Journal of Tropical Ecology

www.cambridge.org/tro

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

Alejandro Farji-Brener[1], Débora Elías Díaz[2], Isabelle Holanda[3], Andrés Sierra Ricaurte[4], Kenneth Barrantes[5] and Pablo José Gutiérrez-Campos[6]

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

Research Article

Cite this article: Farji-Brener A, Díaz DE, Holanda I, Ricaurte AS, Barrantes K, and Gutiérrez-Campos PJ (2023). Changes in spinescence across leaf ontogeny support the optimal defence hypothesis in blackberries (*Rubus adenotrichos*). *Journal of Tropical Ecology*. **39**(e30), 1–5. doi: https://doi.org/10.1017/S0266467423000202

Received: 20 May 2022
Revised: 31 March 2023
Accepted: 12 June 2023

Keywords:
Costa Rica; herbivory; tropical montane forest; plant physical defences

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

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

© The Author(s), 2023. Published by Cambridge University Press



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 (Kandylis 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 GDBH 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 ± 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

https://doi.org/10.1017/S0266467423000202 Published online by Cambridge University Press

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 x its perimeter ($\pi * 2r$), and the area of the petiole occupied by each prickle was calculated through photos with the software ImageJ ® (Rasband 1997), considering the base of the spine such as a circle ($\pi * r^2$) (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 /cm^2, respectively, $t = 4.6$, mean ± 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 cm^2, mean ± SE, $t = 0.3$, $P = 0.78$).

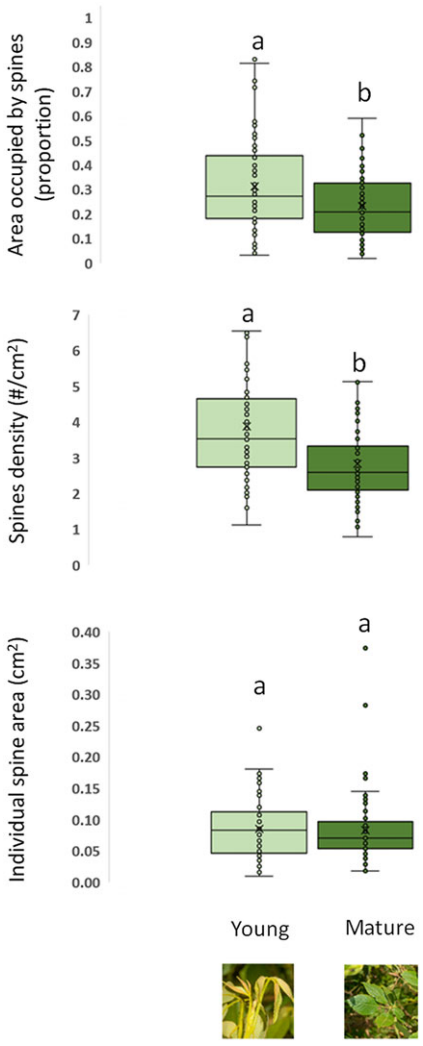


Figure 2. Petiole area occupied by spines (proportion), spine density (#/cm^2), and individual spine area (cm^2) in mature and young petioles. Different letters imply statistical significant differences (paired *t*-test; see text for more details).

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 assignation 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 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

https://doi.org/10.1017/S0266467423000202 Published online by Cambridge University Press

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 assignation of anti-herbivore defences. Our single-species study provides additional support to the ODH, suggesting that herbivore pressure can drive the assignation of plant structural defences as tissues grow.

Acknowledgements. We thank two anonymous reviewers and Kety Huberman for their helpful suggestions. We also thank the Organization for Tropical Studies for its logistical support. This research received no specific grant from any funding agency, commercial or not-for-profit sectors.

Competing interests. The authors declare none.

References

Archibald S and Bond WJ (2003) Growing tall vs growing wide: tree architecture and allometry of Acacia karroo in forest, savanna, and arid environments. *Oikos* **102**(1), 3–14.

Armani M, Charles-Dominique T, Barton KE and Tomlinson KW (2019) Developmental constraints and resource environment shape early emergence and investment in spines in saplings. *Annals of Botany* **124**(7), 1133–1142.

Armani M, Goodale UM, Charles-Dominique T, Barton KE, Yao X and Tomlinson KW (2020) Structural defence is coupled with the leaf economic spectrum across saplings of spiny species. *Oikos* **129**(5), 740–752.

Barto EK and Cipollini D (2005) Testing the optimal defence theory and the growth-differentiation balance hypothesis in *Arabidopsis thaliana*. *Oecologia* **146**(2), 169–178.

Barton KE and Koricheva J (2010) The ontogeny of plant defence and herbivory: characterizing general patterns using meta-analysis. *The American Naturalist* **175**(4), 481–493.

Boege K, Dirzo R, Siemens D and Brown P (2007) Ontogenetic switches from plant resistance to tolerance: minimizing costs with age? *Ecology Letters* **10**(3), 177–187.

Boege K and Marquis RJ (2005) Facing herbivory as you grow up: the ontogeny of resistance in plants. *Trends in Ecology and Evolution* **20**(8), 441–448.

Bryant JP, Reichardt PB, Clausen TP, Provenza FD and Kuropat PJ (1992) *Woody plant-mammal interactions*. In Rosenthal GA and Berenbaum MR (eds). *Herbivores: their interactions with secondary plant metabolites. 2nd ed. Vol. 2: evolutionary and ecological processes*. Academic Press.

Castro J and Cerdas M (2005) *Mora (*Rubus *spp) Cultivo y Manejo Pos-cosecha*. San José, Costa Rica: MAG (Ministerio de Agricultura).

Charles-Dominique T, Barczi JF, Le Roux E and Chamaillé-Jammes S (2017) The architectural design of trees protects them against large herbivores. *Functional Ecology* **31**(9), 1710–1717.

Clark LL and Burns KC (2015) The ontogeny of leaf spines: progressive versus retrogressive heteroblasty in two New Zealand plant species, *New Zealand Journal of Botany* **53**(1), 15–23. https://doi.org/10.1080/0028825X.2014.997254

Coley PD and Barone JA (1996) Herbivory and plant defences in tropical forests. *Annual Review of Ecology and Systematics* **27**(1), 305–335.

Cooper SM and Owen-Smith N (1986) Effects of plant spinescence on large mammalian herbivores. *Oecologia* **68**(3), 446–455.

Cornelissen JH, Lavorel S, Garnier E, Díaz S, Buchmann N, Gurvich DE, ... Poorter H (2003) A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Australian Journal of Botany* **51**(4), 335–380.

Coverdale TC (2019) Defence emergence during early ontogeny reveals important differences between spines, thorns and prickles. *Annals of Botany* **124**(7), iii–iv.

Crofts SB and Stankowich T (2021) Stabbing spines: a review of the biomechanics and evolution of defensive spines. *Integrative and Comparative Biology* **61**(2), 655–667.

Cronin G and Hay ME (1996) Within-plant variation in seaweed palatability and chemical defences: optimal defence theory versus the growth-differentiation balance hypothesis. *Oecologia* **105**(3), 361–368.

Fenner M, Hanley ME and Lawrence R (1999) Comparison of seedling and adult palatability in annual and perennial plants. *Functional Ecology* **13**(4), 546–551.

Fordyce JA and Agrawal AA (2001) The role of plant trichomes and caterpillar group size on growth and defence of the pipevine swallowtail Battus philenor. *Journal of Animal Ecology* **70**(6), 997–1005.

Gowda JH (1996) Spines of *Acacia tortilis*: what do they defend and how? *Oikos*, 279–284.

Gowda JH and Palo RT (2003) Age-related changes in defensive traits of *Acacia tortilis* Hayne. *African Journal of Ecology* **41**, 218–223.

Hammel EB, Grayum MH, Herrera C and Zamora N (2014) Manual De Plantas De Costa Rica. St. Louis, Mo.: Missouri Botanical Garden.

Hanley ME, Lamont BB, Fairbanks MM and Rafferty CM (2007) Plant structural traits and their role in anti-herbivore defence. *Perspectives in Plant Ecology, Evolution and Systematics* **8**(4), 157–178.

Hay ME, Kappel QE and Fenical W (1994) Synergisms in plant defences against herbivores: interactions of chemistry, calcification, and plant quality. *Ecology* **75**(6), 1714–1726.

Herms DA (1999) Physiological and abiotic determinants of competitive ability and herbivore resistance. *Phyton* **39**, 53–64.

Herms DA and Mattson WT (1992) The dilemma of plants: to grow or defend. *The Quarterly Review of Biology*, **67**(3), 283–335.

Herms DA and Mattson WT (1994) Plant growth and defence. *Trends Ecology Evolution* **9**, 488.

Holdridge LR (1967) *Life zone ecology*. San José, Costa Rica: Tropical Science Center, 206 pp.

Kandylis K, Hadjigeorgiou I and Harizanis P (2009) The nutritive value of mulberry leaves (Morus alba) as a feed supplement for sheep. *Tropical Animal Health and Production* **41**(1), 17–24.

Kappelle M, & Horn SP (2016) The Páramo ecosystem of Costa Rica's highlands. *Costa Rican Ecosystems* **744**.

Kariñho-Betancourt E, Agrawal A, Halitschke R and Núñez-Farfán J (2015) Phylogenetic correlations among chemical and physical plant defenses change with ontogeny. *New Phytologist* **206**(2), 796–806.

Kellogg AA, Branaman TJ, Jones NM, Little CZ and Swanson JD (2011) Morphological studies of developing *Rubus prickles* suggest that they are modified glandular trichomes. *Botany* **89**(4), 217–226.

Marquis RJ (1992) A bite is a bite is a bite? Constraints on response to folivory in *Piper arieianum* (Piperaceae). *Ecology* **73**(1), 143–152.

Matsuki S, Sano Y and Koike T (2004) Chemical and physical defence in early and late leaves in three heterophyllous birch species native to northern Japan. *Annals of Botany* **93**(2), 141–147.

McCall AC and Fordyce JA (2010) Can optimal defence theory be used to predict the distribution of plant chemical defences? *Journal of Ecology* **98**, 985–992.

Mithöfer A and Boland W (2012) Plant defence against herbivores: chemical aspects. *Annual Review of Plant Biology* **63**, 431–450.

Moreira X, Abdala-Roberts L, Galmán A, Bartlow AW, Berny-Mier y Teran JC, Carrari E, ... Rasmann S (2020) Ontogenetic consistency in oak defence syndromes. *Journal of Ecology* **108**, 1822–1834.

Moreira X, Zas R and Sampedro L (2012) Differential allocation of constitutive and induced chemical defences in pine tree juveniles: a test of the optimal defence theory. *PLoS One* **7**(3), e34006.

Obeso JR (1997) The induction of spinescence in European holly leaves by browsing ungulates. *Plant Ecology* **129**(2), 149–156.

Ochoa-López S, Damián X, Rebollo R, Fornoni J, Domínguez CA and Boege K (2020) Ontogenetic changes in the targets of natural selection in three plant defences. *New Phytologist* **226**(5), 1480–1491.

Ochoa-López S, Villamil N, Zedillo-Avelleyra P and Boege K (2015) Plant defence as a complex and changing phenotype throughout ontogeny. *Annals of Botany* **116**(5), 797–806.

Ohnmeiss TE and Baldwin IT (2000) Optimal defence theory predicts the ontogeny of an induced nicotine defence. *Ecology* **81**, 1765–1783.

Rasband WS (1997) Image J1: Image processing and analysis in Java. Maryland, National Institute of Health. Available at https://imagej.net/ImageJ1

Rhoades DF (1979) *Evolution of plant chemical defence against herbivores*. In: Rosenthal GA and Janzen DH (eds) Herbivores: their interaction with secondary plant metabolites. New York and London: Academic Press, 4–53.

Wolfson JL and Murdock LL (1990) Growth of Manduca sexta on wounded tomato plants: role of induced proteinase inhibitors. *Entomologia Experimentalis et Applicata* **54**, 257–264.

Woodman RL and Fernandes GW (1991) Differential mechanical defense: herbivory, evapotranspiration, and leaf-hairs. *Oikos* **60**(1), 11–19.

Zangerl AR and Bazzaz FA (1992) Theory and pattern in plant defence allocation. In Fritz RS and Simms EL (eds) *Plant resistance to herbivores and pathogens: ecology, evolution, and genetics*. The University of Chicago Press, Chicago, EEUU, 610 pp.

Zangerl AR and Rutledge CE (1996) The probability of attack and patterns of constitutive and induced defense: a test of optimal defense theory. *The American Naturalist* **147**(4), 599–608.

https://doi.org/10.1017/S0266467423000202 Published online by Cambridge University Press