

Herbivory of native and exotic North-American prairie grasses by nymph *Melanoplus* grasshoppers

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Abstract Although the general interaction between native and exotic plants and specialist insect herbivores has received considerable attention in ecological studies, plant responses to herbivory by generalist insects, as well as feeding preferences of generalist insects, are still poorly understood. Experimental studies comparing leaf damage of native and exotic plants to generalist insects have provided inconsistent results due to irregularities in methodology such as using only one type of experiment or using non-standard measurements. Our study addresses these issues by examining leaf damage caused by generalist *Melanoplus* grasshoppers in morphologically and physiologically similar native (*Andropogon gerardii* and *Bouteloua curtipendula*) and exotic grasses (*Miscanthus sinensis* and *Bothriochloa ischaemum*) in Ohio and Maryland. In a 2-year study, we explored whether exotic grasses sustain less damage than native grasses, and consequently, whether generalist grasshoppers may potentially pose biotic resistance to

exotic grasses. Using a combination of choice and no-choice feeding experiments with intact plants and with clipped leaves under different (field and greenhouse) conditions, we found that overall both herbivory and grasshopper performance (body length) were greater on the exotic grasses compared to native grasses. Our results suggest that exotic grasses which do not share a coevolutionary history with native generalist *Melanoplus* grasshoppers might have lower physical and chemical defenses than native plants. Consequently, *Melanoplus* grasshoppers may provide biotic resistance to these exotic grasses should these plants invade natural areas at the study regions. These results have important applications for predicting the interaction between exotic plants and generalist herbivores in the introduced range, which is critical for understanding factors facilitating plant invasions.

Keywords Coevolutionary history · Herbivory · Leaf damage · Plant–insect interaction · Plant invasion · Plant resistance

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Introduction

The interaction between exotic plants and insect herbivores can be an important mechanism which can affect the establishment of exotic plants and facilitates plant invasion in the introduced range (Blossey and Notzold 1995; Keane and Crawley

2002; Rogers and Siemann 2005; Fornoni 2011). The intensity and pattern of herbivory, as well as plant responses to insect herbivores, could differ between specialist and generalist herbivores (Bossdorf et al. 2004; Ali and Agrawal 2012). Whereas the interaction between specialist herbivores and invasive plants has been well explored (e.g., Zangerl et al. 2008; Zou et al. 2008; Lu and Ding 2010; Wang et al. 2011; Bukovinszky et al. 2014), the impact of native generalist herbivores on the success of invasive species is not easily predicted and needs to be studied further (Bossdorf et al. 2004; Joshi and Vrieling 2005; Tallamy et al. 2010; Schaffner et al. 2011).

Several hypotheses have been proposed to explain and/or predict the interactions between native generalist herbivores and exotic plants in the introduced range. For example, the enemy release hypothesis (ERH) states that exotic plants have escaped from their native enemies (herbivores and pathogens) in the introduced range; as a result, they have reduced herbivore damage compared to native plant species, partially due to feeding preferences of native generalist herbivores for native plant species (Keane and Crawley 2002). The behavioral constraints hypothesis (BCH) also suggests that native insect herbivores will avoid sampling novel and potentially toxic food due to their behavioral avoidance constraints (Lankau et al. 2004). In contrast, the biotic resistance hypothesis (BRH) predicts that introduced plants which do not share coevolutionary history with native herbivores will be less well defended compared to native plants, and native generalist herbivores will prefer to feed on these exotic plants, posing biotic resistance to plant invasions (Maron and Vilà 2001). In other words, if native herbivores cause greater damage to exotic plants, they may limit their spread and abundance, and ultimately prevent potential negative effects from their invasion. None of these hypotheses (ERH, BCH, and BRH), however, are consistently held up during experimental tests.

In the present study, we focus on biotic resistance which generalist insect herbivores can potentially pose to exotic plants. Biotic resistance has commonly been documented in cases of different arthropod and non-arthropod species (Levine et al. 2004; Parker and Hay 2005; Parker et al. 2006; Lind and Parker 2010; Morrison and Hay 2011; Pearson et al. 2011). These studies demonstrated that native generalists prefer to feed on exotic plant species, which can reduce exotic

plants abundance. The strength of biotic resistance, however, varied among herbivores, and the effect of generalist insect herbivores on exotic plants is still uncertain and needs to be further explored (Bossdorf et al. 2004; Tallamy et al. 2010; Maron and Vilà 2001; Ali and Agrawal 2012).

To address this concern, we examined herbivory by a generalist grasshopper on native and exotic grasses and investigated whether these generalist insect herbivores could exhibit similar biotic resistance which had been documented previously for other arthropod and non-arthropod species. In our study, we also address the issue of inconsistency in studies used to test the invasion hypotheses described above by comparing leaf damage between native and exotic plants (e.g., Chun et al. 2010; Atwood and Meyerson 2011; Inderjit 2012). Such inconsistency may be due to measurement of only a single plant trait or using non-standard metrics of herbivore defense, such as, for example, uncommon data conversions or chemical pathways which might differ among plants (Atwood and Meyerson 2011). In addition, different experimental designs across studies, such as conducting experiments in the field or greenhouse, or experiments with clipped leaves alone might limit the data integration across studies (Atwood and Meyerson 2011). Moreover, results from laboratory or common garden experiments alone might not reflect all possible plant responses which occur naturally and therefore should be interpreted carefully (Motheral and Orrock 2010). Han et al. (2008) also suggested that variation of physiological and morphological traits between native and exotic species can affect results of leaf damage more than the location that the plants originated from. We address these concerns in our study, as described below.

Our primary objective was to determine if exotic grasses, which do not share a coevolutionary history with native generalist insect herbivores, sustain more leaf damage compared to native grasses. For our experiments, we chose native (*A. gerardii* and *B. curtipendula*) and exotic, potentially invasive, grasses (*M. sinensis* and *B. ischaemum*), all of which were exposed to herbivory by nymphs of generalist grasshoppers of the *Melanoplus* genus (Orthoptera: Acrididae). Considering that *Melanoplus* grasshoppers do not inhabit any areas in the native range of *Miscanthus* and *Bothriochloa* grasses, and thus do not share coevolutionary history, we hypothesized that exotic

grasses would be less defended against grasshopper herbivory, and therefore would demonstrate greater leaf damage than native grasses. Confirmation of our expectations of greater leaf damage in exotic grasses would indicate that *Melanoplus* grasshoppers can pose biotic resistance to exotic *Miscanthus* and *Bothriochloa* grasses in Ohio and Maryland, should they escape from ornamental settings or cultivation in grasslands. Consequently, this knowledge about plant responses to herbivory and grasshopper feeding preferences will allow us to better understand and predict interactions between exotic plants and generalist insect herbivores in the introduced range.

Methods

To explore our objective, we conducted a 2-year study comparing leaf damage of native and exotic grasses caused by *Melanoplus* grasshoppers. In the preliminary experiments in 2012 with native/exotic plant pairs, (*A. gerardii*/*M. sinensis* ‘*Zebrinus*,’ *B. curtipendula*/*M. sinensis* ‘*Gracillimus*’), we observed greater leaf damage on intact exotic grasses than on native grasses, while clipped leaf segments of grasses did not exhibit significant differences in herbivore damage (online resource 1). In the experiments in 2013, which are the focus of the present paper, we replaced the two cultivars of *M. sinensis* with a wild type of a non-cultivated variety (to eliminate potential similarity between two cultivars in comparison to other species), and added exotic *B. ischaemum*. We followed recent methodological suggestions on choosing experimental design and measurements (Motheral and Orrock 2010; Atwood and Meyerson 2011) and explored a set of standard measures of leaf damage for our grass–grasshopper system using a combination of experiments with intact plants and clipped leaves under field and laboratory conditions. In addition, we quantified grasshopper growth rate separately on native and exotic plants. It has been demonstrated that weight and size measurements of many insect herbivores are good indicators of how host plant quality affects insect fecundity (Beck 1965; Emden 1969; Awmack and Leather 2002; Berner et al. 2005; Adis et al. 2008; Strengbom et al. 2008). Particularly, Adis et al. (2008) found that morphology of grasshoppers *Cornops aquaticum* differs depending on their host plants (*Eichhornia azurea* vs. *E. crassipes*): body size of

grasshoppers feeding on *E. azurea* was larger compared to grasshoppers feeding on presumably poorer in quality *E. crassipes*. Berner et al. (2005) also demonstrated that growth rate of *Omocestus viridulus* grasshoppers were lower on grass with low nitrogen content compared to those on food that was rich in nitrogen. Based on those studies, we measured changes in grasshopper body weight and body length as an additional estimate of grasshopper performance on native and exotic plants and the plants’ nutritional value for these insects.

Study sites

To examine herbivory levels in the field, we established a common garden (12 × 8 m²) at the Western Maryland Research and Education Center (WMREC, 39°30.618’N, 077°44.070’W, Keedysville, MD) in May 2013. The ground was tilled prior to planting and no fertilizer was applied during the period in which the project took place. We removed weeds throughout the experiments and watered the plants when necessary to supplement natural precipitation. The chosen plot was surrounded by a corn field and a plot with sunflowers (at a distance of 6 m from all sides). These sites presumably were attractive to grasshoppers, which were collected adjacent to the common garden.

To replicate the field experiment and to explore whether patterns of leaf damage in response to the same grasshopper genus species would be consistent across sites, we established another similar common garden at the University of Cincinnati Center for Field Studies (UCCFS, 39°17.134’N, 084°44.413’W, Harrison, OH). This second site was treated in the same way as described previously.

To control for possible differences in plant biomass and their potential effect on grasshopper feeding choice, we also repeated the experiments in the greenhouse at the University of Cincinnati (UC) using potted plants. Finally, to explore how cutting plants may affect grasshopper herbivory, we also conducted experiments with clipped leaf segments.

Study organisms

We used grasses of the Andropogoneae tribe of the Poaceae family—these consisted of two native species [*A. gerardii* (big bluestem) and *B. curtipendula* (sideoats grama)] and two exotic, potentially invasive

species [*M. sinensis* (Chinese silver grass) and *B. ischaemum* (yellow bluestem)]. All species are perennial C₄ grasses. Both *A. gerardii* and *B. curtipendula* are widely distributed throughout most of the United States, where they are dominant species in prairie ecosystems. *B. ischaemum* and *M. sinensis* were introduced in the US in late 1800s from Eurasia and Japan respectively, because of their high forage quality (Hickman et al. 2006; Schmidt et al. 2008; Quinn et al. 2010). More recently, *M. sinensis* has also become important as a landscaping plant in the horticultural industry. When escaping cultivation, both exotic species demonstrate high competitive ability and inhibit growth of native grasses, and thus are considered potentially invasive (Schmidt et al. 2008; Quinn et al. 2010). Particularly, *B. ischaemum* raises concern in Great Plains where together with its congener *B. bladhii* it is shown to be competitively superior to common native prairie grasses such as *A. gerardii*, *B. curtipendula*, and *Schizachyrium scoparium* (little bluestem) (Schmidt et al. 2008).

All plant species were reported to be suitable for grasshopper feeding (Alward and Joern 1993; Han et al. 2008; Nabity et al. 2012), and were selected in part because their general morphological similarity in plant structure should provide similar attractiveness for grasshoppers. Some physical traits, however, such as trichome density and leaf toughness differ among these grasses and might act as a deterrent for grasshoppers. For example, mean leaf trichome density and leaf toughness of native *A. gerardii* are 188.33 ± 24.12 (trichomes along a leaf edge of 5 cm) and 232.17 ± 11.17 g respectively, while exotic *M. sinensis* contains 125.66 ± 19.67 trichomes and has leaf toughness of 253.64 ± 12.57 g (online resource 2).

We obtained seeds for our study plants from Prairie Moon Nursery, Winona, MN (*A. gerardii* and *B. curtipendula*), Outsidepride.com, Inc., Independence, OR (*M. sinensis*), and Warner Brothers Seed Company, Lawton, OK (*B. ischaemum*). We planted seeds in the UC greenhouse, and a month later, we transferred potted plants (20–25 cm in height, 8.9 cm² pot) to the study plots in Maryland and Ohio. Other plants were kept at the greenhouse for the experiments with potted plants and clipped leaf segments. To more closely simulate grasshoppers' natural food choices, we offered four plant species (two native and two exotic) together in feeding experiments.

The generalist herbivore used in our study were nymphs of the *Melanoplus* spp. grasshoppers (Acrididae: Orthoptera), presumably *M. differentialis* and *M. femurrubrum*. Nymph grasshopper species were identified based on their size, color, and stripes pattern using the keys of Pfadt (1994). We did not rear the nymphal grasshoppers for species identification in this study. We however maintained identical nymphs collected from the same study sites for other experiments; the emerging adults were *M. differentialis* and *M. femurrubrum*. In addition, the same species as adults were observed later in the season at the same collection sites.

Grasshoppers of the *Melanoplus* genus were chosen because of their wide distribution, abundance at both study plots in Ohio and Maryland, as well as their ecological and agricultural importance (Belovsky and Slade 2000; Branson and Sword 2009).

In addition, the grasshopper mode of feeding makes them ideal for feeding experiments and quantifying leaf damage. When feeding on a plant, grasshoppers examine potential food with palps and antennae (Brown and Smith 1983), and then accept or reject a particular plant based on both its physical (toughness, texture of the leaf surface, shape, moisture content, and color) and chemical characteristics (repellent substances, feeding deterrents, adaptable chemicals) (Williams 1954). Feeding pattern can vary depending on a plant type. When feeding on grasses, as in our experiments, grasshoppers usually move along the leaf edge and chew off leaf segments in long lengths (Chapman 1974). We called each grazed mark “a scar”. Main veins are often left intact, whereas leaf tips can be bit off completely.

Grasshopper nymphs (third and fourth instars) were collected adjacent to the plot and maintained a few hours in an open air aluminum screen cage at the site prior to the field experiments, and a few days in the greenhouse prior to the experiments with potted plants and clipped leaves. During that time grasshoppers were fed a mixture of plants collected from the same location.

Field experiments with intact plants

In June 2013, 48 potted plants (12 pots of each of four species) were arranged and planted in twelve groups of four plants each; each potted plant was of a different species (*A. gerardii*, *B. curtipendula*, *M. sinensis*, and

B. ischaemum). For each plant group, we set up twelve open air aluminum screen cages ($41 \times 41 \times 51 \text{ cm}^3$ Repti Breeze Aluminum Screen Cage, Zoo Med Laboratories, Inc., California, USA) in two rows with 1-m spacing among cages and 2-m spacing between rows. Plants were watered every other day during the first week after planting, and whenever the soil was dry during the following 3 weeks prior to the commencement of the experiments. Plants were not watered during the experiment to simulate natural field conditions. To eliminate any potential effect of seasonal changes on plant resistance to grasshopper herbivory, the experiments at both sites were conducted simultaneously.

All experiments were conducted in July 2013, on the fourth week after planting at the field site. For feeding experiments, three grasshopper nymphs were placed in each cage for 5 days. On the sixth day, the grasshoppers were removed and released. All components of leaf damage were then measured. For each plant, we determined the following: (1) *the total volume of the grazed portion* (TGP), calculated as the sum of (length \times width \times depth of each scar cm^3); and (2) *number of missing tips per plant* (MT). Both these measurements were standardized for number of grasshoppers per cage and number of days of the experiment as (TGP/3 grasshoppers/5 days; MT/3 grasshoppers/5 days). Measurements of the length and the width of scars were taken as maximum values; the depth of scars was estimated as the maximum leaf thickness near the main vein of the leaf, which is often left intact by grasshoppers.

The TGP was chosen as a main measurement of leaf damage, which allowed us to compare relative palatability and suitability of native and exotic grasses for grasshopper feeding. When choosing the procedure to estimate TGP, we originally intended to follow Zou et al. (2008), who calculated the grazed area of leaves from Chinese tallow tree by scanning them and using the ScionImage program. However, this approach proved to be unfeasible for measuring the leaf area of grasses due to the shape, size, and large quantities of leaves per plant. Based on similar patterns of grazed portions among all grasses, we considered our measurements to be the most accurate way to estimate leaf damage within the context of our study. The number of missing tips was additionally used to compare relative activity of grasshoppers on native and exotic grasses, and to estimate whether plant traits, such as leaf

surface and plant architecture, allow grasshoppers to move freely along the plant to the tip of the leaves. These two measurements of leaf damage were also chosen for the analysis of grasshopper herbivory on native and exotic grasses as they were the least correlated variables among four other variables that were initially considered for the analysis (online resource 3).

Greenhouse experiments with potted plants

In July 2013, simultaneously with field experiments in Ohio and Maryland, we conducted two types of feeding experiments with potted plants in the greenhouse of the UC: (1) choice experiment (native and exotic plants were both placed in the same cage), and (2) no-choice experiment (native or exotic plants were placed in separate cages). For the choice experiment, 48 potted plants (the same plant species as those in the field experiments), were similarly arranged in groups of four (one of each species) and were placed in twelve fabric cages (Bioquip, rearing and observation cage, $36 \times 36 \times 61 \text{ cm}^3$, Cat. No. 1466B). For no-choice experiments, potted plants were arranged into two groups: native (*A. gerardii* and *B. curtipendula*) and exotic (*M. sinensis* and *B. ischaemum*). Native and exotic plants were then placed separately in similar fabric cages: two potted plants (one of each species) in each cage.

Grasshoppers ($n = 3$ for choice and $n = 1$ for no-choice trials) were placed in each cage to feed for 5 and 10 days, respectively. A longer period for the no-choice experiment was needed for estimating grasshoppers' growth rates.

Leaf damage in choice and no-choice experiments was estimated by measuring TGP and MT as in the field experiments. Both measurements were also standardized per number of grasshoppers and days of experiments (choice: 3 grasshoppers/5 days; no-choice: 1 grasshopper/10 days). In addition, growth rate of grasshoppers in no-choice experiments was estimated using two measurements: (1) changes in *body weight*: (final weight–initial weight)/initial weight; and (2) changes in *body length*: (final length–initial length)/initial length. Body length was measured as the length from the tip of the head to the tip of the abdomen. These two measurements were chosen following Awmack and Leather (2002) and Berner et al. (2005), as additional indicators of

grasshopper performance on native and exotic plants and, consequently, plant quality. Four dead grasshoppers were found at the end of the no-choice experiment: in one cage with exotic grasses and in three cages with native grasses. These cages were excluded from analysis of both grasshopper growth data and leaf damage data.

Experiment with clipped leaf segments

To eliminate the potential effect of number of leaves and their different sizes on grasshopper choice, we clipped one leaf of the same length (25 cm) and maximum width of 0.7 cm from each plant species (*A. gerardii*, *B. curtipendula*, *M. sinensis*, and *B. ischaemum*). Each leaf was weighted and then four leaves all together were offered to nymph grasshoppers for consumption. The feeding arena consisted of 12 small plastic containers (18 × 11 × 13 cm³ All Living Things[®] Critter Totes, PetSmart, Inc.). The base of the leaves was wrapped with moist filter paper (to keep leaf tissue fresh and attractive for grasshoppers during the experiment) and placed on the bottom of the container. One grasshopper was placed in each container. Another twelve control containers with leaves but without grasshoppers were similarly prepared. The feeding trial lasted 5 h, during which all containers were kept in the greenhouse at 25–26 °C to control conditions of the experiment. Grasshoppers were starved for 24 h prior to all feeding trials and new individuals, which had not been used in the experiments previously, were used for each trial.

After grasshoppers were removed from the containers, leaves were weighed, and the following measurements of leaf damage were taken: (1) *the total leaf biomass consumed* by grasshoppers, following the formula suggested by Waldbauer (1968): $[(1 - (\text{natural loss of aliquot}/\text{initial weight of aliquot}/2)) \times (\text{weight of food introduced} - (\text{weight of uneaten food } (L) + (\text{natural loss of aliquot}/\text{final weight of aliquot} \times L)))]$ g; and (2) *the proportion of the amount of leaf tissue consumed*: absolute amount of leaf tissue consumed/initial amount of leaf tissue offered, g/g. Both measurements were also standardized by the time of the experiments (g/5 h).

Statistical analysis

To compare overall leaf damage between native and exotic plants in each choice experiment, all

measurements were averaged within exotic and within native plants for each cage/container prior to data analysis: [e.g., (grazed portion of *A. gerardii* in cage 1 + grazed portion of *B. curtipendula* in cage 1)/2]. The data for each choice experiment were analyzed separately. Since observations within each cage/container were not independent, the difference between native and exotic plants within a cage was calculated for each measurement. The obtained set of independent values across cages was then tested for a difference from zero using separate one-sample *t* tests for each measurement.

In the no-choice greenhouse experiment, all measurements were similarly averaged within each cage. Each measurement of leaf damage, as well as grasshopper growth rate on each type of plant, was then compared between native and exotic plants using separate one-way fixed-effects ANOVA. One-way ANOVA was also used to compare differences in leaf damage between native and exotic plants at different field sites (WMREC and UCCFS).

For each experiment (field, greenhouse, and experiments with clipped leaf segments), the significance level was adjusted using Bonferroni correction. Normality and homoscedasticity of all data were tested using the Shapiro–Wilk test and Bartlett’s test respectively at $\alpha = 0.05$.

To estimate the potential differences among plant species which might affect leaf damage, the Kruskal–Wallis test followed by post hoc Mann–Whitney’s U test with a Bonferroni correction (due to lack of normality of data and lack of damage in about 25 % plant individuals) was conducted to compare the leaf damage between plant species within native and exotic pairs.

All components of leaf damage were analyzed in R (v.3.1.1).

Results

Field experiments with intact plants

In field experiments, both measures of leaf damage were greater in exotic grasses at both Maryland and Ohio field sites (Table 1; Fig. 1). In total, the volume of grazed leaf tissue of exotic plants was about six times greater than that of native plants at both field sites. In addition, exotic plants contained four times more missing tips than native plants (Table 1).

In addition, field site as a factor did not have a significant effect on any leaf damage trait (TGP: $F_{1,22} = 0.085$; $P = 0.773$; MT: $F_{1,22} = 0.027$; $P = 0.871$). Differences among plant species were significant only for TGP ($\chi^2 = 37.88$, $df = 3$, $P < 0.0001$): *M. sinensis* had the greatest damage. All other comparisons among plant species did not reveal a significant difference.

Greenhouse experiments with potted plants

Similar to the results from field experiments, both measurements of leaf damage were greater in exotic grasses in the choice experiment (Table 1; Fig. 2a, b).

Differences among plant species were detected for TGP only ($\chi^2 = 17.01$, $df = 3$, $P < 0.001$). For this trait, *M. sinensis* sustained the greatest damage. All other comparisons among plant species did not reveal a significant difference. In no-choice experiments, both TGP and MT did not differ between native and exotic plants (Table 1; Fig. 2c, d).

In terms of body mass, the growth rate of grasshoppers did not differ between native and exotic plants; grasshoppers body length, however, were greater on exotic plants (Fig. 3a, b; online resource 4). Overall, the body length of grasshoppers increased during the experiment (0.05 ± 0.02 cm on native plants and 0.29 ± 0.06 cm on exotic plants), whereas

Table 1 Leaf damage in native and exotic plants caused by nymph *Melanoplus* grasshoppers in field experiments

Experiment	Measurements	Plants		Test results	Plant differences
		Native	Exotic		
Field: intact plants, WMREC	Total volume of the grazed portion (cm ³ /day)	0.00046 ± 0.0001	0.00266 ± 0.0005	$t_{11} = 4.07$; $P < 0.001^*$	*
	Missing tips (# per plant/day)	0.036 ± 0.012	0.1693 ± 0.03133	$t_{11} = 4.55$; $P < 0.001^*$	ns
Field: intact plants, UCCFS	Total volume of the grazed portion (cm ³ /day)	0.0004 ± 0.00013	0.00266 ± 0.00053	$t_{11} = 4.02$; $P = 0.001^*$	*
	Missing tips (# per plant/day)	0.03866 ± 0.012	0.18066 ± 0.034	$t_{11} = 3.43$; $P = 0.002^*$	ns
Greenhouse: intact plants, choice	Total volume of the grazed portion (cm ³ /day)	0.002 ± 0.0006	0.00866 ± 0.002	$t_{11} = 2.90$; $P = 0.007^*$	*
	Missing tips (# per plant/day)	0.12466 ± 0.01733	0.36333 ± 0.06466	$t_{11} = 3.61$; $P = 0.002^*$	ns
Greenhouse: intact plants, no-choice	Total volume of the grazed portion (cm ³ /day)	0.003 ± 0.0007	0.004 ± 0.001	$F_{1,22} = 0.69$; $P = 0.417$	*
	Missing tips (# per plant/day)	0.112 ± 0.029	0.266 ± 0.075	$F_{1,22} = 3.59$; $P = 0.0711$	ns
Greenhouse: leaf segments	Total leaf biomass consumed (g/h)	0.002 ± 0.001	0.004 ± 0.001	$t_8 = 0.57$; $P = 0.7102$	ns
	Proportion of leaf biomass consumed (g/g/h)	0.02 ± 0.008	0.03 ± 0.008	$t_8 = 0.27$; $P = 0.6046$	ns

Field experiments were conducted at Western Maryland Research and Education Center (WMREC) and at the University of Cincinnati Center for Field Studies (UCCFS). Experiments with intact potted plants and clipped leaf segments were conducted at the University of Cincinnati greenhouse

Native plants included *Andropogon gerardii* and *Bouteloua curtipendula*; exotic plants included *Miscanthus sinensis* and *Bothriochloa ischaemum*

All values indicate mean ± 1 SE

P values with asterisks (“*”) are significant at the Bonferroni adjusted significance of 0.025. One-sample t tests (for field and choice greenhouse experiments) and one-way ANOVAs (for no-choice greenhouse experiment) were used

Significant differences between plant species in native and exotic pairs indicated as “*”; differences which are not significant “ns”. The Kruskal–Wallis test followed by post hoc Mann–Whitney’s U test with a Bonferroni correction was used

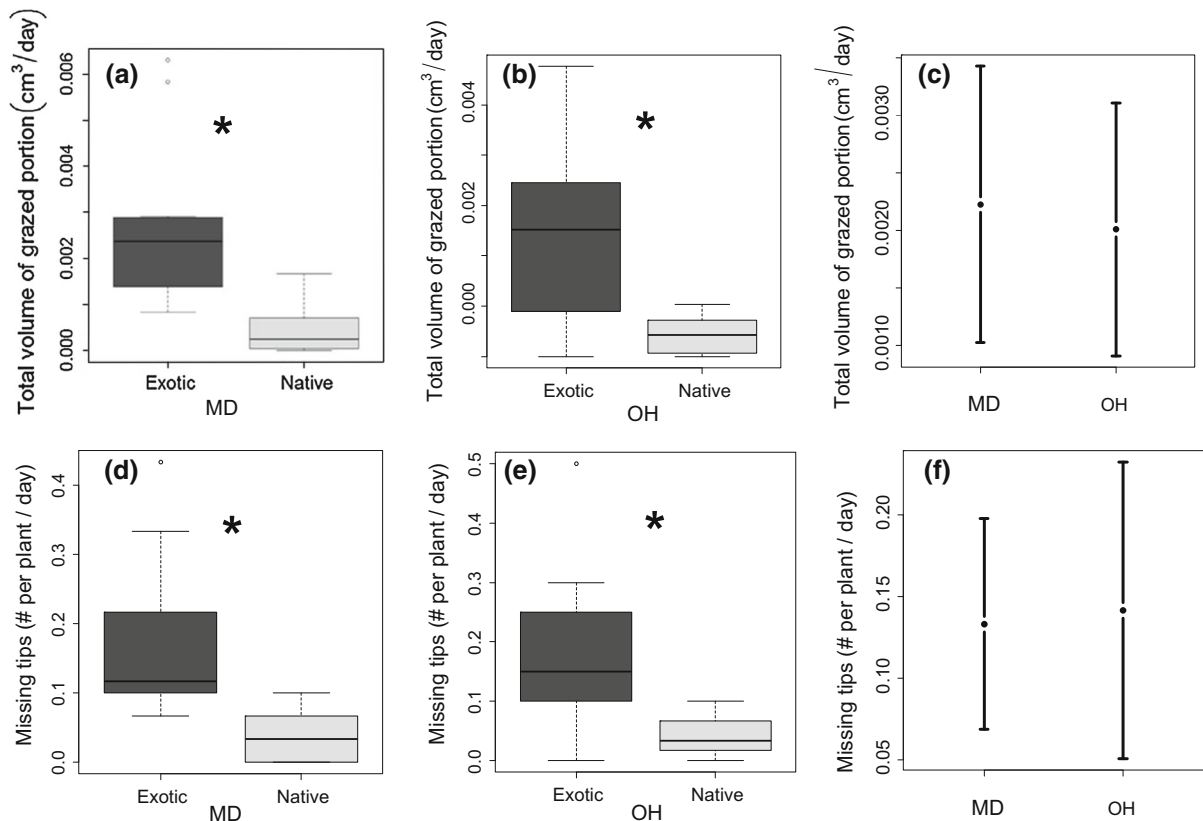


Fig. 1 Two measurements (a–f) of leaf damage of intact exotic *Miscanthus sinensis* and *Bothriochloa ischaemum* (dark gray box plots) and native *Andropogon gerardii* and *Bouteloua curtipendula* plants (light gray box plots) in the field experiments at Western Maryland Research and Education Center (MD) and at the University of Cincinnati Center for Field Studies (OH). Box plots represent measurements of the total volume of grazed portion (cm^3/day) (a, b) and the number of

missing tips per plant per day (d, e) averaged within native and exotic species. The vertical lines represent mean differences in leaf damage between native and exotic plants across sites $\pm 95\%$ confidence intervals (c, f). Asterisks (“**”) indicate significant differences within exotic and native plants at the Bonferroni adjusted significance level of $P = 0.025$ (one-sample t tests were used)

their body weight decreased on both native and exotic plants (online resource 4). Body weight and body length were not strongly correlated ($r = 0.2$; Fig. 3c).

Experiment with clipped leaf segments

In the experiment with clipped leaf segments, we did not observe any differences in leaf damage between native and exotic plants (Table 1; Fig. 4). On average, the total amount of leaves consumed by grasshoppers was 0.002 ± 0.001 g on native plants, and 0.004 ± 0.001 g on exotic plants; which represented no more than 15 % of the initial amount of leaf tissue from both native and exotic plants. There were no detected differences in leaf damage among plant species.

Discussion

Overall, our results demonstrated not only lack of avoidance of the exotic grasses by grasshoppers, but also showed grasshopper preference for exotic grasses in the majority of experiments: exotic grasses sustained an equal or greater level of leaf damage compared to native grasses in all experiments. Our hypothesis of greater leaf damage in exotic grasses compared to natives was supported in field and greenhouse choice experiments, in which the amount and intensity of leaf damage were greater on exotic grasses. However, we did not find support for our hypothesis in the greenhouse no-choice experiment and in the experiment with clipped leaf segments: in these cases, we observed a comparable level of leaf

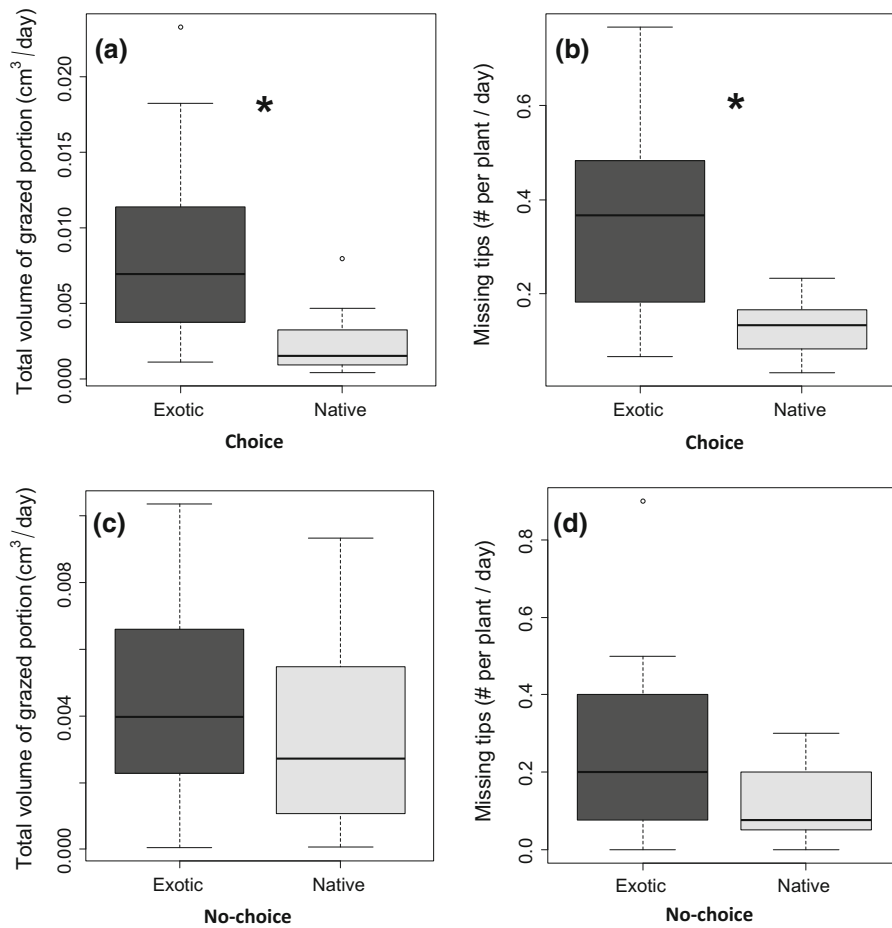


Fig. 2 Two measurements (**a–d**) of leaf damage of intact exotic *Miscanthus sinensis* and *Bothriochloa ischaemum* (dark gray box plots) and native *Andropogon gerardii* and *Bouteloua curtipendula* plants (light gray box plots) in the experiments with potted plants at the University of Cincinnati greenhouse. Box plots represent measurements of the total volume of grazed portion (cm^3/day) and the number of missing tips per plant per

day in the choice (**a, b**) and the no-choice (**c, d**) experiments. All data are averaged within native and exotic species. Asterisks (“**”) indicate significant differences within exotic and native plants at the Bonferroni adjusted significance level of $P = 0.025$ (one-sample t tests and one-way ANOVAs were used for choice and no-choice experiments respectively)

damage in exotic and native plants. Similarly, grasshoppers’ growth rates (in terms of both body weight and body length) on native and exotic plants also suggest that exotic plants did not have a more (if any) inhibitory effect on grasshopper growth than do native plants.

Our discovery of higher or similar levels of leaf damage in exotic plants compared to native plants is consistent with previous studies, indicating that generalist insect herbivores do not avoid feeding on exotics and when available, readily incorporate these plants in their diet (Agrawal and Kotanen 2003; Siemann and Rogers 2003; Lankau et al. 2004; Hull-

Sanders et al. 2007; Zou et al. 2008; Lind and Parker 2010; Fielding and Conn 2011; Fan et al. 2013). Our findings are also consistent with results from similar studies on non-insect invertebrates, where generalist herbivores preferred exotic plants over natives (e.g., Morrison and Hay 2011). However, Lankau et al. (2004) demonstrated that generalist grasshoppers, which preferred exotic plants over natives in laboratory and common garden trials, might not recognize exotics as a potential food in nature because of behavioral constraints. The authors studied invasive Chinese tallow tree (*Sapium sebiferum*), a plant, which morphologically and physiologically differs from

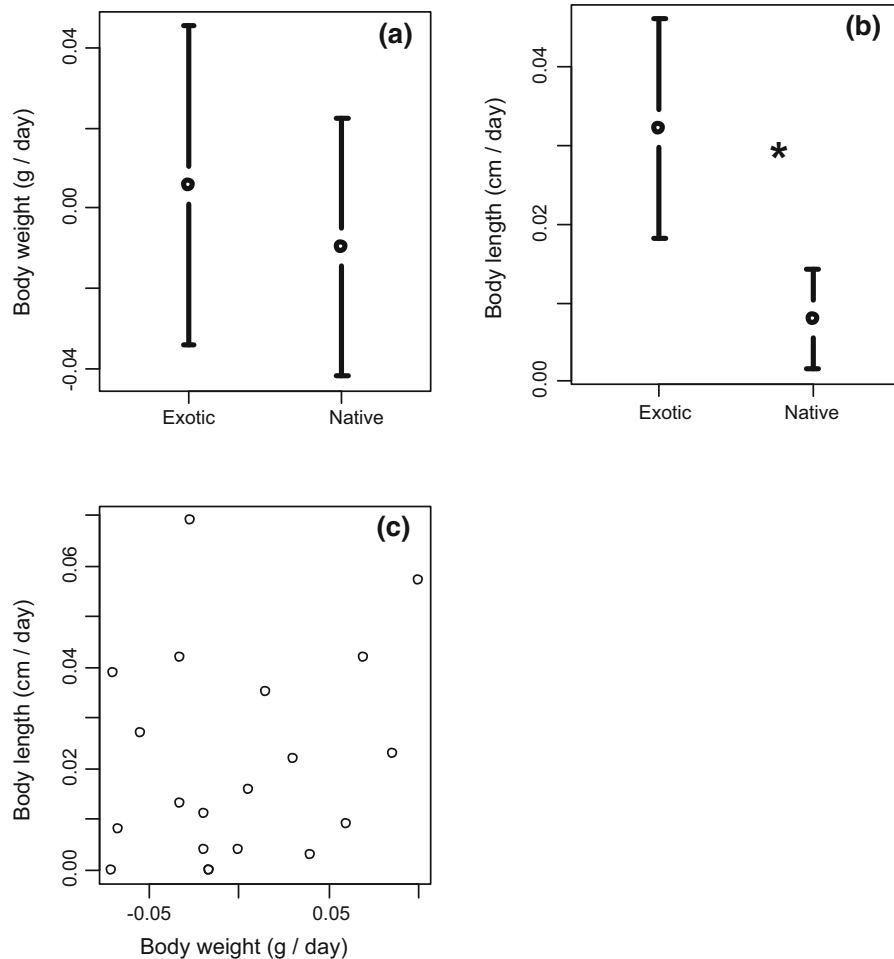


Fig. 3 Growth rates of nymph *Melanoplus* grasshoppers on intact exotic *Miscanthus sinensis* and *Bothriochloa ischaemum* and native *Andropogon gerardii* and *Bouteloua curtipendula* plants in the no-choice experiment with potted plants at the University of Cincinnati greenhouse. **a** Changes in body weight (g/day), **b** changes in body length (cm/day), **c** correlation

between body weight and body length ($r = 0.2$). The vertical lines represent mean values $\pm 95\%$ confidence intervals. Asterisks (“*”) indicate significant differences in grasshopper growth rates on exotic and native plants at the Bonferroni adjusted significance level of $P = 0.025$ (one-way ANOVAs were used)

grasses. Future investigations should explore natural herbivory of the grasses from the current study to see if grasshoppers demonstrate the same behavioral constraints and avoid exotic grasses under natural conditions.

Our results do contrast with some other studies of interactions between exotic plants and native generalist insects (e.g., Han et al. 2008; Jogesh et al. 2008; Tallamy et al. 2010; Schaffner et al. 2011), as well as with some investigations involving non-insect invertebrates (e.g., Motheral and Orrock 2010; Tomas et al. 2011). Some of these authors used laboratory feeding

trials (e.g., Tallamy et al. 2010; Tomas et al. 2011) or focused on congeneric native/exotic plant pairs only (e.g., Jogesh et al. 2008). Congeneric comparisons are critical in understanding differences in resistant traits of native and exotic plants which might facilitate invasion. However, using morphologically and physiologically similar plants, which share common habitat and are not necessarily closely related, should also provide important insight into plant resistance from the insect’s perspective. This way we can observe plant responses under natural feeding behavior of generalist herbivores, as it is unlikely that each feeding

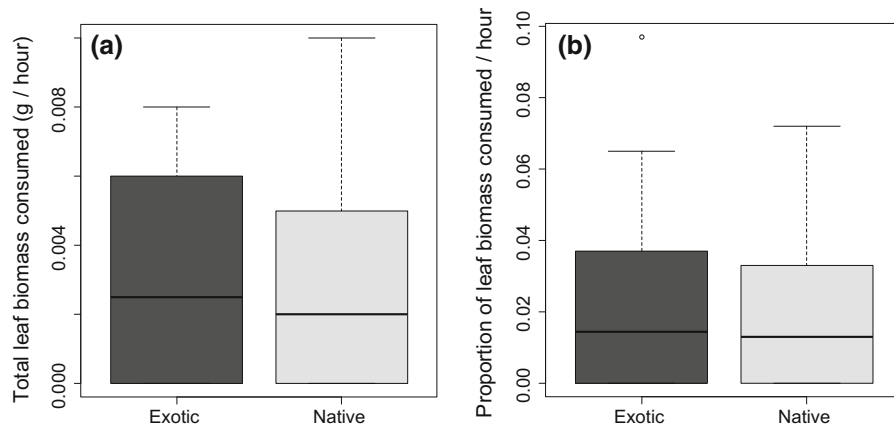


Fig. 4 Two measurements (**a**, **b**) of leaf damage of exotic *Miscanthus sinensis* and *Bothriochloa ischaemum* (dark gray box plots) and native *Andropogon gerardii* and *Bouteloua curtipendula* plants (light gray box plots) in the experiments with clipped leaf segments at the University of Cincinnati greenhouse. Box plots represent measurements of the total leaf

biomass consumed (g/hour) (**a**) and the proportion of leaf biomass consumed per hour (**b**). All data are averaged within native and exotic species. Differences in both measurements were not significant between native and exotic species (one-sample *t* tests were used)

choice of generalist grasshoppers in the field is limited by a choice between two congeneric native/exotic plant species.

As the field experiments more closely simulated natural conditions, our results suggest that grasshoppers do not avoid feeding on exotic plants, and even prefer to feed on them, presumably because of similar or lower level of resistance of exotic plants to herbivory compared to native plants. The lack of avoidance of exotic plants by grasshoppers was supported by the results from the no-choice experiment; when presented separately, both native and exotic plants were similarly utilized by grasshoppers.

Plant resistance traits—i.e., traits that can affect the amount of damage (Rausher 1992)—include the presence of surface wax, spines, trichomes (hairs), leaf toughness (potentially determined by silica content), and synthesis of chemicals (Price et al. 2011). Greater resistance of native grasses to grasshopper herbivory compared to exotic grasses in our study can be due to the following: (1) the hairy leaf surface (glandular hairs on the margin of leaf blade in *B. curtipendula*, and more sparse hairs in *A. gerardii*), and (2) more compact leaves of these native species, which can prevent active foraging of grasshoppers, especially nymphs, considering the size of their legs and mouth parts. In contrast, exotic *M. sinensis* and *B. ischaemum* have smoother leaf surfaces and leaves that are less compact, with larger internodes, which

presumably make it easier for nymph grasshoppers to move and feed on these plants. In addition, *M. sinensis* which sustained the greatest leaf damage in most of our experiments, had less trichome density and leaf toughness especially compared to that of native *B. curtipendula* (online resource 2).

Leaf toughness of *Miscanthus* and *Bothriochloa* apparently does not prevent grasshoppers from feeding. For example, it has been demonstrated in greenhouse experiments with *Miscanthus* plants and the American grasshopper *Schistocerca americana*, an important crop pest, that silica in plant tissue influences cell thickness and also increased the consumption rate of this grasshopper species, although conversion efficiency was reduced (Nabity et al. 2012). In our study, we also found that whenever differences among plant species were detected, *M. sinensis* sustained the greatest level of leaf damage. It would be interesting for future studies to explore whether the same silica component of plant resistance as in *M. sinensis* affects feeding of the *Melanoplus* grasshoppers with regard to *Bothriochloa* plants.

In terms of chemical compounds, Mole and Joern (1994) demonstrated that native North-American grasses of the Poaceae family (including *Andropogon* and *Bouteloua*) possessed neither the strong deterrents nor phagostimulants that could affect grasshopper feeding. A comparison of secondary metabolites of *Miscanthus* and *Bothriochloa* grasses to those found in

Andropogon and *Bouteloua*, and specifically the effect of these metabolites on grasshopper feeding, would be also helpful to better explain the increased leaf damage in these exotic grasses. We can only speculate that the balance of deterrents and phagostimulants in *Miscanthus* and *Bothriochloa* grasses made them attractive to grasshoppers. It is possible that after removal from the plant, the level of resistance of the clipped portion decreased in native plants, which caused them to be as palatable for grasshoppers in our feeding experiments as were leaves clipped from exotic plants. Equal levels of leaf consumption by grasshoppers suggest that leaves of exotic plants either had not changed resistance after they had been clipped, or their resistance levels had decreased but were not lower than those of native plants.

Given that two different grasshopper species were used in this experiment, it is possible that they might exhibit different preferences for native and exotic grasses. However, for the purpose of our experiments, we were interested in general plant responses to herbivory by generalist species under natural conditions. We have not separated *M. differentialis* and *M. femurrubrum* nymph grasshoppers in our experiments as they occur in the same habitats and consume plants of the same genus (e.g., Caswell and Reed 1976). Following studies which used combined *Melanoplus* spp. species in feeding experiments (e.g., Berdahl et al. 1990), we expected these grasshopper species, especially at nymphal stage, to have similar preferences with regard to native and exotic grasses.

Invasive plants often cause environmental and economic problems and their control is often costly (Pimentel et al. 2000, 2005). Knowing how exotic, potentially invasive grasses interact with native generalist herbivores, such as grasshoppers, is critical for predicting invasion in certain areas and/or developing plans for effective control of invasive plants. Our study supports the idea that lack of coevolution between exotic plants and native generalist herbivores can result in decreased resistance of exotic plants to novel native herbivores (Parker et al. 2006), and provides potential support for the BRH hypothesis. However, further testing the BRH, as well as ERH and BCH hypotheses using the proposed grasses–grasshoppers model would require additional investigations, such as exploring plant tolerance and competitive ability of exotic grasses (ERH), and/or obtaining additional data on natural herbivory by

Melanoplus grasshoppers (BCH and BRH). It is also critical to test the effects of herbivory on plant fitness at the population level, as well as to compare data on herbivory at different times of the season. Combined results from such studies will provide more information about interactions between native insect herbivores and their novel host plants from both plant and insect perspectives.

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