

Consequences of herbivory by native ungulates for the reproduction of a savanna tree

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Summary

1 Herbivores may negatively impact plant populations by reducing the survival, growth and reproduction of individual plants.

2 In African savannas, browsing by large mammalian herbivores has been shown repeatedly to reduce adult survival and growth of members of the genus *Acacia*, the dominant trees in these systems. However, the potential costs of mammalian herbivory to *Acacia* reproduction are largely unexplored, and have never been investigated experimentally in African savannas.

3 Using a long-term, large-scale field experiment, we demonstrate that, in the absence of native herbivores, individual trees were twice as likely to reproduce, and those that reproduced produced a greater biomass of seeds. In addition, spine length (an indicator of past herbivory) was correlated with reduced reproduction of *Acacia drepanolobium*, the dominant tree across large areas of East Africa. Browsing by native herbivores triggers the production of longer spines, an induced resistance, and spine length was significantly and negatively related both to the occurrence and magnitude of reproduction.

4 Induced resistance appeared to mitigate the negative effects of herbivory where large herbivores were present: trees with long spines reproduced at levels comparable to trees with similarly long spine lengths in the absence of browsers.

5 Large mammalian herbivores kill and suppress the growth of adult *Acacia*, and therefore often are regarded as critical in maintaining the co-dominance of trees and grasses in African savannas. Our results provide the first experimental evidence that large mammalian herbivores can suppress reproduction in *Acacia*, thus highlighting an additional pathway through which these herbivores might impact population dynamics of this important and widespread genus.

6 In savanna systems, declining populations of large herbivores may trigger a series of complex demographic responses for trees, with potentially strong consequences for the structure and function of savannas.

Key-words: *Acacia*, cost of herbivory, hierarchical model, induced resistance, landscape change, population dynamics, reproduction, savanna, seed production, ungulate

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Introduction

Individual plants can exhibit a multitude of responses to herbivory. Among the most conspicuous of these strategies are induced responses (Karban & Baldwin

1997). Induced responses may constitute an allocation cost, whereby resources that otherwise would be devoted to growth or reproduction are used to defend the individual from attack by herbivores (Baldwin *et al.* 1990; Berenbaum & Zangerl 1994; Redman *et al.* 2001; Gómez & Zamora 2002; Siemens *et al.* 2002; Cipollini *et al.* 2003; Walls *et al.* 2005). Where (and when) herbivores occur, it follows that induced responses should mitigate the

negative effects of herbivory, if not provide a net benefit to individuals (Karban *et al.* 1997). By contrast, induced responses are unnecessary where herbivory is absent, and individuals should instead allocate resources to reproduction or growth.

In addition to the potential cost to plants of generating induced responses, costs of herbivory may arise from the loss of photosynthate and associated compensatory regrowth (i.e. tolerance, *sensu* Strauss & Agrawal 1999). Compensation following herbivory has been demonstrated to reduce growth (Meyer 1998a), survival (Meyer 1998b) and reproduction (Ruohomäki *et al.* 1997) of individual plants. Both induced responses and compensation are hypothesized to be adaptive, in that individuals should be under selection to maximize gains relative to the costs of implementing these strategies. However, for long-lived plants in particular, the combined costs of induced responses and compensatory regrowth may accrue over time to limit energy stores and thus influence the occurrence and magnitude of future reproduction (Ehrlén & van Groenendael 2001).

Trees of the genus *Acacia* are widespread and important components of savanna ecosystems worldwide. In African savannas, *Acacia* abundance has been linked to such important community and ecosystem variables as species diversity (Dean *et al.* 1999), soil-water infiltration (Dougill *et al.* 1998), size and availability of nutrient pools (Hudak *et al.* 2003) and productivity (Belsky 1994). Large mammalian herbivores often are regarded as key regulators of *Acacia* abundance, and their effects upon survival and growth of adult trees have received considerable attention (e.g. Dublin *et al.* 1990; van Langevelde *et al.* 2003; Augustine & McNaughton 2004). However, the impacts of large mammalian herbivores upon other, potentially critical, demographic phenomena (e.g. reproduction) have been largely unexplored, and experimental evidence for these effects is virtually absent (but see, e.g. Miller & Coe 1993; Miller 1994).

Using a large-scale exclusion experiment that manipulates the occurrence of mammalian herbivores (Young *et al.* 1998), we investigated the impacts of these herbivores on the reproduction of *Acacia drepanolobium*, a tree abundant throughout regions of East Africa. *Acacia drepanolobium* is a small (< 6.5 m) tree that defends itself from herbivores with stipular spines (Milewski *et al.* 1991), symbiotic ants (Young *et al.* 1997) and possibly tannins (Ward & Young 2002). In a given year, large herbivores browse and remove shoot tips from 10 to 20% of individual branches at our study site (Young *et al.* 1998), and the trees respond to this browsing in two ways. First, trees exhibit regrowth following loss of shoots and leaves, such that full compensation (measured as total shoot length) occurs within 1 year (Gadd *et al.* 2001). Secondly, trees induce spine growth following browsing (Young & Okello 1998; Young *et al.* 2003; see also Huntzinger *et al.* 2004). As spine removal experiments have demonstrated that the spines of *A. drepanolobium* effectively deter browsing by large herbivores (Milewski *et al.* 1991), we follow Karban &

Baldwin (1997) and regard spine growth as an induced resistance. Importantly, individual trees continue to allocate resources to spine growth upwards of 10 years after being browsed (Young *et al.* 2003; T. P. Young, unpublished data). Thus, in addition to constituting an induced resistance, spine lengths of *A. drepanolobium* integrate the browsing history of an individual tree, and therefore can be regarded as a measure of past herbivory.

Our study uses the results above as a foundation to address two objectives. First, we investigated whether reproduction (both probability of reproduction and seed production) of *A. drepanolobium* differed between exclusion plots and plots accessible to browsers in two separate years. Secondly, we evaluated the extent to which reproduction was correlated with past herbivory, using spine length as a proxy for past herbivory. We assume that differences in herbivory among plots are driven primarily by large mammals, as herbivory by insects (hemipterans, homopterans and orthopterans) does not appear to differ between herbivore treatments (J. R. Goheen, unpublished data). We make no claims as to the relative importance of each potential cost (induced resistance, compensatory regrowth, loss of photosynthate), but we use spine length as an aggregate measure of past herbivory, with its entire attendant costs (see above).

Methods

STUDY SITE AND THE KENYA LONG-TERM EXCLUSION EXPERIMENT (KLEE)

We conducted surveys for reproductive *A. drepanolobium* in conjunction with the Kenya Long-term Exclusion Experiment (KLEE; Young *et al.* 1998) located at the Mpala Research Centre in the Laikipia District of central Kenya (0°17' N, 37°52' E, 1800 m ASL). KLEE occurs on 'black cotton' soils of impeded drainage, where *A. drepanolobium* comprises over 97% of the overstorey vegetation (Young *et al.* 1998). KLEE consists of three blocks separated by 100–250 m; each block is comprised of six 4-ha plots. Each plot is associated with a herbivore treatment, whereby different combinations of large mammalian herbivores have been excluded or permitted access since 1995 using a series of electrified fences. Treatments were assigned to plots using a stratified random design within blocks. For the purposes of this study, we used all three replicates of the following four herbivore treatments:

1. Full fencing that excludes all large (> 15 kg) herbivores.
2. Full fencing that excludes native large herbivores, but cattle are allowed to graze.
3. Unfenced: native herbivores have access but cattle are not allowed to graze.
4. Unfenced: native large herbivores have access and cattle are also allowed to graze.

Full fencing for treatments 1 and 2 consists of a 2.5-m fence with 11 strands of wire, with alternate wires electrified at 3000–7500 V. Treatments 3 and 4 are

unfenced, allowing free access by native large herbivores. At regular intervals, approximately three times per year, cattle are herded through treatments 2 and 4 at densities equivalent to the surrounding ranch (11–15 km⁻²). See Young *et al.* (1997) for further details about the experimental design of KLEE. Native, large herbivores common at our study site that browse on *A. drepanolobium* include elephants (*Loxodonta africana*), giraffes (*Giraffa camelopardalis*), elands (*Taurotragus oryx*) and Grant's gazelles (*Gazella granti*). With the exception of giraffes, all of these herbivores are mixed feeders (*sensu* Hofmann 1973) and switch from browsing to at least partial grazing on grasses following the rains. Cattle do not browse on *A. drepanolobium* (Young & Okello 1998; Odadi 2003), and past studies in our system have detected no effects of cattle on spine growth (Young & Okello 1998; Young *et al.* 2003).

ASSESSING REPRODUCTION AND INDUCED RESISTANCE OF *A. DREPANOLOBIUM*

In 2004, we established two permanent 150 × 10 m transects in each of the 12 plots. Between May and August in 2004 and 2005, we surveyed each *A. drepanolobium* in each transect for reproductive status (i.e. the presence of fruit) while recording its height, stem circumference at 1 m, crown diameter and identity of ant occupant (*Crematogaster sjostedti* or *C. mimosae*). Each individual *A. drepanolobium* is occupied by one and only one species of ant occupant (Young *et al.* 1997; Palmer *et al.* 2000). We collected and analysed these data because many traits of *A. drepanolobium* are correlated with the species of ant occupant (Young *et al.* 1997). We only analysed data for *C. sjostedti* and *C. mimosae* ants. We did not analyse data for trees inhabited by the ant *C. nigriceps*, because this species effectively sterilizes host trees (Stanton *et al.* 1999; Young *et al.* 1997) and induces spine growth in the absence of large mammals (Young *et al.* 2003), nor for trees inhabited by the ant *Tetraponera penzigi*, because this species typically occupies younger (and thus non-reproductive) trees (Young *et al.* 1997; Palmer *et al.* 2000). While native herbivores can be important consumers of *Acacia* fruits and seeds in other savannas (Pellew 1984; Miller 1996), fruit consumption by ungulates appears to be negligible in our system. For a small proportion of trees (< 2%), there was evidence that native herbivores were directly consuming fruits. In the few instances in which this did occur, < 5% of total fruits were removed. We did not analyse data for trees < 1.3 m tall, as 1.3 m was the minimum height at which we observed reproduction. In total, we analysed data from 1914 trees across the 12 plots.

In July 2004 and 2005, we selected eight reproductive trees per plot to assess seed production. Reproductive trees were selected randomly, subject to the constraints that: (i) four of these trees were occupied by the ant *C. mimosae* and four of these trees were occupied by the ant *C. sjostedti*; and (ii) there was no evidence for direct

consumption of fruits. Fruits of each selected tree were monitored every 2–3 days following maturation of fruit. Fruits were designated as 'mature' when they began to dry. The majority of fruits on an individual typically dehisce within *c.* 72 hours of each other, after which the seeds hang from arils attached to the inside walls of pods and are eventually passively dispersed on windy days (J. R. Goheen, personal observation). Seeds were collected from dehiscent pods, sun-dried for 7–10 days and counted. We recorded the total biomass of seeds per tree to the nearest 0.01 g using an electronic balance. In 2005, seeds from two sample trees were ruined due to inclement weather and were excluded from analyses.

We used spine lengths as an index of past herbivory (Young & Okello 1998; Young *et al.* 2003). *Acacia drepanolobium* produces one pair of stipular spines at each node of growing shoots following periods of heavy rain. After several weeks of growth, spines are fully lignified, at which time their lengths are fixed (Young & Okello 1998). Between May and August 2005, we measured mature spine lengths on 40 randomly selected reproductive trees in each plot. As spine length depends partly on the height of the branches on which spines occur (Young *et al.* 2003), we measured spine lengths at four predetermined branch heights for each individual. Each individual was divided into quartiles of equal length along its basal stem, based on its height. We measured spines on a single branch with living shoot tips for each quartile. For example, on a 2-m individual, we measured spines on a single branch at four heights: 0.00–0.50 m, 0.51–1.00 m, 1.01–1.50 m and 1.51–2.00 m.

Following the methods of Young *et al.* (2003), we measured to the nearest mm, the first mature spine occurring at least 5 cm from each growing shoot tip and a single spine at the next three nodes more proximate to the tree. If spines at a node differed noticeably in size, we measured both and recorded the average. If no spines were visible at a node, a value of 0.0 cm was recorded. We did not measure spines on individuals with noticeable herbivory on recent shoots. Spine lengths were averaged within and across branches. For inclusion in our analyses, individuals needed to have at least one branch with living shoot tips in at least three of the four predetermined quartiles. This criterion was not met for some trees (*c.* 10%), in which cases these individuals were excluded from subsequent analyses. In total, we measured 4366 spines (mean = 10.25 spines per individual) on 426 trees across 12 plots (mean = 36 trees per plot).

STATISTICAL ANALYSES

We used multivariate analysis of variance (MANOVA) to analyse the effects of herbivore treatment (native herbivore exclusion, cattle exclusion) on the proportion of reproductive *A. drepanolobium* trees per plot. Proportions were calculated using all 1914 trees in both years and were arcsine transformed. Our models specified occurrence of native herbivores, occurrence of cattle

and block as fixed effects (random effects are not permitted in MANOVA; Scheiner 2001). Occurrence of native herbivores was crossed with occurrence of cattle. Probability of reproduction (i.e. the proportion of fruiting trees per plot) did not differ between years, so we pooled data between 2004 and 2005 in this analysis. We distinguished between trees occupied by *C. mimosae* and those occupied by *C. sjostedti*, so that the proportion of *C. mimosae*- and *C. sjostedti*-occupied trees each were treated as a dependent variable in the MANOVA. We checked that univariate responses were normal, but we did not test for multivariate normality directly. When the overall MANOVA was statistically significant for an effect, we used one-tailed, univariate ANOVAs to assess the consistency in tree reproduction between ant occupants.

We used mixed-model analysis of variance (ANOVA) to analyse the effects of herbivore treatment upon the mean seed production among reproductive trees per plot (for the 96 and 94 trees selected in 2004 and 2005, respectively) and mean spine length per plot (in 2005 only). We log-transformed seed biomass to meet ANOVA assumptions. Seed production was significantly higher in 2005 than in 2004 (independent samples *t*-test; $t = -2.18$, d.f. = 22, $P = 0.04$), so we performed a separate analysis for seed production in each year. For each of the three ANOVAs, fixed effects (native herbivore occurrence and cattle occurrence) were crossed, and block was specified as random. In each analysis, we pooled data between ant occupants, as neither seed production nor mean spine length differed between *C. mimosae*- and *C. sjostedti*-occupied trees (Mann–Whitney *U*-tests, $P > 0.30$ for all). Distinguishing between trees occupied by each ant species did not affect the outcome of our analyses.

We built a series of models to explain variation in the probability and magnitude of reproduction for individual trees in 2005. For each of the 426 trees for which we measured spines, we constructed logistic models for the log-odds of reproduction (logit link) from a suite of predictor variables including mean spine length, ant occupant, and three metrics of tree size (height, circumference and crown breadth). For each of the 94 reproductive trees from which we collected and weighed seeds, we modelled the log biomass of seeds as linear functions of these same predictor variables.

Our set of candidate models for both probability and magnitude of reproduction comprised every combination of the tree-level main effects. For logistic models of probability of reproduction, we used Akaike's Information Criterion (AIC) to evaluate support for the models; for linear models of seed production, we used AIC corrected for small sample sizes (AIC_c; Burnham & Anderson 2002). We subtracted the minimum AIC or AIC_c value from each candidate set of models from each model in its associated set. The resulting differences (Δ_i 's) were used to make inferences about the 'best' model, as models with $\Delta_i \leq 4$ are those with strong empirical support (Burnham & Anderson

2002). Inclusion of interaction terms did not improve support for any of the models. No strong collinearity was detected among predictors (tolerance > 0.50, variance inflation factor < 1.97 for all pairs of predictor variables). We calculated the Akaike weight (Burnham & Anderson 2002) as $w_i = \exp(-1/2\Delta_i) / \sum_{g=1} \exp(-1/2\Delta_g)$ for each model in both sets of candidate models, where g is the number of models in each set. Because these are normalized to sum to 1.00, w_i reflects the relative likelihood of model i , given the data and a candidate set of models.

As we used trees as sampling units in calculating AIC values, the same herbivore treatment was assigned to all trees within the same plot. This could violate assumptions of independence if either predictor or response variables are correlated among trees within a plot. Therefore, we constructed hierarchical generalized linear models (HGLMs; Raudenbusch & Bryk 2002) that included variables from our lowest-AIC model from each of the candidate sets (see Moore & Swihart (2005) for a recent example of this approach in an ecological context). HGLMs are useful statistical tools when response variables (e.g. occurrence of reproduction) are associated with level-1 (tree-level) predictors (e.g. spine lengths) that themselves are functions of level-2 (plot-level) predictors (e.g. herbivore treatment), or when data points are not independent because of their proximity in space. In addition, HGLMs allow the partitioning of variance between fixed effects of trees, fixed effects of plots, and random effects associated with trees and plots, permitting parameter estimation with unbiased estimates of standard errors. Finally, HGLMs are valuable in identifying appropriate sampling units for analysis when nested data structures exist (e.g. trees within plots). We did not include occurrence of cattle as a level-2 predictor, because neither reproduction nor spine lengths varied with the occurrence of cattle (see below). Therefore, the level-2 predictor 'herbivore treatment' applies only to the occurrence of native herbivores. See Appendix S1 in Supplementary Material for further details on HGLMs.

Results

The proportion of trees reproducing in plots from which native herbivores had been excluded (i.e. plots accessible by no large herbivores or accessible only by cattle; hereafter 'exclusion plots') was twice that of trees in plots to which native herbivores had access (i.e. plots accessible by all large herbivores or accessible only by native herbivores, hereafter 'open plots'; reproduction in exclusion plots, $35.9\% \pm 2.7$; in open plots, $17.8\% \pm 3.0$; mean \pm SE; Fig. 1a, Table 1). The effect of native herbivore exclusion upon the proportion of reproducing trees was statistically significant for trees occupied by each ant species (*C. sjostedti*, $F_{1,22} = 12.04$, $P < 0.01$; *C. mimosae*, $F_{1,22} = 11.49$, $P < 0.01$). For reproductive trees, seed production in exclusion plots was 60–100% greater than seed production in open plots (2004 – log

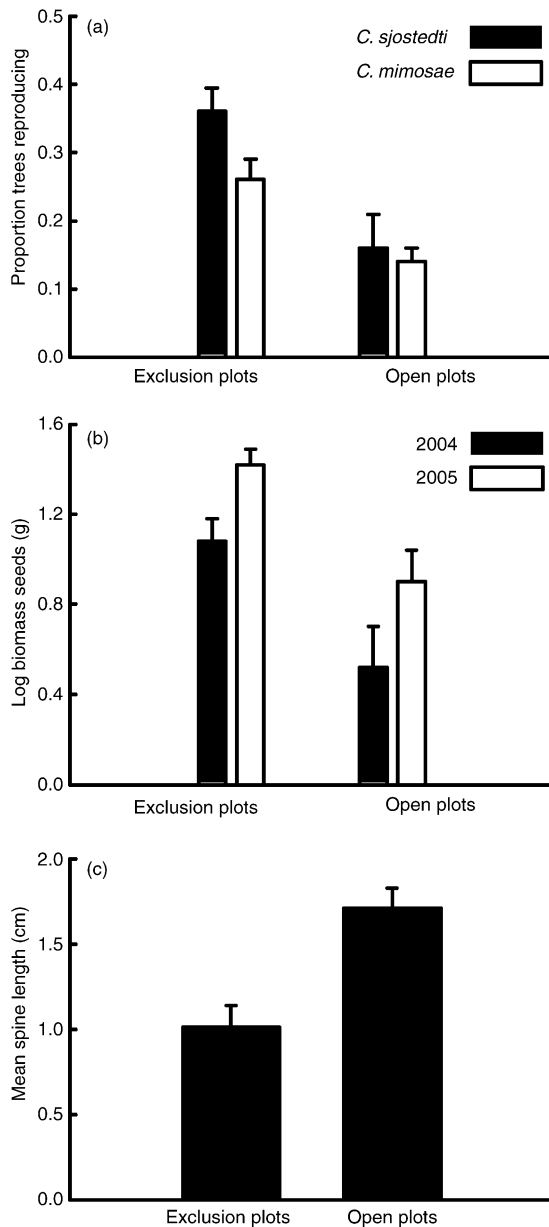


Fig. 1 Reproduction and mean spine lengths for *A. drepanolobium* in plots accessible to native herbivores (open plots) and plots from which native herbivores have been excluded for the past 10 years (exclusion plots). All bars represent mean values + 1 SE. (a) Average proportion of trees reproducing across plots, segregated by ant occupant and pooled between 2004 and 2005. (b) Average seed production for reproductive trees across plots in 2004 and 2005. (c) Spine lengths for trees averaged across plots, measured in 2005.

biomass seeds in exclusion plots, $1.08 \text{ g} \pm 0.10$; in open plots, $0.52 \text{ g} \pm 0.18$; 2005 – log biomass seeds in exclusion plots, $1.42 \text{ g} \pm 0.07$; in open plots, $0.90 \text{ g} \pm 0.14$; mean \pm SE; Fig. 1b and Table 1). Consistent with prior studies, herbivory (and thus spine lengths of trees) was reduced significantly by the exclusion of native herbivores (mean spine length in exclusion plots, $1.01 \text{ cm} \pm 0.13$; in open plots, $1.71 \text{ cm} \pm 0.12$; mean + SE; Fig. 1c, Table 1). Block effects accounted for $\leq 40\%$ of the variation in seed reproduction and mean spine length in each ANOVA.

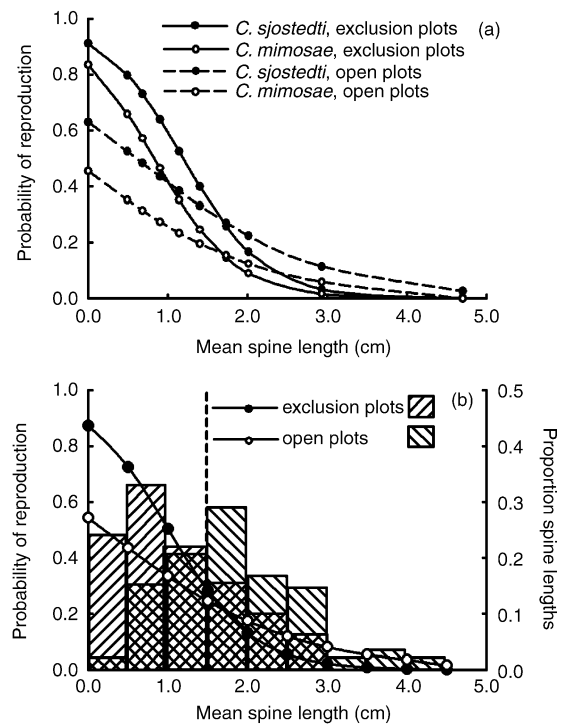


Fig. 2 (a) The predicted probability of *A. drepanolobium* reproduction based on a fitted HGLM as a function of herbivore treatment, spine length and ant occupant (height and crown breadth are held constant at their grand means). See Table 3 for coefficients. (b) The predicted probability of reproduction as a function of herbivore treatment and spine length, averaged across ant occupant. Frequency histograms represent spine lengths of individual trees in exclusion plots and open plots. The dashed vertical line represents the threshold (1.5 cm) beyond which probability of reproduction as a function of spine length did not differ between exclusion and open plots. Note that the majority of trees in exclusion plots have spine lengths below this threshold, while the majority of trees in open plots have spine lengths above this threshold.

In the tree-level analyses, fits and concordance of logistic models with strong support were good (mean Nagelkerke's $R^2 = 0.68$, mean Somer's $D = 0.87$), while fits of linear models with strong support were modest (mean adjusted $R^2 = 0.26$; Table 2). There was a negative relationship between spine length and reproduction for both probability of reproduction and seed production, as each model receiving strong support for both probability of reproduction and seed production included spine length as a predictor (Table 2). Models that did not include spine length as a predictor received essentially no support for probability of reproduction (for models without spine length, all $w_i < 0.001$) or seed production (for models without spine length, mean $w_i < 0.001$).

Results from hierarchical models agreed with those from tree-level analyses. Probability of reproduction was strongly and negatively correlated with a tree's history of herbivory (spine length) across treatments and plots ($P < 0.001$; Fig. 2, Table 3). Probability of reproduction was marginally higher for trees occupied by *C.*

Table 1 Effects of herbivore treatment (native herbivore presence vs. absence, cattle presence vs. absence) on proportion of trees reproducing, seed production, and mean spine lengths. Effects of herbivore treatment on proportion of trees reproducing were analysed using MANOVA on arcsine-transformed data. Effects of herbivore treatment on seed production and mean spine lengths were analysed using ANOVA on log-transformed data

	Factor	Wilk's λ	F	d.f.	P
Proportion reproducing (2004 and 2005)	Native herbivores	0.41	7.71	3,16	< 0.01
	Cattle	0.72	2.08	3,16	0.15
	Native herbivores \times cattle	0.87	0.80	3,11	0.51
	Block	0.49	2.30	6,32	0.06
Seed production (2004)	Native herbivores	NA	9.67	1,6	0.02
	Cattle	NA	0.27	1,6	0.62
	Native herbivores \times cattle	NA	0.27	1,6	0.62
	Block	NA	2.81	2,6	0.14
Seed production (2005)	Native herbivores	NA	17.44	1,6	< 0.01
	Cattle	NA	2.17	1,6	0.19
	Native herbivores \times cattle	NA	0.22	1,6	0.65
	Block	NA	3.79	2,6	0.09
Mean spine length (2005)	Native herbivores	NA	15.00	1,6	< 0.01
	Cattle	NA	2.16	1,6	0.19
	Native herbivores \times cattle	NA	0.32	1,6	0.59
	Block	NA	0.61	2,6	0.57

Table 2 Model selection results for probability of reproduction (logistic models) and seed production (linear models) for tree-level predictors. Models with strong levels of support ($\Delta_i \leq 4$) are presented. Akaike weights (w_i) represent the relative likelihood of a particular model, given a set of candidate models. R^2 values represent adjusted Nagelkerke's R^2 for logistic models and adjusted R^2 for linear models. Somer's D is calculated for logistic models as a measure of concordance between predicted and observed reproduction. Linear models for seed production are compared using AIC corrected for small sample sizes (AIC_c)

	AIC	Number of parameters	Δ_i	w_i	R^2	Somer's D
Probability of reproduction						
Spine length + ant + crown + height	269.13	5	0	0.58	0.68	0.87
Spine length + ant + crown + height + circumference	271.12	6	1.99	0.21	0.68	0.87
Spine length + ant + height	271.32	4	2.19	0.19	0.68	0.86
Seed production						
Spine length + crown	-96.08	3	0	0.32	0.27	NA
Spine length + crown + ant	-94.07	4	2.01	0.11	0.27	NA
Spine length + crown + height	-93.95	4	2.13	0.11	0.27	NA
Spine length + crown + circumference	-93.90	4	2.18	0.11	0.24	NA
Spine length + circumference	-92.35	3	3.73	0.05	0.26	NA
Spine length	-92.14	2	3.95	0.05	0.24	NA

sjostedti (using trees with spine lengths = 0.0, predicted probability of reproduction for *C. sjostedti*-occupied tree = $77\% \pm 4.1$, for *C. mimosae*-occupied tree = $65\% \pm 4.0$, mean \pm SE, $P = 0.10$; Fig. 2, Table 3), and probability of reproduction increased with increasing crown breadth ($P = 0.04$; Table 3).

Both the intercepts (γ_{00} and γ_{01}) and slopes (γ_{10} and γ_{11}) of the relationship between probability of reproduction and spine length differed as a function of herbivore treatment. For the effect of native herbivores upon probability of reproduction, there was no random plot effect (τ_{0j} c. 0), so we considered tree-level data independent and used individual trees to estimate the average log-odds of reproduction in exclusion plots (γ_{00}) and the difference in log-odds of reproduction in native herbivores access plots (γ_{01}). There were random plot effects on spine length, ant occupant, height and crown breadth (all τ_{0j} 's > 0), so plots were treated as sampling

units in estimating coefficients of predictors (γ_{10} , γ_{20} , γ_{30} and γ_{40}) and the coefficient describing the interaction between herbivore treatment and spine length upon the probability of reproduction (γ_{11}).

Probability of reproduction converged among herbivore treatments with increasing spine lengths. In open plots, individuals with longer-than-average spines were equally likely to reproduce as individuals with similarly long spine lengths in exclusion plots (Fig. 2, Table 3). Conversely, individuals with shorter-than-average spines in exclusion plots were significantly more likely to reproduce relative to individuals with identical spine lengths in open plots (Fig. 2, Table 3). This disparity became more pronounced with decreasing spine lengths. To further illustrate this point, we combined predicted probabilities of reproduction into 0.5-m bins of spine lengths up to 3.0 cm (there were very few trees in native herbivore exclosures with mean spine lengths > 3.0 cm),

Table 3 Results from HGLMs containing tree-level predictors from the lowest-AIC model for log-odds of reproduction* and seed production†. Herbivore treatment and ant occupant were coded as binary variables. γ_{00} represents the average log-odds of reproduction or seed production in exclusion plots for trees with spine lengths of 0.0 and all other quantitative predictors held constant at their grand means. γ_{01} represents the difference in the log-odds of reproduction or seed production in open plots for trees with spine lengths of 0.0 and all other quantitative predictors held constant at their grand means. γ_{q0} is the average slope for level-1 predictor q in exclusion plots, γ_{q1} is the difference in the slope of level-1 predictor q in open plots. For example, γ_{10} represents the change in log-odds of reproduction or seed production for trees in exclusion plots with increasing spine lengths, and γ_{11} represents the change in the log-odds of reproduction or seed production for trees in open plots with increasing spine lengths. Where γ_q 's did not differ between exclusion and open plots, only γ_{q0} s are presented

Fixed effect	Coefficient	SE	d.f.	<i>T</i>	<i>P</i>
Probability of reproduction					
Native herbivores (β_0)					
Intercept (γ_{00})	2.33	0.31	419	7.43	< 0.001
Intercept (γ_{01})	-1.8	0.46	419	-3.91	< 0.001
Mean spine length (β_{1j})					
Slope (γ_{10})	-1.96	0.39	10	-5.07	< 0.001
Slope (γ_{11})	1.07	0.44	10	2.45	0.03
Ant (β_{2j})					
Intercept (γ_{20})	-0.71	0.39	11	-1.08	0.10
Height (β_{3j})					
Slope (γ_{30})	0.43	0.28	11	1.51	0.16
Crown breadth (β_{4j})					
Slope (γ_{40})	0.57	0.24	11	2.39	0.04
Seed production					
Native herbivores (β_0)					
Intercept (γ_{00})	1.72	0.13	10	13.73	< 0.001
Intercept (γ_{01})	-0.55	0.19	10	-2.91	0.01
Mean spine length (β_{1j})					
Intercept (γ_{10})	-0.41	0.09	88	-4.36	< 0.001
Intercept (γ_{11})	0.22	0.12	88	1.74	0.08
Crown breadth (β_{2j})					
Intercept (γ_{20})	0.16	0.06	89	2.83	< 0.01

* $\log[\phi_{ij}/(1 - \phi_{ij})] = \gamma_{00} + \gamma_{01} \times \text{native herbivores}_{ij} + \gamma_{10}(\text{mean spine length}_{ij}) + \gamma_{11} \times \text{herbivore treatment}_{ij}(\text{mean spine length}_{ij}) + \gamma_{20}(\text{ant}_{ij}) + \gamma_{30}(\text{height}_{ij}) + \gamma_{40}(\text{crown}_{ij}) + u_1(\text{spine}_{ij}) + u_2(\text{ant}_{ij}) + u_3(\text{height}_{ij}) + u_4(\text{crown}_{ij}) + r_{ij}$.

† $\log(y_{ij}) = \gamma_{00} + \gamma_{01} \times \text{native herbivores}_{ij} + \gamma_{10}(\text{mean spine length}_{ij}) + \gamma_{11} \times \text{native herbivores}_{ij}(\text{mean spine length}_{ij}) + \gamma_{20}(\text{crown}_{ij}) + u_0 + r_{ij}$.

and then we tested for differences in probability of reproduction for each bin by herbivore treatment using a series of Bonferroni-corrected Mann-Whitney *U*-tests. For individuals with mean spine lengths > 1.5 cm, probability of reproduction was similar between open and exclusion plots (Fig. 2b).

Seed production was correlated negatively with past herbivory (spine length) across treatments and plots ($P < 0.001$; Fig. 3, Table 3), and seed production was correlated positively with crown breadth ($P < 0.01$; Table 3). With respect to the effect of native herbivores upon seed production, there were random effects associated with plots ($\tau_{0j} > 0$), so we treated plots as sampling units in estimating γ_{00} and γ_{01} . Effects of spine length and crown breadth did not vary randomly among plots (τ_{qj} 's *c.* 0), so we treated trees as sampling units in estimating γ_{10} , γ_{20} and γ_{11} . Similar to the results from the logistic HGLM, the intercepts (γ_{00} and γ_{01}) and slopes (γ_{10} and γ_{11}) of the relationships between seed production and spine length differed as a function of herbivore treatment, although the difference between slopes was weak ($P = 0.08$, Fig. 3, Table 3). We combined seed production into 0.5-cm bins of mean spine

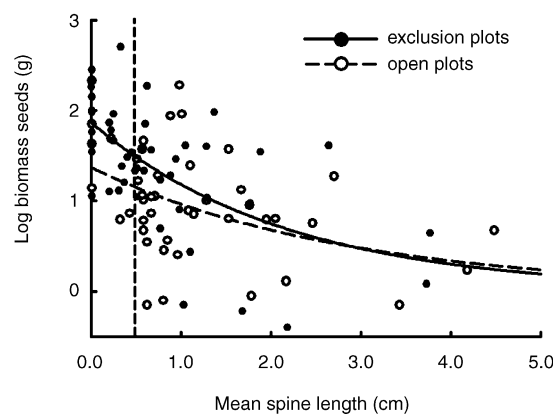


Fig. 3 Seed production based on a fitted HGLM as a function of herbivore treatment and spine length, holding crown breadth constant at its grand mean. Lines represent fitted exponential functions to better display the convergence in seed production among treatments with increasing spine lengths. Exclusion plots: $\log \text{biomass seeds (g)} = 1.87 \times \exp(-0.46 \times \text{spine length})$; open plots: $\log \text{biomass seeds (g)} = 1.37 \times \exp(-0.354 \times \text{spine length})$. See Table 3 for coefficients. The dashed vertical line represents the threshold (0.5 cm) beyond which seed production as a function of spine length did not differ between exclusion and open plots.

lengths up to 2.0 cm (we sampled very few trees reproducing with mean spine lengths > 2.0 cm), and then we tested for differences in seed production for each bin by herbivore treatment (Bonferroni-corrected Mann–Whitney *U*-tests). For individuals with mean spine lengths > 1.0 cm, probability of reproduction was similar between open and exclusion plots (Fig. 3).

Discussion

In our study system, a tree's history of herbivory was strongly and negatively correlated with its reproduction. Ten years of exclusion of native herbivores reduced levels of browsing and resulted in a relaxation of spine growth, such that spines were 39% shorter where mammalian browsers had been excluded. The overall costs of herbivory to reproduction were striking: twice as many trees reproduced when protected from native herbivores compared with trees that were exposed to native herbivores. In addition, seed biomass production of trees exposed to native herbivores was significantly lower than that of trees protected from these herbivores.

Using a long-term, large-scale experiment, we have demonstrated the overall costs of herbivory for the incidence and magnitude of *Acacia* reproduction. However, we are unable to disentangle experimentally the reproductive costs of induced responses, compensatory regrowth, and photosynthate removal. To separate unequivocally each relative effect of herbivory, we would need to: (i) experimentally induce spine growth and quantify its costs in exclusion plots; and (ii) assess costs following experimental reduction of spine lengths in open plots (Agrawal 2000). For trees with short spines, some portion of the suppressed reproduction of *A. drepanolobium* may arise from the short-term costs of tissue removal by native herbivores, as short spines are less effective at deterring browsing than long spines (Cooper & Owen-Smith 1986; Milewski *et al.* 1991; Gowda 1996). The lower intercepts for relationships in open plots (Figs 2 and 3) lend some support to this hypothesis. However, we suggest that the costs of photosynthate removal and associated regrowth may be lower than costs of induced responses for two reasons. First, probability of reproduction and seed production converged between open and exclusion plots with increasing spine lengths of individuals. Trees in open plots are browsed by native herbivores, triggering compensatory regrowth and spine growth. In contrast, trees in exclusion plots have been protected from browsing for 10 years, and the majority of these trees have responded by relaxing spine growth relative to trees in open plots. In spite of this, a small proportion of exclusion trees continue to invest in long spines (i.e. > 1.5 cm); these trees reproduce at an equivalent rate and magnitude as unprotected trees with spines of comparable lengths. We suspect that this small proportion of trees had either experienced relatively more mammalian herbivory prior to fencing, or more recent herbivory prior to fencing,

or possibly there were genotypic differences among trees (Fordyce 2006).

Secondly, Gadd *et al.* (2001) demonstrated that *A. drepanolobium* exhibit increased regrowth following shoot and leaf removal, compared with control branches. Within 1 year of simulated browsing, individuals had compensated fully for removal of vegetative tissue, suggesting that any potential costs of photosynthate removal and compensation should be expressed over a relatively brief period of time. In contrast, Young *et al.* (2003) have documented the protracted relaxation of spine growth following browsing, such that some individuals continued to allocate resources to long spines in excess of 10 years after being browsed. Although we did not measure lifetime fitness, our results combined with previous efforts in this system (Gadd *et al.* 2001; Young *et al.* 2003), suggest the possibility that native herbivores may suppress reproduction of *A. drepanolobium* by inducing spine growth.

The trend towards increased probability of reproduction observed for *C. sjostedti*-relative to *C. mimosae*-occupied trees suggests that *A. drepanolobium* may experience a trade-off in biotic defences and reproduction. These two ant species differ strongly in both use of host-plant extrafloral nectar and in levels of aggression towards herbivores (T. M. Palmer *et al.*, unpublished manuscript). While host plants occupied by the highly aggressive and nectar-dependent *C. mimosae* produce large quantities of nectar, host plants occupied by the non-aggressive and more predatory (and less nectar-dependent) *C. sjostedti* are characterized by nectaries that are largely inactive (T. M. Palmer *et al.*, unpublished manuscript). Our results imply that the increased allocation to nectar production by *C. mimosae*-occupied plants may come at a cost for reproduction relative to host plants occupied by *C. sjostedti*. This perspective is consistent with prior observations that *A. drepanolobium* reduces investment in its symbiont ants (extrafloral nectaries and swollen thorn domatia) in the absence of large herbivores (Huntzinger *et al.* 2004; T. M. Palmer *et al.*, unpublished manuscript). Maintaining abundant populations of defensive ants in the presence of large herbivores may further suppress host plant reproduction in this system.

The extent to which native herbivores may influence populations of *A. drepanolobium* by suppressing reproduction remains an open question, and one that depends ultimately on the importance of seed production relative to other demographic rates and phenomena (Gómez 2005). Further, reproduction should have the greatest impacts on plant population dynamics when recruitment is limited most by propagule pressure (e.g. Eriksson & Ehrlén 1992) rather than microsite (e.g. Eskelinen & Virtanen 2005) or predator (e.g. Orrock *et al.* 2006) limitation. Currently, we are in the process of investigating how heightened seed production may influence recruitment and abundance of *A. drepanolobium*.

The suppression of *Acacia* reproduction by native herbivores might exacerbate the well-established

negative effects that these herbivores have on survival and growth of adult trees. In African savannas, mammalian herbivores kill mature trees, and therefore are often considered crucial in maintaining the balance between co-dominating trees and grasses in these systems (Laws 1970; Owen-Smith 1988; Bond & Loffell 2001). While the impact of mammalian herbivores on mature trees is undeniable, many ecologists have speculated that other, less visually obvious, phenomena (i.e. reproduction, seedling survival and recruitment) might be equally important as or more important than adult survival in dictating tree abundance (Prins & Van der Jeugd 1993; Midgley & Bond 2001; Goheen *et al.* 2004; Sankaran *et al.* 2004). Our results reveal how mammalian herbivores can have profound consequences for the reproduction of savanna trees. We expect that the suppression of *Acacia* reproduction by mammalian herbivores could prove critical in regulating the abundance of these important components of savanna ecosystems.

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Supplementary material

The following supplementary material is available for this article:

Appendix S1 Formulation of hierarchical generalized linear models (HGLMs) used in this study.

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