Title: Divergence in herbivore resistance during the invasion of a global plant invader

Authors

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Abstract

Premise: Adaptation to novel biotic and abiotic environments has often been assumed in successful invasions. However, invasive species often occupy broad climatic niches within their native and introduced ranges, and a lack of invasion history and sampling bias may obscure the ability to establish how traits have changed in invasive populations from those of the origin population.

Methods: We tested heritable differences in herbivore resistance of the global invasive species, *Reynoutria japonica* (Japanese knotweed) by using 128 populations from both introduced (Europe and North America) and native ranges (China; Japan) in two replicated common gardens in the native range, we also explored associations between climatic and herbivore environments of the source habitats with variation in herbivore resistance within and among ranges.

Results: We found that introduced European and North American populations were similar in all foliar resistance traits to that of native Japanese populations, but different from that of the native Chinese populations in both common gardens. On the whole, Chinese populations tended to be taller, exhibited higher LDMC and leaf C:N ratio, but lower leaf thickness, as well as leaf flavonoids compared with introduced European, North American, and native Japanese populations. In the native range, we found herbivore resistance was strongly associated with climate and herbivore pressure at the collection habitats, however, considerably few local adaptation patterns were detected in introduced European and North American populations.

Synthesis: Our results indicate that the introduction of certain genotypes (i.e. founder effects) played an important role in biogeographic divergence in herbivore resistance. All-purpose genotype and lack of genetically based differentiations to biotic and abiotic environmental factors might together lead to the successful invasion of *Reynoutria*

japonica in the introduced ranges.

Keywords: *Reynoutria japonica*, common gardens, plant invasion, rapid adaptive evolution, plant defense, plant-herbivore interactions, plant-climate interactions

1. Introduction

The number of invasive plant species has increased dramatically over the past two centuries (Seebens et al., 2017), causing exceedingly negative impacts on environment (Powell et al., 2013; Bellard et al., 2016; Castro-Díez et al., 2019) and economy (Bradshaw et al., 2016; Diagne et al., 2021). In the context of globalization and climate change, one-sixth of the world's land surface is expected to be highly vulnerable to invasion (Early et al., 2016), and the number of new invasive species is likely to increase further (Seebens et al., 2017, 2021) Thus, a comprehensive understanding of the mechanisms that underlie the successful invasion of alien species is an urgent issue in ecology and evolution (Kempel et al., 2013; Pyšek et al., 2020).

Many hypotheses predict that increased performance of invasive populations is driven by adaptation to novel abiotic or biotic conditions, such as fewer natural enemies or a more benign environmental conditions in the introduced ranges (Cripps et al., 2006; Mitchell et al., 2006; Montti et al., 2016). The enemy release hypothesis (ERH) states that plant species would experience a decrease in regulation by herbivores and other natural enemies, resulting in a rapid increase in performance (Keane and Crawley, 2002). Since resistance can be costly (Koricheva, 2002), shifts in enemy pressure can lead to rapid evolutionary changes in growth and defense allocation (Bossdorf et al., 2005; Buswell et al., 2011; Li et al., 2022). The evolution of increased competitive ability (EICA) hypothesis predicts that invasive populations may have less herbivore resistance by allocating fewer resources to costly herbivore defences (Blossey and Nötzold, 1995; Joshi and Vrieling, 2005; Lin et al., 2015). Despite strong evidence for enemy release (Keane and Crawley, 2002; Liu et al., 2006; Castells et al., 2013; Rotter et al., 2019; Xiao et al., 2020), especially from specialists, the evidence in support of the prediction of EICA is mixed (Bossdorf et al., 2005; Felker-Quinn et al., 2013; Rotter

and Holeski, 2018). For example, lower resistance against herbivory has been observed in several invasive species (Siemann and Rogers, 2001; Jakobs et al., 2004; Huang and Ding, 2016), while similar or even higher resistance was documented in others (Genton et al., 2005; Lewis et al., 2006; Alba et al., 2011; Bhattarai et al., 2017; Gruntman et al., 2017; Lin et al., 2019). These mixed results have also been suggested to be related to the costs required for different types of resistance (Koricheva, 2002; Neilson et al., 2013), as well as changes in herbivore community composition experienced by invasive plants upon arrival in the invasive range (Müller-Schärer et al., 2004, the shifting defence hypothesis (SDH); Cripps et al., 2006).

Climate, as well, often varies between native and introduced ranges of invasive species (Early and Sax, 2014; Bocsi et al., 2016), and adaptation may occur in response to altered environmental conditions (Buswell et al., 2011; Alexander et al., 2012; Lee and Kotanen, 2015). Heritable differences in traits between native and introduced plant populations across similar environmental gradients suggest rapid adaptation to climate after introduction (Etterson et al., 2008; Alexander, 2013; Woods and Sultan, 2022). In the introduced ranges, climates can directly affect plant defenses by imposing physiological constraints as well as determining resource availability (Coley, 1985; Wright et al., 2004). Climate factors can also influence the abundance, and diversity of natural herbivores in plant communities (Maron et al., 2014; Anstett et al., 2016; Zhang et al., 2016), thus indirectly driving selection for certain plant traits (Loughnan and Williams, 2019). Therefore, understanding the impact of natural enemy release and climatic factors on invasive populations' herbivore resistance is critical to understanding mechanisms for invasion success, as well as predicting which habitats are most vulnerable to future invasions. Nevertheless, the associations between climate and herbivores within native and introduced populations have rarely been explored

simultaneously in common garden experiments (Xiao et al., 2020).

Although comparisons have been frequently made between native and introduced populations, conclusions from such studies can be ambiguous due to several shortcomings (van Kleunen et al., 2018; Woods and Sultan, 2022). First, lack of invasion history and sampling biases may obscure the ability to establish how traits have changed in invasive populations compared to origin populations (Cano et al., 2009; Yang et al., 2014; Bukovinszky et al., 2014). This can be challenging because invasive species often occupy broad climatic niches within their native as well as in the introduced range (Brandenburger et al., 2019, 2020; Liu et al., 2020). Secondly, the conditions of a given common garden may not elicit important differences between native and introduced populations, and studies have shown that growth and defense traits vary among gardens since the geographical location of the common garden determines abiotic and biotic environmental conditions (e.g. climate, interacting species, etc.; Maron et al., 2004; Moloney et al., 2009; Qin et al., 2013; Yang et al., 2021). Common garden comparisons in the native range will be more informative since both specialists and generalists' herbivores are expected to exist in the native range, but specialists might not be found in the invasive range (Maron et al., 2004).

In this study we ask if the introduced plant populations have evolved decreased herbivore resistance compared to the native populations and whether changes in defense traits are related to herbivore pressure and climatic factors at the collection sites. We compared the biogeographic divergence in herbivore resistance and the potential drivers for the differences between native and introduced populations of the perennial Japanese knotweeds (*Reynoutria japonica*) by growing 128 populations, 55 from the native range in China and Japan, along with 73 from the invasive ranges in Europe and North America, in two common gardens with differed climate factors in the native range.

The worldwide distribution and relatively clear invasion history provide an excellent opportunity for us to explore the trait divergence and its potential mechanisms during the invasion process. We addressed the following hypotheses: (1) introduced populations have lower herbivore resistance than native populations. (2) Traits of native populations are more strongly affected by biotic and abiotic factors at the sampling sites than those of introduced populations.

2. Materials and methods

2.1 Study system

Reynoutria japonica (Japanese knotweed) is native to eastern Asia and introduced to Europe in the early 1840s along with its sister species *R. sachalinensis* (Bailey and Conolly, 2000), and to North America in 1870s as ornamental, then widely naturalized in both regions (Barney, 2006; Shimoda and Yamasaki, 2016; Del Tredici, 2017). *R. japonica*, *R. sachalinensis*, and their hybrid *R.* × *bohemica* spread rapidly once introduced, especially along river banks or roadsides, forming dense stands and often spreading hundreds of square meters (Bímová et al., 2004; Tiébré et al., 2008; Rouifed et al., 2014). Knotweed invasion seriously threatens biodiversity and integrity of ecosystems (Stoll et al., 2012; Mincheva et al., 2014) and cause substantial economic damage as well (Reinhardt et al., 2003; DEFRA, 2003). It has become a major environmental threat in Europe and North America and is listed among the 100 world's worst invasive alien species (Lowe et al., 2000).

2.2 Sample collection

We conducted a cross-latitudinal survey of 150 Japanese knotweeds (*R. japonica* and the hybrid *R.* × *bohemica*) populations at field sites in the native range of China

(23.29°N to 36.86°N) and the introduced range of North America (34.24°N to 44.94°N) and Europe (44.67°N to 59.94°N). In China, we sampled from Xunwu in Guangdong to Zouping in Shandong between 8 July-16 August in 2020. In Europe, we sampled from Carmagnola, Italy to Uppsala, Sweden between 30 May-16 June 2019. In the United States, we sampled from Commerce, Georgia to Milford, Maine between 2-19 June 2019 (Fig. 1, Table S1).

In each range, we surveyed 50 populations along a 2000 km transect (sampling approximately every 40 km). At each site, we identified the taxa (*R. japonica* or *R.* × *bohemica*) based on morphological characters (Bailey et al. 2009), measured the width and length of the population and laid a 30 m transect for sampling. We selected five knotweed stems at regular intervals along the transect at 2, 8, 14, 20 and 26 m. When a population was smaller than ~28 m in length, we reduced the distance between stems, but no less than one meter.

We also collected six native source *R. japonica* populations Nagasaki, Japan (32.81°N to 33.33°N) from 28 April to 4 May 2021 which was identified as the potential source region of the European and North American populations (Bailey and Conolly, 2000; Del Tredici, 2017). For Japanese populations, we collected rhizomes from 5 to 8 individuals at each site separated by at least 6 m. The phylogenetic structure based on chloroplast genes indicated that those Japanese populations were the native source populations of the introduced knotweeds in Europe and North America, while Chinese populations dispersed from Japanese ancestral lineage around 2.0-3.5 Ma (Zhang et al. unpublished data).

We stored the Chinese rhizomes at 4 °C for 2 weeks before planting them in pots. We imported and grew rhizomes from Japanese, European, and North American populations in a quarantine glasshouse at Xishuangbanna Tropical Botanical Garden,

Chinese Academy of Sciences (Yunnan, China) before we set up the common garden experiment. Due to legal restrictions, only one rhizome from each of the North America populations were successfully imported till we set up the experiment.

2.3 Common garden experiment set up

We established two common gardens, one at Fudan University in Shanghai (31°20′N, 121°30′E) and another at Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences in Yunnan (21°41′N, 101°25′E), with *R. japonica* rhizomes of 55 populations from native (5 populations from Japan; 50 populations from China) and 73 populations from the introduced range (46 populations from Europe; 27 populations from North America) (Fig1, Table S1). Both common gardens were set up on open flat ground areas, with weed mats to cover the ground to prevent weeds in each of the common gardens.

Before planting the rhizomes, we cut rhizomes from each of the individuals and then kept them under 4 °C for at least 4 weeks to facilitate sprouting success. We removed all fine roots and cut them to about the same size of 3-10g (with at least one node) and weighed the fresh weight (± 0.1 g) for each of the rhizomes. On 11 March 2022, all rhizomes were treated with fungicide and then planted separately into 10.8 L plastic pots filled with 3.2 kg potting soil (Pindstrup Mosebrug A/S, Denmark) in both gardens. Due to rhizome availability, we had 518 (27 individuals from 27 populations of North America; 218 individuals from 46 populations of Europe; 239 individuals from 50 populations of China, and 34 individuals from 5 populations of Japan) individuals in Shanghai garden, and 463 (16 individuals from 16 populations of North America; 190 individuals from 46 populations of Europe; 232 individuals from 50 populations of China and 25 individuals from 5 populations of Japan) *R. japonica* individuals in

Xishuangbanna garden. After transplantation, we randomly distributed all pots into 5 blocks, with 1 individual from each of the populations in each block whenever possible. In each of the gardens, the distance between pots was at least 90 cm to avoid aboveground competition. We added 10 g Osmocote fertilizer twice (Everris International B.V., Heerlen, Netherlands) to each pot at the beginning (11 March 2022) and in the middle (16 August 2022) of the experiment. During the experiment, we checked the plants every day and watered all plants when the potting soil became dry. We put a tray under each of the pots to avoid loss of water and nutrients during watering. The pots, soils, and fertilizers used in the two common gardens were identical.

2.4 Measurements of foliar traits and herbivore damage

We quantified plant traits related to herbivore resistance (leaf toughness, lignin, flavonoids, alkaloids), and also quantified the natural herbivore damage of each individual under the natural herbivore conditions in both common gardens in the middle of the growing season (Figure S1). Previous studies have shown that other leaf traits, such as SLA, leaf thickness, leaf dry matter content, and leaf C:N ratio are also related to herbivore resistance (Feeny, 1976; Elger and Willby, 2003; Schmitz, 2008; Feng et al., 2011; Lin et al., 2015; Rotter et al., 2019). Therefore, we also measured those traits in this study.

We measured the length of the tallest shoot in each pot from the soil surface (±0.1 cm) and recorded it as the plant height of each individual. Then we sampled five leaves (1, 2, 4, 5, 6 leaves on the tallest shoot, counting from most recent fully developed leaves) to determine plant leaf traits. The fresh weight of five leaves from each plant were quickly weighed (± 0.001g). Leaf thickness and toughness of each plant was then been determined by using the mean values of each of the five leaves. Specifically, leaf

thickness was measured with a digital micrometer (Digimatic Outside Micrometer, Mitutoyo, Japan), and leaf toughness with a penetrometer (FA10, SAUTER, Balingen, Germany) in Shanghai garden and with a mechanical testing machine (ZQ990A, Dongguan Zhiqu Precision Instrument Co., Ltd, China) in Xishuangbanna garden. Then we scanned the five leaves of the same plant using a scanner (Epson Perfection V330 Photo Scanner; Epson, Suwa, Japan) and calculated the leaf area by Fiji ImageJ v.1.53c software (NIH, Bethesda, MD, USA). We dried all leaves at 60°C for 72h to determine leaf dry mass. We calculated specific leaf area (SLA) as the ratio of leaf area to leaf dry mass, and leaf dry matter content (LDMC) as the ratio of leaf dry mass to leaf fresh mass.

We also quantified the herbivore damage by measuring the percentage of leaf area eaten for each individual plants two days before the leaf trait measurements. We further subdivided the leaf area eaten into beetles and caterpillars' damage based on chewing modes and leaf notch types (Figure S2; personal observation). All measurements were carried out from July 14 to 21, 2022 in Xishuangbanna garden and from July 24 to 31, 2022 in Shanghai garden.

2.5 Leaf secondary chemical compounds analyses

We quantified leaf chemistry traits using the dried samples of five leaves from each plant. We measured leaf total N, C, lignin, and leaf secondary metabolites (alkaloids and total flavonoids) of the dried leaves after grinding samples to the required particle size with a ball mill (MM400, Retsch, Germany). We measured total C and N with an organic elemental analyzer (FlashSmart™ Elemental Analyzer, Thermo-Fisher Scientific, USA) via thermal combustion and TCD/IR detection of CO_2/N_2. We measured leaf lignin, alkaloids and total flavonoids using the MZS-1-G, SWJ-1-Y and

LHT-1-G test kits (Suzhou Comin Biotechnology Co., Ltd., Suzhou, China), respectively. In total, we measured 981 individual plants sampled from both common gardens (518 and 463 individuals in Shanghai and Xishuangbanna garden, respectively).

2.6 Historical climatic and herbivore pressure of collection habitats

We extracted climatic data for 1970-2000 from the WorldClim database (Fick and Hijmans, 2017), and focused specifically on eight climate variables considering that they represent more biologically meaningful descriptions of the conditions that plants experienced during the growing season and overwintering relative to annual averages (Keller et al., 2009). The eight variables were mean annual temperature (bio1), maximum temperature of warmest month (bio4), minimum temperature of coldest month (bio5), temperature seasonality (bio6), mean annual precipitation (bio12), precipitation of the wettest month (bio13), precipitation of the driest month (bio14), precipitation seasonality (bio15).

We estimated the extent of herbivore pressure suffered by *R. japonica* (i.e., ratio of leaf area eaten) in field during sampling (Irimia et al. unpublished data).

2.7 Statistical analyses

We performed all analyses with R version 4.2.1 (R Core Team, 2022). We used linear mixed models to examine the effects of range, garden, and their interactions on plant traits and herbivore damage. We treated range (China, Japan, Europe, North America), garden (Shanghai, Xishuangbanna) and their interactions as fixed effects, while population and block as random effects. We also used initial rhizome weight as a covariate to account for the variances in initial size. The significance of fixed effects in the mixed models was assessed by the Type III Wald chi-squared test using the *car*

package (Fox and Weisberg, 2018). For the indicator that displayed a significant garden or range effect ($P < 0.05$), we conducted a Tukey post hoc test with the *emmeans* () and the *pairs* () functions (*emmeans* package, Lenth, 2018). We *log*-transformed herbivore damage data to normalize the distribution of residuals when necessary.

To identify the relationship between leaf traits and herbivore damage, we performed spearman correlation analysis to calculate the correlation coefficient between trait mean and herbivore damage mean of each population within each garden using the *Hmisc* package (Hauke and Kossowski, 2011).

To evaluate whether abiotic and herbivore pressure factors of each population's collection sites explain traits associated with herbivore resistance of native and invasive populations, we conducted the following analyses. We firstly summarized climate variables of populations' collection sites by conducting a principal component analyses (PCA) using the *prcomp* function. We then performed population-level simple linear regression analyses (i.e. with population means) by treating first principle component of climate or field leaf damage as a fixed effect on traits associated with herbivore resistance of native and introduced populations in common gardens, respectively (Moreira et al., 2018; Galmán et al., 2021).

3. Results

3.1 Differences in herbivore damage among ranges

Herbivore damage differed remarkably in the two common gardens (Fig.2). The percentage of leaf area eaten beetles and caterpillars in Xishuangbanna were 3.1 times and 18.9 times higher than that of Shanghai garden, respectively (Fig.2).

In Shanghai, Leaf area eaten by caterpillars of Chinese ('CN' hereafter) populations were higher than that of European ('EU' hereafter) and North American

('NA' hereafter) populations (Fig.2c). In Xishuangbanna, there was no pronounced difference in percentage of leaf area eaten among ranges (Fig.2a). The CN populations suffered higher beetle damage compared to EU and NA populations, and lower caterpillar damage compared to EU populations (Fig.2bc). No significant differences were detected in caterpillar and beetle damages among EU, NA populations, and Japanese ('JA' hereafter) populations (Fig.2).

3.2 Differences in herbivore resistance traits among ranges

In Shanghai, CN populations were taller, exhibited higher LDMC and leaf C:N ratio, but lower leaf thickness as well as leaf flavonoids compared with introduced EU, NA, and native JA populations (except leaf flavonoids). CN populations also displayed lower SLA but higher leaf toughness compared with the EU population. In Xishuangbanna, CN populations were taller, displayed higher LDMC, leaf C:N ratio (except EU populations), and thinner leaves compared with introduced EU and NA populations, taller and thinner leaves than JA populations (Fig.3). SLA, leaf toughness, lignin, alkaloids and flavonoids content did not differ among ranges within Xishuangbanna gardens (Fig.3). No significant differences were detected in all traits between JA populations and EU and NA populations in both common gardens (Fig.3).

3.3 Correlation between leaf resistance traits and herbivore damage

Herbivore damage levels related to different plant traits in different gardens (Fig.4, Table S2). In Shanghai, the percentage of leaf area eaten by caterpillars were positively correlated with height, LDMC and leaf C:N ratio, but were negatively related to leaf thickness and flavonoids content (Fig.4, Table S2). Populations with larger SLA tended to suffer less beetles damage (Fig.4b, Table S2).

In Xishuangbanna, the percentage of leaf area eaten was positively correlated with plant height and LDMC (Table S2). There were also differences in plant traits that affect feeding by different insect types. Plants were taller, with larger LDMC, lower leaf thickness and flavonoids content suffered from more beetle feeding (Fig.4, Table S2). Plants that are smaller and have thicker leaves were more susceptible to caterpillar feeding (Fig.4, Table S2).

3.4 Effects of climate and herbivore pressure of collection habitats on plant traits

The first principal component ('climate PC1' hereafter) explained more than 54% of the variance in the eight climatic variables across all populations, and was negatively related to the mean annual temperature, minimum temperature of coldest month and precipitation of the wettest month (Fig.5, Table S3). Compared with the introduction areas of Europe and North America, the native ranges of China and Japan have higher temperature, precipitation, as well as higher seasonality of temperature and precipitation (Fig.5).

Significant correlations between climatic PCs and plant traits, herbivore damage of collection habitats and plant traits were pervasive within native populations, however, considerably fewer significant correlations were detected with climatic PCs, herbivore damage of collection habitats, and plant traits within introduced populations (Fig.6, Fig.7).

In terms of the impact of climate at the collection sites on plant traits, SLA and leaf flavonoids content of native populations were positively related to climate PC1, while leaf thickness and C:N ratio were negatively correlated with climate PC1 in both common gardens (Fig. 6). The LDMC of the native populations also decreased with the increase of climate PC1 in Xishuangbanna garden (Fig. 6c). We only detected that the

leaf flavonoids content of the introduced populations increased with the increase of climate PC1 within Xishuangbanna garden (Fig. 6f). Specifically, native populations from warmer and humid sites tended to have higher LDMC, leaf C:N ratio, and thicker leaves as well as lower SLA and leaf flavonoids content (Fig. 5, Fig. 6, Table S3). In Xishuangbanna, introduced populations from warm and humid areas tend to have higher leaf flavonoids content (Fig. 5, Fig. 6f, Table S3).

The relationships between plant traits and herbivore pressure of collection habitats showed that, knotweeds of CN populations from sites with severe herbivory damage tended to decreased SLA and leaf flavonoids content in both common gardens, but increased LDMC in Xishuangbanna garden (Fig. 7c). Introduced populations facing severe herbivory at sampling sites tended to be taller within Xishuangbanna common garden (Fig. 7a).

4. Discussion

Divergence in importance ecological traits are frequently inferred in successful invasions, however, the mechanisms underlying the shifts have been ambiguous. In this study, we tested heritable differences in herbivore resistance of *R. japonica* by using 128 populations from both introduced and native ranges in two replicate common gardens in the native range. We also explored how climate and herbivory of the collection habitats predicted variation in herbivore resistance among populations within each of the ranges. Our results showed that introduced European and North American populations were similar in herbivore resistance traits to the putative native source Japanese populations, and together different from native Chinese populations. Herbivore resistance of native populations was strongly affected by the climate and

herbivory at the collection habitats, but only weaker local adaptation patterns were found in invasive populations.

4.1 Differences in herbivore resistance among ranges

The EICA hypothesis predicted that the costly defense traits of introduced populations will be lower due to release from native herbivores (Blossey and Notzold, 1995). In this study, we found that introduced European and North American populations were significantly diverged in most foliar traits from the native Chinese populations, however, no differences in those traits were detected when compared to the putative native source Japanese populations. Our results showed the importance of identifying the source populations within the native range to determine if invasive plants have evolved during their invasion (Colautti and Lau, 2016; Brandenburger et al., 2020).

In our study, we found no significant differences in herbivore resistance traits, such as leaf C:N ratio, leaf toughness, leaf flavonoids, leaf lignin, or alkaloids between introduced European, North American, and native Japanese populations in both Shanghai and Xishuangbanna gardens. Similar patterns existed when we looked at herbivore damage of plants grown under two different natural herbivore environments in both gardens in the native range. Our results did not support the EICA prediction that invasive populations have lower herbivore resistance than native populations (Blossey and Notzold, 1995). No difference or even higher resistance of invasive populations are also found in other invasive species within common garden experiment, for example, in *Chromolaena odorata* (Liao et al., 2014), *Brassica nigra* (Oduor et al., 2011), and in *Verbascum Thapsus* (Endriss et al., 2018). Similar or higher resistance and performance of introduced Japanese populations of *R. japonica* was also documented by Rouifed et

al. (2018), in which they found that introduced populations did not differ in composition of secondary metabolites, stem stiffness, leaf thickness, aboveground biomass and height, but were higher in leaf toughness and belowground biomass to the native *R. japonica* populations when grown under similar condition in the introduced range. As the Japanese populations used in our experiments were likely from the source region of the introduced European and North American populations, our results thus provide evidence that the introduction of certain genotypes (i.e. founder effects) played an important role in the invasion success of *R. japonica*.

Our study found that the herbivore damage varied substantially in two different gardens, and related to different foliar traits under different environments. For instance, in Shanghai, herbivore damage was strongly associated with height, SLA, LDMC, leaf thickness, leaf C:N ratio and flavonoids content. While in Xishuangbanna garden, the damage rates were strongly associated with height, LDMC, leaf thickness and flavonoids content. It should be noted that the herbivore diversity, abundance, and feeding guild differed significantly among the two common gardens (Joosten and Veen, 2011). Therefore, the different plant responses between the two gardens might be due to the feeding preferences of the different types of herbivores (Van der Meijden, 1996; Bossdorf et al., 2004; Yang et al., 2014). In fact, in Xishuangbanna garden, we found that damage by beetles was associated with different traits than damage by caterpillars. The beetles prefer taller plants, thinner leaves with lower flavonoids, and higher LDMC. The caterpillars prefer leaves that are thicker and plants are smaller. Besides, the climate, average temperature, precipitation, and solar radiation were different between the two common gardens during the experiment (Table S4). And leaf traits could also play an important role in other aspects such as enhance plant resistance to abiotic stress like ultraviolet radiation and drought (Levitt and Lovett, 1985; Strauss and Agrawal, 1999;

Harborne and Williams, 2000; Peter Constabel et al., 2014; Barton and Boege, 2017; Li et al., 2022). Our results thus indicate the importance of using multiple common gardens in the native range when testing the herbivore resistance of the invasive plants (Maron et al. 2004; Qin et al., 2013; Yang et al. 2014; Bossdorf et al. 2015; Yang et al., 2021).

4.2 Effect of climatic factors and herbivore damage of collection habitats on plant traits

In our study, we found significant differences in temperature and precipitation between native and introduced Japanese population collection areas (Fig.5). Our results suggested that *R. japonica* may have shifted niches during the invasion of Europe and North America. Niche shifting has been reported in other invasive species (Gallagher et al., 2010; Early and Sax, 2014; Atwater et al., 2018; Bates and Bertelsmeier, 2021). For example, *Centaurea stoebe* (Broennimann et al., 2007), *Ulex europaeus* (Christina et al., 2020) and *Rumex* species (Carlin et al., 2023) appear to have shifted their climate niche from the native niche in the non-native ranges.

We found that climate and herbivore damage of the collection sites predicted traits in both common gardens for plants from the native range. In comparison, we only detect few significant relationships between traits in common garden and environmental characteristics of the source sites for plants from the introduced ranges. A large body of studies have documented the development of parallel clinal patterns in ecological traits among populations of native and introduced ranges (Agrawal et al., 2015; Leger and Rice, 2007; Etterson et al., 2008; Rosche et al., 2019; Hodgins et al., 2020). Others have shown unparalleled (Bhattarai et al., 2017; Yang et al., 2021; Woods and Sultan, 2022), or no clinal patterns (Endriss et al., 2018; Sun and Roderick, 2019) in successful

invasions. Our results provide little evidence that genetic differentiation occurred in foliar traits for populations among introduced European or North American ranges.

Our study found a lack of genetic differentiations in response to climate and herbivore pressures in the introduced European and North American populations. These findings might be due to several reasons. First, the introduction of an all-purpose genotype (Baker, 1965) from Japan, as the majority of invasive *R. japonica* populations are thought to be descendants of a single clone (Hollingsworth and Bailey, 2000; Richards et al., 2012; Gaskin et al., 2014; Groeneveld et al., 2014; Zhang et al., 2016). If the single genotype has a higher fitness, then there is no genetically based variation for selection to act on even in the face of very different biotic and abiotic factors in introduced ranges (Richards et al., 2006). Second, adaptation challenges will not occur if traits in the native range can be co-opted for different uses in the introduced range (i.e., a process termed exaptation; Hufbauer et al., 2012). Third, insufficient time for adaptation to occur. Although rapid evolutionary changes have frequently been reported, examples from the invasion literature show that geographic clines generally develop 50-150 generations after introduction (Moran and Alexander, 2014). *R. japonica* were introduced to Europe about 170 years ago and introduced to North America less than 50 years ago, and began to expand rapidly in the past 100 years in Europe (Bailey and Conolly, 2000), this may suggest that insufficient time for the perennials. However, there is also studies suggesting that mutations may be common enough to result in genetic variation that can be exploited for adaptation, even within a relatively short period of time after an invasion (Lynch and Conery, 2000, Ossowski et al., 2010). Population (and quantitative) genomics approaches based on high-throughput sequencing and genotyping technologies could be used to better understand the genetic architecture behind invasion success in the future (Bock et al., 2015; Estoup et al., 2016).

Since *R. japonica* is a perennial plant, the results of one-year experiments may need to be treated with caution. Furthermore, our study did not clarify whether the herbivore in the two common gardens were specialists or generalists, measuring plant palatability using specialists and generalists will help us better understand the evolution of defensive traits during plant invasion in the future.

5. Conclusion

Our study used replicate common gardens and employed a biogeographical approach with samples spanning an exceptionally large climatic gradient from both introduced and native ranges to test the mechanisms that contribute to the herbivore resistance divergence of invasive species *R. japonica*. Our study shows that the introduction of certain genotypes (i.e. founder effects) might contribute to the biogeographical divergence of herbivore resistance of *R. japonica*. All-purpose-genotype and lack of genetically based differentiations to biotic and abiotic environmental factors might together lead to the successful invasion of *R. japonica* in the introduced ranges. Our study emphasizes the importance of combining invasion history and using multiple common gardens in the native range when testing the mechanisms underlying the resistance divergence in successful invasions.

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Authors' contributions

BL, OB, CLR, MP, ZL and PC designed the experiments. PC, ZL, SW, LZ, YZ, and JB performed the research. PC, ZL and TL analyzed the data and drew the figures. PC, SW, LZ, TL, MP, CLR, OB, ZL and BL contributed to writing and/or revising the paper. All authors read and approved the manuscript.

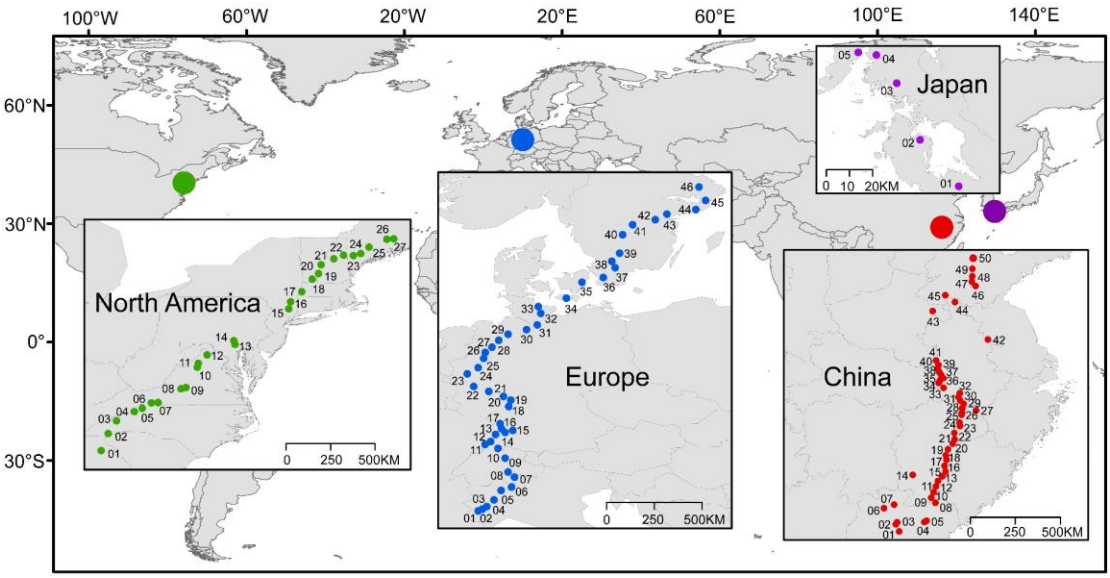

Fig. 1. Sampling sites of *R. japonica* populations in the introduced ranges of North America (N = 27 populations) and Europe (N = 46 populations), and in the native ranges of China (N = 50 populations) and Japan (N = 5 populations). The larger points represent the mean centers of population locations in the four ranges respectively.

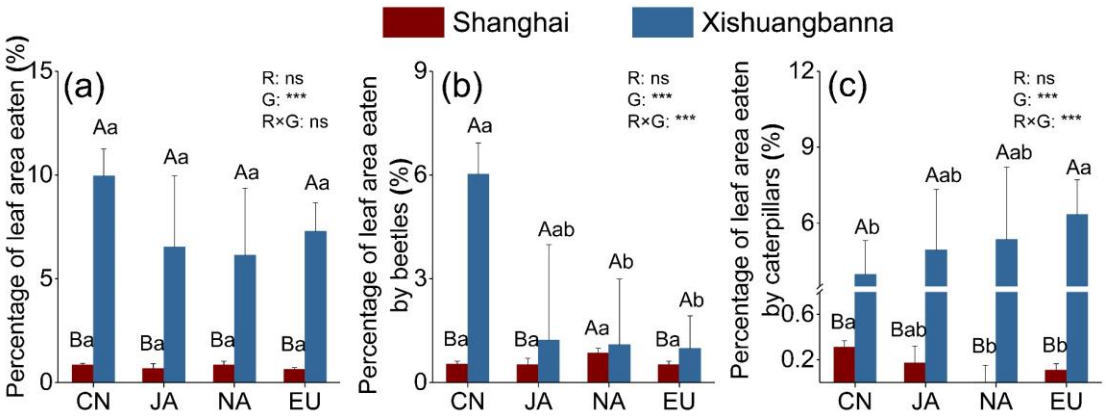

Fig. 2. Regional variation in herbivores damage of *R. japonica* from native China (CN) and Japan (JA) and introduced North America (NA), Europe (EU) ranges. Red represents Shanghai common garden's results, and blue represent the results of Xishuangbanna common garden. The same capital letter means that plants of the same range have no significant differences between two common gardens, and the same lowercase letter means that plants of different origin have no significant differences in the same common garden. Histograms are regional mean comparisons. Bars indicate means + 1 standard error. In each panel, the P-values were reported for the effects of garden (G), plant range (R), and their interactions (G×R) as *chi-square* tested. n.s. =not significant; *, *P*<0.05; **, *P*<0.01; *, *P*<0.001.**

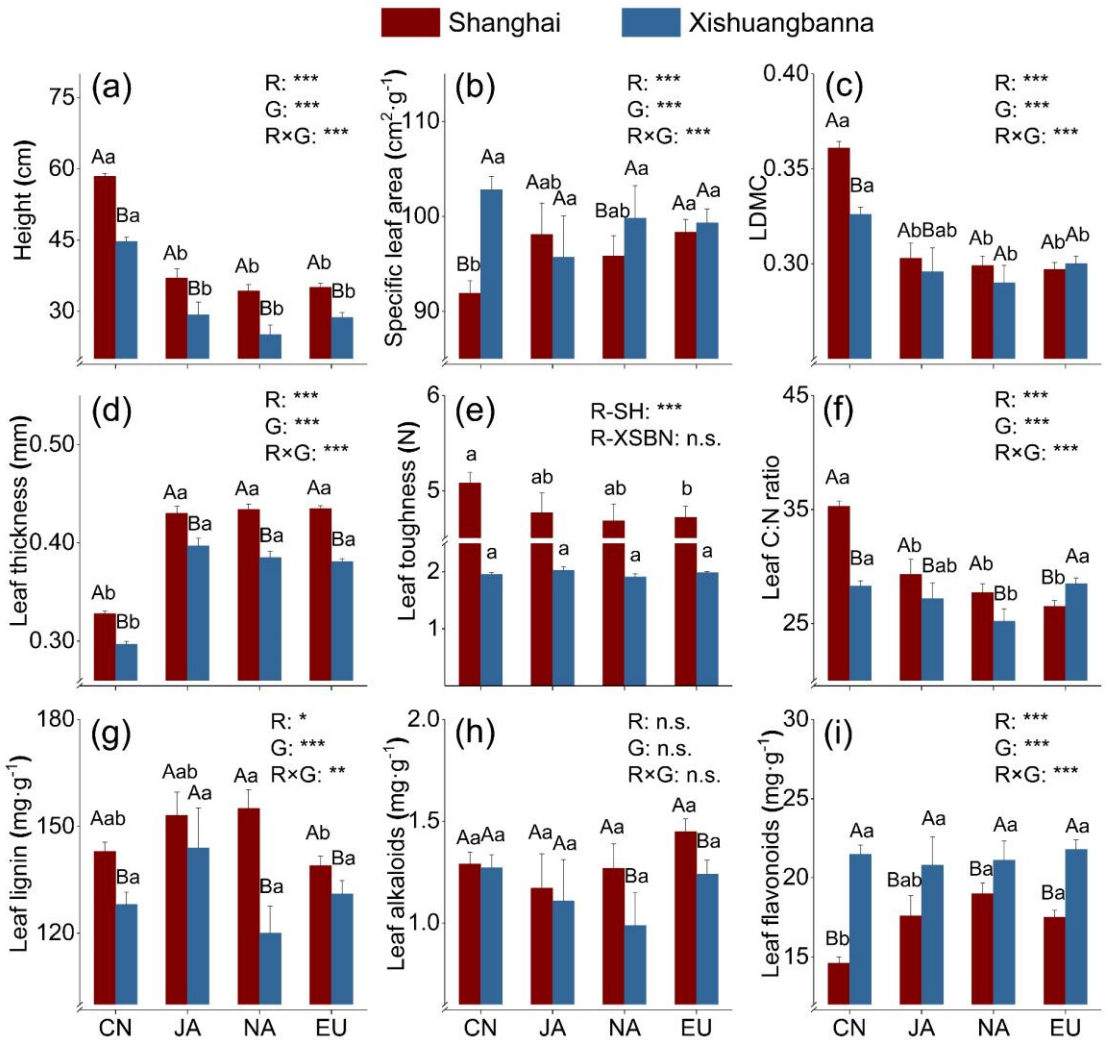

Fig. 3. Regional variation in plant performance of *R. japonica* from native China (CN) and Japan (JA) and introduced North America (NA), Europe (EU) ranges. Red: Shanghai garden, blue: Xishuangbanna garden. The same capital letter means that plants of the same range have no significant differences between two common gardens, and the same lowercase letter means that plants of different origin have no significant differences in the same common garden. Histograms are regional mean comparisons. Bars indicate means + 1 standard error. In each panel, the P-values were reported for the effects of garden (G), plant range (R), and their interactions (G×R) as *chi-square* tested. n.s. =not significant; *, *P*<0.05; **, *P*<0.01; *, *P*<0.001. Leaf toughness were incomparable between two gardens since we used different instruments in measurement.**

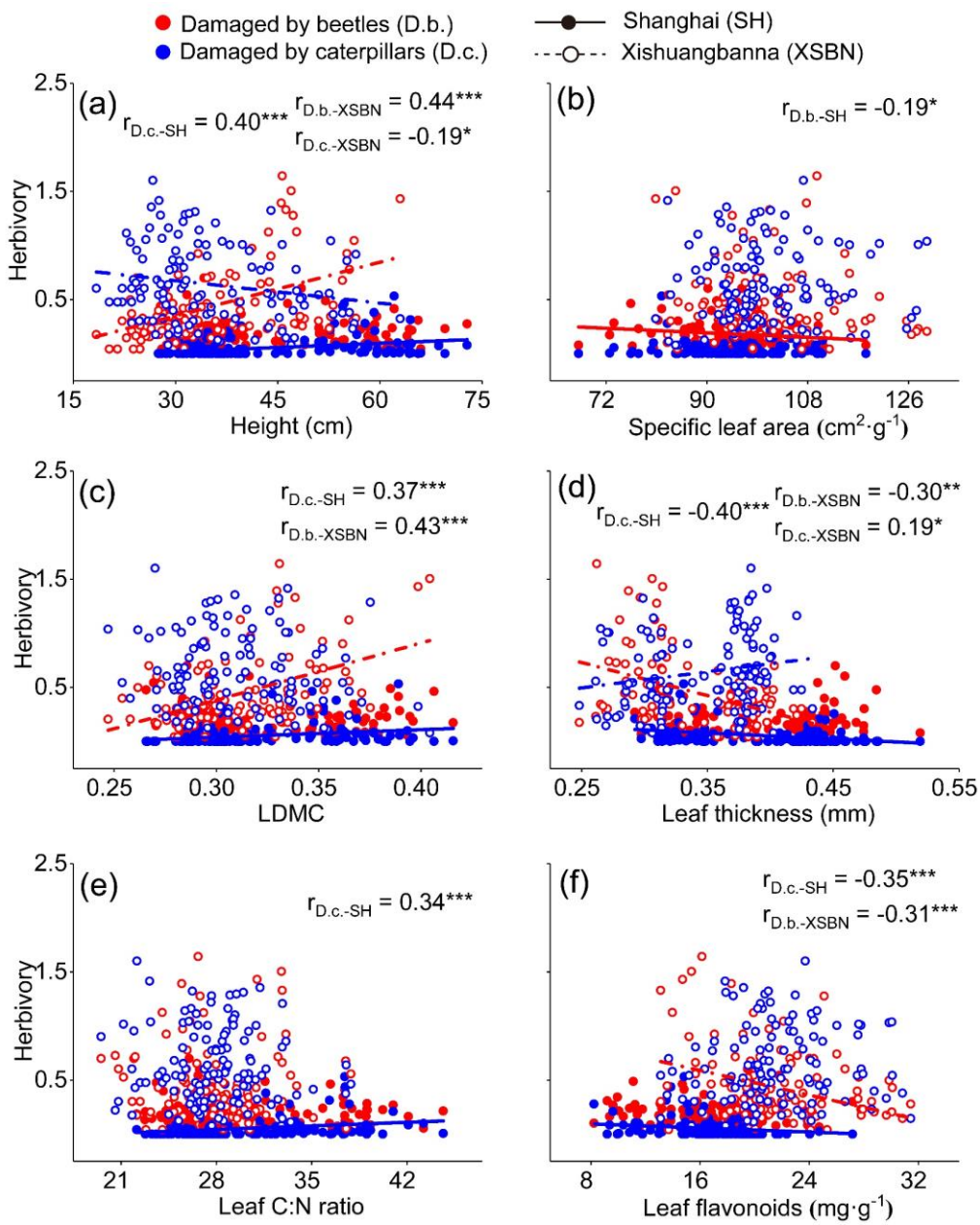

Fig. 4. Relationships between the key plant traits and herbivore damage of *R. japonica* from native and introduced ranges in Shanghai (solid, SH) and Xishuangbanna (hollow, XSBN) common gardens. Red and blue: percentage of leaf area eaten by beetles (D.b.) and caterpillars (D.c.). Trendlines are given only for significant relationships, and correlation coefficients (r) and P-values are shown, *, *P*<0.05; **, *P*<0.01; *, *P*<0.001. Points in the scatterplot represent the mean of populations. The herbivorous data have been *log*-transformed for the convenience of display.**

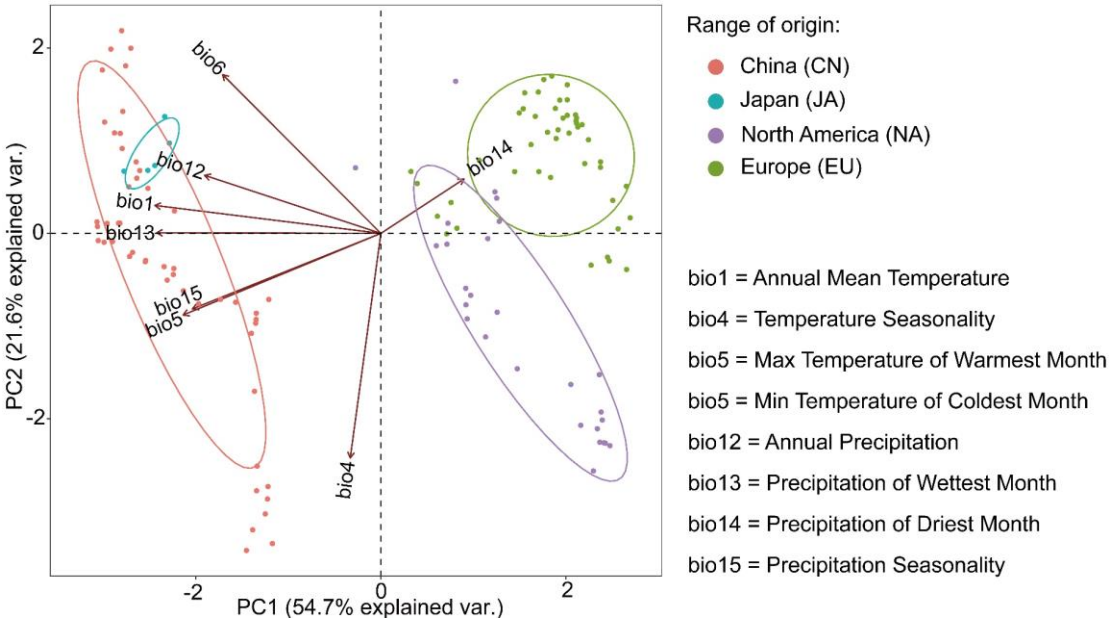

Fig. 5. Principal component analysis (PCA) for climate variables at sample collection sites of *R. japonica* in common gardens. Colored dots indicate sample collection sites. Percentages on the X- and Y-axes indicate the variation explained by each principal component.

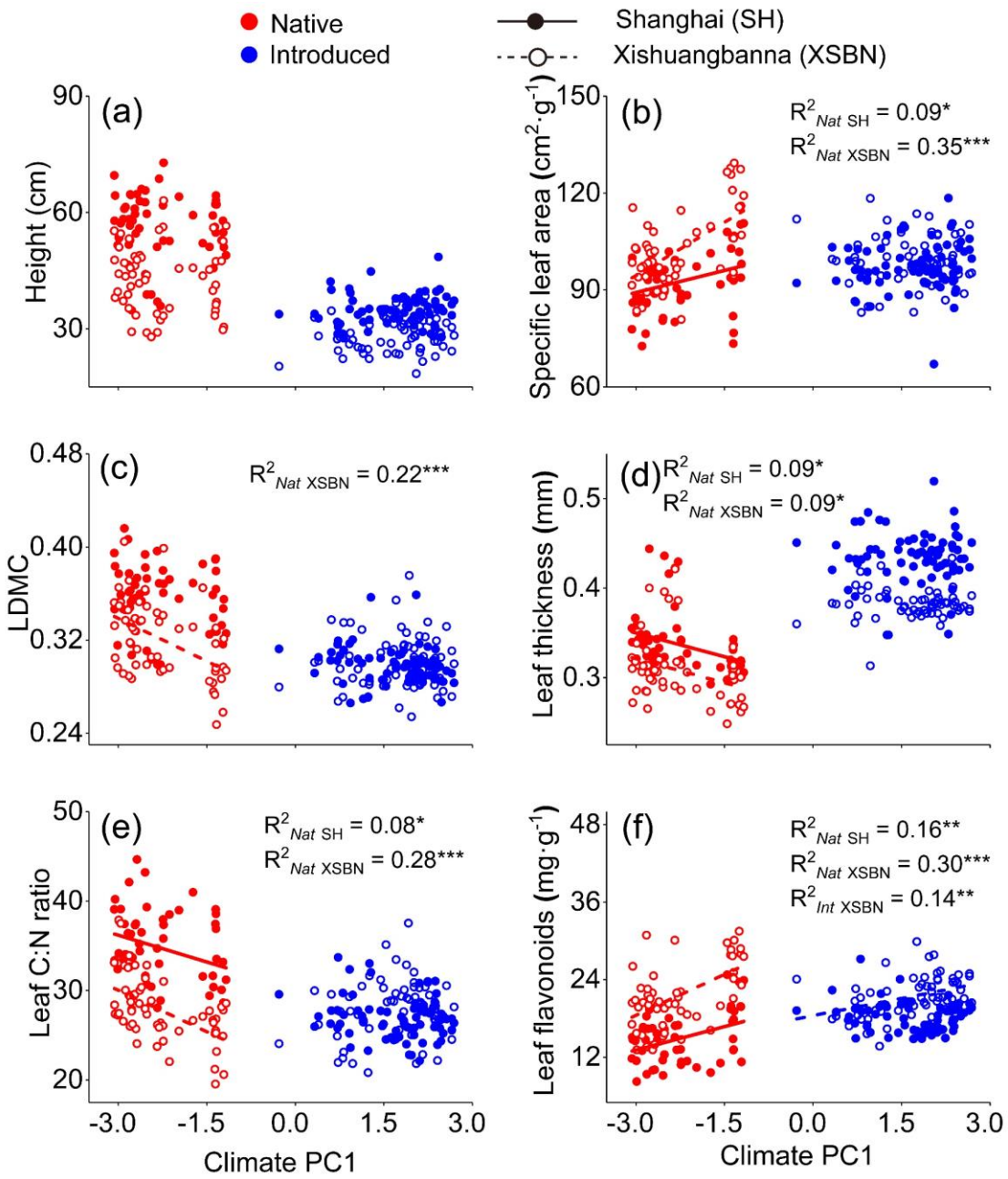

Fig. 6. Relationships between the key plant traits and climate PC1 for native (China and Japan: red) and introduced (North America and Europe: blue) populations in Shanghai (Solid) and Xishuangbanna (hollow) common gardens. Points in the scatterplot represent the mean of populations. Trendlines are given only for significant relationships (*P* < 0.05), *, *P*<0.05; **, *P*<0.01; *, *P*<0.001. Climate PC1 represents the first components of climatic factors by PCA.**

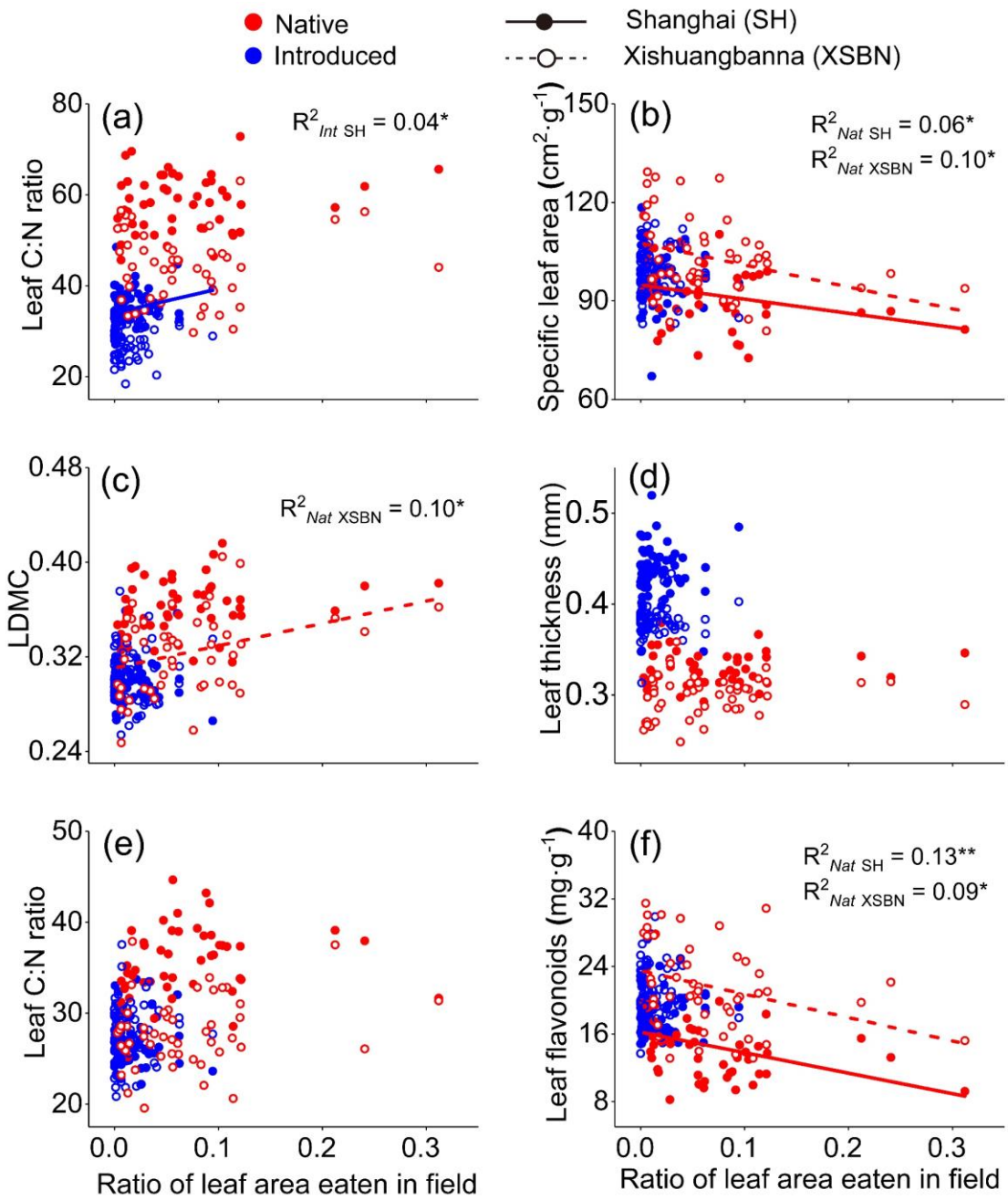

Fig. 7. Relationships between the key plant traits and leaf damage in field for native (China and Japan: red) and introduced (North America and Europe: blue) populations in Shanghai (Solid) and Xishuangbanna (hollow) common gardens. Points in the scatterplot represent the mean of populations. Trendlines are given only for significant relationships (*P* < 0.05), *, *P*<0.05; **, *P*<0.01; *, *P*<0.001.**

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Appendix

Table S1. Sampling locations and common gardens material distribution. SH: Shanghai, XSBN: Xishuangbanna.

Population ID	Collection site	Latitude (°N)	Longitude (°E)	Garden
China (Native)				
CN01	Guangdong	23.29	114.01	SH / XSBN
CN02	Guangdong	23.64	113.84	SH / XSBN
CN03	Guangdong	23.74	113.91	SH / XSBN
CN04	Guangdong	23.75	115.27	SH / XSBN
CN05	Guangdong	23.82	115.38	SH / XSBN
CN06	Guangdong	24.44	113.25	SH / XSBN
CN07	Guangdong	24.62	113.76	SH / XSBN
CN08	Guangdong	24.71	115.82	SH / XSBN
CN09	Jiangxi	24.96	115.61	SH / XSBN
CN10	Jiangxi	25.24	115.73	SH / XSBN
CN11	Jiangxi	25.53	115.84	SH / XSBN
CN12	Jiangxi	25.80	115.94	SH / XSBN
CN13	Jiangxi	26.03	116.16	SH / XSBN
CN14	Jiangxi	26.10	114.69	SH / XSBN
CN15	Jiangxi	26.27	116.32	SH / XSBN
CN16	Jiangxi	26.55	116.27	SH / XSBN
CN17	Jiangxi	26.85	116.37	SH / XSBN
CN18	Jiangxi	27.08	116.34	SH / XSBN
CN19	Jiangxi	27.38	116.45	SH / XSBN
CN20	Jiangxi	27.66	116.68	SH / XSBN
CN21	Jiangxi	27.87	116.78	SH / XSBN
CN22	Jiangxi	28.19	116.76	SH / XSBN
CN23	Jiangxi	28.54	117.05	SH / XSBN
CN24	Jiangxi	28.71	117.02	SH / XSBN
CN25	Jiangxi	29.10	117.12	SH / XSBN
CN26	Jiangxi	29.21	117.15	SH / XSBN
CN27	Jiangxi	29.31	117.85	SH / XSBN
CN28	Jiangxi	29.43	117.16	SH / XSBN
CN29	Jiangxi	29.64	117.22	SH / XSBN
CN30	Anhui	29.81	117.05	SH / XSBN
CN31	Anhui	29.98	116.97	SH / XSBN
CN32	Anhui	30.18	117.04	SH / XSBN
CN33	Anhui	30.44	116.23	SH / XSBN
CN34	Anhui	30.69	115.98	SH / XSBN

CN35	Anhui	30.82	116.05	SH / XSBN
CN36	Anhui	30.92	116.21	SH / XSBN
CN37	Anhui	31.09	116.10	SH / XSBN
CN38	Anhui	31.21	116.02	SH / XSBN
CN39	Anhui	31.40	115.93	SH / XSBN
CN40	Anhui	31.58	115.97	SH / XSBN
CN41	Henan	31.81	115.85	SH / XSBN
CN42	Jiangsu	32.85	118.44	SH / XSBN
CN43	Henan	34.27	115.68	SH / XSBN
CN44	Jiangsu	34.72	116.79	SH / XSBN
CN45	Shandong	35.06	116.31	SH / XSBN
CN46	Shandong	35.52	117.82	SH / XSBN
CN47	Shandong	35.74	117.64	SH / XSBN
CN48	Shandong	36.00	117.65	SH / XSBN
CN49	Shandong	36.38	117.66	SH / XSBN
CN50	Shandong	36.87	117.68	SH / XSBN
Japan (Native)				
JA01	Unzen	32.81	129.92	SH / XSBN
JA02	Saikai	32.99	129.77	SH / XSBN
JA03	Sasebo	33.21	129.68	SH / XSBN
JA04	Hirado	33.32	129.60	SH / XSBN
JA05	Hirado	33.33	129.53	SH / XSBN
North Ameraca (Introduced)				
NA01	Georgia	34.24	-83.46	SH / XSBN
NA02	NorthCarolina	35.10	-83.10	SH / XSBN
NA03	NorthCarolina	35.74	-82.68	SH / XSBN
NA04	NorthCarolina	36.21	-81.78	SH / XSBN
NA05	NorthCarolina	36.38	-81.38	SH / XSBN
NA06	Virginia	36.65	-80.92	SH / XSBN
NA07	Virginia	36.67	-80.57	SH
NA08	Virginia	37.37	-79.41	SH
NA09	Virginia	37.43	-79.16	SH
NA10	Virginia	38.46	-78.59	SH / XSBN
NA11	Virginia	38.65	-78.54	SH
NA12	Virginia	39.08	-78.09	SH / XSBN
NA13	Maryland	39.61	-76.68	SH / XSBN
NA14	NewJersey	41.01	-74.35	SH / XSBN
NA15	NewYork	41.41	-73.96	SH / XSBN
NA16	NewYork	41.77	-73.86	SH
NA17	Massachusetts	42.64	-72.91	SH / XSBN

NA18	Vermont	42.92	-72.77	SH / XSBN
NA19	Vermont	43.20	-72.45	SH / XSBN
NA20	Vermont	43.84	-72.19	SH
NA21	NewHampshire	43.94	-71.68	SH / XSBN
NA22	NewHampshire	44.12	-71.18	SH / XSBN
NA23	Maine	44.11	-70.69	SH
NA24	Maine	44.21	-70.31	SH
NA25	Maine	44.53	-69.89	SH
NA26	Maine	44.94	-68.99	SH
NA27	Maine	44.95	-68.64	SH
Europe (Introduced)				
EU01	Italy	44.67	7.29	SH / XSBN
EU02	Italy	44.75	7.48	SH / XSBN
EU03	Italy	44.88	7.69	SH / XSBN
EU04	Italy	45.19	8.04	SH / XSBN
EU05	Italy	45.64	8.37	SH / XSBN
EU06	Italy	45.80	8.87	SH / XSBN
EU07	Switzerland	46.27	9.00	SH / XSBN
EU08	Switzerland	46.51	8.7	SH / XSBN
EU09	Switzerland	47.16	8.56	SH / XSBN
EU10	Germany	47.62	8.22	SH / XSBN
EU11	Germany	47.81	7.61	SH / XSBN
EU12	Germany	47.94	7.88	SH / XSBN
EU13	Germany	48.28	8.11	SH / XSBN
EU14	Germany	48.37	8.56	SH / XSBN
EU15	Germany	48.47	8.92	SH / XSBN
EU16	Germany	48.56	8.39	SH / XSBN
EU17	Germany	48.79	8.32	SH / XSBN
EU18	Germany	49.59	8.73	SH / XSBN
EU19	Germany	49.90	8.83	SH / XSBN
EU20	Germany	50.07	8.48	SH / XSBN
EU21	Germany	50.31	7.79	SH / XSBN
EU22	Germany	50.54	7.08	SH / XSBN
EU23	Germany	51.14	6.78	SH / XSBN
EU24	Germany	51.43	7.29	SH / XSBN
EU25	Germany	51.87	7.55	SH / XSBN
EU26	Germany	52.15	7.62	SH / XSBN
EU27	Germany	52.39	7.94	SH / XSBN
EU28	Germany	52.72	8.26	SH / XSBN
EU29	Germany	53.01	8.70	SH / XSBN
EU30	Germany	53.22	9.57	SH / XSBN

EU31	Germany	53.45	10.08	SH / XSBN
EU32	Germany	53.99	10.25	SH / XSBN
EU33	Germany	54.31	10.13	SH / XSBN
EU34	Denmark	54.71	11.45	SH / XSBN
EU35	Denmark	55.46	12.19	SH / XSBN
EU36	Sweden	55.68	13.19	SH / XSBN
EU37	Sweden	56.14	13.76	SH / XSBN
EU38	Sweden	56.45	13.60	SH / XSBN
EU39	Sweden	56.83	13.96	SH / XSBN
EU40	Sweden	57.70	14.11	SH / XSBN
EU41	Sweden	58.17	14.58	SH / XSBN
EU42	Sweden	58.41	15.65	SH / XSBN
EU43	Sweden	58.67	16.20	SH / XSBN
EU44	Sweden	58.89	17.56	SH / XSBN
EU45	Sweden	59.32	18.02	SH / XSBN
EU46	Sweden	59.95	17.71	SH / XSBN

Table S2. Spearman's rank correlation coefficient (r) between leaf herbivory and plant traits.

	Height	SLA	LDMC	Leaf thickness	Leaf toughness	Leaf C:N ratio	Leaf lignin	Leaf alkaloids	Leaf flavonoids
Percentage of leaf area eaten [1]	0.17	**-0.20***	0.09	-0.04	0.00	0.05	-0.12	0.07	-0.14
Percentage of leaf area eaten by beetles [1]	0.01	**-0.19***	-0.04	0.13	-0.06	-0.11	-0.11	0.10	-0.07
Percentage of leaf area eaten by caterpillars [1]	**0.40*****	-0.05	**0.37*****	**-0.40*****	0.12	**0.34*****	-0.14	0.05	**-0.35*****
Percentage of leaves eaten [2]	**0.18***	-0.09	**0.25****	-0.11	-0.18	-0.03	0.17	0.02	-0.18
Percentage of leaf area eaten by beetles [2]	**0.44*****	-0.17	**0.43*****	**-0.30****	-0.13	-0.06	0.06	0.00	**-0.31*****
Percentage of leaf area eaten by caterpillars [2]	**-0.19***	0.03	-0.13	**0.19***	-0.08	-0.03	0.15	0.03	0.11

[1] Data were from Shanghai common garden, [2] Data were from Xishuangbanna common garden.

Asterisk indicates a significant correlation: *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

Table S3. Summary of PCA of eight climate variables. The first components explained 54% of climatic variation among native and introduced ranges' localities. Climate variables with stronger loadings (> 0.40) on climate PC1 are shown in bold.

	PC1 climate
Eigenvalues	4.378
Percent variance	54.73
Loadings	
Mean annual temperature (bio1)	**-0.461**
Maximum temperature of warmest month (bio4)	-0.063
Minimum temperature of coldest month (bio5)	**-0.404**
Temperature seasonality (bio6)	-0.323
Mean annual precipitation (bio12)	-0.360
Precipitation of the wettest month (bio13)	**-0.458**
Precipitation of the driest month (bio14)	0.170
Precipitation seasonality (bio15)	-0.385

Table S4. The average temperature, precipitation and solar radiation monthly in Shanghai and Xishuangbanna during experiments (March - October) (Data from http://www.nmic.cn/; He et al., 2010; Song et al., 2010; Yu et al., 2021).

	Average temperature (°C)	Average precipitation (mm)	Solar radiation (MJ m^{-1})
Shanghai	20.3	115.9	453.7
Xishuangbanna	24.1	170.7	466.0

Reference:

He F F, Xue J, Mu H Z. Estimation of total solar radiation and its temporal and spatial distributions over Shanghai area[J]. Resour Sci, 2001, 32: 693-700.

Song F Q, Zhao J B, Zhang Y P, et al. Effects of the regional climate change on the plant growth trend in Xishuangbanna[J]. Acta Botanica Yunnanica, 2010, 32(6): 547-553.

Hui Y, Qinghai S, Yiping Z, et al. Long-term variation characteristics of radiation in the tropical seasonal rainforest in Xishuangbanna, southwestern China[J]. Journal of Beijing Forestry University, 2021, 43(4): 56-67.

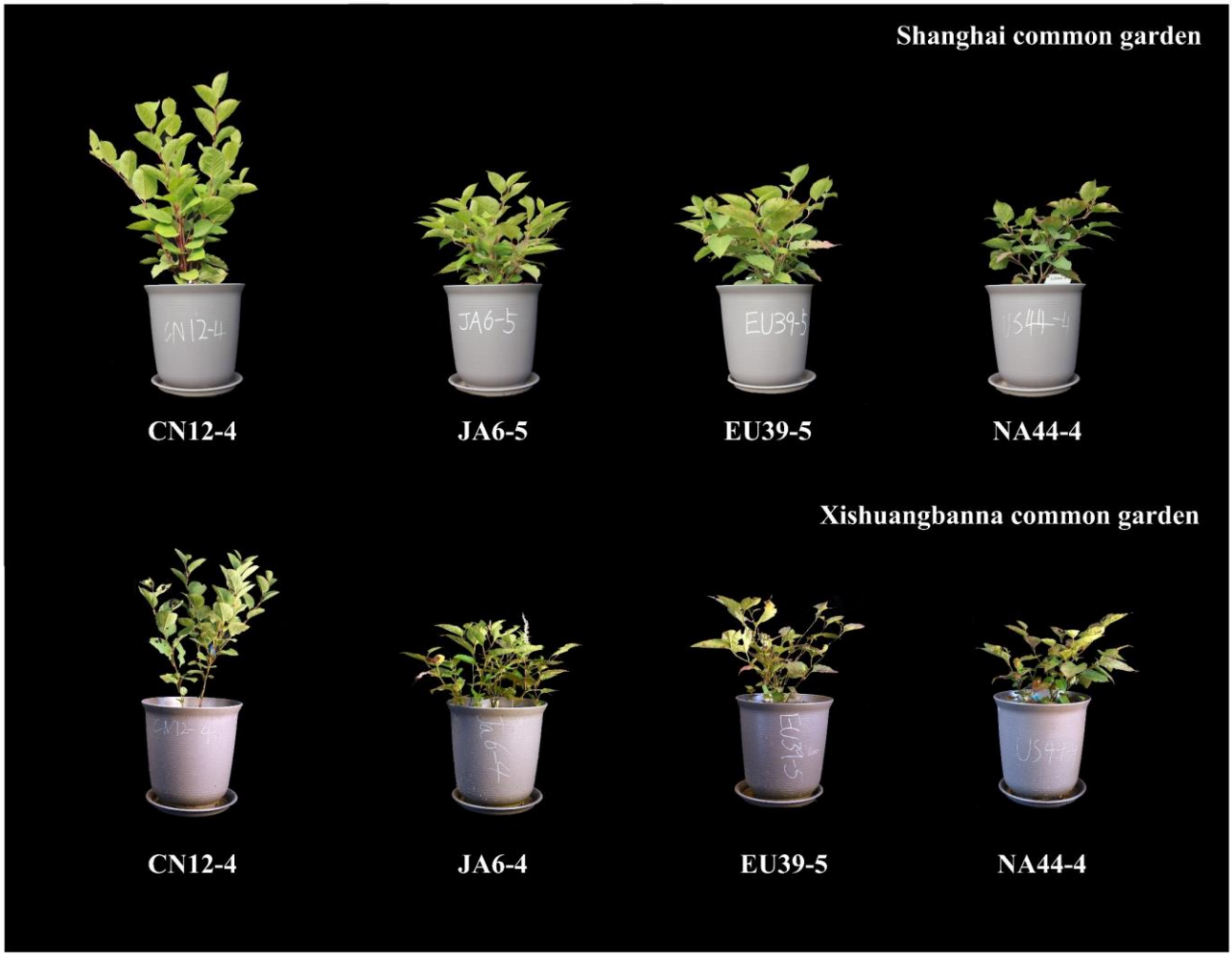

Figure S1. Photos shows the growth of *R. japonica* in Shanghai and Xishuangbanna common gardens in mid-July.

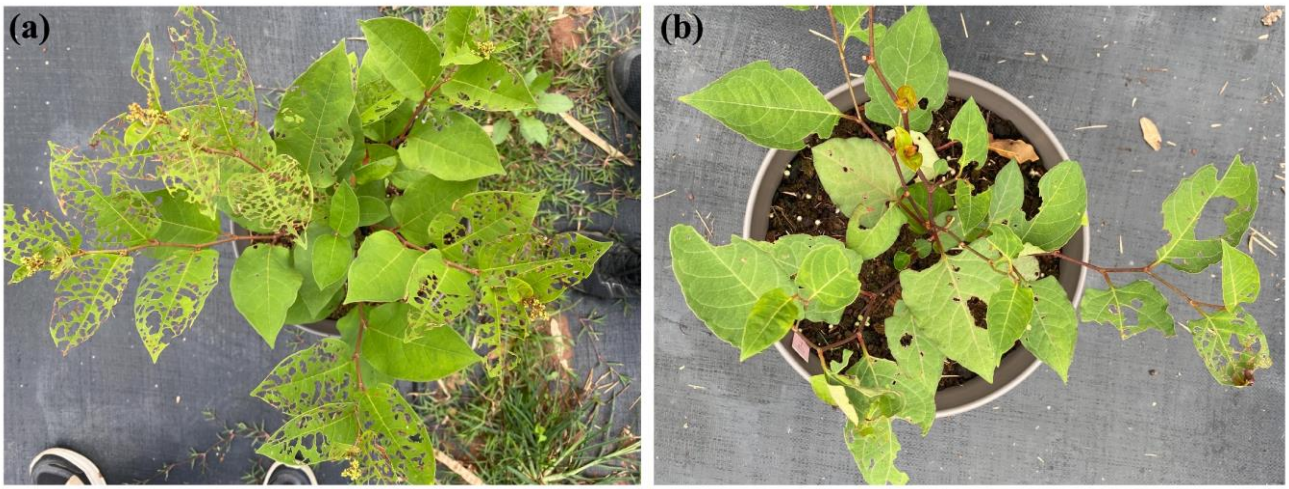

Figure S2. Leaf notches caused by beetles (a) and caterpillars (b).