Within-plant variation in defences in response to simulated herbivory in a semi-arid southern African savannah

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Abstract

Within-plant spatial variation in herbivore pressure can induce localized antiherbivory defence responses. We tested this hypothesis by studying branch-specific responses of *Acacia robusta*, *Dichrostachys cinerea* and *Ziziphus mucronata* to simulated mammalian herbivory. Herbivory was simulated by clipping the terminal shoots (3 cm from tip) of tree branchlets, allowing them one year of regrowth and then comparing their spine length and density and condensed tannins with those of adjacent unclipped branchlets. Condensed tannins concentrations were higher in clipped branchlets than in unclipped branchlets in all three woody species ($P < 0.05$). Spine length was higher in clipped branchlets than in unclipped branchlets in *A. robusta* ($P < 0.05$) but was similar in both *D. cinerea* and *Z. mucronata* ($P > 0.05$). Spine density was double in clipped branchlets as compared to the unclipped branchlets in *Z. mucronata* ($P < 0.05$) but was similar in both *A. robusta* and *D. cinerea* ($P > 0.05$). We found evidence of within-plant variation in condensed tannins concentration and spine length and density in response to simulated herbivory in the three woody species.

Key words: condensed tannins, resprouts, shoot clipping, spine density, spine length

Résumé

La variation spatiale, dans un végétal, de la pression des herbivores peut induire des réponses défensives localisées contre ceux-ci. Nous avons testé cette hypothèse en étudiant les réponses spécifiques des branches d'*Acacia robusta*, *Dichrostachys cinerea* et *Ziziphus mucronata* pour simuler le broutage par des mammifères. Nous avons simulé cette herbivorie en coupant les pousses terminales (à 3 cm de l'extrémité) de trois rameaux, que nous avons ensuite laissé repousser pendant un an avant de comparer la longueur et la densité de leurs épines et les tanins condensés avec ceux d'autres rameaux voisins non coupés. Les concentrations en tanins condensés étaient plus élevées dans les rameaux coupés que dans les non coupés chez toutes les espèces ligneuses ($P < 0.05$). La longueur des épines était plus grande sur les rameaux coupés que sur les non coupés chez *A. robusta* ($P < 0.05$) mais était semblable chez *D. cinerea* et *Z. mucronata* ($P > 0.05$). La densité des épines était deux fois plus grande chez les rameaux coupés que chez les non coupés chez *Z. mucronata* ($P < 0.05$), mais elle était semblable chez *A. robusta* et *D. cinerea* ($P > 0.05$). Nous avons donc trouvé chez ces trois espèces ligneuses des preuves de variations de la condensation en tanins condensés et de la longueur et la densité des épines en réponse à une herbivorie simulée.

Introduction

Woody plant responses to herbivory have largely been considered to be systemic, occurring throughout the entire individual plant (Karban, 2011). For instance, Haukioja & Neuvonen (1985) found experimental damage to one part of the plant to increase defences throughout birch trees. However, Young, Stanton & Christian (2003) reported branch-specific responses to herbivory in spine length of *Acacia drepanolobium*. Based on the findings of Young, Stanton & Christian (2003), it can be hypothesized that within-plant spatial variation in herbivore pressure can induce localized antiherbivory

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defence responses. However, this hypothesis requires further empirical testing.

Most studies on induced plant defences in woody plants have considered between plant and not within-plant variations in condensed tannins contents and spinescence (e.g. Rohner & Ward, 1997; Gowda & Palo, 2003; Rooke *et al.*, 2004; Milewski & Madden, 2006). Herbivory is considered costly because the plant increases its resource allocation to defence (Karban & Baldwin, 1997; Stamp, 2003). In the absence of herbivory, a plant is expected to allocate more resources to growth and reproduction, rather than to defence (Karban, 2011). Chemical (e.g. condensed tannins concentration) and physical (e.g. spinescence – spine length and density) defences are quickly activated after herbivore damage to increase plant protection (Eyles *et al.*, 2010). Localized responses to defoliation are potentially advantageous because resources will only be mobilized to defend the affected plant parts. Baldwin (1998) argued that strategies based on induced defences were cost-saving because their associated costs only materialized when functionally necessary. Herbivores have been observed to move away from locally damaged plant parts and to preferentially feed on undamaged tissues (Edwards & Wratten, 1983). Condensed tannins concentration and plant spinescence in woody plants increase in response to herbivory (Bryant *et al.*, 1991; Milewski, Young & Madden, 1991; Ward & Young, 2002; Young, Stanton & Christian, 2003). For instance, simulated herbivory increased spinescence in *A. tortilis* (Gowda, 1997). Defences that are locally induced and expressed may be advantageous in systems where certain portions of the plant are consistently at greater risk of herbivory (Young, Stanton & Christian, 2003). For example, locally inducible increased spine length in *Acacia* spp. may have evolved because upper canopy branches are consistently out of reach of herbivores.

We carried out a controlled and replicated simulated herbivory experiment to investigate the changes in condensed tannins concentration, spine length and density of *Acacia robusta* Burch, *Dichrostachys cinerea* (L.) Wight and Arn. and *Ziziphus mucronata* Willd. in response to localized defoliation in a semi-arid southern African savannah. *Acacia robusta*, *D. cinerea* and *Z. mucronata* are widely distributed and frequently browsed by large mammalian herbivores in the semi-arid southern Africa savannah. We hypothesized that condensed tannins concentration, spine length and density will increase in clipped as opposed to unclipped branchlets within a woody plant.

Materials and methods

Study site description

The field research was carried out from February 2012 to February 2013 at the 78 ha National University of Science and Technology in Bulawayo, Zimbabwe (20°08′S, 28°36′E, 1341 m a.s.l.). The area was fenced in 1993, and since then, it has not been grazed by mammalian herbivores or affected by fire. It experiences three climatic seasons: a hot wet period from November to April, a cool dry period from May to July and a hot dry period from August to October. The rainy season is from November to April, and the mean annual rainfall is 600 mm, with a range from 325 to 915 mm (Mlambo, Mwenje & Nyathi, 2007). The mean annual temperature is 23.6°C. October is the hottest month, and July is the coldest. The site has a slope angle of 3–5°, and the soil is Chromic Luvisol developed from granite with a sandy loam texture (Mlambo, Mwenje & Nyathi, 2007). The vegetation is deciduous open woodland with a tree crown cover of 30–40%. Some of the common woody species include the three study species *Acacia robusta* Burch, *Dichrostachys cinerea* (L.) Wight and Arn. and *Ziziphus mucronata* Willd.

Study species

Acacia robusta Burch, *Dichrostachys cinerea* (L.) Wight and Arn. and *Ziziphus mucronata* Willd. are multistemmed. Tree branches were classified as main stem (emerging from the ground), large branches (those emerging from the main stem and normally >1 cm in diameter) and branchlets (3 cm from the shoot terminals where clipping was performed). *Dichrostachy cinerea* had six large branches (SD = 2.10, n = 7) and 37 branchlets (SD = 11.76, n = 7), *A. robusta* three large branches (SD = 1.07, n = 8) and 19 branchlets (SD = 4.23, n = 8) and *Z. mucronata* eight large branches (SD = 2.87, n = 4) and 53 branchlets (SD = 16.39, n = 4). In *D. cinerea*, the diameter at base for main stem, large branches and branchlets were 3.82 cm (SD = 1.01, n = 7), 1.10 cm (SD = 0.47, n = 14) and 0.3 cm (SD = 0.11, n = 21), respectively. In *A. robusta*, the diameter at base for main stem, large branches and branchlets were 4.77 cm (SD = 1.87, n = 8), 2.10 cm (SD = 0.87, n = 12) and 0.4 cm (SD = 0.21, n = 14), respectively. In *Z. mucronata*, the diameter at base for main stem, large branches and branchlets were 4.45 cm (SD = 2.21, n = 4), 2.10 cm

(SD = 0.67, n = 7) and 0.2 cm (SD = 0.16, n = 13), respectively. Tree height was 3.5 m (SD = 1.92, n = 7), 4.6 m (SD = 2.04, n = 8) and 5.21 m (SD = 2.94, n = 4) for *D. cinerea*, *A. robusta* and *Z. mucronata*, respectively. The three woody species depend on spines and leaf chemistry for antiherbivory defences. *Acacia robusta* has paired straight stipular spines, *Z. mucronata* paired straight and curved and *D. cinerea* transformed lateral shoots forming spines. The spines on *D. cinerea* are sharp-tipped short shoots that have stopped growing at an early stage of development and have leaf-bearing nodes. In *A. robusta* and *Z. mucronata*, the spines are produced and fully lignify during the growth season.

Spine measurements

Three 80 m × 30 m belt transects were set out within a flat and uniform area of *Acacia* dominated woodland. All *A. robusta*, *D. cinerea* and *Z. mucronata* trees within each belt transect were identified. For *A. robusta*, six, nine and five trees were identified in each belt transect, respectively. For *D. cinerea*, twelve, ten and fifteen trees were identified in each belt transect, respectively. For *Z. mucronata*, four, three and six trees were identified in each belt transect, respectively. In each belt transect, within a species, all identified trees were allocated numbers, and then, three randomly selected by drawing their numbers from a box. Terminal shoots were then clipped (3 cm from tip) from ten branchlets per plant in February 2012, in the middle of the rainy season when the trees had foliage, to simulate browsing by mammalian browsers, using garden shears. Individual tree branchlets were treated as autonomous (Riba, 1998). Branchlets at a height of between 1 m and 1.5 m were sampled because they were well within the reach of many large mammals. Each of the clipped branchlet was marked with white primer paint over the bark. In February 2013 (1 year after clipping), the trees were revisited and new shoots that emerged from the clipped branchlets (hereafter referred to as resprouts) were identified. These new shoots arose from axillary buds just below the damaged shoot tip. The spines on these resprouts were counted, and spine density expressed as the number of spines per cm, while spine lengths were measured to the nearest mm. New shoots from unclipped branchlets within the same height and of similar size (hereafter referred to as nonresprouts) were selected, and their density and length determined in a similar manner.

Condensed tannins

About 20 g of fresh leaves of both resprouts and nonresprouts was collected per branchlet per plant and air-dried in the shade. The leaf samples were then oven-dried at 60°C to constant weight in the laboratory and then ground in a Wiley mill to pass through a 1-mm sieve. Condensed tannins were extracted from the dry leaf powder with 20 ml of 50% (v/v) methanol/water in an ultrasonic bath for 15 min. The samples were diluted to 1 mg ml^{-1} and centrifuged at 200 g for 15 min to decant the extract. Condensed tannins were assessed by the butanol method (Waterman & Mole, 1994), but the extract was diluted to a final concentration of 1.0 mg ml^{-1}. Absorbance was measured at 550 nm using a spectrophotometer.

Statistical analysis

All data were normally distributed (Kolmogorov–Smirnov test) (SPSS, 2002). Data on spine length, spine density and condensed tannins concentrations were averaged for the ten branchlets per plant and then subjected to a paired *t*-test (resprouts versus nonresprouts) using SPSS (SPSS, 2002) to test for the response of the woody species to simulated herbivory.

Results

In *A. robusta* ($t = 4.945$, DF = 8, $P = 0.08$), spine length of resprouts was twice that of nonresprouts, while in *D. cinerea* ($t = -0.240$, DF = 8, $P = 0.833$) and *Z. mucronata* ($t = -0.200$, DF = 8, $P = 0.854$), it was similar (Fig. 1).

Resprouts spine density was double that of nonresprouts in *Z. mucronata* ($t = 7.319$, DF = 8, $P < 0.05$) but similar in *A. robusta* ($t = 1.178$, DF = 8, $P = 0.304$) and *D. cinerea* ($t = 1.094$, DF = 8, $P = 0.354$) (Fig. 2).

The condensed tannins concentration of the resprouts was higher than the nonresprouts in all the three woody species (*A. robusta*: $t = 13.71$, DF = 8, $P < 0.05$; *Z. mucronata*: $t = 127.31$, DF = 8, $P < 0.001$; *D. cinerea*: $t = 23.00$, DF = 8, $P < 0.01$) (Fig. 3).

Discussion

Our study supported the hypothesis that woody species produce localized branch-specific antiherbivory responses to defoliation. Young, Stanton & Christian (2003) reported
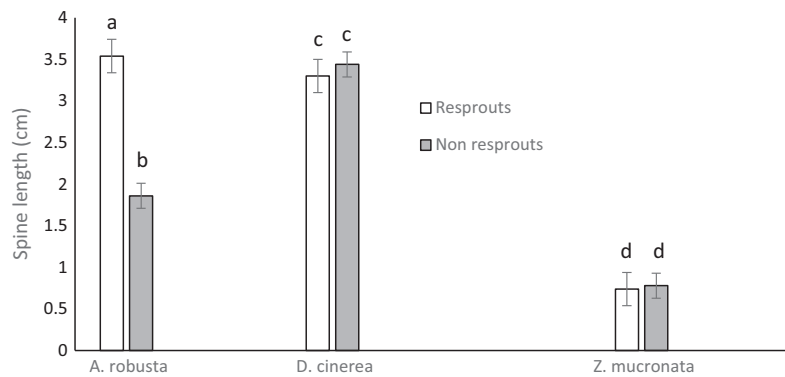
Fig 1 Mean (±SE) spine length of resprouts and non respouts of *A. robusta, D. cinerea* and Z. *mucronata*. Different letters indicate significant differences between the resprouts and non resprouts within a species (paired *t*-test: $P < 0.05$)
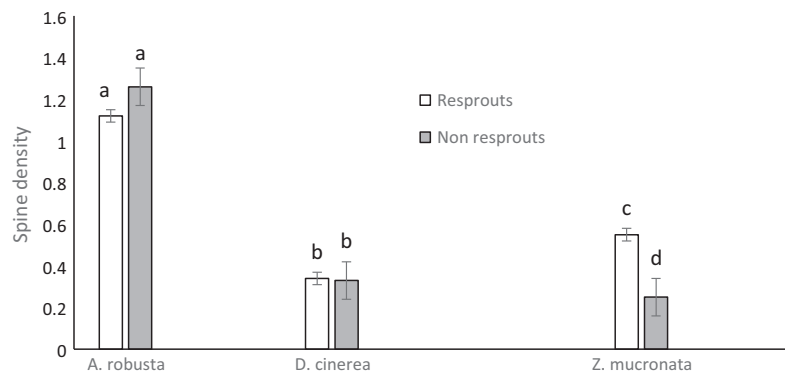

Fig 2 Mean (±SE) spine density (number of spines per cm) of resprouts and non respouts of *A. robusta, D. cinerea* and *Z. mucronata*. Different letters indicate significant differences between the resprouts and non resprouts within a species (paired *t*-test: $P < 0.05$)
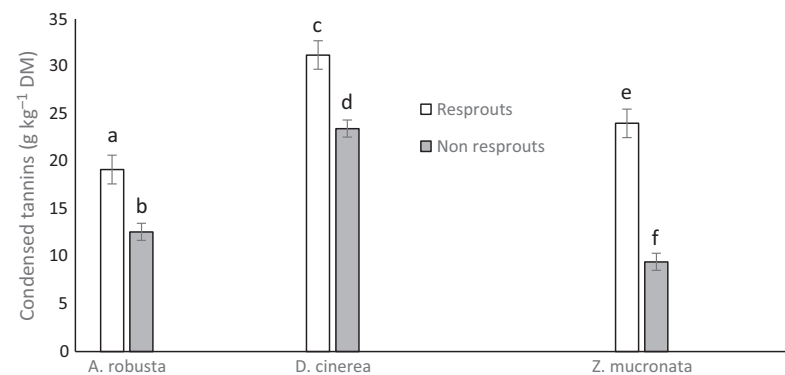

Fig 3 Mean (±SE) condensed tannins (g kg^{-1} DM) of resprouts and non respouts of *A. robusta, D. cinerea* and *Z. mucronata*. Different letters indicate significant differences between the resprouts and non resprouts within a species (paired *t*-test: $P < 0.05$)

the induction of increased spine length in *A. drepanolobium* as extremely local with adjacent branches not responding to experimental shoot removal with increased spine lengths. Other researchers have also reported spines on taller branches as responding independently from lower branches, even on the same trees (Young, 1987; Milewski,

Young & Madden, 1991; Young & Okello, 1998). Tuomi *et al.* (1988) found the induced defence responses to be stronger in the parts of the plant closer to the site of defoliation. Rooke *et al.* (2004) reported within-plant differences in condensed tannins of woody plants between lower and higher tree branches. The three study species were highly sectored through branching such that the exchange of nutrients, secondary chemicals and hormones that mediate plant–herbivore interactions was limited to those plant tissues that shared active vascular connections (Orians & Jones, 2001; Schittko & Baldwin, 2003). Karban (2011) reported plant responses to herbivory as highly localized with little integration at the scale of the whole plant. Karban & Adler (1996) suggested that localized, branch-specific induction of increased spine length did not evolve under the selective pressure of temporal variation in the presence or absence of herbivores.

Rohner & Ward (1997) suggested that inducible physical defences may be common in many *Acacia* species and perhaps also in other spinescent plants of arid and semi-arid regions. Spine length was twice in *A. robusta* with density doubling in *Z. mucronata* in response to simulated herbivory. However, shoot clipping did not illicit a response in *D. cinerea* in any of the two measured physical defence parameters. This could be attributed to the different origins of *D. cinerea* spines, which are considered as transformed lateral shoots. Rooke *et al.* (2004) found induced responses of spines to depend on their origin.

In this study, large mammalian herbivores have been excluded for more than twenty years. Young, Stanton & Christian (2003) demonstrated that simulated large mammal browsing induces greater spine length on trees that had relaxed spine length after several years of herbivore exclusion.

In all the woody species, the condensed tannins concentration increased in response to shoot clipping. Clipping the terminal 3 cm of shoots can be considered as simulating less severe browsing. Ward & Young (2002) proposed that less severe browsing induces the production of condensed tannins, while more severe browsing induces growth responses with high carbohydrates demands, which would limit the production of condensed tannins. For example, heavily browsed *A. nigrescens* was found to have greatly reduced condensed tannin concentrations, creating a positive feedback loop between the plants and the herbivores (du Toit, Bryant & Frisby, 1990; Fornara & du Toit, 2007; Skarpe & Hester, 2008).

Although the limited number of species precludes generalizations to be made, our results show that woody plants respond to herbivory through localized, branch-specific induced increases in condensed tannins and spinescence.

Acknowledgements

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