Effects of florivory on the anatomy, histochemistry and resource production of flowers of Senna aversiflora (Herb.) H.S.Irwin & Barneby

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Abstract

Florivory directly affects floral structures, especially petals and anthers. The physical damage to these whorls can alter the characteristics of the flowers, compromise their functions and, consequently, impact fertility and reduce the reproductive success of the species. We provide the floral anatomical description of *Senna aversiflor*a (Herb.) H.S.Irwin & Barneby. We measured various anatomical traits of petals and quantified the levels of chemical compounds and the pollen produced by intact and damaged flowers in order to identify characters associated with the plant-florivore interaction. We found that the epidermis (adaxial and abaxial surfaces) and mesophyll of the petals of healthy flowers was thicker when compared to damaged flowers. We infer that the smaller thickness of traits associated with the absence of characters with deterrent effect on herbivores and greater production of attractive/nutritive chemical compounds in relation to defense compounds contribute to make the species highly susceptible to florivory. Pollen production in damaged flowers did not differ between the different stages of floral development. However, florivory has a negative effect on the amount of pollen produced. Damaged flowers had less pollen than healthy flowers. We conclude that florivory in *S. aversiflora* exerts significant pressure on petal anatomy and resource production by flowers.

3.2.1 Floral bud

In the basal region, below the receptacle, the sections of the peduncle of the flower bud showed a flat circular shape (Figure 2A) with undulated uniseriate epidermis. Adjacent to the epidermis, the cortical region is filled by 2-4 layers of fundamental parenchyma, and internally there is a thick continuous layer of sclerenchyma forming a ring that surrounds the entire phloem (Figure 2A). The vascular system has seven collateral bundles: three larger ones facing the adaxial face and four smaller ones positioned abaxially. The medullary parenchyma is well developed, with larger cells in relation to the cortical parenchyma, and with lignified cell walls (Figure 2A).

Above the peduncle, the calyx has two short sepals (Figure 2B) positioned externally to the others that are more elongated. Internally to the sepals, there are petals that are thinner in relation to the sepals (Figure 2C), creating a wrap protecting the reproductive whorls. Sections of the median part of the bud showed how the androecium was arranged on the bud axis: the pollinating anthers located abaxially (Figure 2D) and the feeding anthers adaxially (Figure 2E). In this part of the bud, it was possible to reach only one staminode that is placed to the left of the feeding anthers (Figure 2F). The gynoecium is positioned in the center of the bud (Figure 2G).

3.2.2 Calyx

The calyx of *S. aversiflora* is heteromorphic, consisting of five sepals, two smaller external ones and three larger internal ones. In paradermal section, the sepals present an epidermis with irregular cell shapes, with anticlinal walls varying from curved to sinuous on both surfaces (Figure 3A-B), a striated cuticle (Figure 3C), non-glandular trichomes restricted to the margins (Figure 3D), and glandular trichomes at the base. Sepals are hypoestomatic with plenty anisocytic (Figure 3A-B) and tetracytic stomata. Stomata occur mostly in the basal and median region of the sepals, being rare or absent at the apex (Figure 3D-E). In cross-section, the epidermis of both the adaxial and abaxial surface is uniseriate with cell shapes ranging from oval to rectangular, and adjacent to the epidermis, there is a homogeneous mesophyll (Figure 3F) with circular cells of different sizes and few intercellular spaces (Figure 3G). Collateral vascular bundles occur sparsely along the mesophyll (Figure 3 F-H).

3.3.3 Corolla

The corolla of *S. aversiflora* is composed of five petals that differed morphologically into, consisting of three groups: concave petals, smaller non-concave petals, and larger non-concave petals (Figure 4A). Internally, consistent anatomical patterns are observed, with few variations in the analyzed traits. In paradermal section, the concave petals present varied cell shapes and anticlinal wall contours: on the adaxial surface, in the basal region, the cells are polygonal with curved and straight anticlinal walls (Figure 4B) while in the median and apical regions, the cells are irregular and have sinuous anticlinal walls (Figure B). In turn, on the abaxial surface, the anticlinal walls are evenly sinuous throughout the petal (Figure 4C). The other petals present irregular cell shapes with sinuous anticlinal walls on both surfaces (4D-O). Stomata are distributed on both epidermal surfaces (4H-I), with the tetracytic and anisocytic types being most commonly observed (Figure 4I), and the anomocytic type the more rare. The sinuosity of the epidermal cells forms crests and valleys and between these points we observed the formation of bordered pit chambers in all petals, more conspicuously in the smaller non-concave petals (Figure 4J, K-L). Simple trichomes are present in all petals, sparsely distributed in the basal region and more densely on the veins (Figure 4M-N). The petals are glabrous in the apical region. Sessile glandular trichomes are rarely found, being observed only by SEM (Figure 4O).

In cross section, the petals have a uniseriate epidermis on both surfaces, with cells of tabular to oval shapes (Figure 5A), simple trichomes sparsely distributed on the epidermis (Figure 5B), and rare sessile glandular trichomes (Figure 5C). The mesophyll is homogeneous, consisting only of braciform cells (Figure 5D). The cells in the concave petals have visually shorter arms that connect to each other forming a kind of network with visually smaller intercellular spaces (Figure 5A and D) in relation to the other petals. In the latter, the cellular arms are longer and more loosely connected, leaving intercellular spaces that are visually larger in relation to the concave petals (Figure 5E). The petals have planoconvex veins (Figure 5I), except the concave petals, whose lateral veins are biconvex (Figure 5J). The vascular system is collateral, formed by a single central bundle that occurs in the center of the veins (Figure 5I-J).

3.3.4 Androecium

Senna aversiflora has heterantherous flowers. The androecium is formed by three larger pollinating anthers, four smaller feeding anthers, and three staminodes. Although the external morphology differs, the internal structural organization of the anthers shows a pattern (Figure 6A-B). In cross section, the anthers are composed of a pair of thecae (Figure 6A-B). The exothecium is papillose and uniseriate (Figure 6C), glabrous, and covered by a striated cuticle. Adjacent to the epidermis, there is a fibrous endothelium (Figure 6C) and, more internally, 1-2 extracts of parenchymal tissues followed by the collapsed tapetum (Figure 6C). The lobes are separated by connective tissue and within each lobe, the parenchymal tissue separates the lobes into two locules (totaling four locules). In the central region of each locule, the parenchymal tissue protrudes vertically and at its end forms a convoluted swelling (Figure 6D). The vascular tissue is located in the central region of the connective site (Figure 6D) consisting of three collateral bundles, one smaller central one and two lateral ones (Figure 6E). The filament has a convex concave shape (Figure 6F) with a uniseriate epidermis and the cortical region consists of 1-2 layers of collenchyma and 5-7 layers of parenchyma. The vascular system is collateral, formed by a single U-shaped central bundle (Figure 6F). Analyses of light microscopy images showed no observable structural modifications between intact and damaged anthers.

3. 3. 5 Gynoecium

The gynoecium of *S. aversiflora* is formed by a single carpel with an ovary varying in shape from linear to curved, with a reduced style (Figure 7A). In cross section, the ovary is a rectangle with dilated angles and a slight narrowing of the sides (Figure 7B). The epidermis is composed of uniseriate tabular cells with thickened cell walls and simple trichomes that are densely distributed throughout the epidermis. A hypodermis is found adjacent to the epidermal tissue, with visually larger cells than the epidermis. Internally to the hypodermis, the filling tissue consists of parenchyma with compressed cells, without intercellular spaces. Two conspicuously smaller cell layers occur more internally towards the locule, and adjacent to these layers, we observed the parietal tissue with 1-3 layers of elongated and juxtaposed cells (Figure 7C).

The ovule is placed in the center of the locule. In longitudinal section, the ovule is seen located at the base of the ovary (Figure 7D). The ovary is continuous with the style (Figure 7E). As the style approaches the stigma, the locule narrows (Figure 7F). The vascular system is collateral, formed by four V-shaped bundles arranged at the angles of the ovary and the style (Figure 7G). The stigma is obovate with a uniseriate epidermis and hypodermis, fundamental parenchyma filling the central region, and vascular bundles reduced to two U-shaped bundles located at the ends of the stigma (Figure 7H-I). At one end, the vascular bundle surrounds the slit of the stigma (Figure 7I).

3.4 Analysis of anatomical traits of intact and damaged flowers

The thickness of the adaxial and abaxial cuticle of intact and damaged flowers did not differ statistically. In turn, the median thickness of the adaxial and abaxial epidermis and median thickness of the mesophyll differed significantly between intact and damaged flowers according to the non-parametric Kruskau-Wallis test. Thus, intact flowers had thicker tissues than damaged flowers ($p = 0.05$) (Table 2).

The structural organization of the mesophyll did not differ between intact and damaged flowers, except for the intercellular spaces described above. However, we observed that while the mesophyll of intact flowers remained expanded and linear (Figure 5F), damaged flowers had constrictions with undulations on the epidermis, forming depressions along the mesophyll (Figure 5G-H). These differences in the conformation of the mesophyll can be seen in Figure 5. Mesophyll thickness also differed statistically between intact and damaged flowers: the median thickness of the mesophyll of intact flowers was significantly larger than that of damaged flowers.

3.5 Histochemistry of intact and damaged flowers

Based on a control floral section (Figure 8A), all intact and damaged flowers analyzed showed a positive reaction for the presence of alkaloids (Figure 8B-C). However, the petals of intact flowers showed a negative reaction, while all floral whorls of the petals of damaged flowers showed a positive reaction for the presence of these compounds. Only damaged flowers had phenolic compounds, exclusively in the anthers damaged by florivores (Figure 8 D). Intact and damaged flowers showed a positive reaction for proteins (Figure 8E-F) in all floral whorls and in epidermal appendages, such as glandular trichomes. Starch was detected in the petals and anthers of damaged flowers (Figure 8G), but only in the petals of intact flowers. Pectins were identified in the cell walls of the sepals (Figure 8H), petals, anthers, and ovaries of damaged flowers (Figure 8I), but only in the sepals and anthers of intact flowers. Tannins were detected in the anthers of damaged flowers, but not identified in any part of intact flowers. Lipid substances occurred in the sepals and anthers of both intact and damaged flowers (Figure 8J-L), but not in other whorls. Reducing sugars (glucose and fructose) were found in intact and damaged flowers (Figure 8M-O). The tests for essential oils and oleoresins were negative.

3.5.1 Percentage of chemical compounds in intact and damaged flowers

The percentages of nutritional substances were higher than those of defense substances in both intact and damaged flowers (Figure 9). The petals of damaged flowers presented approximately 100% of nutritional substances, which include pectins, proteins, lipids and sugars. The anthers of damaged flowers also presented a high percentage of these compounds, with 60% in relation to the defense compounds. Low detection of alkaloids and phenolic compounds was observed in all plant organs of intact and damaged flowers. Higher percentages of defense compounds were found only in the anthers in relation to the other whorls: 28% in intact flowers and 38% in damaged flowers.

3.6 Resource production in intact and damaged flowers

Intact flowers produced a significantly higher amount of pollen compared to damaged flowers ($p = 0.01$) (Figure 10a). The amount of pollen did not differ significantly between the initial, intermediate, and pre-anthesis stages of floral development ($p = 0.8280$) (Figure 10b).

4. DISCUSSION

4.1 Floral anatomy

The diversity of epicuticular waxes found in *S. aversiflora* is important because waxes are considered taxonomically valuable characters (Barthlott et al., 1998), especially helpful in the classification of*Senna* species of the series *Bacillares* (Souto et al., 2022). Furthermore, waxes play an important ecological role in plants, protecting them against biotic and abiotic stresses, including radiation, water loss, microorganisms, and insects (Ahmad et al., 2015; Lewandowska et al., 2020). Waxes are also associated with insect-plant interactions. Crystalloids, for example, help to prevent insects to attach to plant organs (Gorb & Gorb, 2003), as demonstrated by Abbas et al. (2023) in a study with *Citrus* L, in which cultivars with a higher amount of waxes had lower densities of Lepidoptera larvae. However, despite the presence and diversity of waxes in *S. aversiflora* , they do not seem to hinder florivory because all floral whorls of this species are widely consumed.

The ornamentation of epidermal surfaces can be phylogenetically useful for the delimitation of groups and has an important functional role in ecological interactions. Conical and/or papillose and lobular epidermal surfaces are commonly found in Fabaceae groups such as Papilionoideae, Mimosoideae, and Caesalpinioideae, which includes *Cassia* L and*Senna* Mill. species (Odeja et al., 2009). The patterns observed are a rugose tabular epidermis with striations in *Cassia emarginata* L. and a papillose conical epidermis in *Senna alata* (L.) Roxb (Odeja et al., 2009).

The sinuous anticlinal walls observed in the epidermis of *S. aversiflora* are similar to those observed in species with different pollination syndromes (Kraaij & Van de Kooi, 2019). The straight conformation of the anticlinal walls at the base of the concave petal in *S. aversiflora* suggests a functional role in pollination, by ricochet or looping. Pollen is released after the vibration of the flowers by the bees and ejected towards the concave petal, where it collides (ricochets) or runs (makes a loop) through its entire surface to the apex, being then deposited on the back of the pollinator (Westerkamp, 2004; Almeida et al., 2013; Amorim et al., 2017). Considering that straight walls configure a more leveled epidermis, we believe that this is an attribute that facilitates the path of pollen grains when bees visiting *S. aversiflora* flowers vibrated and pollen is released.

Flowers usually have petals with papillose epidermal cells, a characteristic also associated with the attraction and interaction with pollinators (Odeja et al., 2009; Costa et al., 2016; Bailes & Glover, 2018; Cavallini-Speisser et al., 2021). According to Costa et al. (2016), several bee-pollinated flowers have a papillose epidermis that can facilitate the landing and movement of bees in flowers. However, even though *S. aversiflora* is pollinated by bees, papillose epidermal cells were not observed in the species. The absence of papillae may be related to the aforementioned ricochet or looping pollination system. A papillose epidermis would probably make it difficult for pollen to slide on the petals.

Regarding the striated cuticle observed in the petals by scanning and light microscopy in *S. aversiflora* , it is known that this character also has a role in the interaction between flowers and pollinators. According to Whitney et al. (2009), regular folds in the waxy cuticle of the epidermis of the petals may cause the diffraction of light and serial propagation of bright reflections, working as a clue in the petals perceptible to pollinators.

The presence of few trichomes in the petals may be related to the distribution of florivores in *S. aversiflora* , since pubescence is a character that promotes herbivore deterrence (Hnaley et al., 2007). The high density of simple trichomes in plant organs creates an obstacle for insect movement and feeding (Dai et al., 2010; Kaur & Kariyat, 2023). The mesophyll of flowers, including those of several species of Fabaceae, is commonly homogeneous with braciform cells (Costa et al., 2016), as observed in *S. aversiflora* . The organization of braciform cells in the mesophyll form large air cavities. These cavities, according to Van der Kooi et al. (2019), create differences in the refractive indices of petal tissues and strong reflection and scattering of light in relation to other flowers. This dynamic has an effect on the appearance of petals by reflecting or diffusing light (Cavallini-Speisser et al., 2021).

We associate the papillae found on the epidermis of the anthers to a better adherence of the pollinators. *Senna aversiflora* has flowers that are buzz pollinated by pollen-collecting bees that cling to the anthers (Pritchard & Vallejo-Marín, 2020), and it has been proposed that papillae facilitate the landing and movement of pollinators on flowers (Aronne et al., 2012; Costa et al., 2016). The fibrous endothelium gives rigidity to the anthers and consequently protection to the pollen grains inside. The fibrous anthers of *S. aversiflora* may also contribute to pollination by the mechanical support provided, being this one of the most important functional roles of lignin in plants (Yadav, 2018). The bees that pollinate *S. aversiflora* are large and, according to Dulberger (1981), the need to protect the gynoecium from the action of pollinators is a possible explanation for the existence of enantiostyly, a floral polymorphism observed in*Senna* species. In this sense, the existence of a lignified endothelium in the gynoecium, observed in our study, may provide resistance against the weight of the bees and their vibration during visits.

4.2 Influence of florivory on anatomical traits

Our results corroborated the proposed hypothesis on the thickness of anatomical traits, as we found that damaged flowers had less thick traits, except for the cuticle. Studies on leaf herbivory indicate that cuticle thickness generally functions as a mechanical barrier that hinders the penetration of the oral tract of herbivores into the leaf (Petters, 2002. Corrêa et al., 2008; Mostafa et al., 2022; Demis, 2024). The epidermis of damaged flowers was less thick on both surfaces than that of intact flowers, probably due to the stress caused by florivory, including loss of biomass and water, consequently triggering a retraction in cell volume.

The mesophyll of the petals of damaged flowers was also less thick than that of intact flowers. In the case of leaves, the number of mesophyll layers in conjunction with other structural traits such as the presence of a lignified epidermis, a thickened hypodermis, and sclerenchyma are considered potential barriers against insects (Correa et al., 2008; Caldwell et al., 2016). The absence or reduction of these traits are opportune for florivory. It has been observed that higher densities of external chewers are associated with the absence of a thickened hypodermis in flowering plants, including species of *Acacia* Mill., and *Pultenaea* Sm. of the family Fabaceae (Peeters, 2002). Further, it has been demonstrated that the proportion of damage caused by chewers decrease with increasing leaf thickness in four tree species (Martini et al., 2022).

Deformations in the petals of damaged flower also include collapsed mesophyll tissue and invaginations in the epidermis, unlike intact flowers whose mesophylls are expanded and rectilinear. Changes similar to the ones observed in the petals of *S. aversiflora* are commonly seen in leaf galls (Nobrega et al., 2023). The present results are the first record of these changes in floral whorls.

4.3 Histochemistry

The hypothesis that damaged flowers have lower concentration of defense compounds was not confirmed, since both intact and damaged flowers showed high percentages of nutritional compounds in relation to defense compounds. Phenolic compounds and alkaloids, although present in the flowers of *S. aversiflora* , occurred in low concentrations. Phenolic compounds are especially involved in the defense and resistance of plants against pathogens, including bacteria, viruses and fungi. They also play a protective role against insect herbivory because they cause oxidative damage to the herbivores and affect their growth, development, and reproduction (Kumar et al., 2020). Alkaloids, in turn, are considered toxic to herbivores (see Wari et al., 2021). The studies by Adler et al. (2011) on the direct and indirect effects of alkaloids on plant suitability for herbivory and pollination showed that alkaloids directly reduced bud herbivory, but did not have strong effects on pollination. The fact that *S. aversiflora* had low levels of alkaloids means that the flowers of this species are vulnerable to herbivore attacks.

As for attraction and nutritional compounds, the flowers and buds of*S. aversiflora* are rich in proteins, sugars, lipids, and carbohydrates. According to Erb et al. (2021), the primary metabolites produced by plants influence and play a positive role on herbivory by increasing the essential nutrients transferred across trophic levels. Host signals, including the primary and secondary metabolites and the nutritional value of the plant part consumed, determine the feeding preferences of herbivores. Sugars, for example, can inhibit the aversive taste of secondary metabolites and make tissues palatable (Machado et al., 2021), while proteins and carbohydrates favor the growth of herbivores (Le Gall et al., 2014). Le Gall et al. (2014) found that higher concentration of carbohydrates and proteins had significant positive effects on the body mass gain of nymphs of the grasshopper*Melanoplus differentialis* (Thomas). The availability of macronutrients in *S. aversiflora* makes it an attractive source of food, which possibly explains this strong antagonistic interaction in the species.

4.4 Resource production

Damage to flowers of *S. aversiflora* at different stages of floral development did not interfere with the amount of pollen produced, although lower values were found in damaged flowers than in intact flowers. Florivory in the initial developmental stage of the buds could affect anther development and pollen production, leading to the abortion of the complete floral structure due to the lack of resource to allocate in deformed flowers. In the intermediate or pre-anthesis stage of floral development, florivory can affect the amount of resource through the loss of pollen itself due to the partial or total destruction of the anthers. According to Carper et al. (2016), florivory has the potential to strongly affect the male function of the plant. Haas & Lortie (2020) add that partial florivory can reduce pollen deposition because damage to flowers reduces the frequency of pollinator visits.

Studies on the influence of florivory on pollen production are scarce in*Senna* species. An investigation similar to the one carried out here with *S. aversiflora* on florivory at different stages of floral development was conducted with *Iris gracilipes* A. Gray by Oguro & Sakai (2009). The authors focused more on the effects of florivory on female reproduction, which was negatively affected (Oguro & Sakai, 2009). A study on leaf herbivory in a species of Curcubitaceae revealed that simulated herbivory in 50% of the leaves had a strong effect on pollen production per flower, leading to a considerable reduction in the number of pollen grains produced by damaged plants compared to control plants (Avila-Skar et al., 2003).

The characterization of the floral anatomy of *S. aversiflora* provided novel information, revealing characteristics that are important for the taxonomic classification of the species as well as in the understanding of its antagonistic and mutualistic ecological relationships. The main anatomical traits that possibly explain why *S. aversiflora* flowers, and especially its petals, are highly susceptible to florivory are the thin cuticle, low density of trichomes on the surface of the petals, absence of papillae on the epidermis, absence of sclerenchyma and crystals in the mesophyll, and high concentrations of nutritive chemical compounds. These characteristics make the flowers more palatable and accessible to florivores. Finally, we conclude that florivory has significant impacts on the reproduction of *S. aversiflora,* decreasing the amount of pollen and volume of petal tissues. These impacts underscore the importance of the selective pressure of florivory shaping the evolution of plants, promoting defense and regeneration strategies to ensure the continuity of reproduction.

Author contributions

Edinalva Vital, Natan Almeida and Emília Arruda delimited the general idea of the work. All practical steps of the work and the writing of the original draft, including the conceptual, methodological and discursive writing, were carried out by the first author. The co-authors Natan Almeida, Ana Virgínia and Emília Arruda contributed to the supervision and formal analysis of the manuscript.

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

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