INSIGHTS

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Drought-induced reductions in plant defenses: Insights from extrafloral nectaries in the Caatinga dry forest

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Abstract

Extrafloral nectaries (EFNs) are anti-herbivory defense-related glands. We measured morphological and anatomical EFN traits in *Pityrocarpa moniliformis* trees along a rainfall gradient in Caatinga dry forest. We observed a reduction in structural EFN traits as rainfall decreased. We conclude that this reduction is a cost-saving strategy, probably mediated by ants.

KEYWORDS
ant–plant interactions, facultative mutualism, herbivory, indirect defense, intraspecific trait variation, phenotypic plasticity, precipitation, resource availability hypothesis

1 | INTRODUCTION

Many plants evolved a wide array of morphological and physiological strategies to protect themselves against herbivores (Mundim & Pringle, 2018). Some strategies involve resource allocation to physical and chemical traits and are considered direct defenses (Agrawal, 2007), whereas others require ecological interactions with herbivores enemies to be expressed and are named indirect defenses (Kessler & Heil, 2011). However, abiotic factors (e.g., climate, light, soil nutrients) also exert great pressure on plants and thus both biotic and abiotic factor might influence plant defensive traits (Abdala-Roberts et al., 2016; Hahn et al., 2019; López-Goldar et al., 2020).

The resource availability hypothesis extended to intraspecific variation (hereafter, RAH$_{intra}$) addresses *intra-specific variation* in plant defenses in populations along environmental gradients (Hahn & Maron, 2016). RAH$_{intra}$ predicts that plant growth rate and defense should be under increasing selective pressure as resource availability increases (Hahn & Maron, 2016). At the same time, while plants show greater productivity (high tissue quality and biomass) in high-resource environments, they may also attract herbivores (Koricheva et al., 1998). Therefore, plants in higher resource environments should be under higher herbivory damage (Cyr & Pace, 1993; Gely et al., 2020), leading to greater selective pressure on plant resistance (Karban, 2011; López-Goldar et al., 2020).

Extrafloral nectaries (EFNs) are an example of indirect plant defenses, in which a carbohydrate-rich food resource is produced predominantly to attract ants, which in turn help to protect plants from herbivores (Del-Claro et al., 2016; Fagundes et al., 2017; Heil, 2015). Because extrafloral nectar is composed of water, sugar, and amino acids, the expression of EFNs can be costly, which is, therefore, predicted to decrease in water-stressed environments (Newman & Wagner, 2013; Yamawo et al., 2012). Maintaining extrafloral nectar production during water deficits might be disadvantageous, particularly not only if plants are under low herbivory pressure (Fagundes et al., 2017; Rios et al., 2008) as predicted by RAH_{intra} but also if attendant-ants are less abundant and aggressive (Câmara et al., 2018; Oliveira et al., 2021). In such environments, plants might be expected to reduce the number (Nogueira et al., 2012; Rudgers & Strauss, 2004) and size of EFNs (Rios et al., 2008; Rudgers, 2004), as well as the amount of nectar secreted (Rios et al., 2008). The opposite would be true in wetter environments, where plants are under high herbivore pressure, and traits important for attracting ants, such as gland size and nectar secretion, should be favored (Rudgers, 2004; Rudgers & Strauss, 2004). Thus, the phenotypic mean of EFN traits among populations under different selection regimes (water availability, herbivory pressure, and ant protection) should differ.

Here, we assess whether EFN traits in *Pityrocarpa moniliformis* (Benth.) Luckow & R. W. Jobson (Fabaceae) change along a rainfall gradient in the Brazilian Caatinga – the largest patch of seasonally dry tropical forest globally (Silva et al., 2017). We attempted to extend the RAH_{intra} by including indirect defenses, which involves tri-trophic interactions (plant–herbivore–ants) rather than plant–herbivore interactions along abiotic gradient. *P. moniliformis* is widely distributed along a natural rainfall gradient (555 mm to 940 mm), in one of the largest protected areas of Caatinga dry forest, providing a fortuitous opportunity to assess the impact of rainfall on EFN traits. According to RAH_{intra}, *P. moniliformis* plants should invest in growth and defense in wetter sites. We focus on the predictions related to anatomical and morphological traits along a rainfall gradient. Extrinsic factors such as biotic (ant and herbivore presence) and abiotic (water availability) changes associated with the geographic distribution of plants can impact directly or indirectly EFN structure (Nogueira, Guimarães, et al., 2012; Nogueira et al., 2015). Thus, we expect that morphological (size) and anatomical (nectar secretion-related traits) EFN attributes will increase as rainfall increases. However, previous reports of herbivory and ant distribution patterns along the rainfall gradient in our study region show contrasting pressure on EFN traits. Andrade et al. (2020) showed that Caatinga plants might be under higher attack from chewing-insects in drier sites where leaf damage was twice as great as in wetter sites, which counters RAH_{intra} prediction of higher herbivory under higher resource availability (wetter sites, see Figure 1a). In this scenario, EFN traits would be favored in drier environments. On the other hand, Oliveira et al. (2021) found that more aggressive ants interact more frequently with *P. moniliformis* plants in wetter rather than drier sites, which agree with RAH_{intra} prediction of higher defense under higher resource availability (Figure 1b). In this case, EFN traits

would be favored in wetter environments (i.e., inverse responses of herbivores and ants along a rainfall gradient). Thus, it is unclear what the ultimate outcome of these abiotic and opposing biotic factors are along rainfall gradients in Caatinga dry forest. By investigating the changes in EFN traits along a rainfall gradient, we hope to improve our understanding of the interplay between abiotic and biotic factors that shape indirect-defense-related traits.

2 | METHODS

This study was conducted in Catimbau National Park (Catimbau NP, 8°24'00'' and 8°36'35'' S; 37°0'30'' and 37°1'40'' W) in Northeast Brazil, where *P. moniliformis* is the most abundant and widely distributed woody plant species (Rito et al., 2017). *P. moniliformis* presents a single EFN per leaf, located on the rachis between the first pair of pinna (Figure S1a). Its EFNs are plate- or dish-shaped, thus presenting a concave secretory surface that contains nectar droplets (Figure S1b). Anatomically, these EFNs consist of an external cuticle, a layer of epidermis and a secretory parenchyma that forms most of the gland's secretory tissue (Figure S1c). Vascular bundles are found near the base of the gland (Figure S1d), irrigating independently the nectary and also the surrounding tissues (Melo et al., 2010). Individuals of *P. moniliformis* were selected in seven permanent 0.1-ha plots (20 × 50 m), separated by a minimum of 2 km, which fall along a steep rainfall gradient (555–940 mm y^{-1}, see more details on our permanent plots at www.peldcatimbau.org). To characterise the rainfall gradient, mean annual rainfall within each plot was obtained from the WorldClim data base (www.worldclim.com.br) at a spatial resolution of 1 km. Because the rainfall and aridity indices were highly correlated ($r > .98$), as observed by previous studies conducted in Catimbau NP (Arnan et al., 2018; Oliveira et al., 2021; Rito et al., 2017), we retained rainfall only as our measure of water deficit.

To evaluate intraspecific variation in EFN size, 10 adult plants were randomly selected in each of our seven plots during the wet season (March to June) of 2015, when EFNs activity is high. All plants with a similar height (≥ 1 m and <2 m and diameter basal height ≥3 cm) and architecture (number of branches) were selected to control for possible ontogenetic effects that might influence anatomical and morphological EFN traits. We selected one branch per individual and collected a terminal and sun-exposed leaf from the second ramification from the trunk to tip to control for leaf age (Sfair et al., 2018). All sampled leaves presented active EFNs (i.e., extrafloral nectar was being secreted, which was possible to see with the naked eye). It is important to highlight that leaf area and number of branches of *P. moniliformis* did not differ along the rainfall gradient (Sfair et al., 2018), which may influence EFN size or the amount of EFNs per tree. The maximum length and diameter of each EFN was obtained with a digital caliper. We estimated EFN size as an elliptical area (EFN size = $EFN_{length} \times EFN_{diameter} \times \pi$).

To evaluate intraspecific variation in the anatomical traits related to EFN size/secretion, we selected 2–3 adult plants out of the 10 individuals selected previously for EFN size measurements in each

FIGURE 1 Conceptual model of the mechanisms driving extrafloral nectarie (EFN) traits in *Pityrocarpa moniliformis* along a rainfall gradient in Caatinga dry forest. (a) Plants in drier environment may present increased investment in EFN traits if leaf damage by chewing insects is higher. (b) Following the resource availability hypothesis extended to instraspecific variation (RAH$_{intra}$), plants in wetter environments may exhibit greater investments in EFN traits if attendant-ants are effective in protecting plants against herbivores

of our seven plots also during the wet season of 2015. From each individual, 10 EFNs from mature leaves (one EFN per leaf from 17 plants, giving a total of 170 EFN measurements) were fixed in FAA 50 (formaldehyde, 50% ethanol, glacial acetic acid) and dehydrated in a graded ethanol series (Johansen, 1940). For anatomical analysis with light microscopy, the EFN samples were infiltrated and embedded in paraffin. Longitudinal and transversal sections (10–12μm) were cut with a rotary microtome Leica Reichert-Jung and Autocut 2040 in the Microscopy Department of North-Eastern Strategic Technologies Center (CETENE) and stained with safranin and Astra blue (Kraus et al., 1998). Micrometry and photomicrography of treated nectary tissues was accomplished using a Leica DM500 microscope with Leica LAS EZ software. We then measured the two anatomical traits using the ImageJ software: (1) secretory parenchyma thickness (μm), which is important for nectar storage and/or the nectar secretion process; and (2) density of secretory cells (number of cells/mm^2), which contributes to nectar exudation. Finally, we calculated the mean parenchyma thickness and density of secretory cells.

We performed a principal component analysis (PCA) to characterize EFN structure in *P. moniliformis* in terms of our morphological and anatomical measurements. All EFN traits were standardized prior to the analysis. We then modelled the effect of rainfall on the first two axes of the PCA (i.e., EFN traits) using linear regression. The PCA and liner regression analyses were carried out using R (R Core Team, 2019).

3 | RESULTS

The two first PCA axes accounted for 98% of the total variability in EFN traits. The first PCA axis represented a gradient of increasing parenchyma thickness, density of secretory cells, and EFN size (loadings of 0.64, 0.60, and 0.48, respectively) and explained 78.3% of the total variance (Figure 2a). The second PCA axis represented a contrast between EFN size (loading of 0.86) and anatomical traits (parenchyma thickness, loading of −0.21, and density of secretory cells, loading of −0.46) and captured a further 21% of the total variance in the data set (Figure 2a). In Figure 2a, it is also possible to observe that plots were split up according to EFN traits. In general, the first PCA axis shows that the drier plots presented the lowest

values of anatomical traits and EFN size when compared to the wetter plots. This is supported by the fact that the first PCA axis was significantly and positively related to increasing rainfall (slope = 0.008, R_{adj}^2 = 72.4, F_6 = 16.7, p = .009, Figure 2b). Interestingly, the second PCA axis indicated that the two wettest plots (P30 and P17) showed the greatest contrasts between anatomical traits and EFN size (see Table S1), with *P30* being mostly influenced by anatomical traits while *P17* was mostly influenced by EFN size (Figure 2a). The second PCA axis was not significantly related to rainfall (slope = 0.001, F_6 = 0.77, p = .421, Figure 2c).

4 | DISCUSSION

The phenotypic variation in EFN traits across *P. moniliformis* populations in Caatinga dry forest suggests that rainfall is an important driver of indirect biotic defense allocation. Our results corroborate with the resource availability hypothesis extended to intraspecific variation (RAH$_{intra}$), which predicts that EFN traits would be favored in wetter environments. Although parenchyma thickness and density of secretory cells decreased 250% in sites with <600 mm annual rainfall, EFN size decreased almost fourfold in drier sites.

Although we obtain a snapshot of EFN trait variation among *P. moniliformis* populations in Catimbau NP, it is likely that the reductions in this indirect biotic defense trait is a result of the cost-benefit trade-off in drier environments. This result is in accordance with RAH$_{intra}$, which proposes that plant investment in defense decreases in lower resource environments. Reduction in EFN traits in drier environments has been suggested to result from a physiological

limitation (Newman & Wagner, 2013)—given that many EFNs are modified stoma that are permanently open (Heil, 2011), and are susceptible to water loss (as observed for *P. moniliformis*, see figure S1C, D). Interestingly, Oliveira et al. (2021) showed that EFN production in *P. moniliformis* does not vary with rainfall. A positive trend between EFN traits and rainfall might suggest an adaptive physiological process. Plants experiencing drought might adjust their anatomical (e.g., decreasing parenchyma thickness and density of secretory cells) and morphological (e.g., reducing surface area) EFN traits to optimize water use and maintain EFN production with reducing rainfall. Furthermore, we cannot rule out factors other than rainfall are important for shaping EFN traits in plant populations such as soil nutrients, light and soil water availability.

EFN traits are also likely to be a result of water availability direct impact on mutualistic partners, especially between attendant-ants (Leal & Peixoto, 2017; Nogueira et al., 2012, 2020; Pringle et al., 2013). Although plants in Caatinga dry forest are likely to be susceptible to damage from leaf-chewing insects in drier environments (Andrade et al., 2020), attendant-ant species visiting EFN-bearing plants decrease markedly with water scarcity (Câmara et al., 2018) and vary thought time (Câmara et al., 2019). In fact, *P. moniliformis* visitations by attendant-ants were reduced both quantitatively (number of ant interactions per branch) and qualitatively (protection effectiveness) in drier environments in Catimbau NP (Oliveira et al., 2021). Perhaps ants are playing an important role in shaping anatomical and morphological EFN traits as rainfall increase in Caatinga dry forest. However, although previous studies focused on herbivory (Andrade et al., 2020) and ant defense (Oliveira et al., 2021) in Catimbau NP, our study only showed the variation in EFN traits
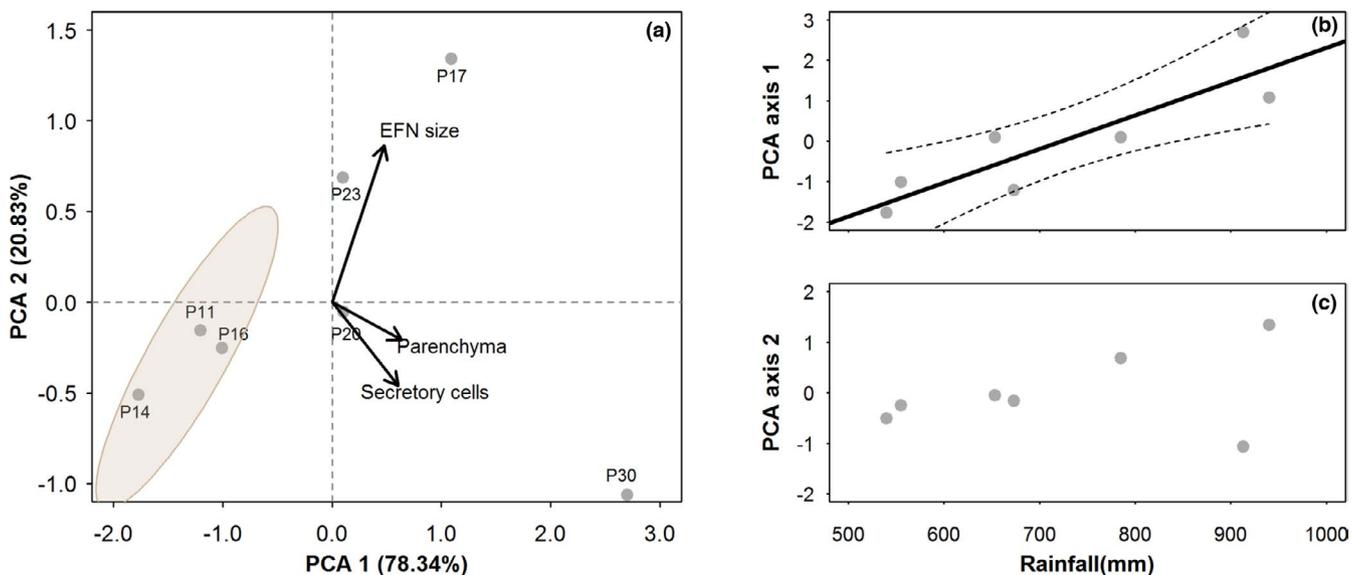

FIGURE 2 Variation in the extrafloral nectarie (EFN) traits of *Pityrocarpa moniliformis* along a rainfall gradient in Catimbau National Park (Pernambuco, NE Brazil). Principal component analysis (PCA) of anatomical (parenchyma thickness and density of secretory cells) and morphological (size) EFN traits *of Pityrocarpa moniliformis* used to calculate the EFN traits (a). Ellipse areas represents drier plots. Relationship between the first PCA axis (b) and the second PCA axis (c) (structural EFN traits) with rainfall. Dots represent the studied plots. Dashed lines represent 95% confidence intervals

along a rainfall gradient. This leaves a gap in the understanding of the sources of variation and function of EFN-related traits. A better and deeper understanding of plasticity in EFN secretion versus canalization in anatomical traits is still needed from a phenotypic integration perspective. This would allow predicting how EFN variation as a complex trait sheds light on the effects of EFN on ant–plant–herbivore interactions along environmental gradients.

In conclusion, we found that rainfall acts as a predictor of *P. moniliformis* plant indirect biotic defense strategies in Caatinga dry forest. Our findings suggest that EFN traits, such as parenchyma thickness, density of secretory cells and EFN size, are favored in wetter sites, whereas populations in which water is a limited resource, plant investment in biotic defenses is reduced. We therefore urge further studies to explore the interplay between abiotic and biotic factors driving EFN traits and the consequences for plants fitness, as well as the possible trade-offs between direct and indirect biotic defenses across plants populations.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are openly available in the Dryad Digital Repository: https://doi.org/10.5061/dryad.5mkkwh773 (Câmara et al., 2021).

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