

Changes in spinescence across leaf ontogeny support the optimal defence hypothesis in blackberries (*Rubus adenotrichos*)

Research Article

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Corresponding author:

Alejandro Farji-Brener;
Email: alefarji@yahoo.com

Alejandro Farji-Brener¹ , Débora Elías Díaz² , Isabelle Holanda³ ,
Andrés Sierra Ricaurte⁴ , Kenneth Barrantes⁵  and Pablo José Gutiérrez-Campos⁶ 

¹LIHO, Inibioma, Conicet-Universidad Nacional del Comahue, Argentina; ²Universidad de El Salvador, Facultad Multidisciplinaria de Occidente, El Salvador; ³Laboratorio de interacción planta-animal, Universidad Federal de Pernambuco, Brasil; ⁴Universidad Nacional de Colombia, Colombia; ⁵Universidad de Costa Rica, Costa Rica and ⁶Universidad Nacional de Costa Rica, Costa Rica

Abstract

Hypotheses based on allocation theory and herbivore selection offer opposite predictions about how defence levels against herbivores change as the plant tissue grows. The growth differentiation balance hypothesis (GDBH) assumes that defences will be resource-limited in immature tissues and predict that defence levels increase as the plant tissue grows. Conversely, the optimal defence hypothesis (ODH) proposes that plants would have the highest level of defences in the parts that have the highest value in terms of fitness and/or are more frequently attacked by herbivores, such as young tissues. We examine whether spinescence in the shrub *Rubus adenotrichos* (blackberry) change as the leaf grows, and if this change is consistent with the GDBH or the ODH. We compare the petiole area occupied by prickles, the prickles density and the individual prickle area in mature versus young petioles from *Rubus adenotrichos*. Our results show that, in *R. adenotrichos*, young tissues are more protected than mature tissues. Prickles density and the petiole area occupied by prickles were up to 25% higher in young petioles than in mature ones. These results support the ODH, reinforcing the idea that extrinsic factors such as herbivores pressure might drive the change of structural defences level across leaf ontogeny.

Introduction

Herbivory can reduce plant fitness and thereby influence the selection of defence traits (Coley and Barone 1996; Marquis 1992). However, the level and kind of defences against herbivores often change dramatically through plant development depending on internal and external constraints (Boege et al. 2007). Several studies have found variation in physical as well as in chemical plant defences across ontogenetic stages in both individual shoots/leaves or entire plants (Barton and Koricheva 2010; Kariñho-Betancourt et al. 2015). Understanding why defences change as plants grow might provide key insights into the role of allocation of resources and herbivory pressure on the ecology and evolution of plant traits.

Two current hypotheses, based on allocation theory and herbivore selection, attempt to explain the ontogenetic changes in plant defences against herbivory and offer opposite predictions about the general directionality of those patterns. The growth differentiation balance hypothesis (GDBH) assumes that plants need to maintain a balance between resources used for growth and for differentiation, which includes defence production (Herms and Mattson 1992). Since defence production is often costly, plants that allocate resources to the defence of young leaves will grow slower than they otherwise might (Herms 1999; Herms and Mattson 1994). Consequently, the GDBH assumes that defences will be resource-limited in immature tissues and predict that defence levels increase as plants grow/leaves mature (Herms and Mattson 1992, 1994). In contrast, the optimal defence hypothesis (ODH) proposes that extrinsic factors such as selection by herbivores are key to determining where and when to assign defences (Bryant et al. 1992; Rhoades 1979). The ODH assumes that plants would have the highest levels of defences in those parts that have the highest value in terms of fitness and/or are more frequently attacked by herbivores, such as young tissues (Zangerl and Bazzaz 1992; Zangerl and Rutledge 1996). Consequently, the ODH predicts that defence levels will decrease as plants or tissues grow (Bryant et al. 1992). Given that previous research partly supports both predictions (Barto and Cipollini, 2005; Barton and Koricheva 2010; Cronin and Hay 1996), more studies are still necessary that include different types of defences to better determine which pattern is more common and the conditions under which one or the other is favoured.

Ontogenetic changes at the plant level and at chemical defences have been more studied than changes at the organ level and in physical defences. For example, recent works often focus only



Figure 1. The Cuericí Biological Field Station, Costa Rica, where the study was carried out (A), shrub of *Rubus adenotrichos* (B), mature (C) and young (D) petioles with their leaves, and a detail of the spines in the petiole (E).

at plant level (Barton and Koricheva 2010; Boege et al. 2007; Boege and Marquis 2005; Hanley et al. 2007; McCall and Fordyce 2010; Moreira et al. 2012, 2020; Ochoa-López et al. 2015, 2020; Ohnmeiss and Baldwin 2000), and in the review of Boege and Marquis (2005), only one of 17 reviewed papers included a structural component (Wolfson and Murdock, 1990). Hence, the study of how physical defences change as the plant tissue grows will help to determine whether the GBDH or OD hypotheses can explain ontogenetic changes both at plant and organ level. Moreover, structural plant defences are not all the same, given that they can differ in their costs and developmental timing (Armani et al. 2020, 2019; Coverdale 2019). Sharp projections from plants, commonly known as spinescence, play a key role in plant defence but can arise from different plant tissue and/or organ (Cooper & Owen-Smith 1986; Cornelissen et al. 2003; Crofts, and Stankowich 2021; Gowda 1996; Hanley et al. 2007; Obeso 1997). Consequently, their emergence during plant growth and their importance as defence may be constrained by the development of those plant parts (Armani et al. 2019, 2020; Clark and Burns 2015). Since spines arise from modified leaves, thorns from twigs or branches, and prickles from epidermal tissue, spines may emerge earliest in conjunction with the first growth of leaves, prickles shortly thereafter due to a slightly longer developmental period, and thorns only after primary stem growth and branching (Coverdale 2019). Thus, the study of how different types of spinescence change as tissue grow may contribute to understand the relationship between plant growth and defence assignation (Hanley et al. 2007).

The shrub *Rubus adenotrichos* (blackberry) is a good model to test how physical defences such as prickles change as plants grow. First, this species shows high abundance of prickles on petioles and stems (Hammel et al. 2014). Second, their leaves have high nutritional value and are preferred forage for several vertebrate herbivores (Kandylyis et al. 2009). Consequently, physical defences such as prickles might play a key role in reducing foliar damage. Finally, young and mature petioles that sustain young and mature leaves are easy to distinguish and are located near each other allowing comparisons within the same plant. Here, we focus on

within-plant variation in defence (e.g., Barto and Cipollini 2005), and determine whether the petiole area occupied by prickles and the prickles density in the shrub *R. adenotrichos* change as the leaves grow, and if this change is consistent with the GBDH or with the ODH.

Material and methods

Study area and species

This study was carried out in the Cuericí Biological Field Station, Costa Rica (9°33'30" N, 83°39'42" W, Figure 1A). The elevation is ca 2800 m, the mean annual temperature is 8°C, the mean annual precipitation is 6500 mm, and the vegetation is classified as montane wet forest (Holdridge 1967). Sampling was conducted on *Rubus adenotrichos* (blackberry) shrubs that surrounded the Biological Station (Figure 1B). Hybrid Andean blackberries are native from Mexico to Ecuador and are widely cultivated in the south of Costa Rica for their edible polyphenolics-rich fruits, which are eaten fresh or consumed as juice, jelly and wine (Castro and Cerdas 2005). *R. adenotrichos* is a shrub up to 5 m tall, with copious hairs and scattered curved prickles in its stems and petioles. Leaves are compound, with three or five leaflets. Flowers are white or pink, and fruits are red or black (Hammel et al. 2014, Figure 1C, D). The main vertebrate herbivores in the region are tapir (*Tapirus* spp) and rabbits (*Sylvilagus* spp) (Kappelle and Horn 2016).

Methods

Sampling

In the summer of 2022 (January and February), we randomly selected 51 plants of *R. adenotrichos* with 2 ± 0.5 m in height (mean \pm SE) that were, at least, 5 m apart from each other. All individuals were located in an open field, receiving full sun. We covered a total sampling area of approximately 2 ha. In each individual, we randomly selected one or two young and mature petioles that emerged from the same stem. Young and mature petioles and their leaves were easily distinguished because of their

colour (light green for young leaves and dark green for mature leaves (Figure 1C and 1D), form, and location (terminal for young and more basal for mature). We sampled a total of 144 petioles, 72 young and 72 mature.

Trait measurements

We measured the length and the diameter of each petiole with callipers, the total number of prickles along the petiole, and the area of the petiole occupied by each individual prickle. The area of each petiole was estimated as its length \times its perimeter ($\pi \times 2r$), and the area of the petiole occupied by a prickle was calculated through photos with the software ImageJ[®] (Rasband 1997), considering the base of the spine such as a circle ($\pi \times r^2$) (Figure 1E). Finally, prickles density was calculated as # prickles/petiole length, and the petiole area covered by prickles was calculated as the ratio of prickles area versus petiole area. The mean area of an individual prickle, the petiole area covered by prickles and prickles density were compared between young and mature petioles using paired t-tests.

Results

Young and mature petioles differed in their area covered by prickles and prickles density, but individual prickles showed similar area (Figure 2). The area of petiole covered by prickles (proportion) was higher in young than in mature tissues (0.31 ± 0.0 vs. 0.23 ± 0.02 , $t = 2.8$, $P < 0.001$). Accordingly, prickles density was higher in young than in mature tissues (3.9 ± 0.2 vs. 2.8 ± 0.1 prickles/cm², respectively, $t = 4.6$, mean \pm SE, $P < 0.001$). Finally, the mean area of an individual prickle was similar between young and mature tissues (0.085 ± 0.006 vs. 0.083 ± 0.006 cm², mean \pm SE, $t = 0.3$, $P = 0.78$).

Discussion

Internal restrictions, such as the need for growth or differentiation, and external factors, such as natural enemies, can both affect how anti-herbivory defences change as the plant tissue grows. As explained before, the ODH predicts that young tissues should be more defended relative to older tissues because the former are more vulnerable and more valuable to plant fitness. In contrast, the GDBH predicts that young tissues should be less defended than mature tissues because growth processes precede differentiation processes. Here, we found evidence that supports the ODH, illustrating how herbivory pressure might drive the assignment of plant defences.

Our comparative results suggest that, in *R. adenotrichos*, young tissues are more protected than mature tissues because prickles density and the petiole area occupied by prickles were up to 25% higher in young than in mature petioles. Since the area of petiole occupied by an individual prickle was similar between young and mature petioles, our results suggest that the production of prickles precedes the elongation of the petiole, resulting in higher prickles density in earlier stages of petiole development. Several comparative and experimental studies demonstrated that spinescence are an effective defence against vertebrate herbivores that often prefer younger plant tissues (Fenner et al. 1999; Hanley et al. 2007). For example, in East Africa, the large thorns of *Acacia tortilis* protect young leaves and axillary meristems from herbivory by goats (Gowda 1996; Gowda and Palo 2003), and shrubs of *Ilex aquifolium* with exceptionally spiny leaves are much less likely to suffer herbivory by ungulates than neighbouring less

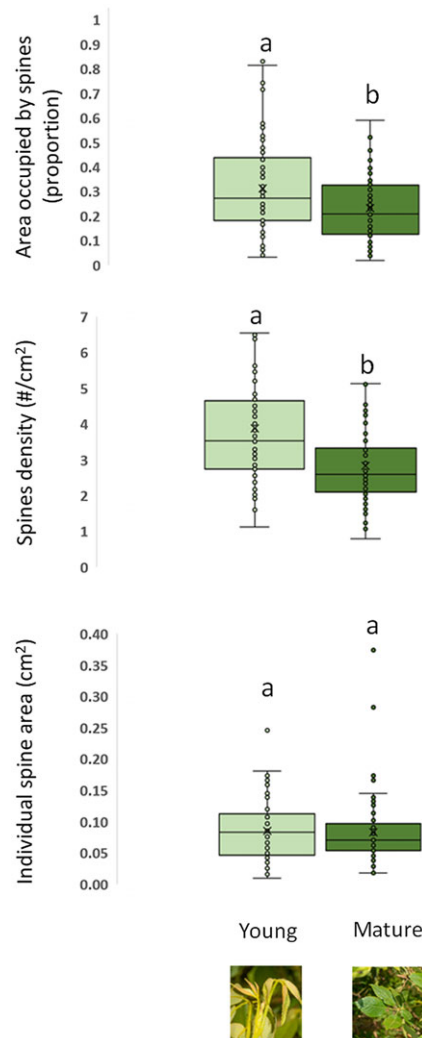


Figure 2. Petiole area occupied by spines (proportion), spine density (#/cm²), and individual spine area (cm²) in mature and young petioles. Different letters imply statistical significant differences (paired t-test; see text for more details).

spiny plants (Obeso 1997). It is known that herbivores choice not only is driven by spines but also relies on many other plants traits. However, and given that the large body of evidence from previous works, it seems logical to assume that young tissues of *R. adenotrichos* could more preferred by herbivores and that prickles could be an effective anti-herbivory defence.

A higher prickles density in early stages of leaf development is also documented in other plant species and in similar physical defences, like trichomes (Kellogg et al. 2011). For example, young leaves of *Verbascum thapsus* show higher hair density and are less eaten than mature leaves (Woodman and Fernandes, 1991). Similarly, a high initial trichrome density in *Aristolochia californica* helps protect its emerging leaves from herbivory (Fordyce and Agrawal, 2001), and trichome density decreased with age in two Japanese Birch species (Matsuki et al. 2004). Taken together, all this evidence suggests that structural defences such as spinescence may play a relevant role against vertebrate herbivore in young leaves, in which the loss of photosynthetic tissue represents a higher cost than in mature ones (Barton and Koricheva 2010; Hanley et al. 2007; Ochoa-López et al. 2015, 2020). Moreover, this structural defence in young tissues could also reduce the herbivory

of the entire plant. Shrubs like blackberry tend to privilege lateral growth over height growth. Consequently, the plant forms a structure so that younger, softer tissues are also in the external part of the plant and therefore more exposed to vertebrates. A higher density of spinescence in younger tissues not only can protect the more exposed new leaves but could also act as a spinescence shield protecting the most internal part of the shrub (Archibald and Bond 2003).

Despite the fact that vertebrate herbivores have been considered a key selective force driving the evolution of spinescence (Charles-Dominique et al. 2017; Cooper and Owen-Smith 1986; Coverdale 2019; Hanley et al. 2007), structural plant defences and ontogenetic changes at organ level have been less studied than chemical defences and changes at plant level (Barton and Koricheva 2010; Hay et al. 1994; Mithöfer and Boland 2012). Therefore, the study of how less known structural defences like prickles change as plant tissues grow will help to better understand the relationship between ontogeny and the assignment of anti-herbivore defences. Our single-species study provides additional support to the ODH, suggesting that herbivore pressure can drive the assignment of plant structural defences as tissues grow.

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Competing interests. The authors declare none.

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