Blue Grama Grass Genotype Affects Palatability and Preference by Semi-arid Steppe Grasshoppers

Ava M. Hoffman  
*Colorado State University*

Holly Perretta  
*Colorado State University*

Nathan P. LeMoine  
*Marquette University*, nathan.lemoine@marquette.edu

Melinda D. Smith  
*Colorado State University*

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Blue Grama Grass Genotype Affects Palatability and Preference by Semi-arid Steppe Grasshoppers

Ava M. Hoffman
Colorado State University Department of Biology
Colorado State University Graduate Degree Program in Ecology
Holly Perretta
Colorado State University Department of Biology
Nathan P. Lemoine
Colorado State University Department of Biology
Colorado State University Graduate Degree Program in Ecology
Melinda D. Smith
Marquette University, Department of Biological Sciences
Colorado State University Department of Biology
Colorado State University Graduate Degree Program in Ecology
Highlights

- Plant palatability and preference by grasshopper herbivores differs between cultivar and wild type blue grama grass.
- Cultivar plants were larger and had lower water content, but silica content did not differ.
- Surprisingly, cultivar plants had reduced nutritive value (higher C:N) which indicates other drivers for palatability and preference.

Abstract

The semi-arid shortgrass steppe ecosystem of North America is dominated by blue grama grass (*Bouteloua gracilis*), a species with substantial intraspecific variability, ecological significance, and economic value. Yet no studies have addressed within species differences in blue grama palatability or insect herbivore preference with respect to plant traits. We performed an experimental study to test the palatability and preference of two blue grama genotypes, wild type versus cultivar, by grasshopper herbivores in the Gomphocerinae subfamily. We found strong evidence that cultivar blue grama was more palatable than wild type and that grasshoppers preferred cultivar plants. Although we could not detect differences in silica content between the two types, we found that cultivar plants were larger, had lower water content, and surprisingly, had reduced nutrient value (greater C:N). These results suggest that intraspecific variation in blue grama size and water content could influence feeding choices by this group of grasshoppers. Conservation managers will have to consider such variation when considering how remnant and restored prairies might be affected by these arthropod herbivores.

Keywords

Dominant species, Shortgrass steppe, Herbivory, Genetic diversity, Cultivar, Accession

1. Introduction

Biodiversity enhances ecosystem function across many biomes (Cardinale et al., 2006). The additional dimension of intraspecific diversity among genotypes can also contribute substantially to greater ecosystem productivity, stability, and trophic diversity (Crutsinger et al., 2006; Koricheva and Hayes, 2018). In some cases, plant intraspecific diversity can lead to more diverse arthropod communities because arthropods have preferences for host genotypes (Ballhorn et al., 2011; Kotowska et al., 2010). Among plants, dominant, abundant, or foundational plant species and their genetic and functional diversity might play an important role in arthropod herbivory compared to rare species by providing proportionally more food and shelter (Bernhardsson et al., 2013; Busby et al., 2015; Robinson et al., 2012; Tomas et al., 2011; Whitham et al., 2006). However, dominant species are not equally studied, leaving gaps in understanding of how arthropod herbivory varies among plant genotypes. In particular, little is known about how grass intraspecific diversity and herbivory are related.

Functional differences among genotypes of dominant grasses may lead to different plant palatability and arthropod preference. While prairie grasses are considered to have lower chemical defenses (Whiles and Charlton, 2006), other factors like plant size, water content, nutritional value, or silica-based defenses could play an important role (Heidorn and Joern, 1984). Specifically, greater plant
palatability and preference could stem from selective intake of larger plants (Heidorn and Joern, 1984) due to their lower investment in defense (Carmona et al., 2011; Herms and Mattson, 1992) or greater accessibility despite defenses (Strauss et al., 2015). Plants with greater water content may be attractive to herbivores from dry environments (Scriber, 1978), but grasshoppers may also prefer wilted plants (Lewis, 1982, 1979) or have no preference for water content (Pérez-Harguindeguy et al., 2003). Greater plant palatability is sometimes found in more nutritious leaves with lower C:N ratios and/or high N content (Pérez-Harguindeguy et al., 2003). Herbivores might also balance nutritive quality against grass defenses, such as silica content (Hartley and DeGabriel, 2016; While and Charlton, 2006), although increased silica content may actually increase consumption rates (Nabity et al., 2012). All of these functional traits are known to vary among grass genotypes (Aspinwall et al., 2013; Soininen et al., 2013) and could therefore influence plant palatability or herbivore preference.

Among functionally distinct plant genotypes, considerable variation for palatability can be found between wild and cultivated plant varieties. For example, maize cultivars and wild relatives differed strongly in volatile defenses against arthropod herbivores (Gouinguené et al., 2001). Although native herbivores might sometimes prefer a wild type plant food source with which they have closely coevolved (Branson and Sword, 2009), cultivated plants can also experience relaxed selection for herbivore defenses, as demonstrated by a growth and defense tradeoff in maize (Rosenthal and Dirzo, 1997). Thus, we might expect substantial differences in grass palatability and preference by arthropod herbivores when comparing cultivated and wild types.

In this study, we tested how intraspecific diversity of blue grama grass, the dominant grass species of the shortgrass steppe ecoregion, would affect plant palatability and preference by grasshopper herbivores. We used two phenotypically distinct genotypes, one wild type genotype and one cultivar genotype, to assess plant palatability and preference by the Gomphocerinae grasshopper subfamily. We predicted that intraspecific diversity would lead to differences in plant palatability and preference between our two genotypes, with the preferred genotype presenting greater size/mass, water content, lower C:N, and lower silica content.

2. Materials and methods

2.1. Study system

Blue grama (Bouteloua gracilis) is the dominant grass species of the shortgrass steppe, comprising up to 90% of cover and annual production in undisturbed prairie (Lauenroth and Sala, 1992; Milchunas et al., 1989). This species is widely considered grazing and herbivore tolerant (Augustine et al., 2010; Lauenroth and Sala, 1992; Milchunas et al., 1998) across many genotypes (Kotanen and Bergelson, 2000). It can be found throughout much of North America outside of its areas of dominance (eastern Colorado and New Mexico, Lauenroth and Burke, 2008), with substantial genetic and phenotypic diversity across the species (Aguado-Santacruz et al., 2004; Butterfield and Wood, 2015; McMillan, 1959; Medina-Roldán et al., 2013; Phan et al., 2003; Smith et al., 2009). Wild type and cultivar blue grama also differ substantially in traits like seed yield, which may be under selection for landscaping or prairie restoration purposes (Carr and Rea, 2014; Phan et al., 2003).

We compared a wild type blue grama genotype from the Central Plains Experimental Range (CPER) in Northeastern Colorado (40.83583, −104.7631) to the commercial blue grama cultivar “Blonde
Ambition” due to differences between wild type and commercial genotypes. For wild type blue grama, we extracted a dormant clone on 5 April 2016 with roots intact, placed the clone in ProMix potting media in a 3.8 L pot, and watered to prevent drying; shoots emerged in approximately three months. Cultivar blue grama “Blonde Ambition” was obtained in early June from Harmony Gardens, Inc. in Fort Collins, CO, USA in a similar pot and similar, nutrient abundant media. Each individual clone was approximately 6 cm² and plants were at a similar phenological stage (onset of flowering) once assays began. The many individual tillers found within these clones were amenable to separation into different assays. Individual blue grama tillers tended to be approximately 20 cm tall with leaves up to 4 mm wide. Plants were watered to pot capacity until use in the experimental assays.

2.2. Mesocosm assays
We performed mesocosm assays to determine if plant palatability and preference varied between wild type and cultivar blue grama. Both palatability and preference assays consisted of randomly selected individual tillers (with roots intact) placed in a 15 mL conical tube filled with water and sealed with cotton. Mesocosms (30 L) were filled to the top of the plant conical tubes with 50/50 ProMix (Premier Tech Ltd, Rivière-du-Loup, QC, Canada) and ‘Greens Grade’ potting media (Profile Products, Buffalo Grove, IL, USA) to simulate the consistency of shortgrass steppe soils. Four tillers were placed in each mesocosm (Fig. S1), with palatability assays consisting of all one type with no choice (either cultivar or wild type) and preference assays consisting of a choice between two cultivar and two wild type tillers. In this study, we define palatability as the mass of plant tissue consumed in a no-choice environment. Preference is the difference between cultivar and wild type mass consumed in a choice environment.

We used grasshoppers from the subfamily Gomphocerinae, the dominant group at CPER (Lauenroth and Burke, 2008; Welch and Kondratieff, 1991) as the arthropod herbivores in this study. In dry prairies, this subfamily feeds primarily on blue grama and other grasses (Joern, 1983); *Psoloessa delicatula* comprises over half of all grasshoppers at the CPER, but three other species (*Eritettix simplex*, *Philibostroma quadrimaculatum*, and *Opeia obscura*) are also common (Welch and Kondratieff, 1991). While lack of identification of this grasshopper subfamily to species and developmental stage is a major caveat of this study, our primary goal was to focus broadly on herbivory by this subfamily. Grasshoppers were obtained immediately prior to starting each assay via net sweeps on linear transects at CPER; grasshoppers in the appropriate subfamily were then selected randomly and starved for approximately 24 h. Because grasshopper size is likely to reflect the amount of plant matter consumed (Brown et al., 2004), grasshopper mass was measured before random placement in each mesocosm to account for mesocosm or grasshopper effect. Average grasshopper mass was 100.6 mg. Mesocosms were sealed with window screen mesh. During all assays, one grasshopper was added per mesocosm, where it was allowed to feed for between 63 and 88 h. Time did not strongly affect the mass of plant tissues consumed and was excluded from further analyses (linear regression, $t = -0.812$, adjusted $r^2 = -0.0088$, $p = 0.4221$, $n = 40$). Each palatability mesocosm (5 cultivar, 5 wild type, 10 total grasshoppers) consisted of four tillers of the same type ($n = 40$ total tillers or data points). Preference mesocosms (5, 5 grasshoppers) were similar but consisted of two cultivar tillers and two wild type tillers ($n = 20$ total tillers or data points).
2.3. Plant measurements

Because potted plants were not fully saturated, assay plants required an acclimation period to achieve a consistent mass. We measured masses of wild type and cultivar plants (n = 5 each) every 30 min after being submerged in water to determine if mass had changed significantly. We then tested whether changes between two different time points were significantly different, where a lack of difference indicated that mass and thus plant water content had stabilized. The water mass gained between 90 min and 120 min versus the mass gained between 120 min and 150 min was not significantly different for either cultivar (paired t-test, p = 0.7158, n = 5) or wild type (paired t-test, p = 0.4239, n = 5) (Fig. S2), so plants were acclimated by soaking roots in water for at least 2 h before being weighed for initial mass and starting the assays. All assays took place between 14 and 22 July 2016. Temperature was approximately 34 °C during the day and 20 °C at night. All mesocosms experienced a 16 h photoperiod.

Starting and final plant tissue mass was weighed to determine grasshopper consumption following the assays. Because grasshopper damage can lead to drying of remaining tissue (i.e., leaves falling off), we dried all remaining plant tissues at 62 °C for 48 h prior to weighing. Next, we used scaling equations to back-calculate an initial dry weight from fresh weight for comparison to final dry weight (TEXT S1). For the cultivar, we weighed tillers both fully saturated and dried to obtain the equation (linear regression, t = 20.39, r² = 0.945, p=<0.001, slope = 2.664, n = 25). We repeated this process for wild type (linear regression, t = 18.79, r² = 0.936, p=<0.001, slope = 3.211, n = 25). We calculated the mass consumed as the difference between the calculated starting dry weight of each tiller compared to the measured final dry weight. All fresh weights consisted of leaves and roots cleaned to be free of soil and patted dry.

We quantified plant traits including mass, dry matter content (DMC, inversely related to water content), aboveground C:N, and aboveground silica content to determine if these traits differed between cultivar and wild type blue grama, and ultimately, could link to differences in palatability and preference. For mass and DMC, we used the same individuals measured for the scaling equations above (n = 25). Leaf tissue was pooled uniformly within type for elemental analysis. For C:N ratio quantification, tissue was ground to a fine powder using a mortar and pestle and run through a LECO Tru-SPEC elemental analyzer (LECO Corporation, Saint Joseph, MI, USA) at the EcoCore facility at Colorado State University (n = 10 for each cultivar and wild type). Silica analysis was performed using Inductively Couple Plasma-Atomic Emission Spectroscopy at the Soil, Water, and Plant Testing Laboratory at Colorado State University (n = 6 for cultivar, n = 4 for wild type).

2.4. Statistical analyses

We used different modeling approaches in this study to account for different data structure. Specifically, we used Bayesian linear regression models to account for hierarchical structure in observations of palatability and preference. Bayesian approaches tend to be more appropriate for such data (Gelman, 2006; Kruschke and Vanpaemel, 2015). However, plant mass, DMC, nutrient composition, and silica content data was only observed within wild type and cultivar groups, rendering a Bayesian approach unnecessary.

We used a varying intercept and slope model for preference mesocosms,
Where $i$ is each observation within $j$ type (cultivar or wild type), $y$ is the mass consumed, $g$ is the grasshopper weight, and $m$ is the starting fresh mass of the tiller. Variance in mass consumed was allowed to vary within the model across the two types. In preference mesocosms, the mass consumed was calculated using the same model as above. To calculate preference, we used the same varying intercept and slope model but also calculated a derived parameter ‘difference’ by subtracting the wild type mass difference distribution from the cultivar mass difference distribution (i.e., differenced the posterior distributions). Vague priors were used for all parameters. All models were run simultaneously with four chains using Stan (Gelman et al., 2015), with 50,000 No U-Turn Sampler iterations discarded as warmup and 50,000 iterations retained. All models were compiled within R (R Core Team, 2018). Rhat values ($\sim 1$) and posterior draws within Stan indicated convergence of all parameters (Fig. S3, Fig. S4). Differences between cultivar and wild type traits were determined using Wilcoxon rank sum tests.

3. Results

Our data demonstrate the grasshoppers in this study both preferred cultivar blue grama and found it more palatable. Mass consumed by grasshoppers on wild type blue grama did not differ from zero at the average grasshopper mass and tiller mass (95% credible interval (CrI): 10.29, 10.68, Table 1) whereas cultivar mass consumed was greater than zero (CrI: 12.85, 26.57, Table 1, Fig. 1a). When we differenced the two distributions, there was strong evidence for a difference between mass consumed for cultivar and wild type in palatability assays (CrI: 7.04, 31.95). In other words, between 7.0 and 32.0 mg more tissue was consumed from cultivar plants compared to wild type in these assays. We found evidence that larger grasshoppers consumed more plant material, but only for cultivar plants ($\beta_1$ CrI: 0.012,0.48 for cultivar and −0.51,0.47 for wild type, Table 1). Fresh tiller mass did not affect palatability ($\beta_2$ CrI: 0.012,0.12 for cultivar and −0.059,0.069 for wild type, Table 1).

Table 1. Parameter results from palatability and preference models. Parameter $\beta_1$ represents the slope for mesocosm grasshopper mass. Parameter $\beta_2$ represents the slope for fresh tiller mass. The posterior represents the mass consumed (or the difference in mass consumed) at the average grasshopper mass and fresh tiller mass. All numbers represent the 95% credible interval (CrI).

<table>
<thead>
<tr>
<th>Model</th>
<th>$\beta_1$</th>
<th>$\beta_2$</th>
<th>Posterior</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>palatability (cultivar)</td>
<td>(0.012,0.479)</td>
<td>(-0.012,0.12)</td>
<td>(12.85,26.57)</td>
<td>(10.35,20.22)</td>
</tr>
<tr>
<td>palatability (wild type)</td>
<td>(-0.51,0.47)</td>
<td>(-0.059,0.069)</td>
<td>(-10.29,10.68)</td>
<td>(16.37,31.75)</td>
</tr>
<tr>
<td>preference (cultivar)</td>
<td>(-0.02,0.17)</td>
<td>(-0.063,0.0049)</td>
<td>(13.71,25.09)</td>
<td>(4.85,14.89)</td>
</tr>
<tr>
<td>preference (wild type)</td>
<td>(-0.055,0.14)</td>
<td>(-0.028,0.042)</td>
<td>(1.79,13.14)</td>
<td>(4.94,14.50)</td>
</tr>
<tr>
<td>preference (difference)</td>
<td></td>
<td></td>
<td>(4.02,20.04)</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 1. Plant palatability and preference by grasshoppers varied within blue grama. (a) Posterior 95% credible intervals for palatability of cultivar and wild type blue grama provide evidence that cultivar is more palatable. Distributions shown are calculated at the average grasshopper mass and fresh tiller mass. (b) Posterior 95% credible interval (CrI) for preference of blue grama type at average grasshopper mass and fresh tiller mass. A non-zero positive preference indicates that cultivar was preferred. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Within the preference assays, there was evidence for overall grasshopper preference different from zero (CrI: 4.02, 20.04, Table 1, Fig. 1b). In this case, the positive value indicated that cultivar is preferred. Unlike palatability assays, we did not find evidence that larger grasshoppers consumed more plant material ($\beta_1$ CrI: 0.02, 0.17 for cultivar and $-0.055$, 0.14 for wild type, Table 1). Like plant palatability, fresh tiller mass did not affect preference ($\beta_2$ CrI: 0.063, 0.0049 for cultivar and $-0.028$, 0.042 for wild type, Table 1).

When we performed trait analysis between cultivar and wild type blue grama, we found intraspecific differences in dry mass, DMC, C:N ratio, but not silica content. Analysis of dry mass showed that cultivar blue grama tended to be larger (Wilcoxon rank sum test, $p = 0.0003$, $n = 25$) by approximately 69–162 mg (Fig. 2a). We found that DMC was also greater in cultivar plants (Wilcoxon rank sum test, $p = 0.0005$, $n = 25$) by 20.6–67.3 mg $g^{-1}$ (Fig. 2b). For nutrient content, we found that cultivar plants had greater C:N ratios (Wilcoxon rank sum test, $p < 0.0001$, $n = 10$), indicating wild type plants contained more nitrogen (Fig. 2c). However, we did not detect differences in silica content (Wilcoxon rank sum test, $p = 0.11$, $n = 4$, Fig. 2d).
Fig. 2. Traits varied between cultivar and wild type blue grama. (a) Cultivar blue grama was heavier than wild type, (b) Cultivar had greater DMC than wild type, (c) C:N ratio was greater in cultivar compared to wild type, and (d) no significant differences were detected for % silica content. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

4. Discussion

In this study, we aimed to quantify intraspecific differences in palatability and preference of cultivar and wild type blue grama grass as well as traits that might be linked with such differences. Cultivar blue grama showed greater palatability compared to its wild type counterpart. Although grasshopper density is often low in the shortgrass steppe, grazing pressure by the Acrididae family can remove over 25% of plant production (Belovsky and Slade, 2018). This could indicate that slightly lower palatability in the wild type blue grama genotype has emerged due to coevolution with herbivores consistent with a growth-defense tradeoff (Rosenthal and Dirzo, 1997). Alternatively, cultivar blue grama could have experienced human-caused selection away from herbivore resistance (Chun et al., 2010; Kempel et al., 2011). However, the genotype-palatability relationship may vary based on the taxonomic specificity of the herbivore and the genotypes in question.

When presented with a food choice, grasshoppers showed preference for cultivar blue grama over wild type. Although such differences have not been determined within similar species, grasshoppers have shown mixed preferences, including preference toward non-native grasses (Avanesyan and Culley,
2015) and in contrast, native species (Branson and Sword, 2009). Indeed, differences in preference can emerge within a single grasshopper genus (Behmer and Joern, 2008; Joern, 1983). Although our results are general with respect to the Gomphocerinae subfamily, species and developmental stage of the grasshoppers used might have had substantial effects on our preference findings. This caveat is especially important with a small dataset (n = 20) where we could be overestimating effect size (Lemoine et al., 2016). Nevertheless, our findings combined with recently improved understanding of blue grama genetic diversity (Butterfield and Wood, 2015; Phan et al., 2003) suggest that intraspecific diversity in this dominant plant species can affect behavior by insect herbivores like grasshoppers and warrants further study.

In our palatability assays, we discovered that larger grasshoppers consumed more cultivar plant mass. This might be unsurprising considering that larger grasshoppers have greater metabolic demands (Brown et al., 2004) and consume more plant mass (Moretti et al., 2013). Yet, larger grasshoppers did not consume more wild type plant mass, which could indicate that larger grasshoppers in this study consumed larger or more wilted plants more efficiently. Our findings might also reflect additional trait differences in cultivar leaves (such as lower toughness, Ibanez et al., 2013) or different traits in larger grasshoppers (such as mandibular strength, Deraison et al., 2015). This effect was not seen in preference assays (although once again, this could be the result of a small sample size).

Traits relating to grass palatability and preference differed among cultivar and wild type blue grama. Although we expected that grasshoppers would prefer and find higher water content plants more palatable, we found that grasshoppers preferred greater DMC associated with lower water content in cultivar tillers. While water content is an important component in a food source (Scriber, 1978), greater water content in blue grama might be associated with lower preference by some dry prairie grasshoppers (Heidorn and Joern, 1984) and could be associated with wilting (Lewis, 1982). Although we accounted for plant mass in the model, cultivar tillers tended to be larger, which could have increased foraging encounters (Strauss et al., 2015). It is important to note that mass and DMC were measured on whole plants (including roots), and although differences in root allocation were not measured, it is possible for root mass to vary among blue grama genotypes (Hoffman et al., unpublished data).

In contrast, our results showed the opposite pattern from the majority of literature suggesting preference of more nutritious, higher nitrogen leaves (Descombes et al., 2017; Pérez-Harguindeguy et al., 2003). Although unlikely that grasshoppers were targeting higher C:N ratios, different leaf C:N is preferred by different grasshopper families (Deraison et al., 2015) and species (Behmer and Joern, 2008), often with few generalizable patterns (Joern and Behmer, 1998). Our results might also be obscured if C:N content in both blue grama types was sufficient to satisfy grasshopper protein demands. It is unclear if specific carbon-based compounds increased our C:N ratio and whether these compounds were attractive to this subfamily. Indeed, Heidorn and Joern (1984) suspected that differences in preference by grasshoppers in the shortgrass steppe might be due to specific sugars, amino acids, or fatty acids. Regardless of preference or palatability and our efforts to control potting media type, soil differences may have produced differences in nutrient content in cultivar and wild type plants. Although silica content can be an important factor in palatability or feeding preferences
(Hartley and DeGabriel, 2016), we did not find any differences in silica content (although this might have been due to small sample size or soil differences, Massey et al., 2006).

Overall, our results suggest that larger size and lower water content in cultivar blue grama might lead to increased palatability and preference by Gomphocerinae grasshoppers. However, additional plant traits not examined here could also be important drivers of food choice, such as presence of specific limiting nutrients (Behmer and Joern, 1993), or traits like specific leaf area and cuticle thickness (Pérez-Harguindeguy et al., 2003). Other factors besides preference (such as insect behavior) (Heidorn and Joern, 1984) and grasshopper mandibular strength (Ibanez et al., 2013) might also influence food selection where blue grama is an option. More comprehensive research is needed linking traits and grasshopper herbivory, both in controlled and field studies using dominant species. This is especially true considering extensive intraspecific diversity in blue grama (Aguado-Santacruz et al., 2004; Butterfield and Wood, 2015; Phan et al., 2003) may lead to different community responses outside the two genotypes examined here. Nevertheless, in the context of land use change and needed restoration in the shortgrass steppe (Allred et al., 2015), our results should encourage conservation managers to consider the effects of blue grama genetic diversity on other trophic levels.

Author contributions
AMH, NPL, and MDS conceived and designed the experiments. HP collected the data. AMH and HP analyzed the data. All authors wrote the manuscript.

Conflicts of interest
The authors declare no competing interests.

Data accessibility

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Appendix A. Supplementary data
Help
The following are the Supplementary data to this article:

Download : Download XML file (725B)

Multimedia component 1.

Download : Download Word document (265KB)

Multimedia component 2.
References


