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Authors

Karban, Richard
Takabayashi, Junji

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Chewing and other cues induce grass spines that protect meristems

Richard Karban¹ · Junji Takabayashi²

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Abstract

Grasses frequently have unidirectional hairs, prickles, and spines; these leaf features have been hypothesized to move herbivores and their chewing damage away from grass meristems, which are located basally. Observations of chewing damage to two grasses, *Andropogon virginicus* and *Phragmites australis*, were consistent with this hypothesis as leaf tips received 10× and 2× more damage than bases. Grasshoppers were no more likely to land on leaf tips than bases although they oriented towards the tips after landing. Leaves of *A. virginicus* that were damaged by chewing herbivores had fewer spines than leaves on the same or neighboring plants that lacked damage. This suggests that herbivores chose less spiny leaves. At a larger spatial scale, plants in neighborhoods favorable for grasshoppers had more spines than plants in less favorable neighborhoods. We found no evidence that marginal spines allowed leaves to shed water more rapidly, a potential alternative benefit. The density of spines on new leaves increased following cues of damage. *A. virginicus* leaves produced after an adjacent leaf had been clipped with scissors had 13% more spines than new leaves on unclipped plants. Clipping with scissors failed to increase spine density for new *P. australis* leaves although experimental chewing by caterpillars led to the production of new leaves with 24% more spines than controls. Unchewed new leaves within 20 cm of a chewed neighbor had 13% more spines than controls. Grasses are capable of responding to cues of tissue damage to their own and neighboring leaves, potentially reducing herbivory to meristems.

Keywords Plant behavior · Defense · Resistance · Hairs · Prickles · Unidirectional · Anisotropic · Herbivores · Grasshoppers · Volatile · Plant communication

Introduction

Plants defend themselves against herbivores, and many of these defenses are inducible, expressed only after the plant has received a cue indicating a high risk of attack (Karban 2015). Because many plants can tolerate the loss of some leaf tissue, herbivore feeding serves as a reliable cue of risk that many plants perceive and respond to (Orrock et al. 2015). Our understanding of plant defenses and the cues that

trigger those defenses has increased over the past decades. However, the defenses of grasses and the cues that they use to perceive risk are less well known compared to those of dicotyledonous plants.

This is unfortunate because grasses (Poaceae) are ecologically and economically very important. They are found in most of the earth's biomes and are the dominant vegetation type in environments with relatively little precipitation and warm temperatures (Whittaker 1975). Grasses are the chief source of food for humans as well as domesticated and wild grazing mammals. Unlike most other plants, which have meristems located at the top of shoots, the growing points of grasses are at the base of the culm, frequently at or below ground level (Metcalfe 1960). This morphology makes grasses relatively more tolerant of leaf damage and removal since leaf tissues can be replaced with little fitness loss (McNaughton 1979; van der Meijden et al. 1988; Moore and Johnson 2017). The best studied defensive trait for this family is a high concentration of silica, which may be induced following damage cues and makes chewing grass

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✉ Richard Karban
rkarban@ucdavis.edu

Junji Takabayashi
junji@ecology.kyoto-u.ac.jp

¹ Department of Entomology and Nematology, University of California, Davis, CA 95616, USA

² Center for Ecological Research, Kyoto University, Hirano 2-509-3, Otsu, Shiga 520-2113, Japan

tissues less rewarding (McNaughton and Tarrant 1983; Hartley and DeGabriel 2016). Grasses also possess other morphological traits that discourage grazing and some species benefit from symbiotic associations with other organisms (e.g., endophytic fungi) that provide defense (Vicari and Bazely 1993; Moore and Johnson 2017).

Grasses frequently have hairs, prickles, and spines along leaf margins and other surfaces that are unidirectional, pointing towards the leaf tips (Metcalf 1960). The terminology for these features varies among different authors (Bell 2008). From a human perspective, these features make it easy to slide your fingers from the base to the tip and difficult to move in the opposite direction. Recently, Vermeij (2015) proposed that unidirectional surface features may direct small herbivores away from valuable meristems and thereby protect grasses from fitness losses associated with herbivory. This hypothesis was generally supported in the one study that tested it; *Avena barbata* and several other grass species in California received more chewing damage at leaf tips than at leaf bases and a small generalist caterpillar was more likely to turn in the same direction as the hairs on several grasses (Karban et al. unpubl.). Under some circumstances, individuals of *A. barbata* with unidirectional hairs produced more seeds than individuals that lacked these hairs, although this result varied over time and space. In general, demonstrating a clear fitness benefit of putative plant defenses has been far more difficult than finding evidence that they are associated with reduced damage (Marquis 1992a; Agrawal et al. 2012).

Spines and prickles along leaf margins may also have other functions and consequences. They may trap or puncture small herbivores (e.g., Eisner et al. 1998) or make walking over leaf surfaces difficult (e.g., Wheeler and Krimmel 2015). Unidirectional marginal spines shed water more quickly and may reduce damage by pathogens (Field et al. 2005, Vermeij 2015). Leaves of many tropical plants are pointed and these ‘drip-tips’ have been found to allow leaves to dry more quickly and support smaller loads of epiphytes and plant pathogens (Dean and Smith 1978; Ivey and De Silva 2001; Meng et al. 2014).

Plant traits will be most valuable as defenses if herbivores recognize those traits and avoid individuals that express them (Karban 2011; Rubin et al. 2015). Furthermore, plant defenses will be most valuable if they protect those tissues that are most closely related to fitness. From the plant’s point of view, the distribution of damage can be more important than the amount of tissue removed (Marquis 1992b; Mauricio et al. 1993; Meyer 1998). For these reasons, Vermeij (2015) argued that studies that examined the behavior of herbivores were required to test his hypothesis about the adaptive value of unidirectional hairs and spines. However, behavioral observations are not often included in studies of putative plant defenses.

Many plants respond to cues of damage by priming or inducing resistance mechanisms (see above) and these responses can include spines and other external defenses (Young 1987; Barton 2016; Kato et al. 2017). However, it is not known whether grasses produce more unidirectional spines and prickles when risk of damage is high. Induced responses are most likely for leaves that expand following perception of the cue. Damage to a plant’s own tissues is probably the most common (and reliable) cue to predict future attack, although growing evidence indicates that many plants, including some grasses, respond to volatile cues emitted by damaged neighbors (Engelberth et al. 2004, Ramadan et al. 2011; Karban et al. 2014). It is unknown if volatile cues from damaged neighbors will elicit denser or more effective grass spines.

In this study, we asked several related questions about unidirectional spines on the leaf margins of two grass species. (1) Is damage by chewing herbivores more common at leaf tips or bases? (2) Are grasshoppers more likely to land on leaf tips or bases? Once they have landed do grasshoppers orient up or down? (3) Do leaves with fewer marginal spines receive more chewing damage? (4) Do leaves with more spines shed water more rapidly perhaps to reduce suitability for pathogens? (5) Does experimental clipping induce a higher density of leaf spines? (6) Do volatiles from experimentally clipped neighbors induce a higher density of leaf spines?

Methods

Study site and system

This work was conducted at the Center for Ecological Research of Kyoto University, located in Otsu, Japan (34.971, 135.958). Observations and experiments were performed in two fallow fields that had been leveled for agriculture. Sites were chosen that had relatively high levels of chewing damage and high abundances of grasshoppers.

Two of the most common grasses at this study site were the native perennial, *Phragmites australis*, and the perennial, *Andropogon virginicus*, which was introduced from North America but has spread widely throughout East Asia, Australia, and South America. Both grasses experienced high levels of herbivory by grasshoppers including *Conocephalus maculatus* (Tettigoniidae), *Gonista bicolor* (Acrididae), *Aiolopus thalassinus tamulus* (Acrididae), and *Phaneroptera falcata* (Tettigoniidae). In addition, *A. virginicus* was attacked by *Pelopidas mathias* (Lepidoptera: Hesperidae), and *P. australis* hosted unidentified Noctuid caterpillars.

These two grass species were selected for study because they had conspicuous unidirectional spines (prickle-hairs sensu Metcalf 1960) that could be counted readily and

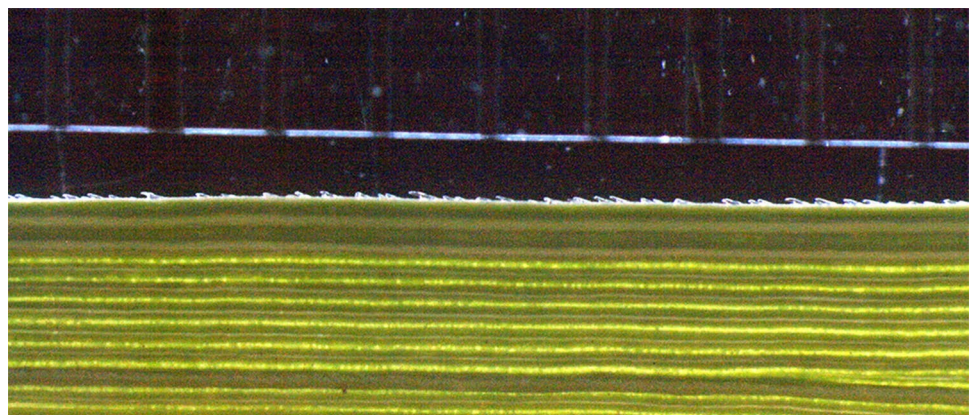
unambiguously using a dissecting microscope. *A. virginicus* has spines along the leaf margins that have been described as robust and pointed structures with swollen bases oriented in one direction towards the tip (Hilu 1984; see figs in Hilu). Hilu noted that the frequency of these features was variable and that they may be absent from some individuals. We observed rows of prickles and hairs that were on the upper surface of most leaves and were parallel to the veins. *P. australis* has strong spines approximately 0.5 mm in length that stick out from the leaf margins about 0.1 mm (Fig. 1). Metcalfe (1960, p. 385) also described prickle-hairs between the veins although the leaves at this study site did not have these.

In this study, we quantified the density of spines along the leaf margins. To do so, we first scanned the leaves using a dissecting microscope to locate the region along the leaf edge that had the maximum spine density. Spines were generally less dense at the tip and the very base of the leaf. We counted the number of spines per cm of leaf edge over the region with maximum spine density. Because spines and prickles were less reliably counted on the leaf surface compared to the edge, no attempt was made to quantify their density on the leaf surface. Grasshoppers generally began feeding at the leaf margin so that a measurement at this location was likely to be particularly relevant.

Leaf position and the distribution of chewing damage

To determine the distribution of leaf damage within leaves, we haphazardly selected leaves that had visual chewing damage at the field site on September 14, 2017. We removed those leaves and divided them into three equal sized lengths that we termed tip, middle, and base. We recorded whether chewing damage occurred in each of these three leaf positions for 40 leaves of *A. virginicus* and for 40 leaves of *P. australis*. We tested for an association between leaf position (tip, mid, base) and chewing damage for each species using a G-test.

Fig. 1 Spines on the leaf margin of *Phragmites australis*. The ruled marks are at 1-mm intervals for scale



Grasshopper behavior: leaf position and orientation

We recorded where on *A. virginicus* leaves grasshoppers landed (tip, middle, and base). These observations were conducted opportunistically between 14 and 21 September. A total of 27 observations were recorded. The distribution of landing sites was compared to a null expectation that grasshoppers were equally likely to land on each leaf position. The distribution of observed landing sites was compared to this null expectation using a G-test. Although grasshopper species was recorded, there were not sufficient observations to analyze the species individually. We did not have sufficient observations of grasshoppers landing on *P. australis* to conduct a similar analysis for this species.

We recorded the orientation of the head of grasshoppers with respect to the leaf they landed on (i.e., were they oriented towards the tip or towards the base?). One grasshopper landed perpendicular to the leaf blade, and this observation was not included in the analysis. We compared the distribution of landing orientation (up or down) to a null expectation that these two would be equal using a G-test.

Associations between chewing damage and unidirectional spines

To examine relationships between leaf spines and damage, we compared the density of spines on the margins of leaves that had chewing damage, undamaged leaves that were adjacent to these damaged leaves on the same plant, and undamaged leaves on neighboring plants that were free of damage. We haphazardly selected 36 triplets of leaves of *A. virginicus* that fit these three damage categories. The leaf on the neighboring plant was matched for size and ontogeny with the damaged leaf. Leaves were selected on September 29, 2017, and spines were counted using a dissecting microscope as described above. The association between density of spines and the three damage states (damaged, undamaged adjacent to damage, undamaged with a damaged neighbor) was evaluated using a 2-way least squares model

with damage state and plant as predictor variables in JMP Pro 13.0. We expected that the density of spines on damaged leaves would differ from undamaged leaves on the same plant and that damaged leaves would differ from undamaged leaves on neighboring plants and these two hypotheses were tested using a priori contrasts. We conducted a similar analysis for 30 *P. australis* leaves with chewing damage on 24 September.

Spine densities at a neighborhood scale

It became apparent that some areas of the field site had much higher densities of grasshoppers and chewing damage than others. The level fields were terraced into the landscape, which was higher in the north. Those sites that we chose for the observations described above were all south-facing field edges and embankments that separated the terraced level fields. These south-facing areas consistently had higher numbers of grasshoppers, while north-facing areas had few grasshoppers and little damage. We hypothesized that at this landscape scale, neighborhoods with high levels of damage would have leaves with higher densities of spines and neighborhoods with low levels of damage would have leaves with lower densities of spines. This expectation was based on the hypothesis that individuals with more spines would be favored by sorting and/or by local adaptation in neighborhoods where conditions were consistently favorable for grasshoppers.

We tested this hypothesis by comparing levels of damage at this larger spatial scale and number of spines. We selected 60 individuals of *A. virginicus* along the edges of one of the study fields such that roughly half were on north-facing slopes and roughly half were on south-facing slopes. The focal plants had no visible chewing damage. We estimated the risk of damage in the neighborhood of each focal plant by examining 10 conspecifics that grew within a radius of 50 cm. The number of neighbors with chewing damage served as an index of risk of damage in that neighborhood. The density of spines on each focal plant was estimated as described above. Since these data fit the assumptions of ANOVA, we used a linear model with the neighborhood damage index (number of neighbors with damage out of 10 neighbors) as the predictor and density of spines as the response variable. We conducted a second analysis with north and south as a covariate to separate effects due to this orientation in the field from additional effects of risk of damage.

Spines and rates of drying

We hypothesized that unidirectional hairs might allow leaves to dry more quickly and that this microhabitat could deter leaf pathogens. We sprayed fully expanded leaves of *A.*

virginicus with water as a fine mist (0.625 ml per leaf) on October 17 and 26, 2017. The upper surface of each sprayed leaf was west-facing and all plants were on the west-facing slope on the edge of a terraced field; as such, the aspect and growing conditions of all individuals were similar. After 30–40 min approximately half of the leaves had dried and we collected 40 wet leaves and 40 that had no visible signs of water droplets. We counted the density of spines on these leaves using a dissecting microscope and compared the mean density of marginal spines of wet and dry leaves using a t-test.

Induction of spines on new leaves

To examine intra- and inter-plant induction of spines on new leaves, we selected 60 *A. virginicus* individuals on September 15, 2017 that had three leaves and marked the most recently expanded leaf with tape. Half were assigned to be experimentally clipped with scissors. We removed 5 cm of the edge of the marked leaf blade, over to, but not including, the main vein. We also marked a recently expanded leaf blade of another neighboring plant that was within 5 cm of the experimental leaf. This neighboring leaf grew from a different root crown than the experimental leaf. On 10 November we removed the leaf that had been produced following the experimental clipping treatments (there was generally only one new leaf) and counted the density of marginal spines on this new leaf. We also removed and counted marginal spines on the newly produced leaf of the neighboring plant. We compared the density of spines on the leaves produced since our treatments using a linear model in JMP Pro 13.0. We tested the hypotheses that clipping induced more spines on new leaves and that volatile communication between clipped plants and neighbors resulted in more spines using a priori contrasts.

We conducted a similar experiment with *P. australis* by selecting 36 individuals with no evidence of chewing damage and marking the most recently expanded leaf. We clipped a 5-cm section of the leaf edge for half of those marked leaves and designated the marked leaf on 36 unclipped plants as controls. On 6 and 7 November we collected the marked leaves and all of the leaves above the mark that had expanded since the treatments were applied and counted the density of marginal spines on these leaves. We calculated the mean difference in spine density for the newly expanded leaves compared to each marked leaf as follows:

$$\text{Mean difference in spines post-treatment} = \frac{\sum (\text{Spine density of each new leaf} - \text{Spine density of marked leaf})}{\text{Number of new leaves}}$$

This estimated the density of spines on the new leaves standardized for the density of spines on that plant before the treatment. We compared this standardized estimate of spine density for new leaves on clipped and unclipped control plants using a least squares linear model in JMP Pro 13.0. We were not able to calculate this standardized measure for *A. virginicus* because the leaves that had been present before treatment had already senesced for many of the plants. *P. australis* holds multiple leaves over a longer period than *A. virginicus*.

We tested the hypothesis that cues from leaves damaged by actual herbivores caused production of more spines. Although grasshoppers are the most common herbivores in our system, they are difficult to manipulate experimentally. Some of the chewing damage to *P. australis* leaves was caused by noctuid caterpillars which are more easily manipulated. We moved caterpillars to new leaves and allowed them to feed and thereby cause naturalistic damage. We imposed chewing damage to 15 plants in this

manner between 15 September and 10 October. Plants where the caterpillar failed to feed were not included. For each of these experimental plants that were chewed by caterpillars, we established two controls within a few meters that had no signs of chewing damage. We also marked the most recently expanded leaf without chewing damage on a neighboring stem if one was available within 20 cm of either the chewed or control leaves. Newly produced leaves of these plants were collected and counted on 9 November and the standardized number of spines post-treatment was calculated as described above. A general linear model was used to evaluate the effect of treatments on number of spines post-treatment with plant identity included in the model as a blocking factor. A priori contrasts tested for effects of caterpillar feeding on induction of new spines (caterpillar vs. control), and for a cue caused by caterpillar feeding that induced spine production in neighbors (near caterpillar vs. [control + near control]).

Fig. 2 The distribution of chewing damage and grasshopper activities. **a** Chewing damage to tip, middle, and basal sections of 40 leaves of *Andropogon virginicus*. Dark bars indicate chewing damage and open bars indicate no damage. **b** Chewing damage to tip, middle, and basal sections of 40 leaves of *Phragmites australis*. Dark bars indicate chewing damage and open bars indicate no damage. **c** The leaf position chosen by grasshoppers that landed on leaves of *Andropogon virginicus*. **d** The orientation chosen by grasshoppers that landed on leaves of *Andropogon virginicus*. The up direction is the same as the direction of leaf hairs and spines

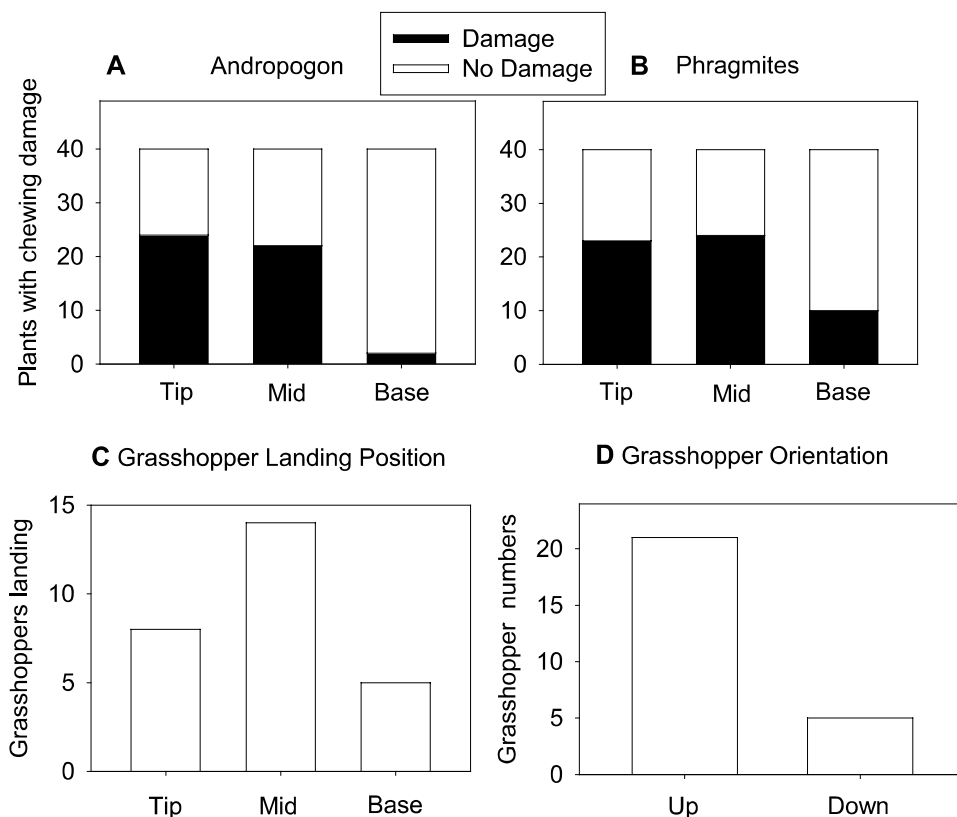


Fig. 3 Density of spines and levels of chewing damage. **a** Mean number of spines (± 1 se) for *A. virginicus* leaves with chewing damage, undamaged leaves on the same plant, and undamaged leaves on an adjacent neighboring plant. **b** Mean number of spines (± 1 se) for *P. australis* leaves with chewing damage, undamaged leaves on the same plant, and undamaged leaves on an adjacent neighboring plant. **c** Relationship between risk of chewing damage in the neighborhood and the density of spines for *A. virginicus*. The neighborhood damage index is the number of plants within 50 cm with chewing damage out of a sample of 10 possible plants. The line shows the best fit linear model

Results

Leaf position and the distribution of chewing damage

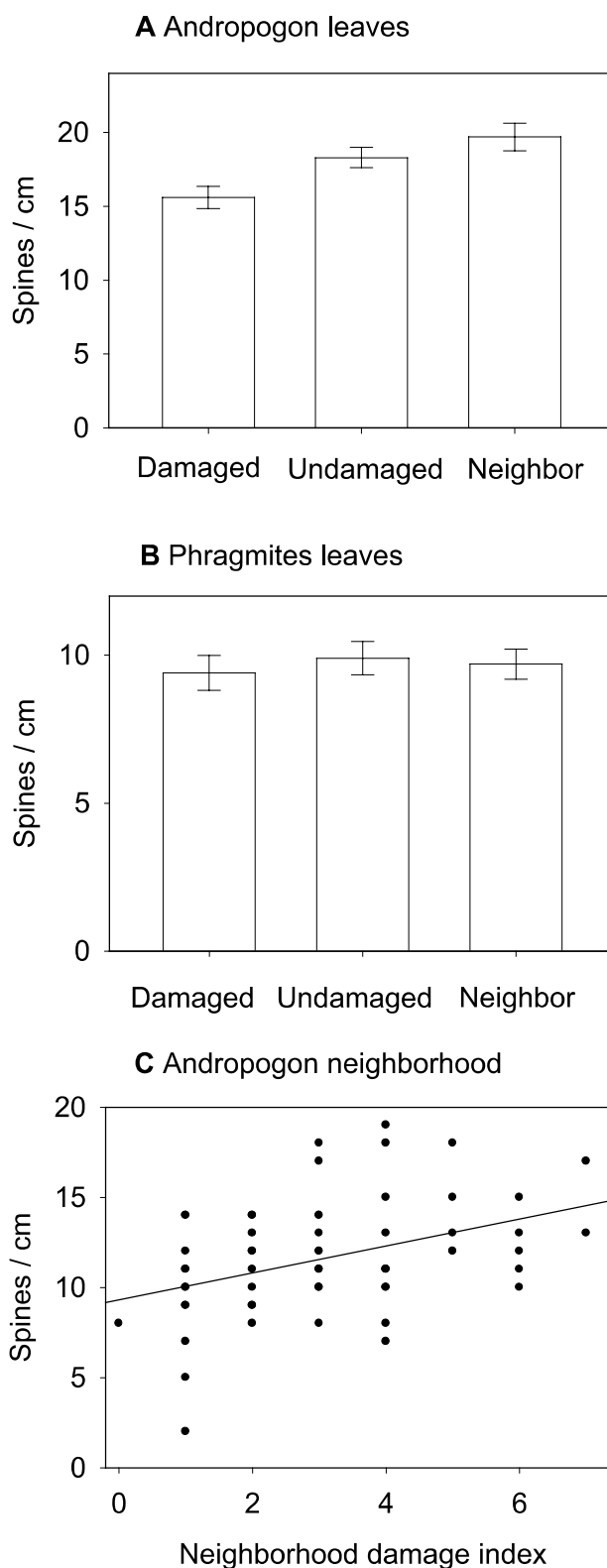
Chewing insects were more likely to damage the tip and middle third of *A. virginicus* leaves than they were to damage the basal third (Fig. 2a, $G = 36.75$, $df = 2$, $P < 0.001$). The basal region received damage for only 2/40 leaves that were sampled. Chewing insects were also more likely to damage the tip and middle third of *P. australis* leaves compared to the basal third (Fig. 2b, $G = 12.68$, $df = 2$, $P = 0.002$). For this species 10/40 leaves had damage to the base.

Grasshopper behavior: leaf position and orientation

Grasshoppers were common at the study site and often moved between plants. Grasshoppers most often landed in the middle of leaves of *A. virginicus* although they were not significantly more likely to choose one leaf position (tip, middle, base) when landing on a new grass blade (Fig. 2c, $G = 2.31$, $df = 2$, $P = 0.32$). However, they did orient upwards when they landed on a new leaf more often than would be expected by chance (Fig. 2d, $G = 5.58$, $df = 1$, $P = 0.02$). Of 26 landing, 21 were pointing upwards on the leaf, in the same direction that the spines and hairs pointed. For 2 of the 5 cases where they initially oriented down towards the leaf base, they quickly changed orientation so that they faced towards the tip.

Associations between chewing damage and unidirectional spines

Chewing damage was associated with fewer spines on leaves of *A. virginicus* (Fig. 3a, $F_{2,104} = 6.95$, $P = 0.002$). Undamaged leaves on plants with chewing damage had 17% greater density of spines than leaves with chewing damage (Fig. 3a, a priori contrast $F_{1,104} = 5.78$, $P = 0.02$). Undamaged leaves on neighboring plants had 26% more spines than leaves with chewing damage (Fig. 3a, a priori contrast $F_{1,104} = 13.46$, $P < 0.001$).



Chewing damage was not associated with a difference in the density of spines for *P. australis* (Fig. 3b, $F_{2,86} = 0.18$, $P = 0.83$).

Spine densities at a neighborhood scale

At a larger spatial scale, areas with higher densities of grasshoppers and higher levels of chewing damage had *A. virginicus* plants with more spines (Fig. 3c, spines = 0.75 neighborhood damage index + 9.32, slope se = 0.22, $R^2 = 0.16$, $df = 1.58$, $P = 0.001$). A covariate for orientation (north- or south-facing) was significant and the effect of neighborhood was also still significant in the model that included this covariate (spines = 0.76 neighborhood damage index + 1.06 [N/S] + 8.98; coefficient for neighborhood damage index se = 0.21, $t = 3.58$, $P = 0.001$, coefficient for N/S se = 0.38, $t = 2.82$, $P = 0.007$; LS mean ± 1 se for plants of north-facing slopes = 10.27 ± 0.61 spines, LS mean ± 1 se for south-facing slopes = 12.40 ± 0.45).

Spines and rates of drying

Contrary to our a priori hypothesis, water droplets coalesced along the parallel rows of prickly-hairs on *A. virginicus* leaves that had been sprayed. Leaves that retained water droplets had 16% higher densities of marginal spines compared to leaves that were dry (Fig. 4, $t = 2.36$, $df = 78$, $P = 0.02$).

Induction of spines on new leaves

Experimental clipping in September affected the number of marginal spines on new *A. virginicus* leaves that were produced in the months that followed (Fig. 5a, $F_{3,113} = 3.12$, $P = 0.03$). New leaves on plants that had been clipped had 13% more spines than new leaves on unclipped control

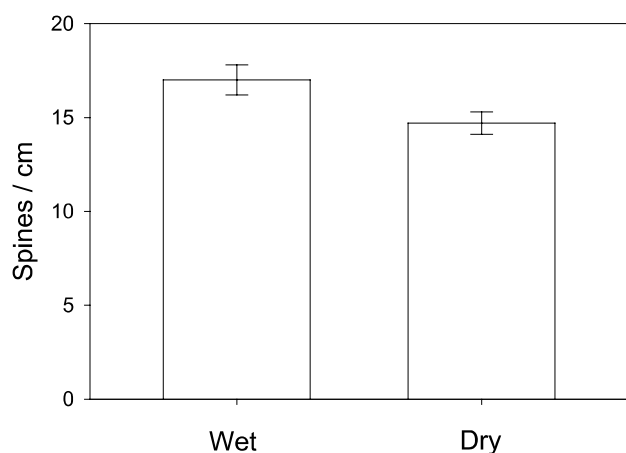


Fig. 4 Density of spines and rate of leaf drying for *A. virginicus*. Vertical bars indicate the mean number of spines/cm + 1 se for leaves that had water droplets on the adaxial surface (wet) and leaves with no visible water droplets (dry)

plants (a priori contrast, $F_{1,113} = 4.52$, $P = 0.04$). There was no indication that neighboring plants within 5 cm of clipping produced more spines than controls (a priori contrast, $F_{1,113} = 0.76$, $P = 0.39$) nor more spines than plants within 5 cm of controls (a priori contrast, $F_{1,113} = 1.78$, $P = 0.18$).

Experimental clipping of *P. australis* with scissors failed to affect the standardized number of spines on leaves produced post-treatment (Fig. 5b, $F_{1,33} = 0.13$, $P = 0.72$). In contrast, chewing by caterpillars affected the number of spines on leaves produced post-treatment ($F_{3,62} = 5.10$, $P = 0.003$). Chewing by caterpillars was associated with new leaves that had an average of 3.7 (24%) more marginal spines per cm than unclipped controls (Fig. 5c, a priori contrast $F_{1,62} = 11.54$, $P = 0.001$). Unchewed leaves on plants that were near to leaves that were chewed by caterpillars had an average of 2.2 (13%) more marginal spines than controls (Fig. 5c, a priori contrast $F_{1,62} = 5.35$, $P = 0.02$).

Discussion

Unidirectional spines as a defense

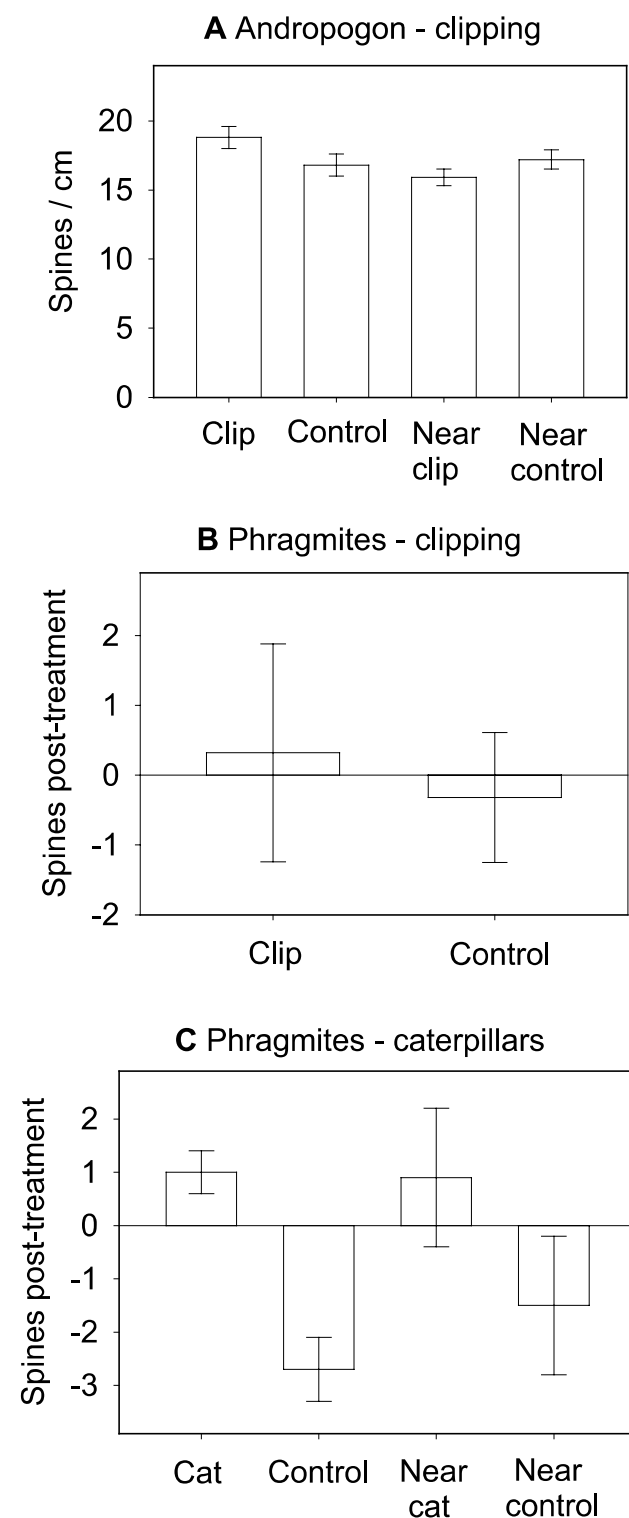
Unidirectional hairs, prickles, and spines are common features on the leaves of many plants, including grasses (Metcalf 1960; Vermeij 2015, Karban et al. unpubl.). They have been hypothesized to act as ratchets, leading small herbivores towards leaf tips. This unidirectional movement has been predicted to result in less damage near leaf bases where valuable meristems of many grasses are located. Patterns of damage to both of the grasses examined in this study were consistent with this prediction (Fig. 2a, b), similar to results from several species in California (Karbon et al. unpubl.). However, this pattern of damage could result from several unrelated mechanisms. Observations of grasshoppers that flew to, and alighted on, blades of *A. virginicus* indicated that the basal third of the blade was not completely avoided at this stage in the encounter sequence (Fig. 2c). However, grasshoppers were strongly biased towards orienting in the direction of the leaf tip during the landing process (Fig. 2d) and were more likely to move towards the tip as a result of this orientation (data not shown). The comparison between initial landing and subsequent orientation suggests that grasshoppers are choosing to preferentially orient towards the leaf tips. There are many reasons why grasshoppers may orient upwards following landing; this orientation may allow them to better observe their environment and to quickly jump from their current perch if they perceive threats. However, these observations about their orientation are consistent with the hypothesis that unidirectional leaf features may be involved.

Fig. 5 Induction of spines on new leaves. **a** Mean density (± 1 se) of spines for new leaves produced on plants that were experimentally clipped with scissors, unclipped controls, unclipped plants within 5 cm of clipped leaves, and unclipped plants within 5 cm of unclipped controls. **b** Mean density of spines per cm (± 1 se) for leaves produced post-treatment standardized by pre-treatment density for each plant. Clipped plants were experimentally damaged with scissors. **c** Mean density of spines per cm (± 1 se) for leaves produced post-treatment standardized by the pre-treatment density for each plant. Plants were either experimentally damaged by caterpillars, undamaged controls, or neighbors within 20 cm of a caterpillar-damaged or control leaf

Unidirectional surface features may be useful as plant defenses if they result in less damage to vulnerable tissues and if this effect results in enhanced plant fitness. In a previous common garden experiment that compared seed production of *Avena barbata* morphs with and without unidirectional hairs, the presence of hairs was associated with increased fitness in some years and some sites (Karban et al. unpubl.). For *Andropogon virginicus* in this study, leaves with a higher density of marginal spines had less chewing damage than undamaged leaves on the same plants and undamaged leaves on neighboring plants (Fig. 3a). One possible explanation for this association was that herbivores selected leaves that had fewer spines as desirable sites for feeding. A negative association between putative plant defenses and herbivore damage has been commonly observed in many other systems (e.g., Feeny 1976; Agrawal et al. 2012). Other causal mechanisms involving either induced plant responses or herbivore choice could also have produced the correlation in this case.

At larger spatial scales, this association was reversed (Fig. 3c). Individuals of *A. virginicus* that were in environments that were predictably (and presumably consistently) favorable for herbivores, such as south-facing embankments, had higher densities of spines. Individuals in locations that were unfavorable for herbivores such as north-facing embankments had lower densities of spines. One possible explanation for these site-specific differences is greater selection for spines where herbivore pressure was consistently higher or by greater induction of spines on those individuals that had previously been attacked by herbivores (Fig. 5). Other studies have reported higher levels of defenses at sites that are more favorable for herbivores (e.g., Louda and Rodman 1996; Sakata et al. 2017). Since this inference is based on a correlation, other explanations are also possible; for example, differences in sunlight may drive morphological differences.

These conflicting results provide a striking example of the importance of scale in ecology (Levin 1992; Hewitt et al. 2007). At the scale of individual leaves, risk of damage is unpredictable until a bout of actual herbivory.



Under these conditions, selection may favor plants that do not invest in costly additional spines until they receive a reliable cue, such as tissue loss. At the scale of the neighborhood, the risk of damage is more predictable since some areas consistently had high herbivore pressure and

others consistently had low herbivore pressure. Consistent herbivore pressure may select for plants that invest in defenses more routinely than those growing in neighborhoods with predictably lower risk.

Unidirectional hairs, prickles, and spines may serve other functions as well. Contrary to reports for other species (e.g., Eisner et al. 1998; Wheeler and Krimmel 2015), we did not observe any insects impaled or attached to the grass leaves in our study. They have also been proposed to provide impede attack by plant pathogens by facilitating drainage of water from leaf surfaces (see "Introduction"). Wet surfaces provide favorable habitats for the growth of fungi and epiphylls. This potential benefit cannot explain the presence of unidirectional features in this system because leaves with more marginal spines dried more slowly rather than faster (Fig. 4).

Cues that induced greater spine density

Plants respond to a wide variety of cues that reliably predict risk of attack by adjusting the levels and characteristics of their defenses (Karban 2015). Actual damage to their own tissues represents the most commonly reported and certainly one of the most reliable sources of information about risk of future attack. *A. virginicus* and *P. australis* responded differently to simulated or real herbivory. New leaves of *A. virginicus* that were produced on plants that were experimentally clipped with scissors had more marginal spines than newly produced leaves on unclipped control plants (Fig. 5a). It was not possible to experimentally clip leaves of this species using actual herbivores. New leaves of *P. australis* that were produced following clipping with scissors did not differ from new leaves on undamaged control plants (Fig. 5b). However, new leaves of *P. australis* that were produced following experimental clipping by caterpillars had more marginal spines relative to controls (Fig. 5c). Other workers have found that plants are more responsive to cues associated with actual herbivores compared to those from artificial mechanical damage (see Barton 2016 for a review). Herbivore saliva and oral secretions contain elicitors that many plants perceive and respond to (Felton and Tumlinson 2008).

Many plants, including some grasses, have been found to perceive volatile cues emitted by damaged neighbors and to prime or induce their own defenses (Karban et al. 2014). We found no evidence that *A. virginicus* was sensitive to volatile cues emitted by experimentally damaged neighbors (Fig. 5a). Damage to a plant's own tissues is probably a more accurate predictor of future damage than cues from neighbors so this negative result was not surprising. For *P. australis*, new leaves on plants that had been experimentally clipped with scissors failed to become spinier (Fig. 5b). Therefore, it was surprising that leaves of plants

with neighbors that had been clipped by caterpillars did have more spines (Fig. 5c). It is possible that feeding by actual caterpillars caused plants to emit different or more effective cues than clipping with scissors (see above). It would have been informative to compare responses of new leaves on plants clipped with scissors, plants chewed by caterpillars, and unclipped controls. However, this comparison was not possible because the availability of caterpillars and the difficulty of getting them to feed on experimental tissues greatly limited our ability to create these treatments. It was also interesting that control leaves on plants that were not chewed by caterpillars produced fewer spines than pre-treatment leaves on the same plants (Fig. 5c, left). This comparison was not planned a priori and therefore inferential statistics were not conducted although this pattern suggests that production of spines may be costly in the absence of herbivory.

The new leaves on plants without chewing damage that were within 20 cm of a neighbor that had been fed upon by caterpillars exhibited an increased density of spines. In many cases, the experimentally damaged leaf touched the unattacked neighboring leaf although the two leaves originated from different root crowns. We did not determine if the neighboring root crowns shared rhizomatous or mycorrhizal connections (Stuefer et al. 2004; Babikova et al. 2013); as such we do not know if the cue responsible for the communication we observed was a volatile or moved through the vasculature or symbionts of the plant. In summary, these results indicate that *A. virginicus* responds to experimental damage by producing leaves with more spines, although this species gave no indication of responding to volatile cues from damaged neighbors. *P. australis* was not found to endow newly produced leaves with more spines following clipping with scissors but did show greater spine production when it received cues from neighbors damaged by caterpillars.

Conclusion

Unidirectional hairs, prickles, and leaf spines are common on many plants, including many grasses. They may direct insect herbivores towards leaf tips and away from valuable meristems. Although these experiments considered herbivory only by insects, grazing mammals may also be directed away from grass meristems. These unidirectional surface features may allow grasses to be grazed by herbivores without suffering much risk of damaged meristems. As such, unidirectional features of grasses may be common, but unrecognized, defenses that make their relationship with wild and domesticated herbivores possible.

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