Intensity of herbivory correlates with stronger constitutive and weaker induced defenses for non-native plant species – another mechanism for EICA?

Xiao Sun[1], Yumei Sun[2], Xueyao Cao[2], Xincong Zhai[2], Ray Callaway[3], Jinlong Wan[4], S. Luke Flory[5], Wei Huang[6], and Jianqing Ding[1]

[1]Henan University
[2]Affiliation not available
[3]University of Montana
[4]Wuhan Botanical Garden Chinese Academy of Sciences
[5]University of Florida
[6]Key Laboratory of Aquatic Botany and Watershed Ecology

January 3, 2023

Abstract

Non-native plants are typically released from specialist enemies in new ranges, but continue to be attacked by generalists, but whether they shift relative allocation to constitutive or induced defenses is unknown. We compared herbivory on co-occurring native and non-native species and also constitutive and induced defenses. Non-natives suffered less damage than natives and constitutive defenses of non-natives was lower than that of native congeners, whereas induced defense was the opposite. The strength of constitutive defenses for a species was correlated with the intensity of herbivory experienced, for non-natives, whereas induced defenses showed the reverse. The defenses of natives were not related to herbivory pressure. Finally, the strength of induced defenses correlated positively with growth, suggesting a novel mechanism for the evolution of increased competitive ability. These results expand our understanding of fundamental tradeoffs in constitutive and induced defenses and provide novel insight into how herbivory pressure affects defense allocation.

Introduction

Non-native plant invasions have provided remarkable insights into rapid adaptation in defense allocation (Callaway & Maron, 2006; Waller et al., 2020). In part, this is because non-native plant species are typically released from specialist enemies in introduced ranges but continue to be attacked by generalists - the Enemy Release Hypothesis (Keane & Crawley, 2002). Thus, the most predictable change in herbivore communities in non-native ranges, at least until biological control agents are introduced, is the loss of specialists and disproportionate exposure to naive generalists. Generalist-dominated communities in non-native ranges commonly correspond with exotic species developing greater qualitative chemical defenses against generalists, relative to conspecifics in the native range - the Shifting Defense Hypothesis (Joshi & Vrieling, 2005; Zhang et al., 2018). These qualitative defenses may be constitutive or induced, but little is known about selection on constitutive versus induced defenses in non-native ranges. Theory and hypotheses that specifically focus on how constitutive and induced defenses might respond to generalists in native and non-native ranges have not led to clear predictions (Orians & Ward, 2010), which is barrier to understanding successful invasion.

Plants are thought to maximize their fitness by balancing resource allocation to defense with other functions (Rotter & Holeski, 2018; Mertens et al., 2021), and this allocation can be affected by herbivore pressure

1

(Stamp, 2003). Constitutive defenses protect plants from attack without a time lag, but constitutive defenses are thought to be costly in the absence of herbivores (Wittstock & Gershenzon, 2002; Ali & Agrawal, 2012; Aljbory & Chen, 2018). Thus, when herbivore pressure is low, plant fitness might be maximized by investing less in constitutive defenses. In contrast, induced defenses appear to be cost-saving strategies where defenses are expressed only in response to herbivore pressure (Gatehouse, 2002; Agrawal & Hastings, 2019).

From the "quagmire" (Stamp, 2003) of hypotheses for defense allocation in plants, a few possibilities emerge for how induced and constitutive defenses might evolve in plant species in their non-native ranges. In a review of invasive plants, Orians & Ward (2010) suggested that induced defense should evolve to be more prevalent in non-native ranges where the cost of defense is high and plants are attacked infrequently (Bixenmann et al., 2016; Agrawal & Karban, 1999). Different non-native species often experience very different degrees of generalist herbivore pressure (Zou, 2008; Sims-Chilton et al., 2009), providing opportunities to test Orian & Ward's idea. For example, a common garden survey found that different non-native species suffered from 0% to more than 40% leaf herbivory from generalists (Agrawal & Kotanen, 2003). Variation in herbivore pressure should lead to variation in defense traits (Coverdale & Agrawal, 2022; Wan et al., 2022) and less intense and less frequent herbivory is theorized to select for allocation to induced defenses (Bixenmann et al., 2016). However, how this variation in generalist herbivore pressure affects any potential tradeoffs in constitutive vs. induced defensive strategies among non-native plant species is unknown.

Growth-defense tradeoffs appear to derive from plasticity or adaptive variation in allocation that maintains fitness in a variable environment (Monson et al., 2022). Increased investment in defense may result in reduced growth (Hahn et al., 2021), leading to differential investment in growth or defense by species based on their evolutionary responses to environmental conditions - the Resource Availability Hypothesis (Coley et al., 1985). Most studies have focused on how variation in herbivore pressure across geographical clines shapes evolution of plant defense (Woods et al., 2012; Moreira et al., 2018). However, these studies have not generally considered how biogeographical shifts in defense strategies might constrain growth.

Non-native species provide a good system for studying induced and constitutive defenses. If non-native species are attacked less by generalists than native species, we can test the prediction that (i) non-natives increase expression of cheaper induced defenses and decrease expression of expensive constitutive defenses (Figure S1A and S1B). If non-native species are attacked disproportionately by generalists, then this disentangling of generalist and specialist effects might permit a cleaner opportunity to test another prediction (ii), that the herbivore pressure a species experiences will increase expression of constitutive defenses and decrease induced defenses (Figure S1A and S1B). We can then test the prediction (iii) that allocation to less expensive induced defense allow allocation to greater growth (Figure S1C).

To explore these predictions, we first carried out large spatial scale measurements of herbivore intensity on 27 non-native and 59 co-occurring native species in a field survey in northern China (Table S1, S2 and S3). We then quantified herbivore intensity on 12 pairs of non-native species and native congeners in a common garden. Finally, we conducted measurements of constitutive and induced defenses as well as plant growth on these pairs of common garden species (Table S4).

Materials and Methods

Herbivore pressure in the field

To compare herbivore pressures on different non-native and native plant species under natural conditions, we conducted a field survey in abandoned agricultural fields at 28 sites, ranging from 34°00' to 38°54' N and 112°55' to 119°44' E in northern China in 2018 (Table S1).

At each site, we selected the three tallest plants of each non-native species and co-occurring native species in a 5 x 10 m plot. To determine herbivore pressure, we calculated the percentage of damaged leaves by counting 10-50 leaves starting from the top of each plant and recording the proportion of damaged leaves (Table S2 and S3).

To determine whether herbivore biomass correlated with leaf damage, we recorded the number of each

herbivore species on each non-native plant. Native plants were not measured as these measurements were simply to correlate damage with insect biomass. To obtain the mean biomass of each herbivore category, we carried out a field survey at eight sites in 2019. We collected all herbivores on non-native species and determined the fresh weight of individual insects to the nearest 0.01g as described in Supplementary Methods 1.

Herbivore pressure in the common garden

We conducted a common garden experiment at Henan University, Kaifeng, China in 2020 (34°30' N, 114°10' E). We selected the 12 most common non-native herbaceous plant species in the field survey and compared herbivory on these species and 12 co-planted native congeners (Table S4). Specialist herbivores were absent in our system. Thus, these species provided an explicit test of how non-native plant species cope with generalist herbivores (see Müller-Schärer et al., 2004; Joshi & Vrieling, 2005).

In the field survey, we found no significant effect of site on leaf damage for most non-native species or native congeners (Table S2 and S3). Therefore, for controlled experiments we collected seeds from 2 to 3 individuals of each non-native species and their native congeners at each site and mixed them. We sowed seeds in trays (20×30 cm) and placed them in the greenhouse. Similar sized seedlings of all species (about 5 cm in height) were used for the following experiments.

We removed standing vegetation by hand from an agricultural field at Henan University and established 168 1×1 m plots grouped into 6 blocks. Plots within each block were separated by 1.5 m and blocks were separated by 3 m. Then, the 28 plots within each block were randomly assigned to one of 14 pairs of non-native species and their native congeners. We transplanted four seedlings into each plot spaced 25 cm apart. All plants were exposed to natural herbivores.

Three months after planting, we recorded herbivore abundance and measured herbivore biomass as described above and also evaluated the percentage of damaged leaf area instead of the percentage of damaged leaves for all plants. We clipped 20 leaves from each plant and calculated damaged leaf area and total leaf area using Image Proexpress V.6.0 (Media Cybernetics, Inc., Bethesda, MD, USA). We used the average of herbivore biomass and percentage of damaged leaf area of the four plants in each plot for analysis.

Constitutive and induced bioassay defense

We used the same 12 pairs of non-native species and their native congeners for assessment of constitutive and induced defenses. We sowed the seeds of each species separately into 96-cell trays and then transplanted similar sized plants of each species individually into pots (15 cm diameter, 18 cm height) filled with 50% potting media (Pindstrup, Denmark) and 50% topsoil collected from the common garden. Greenhouse conditions were as described above.

When plants had 20 leaves, we started the herbivory treatment and bioassay. As the non-native plant species were mainly attacked by Lepidoptera in the field survey (Figure S2A) and common garden experiment (Figure S2B), thus we applied *Spodoptera littoralis* (Lepidoptera: Noctuidae), a species that feeds on more than 40 plant families (Kempel et al., 2011), as the generalist herbivore. We obtained eggs of *S. littoralis* from Keyun Biological Control Co., Ltd., China and fed them on bean-based artificial diet. The newly emerged *S. littoralis* larvae from the next generation were used to quantify the constitutive defense of pairs of non-native species and their native congeners. We measured larval weight gain on leaves harvested from healthy undamaged plants. We clipped one fully expanded leaf from the top of plants and placed it on a Petri dish (9 cm diameter) with moist filter paper. Then, we added a newly emerged pre–weighed larva (weight$_1$) into the Petri dish. We replaced each leaf with a fresh one from another healthy plant every day. After four days we re-weighed larva and calculated weight gain for 10 replicates.

To quantify induced defenses of pairs of non-native species and native congeners, we measured larval weight gain on leaves that were harvested from plants previously damaged by herbivores. The initial damage level was at roughly 10%, which was consistent with the percentage of leaf damage in common garden experiment (11.5±1.2 %). We then added 2-6 second instar larvae to the two leaves in the middle of each plant, depending

on the size of the different plant species, and covered them with a mesh bag (0.8 mm openings). After two days we removed larvae. Plants were grown 4-8 days to produce new leaves and then we followed the same protocol as above with 10 replicates.

Constitutive and induced chemical defense

To test the contribution of secondary metabolites to constitutive and induced defenses, we measured chemicals in leaves that were harvested from previously healthy and herbivore-damaged plants. Terpenoids and phenols are important secondary metabolites for defense against a wide range of herbivores (Mithöfer & Boland, 2012). Thus, we used the same non-native species and their native congeners used in the bioassay to measure total phenolics and total triterpenoids in leaves harvested from healthy and herbivore-damaged plants.

Leaves were flash frozen in liquid nitrogen and stored at -80°C for analysis. Total phenolic concentration was calculated as described in Supplementary Methods 2.

Growth rates

To evaluate the relationship between the strength of induced defenses and plant growth, we measured the relative growth rate of non-native and native plants. Two weeks after transplanting, we harvested ten similar-sized plants for each species and measured total dry biomass ($weight_1$). After six more weeks, we harvested conspecifics and determined total dry biomass ($weight_2$). We calculated relative growth rate (RGR) of each species as: RGR = (weight2 - weight1) / 42 days. There were 12 replicates for each combination of $weight_1$ plants and $weight_2$ plants for each non-native species and native congeners.

Statistical analysis

Herbivore pressure - To test for difference in herbivore pressure (binary data of undamaged *vs.* damaged leaves in a cbind matrix) among the non-native plant species in the field survey, we used a Wald Chi-square test applied on a Generalized Linear Mixed Model (GLMM) with a binomial distribution. Site was a random effect. We used a bootstrap method to test whether results differed when the number of native plant species was equal to the number of non-native species. We tested for difference in herbivore pressure (logit-transformed percentage of damaged leaf area) among the non-native plant species in the common garden experiment using a Wald Chi-square test applied on a Linear Mixed Model (LMM) with blocks as random effects. We conducted same analyses for native species. To test for differences in herbivore pressure (binary data of undamaged*vs.* damaged leaves in a cbind matrix) between the non-natives and natives in the field survey, we used a Wald Chi-square test applied on a GLMM with a binomial distribution. Site and species nested in origin as random effects. We also tested for differences in herbivore pressure (logit-transformed percentage of damaged leaf area) in the common garden experiment using LMM with blocks and species nested in origin as random effects. Furthermore, we used GLMM with binomial distribution that included random terms for sites and species to test whether percentage of damaged leaves (binary data of undamaged *vs.* damaged leaves in a cbind matrix) depended on herbivore biomass in the field survey and used LMM that included random terms for block and species to test whether percentage of damaged leaf area (logit-transformed) depended on herbivore biomass in the common garden experiment. Finally, for non-native species and their native congeners, in both the field survey and common garden experiment, we used LM to examine the relationship between leaf damage and herbivore biomass for the two experiments using mean values for species.

Constitutive and induced defenses - We assessed constitutive and induced defenses using larval weight gain and chemical contents for each species. For constitutive defense, we used larval weight gain on the leaves of plants that had not been previously attacked. For induced defense, we calculated the larval weight gain on the leaves of previously attacked plants minus the mean of larval weight gain on the leaves of un-attacked plants. Constitutive and induced defenses expressed by chemicals were evaluated using the same methods. We used the percentage of herbivore-damaged leaf area for each species in the common garden experiment as herbivore pressure. To evaluate relationships among constitutive defense, induced defense and herbivore pressure, we

4

carried out Pearson correlations in which we multiplied larval weight with -1 since higher larval weight gain indicates lower defense. Mean values per species were used for above analyses and non-native and native species were analyzed separately. To test constitutive and induced defense between non-native and native species we used LMM with species as a random effect. Finally, to test whether changes in chemicals might underly changes in herbivore growth, we conducted Pearson correlations across both herbivory treatments and all species to examine the dependence of larval weight gain on phenolics or triterpenoids using mean values per species.

Strength of growth and induced defense - To test for differences in relative growth rate between non-native plant species and native congeners, we used a LMM with species nested within origin as random effects. Furthermore, we calculated difference in induced defense in term of larval weight gain and difference in plant growth rate between non-native species and corresponding native congeners. We then used Pearson correlation to evaluate relationship between difference in induced defense and difference in plant growth rate.

Homogeneity of variances and normality of distributions of data were checked before data analysis and P-values were corrected by False Discovery Rate (FDR) (Benjamini & Hochberg, 1995). All statistics were carried out using R (version 4.0.5) with the 'car', 'lme4', and 'RVAideMemoire' packages (Bates, 2014).

Results

Herbivore pressure on native and non-native plant species

In the field survey of 28 sites, 396 individuals of 27 non-native plant species and 678 individuals of 59 native plant species were evaluated. There were large differences among non-native plant species ($\chi^2 = 3744$, P < 0.001, Figure 1A) and among native species ($\chi^2 = 3025$, P < 0.001, Figure 1B) in herbivore damage, with non-native plant species damaged 46.2% less than natives ($\chi^2 = 22.63$, P<0.001, Figure 1C).

In the common garden experiment, herbivore damage varied by over an order of magnitude among non-native species ($\chi^2 = 198.43$, P < 0.001, Figure 1D) and among native species ($\chi^2 = 71.22$, P < 0.001, Figure 1E). Herbivore damage on non-native species was 41.5% less than on natives ($\chi^2 = 6.53$, P = 0.011, Figure 1F).

Non-native plant species with higher herbivore biomass on them received more leaf damage in the field survey (r = 0.97, P < 0.001, Figure S3A) and in the common garden (r = 0.38, P < 0.001, Figure S3B). Furthermore, there was a strong positive linear relationship between herbivore damage on particular species in the field survey and those species in the common garden experiment (r = 0.88, P < 0.001, Figure S4).

Herbivore pressure and constitutive and induced defense

Assessed by *S. littoralis* larval weight gain, constitutive defense (-1 × larval weight gain on healthy plants) was positively correlated with herbivore pressure (assessed as described above) across all 12 non-native plant species ($\chi^2 = 0.00$, P = 0.020), whereas there was no correlation between constitutive defense and herbivore pressure across the 12 native congeners ($\chi^2 = 0.32$, P = 0.306, Figure 2A). Induced defense [-1 × (larval weight gain on previously damaged plants - larval weight gain on healthy plants)] was negatively correlated with herbivore pressure for non-native plant species ($\chi^2 = -0.69$, P = 0.014), whereas there was no correlation between induced defense and herbivore pressure for native congeners ($\chi^2 = -0.49$, P = 0.106) (Figure 2B). Overall, constitutive defense of non-native plant species was 38.7% lower than that of native congeners ($\chi^2 = 4.28$, P = 0.039) (Figure 2A), while induced defense of non-native plant species was 55.3% higher than that of native congeners ($\chi^2 = 5.56$, P = 0.018) (Figure 2B).

For chemical defenses assessed by phenolics, results were similar to that assessed by larval weight gain. Constitutive defense (phenolics in healthy plants) ($\chi^2 = 0.76$, P = 0.004) and induced defense (phenolics in previously attacked plants - phenolics in non-attacked plants) ($\chi^2 = -0.84$, P < 0.001) were positively and negatively correlated with herbivore pressure, respectively, across all 12 non-native plant species (Figure 2C and 2D). Whereas, for native congeners, defense assessed by phenolic content showed no correlation between constitutive defense and herbivore pressure ($\chi^2 = -0.20$, P = 0.525) or between induced defense and herbivore pressure ($\chi^2 = -0.18$, P = 0.571, Figure 2C and 2D). Overall, constitutive defense of non-native

5

plant species, measured as phenolic content, was equal to that of native congeners ($\chi^2 = 0.02$, P = 0.885, Figure 2C), whereas induced defense was higher than that of native congeners ($\chi^2 = 163.20$, P < 0.001, Figure 2D). However, for defense assessed by triterpenoids, there was no relationship between constitutive defense and herbivore pressure (non-natives, $\chi^2 = -0.08$, P = 0.807; natives, $\chi^2 = -0.20$, P = 0.527), or between induced defense and herbivore pressure (non-natives, $\chi^2 = -0.07$, P = 0.820; natives, $\chi^2 = 0.01$, P = 0.970) for non-native plant species and native plant species (Figure 2E and 2F). Constitutive defense ($\chi^2 = 0.03$, P = 0.852) and induced defense ($\chi^2 = 2.15$, P = 0.143) of non-native plant species, measured as triterpenoids, was equal to that of native congeners (Figure 2E and 2F).

Across all herbivory treatments (leaves from un-attacked and attacked plants) and plant species (non-natives and natives), larval weight gain was negatively correlated with phenolic content (r = -0.29, P = 0.047, Figure 3A) and with triterpenoid content (r = -0.29, P = 0.044, Figure 3B).

Tradeoff between constitutive and induced defense

For defense assessed by larval weight gain, plant species that had higher constitutive defense had lower induced defense, resulting in a negative correlation across the 12 non-native plant species (r = -0.58, P = 0.048) and the native congeners combined (r = -0.85, P < 0.001) (Figure 4A). This was similar for non-native plant species when defense was assessed by phenolic content (r = -0.76, P = 0.004), but not for native congeners (r = 0.05, P = 0.881) (Figure 4B).

Strength of induced defense and growth

For the 12 pairs of non-native plant species and native congeners, RGR of non-native plant species was 1.6 times higher than that of native congeners ($\chi^2 = 19.08$, P < 0.001) (Figure 5A, Figure S5). This variation in RGR was strongly positively correlated with variation in the strength of induced defense as measured by larval weight gain (r = 0.72, P = 0.019) (Figure 5B).

Discussion

Our results make two key conceptual contributions to understanding allocation of plant defenses. First, we showed that non-native species, which presumably have escaped from specialist natural enemies, had lower constitutive defenses and higher induced defenses, which correlated with higher growth rates. Second, non-native plant species, ostensibly exposed primarily to generalists, showed a strong positive relationship between herbivore pressure and constitutive defense, and a strong negative relationship between herbivore pressure experienced by a species and the intensity of induced defenses exhibited by that species. These findings expand our understanding of constitutive vs. induced defense allocation associated with herbivore pressure on non-native plants.

Integrating these results suggests that reduced herbivore attack (Figure 1C and 1F; i.e., escape from enemies) favored allocation to induced defenses (Figure 2B) instead of constitutive defenses (Figure 2A). Our results also suggest that induced defenses are a "cheaper" overall strategy which favors higher growth rates (Figure 5). It appears that the generalist-dominated herbivore community that attacked non-native plant species drove a strong positive relationship between the intensity of herbivore pressure and allocation to constitutive defense among our target species (Figure 2A and 2C). This supports long-held theory that has had minimal empirical support - intense or consistent herbivory should select for more expensive constitutive defenses over cheaper induced defenses (Ito & Sakai, 2009).

Generalist herbivore pressure

Native generalist herbivores often avoid non-native plant species or do not suppress them (Schaffner et al., 2011; Sedio et al., 2020). For example, Cappuccino & Carpenter found that non-native invasive plants experienced 96% less leaf damage than non-native species that were not classified as invasive (Cappuccino & Carpenter, 2005). One hypothesis for this pattern is the novelty of biochemicals, in their new ranges, produced by non-native plants (Schaffner et al., 2011; Callaway & Aschehoug, 2000). Cappuccino & Arnason (2006) found that a large suite of invasive species had more unique secondary chemicals than exotic plants,

6

relative to native species. These, and other studies (see review by Inderjit et al. 2021), indicate that unusual or novel biochemicals of some non-native species may be why native herbivores avoid them (Inderjit et al., 2021).

Defense strategies

Intense and predictable herbivory is thought to select for constitutive plant defenses (Kalske & Kessler, 2020). Induced defenses appear to be adaptive to less intense and infrequent herbivory (Agrawal & Karban, 1999; Ito & Sakai, 2009) as a cost-saving alternative to constitutive defense (Detto & Xu, 2020). Relevant literature on invasives is minimal, but the invasive genotype *Lespedeza cuneata* exhibited greater induced defense but lower constitutive defense than ancestral native genotypes (Beaton et al., 2011) as might be predicted by reduced or more sporadic herbivory in the non-native range. In contrast, non-native genotypes of *Alternanthera philoxeroides* have higher constitutive defenses and lower induced defenses (Liu et al., 2020). Clearly, the literature on evolutionary changes in constitutive or induced defenses by exotic plants is limited.

Other recent results indicate that secondary metabolism plays key roles in the interactions between non-native plants and herbivores (Tian et al., 2021; Yu et al., 2022). Phenolics inhibit the digestion of protein (Rehman et al., 2012) and have been central in many studies of plant-defense mechanisms (e.g., Kumar et al., 2020). We showed that weight gain of a generalist herbivore was negatively correlated with phenolic concentration across all species and herbivory treatments, suggesting that defense might derive from variation in constitutive and induced phenolic concentrations. Triterpenes are terpenoids, also abundant in plants (Kumar et al., 2020), and larval herbivore weight gain was negatively correlated with triterpenoid content across our native species. However, triterpenoid content did not correspond with herbivore pressure. Triterpenes have many other functions (González-Coloma et al., 2011), and the functional diversification of triterpenes may have masked simple responses to herbivores.

Cost-benefit allocation

Optimal defense theory assumes that organisms are under strong natural selection to allocate resources to optimize their cost-benefit ratio in terms of fitness (Stamp, 2003; Alba et al., 2012). Much of this research has focused on a proposed evolutionary tradeoff where invasive plants experience relaxed selection on herbivore defense and evolve greater allocation to growth and competitive ability - the Evolution of Increased Competitive Ability hypothesis (EICA) (Zhang et al., 2020; Callaway et al., 2022). Generalist herbivory can mediate defensive strategies and resource allocation (Müller-Schärer et al., 2004). Thus, we suggest that the costs and benefits of induced defenses should differ in low-versus high-damage risk environments. Such damage pressure-dependent defensive strategies could drive strong selection on developmental defensive strategies (Maron et al., 2019) in ways that optimize plant defense and maximize plant fitness components for each non-native species. In this context, constitutive defense was lower whereas induced defense was higher for non-native species than native congeners, respectively. The striking positive relationship between herbivory experienced by different non-native species in the field and constitutive defense, and negative relationship between herbivory experienced by non-native species and induced defense imply that introduced plants may adopt a cost saving strategy between constitutive defense and induced defense in response to generalist pressure.

Plant species commonly show a strong trade-off between defense and growth (Lazzarin et al., 2021), but herbivore-driven changes in defensive strategy might alter the cost-benefit ratios of defense and growth allocation. Maintaining constitutive defense at high levels appears to require plants to invest substantial resources, potentially increasing the total cost of chemical defenses. If induced defenses are less costly than constitutive defenses, perhaps relaxed selection on constitutive defense could allow greater growth, and such a tradeoff might contribute to the dominance of some non-native species. To our knowledge, no study of EICA has experimentally integrated potential increased growth tradeoffs of non-native plants derived from a shift from constitutive to induced defenses. Our results support the defense strategies that have proposed such tradeoffs between constitutive defense and induced defense where cheaper induced defenses allow greater growth and reproduction (Mumm & Hilker, 2006; Mauch-Mani et al., 2017). Altogether, the results suggest

that the defense strategy of stronger protection against generalist herbivores is a factor contributing to invasion success.

An important caveat is that we only used one generalist herbivore, *S. littoralis* , to explore the defensive strategy of non-native plants. Other studies indicate that generalist preferences and impacts can vary a great deal among herbivore species (Inderjit et al., 2021). Our non-native plants were occupied by many species of generalists in the field, thus, to better understand patterns of defense strategies when non-native plants face generalist herbivore pressure, several generalist herbivore species should be included in future testing of plant defenses. We emphasize that our binary approach to herbivore diet (generalist vs. specialist) was heuristic, and thus too simplistic (Hardy et al., 2020), but appeared to have allowed us to detect some fundamental ecological phenomena. Finally, the best tests of hypotheses such as ours compare the same species in native and non-native ranges (Sheng et al., 2022), and it should be noted that studying defense-related tradeoffs among native and non-native species in the same non-native range provides strong, but not the best, evidence for defense adaptations (Van Kleunen et al., 2010). However, including many non-native and native congeners increases the strength of our results.

Integrating herbivore-related hypotheses for non-native species invasion

Interactions between non-native plants and herbivores have improved our understanding of plant-herbivore interactions, underlying mechanisms, and rapid evolution of these dynamics (Müller-Schärer et al., 2004; Lin et al., 2021). Two key hypotheses derived from non-native invasions are the Enemy Release Hypothesis - that non-native species escape much of the herbivory experienced in their native ranges, primarily by specialists, and the related Shifting Defense Hypothesis - that non-native invaders increase defenses, chiefly qualitative, against generalists. However, we do not know how constitutive and induced defenses might respond to generalist-dominated herbivore communities or the intensity of attack by these communities. Our results show a shift to induced defenses by non-native plants, and that as herbivore pressure intensifies on non-native species, so does the strength of their constitutive defenses. Finally, our results suggest new ways to consider the Evolution of Increased Competitive Ability Hypothesis - perhaps the "cheaper" induced strategy adopted by non-natives allows more allocation to growth and competitive ability. Thus, our results suggest that all three hypotheses may be surprisingly integrated (Figure 6). These results expand our understanding of fundamental tradeoffs in constitutive and induced defenses and provide novel insight into how variation in herbivore communities might affect defense allocation in plants.

Competing interest statement : The authors have declared that there is no conflict of interests.

References

Agrawal, A.A. & Hastings, A.P. (2019) Trade–offs constrain the evolution of an inducible defense within but not between plant species. *Ecology* , 100, e02857.

Agrawal, A.A. & Karban, R. (1999) Why induced defenses may be favored over constitutive strategies in plants. In: *The Ecology and Evolution of Inducible Defenses* (eds Tollrian, R. & Harvell, C.D.). Princeton University Press, Princeton, NJ, pp. 45–61.

Agrawal, A.A. & Kotanen, P.M. (2003) Herbivores and the success of exotic plants: a phylogenetically controlled experiment. *Ecol .Lett* ., 6, 712–715.

Alba, C., Bowers, M.D. & Hufbauer, R. (2012) Combining optimal defense theory and the evolutionary dilemma model to refine predictions regarding plant invasion. *Ecology* , 93, 1912–1921.

Ali, J.G. & Agrawal, A.A. (2012) Specialist versus generalist insect herbivores and plant defense. *Trends Plant Sci* ., 17, 293–302.

Aljbory, Z. & Chen, M.S. (2018). Indirect plant defense against insect herbivores: a review. *Insect Sci* ., 25, 2–23.

Bates, D., Mäechler, M., Bolker, B.M. & Walker, S.C. (2014) Fitting linear mixed-effects models using Lme4. *J . Stat . Softw* ., 67, 1–48.

Beaton, L.L., Van Zandt, P.A., Esselman, E.J. & Knight, T.M. (2011) Comparison of the herbivore defense and competitive ability of ancestral and modern genotypes of an invasive plant, *Lespedeza cuneata . Oikos* , 120, 1413–1419.

Benjamini, Y. & Hochberg, Y. (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J .R . Stat . Soc . B* ., 57, 289–300.

Bixenmann, R.J., Coley, P.D., Weinhold, A. & Kursar, T.A. (2016) High herbivore pressure favors constitutive over induced defense. *Ecol . Evol* ., 6, 6037–6049.

Callaway, R.M. & Aschehoug, E.T. (2000) Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. *Science* , 290, 521–523.

Callaway, R.M. & Maron J.L. (2006) What have exotic plant invasions taught us over the past 20 years? *Trends Ecol. Evol.* , 21, 369–374.

Callaway, R.M., Lucero, J.E., Hierro, J.L. & Lortie, C.J. (2022) The EICA is dead? Long live the EICA! *Ecol . Lett* ., 25, 2289–2302.

Cappuccino, N. & Carpenter, D. (2005) Invasive exotic plants suffer less herbivory than non–invasive exotic plants. *Biol . Lett* ., 1, 435–438.

Cappuccino, N. & Arnason, J.T. (2006) Novel chemistry of invasive exotic plants. *Biol . Lett* ., 2, 189–193.

Coley, P.D., Bryant, J.P. & Chapin III, F.S. (1985) Resource availability and plant antiherbivore defense. *Science* , 230, 895–899.

Coverdale, T.C. & Agrawal, A.A. (2022) Experimental insect suppression causes loss of induced, but not constitutive, resistance in *Solanum carolinense . Ecology* , 103, e3786.

Detto, M. & Xu, X. (2020) Optimal leaf life strategies determine $V_{c,max}$ dynamic during ontogeny. *New Phytol* ., 228, 361–375.

Gatehouse, J.A. (2002) Plant resistance towards insect herbivores: a dynamic interaction. *New Phytol* ., 156, 145–169.

González-Coloma, A., López-Balboa, C., Santana, O. & Fraga, R. (2011) Triterpene-based plant defenses. *Phytochem . Rev* ., 10, 245–260.

Hahn, P.G., Keefover-Ring, K., Nguyen, L. & Maron, J.L. (2021) Intraspecific correlations between growth and defense vary with resource availability and differ within and among populations. *Funct . Ecol* ., 35, 387–396.

Hardy, N.B., Kaczvinsky, C., Bird, G. & Normark, B.B. (2020) What we don't know about diet-breadth evolution in herbivorous insects. *Annu . Rev .Ecol . Evol . S* ., 51, 103–122.

Ito, K. & Sakai, S. (2009) Optimal defense strategy against herbivory in plants: Conditions selecting for induced defense, constitutive defense, and no–defense. *J .Theor . Biol* ., 260, 453–459.

Joshi, J. & Vrieling, K. (2005) The enemy release and EICA hypothesis revisited: incorporating the fundamental difference between specialist and generalist herbivores. *Ecol . Lett* ., 8, 704–714.

Kalske, A. & Kessler, A. (2020) Population–wide shifts in herbivore resistance strategies over succession. *Ecology* , 101, e03157.

Keane, R.M. & Crawley, M.J. (2002) Exotic plant invasions and the enemy release hypothesis. *Trends Ecol . Evol* ., 17, 164–170.

Kempel, A., Schaedler, M., Chrobock, T., Fischer, M. & van Kleunen, M. (2011) Tradeoffs associated with constitutive and induced plant resistance against herbivory.*P . Natl . Acad .Sci . USA* , 108, 5685–5689.

Kumar, S., Abedin, M., Singh, A. K. & Das, S. (2020) Role of phenolic compounds in plant-defensive mechanisms. In: *Plant Phenolics in Sustainable Agriculture Singapore* (eds Lone, R., Shuab, R., Kamili, A.N.). Springer, Singapore, pp. 517–532.

Lazzarin, M., Meisenburg, M Meijer, D., Van Ieperen, W., Marcelis, L.F.M., Kappers, I.F. *et al* . (2021) LEDs make it resilient: effects on plant growth and defense. *Trends Plant Sci* ., 26, 496–508.

Lin, T., Vrieling, K., Laplanche, D., Klinkhamer, P.G., Lou, Y. Bekooy, L. *et al* . (2021). Evolutionary changes in an invasive plant support the defensive role of plant volatiles. *Curr .Biol* ., 31, 3450–3456.

Liu, M., Pan, X., Zhang, Z., Kleunen, M.V. & Li, B. (2020) Testing the shifting defense hypothesis for constitutive and induced resistance and tolerance.*J . Pest Sci* ., 93, 264–355.

Maron, J.L., Agrawal, A.A. & Schemske, D.W. (2019) Plant–herbivore coevolution and plant speciation. *Ecology* , 100, e02704.

Mauch-Mani, B., Baccelli, I., Luna, E. & Flors, V. (2017) Defense priming: an adaptive part of induced resistance. *Annu . Rev . Plant Biol* ., 68, 485–512.

Mertens, D., Fernandez de Bobadilla, M., Rusman, Q., Bloem, J., Douma, J.C. & Poelman, E.H. (2021) Plant defence to sequential attack is adapted to prevalent herbivores.*Nat . Plants* , 7, 1347–1353.

Mithofer, A. & Boland, W. (2012) Plant defense against herbivores: chemical aspects. *Annu . Rev . Ecol . Evol .S* ., 63, 431–450.

Monson, R.K., Trowbridge, A.M. Lindroth, R.L. & Lerdau, M.T. (2022) Coordinated resource allocation to plant growth–defense tradeoffs. *New Phytol* ., 233, 1051–1066.

Moreira, X., Petry, W.K., Mooney, K.A., Rasmann, S. & Abdala-Roberts, L. (2018) Elevational gradients in plant defences and insect herbivory: recent advances in the field and prospects for future research. *Ecography* , 41, 1485–1496.

Muller-Scharer, H., Schaffner, U. & Steinger, T. (2004) Evolution in invasive plants: implications for biological control. *Trends Ecol . Evol* . 19, 417–422.

Mumm, R. & Hilker, M. (2006) Direct and indirect chemical defence of pine against folivorous insects.*Trends Plant Sci* ., 11, 351–358.

Orians, C.M. & Ward, D. (2010) Evolution of plant defenses in nonindigenous environments. *Annu . Rev . Entomol* ., 55, 439–459.

Rehman, F., Khan, F.A. & Badruddin, S. M.A. (2012) Role of phenolics in plant defense against insect herbivory. In: *Chemistry of phytopotentials: health, energy and environmental perspectives* (eds Khemani, L.D., Srivastava, M.M., Srivastava, S.). Springer, Berlin, Heidelberg, pp. 309–313.

Rotter, M.C. & Holeski, L.M. (2018) A meta–analysis of the evolution of increased competitive ability hypothesis: genetic-based trait variation and herbivory resistance trade-offs. *Biol .Invasions* , 20, 2647–2660.

Schaffner, U., Ridenour, W.M., Wolf, V.C., Bassett, T., Muller, C., Muller-Scharer, H. *et al* . (2011) Plant invasions, generalist herbivores, and novel defense weapons. *Ecology* , 92, 829–835.

Sedio, B.E., Devaney, J.L., Pullen, J., Parker, G.G., Wright, S.J. & Parker, J.D. (2020) Chemical novelty facilitates herbivore resistance and biological invasions in some introduced plant species. *Ecol . Evol* ., 10, 8770–8792.

Sheng, M., Rosche, C., Al-Gharaibeh, M., Bullington, L.S., Callaway, R.M., Clark, T. *et al* . (2022). Acquisition and evolution of enhanced mutualism–an underappreciated mechanism for invasive success?*ISME J* ., 16, 2467–2478.

Simberloff, D., Kaur, H., Kalisz, S. & Bezemer, T.M. (2021) Novel chemicals engender myriad invasion mechanisms. *New Phytol* ., 232, 1184–1200.

Sims-Chilton, N.M., Zalucki, M.P. & Buckley, Y.M. (2009) Patchy herbivore and pathogen damage throughout the introduced Australian range of groundsel bush,*Baccharis halimifolia* , is influenced by rainfall, elevation, temperature, plant density and size. *Biol . Control* , 50, 13–20.

Stamp, N. (2003) Out of the quagmire of plant defense hypotheses. *Q . Rev . Biol* ., 78, 23–55.

Tian, B, Pei, Y., Huang, W., Ding, J. & Siemann, E. (2021) Increasing flavonoid concentrations in root exudates enhance associations between arbuscular mycorrhizal fungi and an invasive plant. *ISME J* ., 15, 1919-1930.

Van Kleunen, M., Dawson, W., Schlaepfer, D., Jeschke, J.M. & Fischer, M. (2010). Are invaders different? A conceptual framework of comparative approaches for assessing determinants of invasiveness. *Ecol . Lett* ., 13, 947–958.

Waller, L.P., Allen, W.J., Barratt, B.I.P., Condron, L.M., Franca, F.M., Hunt, J. E. *et al* . (2020) Biotic interactions drive ecosystem responses to exotic plant invaders. *Science* , 368, 967–972.

Wan, J., Yi, J., Tao, Z., Ren, Z., Otieno, E. O., Tian, B. *et al* . (2022). Species-specific plant-mediated effects between herbivores converge at high damage intensity. *Ecology* , 103, e3647.

Wittstock, U. & Gershenzon, J. (2002) Constitutive plant toxins and their role in defense against herbivores and pathogens. *Curr .Opin . Plant . Biol* ., 5, 300–307.

Woods, E.C., Hastings, A.P., Turley, N.E., Heard, S.B. & Agrawal, A.A. (2012) Adaptive geographical clines in the growth and defense of a native plant. *Ecol .Monogr* ., 82, 149–168.

Yu, H., He, Y., Zhang, W., Chen, L., Zhang, J., Zhang, X. *et al* . (2022) Greater chemical signaling in root exudates enhances soil mutualistic associations in invasive plants compared to natives. *New Phytol* ., 236, 1140-1153.

Zhang, Z., Liu, Y., Yuan, L., Weber, E. & Kleunen, M.V. (2020) Effect of allelopathy on plant performance: a meta–analysis. *Ecol . Lett* ., 24, 348–362.

Zhang, Z., Pan, X., Blumenthal, D., Van Kleunen, M., Liu, M. & Li, B. (2018) Contrasting effects of specialist and generalist herbivores on resistance evolution in invasive plants.*Ecology* , 99, 866–875.

Zou, J., Siemann, E., Rogers, W. E. & DeWalt, S.J. (2008) Decreased resistance and increased tolerance to native herbivores of the invasive plant *Sapium sebiferum* . *Ecography* , 31, 663–671.

Figures

Hosted file

`image1.emf` available at https://authorea.com/users/571956/articles/617053-intensity-of-herbivory-correlates-with-stronger-constitutive-and-weaker-induced-defenses-for-non-native-plant-species-another-mechanism-for-eica

Figure 1. Herbivore pressure on non-native and native plant species. Percentage of damaged leaves on non-native (A) and co-occurring native (B) species in the field survey, and percentage of damaged leaf area

on non-native species (D) and native congeners (E) in the common garden experiment. Differences in the percentage of damaged leaves on non-native and co-occurring native species in the field (C) and in the percentage of damaged leaf area on non-native species and native congeners in the common garden (F). Boxplots represent the interquartile range and median, and points represent outliers. There were 27 non-native plant species measured (n = 3 to 66 individuals per species depending on occurrence in the field) and 59 non-native plant species measured (n = 3 to 54 individuals per species depending on occurrence) in the field survey across 28 sites. The most common 12 non-native plant species and their native congeners were used in the common garden experiment (n = 6 per species). * indicate p <0.05 and *** indicate p <0.001.

Hosted file

`image2.emf` available at https://authorea.com/users/571956/articles/617053-intensity-of-herbivory-correlates-with-stronger-constitutive-and-weaker-induced-defenses-for-non-native-plant-species-another-mechanism-for-eica

Figure 2. Correlations between constitutive or induced defenses and herbivore pressure across 12 non-native plant species and 12 native plant species. Relationships between the percentage of damaged leaf area in the common garden experiment and constitutive defense assessed by generalist *Spodoptera littoralis* larval weight gain on the leaves of healthy plants (A) and assessed by phenolics (C) and triterpenoids (E) contents in the leaves of healthy plants. Relationships between the percentage of damaged leaf area in the common garden experiment and induced defense assessed by difference in *S. littoralis* larval weight gain on the leaves between healthy and damaged plants [-1 x (larval weight gain on the leaves of damaged plant – average of larval weight gain on the leaves of healthy plant), B], and assessed by differences in phenolics in the leaves between healthy and damaged plants (content in the leaves of damaged plant – average of content in the leaves of healthy plant, D) and difference in triterpenoids on the leaves between healthy and damaged plants (content in the leaves of damaged plant - average of content in the leaves of healthy plant, F). Each point represents individual non-native species (red points) and native congeners (blue points). Lines indicate significant linear relationships. The embedding boxplots represents the comparison for each group of species overall. * indicate p <0.05 and *** indicate p <0.001.
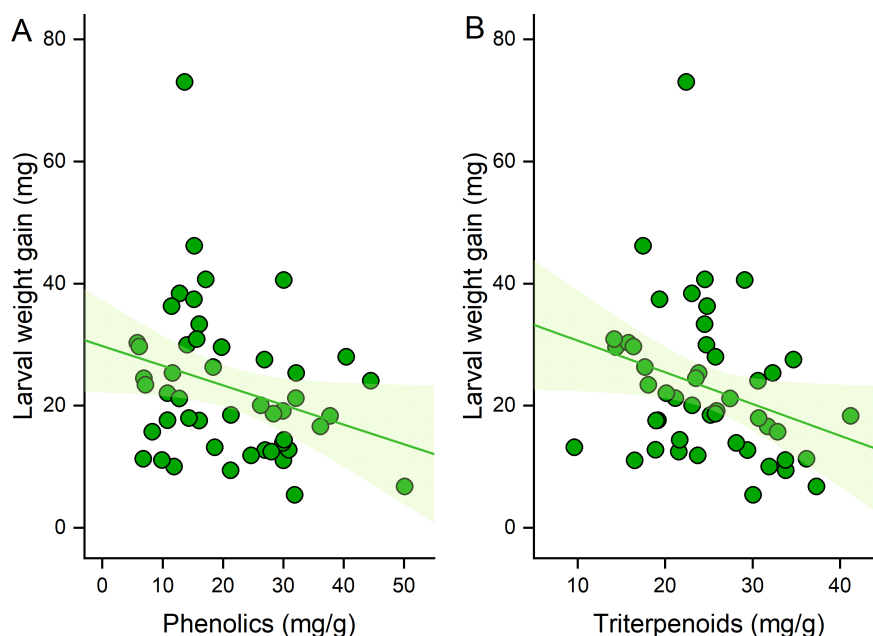

Figure 3. Relationships between the growth of *Spodoptera littoralis* larvae and leaf secondary chemicals across all species (12 pairs of non-native plants and native congeners) and herbivory treatments (healthy

plants and larvae-damaged plants). Relationship between larval weight gain and leaf phenolic content (A) and leaf triterpenoid content (B). Data points represent individual non-natives and native species. Lines indicate significant linear relationships.

Hosted file

`image4.emf` available at https://authorea.com/users/571956/articles/617053-intensity-of-herbivory-correlates-with-stronger-constitutive-and-weaker-induced-defenses-for-non-native-plant-species-another-mechanism-for-eica

Figure 4. Trade-off between constitutive defense and induced defense across 12 pairs of non-native plants and native congeners. Relationship between constitutive defense (-1 × larval weight gain on the leaves of healthy plant) and induced defense [-1 × (larva weight gain on the leaves of damaged plant – average of larval weight gain on the leaves of healthy plant)] assessed by generalist *Spodoptera littoralis* larval growth (A). Relationship between constitutive defense (content in the leaves of healthy plant) and induced defense (content in the leaves of damaged plant - average of content in the leaves of healthy plant) assessed by phenolics (B). Blue points represent individual non-native species, and red points represent individual native species. Lines indicate significant linear relationships.
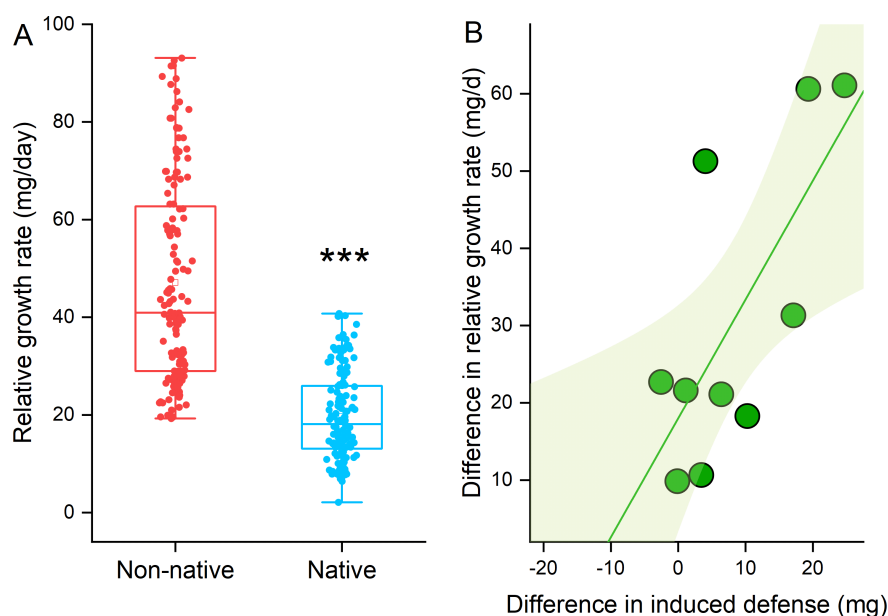

Figure 5. Relative growth rates of non-native species and native congeners (A). Relationship between the difference in the relative growth rate between non-native species and its corresponding native congener and the difference in the induced defense between non-native species and its corresponding native congener (B). Induced defense was assessed by difference in *Spodoptera littoralis* larval weight gain on the leaves between healthy and damaged plants [-1 × (larval weight gain on the leaves of damaged plant – average of larval weight gain on the leaves of healthy plant)]. Each point represents a non-native species and its native congener. Lines indicate significant linear relationships. *** indicates $p < 0.001$.

Hosted file

`image6.emf` available at https://authorea.com/users/571956/articles/617053-intensity-of-herbivory-correlates-with-stronger-constitutive-and-weaker-induced-defenses-for-non-native-plant-species-another-mechanism-for-eica

Figure 6. Appropriation of ancient Chinese folk mythology to illustrate integration of the ERH, SDH, and

EICA hypotheses with variation in defense allocation. Sun Wukong, the monkey king (representing a plant species), possesses inherent strength that is limited by the incantation of the golden hoop (A) - specialist herbivores - which keeps him under control. However, when Sun Wukong undertakes a pilgrimage to the west (B) - non-native introduction - the golden hoop is no longer required, allowing a rapid increase in defense capacity (C) - the golden cudgel he acquired on his journey - against the now predominant generalist herbivores. Simultaneously, removing the golden hoop allows a strong and consistent response to *variation* in generalist herbivore pressure, resulting in a positive correlation between herbivore pressure and allocation to cheaper induced defense (D). This reallocation leads to greater growth consistent with the Evolution of Increased Competitive Ability Hypothesis (E).
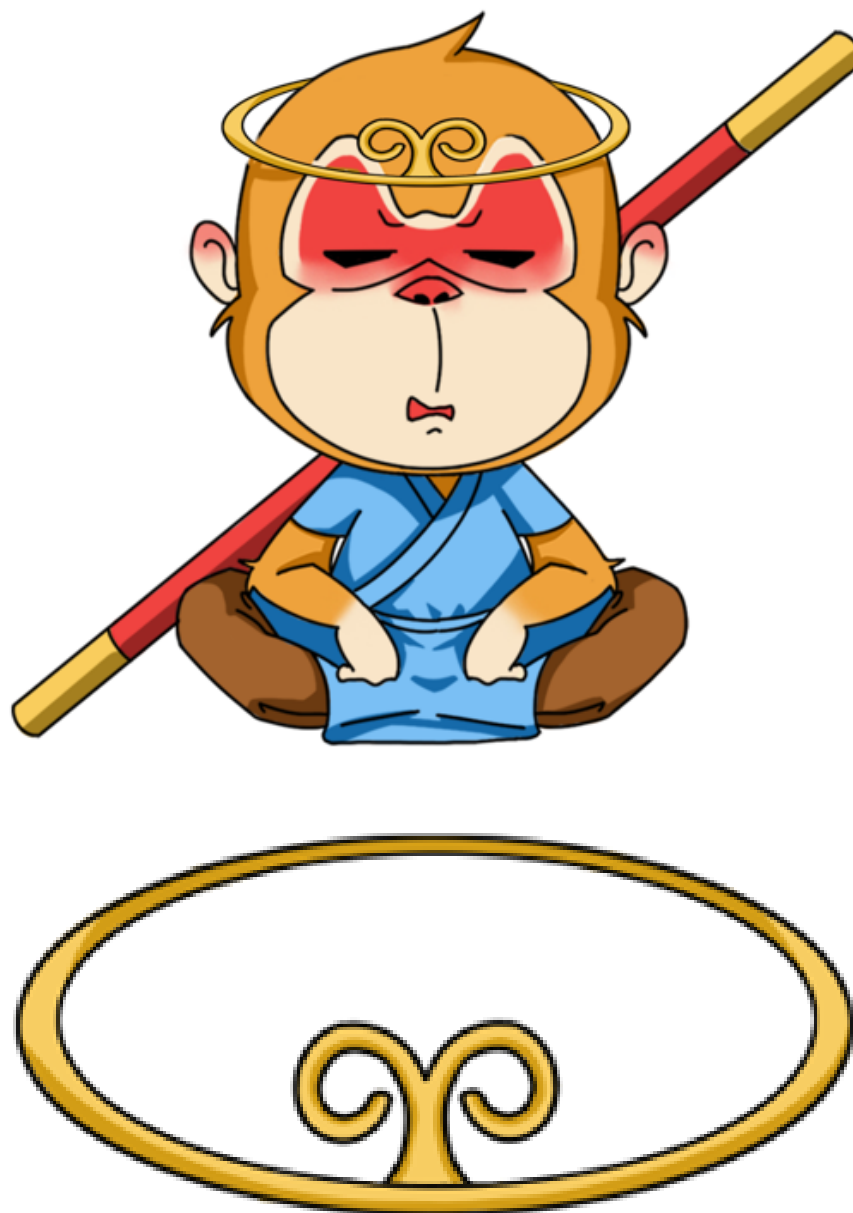
: Sun Wukong. : The golden hoop. : Generalist herbivore. : specialist herbivore. : Golden cudgel, plant defense. ERH: Enemy Release Hypothesis. SDH: Shifting Defense Hypothesis. EICA: Evolution of Increased Competitive Ability Hypothesis.

Supporting Information

Herbivore pressure correlates with stronger constitutive and weaker induced defenses for non-native plant species

Xiao Sun[1], Yumei Sun[1], Xueyao Cao[1], Xincong Zhai[1], Ragan M. Callaway[2], Jinlong Wan[3,4], S. Luke Flory[5], Wei Huang[3,4]*, Jianqing Ding[1]

[1] State Key Laboratory of Crop Stress Adaptation and Improvement, School of Life Sciences, Henan University, Kaifeng 475004, China

[2] Division of Biological Sciences and Institute on Ecosystems, University of Montana, Missoula 59812, USA

[3] Key Laboratory of Aquatic Botany and Watershed Ecology, Wuhan Botanical Garden, Chinese Academy of Sciences, Wuhan 430074, China

[4] Core Botanical Garden, Chinese Academy of Sciences, Wuhan 430074, China

[5] Agronomy Department, University of Florida, Gainesville 32611, USA

*Correspondence: Wei Huang, huangwei0519@wbgcas.cn

This file contains the following Supplementary Information:

- Supplementary Methods: Appendix Methods 1-2
- Supplementary Figures: Appendix Figure S1-S5
- Supplementary Tables: Appendix Table S1-S4

Supplementary Methods 1: Evaluate herbivore biomass in the field survey

We used herbivore biomass to represent herbivore pressure (Allen et al., 2021) as higher herbivore abundance may not always translate to greater herbivore damage if feeding by particular herbivore species is relatively minor (e.g., aphids and beetles) or extensive (e.g., larvae). To determine whether the herbivore biomass correlated with leaf damage, we checked all leaves and recorded the number of each herbivore species on each non-native plant. We classified observed herbivores into five orders (nine categories), including Lepidoptera (larvae), Orthopter, Thysanoptera, Hemiptera (leafhoppers, stinkbugs, whiteflies, and aphids), and Coleoptera (beetles and herbivorous ladybirds) since some larvae were difficult to identify to species. A

total of 20,723 insects on non-native plants were counted (Native plants were not measured as these measurements were primarily to correlate damage to insect biomass). Then, we evaluated herbivore biomass on each plant by multiplying abundance and mean herbivore biomass of each category and then summing herbivore biomass of all categories. To obtain the mean biomass of each herbivore category, we carried out a field survey at eight sites in 2019. We collected all herbivores on non-native species, stored them in plastic tubes with leaves of host plants and determined the fresh weight of individual insects to the nearest 0.01g in the lab. The mean biomass of each herbivore category was as follows: Lepidoptera, 0.25 g/ individual; Orthoptera, 0.45 g/ individual; Thysanoptera, 0.01g/30 individuals; leafhopper, 0.03g/ individual; stinkbug, 0.19 g/ individual; whitefly, 0.01 g/30 individuals; aphid, 0.01 g/5 individuals; leaf beetle, 0.05 g/ individual; ladybird, 0.04 g/ individual.

Supplementary Methods 2: Chemical analysis of total phenolics and total triterpenoid

Leaves were flash frozen in liquid nitrogen and stored at -80°C for analysis. Total phenolic concentration was calculated with the modified Folin-Ciocalteau method (Singleton & Rossi, 1964). Briefly, an aliquot of 1 mL of free phenolic acid extract was added to 5 mL of water, 1.0 mL Folin-Ciocalteau reagent, and 3 ml of saturated sodium carbonate solution (7.5%) and mixed in a screw-top test tube. The absorbance was measured at 765 nm with a spectrophotometer, after incubation for 2 h at room temperature. Quantification was based on the standard curve, established with 0, 50, 100, 150, 200 and 250 mg/L of gallic acid, and the results were expressed as gallic acid equivalent in milligrams per gram dry weight (mg GAE/g DW). Total triterpenoid concentration was determined via vanillin-glacial acetic acid-perchloric acid spectrophotometry using oleanolic acid as the standard (Fan & He, 2006). Briefly, total triterpenoids were extracted by ultrasonic extraction using anhydrous ethanol as a solvent. Oleanolic acid was used as the reference, 5% vanillin-acetic acid glacial solution and perchlorate acid were used as the chromogenic agent, followed by water bath at 70°C for 15min, and the absorbance was measured at 560 nm. Because the amount of one leaf was not sufficient for each chemical analysis, we gathered leaves from six plants as a biological replication. There were 12 replicates for each combination of health plant and herbivory treatment for each non-native species or their native congeners.

References

Allen, W.J., Waller, L.P., Barratt, B.I., Dickie, I.A. & Tylianakis, J.M. (2021) Exotic plants accumulate and share herbivores yet dominate communities via rapid growth. *Nat. Commun* ., 12, 1–12.

Singleton, V. & Rossi, J.A. (1964) Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am . J .Enol . Viticult* ., 16, 144–158.

Fan, J.P. & He, C.H. (2006) Simultaneous quantification of three major bioactive triterpene acids in the leaves of *Diospyros kaki* by high–performance liquid chromatography method. *J .Pharmaceut . Biomed* ., 41, 950–956

Hosted file

image12.emf available at https://authorea.com/users/571956/articles/617053-intensity-of-herbivory-correlates-with-stronger-constitutive-and-weaker-induced-defenses-for-non-native-plant-species-another-mechanism-for-eica

Figure S1. Predictions for how herbivore pressure affects plant defense and growth traits. Plant species under higher herbivore pressure (putatively natives, blue line) are predicted to have higher mean constitutive defense and lower mean induced defense than plants under lower herbivore pressure (putatively non-natives, red line). Also, differences in herbivore pressure among species should correlate with increasing constitutive defense and decreasing induced defenses for all species, illustrated by the slopes of the lines (A and B). These defense responses should lead to growth-defense trade-offs in which greater allocation to less expensive induced defense allows allocation to greater growth (C).

Hosted file

Figure S2. Total herbivore biomass collected on the non-native plants in the field survey (A) and common garden experiment (B). We classified herbivores in the field survey and common garden experiment into five orders (nine categories), including larva Lepidoptera, Orthopter, Thysanoptera, Hemiptera (leafhopper, stinkbug, whitefly, and aphid), and Coleoptera (beetle and ladybird) since larvae of some species were difficult to identify to species. The values are the total herbivore biomass in each category.
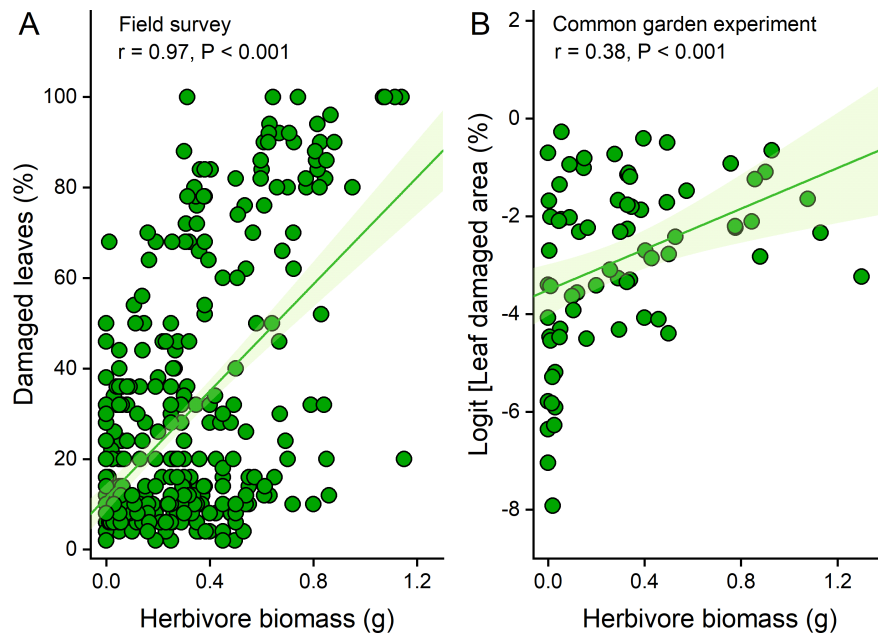

Figure S3. Relationship between herbivore biomass and leaf damage for non-native plant species in the field survey (A) and in the common garden experiment (B). Data points represent individual plants (n = 396 in the field survey, n = 84 in the common garden experiment). Lines through points indicate significant linear relationships.
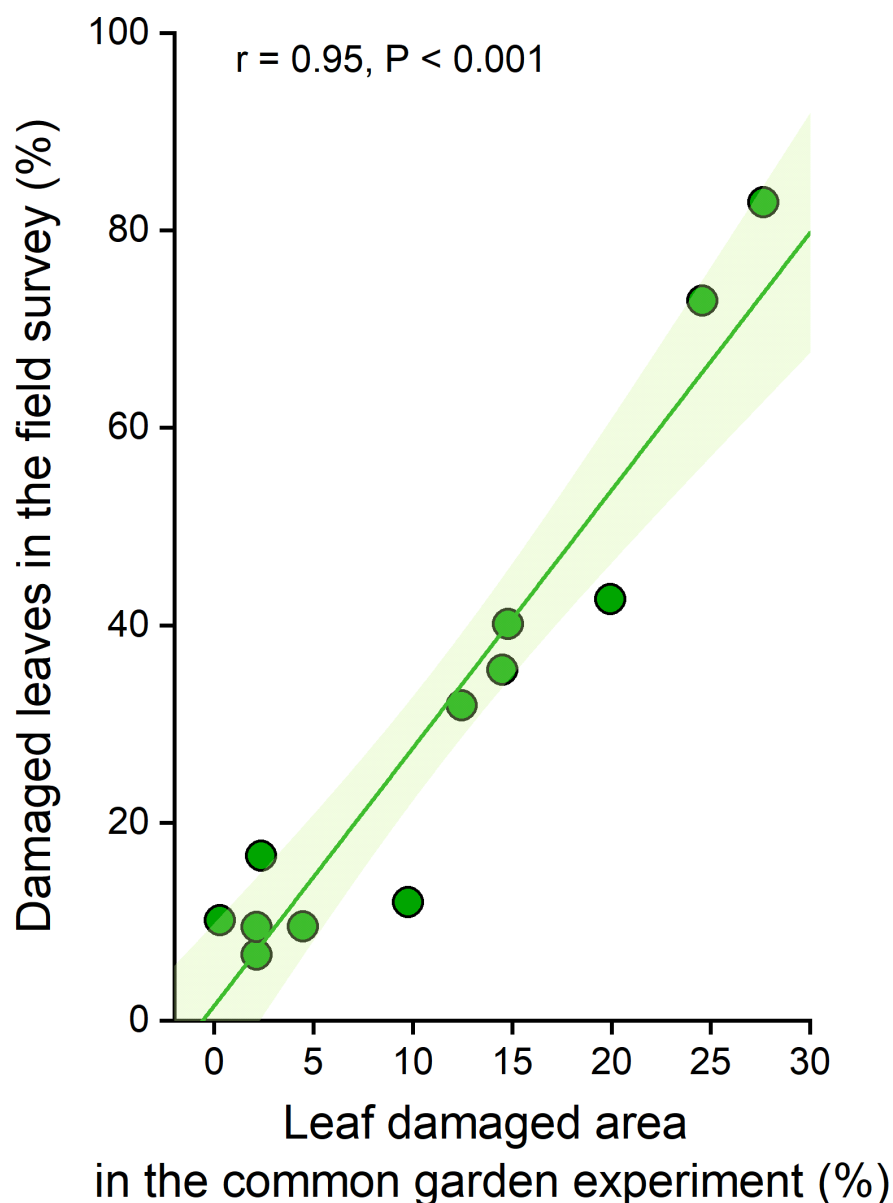
Figure S4. Relationship between percentage of damaged leaves in the field survey and percentage of damaged leaf area in the common garden experiment for non-native species. Each points represent individual non-native species. The line through points indicates a significant linear relationship.

Hosted file

`image16.emf` available at https://authorea.com/users/571956/articles/617053-intensity-of-herbivory-correlates-with-stronger-constitutive-and-weaker-induced-defenses-for-non-native-plant-species-another-mechanism-for-eica

Figure S5. Pairs of non-native plants and native congeners at the end of experiment. Left, native plants; right, non-native plants. a,*Amaranthus tricolor vs. Amaranthus retroflexus* ; b,*Xanthium sibiricum vs. Xanthium italicum* ; c, *Bidens tripartite vs. Bidens frondose* ; d, *Solidago decurrens vs . Solidago canadensis* ; e, *Rhus chinensis vs . Rhus typhina* ; f, *Phytolacca acinosevs . Phytolacca americana* ; g, *Amaranthus*

blitum vs . *Amaranthus spinosus* .

Table S1. Locations of 28 sites for the field survey in northern China. Codes of the sites with asterisks indicate those surveyed again for evaluation of herbivore biomass.

Number	Code	Site	Province	Longitude	Latitude
1	CZ1	Cangzhou	Hebei	38°11'N	116°43'E
2	CZ2	Cangzhou	Hebei	38°54'N	116°09'E
3	HD	Handan	Hebei	36°13'N	114°23'E
4	HS1*	Hengshui	Hebei	37°51'N	115°41'E
5	HS2	Hengshui	Hebei	37°35'N	115°37'E
6	XT1	Xingtai	Hebei	36°54'N	114°29'E
7	XT2*	Xingtai	Hebei	36°52'N	114°24'E
8	XT3	Xingtai	Hebei	36°54'N	114°24'E
9	DF1	Dengfeng	Henan	34°27'N	112°58'E
10	DF2	Dengfeng	Henan	34°32'N	112°55'E
11	GY*	Gongyi	Henan	34°48'N	113°01'E
12	KF1*	Kaifeng	Henan	34°52'N	114°18'E
13	KF2	Kaifeng	Henan	34°49'N	114°16'E
14	SQ*	Shangqiu	Henan	34°08'N	115°25'E
15	XX*	Xinxiang	Henan	35°06'N	114°16'E
16	ZK*	Zhoukou	Henan	34°00'N	114°24'E
17	XZ1	Xuzhou	Jiangsu	34°26'N	117°08'E
18	XZ2	Xuzhou	Jiangsu	34°29'N	117°08'E
19	BZ*	Binzhou	Shandong	36°58'N	117°38'E
20	DZ	Dezhou	Shandong	36°50'N	116°41'E
21	JN	Jinan	Shandong	36°53'N	117°30'E
22	JN1	Jining	Shandong	34°45'N	117°08'E
23	JN2	Jining	Shandong	35°39'N	116°57'E
24	JN3	Jining	Shandong	35°47'N	117°04'E
25	LW	Laiwu	Shandong	36°13'N	117°27'E
26	LY	Linyi	Shandong	36°05'N	118°38'E
27	WF	Weifang	Shandong	36°33'N	119°44'E
28	ZB	Zibo	Shandong	36°51'N	118°19'E

Table S2. Occurrence and herbivore pressure of 27 non-native plant species in 28 sites in field survey across North China. Species with asterisks were used in the common garden experiment, bioassay and chemical analysis. The difference in the percentage of damaged leaf (binary data for undamaged *vs.* damaged leaves in a cbind matrix) among sites was tested using a Wald Chi-square test applied on a Generalized Linear Model (GLM) with a binomial distribution. Each non-native species was analyzed separately.

Non-native species	Number of measured individuals	Frequency of occurrence (%)	Number of insects	Difference
				Df
*Erigeron canadensis**	66	78.6	4131	21
*Ipomoea cairica**	36	42.9	355	11
*Bidens frondosa**	30	35.7	1747	9
*Amaranthus spinosus**	27	32.1	1396	8
*Bidens pilosa**	27	32.1	1708	8
*Amaranthus retroflexus**	18	21.4	696	5
*Aster subulatus**	15	17.9	19	4

20

Non-native species	Number of measured individuals	Frequency of occurrence (%)	Number of insects	Differenc
*Datura stramonium**	15	17.9	1098	4
*Hibiscus trionum**	15	17.9	1105	4
*Abutilon theophrasti**	12	14.3	3066	3
*Phytolacca americana**	12	14.3	1329	3
*Rhus typhina**	12	14.3	2	3
*Solidago canadensis**	12	14.3	1122	3
*Xanthium italicum**	12	14.3	18	3
Cyperus rotundus	9	10.7	8	
Erigeron bonariensis	9	10.7	15	
Flaveria bidentis	9	10.7	12	
Veronica didyma	9	10.7	0	
Veronica persica	9	10.7	0	
Ambrosia artemisiifolia	6	7.1	1370	
Cosmos bipinnata	6	7.1	173	
Daucus carota	6	7.1	4	
Helianthus tuberosus	6	7.1	243	
Lactuca seriola	6	7.1	648	
Solanum aculeatissimum	6	7.1	5	
Erigeron annuus	3	3.6	320	
Sonchus asper	3	3.6	133	

Table S3. Number and frequency of occurrence 59 native plant species in 28 sites in a field survey across North China. Species with asterisks were used in the common garden experiment, bioassay and chemical analysis. The difference in the percentage of damaged leaf (binary data of undamaged *vs.* damaged leaves in a cbind matrix) among sites was tested using a Wald Chi-square test applied on a Generalized Linear Model (GLM) with a binomial distribution. Each non-native species was analyzed separately. Each native species was analyzed separately.

Non-native species	Number of measured individuals	Frequency of occurrence (%)	Difference in herbivore
			Df
Xanthium sibiricum	54	64.3	17
Bidens biternata	45	53.6	14
Amaranthus blitum	39	46.4	12
Amaranthus tricolor	33	39.3	10
Abutilon indicum	30	35.7	9
Phytolacca acinosa	27	32.1	8
Datura metel	15	17.9	4
Erigeron acer	12	14.3	3
Rhus chinensis	12	14.3	3
Solidago decurrens	12	14.3	3
Aster indicus	9	10.7	2
Bidens tripartita	9	10.7	2
Hibiscus mutabilis	9	10.7	2
Ipomoea aquatica	9	10.7	2
Setaria viridis	45	53.6	
Cirsium arvense	42	50.0	
Taraxacum mongolicum	42	50.0	
Artemisia argyi	27	32.1	
Eclipta prostrata	15	17.9	

Non-native species	Number of measured individuals	Frequency of occurrence (%)	Difference in herbivore
Sonchus wightianus	12	14.3	
Artemisia sacrorum	9	10.7	
Aster ageratoides	9	10.7	
Cirsium japonicum	9	10.7	
Lactuca indica	9	10.7	
Alternanthera sessilis	6	7.1	
Chloris virgata	6	7.1	
Digitaria sanguinalis	6	7.1	
Eupatorium chinense	6	7.1	
Eupatorium fortunei	6	7.1	
Imperata cylindrica	6	7.1	
Ixeris chinensis	6	7.1	
Plantago asiatica	6	7.1	
Portulaca oleracea	6	7.1	
Pulsatilla chinensis	6	7.1	
Solanum nigrum	6	7.1	
Sonchus oleraceus	6	7.1	
Cynodon dactylon	6	7.1	
Artemisia carvifolia	3	3.6	
Artemisia scoparia	3	3.6	
Bolboschoenus yagara	3	3.6	
Bothriospermum chinense	3	3.6	
Carduus nutans	3	3.6	
Chrysanthemum lavandulifolium	3	3.6	
Coreopsis basalis	3	3.6	
Cyperus rotundus	3	3.6	
Elephantopus scaber	3	3.6	
Euphorbia thymifolia	3	3.6	
Gaillardia pulchella	3	3.6	
Helianthus annuus	3	3.6	
Hydrocotyle sibthorpioides	3	3.6	
Ixeris japonica	3	3.6	
Lactuca raddeana	3	3.6	
Rehmannia glutinosa	3	3.6	
Salvia plebeia	3	3.6	
Silene conoidea	3	3.6	
Torilis scabra	3	3.6	
Tribulus terrestris	3	3.6	
Trifolium repens	3	3.6	
Trigonotis peduncularis	3	3.6	

Table S4. List of the 12 non-native plant species and their native congeners used in the common garden experiment, bioassay and chemical analysis.

Number	Family	Genus	Native	Growth type	Life cycle	Non-native	Growth type	Life cycle	Or
1	Asteraceae	Aster	*Aster ageratoides*	herbaceous	Annual	*Aster subulatus*	herbaceous	annual	No Ar

Number	Family	Genus	Native	Growth type	Life cycle	Non-native	Growth type	Life cycle	Or
2	Asteraceae	Bidens	*Bidens tripartite*	herbaceous	Annual	*Bidens frondosa*	herbaceous	annual	No An *As su*
3	Asteraceae	Bidens	*Bidens biternata*	herbaceous	Annual	*Bidens pilosa*	herbaceous	annual	An
4	Asteraceae	Erigeron	*Erigeron acer*	herbaceous	Annual	*Erigeron canadensis*	herbaceous	annual	No An
5	Asteraceae	Solidago	*Solidago decurrens*	herbaceous	Annual	*Solidago canadensis*	herbaceous	annual	No An
6	Asteraceae	Xanthium	*Xanthium sibiricum*	herbaceous	Annual	*Xanthium italicum*	herbaceous	annual	An
7	Amaranthaceae	Amaranthus	*Amaranthus tricolor*	herbaceous	Annual	*Amaranthus retroflexus*	herbaceous	annual	An
8	Amaranthaceae	Amaranthus	*Amaranthus blitum*	herbaceous	Annual	*Amaranthus spinosus*	herbaceous	annual	So An
9	Solanaceae	Datura	*Datura metel*	herbaceous	Annual	*Datura stramonium*	herbaceous	annual	So As
10	Convolvulaceae	Ipomoea	*Ipomoea aquatica*	herbaceous	Annual	*Ipomoea cairica*	herbaceous	annual	As
11	Phytolaecaceae	Phytolacca	*Phytolacca acinosa*	herbaceous	Perennial	*Phytolacca americana*	herbaceous	perennial	No An
12	Malvaceae	Abutilon	*Abutilon indicum*	herbaceous	Annual	*Abutilon theophrasti*	herbaceous	annual	So As