A native plant competitor mediates the impact of above- and belowground damage on an invasive tree

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Abstract. Plant competition may mediate the impacts of herbivory on invasive plant species through effects on plant growth and defense. This may predictably depend on whether herbivory occurs above or below ground and on relative plant competitive ability. We simulated the potential impact of above- or belowground damage by biocontrol agents on the growth of a woody invader (Chinese tallow tree, Triadica sebifera) through artificial herbivory, with or without competition with a native grass, little bluestem (Schizachyrium scoparium). We measured two defense responses of Triadica through quantifying constitutive and induced extrafloral nectar production and tolerance of above- and belowground damage (root and shoot biomass regrowth). We examined genetic variation in plant growth and defense across native (China) and invasive (United States) Triadica populations. Without competition, aboveground damage had a greater impact than belowground damage on Triadica performance, whereas with competition and above- and belowground damage impacted Triadica similarly. Whole plant tolerance to damage below ground was negatively associated with tolerance to grass competitors indicating tradeoffs in the ability to tolerate herbivory vs. compete. Competition reduced investment in defensive extrafloral nectar (EFN) production. Aboveground damage inhibited rather than induced EFN production while belowground plant damage did not impact aboveground nectar production. We found some support for the evolution of increased competitive ability hypothesis for invasive plants as United States plants were larger than native China plants and were more plastic in their response to biotic stressors than China plants (they altered their root to shoot ratios dependent on herbivory and competition treatments). Our results indicate that habitat type and the presence of competitors may be a larger determinant of herbivory impact than feeding mode and suggest that integrated pest management strategies including competitive dynamics of recipient communities should be incorporated into biological control agent evaluation at earlier stages.

Key words: aboveground–belowground interactions; biological control; Chinese tallow tree, Triadica sebifera; competition; extrafloral nectar; little bluestem, Schizachyrium scoparium; resistance; tolerance.

INTRODUCTION

Herbivory and competition interact to influence plant performance (e.g., Agrawal 2004) and can promote species coexistence or exclusion (e.g., Carson and Root 2000, Hanley and Sykes 2009, Kim et al. 2013). This can occur, for example, through herbivory reducing the ability of plants to compete or by plant competitors inhibiting the ability of plants to defend against herbivores (e.g., Hambäck and Beckerman 2003). Fundamentally, this tension between plant competitive ability and defense against herbivory can be seen as a phenotypic response to a reduction in plant resources. However, for some plant defenses, including tolerating some level of herbivore damage without reductions in plant fitness, it has been hypothesized that both high and low resource levels will improve plant defense (e.g., compensatory continuum hypothesis [Maschinski and Whitham 1989] and growth rate model [Hilbert et al. 1981]). There has been support both for and against these scenarios (e.g., Hawkes and Sullivan 2001, Lieurance and Cipollini 2013), which may be due to differences in the limiting resource for plants and where herbivory occurs, and for many plants, the presence and type of competitors.

Specifically, simple models of plant resource competition with two limiting resources (in this case above- and belowground resources, e.g., light and nitrogen), predict that where herbivores feed on target plants can shift the balance of plant competitive dynamics (resource competition model; Siemann and Weisser 2004) and the ability of plants to tolerate herbivory (limiting resource model, LRM; Wise and Abrahamson 2005, 2007). For example, in a simple two-species model of resource competition, coexistence is assumed because the species differ in their competitive ability for different resources (Siemann and Weisser 2004). In this case, the resource competition
model predicts that aboveground damage on the putatively better aboveground competitor decreases the potential for coexistence, as damage above ground reduces or removes that species’ aboveground competitive advantage, while damage below ground should have less of an impact on plant success. This focus on plant competitors, and their most limiting resource, contrasts the LRM, which predicts that damage that inhibits the ability of plants to uptake their most limiting resource, independent of plant competitors (or perhaps, encompassing all plant competitors), will reduce that plant’s ability to compensate (e.g., Wise and Abrahamson 2007). However, only a handful of studies have examined plant tolerance responses across different competition regimes or with different herbivory types (e.g., apical vs. foliar damage; Dahlgren and Lehtilä 2015), and integration of these models is lacking.

Biological control of weedy or invasive plants could inform our understanding of herbivory–resource competition interactions. Whereas classical biological control of weeds considers host plant specificity and traditionally focuses on biocontrol impact in artificial monocultures where the target weed is experimentally isolated from its recipient plant community (i.e., without competitors from the invasive range), a greater awareness of recipient communities has been shown to improve control. For example, biological control of purple loosestrife (Lythrum salicaria) by Galerucella beetles was more likely to be successful in sites with lower vs. higher fertility (Hovick and Carson 2015), potentially indicating reduced tolerance of plants at lower fertility. Moreover, it is known that the presence of plant competitors can reduce the negative effect of invaders through providing biotic resistance to invasion and reducing invader establishment and/or negative effect (Levine et al. 2004). Plant competitors may also inhibit the ability of plants to defend against introduced control agents and magnify those agents’ impacts. For example, the combined effects of native herbivory and competition by native thistle (Cirsium altissimum) resulted in strong biotic resistance to exotic thistle (C. vulgaris), and reduced invasive impact to the community (Suwa and Louda 2012). Indeed, the mechanisms underlying plant competitive and invasive success may make some biocontrol agents especially effective for particular competitive scenarios. One such case could be tree/grass competition (e.g., woody encroachment into grasslands), where we would predict that damage above ground would be more effective than damage below ground in reducing tree performance. This is because fine-rooted grasses can often exploit belowground soil resources at a greater rate than woody trees, that in contrast, eventually dominate aboveground resource capture of light (e.g., Picon-Cochard et al. 2006).

Chinese tallow tree (Triadica sebifera, Euphorbiaceae; Triadica hereafter) is a devastating invader of the southeastern United States and can quickly dominate natural areas including roadsides, forests, and endangered tallgrass prairies. Several biocontrol agents are currently being tested for Triadica, including two multivoltine insects: an aboveground-feeding caterpillar (Gadirtha fusca; previously G. inexacta) and a flea beetle (Bikasha collaris) that has both an aboveground feeding adult stage and a belowground feeding larval stage (e.g., Wang et al. 2012). Triadica is extremely tolerant of herbivory, although native (Chinese) vs. invasive (United States) populations vary in the specificity of their tolerance response to different aboveground-feeding herbivores (Carrillo et al. 2014). Increasing evidence of evolved increased tolerance of herbivory in invasive plant populations compared to their native counterparts (e.g., Carrillo et al. 2014 [T. sebifera]; Joshi and Telboger 2012 [Lythrum salicaria]; Wang et al. 2011 [T. sebifera]) necessitates a greater understanding of this defense response to above- and belowground forms of damage, and its interaction with the presence of plant competitors. Invasive populations of Triadica have reduced direct, chemical defenses (Wang et al. 2011, 2012), and reduced, yet retained, indirect defenses against herbivores (extrafloral nectar, EFN; Carrillo et al. 2012b). EFN can attract predaceous ants which can provide protection to plants (e.g., Heil 2015) and could potentially reduce population levels of biocontrol agents. It is unclear how EFN production will be impacted by plant competition. Importantly, evaluating potential biocontrol agent impacts solely with native populations would likely overestimate the effects of biocontrol, therefore including invasive populations is necessary for accurate management as they may differ in defense syndromes due to divergent evolutionary history (e.g., Agrawal and Fishbein 2006). Although we predict that broad niche differences in resource utilization will determine target plant responses, genetic variation in competitive ability and resistance and tolerance to above- and belowground herbivory may be important for target plant performance (Agrawal 2004). Therefore, we include both native and invasive populations of Triadica to examine evolved differences in competitive ability and herbivore defense that may impact biocontrol success.

We evaluated the potential relative success of above- or belowground damage on the invasive success of a woody invader, Triadica, by simulating above- or belowground herbivory when in competition with a native grass, little bluestem (Schizachyrium scoparium) or when grown singly. We also examined how damage and competition jointly influenced plant tolerance responses and the production of extrafloral nectar in Triadica as this may impact future biocontrol success. We hypothesize that, in accordance with the resource competition model (Siemann and Weisser 2004), plants that are superior light competitors but weaker soil-resource competitors will be more impacted by aboveground feeding agents than belowground feeding agents when competing with a superior belowground competitor. We specifically predict that Triadica, a superior light competitor, will be more impacted by aboveground feeding biocontrol agents when in competition with little bluestem grass, a putatively
better soil-resource competitor. Due to reduced resource availability, we predict that competition with grass will reduce the defensive (EFN) response of plants, but that in general damage will induce defensive (EFN) responses.

**MATERIALS AND METHODS**

**Study system**

_Triadica sebifera_ (L.) Small (Euphorbiaceae) is a dominant, non-clonal tree species across the southeastern United States where it commonly occurs along roadsides, along waterways, bottomland hardwood forests, and endangered coastal prairies. _Triadica_ is virtually free of herbivore pests in its introduced range, but is attacked by a diversity of specialist and generalist herbivores across multiple feeding guilds both above and below ground in its native range (e.g., Huang et al. 2014). Little bluestem grass, *Schizachyrium scoparium*, is dominant in coastal prairie remnants and is widely distributed throughout the United States across a range of conditions. It is the most likely competitor for _Triadica_ invading coastal prairies (Bruce, Cameron, and Harcombe 1995) and is frequently used for revegetation or restoration seed mixes (USDA PLANTS database). Therefore, results for its competitive ability against invasive _Triadica_ may be generalizable to other grass–tree interactions across the United States.

We grew native and invasive _Triadica_ populations (six populations each) with or without competition with little bluestem and with or without simulated above- or belowground herbivory to determine the effects of competition, aboveground herbivory, belowground herbivory, and their interactions on plant growth and defense (2 × 2 × 2 = 8 treatment combinations per each of 12 plant populations). _Triadica_ propagates by seed and we collected _Triadica_ seeds in late November/early December 2010 across the native (China) and introduced (United States) ranges. We sampled six populations each (five to six trees within each population) from across the native and introduced range and kept seeds in refrigerated storage until spring 2011. We planted seeds on 5 April 2011 in forestry “cone-tainers” (Stuewe & Sons, Tangent, OR, USA) after removing the non-provisioning waxy seed coat by scrubbing with a mild detergent, and grew the seedlings in a climate controlled greenhouse until we began the experiment in spring 2011. We planted seeds in late November/early December 2010 across the soil surface of 1-gallon pots (1 gallon = ~3.8 L) equipped with drip-emitter irrigation and containing a peat-based soil mixture (Pro-mix, Premier Tech Horticulture, Quakertown, PA, USA). *Schizachyrium scoparium* was allowed to grow for 1 week before we transplanted _Triadica_ seedlings directly into the center of the pots. Before transplanting, we recorded _Triadica_ seedling height, number of leaves, and after washing all the soil from the roots, the length of the roots. With scissors, we cut 90% of the aboveground tissue by height (leaf clip treatment, Fig. 1A), cut 90% of the belowground tissue by length (root clip treatment), cut 90% of both aboveground and belowground tissue (both), or did not cut them (control), to simulate outbreak herbivory conditions. We sacrificed some realism and potential relevance by utilizing artificial herbivory to control and impose simultaneous and equivalent amounts of relative damage above and below ground. This type of simultaneous control over damage amounts above and below ground would be effectively impossible with real herbivores feeding naturally on plants that likely vary in resistance across populations and above and below ground, and where belowground damage is hard to observe. The aboveground damage treatment represented complete defoliation but left some stem tissue that could potentially function in photosynthesis. We cleaned scissors between each plant with a 10% ethanol rinse and a kimwipe (Kimtech Science, Kimberly Clark). After we recorded a seedling’s height and root length and had simulated herbivory, we immediately transplanted the seedling into either a competition pot or a no-competition control pot. For each of the six populations from either the native or introduced ranges, we planted four replicates per treatment combination when possible. However, some populations did not have enough seeds germinate to include in all possible combinations, so our total number of plants equaled 380 (vs. 384 for a fully balanced design). We dried and weighed the biomass of the removed above- and belowground tissue to quantify amount of tissue removed.

We used this high level of herbivory for several reasons. First, the amount of aboveground damage approximates a single bout of intense foliar defoliation, which is possible in the native range from a variety of herbivores (J. Ding, personal communication), including _Gadirtha fisca_, a potential biocontrol agent of _Triadica_ (Pogue 2014). Second, attempts to mechanically control _Triadica_ include cutting or burning the aboveground portion of the tree and some attempt at root severing; despite these efforts, _Triadica_ notoriously recovers from massive tissue damage and tissue removal. Third, although it is unclear what typical herbivore damage rates are belowground for _Triadica_, reductions in root chemical defenses suggest invasive populations may be especially poorly defended against potential biocontrol agents (Huang et al. 2012). In this regard, our experimental damage treatment represents a best case scenario for feeding by potential biocontrol agents.

Two weeks after the artificial herbivory treatments and after transplanting the seedlings, we recorded the height and leaf number of the _Triadica_ seedling and the number of extrafloral nectaries producing EFN. We recorded this same data again in early September before harvesting the plants 9 weeks post-herbivory treatments. We separated leaf, stem, and root mass of _Triadica_, and above- and belowground grass leaves and roots, then dried them and recorded their masses.

**Statistical analyses**

We examined differences in plant biomass above and below ground (combined, separately, and the ratio of the two), final plant height and height growth (calculated as
the relative percentage change in height from the start of our experimental treatments until the end of the experiment, and EFN (the number of nectaries actively producing EFN/leaf) across our competition and artificial herbivory treatments and among native and invasive populations with a mixed model ANOVA. The model included competition (grass or no grass), aboveground herbivory (leaf clip or control), belowground herbivory (root clip or control), and plant population origin (China or United States) and their interactions as fixed factors. We included plant population nested within plant population origin as a random factor and conservatively estimated differences between origins using the variation among populations for the main effect of origin and interactive effects including the origin term. *Triadica* plant mass (above-, belowground, and total) was log transformed to meet assumptions of normality and homogeneity of variance. Plant seedlings were randomly assigned to experimental treatments, however a statistical association in initial plant height and experimental treatment existed such that plants in our grass treatments and belowground damage treatments began the experiment shorter than our other plants, independent of plant population. To control for this potential bias, we initially included starting plant height as a covariate in our model. However, the inclusion of this covariate did not change the significance of any of the terms of our model except for one interactive term for total plant mass, so we excluded the covariate from our final analyses. When significant interactive effects occurred, we examined differences among treatments combinations using adjusted means partial difference tests ($P < 0.05$).

We examined whether native vs. invasive populations differed in their tolerance of competition, leaf clipping and root clipping with a mixed model ANOVA. We first determined plant tolerances to each type of stress by calculating the log response ratio of plant biomass (above-, belowground, and whole plant biomass) to that treatment type for each of our 12 populations, i.e., tolerance to herbivory relative to undamaged controls and or tolerance to competition relative to non-competing control. We determined plant tolerance by modifying the approach of Hawkes and Sullivan (2001), who calculated the compensatory responses of plants as log response ratios (LRRs) equivalent of treatment effect sizes. To do this, we calculated LRRs for aboveground, belowground, and total plant biomass for each population using populations means within each treatment (LRRs = 108 total, 9 per population [3 main effects x 3 biomass types])

$$
\text{LRR}_{\text{LC}} = \ln (Y_{\text{LCG}}) + \ln (Y_{\text{LCNG}}) + \ln (Y_{\text{B,G}}) + \ln (Y_{\text{B,NG}}) - \ln (Y_{\text{RC,G}}) - \ln (Y_{\text{RC,NG}})
$$

where $Y$ represents the sample mean, the subscripts LC, RC, B, and CO refer to leaf clip, root clip, both, and control treatments, respectively, and the subscripts $g$ and ng refer to grass and no grass, respectively.

A positive LRR indicates overcompensation for a population, e.g. biomass greater than undamaged plants. Negative $\text{LRR}_{\text{LC}}$ or $\text{LRR}_{\text{RC}}$ values indicate undercompensation for populations, e.g. biomass less than undamaged plants, with more negative values showing less tolerant populations compared to populations with $\text{LRR}_{\text{LC}}$ or $\text{LRR}_{\text{RC}}$ values closer to zero. Similarly, for $\text{LRR}_{G}$, more positive values indicate greater competitive tolerance while more negative values indicate less tolerance of competition within a population. A zero LRR would indicate full compensation.

We performed an ANOVA on the LRRs with plant population origin (China or United States) as a main effect to detect if native vs. invasive populations differed from each other in their aboveground, belowground, or whole plant tolerance response. We did this for either above- or belowground damage, or grass competitors (but not their interactive effects). We also examined correlations among the different tolerance biomass responses (tolerance to aboveground damage, tolerance to belowground damage, and tolerance to grass competitors) using these LRRs both within and across native and invasive populations and within biomass types (for above- vs. belowground biomass, and whole plant biomass across treatments). This analysis was used to detect tradeoffs in plant tolerance to multiple stressors, e.g. does tolerance above ground trade off with tolerance below ground? Or, does tolerance to competitors reduce tolerance to herbivory and does this depend on plant population origin?

Next, we performed an ANOVA with plant origin and tolerance type as main effects and plant population as a random effect on the LRRs for whole plant tolerance with or without a grass competitor. In this analysis, tolerance type had four possible designations: leaf clip in grass, root clip in grass, leaf clip without grass, root clip without grass, calculated as follows:

$$
\text{LRR}_{\text{LC,CO,G}} = \ln (Y_{\text{LC,G}}) + \ln (Y_{\text{CO,G}}) - \ln (Y_{\text{B,G}}) - \ln (Y_{\text{B,NG}})
$$

$$
\text{LRR}_{\text{RC,CO,G}} = \ln (Y_{\text{RC,G}}) + \ln (Y_{\text{CO,G}}) - \ln (Y_{\text{B,G}}) - \ln (Y_{\text{B,NG}})
$$

$$
\text{LRR}_{\text{LC,CO,NG}} = \ln (Y_{\text{LC,NG}}) + \ln (Y_{\text{CO,NG}}) - \ln (Y_{\text{B,G}}) - \ln (Y_{\text{B,NG}})
$$

$$
\text{LRR}_{\text{RC,CO,NG}} = \ln (Y_{\text{RC,NG}}) + \ln (Y_{\text{CO,NG}}) - \ln (Y_{\text{B,G}}) - \ln (Y_{\text{B,NG}})
$$

$$
\text{LRR}_{\text{LC,B,NG}} = \ln (Y_{\text{LC,NG}}) + \ln (Y_{\text{B,NG}}) - \ln (Y_{\text{RC,G}}) - \ln (Y_{\text{RC,NG}})
$$

$$
\text{LRR}_{\text{RC,B,NG}} = \ln (Y_{\text{RC,NG}}) + \ln (Y_{\text{B,NG}}) - \ln (Y_{\text{RC,G}}) - \ln (Y_{\text{RC,NG}})
$$

$$
\text{LRR}_{\text{LS,NG}} = \ln (Y_{\text{LS}}) + \ln (Y_{\text{NG}}) - \ln (Y_{\text{LS}}) - \ln (Y_{\text{NG}})
$$
Results

Effects of plant competition

Competition with grass reduced total biomass of *Triadica* plants by 77%, aboveground biomass by 78%, and belowground biomass by 77% (Table 1, Fig. 1A). The main effect of competition reduced final plant height and decreased height growth (Fig. 1B; relative percentage change in height, grass vs. no grass 242% ± 16% vs. 458% ± 17%, respectively; all means shown with standard error). In general, competition increased the relative amount of biomass allocated belowground compared to biomass allocated aboveground, i.e., root:shoot ratio (grass vs. no grass, 1.09 ± 0.03 vs. 0.88 ± 0.03, respectively; Table 1, Fig. 1A). Competition reduced the number of active extrafloral nectaries per leaf by 74% and the total number of active extrafloral nectaries by 85% (EFN/leaf, Table 1; total EFN, Fig. 2B).
PLANT COMPETITION MEDIATES DAMAGE IMPACT

Effects of aboveground herbivory

Simulated aboveground herbivory (leaf clipping) reduced total biomass of *Triadica* plants by 85%, aboveground biomass by 86%, and belowground biomass by 84% (Table 1, Figs. 1A and 2A). The main effect of leaf clipping reduced final plant height but increased relative height growth (Fig. 1B; relative percentage change in height, leaf clip vs. control was 614% ± 18% vs. 85% ± 16%, *F*<sub>1</sub>, 309 = 105.42, *P* < 0.0001). Leaf clipping increased root:shoot ratio (Table 1; leaf clip vs. control, 1.14 ± 0.03: 0.83 ± 0.03). The main effect of leaf clipping reduced EFN/leaf by 89% and total EFN produced by plants by 91% (Table 1, Fig. 2B).

**Effects of belowground herbivory**

Simulated belowground herbivory (root clipping) reduced total biomass of *Triadica* plants by 42%, aboveground biomass by 36%, and belowground biomass by 49% (Table 1, Figs. 1A and 2B). The main effect of root clipping reduced final plant height and decreased height growth (Fig. 1B; relative percentage change in height for root clip vs. control, 298% ± 17% vs. 402% ± 16%, respectively, *F*<sub>1</sub>, 309 = 24.66, *P* < 0.0001). Root clipping decreased root:shoot ratio (Table 1; root clip vs. control, 0.86 ± 0.03 vs. 1.12 ± 0.03, respectively). Root clipping did not affect EFN/leaf production or total EFN (Table 1, Fig. 2B; EFN/leaf root clip vs. control, 0.93 ± 0.12 vs. 0.65 ± 0.13, respectively).

**Interactions among treatments**

Simulated above- and belowground herbivory interacted to negatively impact belowground biomass of *Triadica*, such that *Triadica* plants were largest in

### Table 1. ANOVA of *Triadica* response to simulated herbivory and competition with grass.

<table>
<thead>
<tr>
<th>Term</th>
<th>df</th>
<th>Mass* (g)</th>
<th>Aboveground mass* (g)</th>
<th>Belowground mass* (g)</th>
<th>Root:shoot*</th>
<th>EFN/leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Origin (O)</td>
<td>1, 10</td>
<td>8.34 (0.0162)</td>
<td>8.91 (0.0137)</td>
<td>8.42 (0.0158)</td>
<td>0.11 (0.7463)</td>
<td>0.00 (0.9776)</td>
</tr>
<tr>
<td>Grass comp. (G)</td>
<td>1, 298</td>
<td>440.16 (&lt;0.0001)</td>
<td>460.64 (&lt;0.0001)</td>
<td>339.57 (&lt;0.0001)</td>
<td>14.60 (0.0002)</td>
<td>26.65 (&lt;0.0001)</td>
</tr>
<tr>
<td>Leaf clip (LC)</td>
<td>1, 298</td>
<td>565.66 (&lt;0.0001)</td>
<td>623.84 (&lt;0.0001)</td>
<td>414.40 (&lt;0.0001)</td>
<td>34.84 (&lt;0.0001)</td>
<td>50.61 (&lt;0.0001)</td>
</tr>
<tr>
<td>Root clip (RC)</td>
<td>1, 298</td>
<td>96.19 (&lt;0.0001)</td>
<td>6371. (&lt;0.0001)</td>
<td>114.50 (&lt;0.0001)</td>
<td>30.23 (&lt;0.0001)</td>
<td>2.44 (0.1195)</td>
</tr>
<tr>
<td>O × G</td>
<td>1, 10</td>
<td>2.78 (0.1262)</td>
<td>2.30 (0.1604)</td>
<td>1.61 (0.2333)</td>
<td>0.77 (0.4018)</td>
<td>0.19 (0.6683)</td>
</tr>
<tr>
<td>O × LC</td>
<td>1, 10</td>
<td>0.23 (0.6420)</td>
<td>0.13 (0.7300)</td>
<td>1.18 (0.3028)</td>
<td>2.84 (0.1227)</td>
<td>0.23 (0.6390)</td>
</tr>
<tr>
<td>O × RC</td>
<td>1, 10</td>
<td>0.17 (0.6847)</td>
<td>0.01 (0.9086)</td>
<td>0.21 (0.6592)</td>
<td>0.40 (0.5144)</td>
<td>0.15 (0.7098)</td>
</tr>
<tr>
<td>G × LC</td>
<td>1, 298</td>
<td>22.80 (&lt;0.0001)</td>
<td>13.09 (0.0003)</td>
<td>32.66 (&lt;0.0001)</td>
<td>19.00 (&lt;0.0001)</td>
<td>12.87 (0.0004)</td>
</tr>
<tr>
<td>G × RC</td>
<td>1, 298</td>
<td>5.20 (0.0233)</td>
<td>2.52 (0.1131)</td>
<td>6.13 (0.0138)</td>
<td>1.07 (0.3016)</td>
<td>1.62 (0.2044)</td>
</tr>
<tr>
<td>RC × LC</td>
<td>1, 298</td>
<td>1.29 (0.2576)</td>
<td>1.60 (0.2069)</td>
<td>0.99 (0.3210)</td>
<td>0.30 (0.5840)</td>
<td>1.47 (0.2257)</td>
</tr>
<tr>
<td>O × G × LC</td>
<td>1, 10</td>
<td>0.13 (0.7293)</td>
<td>0.37 (0.5560)</td>
<td>0.05 (0.8217)</td>
<td>0.01 (0.9051)</td>
<td>0.60 (0.4547)</td>
</tr>
<tr>
<td>O × G × RC</td>
<td>1, 10</td>
<td>2.35 (0.1566)</td>
<td>5.50 (0.0410)</td>
<td>0.36 (0.5644)</td>
<td>8.00 (0.0179)</td>
<td>0.63 (0.4466)</td>
</tr>
<tr>
<td>O × LC × RC</td>
<td>1, 10</td>
<td>0.08 (0.7767)</td>
<td>0.00 (0.9545)</td>
<td>0.02 (0.8894)</td>
<td>0.00 (0.9779)</td>
<td>0.01 (0.9447)</td>
</tr>
<tr>
<td>G × LC × RC</td>
<td>1, 298</td>
<td>0.00 (0.9704)</td>
<td>0.10 (0.7553)</td>
<td>0.07 (0.7850)</td>
<td>1.12 (0.2913)</td>
<td>1.99 (0.1590)</td>
</tr>
<tr>
<td>O × G × LC × RC</td>
<td>1, 10</td>
<td>2.77 (0.1268)</td>
<td>3.89 (0.0768)</td>
<td>0.39 (0.5459)</td>
<td>7.53 (0.0207)</td>
<td>0.04 (0.8370)</td>
</tr>
</tbody>
</table>

**Notes:** Total mass and above- and belowground biomass were log transformed. EFN refers to extrafloral nectaries. F-values reported with p-values in parentheses. Bolded values indicate significance at *P* < 0.05.

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**Fig. 2.** (A) Total *Triadica* biomass (back transformed) across leaf clip and grass competition treatments. Bars with the same letters were not significantly different in post hoc tests (*P* < 0.05). All means are shown with SE. (B) Reductions in extrafloral nectar production due to main effects of grass competitors, leaf clip, or root clip. ** *P* < 0.0001.
control treatments, followed by root clip only, leaf clip only, and root clip and leaf clip plants (Table 1, Figs. 1A, B and 2). However, leaf clip only plants had the highest relative growth rate for height, followed by root clip/leaf clip plants, and then by control and root clip only plants, which did not differ from each other (Fig. 1B, leaf clip × root clip: F1,309 = 16.78, P < 0.0001). Simulated aboveground herbivory interacted with competition such that their negative effects on total, above-, and belowground biomass were less than additive (Table 1, Fig. 1A). Likewise, belowground herbivory interacted with competition to impact total and belowground biomass, but not aboveground biomass (Table 1). Simulated aboveground herbivory and competition interacted to affect root:shoot such that it was significantly higher in the leaf clip and grass treatment compared to the all other treatments (Table 1; leaf clip and grass, no root clip vs. control and grass, no root clip: 1.55±0.06 vs. 0.93±0.05, respectively). Correspondingly, a significant interaction for leaf clip and competition existed for plant height growth (Fig. 1B; leaf clip × competition: F1,309 = 23.21, P < 0.0001), as plants attained a greater percentage of their initial experimental height when clipped above ground and grown without grass (773%±25%), followed by leaf clip/grass (456%±22%), then control/no grass (143%±20%), and finally by control/grass (28%±20%). A significant interaction existed among plant population origin, competition, and simulated belowground herbivory for root:shoot (Table 1, Fig. 3). The four-way interaction including simulated aboveground herbivory was also significant for root:shoot (Table 1). Simulated aboveground herbivory and competition with grass interacted to impact the number of active extrafloral nectaries produced per leaf (EFN/leaf, Table 1) such that the combined reductive effects on EFN were less than additive but bounded by zero.

**Effects of plant origin**

Plants from the native range (China) were smaller (less biomass) than plants from the invasive range (United States), both above and below ground (Table 1, Fig. 1A). Plants from the native vs. the introduced ranges did not differ overall in root:shoot nor in the EFN/leaf they produced (Table 1), nor plant height. United States and China plants did not differ in their tolerance responses (biomass to simulated above- or belowground herbivory, the combination of the two, nor any combination of simulated herbivory with grass (Appendix S1) with one exception: the aboveground tolerance response of United States populations was greater than China populations to the combined stress of root clipping and grass competitors (F1,9 = 7.17, P = 0.03; Appendix S1).

**Tolerance and tradeoffs across responses above and below ground**

*Triadica* tolerated belowground damage better than aboveground damage in terms of biomass (Figs. 1A, B and 4). Tolerance to aboveground damage was greater for *Triadica* plants growing with grass (Fig. 4). This is because within grass treatments, damaged plants were closer in terms of biomass to non-damaged plants than damaged plants were to non-damaged plants in the no-grass treatments (Fig. 4). *Triadica* had greater tolerance to belowground herbivory without grass competitors (Fig. 4).

Across all populations (native China and invasive United States together), most tolerance responses to leaf clipping, root clipping, and grass competitors were not correlated, e.g., aboveground biomass response to root clipping was unrelated to aboveground biomass response to root clipping (r = −0.009, P = 0.98; other non-correlated tolerance responses not reported). Some tolerance responses were positively correlated, typically

![Fig. 3. Root:shoot ratio of native (China) and invasive (United States) populations of *Triadica* across root clip and grass competition treatments. Back-transformed means ± SE are shown. Hatching indicates grass treatments. Bars marked with the same uppercase letters were not significantly different in post hoc tests (P < 0.05).](image3)

![Fig. 4. Tolerance scores for the effect of leaf clipping vs. root clipping with or without a grass competitor. Means with SE show log response ratios for individual population whole plant biomass. Hatching indicates grass treatments. Bars marked with the same uppercase letters were not significantly different in post hoc tests (P < 0.05).](image4)
when experiencing the same stress: i.e., aboveground biomass in response to leaf clipping was positively correlated with belowground biomass response to leaf clipping \( (r = 0.90, P < 0.0001) \), aboveground biomass response to grass competitors was positively correlated with belowground biomass responses in to grass competitors \( (r = 0.69, P = 0.01) \), and aboveground biomass response to root clipping was positively correlated with belowground biomass response to root clipping \( (r = 0.74, P = 0.01) \). A negative trend existed between the aboveground biomass responses of plants to grass competitors and the aboveground biomass responses of plants to root clipping \( (r = -0.59, P = 0.05) \). Likewise, aboveground and belowground biomass responses to root clipping were both negatively correlated with belowground biomass response to grass competitors \( (r = -0.79, P = 0.002 \) and \( r = -0.86, P = 0.0002 \), respectively).

Within either United States or Chinese populations, these tolerance patterns were mostly consistent but with some exceptions. Within United States, but not China populations, plant biomass response above ground was positively correlated to plant biomass response below ground when competing with grass (US, \( r = 0.69, P = 0.01 \); China, \( r = 0.50, P = 0.34 \)). Also, within United States but not China populations, when competing with grass, plant biomass response aboveground was negatively correlated to plant biomass response belowground with root clipping (US, \( r = -0.86, P = 0.02 \); China, \( r = -0.04, P = 0.96 \)). Likewise, with root clipping, plant biomass response aboveground was negatively correlated with plant biomass response belowground with leaf clipping for United States, but not China populations (US, \( r = -0.85, P = 0.03 \); China, \( r = -0.39, P = 0.56 \)). In three cases, the correlation across populations was driven by significant associations for United States but not China populations: above- vs. belowground biomass in response to grass competitors (US, \( r = 0.97, P = 0.0003 \); China, \( r = 0.50, P = 0.34 \)), above- vs. belowground biomass in response to root clipping (US, \( r = 0.85, P = 0.03 \); China, \( r = 0.72, P = 0.20 \)), and belowground biomass in response to grass vs. belowground biomass in response to root clipping (US, \( r = -0.95, P = 0.001 \); China, \( r = -0.76, P = 0.16 \)).

For whole plant biomass responses, tolerance of grass competitors was negatively correlated with tolerance to root clipping across all populations. This was driven by a significant association within United States but not China populations (Fig. 5; US, \( r = -0.90, P = 0.02 \); China, \( r = -0.80, P = 0.12 \)).

**Discussion**

We found that plant competition will likely mediate the impact of above- vs. belowground damage on plant success. As predicted by the resource competition model, we found that aboveground damage had a greater impact than belowground damage on *Triadica* in terms of biomass reductions (Fig. 1A), so we recommend prioritizing aboveground biocontrol agents for woody invasives over those that feed only below ground. Competition with the relatively better belowground competitor (little bluestem grass) magnified these effects, with plant biomass higher but herbivory tolerance lower in the no-competition environments (Figs. 1A and 5). The greater impact of above- vs. belowground damage for *Triadica* contrasts a recent meta-analysis of plant responses to belowground herbivory, which found similar impacts and biomass responses to above- and belowground damage for woody plants (Zvereva and Kozlov 2012). However, within woody plants, that study predominantly considered evergreen trees (primarily leaf-bearing citrus; Zvereva and Kozlov 2012), which may explain the difference we observed. For a deciduous tree such as *Triadica*, the loss of leaves may be comparatively more harmful in terms of resource capture, as the seasonal window for photosynthesis is shorter. In our study, although belowground damage did not have as great an impact on plant success as aboveground damage, competition with a grass competitor magnified the impacts of belowground damage on plant biomass (Fig. 1A). *Triadica* could almost completely compensate in terms of biomass for the loss of 90% of its root tissue compared to controls when not competing with grass, however, *Triadica* had reduced compensatory ability belowground when grass was present (Fig. 4). Indeed, we detected a tradeoff between the ability of *Triadica* to tolerate root clipping and to tolerate grass competitors in terms of biomass produced (Fig. 5). This suggests evolutionary tradeoffs in the ability to endure belowground herbivory when coexisting with good belowground competitors, and may indicate that plants can specialize on either competitive or defensive ability. For *Triadica* or
other woody invaders expanding into open vegetation gaps or disturbed open ground, we predict that the combined effects of integrated management through the addition of both competitors and herbivores will be greater than either alone.

The compensatory continuum hypothesis predicts that plants in low-resource environments will be less able to compensate for herbivory than plants in high-resource environments (e.g., Grime 1977 and reviewed in Wise and Abrahamsom 2007). We did not find this to be the case due to differences in plant size between the high and low competition environments. Specifically, we found that, in general, plants were larger in the putatively low competition environments. However, tolerance to aboveground damage was greater for *Triadica* plants growing in the low-resource, grass-competition environment, as plants damaged and competing with grass were closer in size to non-damaged control plants than damaged plants were to non-damaged controls in the no-grass treatment (Figs. 1A and 4). Put another way, despite absolute biomass being larger in the high-resource, no-competition environment, the proportional change in biomass was smaller for plants growing with grass than plants without grass, leading to higher tolerance of aboveground damage compared to controls within that treatment (Figs. 1 and 4).

In contrast, another study examining compensatory growth in two woody species (a tree, *Brosimum alliacastrum*, and a liana, *Vitis tiliifolia*) found similar rates of compensatory growth in terms of biomass across light resource conditions (Ballina-Gómez et al. 2010), as biomass responses to light were similar in damaged vs. undamaged plants within each species. More comparable to our study, Aranda et al. (2015) found that competition with pasture grasses reduced growth of seedlings of the invasive tree, *Gleditsia triacanthos* (honey locust), greater than defoliation alone, and that the negative impact of defoliation was greater in the absence of competition. These results, and our findings, better support the limiting resource model (LRM) of plant tolerance of herbivory (Wise and Abrahamsom 2005, 2007), as plants were more likely limited by above- than belowground resources in the no-competition environment, leading to reduced tolerance to aboveground damage compared to the grass-competition environment where plants were likely limited belowground. Competition with grass can reduce both above- and belowground resources available to trees but it is likely that belowground competition reduced belowground resources more in our experiment than aboveground resources, as has been demonstrated in other tree-grass systems (e.g., Picon-Cochard et al. 2006). In the LRM, high levels of belowground resources lead to relative aboveground resource limitation for plants (Wise and Abrahamsom 2005). Aboveground herbivory under this scenario would be more detrimental for plants than belowground herbivory and lead to reduced tolerance of aboveground herbivory then in the alternative resource scenario of relatively high aboveground resources with relatively limited belowground resources (Wise and Abrahamsom 2005, 2007).

Competition with grass will likely reduce the ability of *Triadica* to defend against native herbivores and any biocontrol agents that get introduced, as grass competitors reduced the number of active nectaries producing extrafloral nectar (EFN; Table 1, Fig. 2B). Although little is known about the relative investment of plants in EFN or other defense traits across different habitats, a recent study with *Mallotus japonicus* found that some defenses (trichomes, pellucid dots, and EFNs) had higher expression but lower ant attendance in open gaps (which included grasslands) vs. treefall gaps (Yamawo et al. 2014). Native populations of *Triadica* did not produce more EFN (absolute or relative) than invasive populations, contrary to expectations of the evolution of increased competitive ability (EICA) hypothesis. But, evidence for EICA in *Triadica* for EFN production is equivocal. One study showed greater constitutive and induced EFN production by native compared to invasive populations (Carrillo et al. 2012a), while others showed decreased constitutive but increased induced EFN for native populations (Wang et al. 2013), or equivalent EFN production across populations (Carrillo et al. 2012b). EFN is inducible in both native and invasive populations of *Triadica* in response to chewing herbivory and clipping (e.g., Carrillo et al. 2012a, b) and several ant species tend EFN in the invasive range of *Triadica*, including the highly aggressive red imported fire ant, *Solenopsis invicta* (J. Carrillo, personal observation). In this study, we saw inhibition of EFN in response to both grass competitors and the high levels of damage inflicted by aboveground clipping, likely in response to the reduced biomass available to produce new leaves and nectaries. Interestingly, belowground damage did not induce EFN production (nor inhibit it), which intuitively makes sense from a defense allocation perspective if EFN defenses target aboveground herbivores only. However, we have previously shown that belowground herbivory by larvae of the specialist flea beetle (*Bikasha collaris*, a potential biocontrol agent for *Triadica*), does induce EFN, indicating there is some specificity to this defense response (Huang et al. 2015). Intriguingly, *B. collaris* larvae co-occur with aboveground feeding adult beetles, so belowground herbivory in that instance may be a reliable indicator of aboveground herbivory and vice versa (Huang et al. 2012).

Invasive (United States) populations were larger than native (China) populations (Fig. 1), providing additional support for the EICA hypothesis in invasive plants (Blossey and Nötzel 1995). Across all populations, tolerance responses to grass competitors for plant biomass was negatively correlated with tolerance responses to root clipping for plant biomass, but this was driven by a significant association within United States but not China populations (Fig. 5). Additionally, invasive United
States populations tolerated the combined stress of belowground damage and competition with grass better than native China populations, indicating evolved differences in their responses to biotic interactions. Invasive populations appear to be more plastic in their response to biotic stressors, as they invested more in roots in undamaged controls when in competition with grass than China plants, but less than China plants when roots were clipped (Fig. 3). Others have shown that plant responses to the individual and simultaneous stress of competition and herbivory can be phenotypically plastic and experience differential selection due to these stress combination (Boege 2010); our results show that genetic variation exists in the degree of plasticity that may be selected by biotic environment (e.g., native range vs. introduced range).

Tolerance in invasive plants may reduce the effectiveness of biocontrol agents (Wang et al. 2011). This is because for highly tolerant plant populations, biocontrol agents may be able to reach high population densities but not reduce the biomass or reproductive fitness of target plants. In our study, we found that tolerance was higher with competition, but competition directly reduced plant biomass, both above and below ground, and increased the relative amount of biomass *Triadica* allocated belowground (root:shoot; Table 1, Fig. 1A). It may be that competition with native grass or other co-occurring plants may ameliorate the potential positive effects of increased tolerance for invasive plant populations. In another weedy system, de la Pena and Bonte (2014) found that dandelion plants (*Taraxacum officinale*) overcompensated with growth both above and below ground after aboveground herbivory by a locust, while belowground herbivory by root-knot nematodes had no effect on plant growth. When the two herbivores fed together, the production of plant trichomes was inhibited leading to greater leaf damage by locusts and combined herbivore feeding may have also inhibited the compensatory growth response of plants. Antagonistic interactions among biocontrol agents have been extensively reported elsewhere (Stephens et al. 2015); directed choice of agents that target valuable plant parts could decrease indiscriminate release of multiple agents and potentially reduce antagonistic interaction among insect herbivores used to control plants.

Our study is limited by the fact that we simulated herbivory on young tree seedlings instead of using natural herbivores across a range of plant stages. Plants, including *Triadica*, can use specific herbivore cues for both induced resistance (e.g. Carrillo et al. 2012b) and tolerance responses (Korpita, Gomez, and Orians 2012; Carrillo et al. 2014), suggesting herbivore identity is important to plant responses. However, for large scale experiments such as this one where competition and plant anti-herbivore traits are likely to interact, precisely controlling damage rates above and below ground is more feasible with simulated herbivory. Moreover, simulated herbivory is an invaluable tool in weed biological control in identifying vulnerable plant parts, e.g., roots or shoots, to target and in the selection insect biocontrol agents (Raghu and Dhileepan 2005). Although all tests to date of biocontrol agents for *Triadica* have focused on the seedling and sapling stage (e.g., Huang et al. 2010, Wang et al. 2012), the agents under consideration feed on all life stages of *Triadica* in its native range (E. Siemann, personal observation) and reproduction of trees may be especially impacted by herbivory at later life stages (e.g., Massad 2013).

Encouragingly, we found that competition with grass might be able to increase the impact of biocontrol agents on invasive, woody *Triadica*, and directly suppressed biomass of *Triadica*. We assert that invasive species management should integrate competitive dynamics of the target species to better predict the efficacy of agents within specific communities, and reduce potential non-target effects and the release of potentially ineffective agents. For *Triadica* and other woody invasive plants, we recommend a combination of management approaches, including the restoration or addition of native competitors, particularly native grasses or forbs that are relatively good belowground competitors and the introduction of a suitable biocontrol agent that targets the invasive plant’s area of competitive superiority, e.g. aboveground resource capture. Alternately, for a weedy or invasive grass or herbaceous species, including woody native species that are superior competitors for light and/or prioritizing belowground biocontrol agents in restoration efforts could prove successful. These guidelines provide a contracted field of potential agents and plant competitors from which to choose from in initiating or maintaining a management program which recognizes the context dependency of competitive/herbivore interactions (Schädler et al. 2007, Jing et al. 2015) and answers calls within the field to reduce “lottery” approaches to agent introductions (Stephens et al. 2015). Woody plants severely threaten grasslands and invade disproportionately into disturbed and open habitats (e.g., Zalba and Villamil 2002); utilizing multiple top-down and bottom-up approaches will be necessary to reduce their and other invasive species’ ecological impacts.

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**Supporting Information**

Additional supporting information may be found in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/eap.1359/suppinfo

**Data Availability**

Data associated with this paper have been deposited in Dryad: http://dx.doi.org/10.5061/dryad.j72c0