

Feeding preferences of *Melanoplus femurrubrum* grasshoppers on native and exotic grasses: behavioral and molecular approaches

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Abstract

Generalist insect herbivores, such as grasshoppers, may either avoid feeding on exotic plants, potentially enabling these plants to become invasive in the introduced range, or insects may incorporate exotic plants into their diet, contributing to the biotic resistance of native communities and potentially preventing plant invasions. Accurate determination of insect diet preferences with regard to native and exotic plants can be challenging, but this information is critical for understanding the interaction between native herbivores and exotic plants, and ultimately the mechanisms underlying plant invasions. To address this, we combined behavioral and molecular approaches to accurately compare food consumption of the polyphagous red-legged grasshopper, *Melanoplus femurrubrum* (De Geer) (Orthoptera: Acrididae), on native [*Andropogon gerardii* Vitman and *Bouteloua curtipendula* (Michx.) Torr.] and exotic, potentially invasive grasses [*Miscanthus sinensis* Andersson and *Bothriochloa ischaemum* (L.) Keng] (all Poaceae). We found that *M. femurrubrum* grasshoppers demonstrated strong feeding preferences toward exotic grasses in experiments with intact plants under both field and greenhouse conditions, but they showed no preference in experiments with clipped leaves. Additionally, we sampled the gut contents of *M. femurrubrum* collected in the field and identified the ingested plant species based on DNA sequences for the non-coding region of the chloroplast *trnL* (UAA) gene. We found that exotic plants were prevalent in the gut contents of grasshoppers collected at study sites in Ohio and Maryland, USA. These results suggest that the generalist herbivore *M. femurrubrum* does not avoid feeding on exotic grasses with which they do not share coevolutionary history. In addition, by demonstrating greater food consumption of exotic plants, these grasshoppers potentially provide biotic resistance should these grasses escape cultivation and become invasive in the introduced range.

Introduction

Understanding the feeding preferences of generalist insect herbivores is critical for determining plant–insect trophic interactions, especially with regard to exotic plants which potentially can become invasive in the introduced range. For example, evidence of avoidance of exotic plants by generalist insect herbivores may provide a better understanding of mechanisms facilitating invasion, such as the release of exotic plants from their native enemies (the ‘enemy release hypothesis’; Keane & Crawley, 2002). On

the contrary, if generalist insect herbivores do not avoid feeding on exotic plants and/or even show a preference for them over native plants (due in part to an inability of the exotic plants to defend themselves against evolutionarily novel enemies), such insect feeding behavior can contribute to biotic resistance of native communities to exotic plants and potentially prevent plant invasion (the ‘biotic resistance hypothesis’; Parker & Hay, 2005; Parker et al., 2006). In addition, generalist insects may effectively utilize both native and exotic plants if these plants are congeners and thus share morphological and physiological characteristics (‘Darwin’s naturalization hypothesis’; Lambdon & Hulme, 2006). Consequently, determining the trophic interactions among generalist insect herbivores, and native

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and exotic plants is important for a better understanding of the mechanisms underlying the process of plant invasion.

The red-legged grasshopper, *Melanoplus femurrubrum* (De Geer) (Orthoptera: Acrididae), is a polyphagous insect herbivore that is a convenient organism with which to study insect feeding preferences: it is relatively easy to collect, identify, and maintain under laboratory conditions. This grasshopper species is widely distributed throughout North America, including Ohio and Maryland (USA), and is a known crop pest. *Melanoplus femurrubrum* consumes a wide range of plants including grasses and broad-leaved plants such as legumes, dandelion, chicory, goldenrod, Kentucky bluegrass, barley, oats, smooth brome, and timothy (Pfadt, 1994; Lamp et al., 2007). Food selection is driven by nutrition needs of a grasshopper (Joern, 1983). Although grasses have a lower nutritional quality (specifically for nitrogen content) compared to forbs, feeding on grasses allows grasshoppers to avoid detoxification processes (Bernays & Chapman, 1978; Joern, 1983). *Melanoplus* species, actively feed on warm-season grasses such as *Andropogon gerardii* Vitman (Whipple et al., 2009; Loaiza et al., 2011), *Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths (e.g., Alward & Joern, 1993), *Bothriochloa bladhii* (Retz.) ST Blake (e.g., Avanesyan, 2014), *Miscanthus sinensis* Andersson (Nabity et al., 2012), as well as on cold-season grasses such as *Bromus inermis* Leyss. and *Poa pratensis* L. (Whipple et al., 2009). *Melanoplus* grasshoppers are dominant insect herbivores in grasslands; the intensity of grasshopper food consumption of important tallgrass prairie plants influences the changes in nutrient cycling, and consequently, can either increase or decrease plant productivity (Joern, 1983; Belovsky & Slade, 2000) and potentially interactions among plant species. Consequently, knowledge of the dietary preferences of grasshoppers for native and exotic grasses is especially important: many exotic grasses, such as *Bothriochloa* species, which are widely planted because of their high forage quality, demonstrate high competitive ability and may inhibit growth of some economically and ecologically important native grasses (Schmidt et al., 2008).

Although field experiments and observations of natural grasshopper herbivory are informative of grasshoppers' food choices under natural conditions, it is critical to accurately confirm food ingestion, for example, by an analysis of insect gut contents (Garcia-Robledo et al., 2013). Analysis of grasshopper gut contents has an advantage compared to feeding experiments as grasshoppers do not sustain outside influences in their food selection, such as manipulations or the presence of a researcher (Mulkern, 1967). Using DNA barcoding to analyze insect gut contents has been shown to be a highly effective method in

determining insect diet and trophic interactions (Garcia-Robledo et al., 2013; Avanesyan, 2014; Kress et al., 2014). This method is the most advantageous and accurate when DNA sequences from ingested food are compared to a reference DNA barcode library of diet items (Kress et al., 2014). Although there have been some studies on grasshopper feeding on native vs. exotic plants (Porter & Redak, 1997; Siemann & Rogers, 2003; Jogesh et al., 2008; Branson & Sword, 2009; Fielding & Conn, 2011; Fan et al., 2013), only a few studies conducted either microscopical examination of grasshopper gut contents (Joern, 1979, 1983) or plant DNA extraction from grasshopper guts (Matheson et al., 2008) and feces (Valentini et al., 2009). Studies that involved molecular analysis of grasshopper gut contents and feces, used this method to detect ingested food plants only; to our best knowledge, there have been no studies that utilized molecular approaches to determine grasshopper feeding preferences on native and exotic plants, which is critical for understanding the interaction of generalist grasshoppers with exotic plants under natural conditions. In addition to directly addressing this issue, we also investigated the prevalence of native and exotic plants (including grasses) in the gut contents of grasshoppers collected in the field, using a previously developed, PCR-based method to detect plant meals from grasshopper guts (Avanesyan, 2014).

Here, we report the results of a study on grasshopper diet preferences with regard to native and exotic plants using behavioral and molecular approaches. In this study, we were ultimately interested whether generalist insect herbivores, such as *M. femurrubrum* grasshoppers, may limit spread of exotic grasses, should they escape cultivation and become highly invasive in tallgrass prairie. Our main research questions were: do *M. femurrubrum* grasshoppers incorporate exotic grasses in their diet? If so, do they prefer to feed on exotic rather than on native grasses? To more accurately investigate the feeding preferences of *M. femurrubrum* on native and exotic grasses, we incorporated methodological recommendations from previous studies (Mulkern, 1967; Harvey & Fortuna, 2012; Garcia-Robledo et al., 2013) and explored our research questions under experimental and natural field conditions. In feeding experiments, we used two native [*A. gerardii* and *Bouteloua curtipendula* (Michx.) Torr.] and two exotic, potentially invasive, grasses [*M. sinensis* and *Bothriochloa ischaemum* (L.) Keng] (all Poaceae). We chose morphologically and physiologically similar, closely related C₄ warm-season grasses to minimize the effect of any potential physical and physiological differences among plant species on grasshopper behavior. In addition, our choice of grasses was also influenced by known grasshopper attractiveness (especially that of *M. femurrubrum*) to

tall vertical patterns which correspond to upright blades of grasses (Mulkern, 1967); thus we could stimulate the feeding activity of grasshoppers in the enclosures. We conducted field and greenhouse experiments simultaneously to eliminate any potential effects of seasonal changes on grasshopper behavior (Mulkern, 1967). For molecular confirmation of grasshopper diet under natural field conditions, we used plants collected from the field sites, and which also included grasses of Poaceae family (up to 50% of all collected plants). Using a molecular approach we explored the diet preferences of *M. femurrubrum* grasshoppers for native and exotic plants under natural field conditions. We were also interested in exploring whether grasshopper natural food choice is similar to that observed under experimental conditions.

We hypothesized that *M. femurrubrum* grasshoppers would not avoid exotic grasses and might even prefer to feed on them. Our expectations of lack of avoidance of exotic grasses by this grasshopper species were based on (1) highly polyphagous feeding behavior of *Melanoplus* grasshoppers and their ability to switch often between foods (Bernays & Chapman, 1994), (2) their consumption of exotic plants demonstrated by previous studies (Fielding & Conn, 2011; Fan et al., 2013), (3) lack of coevolutionary history between *M. femurrubrum* grasshoppers and exotic *Miscanthus* and *Bothriochloa* grasses, and (4) similar morphological and physiological characteristics of our study grasses, which presumably would provide similar attractiveness for grasshoppers. As such, we expected that gut contents of *M. femurrubrum* grasshoppers collected in the field would contain a similar or greater proportion of exotic plants compared to native plants. To explore our research questions, we conducted a 1-year study which utilized field and greenhouse feeding experiments with intact plants and clipped leaves, as well as detecting plant DNA within grasshopper gut contents to more accurately determine grasshopper food choices under experimental and natural conditions.

Materials and methods

Feeding experiments with intact plants in the field

To examine grasshopper feeding preferences on intact plants, we obtained seeds for *A. gerardii* and *B. curtipendula* (Prairie Moon Nursery, Winona, MN, USA), *M. sinensis* (Outsidepride.com, Independence, OR, USA), and *B. ischaemum* (Warner Brothers Seed Company, Lawton, OK, USA); we planted these seeds in the University of Cincinnati greenhouse in May 2013.

We then established two field study plots each of 90 m² area at the University of Cincinnati Center for Field Studies (UCCFS; 39°17.134'N, 084°44.413'W; Harrison, OH,

USA) and at the Western Maryland Research and Education Center (WMREC; 39°30.618'N, 077°44.070'W; Keedysville, MD, USA). The second field site (WMREC) was used to repeat the field experiment under similar climate and soil conditions and to explore whether *M. femurrubrum* grasshoppers from another population would demonstrate similar feeding preferences.

In the end of July 2013, we arranged 144 potted plants grown from the seeds at the University of Cincinnati greenhouse (plants were 20–25 cm high in 9-cm square pots) in three groups of 48 (each group of 48 contained 12 pots of each plant species): two groups were transferred to the field plots (one per field site), and one group was kept in the greenhouse. In addition, extra plants of each species were grown separately in the greenhouse to be used for experiments with clipped leaves (described below). In the field at each site, the potted plants were arranged and planted in 12 groups of four with ca. 1 m spacing between the groups; each potted plant within the group represented a different species (two native and two exotic species per group). Plants were watered only during the first 2 days after planting, and then plants were allowed to grow under natural field conditions.

All experiments were conducted in September 2013, on the 5th week after transplanting plants to the field. On the day before the experiments, we set up an open air Repti Breeze aluminum screen cage (40 × 40 × 50 cm; Zoo Med Laboratories, San Luis Obispo, CA, USA) around each plant group. Male *M. femurrubrum* grasshoppers were collected near the study plots on the 1st day of the experiment and were immediately weighed and placed in the cages (two males per cage). We intentionally did not starve grasshoppers before the feeding trials, as it has been demonstrated that the longer grasshoppers are starved, the more likely they are to choose unpalatable food (Bernays & Chapman, 1994).

Feeding experiment with intact plants in the greenhouse

In the greenhouse experiment, plants were watered daily. On the day before the experiment, grasshoppers were collected at the UCCFS and kept overnight in the greenhouse in an open air aluminum screen cage and fed a mixture of plants collected on the same collection site. On the 1st day of the experiments, 48 potted plants were arranged in 12 groups of four, as described previously for the field experiment; each plant group and two male grasshoppers were then placed in a fabric rearing and observation cage (35 × 35 × 60 cm; Bioquip, Rancho Dominguez, CA, USA) in the greenhouse. Both field and greenhouse experiments lasted 5 days. On the 6th day, all grasshoppers were removed from the cages and released.

Choice feeding experiment with clipped leaves

To estimate food consumption of grasshoppers on native and exotic plants, the choice experiment with clipped leaves was conducted in the greenhouse in September 2013 in small plastic containers (18 × 11.5 × 12.5 cm; All Living Things® Critter Totes, PetSmart Store Support Group, Inc., USA, <http://www.petsmart.com>). We clipped one leaf of 25 cm length from each plant species, weighed it (weight range 0.07–0.16 g), and placed all four leaves in each container. To keep leaves hydrated during the experiments, we wrapped their bases with a moist paper towel. Following Siemann & Rogers (2003), grasshoppers were starved for 24 h prior to feeding on clipped leaves. Unlike experiments with intact plants, it was critical in the experiments with clipped leaves to use a short period of starvation which would not affect palatability of plants for grasshoppers but would eliminate any plant contents from grasshopper guts. This allowed us to stimulate simultaneous feeding of all grasshoppers during the short experimental period. One male grasshopper was then placed in each container for 3.5 h. The same number of ‘control’ containers (without grasshoppers) were similarly prepared for choice (n = 10) and no-choice (n = 9) experiments. All containers were kept in the greenhouse during the experiments at 25–26 °C.

In 3.5 h after the beginning of the experiment, all grasshoppers were removed and the fresh weight of remaining leaves was measured. The remaining leaves from the feeding experiment, as well as the control leaves, were air dried at room temperature for several weeks. Leaf dry weight was then measured.

No-choice feeding experiment with clipped leaves

To estimate digestibility and assimilation of the ingested food by grasshoppers, the no-choice experiment was conducted similar to the previous choice experiments but with two modifications: (1) in each container, we placed two leaves from either native or exotic plants only, and (2) after removing grasshoppers from containers, any grasshopper fecal material was air dried at room temperature and then measured. The preparation of ‘control containers’, as well as the duration of the experiment, were identical to that in the choice experiment.

Molecular confirmation of diet

To estimate diet preferences of *M. femurrubrum* grasshoppers under natural conditions, when a wider choice of food plants would be available than in the feeding experiments, we investigated the prevalence of exotic and native host plants in grasshopper gut contents. Both *M. femurrubrum* grasshoppers and reference plants (one individual per plant species was clipped at the ground) from two nat-

ural field sites in Ohio (18 × 10 m², 39°17.266′N, 084°44.426′W, Harrison) and Maryland (60 × 12 m², 39°30.783′N, 077°43.968′W, Keedysville) which were located about 100 m from the study plots on which we conducted our feeding experiments. Both sites were adjacent to maize and soybean fields (ca. 5 m distant) and presumably were attractive for grasshoppers.

Plants and grasshoppers were immediately frozen at –20 °C prior to DNA extraction. The fragments (ca. 500 bp) of the non-coding region (intron) of the chloroplast *trnL* (UAA) gene were isolated from grasshopper gut contents and from reference plants using a previously developed protocol (Avanesyan, 2014). All PCR products were sequenced using Sanger sequencing at the Beckman Coulter Genomics facility (Danvers, MA, USA). DNA sequences obtained from reference plants were then BLASTed against the National Center for Biotechnology Information (NCBI) GenBank database (<http://www.ncbi.nlm.nih.gov/genbank/>) and plant species were identified using 98–100% match identity. The local reference plant database for each site was created using BioEdit (Hall, 1999). DNA sequences obtained from ingested plants were then BLASTed against the local plant database which we had previously created; ingested plants were then identified using the highest possible (93–100%) match identity. If an ingested plant did not match any of the reference plants (presumably due to grasshopper feeding on a territory adjacent to the study site), the corresponding sequences were blasted directly against NCBI GenBank database for species identification. The origin of ingested plants (native or exotic) was determined using the United States Department of Agriculture’s PLANTS Database (<http://plants.usda.gov>; accessed 17 August 2015).

Measurements and data analysis

In the experiments with intact plants, we estimated grasshopper food consumption on each plant as the total volume (cm³) of the grazed portion (GP): Σ (length × width × depth of each scar). Each grazed mark was called a ‘scar’. All measurements of each scar’s volume were taken as maximum values. Feeding activity was estimated in terms of feeding rate (FR) as [total volume of grazed portion per unit weight of grasshopper (g) per day]. To calculate grasshopper feeding rate, we followed Farrar et al. (1989) and used each grasshopper’s mean weight at the beginning of the experiment. Plants without any evidence of grazing were excluded from analyses of the measurements of feeding rate.

In the choice experiment with clipped portions of leaves, food consumption was estimated in terms of (1) relative consumption rate (food intake per g of insect per h) (Delvi & Pandian, 1972), and (2) fresh-weight con-

sumption index (Waldbauer, 1968). In the no-choice experiment with clipped leaves, food assimilation was calculated in terms of (1) relative assimilation rate (food assimilated per g of insect per h) (Delvi & Pandian, 1972), and (2) approximate digestibility (the amount assimilated/dry weight of food ingested) (Bailey & Mukerji, 1976). Similar to the experiments with intact plants, we used the weight of grasshoppers obtained at the beginning of the experiment for these calculations.

Differences in grasshopper feeding on native and exotic plants were assessed using the two-way fixed effects ANOVA (for field experiments) and a one-way fixed effects ANOVA (for the greenhouse experiments with both intact plants and clipped leaves), using a general linear model (GLM) (all analyses were conducted in R, v.2.15.2). Site (Ohio or Maryland) and plant origin (native or exotic) were fitted as fixed effects. All data (except food assimilation) were \sqrt{x} -transformed to meet the assumptions of normality and homoscedasticity (based on the Shapiro–Wilk test and Bartlett’s test, respectively). A Kruskal–Wallis test followed by a post-hoc Mann–Whitney’s U test (due to lack of normality of data) was used to estimate potential differences among plant species which might affect grasshopper feeding behavior. These tests were also used to detect any difference in grasshopper feeding under field and greenhouse conditions, separately on native and exotic grasses.

The prevalence of native and exotic plants in grasshopper guts was determined using a binomial test. The χ^2 test was used to estimate the difference between the observed proportion of native and exotic plant species within grasshopper gut contents and the proportion of native and exotic plant species in the field (expected proportion).

Results

Feeding experiments with intact plants in the field

Under field conditions, the total volume of the leaf tissue portion grazed by grasshoppers was 7.5× greater for exotic plants than for native plants, whereas the grasshopper feeding rate was 4× greater on exotic plants (Table 1, Appendix 1). Site location (WMREC or UCCFS) did not have an effect on any of the measured variables (Appendix 1). However, a significant plant origin*site interaction was detected in the total volume of the grazed portion (Appendix 1): native grasses were grazed more frequently at the UCCFS than at the WMREC ($\chi^2 = 4.82$, d.f. = 1, $P = 0.028$), whereas grazing on exotic plants did not differ between sites ($\chi^2 = 1.29$, d.f. = 1, $P = 0.26$). The smallest volume of the grazed portion and the lowest feeding rate of grasshoppers at both sites were detected on *B. curtipendula*, which was not grazed at all at the UCCFS (consequently, these plants were excluded from comparisons of feeding rate). All other comparisons among plant species did not reveal a significant difference.

Feeding experiment with intact plants in the greenhouse

When potted plants were offered to grasshoppers in the greenhouse, the total volume of the leaf tissue portion grazed by grasshoppers was 4× greater on exotic plants than on native plants, whereas the feeding rate of grasshoppers did not differ between native and exotic plants (Table 1, Appendix 1). All comparisons among plant species did not reveal a significant difference between plant species (Appendix 2).

Comparisons of grasshopper herbivory under field and greenhouse conditions revealed that both food

Table 1 Mean (\pm SE) values of leaf tissue consumption and feeding activity of *Melanoplus femurrubrum* grasshoppers on native and exotic intact plants

Site ¹	Parameter	Plants ³		P
		Native	Exotic	
WMREC	Total volume of the grazed portion (cm ³)	0.002 \pm 0.001	0.015 \pm 0.003	0.0016
	Feeding rate ² (cm ³ g ⁻¹ per day)	0.002 \pm 0.001	0.008 \pm 0.002	0.015
UCCFS	Total volume of the grazed portion (cm ³)	0.01 \pm 0.003	0.013 \pm 0.005	0.0016
	Feeding rate (cm ³ g ⁻¹ per day)	0.009 \pm 0.003	0.013 \pm 0.007	0.015
UC greenhouse	Total volume of the grazed portion (cm ³)	0.013 \pm 0.004	0.052 \pm 0.016	0.011
	Feeding rate (cm ³ g ⁻¹ per day)	0.015 \pm 0.003	0.048 \pm 0.014	0.056

¹Feeding experiments with intact plants were conducted in the field at Western Maryland Research and Education Center (WMREC), the University of Cincinnati Center for Field Studies (UCCFS), and at the University of Cincinnati (UC) greenhouse.

²Feeding rate was estimated for plants with at least one grazed mark (scar); undamaged plants were excluded from analysis of this variable.

³Native plants included *Andropogon gerardii* and *Bouteloua curtipendula*; exotic plants included *Miscanthus sinensis* and *Bothriochloa ischaemum*.

consumption and feeding activity of grasshoppers on exotic plants were greater in the greenhouse than in the field (GP: $\chi^2 = 7.44$, d.f. = 1, $P = 0.0063$; FR: $\chi^2 = 9.00$, d.f. = 1, $P = 0.0027$; Figure 1). As for native plants, grasshopper feeding rate only was higher in the greenhouse than in the field ($\chi^2 = 8.03$, d.f. = 1, $P = 0.0045$) (Figure 1).

Choice feeding experiment with clipped leaves

When clipped portions of leaves were offered to grasshoppers in the choice experiment under laboratory conditions, food consumption in terms of both consumption rate and consumption index did not differ between native and exotic plants (Table 2, Appendix 3). In addition, no differences in leaf damage among plant species were observed.

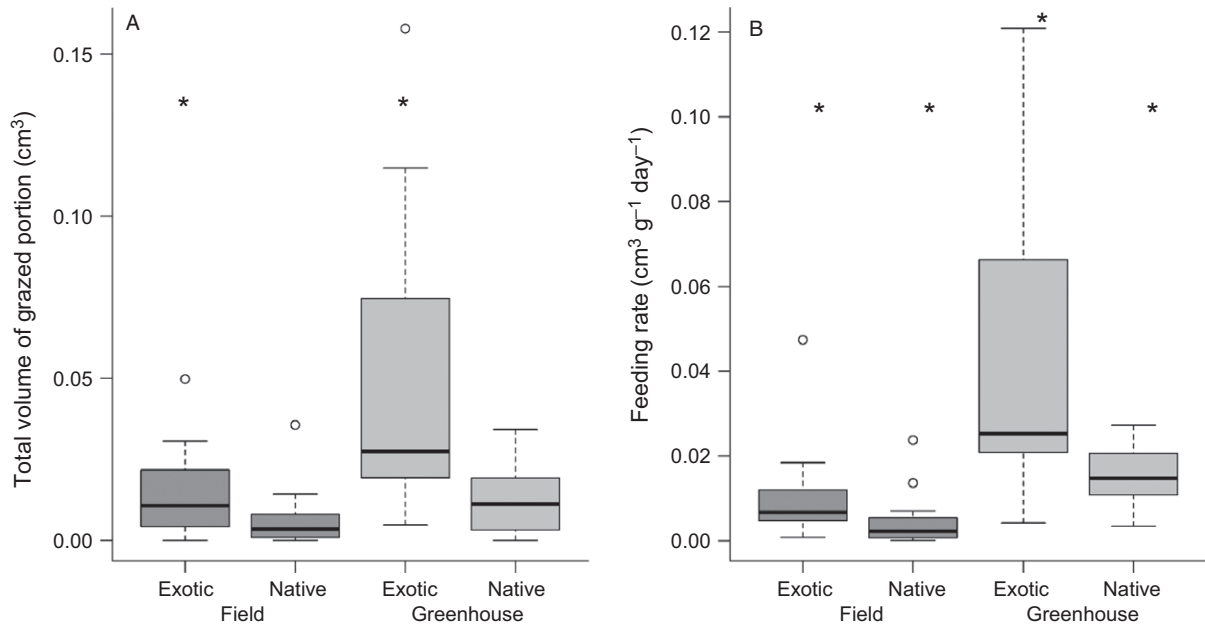


Figure 1 (A) Food consumption and (B) feeding activity of *Melanoplus femurrubrum* grasshoppers on intact exotic *Miscanthus sinensis* and *Bothriochloa ischaemum* and native *Andropogon gerardii* and *Bouteloua curtipendula* plants in the field (dark gray boxplots) and greenhouse (light gray boxplots) experiments. Field experiments were conducted at Western Maryland Research and Education Center and at the University of Cincinnati Center for Field Studies. Boxplots for field data represent results combined across both sites. Median and first and third quartiles are shown, whiskers indicate minima and maxima, and dots show outliers. Asterisks indicate significant differences within exotic and native plants under field and greenhouse conditions (χ^2 test: $P < 0.05$).

Table 2 Mean (\pm SE) values of food consumption and food assimilation of *Melanoplus femurrubrum* grasshoppers on leaves clipped from native and exotic plants

Experiment ¹	Parameter ²	Plants ³		P
		Native	Exotic	
Choice	Relative consumption rate (g g ⁻¹ h ⁻¹)	0.018 \pm 0.007	0.019 \pm 0.005	0.75
	Consumption index	0.018 \pm 0.007	0.034 \pm 0.006	0.09
No-choice	Assimilation rate (g g ⁻¹ h ⁻¹)	0.018 \pm 0.001	0.009 \pm 0.001	0.10
	The approximate digestibility (g g ⁻¹)	0.87 \pm 0.005	0.567 \pm 0.039	0.029

¹Feeding experiments with clipped leaf portions were conducted at the University of Cincinnati under laboratory conditions.

²All measurements were conducted for grasshoppers which made at least one grazed mark ('scar') on leaves; undamaged leaves were excluded from analysis of these variables.

³Native plants included *Andropogon gerardii* and *Bouteloua curtipendula*; exotic plants included *Miscanthus sinensis* and *Bothriochloa ischaemum*.

No-choice feeding experiment with clipped leaves

We also did not observe any differences in assimilation rate of grasshoppers on native and exotic plants (Table 2, Appendix 3). The approximate digestibility, however, was greater on native plants than on exotics (Table 2, Appendix 3). In addition, there were no differences in leaf damage among the plant species.

Molecular confirmation of diet

DNA sequences for 500 bp fragments of the non-coding region of the chloroplast *trnL* (UAA) gene were obtained from plants ingested by male grasshoppers (24 for UCCFS, 19 for WMREC) and from reference plants collected at the study sites (10 for UCCFS, 20 for WMREC) (Table 3). Plants sampled at the UCCFS consisted of one native and 10 exotic species, whereas seven native and 19 exotic species were identified at the WMREC (Table 3). Grasses comprised 28.6 and 50% of all plant species collected at the WMREC and the UCCFS, respectively. Seven plants which were found in grasshopper gut contents were not present in field surveys at the local study site (one at the UCCFS, six at the WMREC).

The analysis of the prevalence of exotic and native plants in grasshopper guts demonstrated more exotic plant species for grasshoppers collected from both study sites (binomial test; UCCFS: $P < 0.0001$; WMREC: $P = 0.0003$). The difference between the observed proportion of native and exotic plant species within grasshopper gut contents vs. the proportion in the field was not significant for both sites (UCCFS: $\chi^2 = 0$, d.f. = 1, $P = 1$; WMREC: $\chi^2 = 0.59$, d.f. = 1, $P = 0.44$). Among all ingested plants at the UCCFS, the exotic *Cichorium intybus* L. (Asteraceae) was the most common; DNA of this species was found in the gut contents of 37.50% of all dissected grasshoppers at that site (Table 3). At the WMREC, most of the collected grasshoppers (52.6%) ingested the exotic *Hordeum vulgare* L. (Poaceae) (Table 3). Ingested grasses (all exotics except one species) were found in 25 and 63.2% of all dissected grasshoppers collected at the UCCFS and the WMREC, respectively.

Discussion

Our expectations of lack of avoidance of exotic plants by this grasshopper species were confirmed in all feeding experiments: grasshoppers consumed exotic plants in the field and in the greenhouse experiments with intact plants, as well as in the experiments with clipped leaves. We also detected the presence of exotic plant DNA in gut contents of grasshoppers collected in the field. Our hypothesis of greater food consumption of exotic grasses compared to native grasses was supported overall in the field

experiments with intact plants (both, in Ohio and Maryland) and in the greenhouse experiments with potted plants. Plant DNA detection in grasshopper gut contents also revealed greater prevalence of exotic plant species compared to native plant species. However, our hypothesis was not supported in the laboratory feeding trials with clipped leaves: grasshopper food consumption and food assimilation did not differ on the leaves clipped from native vs. exotic grasses. We suggest the following main explanations of our results: (1) lack of coevolutionary history between exotic *B. ischaemum* and *M. sinensis* grasses and native *M. femurrubrum* grasshoppers, (2) physiological similarity among those two exotic grasses and native *A. gerardii* and *B. curtipendula*, and (3) abundance and accessibility of exotic plants for grasshopper feeding on the field sites.

Given that *M. femurrubrum* does not occur in the native range of *B. ischaemum* and *M. sinensis*, a lack of coevolutionary history in this plant-insect system, as suggested by the biotic resistance hypothesis, might have resulted in lower levels of defense of exotic plants (primarily resistance) to this novel herbivore compared to native plants (Parker et al., 2006). In our study, we used native and exotic grasses that exhibited similar morphological and physiological traits. Consequently, differences in physical defense between these grasses were minimal and should not have played a major role in attractiveness or deterrence of *M. femurrubrum* grasshoppers. In the current study, we used adult grasshoppers only, whose feeding could be much less affected by plant physical traits (e.g., hairy leaf surfaces) compared to nymphs (Chapman, 1974). Consequently, chemical defenses more likely affected the palatability of plants for these adult grasshoppers. It has been demonstrated that although gustation in grasshoppers influences their food choice and regulates feeding, actual food ingestion depends on the balance between food phagostimulants and deterrents (Medeiros et al., 2008). Grasses, however, in general, demonstrate a relatively low level of chemical defenses; in the case of the native *Andropogon* and *Bouteloua* grasses examined in this study, neither the strong deterrents nor phagostimulants have been demonstrated (Mole & Joern, 1994). Considering the relatedness and physiological similarity of these native grasses to exotic *Miscanthus* and *Bothriochloa* grasses, we might also expect that the lack of the strong deterrents in exotic grasses would make them more palatable to grasshoppers. In addition, due to a lack of coevolution of *M. femurrubrum* grasshoppers with the exotic grasses used in this study, any deterrents that might still be present in exotic grasses (Bernays et al., 1974; Joern, 1979) might not be as effective against novel grasshoppers as those that may occur in native plants, although further investigation is necessary.

Table 3 Food consumption of adult *Melanoplus femurrubrum* grasshoppers based on DNA sequences of ingested plants based on their gut contents

Site	Plant species	Origin ¹	Presence at study plot ²	Grasshopper feeding choice ^{3,4}	
				No.	%
UCCFS	<i>Alopecurus pratensis</i> L.	Exotic	+	4	16.67
	<i>Cichorium intybus</i> L.	Exotic	+	9	37.50
	<i>Lolium multiflorum</i> Lam.	Exotic	+	2	8.33
	<i>Plantago lanceolata</i> L.	Exotic	+	5	20.83
	<i>Pyrus pyrifolia</i> (Burm. f.) Nakai	Exotic	+	—	—
	<i>Setaria sphacelata</i> (Schumach.) Stapf & C.E. Hubb. ex M.B. Moss	Exotic	+	—	—
	<i>Setaria viridis</i> (L.) P. Beauv.	Exotic	+	—	—
	<i>Sorghum bicolor</i> (L.) Moench	Exotic	+	—	—
	<i>Stellaria media</i> (L.) Vill.	Exotic	—	2	8.33
WMREC	<i>Symphytotrichum novi-belgii</i> (L.) G.L. Nesom	Native	+	2	8.33
	<i>Trifolium repens</i> L.	Exotic	+	—	—
	<i>Allium ampeloprasum</i> L.	Exotic	+	—	—
	<i>Amaranthus spinosus</i> L.	Native	+	—	—
	<i>Arctium lappa</i> L.	Exotic	+	—	—
	<i>Bromus arvensis</i> L.	Exotic	—	1	5.26
	<i>Conyza sumatrensis</i> (Retz.) E. Walker	Native	+	—	—
	<i>Erigeron annuus</i> (L.) Pers.	Native	+	—	—
	<i>Glycine max</i> (L.) Merr.	Exotic	—	1	5.26
	<i>Hordeum vulgare</i> L.	Exotic	+	10	52.63
	<i>Lamium amplexicaule</i> L.	Exotic	+	—	—
	<i>Lobelia kalmii</i> L.	Native	+	—	—
	<i>Morus rubra</i> L.	Native	+	—	—
	<i>Oxalis corniculata</i> L.	Exotic	+	1	5.26
	<i>Panicum dichotomiflorum</i> Michx	Native	+	—	—
	<i>Physalis heterophylla</i> Nees	Native	+	—	—
	<i>Poa pratensis</i> L.	Exotic	—	1	5.26
	<i>Rhamnus davurica</i> Pall.	Exotic	—	3	15.79
	<i>Setaria viridis</i> (L.) P. Beauv.	Exotic	+	—	—
	<i>Sorghum bicolor</i> (L.) Moench	Exotic	+	—	—
<i>Veronica arvensis</i> L.	Exotic	—	1	5.26	
<i>Veronica persica</i> Poir.	Exotic	—	1	5.26	

¹The origin of reference plants (native or exotic) was determined using the PLANTS Database (<http://plants.usda.gov>).

²Presence of plants on the site is denoted as '+'. Absence of plants on the site, as well as plants whose DNA was not detected in grasshopper gut contents, is denoted as '—'.

³Grasshopper feeding choices are displayed in terms of the number of grasshoppers ('No.') and the percentage of grasshoppers consumed a particular plant.

⁴Only grasshopper gut contents with only a single plant species DNA were considered for this analysis. Grasshoppers with mixed plant DNA (11% of all collected grasshoppers) were excluded from the analysis, as the analysis of mixed DNA would require additional molecular techniques, such as cloning (Garcia-Robledo et al., 2013), or computational analysis of mixed sequencing chromatograms (Chang et al., 2012), which was beyond the scope of this study.

Our observation of greater food consumption of grasshoppers on exotic plants in the field enclosures and in the greenhouse was consistent with the results of some previous studies which used similar experimental designs (Lankau et al., 2004; Fielding & Conn, 2011; Fan et al., 2013). In terms of the comparison of grasshopper diet preferences

under experimental and natural conditions, *M. angustipennis* grasshoppers in the study conducted by Lankau et al. (2004) preferentially fed on exotic *Sapium* seedlings in feeding experiments, whereas natural grasshopper herbivory on introduced *Sapium* was low. Lankau et al. (2004) suggested this resulted from behavioral constraints of grasshoppers

toward novel (and potentially toxic) food under natural feeding conditions and the grasshoppers' inability to recognize this suitable food source. In contrast to this prediction, we did not observe behavioral avoidance of exotic plants by *M. femurrubrum* under natural feeding conditions based on the results from our molecular confirmation of grasshopper food choice. Our study grasses were not present on the collection sites, although they are present within Ohio and Maryland. Given that *M. femurrubrum* is a common species in grasslands where closely related native and non-native plants, including our study species, are abundant (Han et al., 2008; Whipple et al., 2009), we suggest that these grasshoppers do not avoid feeding on these exotic grasses under natural conditions. We did observe, however, a higher food consumption and feeding activity of *M. femurrubrum* on exotic plants in the greenhouse, compared to the field experiments; this can be explained by more favorable environmental conditions (especially temperature) for grasshopper feeding in the greenhouse experiment. This suggests that grasshopper preferences for favorable host plants (in this case, these two exotic grasses) may become even stronger under more favorable feeding conditions. Together with increased grasshopper activity in areas of favorable host plants (Mulkern, 1967), this may result in increased leaf damage of exotic plant species in grasslands. Further studies on natural grasshopper herbivory on *Bothriochloa* and *Miscanthus* individual plants in grasslands would be helpful.

Differences in our results between the experiments with intact plants and the experiments with clipped leaves can be explained by manipulations such as cutting, which might cause release of secondary chemicals from the cut portion of the plant and thereby affect plant attractiveness and accessibility for herbivores (Mulkern, 1967; Motheral & Orrock, 2010). This might have resulted in similar grasshopper food consumption on native and exotic grasses and even greater approximate digestibility of native plants; this was observed in the no-choice feeding experiment. Our results contrast with the findings of Whipple et al. (2009) who demonstrated that *Arphia xanthoptera* (Burmeister) and *Dichromorpha viridis* (Scudder) grasshoppers also consumed more clipped leaves of exotic grasses. Whipple et al. (2009) used native warm-season (*A. gerardii* and *B. curtipendula*) and exotic cool-season grasses (*P. pratensis* and *B. inermis*). The authors indicated that phylogenetic and physiological difference between those grasses might affect grasshopper food choice more than plant origin (native or exotic). For this reason, we intentionally used warm-season grasses only which were morphologically and physiologically very similar; such grasses, even if they are not congeners as suggested by Darwin's naturalization hypothesis, are still presumably similarly accessible for generalist grasshoppers, which

apparently do not need to adapt to feed on these grasses, even if it is a novel food plant. Further exploration of grasshopper consumption of cutting leaf portions and digestibility of native and exotic plants with presumably different levels of plant resistance would allow us to better understand how clipping of leaves affects their digestibility in insects; specifically, a molecular approach to this question would be effective (Barkhordar et al., 2013).

The differences in grasshopper feeding on intact plants in light of the similarity in size and mass of the clipped portions of leaves may also suggest that grasshopper preferences toward exotic plants may be influenced by factors, other than physical and chemical plant traits, such as plant abundance and particularly plant biomass (Mulkern, 1967; Bernays & Chapman, 1994; Motheral & Orrock, 2010). It has been demonstrated that grasshoppers often choose the most abundant host plant in the area, even when other plants were more acceptable for feeding; as a result, the more frequently ingested plants may not necessarily be the most preferred for grasshoppers (Mulkern, 1967; Chapman, 1974; Boys, 1981; Bernays & Chapman, 1994). For example, *M. femurrubrum* frequency of ingestion of exotic *P. pratensis* was proportional to its abundance (Mulkern, 1967). In addition to the lack of coevolutionary history of native *M. femurrubrum* grasshopper and exotic plants, and physiological similarity of the study plant species, the grasshopper gut content analysis suggests that the abundance of exotic plants at the study sites (more than 50% of all collected reference plants) might affect grasshopper food choice. We found that the proportion of ingested native and exotic plant species within grasshopper gut contents was similar to the proportion of native and exotic plant species in the field. However, we only estimated the number of native and exotic plant species; further investigations are needed to explore the effect of plant coverage in the field on the proportions of ingested plants within grasshopper gut contents.

In conclusion, lack of avoidance of exotic plants and feeding preference of *M. femurrubrum* grasshoppers toward exotic plants, especially under natural field conditions, suggest that these grasshoppers potentially limit spread of exotic *Bothriochloa* and *Miscanthus*, should these grasses escape cultivation and become invasive. Given that these grasshopper species are among common species in tallgrass prairie, we believe that these generalist insect herbivores would be less likely affected by invasion of these two exotic grasses. This is also important for predicting how numerous trophic interactions including native *M. femurrubrum* grasshoppers and native grasses would be impacted by invasion by exotic *Bothriochloa* and *Miscanthus* (Harvey & Fortuna, 2012). Considering that *M. femurrubrum* is also one of the important agricultural pests, our study can also be extended to explore feeding

preferences of this grasshopper species on crop plants compared to grasses growing at field edges to develop more effective means to suppress crop damage.

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Appendix 1

General linear model parameter estimates of leaf tissue consumption and feeding activity of *Melanoplus femurrubrum* grasshoppers on intact plants in relation to location of the experiment ('Site') and plant origin ('Plant type')

Experiment ¹	Parameter	Fixed effects ^{2,3}	Estimate	SE	t	Pr(> t)
Field	Total volume of the grazed portion	(Intercept)	0.11654	0.01558	7.481	7.76e-09
		Plant type	−0.07531	0.02203	−3.418	0.0016
		Site	−0.02318	0.02203	−1.052	0.30
	Feeding rate	Plant type:site	0.06811	0.03116	2.186	0.035
		(intercept)	0.08657	0.01230	7.036	1.8e-07
		Plant type	−0.04799	0.01846	−2.600	0.015
		Site	0.01387	0.02009	0.690	0.50
Greenhouse	Total volume of the grazed portion	Plant type:site	0.03259	0.02907	1.121	0.27
		(Intercept)	0.20614	0.02689	7.666	4.48e-07
		Plant type	−0.10784	0.03803	−2.836	0.011
	Feeding rate	(Intercept)	0.19991	0.02543	7.862	1.68e-06
		Plant type	−0.08009	0.03844	−2.083	0.056

¹Two sites were utilized for the field experiments: Western Maryland Research and Education Center and University of Cincinnati Center for Field Studies. Greenhouse experiments were conducted at the University of Cincinnati greenhouse.

²Native plants included *Andropogon gerardii* and *Bouteloua curtipendula*; exotic plants included *Miscanthus sinensis* and *Bothriochloa ischaemum*.

³Plants of two types were used in the experiments with intact plants: native and exotic plants.

Appendix 2Mean (\pm SE) values of leaf tissue consumption and feeding activity of *Melanoplus femurrubrum* grasshoppers on native and exotic plant species

Site ¹	Parameter	Plants ^{3,4}			
		Native		Exotic	
		<i>Andropogon gerardii</i>	<i>Bouteloua curtipendula</i>	<i>Miscanthus sinensis</i>	<i>Bothriochloa ischaemum</i>
WMREC	Total volume of the grazed portion (cm ³)	0.005 \pm 0.002	0.001 \pm 0.000*	0.005 \pm 0.002	0.026 \pm 0.006
	Feeding rate ² (cm ³ g ⁻¹ per day)	0.003 \pm 0.001	0.0005 \pm 0.000*	0.003 \pm 0.001	0.014 \pm 0.003
UCCFS	Total volume of the grazed portion (cm ³)	0.019 \pm 0.006	0.000 \pm 0.000*	0.024 \pm 0.009	0.002 \pm 0.001
	Feeding rate (cm ³ g ⁻¹ per day)	0.016 \pm 0.007	0.000 \pm 0.000	0.022 \pm 0.013	0.002 \pm 0.001
UC greenhouse	Total volume of the grazed portion (cm ³)	0.017 \pm 0.007	0.009 \pm 0.005	0.069 \pm 0.029	0.035 \pm 0.017
	Feeding rate (cm ³ g ⁻¹ per day)	0.014 \pm 0.006	0.010 \pm 0.006	0.058 \pm 0.024	0.039 \pm 0.020

¹Feeding experiments with intact plants were conducted in the field at Western Maryland Research and Education Center (WMREC) and at the University of Cincinnati Center for Field Studies (UCCFS), as well as at the University of Cincinnati (UC) greenhouse.

²Feeding rate was estimated for plants with at least one grazed mark ('scar'); undamaged *B. curtipendula* plants at the UCCFS were excluded from analysis of this variable.

³Native plants included *A. gerardii* and *B. curtipendula*; exotic plants included *M. sinensis* and *B. ischaemum*.

⁴Asterisks indicate significant differences at the adjusted significance of 0.008 in pairwise comparisons between plant species.

Appendix 3General linear model parameter estimates of food consumption and food assimilation of *Melanoplus femurrubrum* grasshoppers on clipped leaves in relation to a type of experiment ('Exp') and plant origin ('Plant type')

Parameter	Fixed effect ^{1,2}	Estimate	SE	t	Pr(> t)
Relative consumption rate	(Intercept)	0.134632	0.020013	6.727	1.18e-06
	Plant type	-0.009474	0.029684	-0.319	0.75
	Exp	-0.016378	0.028303	-0.579	0.57
	Plant type:exp	-0.018502	0.039775	-0.465	0.65
Consumption index	(Intercept)	0.180679	0.024428	7.397	2.83e-07
	Plant type	-0.064249	0.036232	-1.773	0.091
	Exp	0.005276	0.034546	0.153	0.88
	Plant type:exp	0.032351	0.048549	0.666	0.51
Assimilation rate	(Intercept)	0.03162	0.01298	2.436	0.029
	Plant type	0.03139	0.01836	1.710	0.11
Approximate digestibility	(Intercept)	0.56704	0.08826	6.424	1.59e-05
	Plant type	0.30328	0.12482	2.430	0.029

¹Experiments of two types (choice and no-choice) were conducted at the University of Cincinnati greenhouse.

²Native plants included *Andropogon gerardii* and *Bouteloua curtipendula*; exotic plants included *Miscanthus sinensis* and *Bothriochloa ischaemum*.