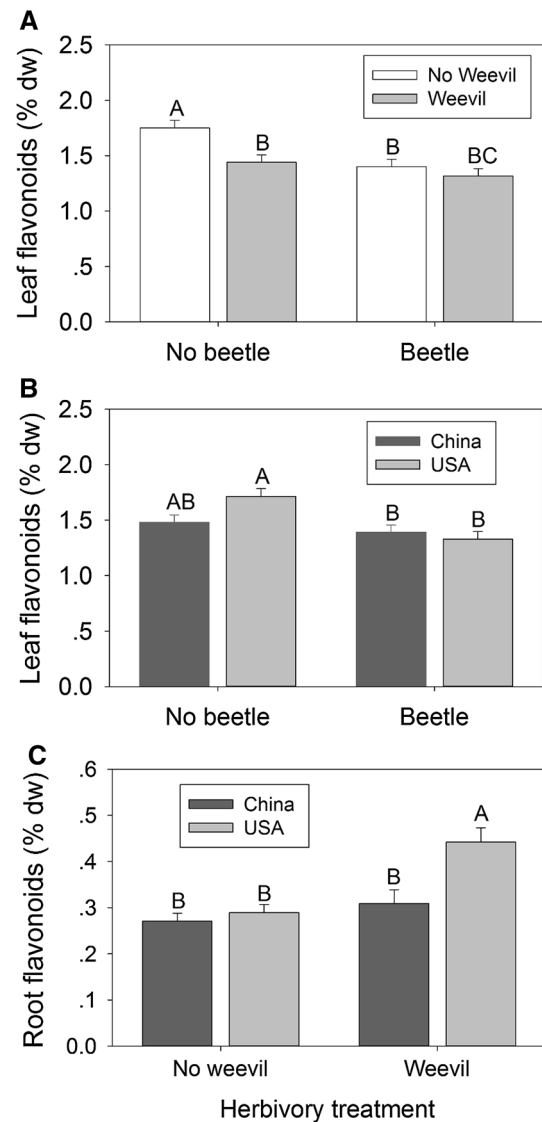


Fig. 4 **a** Effects of BG feeding *Bikasha collaris* beetle herbivory and/or AG feeding *Heterapoderopsis bicallosicollis* weevil herbivory on total flavonoid concentrations in leaves. **b** Effects of BG feeding *B. collaris* beetle herbivory on total flavonoid concentrations in leaves of invasive (USA) and native (China) tallow genotypes. **c** Effects of AG feeding *H. bicallosicollis* weevil herbivory on total flavonoid concentrations in roots of invasive (USA) and native (China) tallow genotypes. Values are means + 1 SE (n = 228). Means with the same letters were not significantly different in post hoc tests

on invasive plants when beetle larvae or weevil adults were absent, the presence of beetles reduced weevil damage and the presence of weevils reduced beetle larval emergence more strongly for invasive plants (Fig. 1a, b). These results suggest that negative feedback between AG-BG interactions was stronger on invasive genotypes. Huang et al. (2012) also found positive feedbacks between *B. collaris* adults and their larval survival were stronger for invasive plants. Some previous studies have suggested that plant

genotypes can greatly affect the performances of AG and BG herbivores as well as their interactive effects (Huang et al. 2012; Singh et al. 2014), and changes in primary or secondary metabolites may contribute to contrasting AG and BG interactions (Haase et al. 2008; Huang et al. 2012; Singh et al. 2014). Varying competitive hierarchies of herbivores among plant genotypes may allow them to coexist within a population and to be more competitive on different plant genotypes (Smith et al. 2008).

The ‘evolution of increased competitive ability’ (EICA) hypothesis states that invasive plants may shift their resources from herbivore defense to growth or reproduction because of the absence of herbivores (Blossey and Nötzold 1995). In our study, both the damage of weevils and beetle larval emergence were higher on US *Triadica* regardless of the presence of the other herbivore (Figs. 2, 3), and we found lower tannins in plants from the introduced range regardless of AG or BG herbivore presence, suggesting that the introduced populations have a lower resistance to AG and BG herbivores, which supports the EICA hypothesis. A similar pattern was reported recently that invasive *Triadica* showed lower resistance to *B. collaris* adults and its conspecific larvae (Huang et al. 2012). A further analysis of resource allocation and chemical responses in invasive plants to AG-BG herbivore interactions may help us to better understand the mechanisms.

Tannins are considered to be an important inducible defense of many plants, such as *Triadica* (Wang et al. 2012), against specialists (Forkner et al. 2004; Müller-Schärer et al. 2004). Tannin concentrations may increase after plants are attacked by specialists, especially those that attack shoots (Huang et al. 2013; Wang et al. 2012). On average, AG weevil herbivory induced the highest tannins in *Triadica* plants compared with BG herbivory or herbivory by both AG-BG insects (Fig. 3a). Some studies documented that AG herbivory may induce local defense in shoots, while BG herbivory may induce systemic defense of plants (Kaplan et al. 2008). However, our study found that tannins in leaves of native plants decreased with BG beetles without significantly affecting root tannins (Fig. 3b), and BG beetles increased tannins in roots of invasive plants and had no effect on shoot tannins (Fig. 3c). Also, the ratio of tannin concentrations in roots to those in leaves was higher for invasive populations with beetles than for invasive plants without beetles or native plants with or without beetles. These results indicate a local transportation of tannins from leaves to roots in invasive populations with BG herbivory. Thus, BG beetles might induce a local response in tannins of invasive genotypes, but neither local or systemic responses were induced in tannins of native genotypes.

We found that the ratio of flavonoid concentrations in roots to those in leaves was higher for invasive populations

with weevils than for invasive plants without weevils or native plants with or without weevils, suggesting that flavonoids were transported from leaves to roots with AG herbivory. Many plants produce flavonoids which may affect feeding or oviposition behaviors of insects (Simmonds 2001). Flavonoids as defensive compounds are considered primarily effective against generalists, while some specialists can sequester flavonoids as a defense against predators or use flavonoids to locate host plants for oviposition and/or feeding (Napal et al. 2010; Treutter 2006). We found that leaf flavonoids decreased with BG beetles (Fig. 4b) and the leaf damage of AG weevils also decreased (Fig. 1a) (especially for invasive genotypes). In contrast, flavonoids in roots increased with AG weevils (Fig. 4c) and beetle survival also decreased (Fig. 2) (especially for invasive genotypes), indicating flavonoids may be positively associated with the performances of weevils but negatively associated with the survival of beetles. Even though root flavonoids have been shown to inhibit the performance of generalist root-feeding beetle larvae (Rao 1990), we do not know whether the flavonoids measured here negatively impact *B. collaris* larval performance. Wang et al. (2012) also found that the performance of the specialist caterpillar *Gadirtha inexacta* was positively related to higher flavonoid concentrations in introduced populations of *Triadica*.

However, the performances of AG-BG herbivores could not be fully explained by changes in tannin and/or flavonoid concentrations in roots and leaves. For example, BG herbivory decreased the damage of AG weevils, but tannin and flavonoid concentrations in leaves also decreased (native genotypes) or did not change (invasive genotypes) (Figs. 3b, 4b). Similarly, AG herbivory inhibited the emergence of BG beetles, but had no significant effects on tannin concentrations in roots (Fig. 3c). BG herbivory can greatly reduce plant growth and fitness, strongly impacting the performances of AG herbivores (Blossey and Hunt-Joshi 2003); similarly, AG herbivory may also strongly affect BG herbivores by direct reductions in root biomass (Milano et al. 2015). We assume that changes in plant growth rate, primary chemicals, such as carbon and nitrogen (Huang et al. 2013), or other secondary chemicals like volatile chemicals (Aratchige et al. 2004) may also mediate interactions between weevils and beetles. As there are trade-offs between different types of secondary chemical defenses and resource allocation between plant growth and defense (Agrawal et al. 2005; Blossey and Nötzold 1995; Zou et al. 2008), comprehensive analyses of the primary and secondary chemical changes in different *Triadica* genotypes are required to fully understand AG and BG herbivore interactions.

In summary, our study provides evidence that genetically differentiated plants (such as native versus invasive genotypes) vary in how they affect AG and BG herbivore

performances and their interactions, suggesting divergent selection on plant defenses plays an important role in mediating AG and BG herbivore interactions. This study may lead to a better understanding of how alien invasive plants affect interactions between AG and BG herbivores in new environments through chemical responses. In addition, it further emphasizes the value of including plant genotypes when investigating AG-BG interactions.

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Author contribution statement All authors conceived the study, XL and WG conducted the fieldwork. XL, ES and JD performed the data analyses and wrote the manuscript. All authors discussed the results and commented on the manuscript.

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