EDITORA VERDE

Revista Verde de Agroecologia e Desenvolvimento Sustentável
Green Journal of Agroecology and Sustainable Development

GVAA GRUPO VERDE DE AGROECOLOGIA E ABELHAS

# Do the edge effects influence epicuticular wax production in *Vismia guianensis* (Aubl.) Choisy (Hypericaceae)?

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A R T I C L E

Received: 21-07-2022
Accepted: 01-02-2023

*Key words:*
Chemical composition
Gas chromatography
Wax quantification

A B S T R A C T

Forest fragmentation leads to various environmental problems. It causes edge effects that can decrease biodiversity, promote the growth of lianas, and affect the production of epicuticular wax. This study focuses on examining the secondary metabolites found in the epicuticular wax of *Vismia guianensis* leaves and investigating the impact of the edge effect on their production. We extracted and quantified the epicuticular wax and analyzed its constituents using gas chromatography coupled with a mass spectrometer. The amount of cuticular wax extracted from *V. guianensis* leaves ranged from 33.69 to 631.57 µg/cm². Individuals at the forest edge had the lowest wax content, averaging 250.96 µg/cm². On the other hand, individuals located in the interior of the forest had the highest values, with an average of 340.67 µg/cm² per surface unit. The analysis of the samples identified and quantified nine long-chain alkanes ($C_{27}$ to $C_{35}$) in the composition of the cuticular wax. The predominant compound in the wax of all individuals was nonacosane alkane (C29). Interestingly, the individuals within the forest fragment exhibited less diversity of compounds compared to those at the forest edge.

## INTRODUCTION

Plant therapeutic agents, such as plant oils and extracts, have been used for various applications in folk medicine (SILVA, 2016). The biosynthesis of secondary metabolites may be influenced by genetic and environmental components (LI et al., 2020). Biotic and abiotic factors of the environment can affect the morphoanatomy and quantitatively and qualitatively influence the production of secondary metabolites (CHAVES 2012; SILVA 2016). Changes in these compounds can affect species' survival strategies. Plants with medicinal potential are the main medicine used in tropical countries (CARDOSO et al., 2019).

Forest fragmentation can interfere with the synthesis of secondary plant metabolites that, by forming edge areas, impact ecological processes, making the environment vulnerable to the invasion of exotic and herbivorous species, in addition to generating microclimatic changes that directly influence the energy balance (DEBINSKI; HOLT, 2000) and favor the development of lianas. Plants located on the forest edges and inside fragmented areas may present a different behavior regarding the secondary metabolites production in response to the environmental pressures suffered (PEIXOTO SOBRINHO et al., 2009). The edge effect can affect diversity, compromising up to 100 meters into the forest (TABANEZ et al., 1997).

Edge effects interfere with the proportions of different aliphatic compounds in epicuticular waxes (BIANCHI; BIANCHI, 1990). Wax plays several roles in plants as repelling insects, conserving moisture and nutrients, and preventing fungal and bacterial contamination. They contain long-chain hydrocarbon compounds with more than 20 carbons (BUKHANOV et al., 2020). Epicuticular waxes make up the outer layer of plants and interact with microorganisms that approach their surface (SALGADO et al., 2020). It may have greater interaction in plants under the edge effects and influence the composition of essential oils. Secondary metabolites have been gaining prominence with the discoveries of their multiple functions in plants, such as structural support, interaction with the environment, growth regulation, and tolerance to water stress (SANTOS, 2015).

In this context, *Vismia guianensis* (Aubl.) Choisy (Hypericaceae family), a species native to Brazil popularly known as “lacre”, undergoes strong extractive pressure for its wood and has several uses in traditional medicine, such as in the treatment of skin diseases, potent laxative, antipyretic, antirheumatic, and antifungal (ALMEIDA-CORTEZ; MELO-DE-PINNA, 2006; SILVA et al., 2018). Wax is a lipid with a great diversity of long-chain alkanes, esters, polyesters, and hydroxy esters of primary alcohols and long-chain fatty acids (ARUNKUMAR et al., 2018).

This species occurs mainly in capoeira and forest edges, measuring between three and seven meters in height (SOUZA; LORENZI, 2012). It is a pioneer species of great importance in the composition of the edge areas of the secondary Atlantic Forest (ALMEIDA-CORTEZ; MELO-DE-PINNA, 2006). This study aimed to record the secondary metabolites present in the epicuticular wax of *Vismia guianensis* leaves and to evaluate the edge effect on the production of these metabolites.

## MATERIAL AND METHODS

The work was carried out in the Camocim fragment, at the Tapacurá Ecological Station (TES), in the municipality of São Lourenço da Mata, Pernambuco, in areas of the edge and interior of the forest (Figure 1). The TES (08º04'S and 35º12'W) occupies an area of approximately 776 hectares, with an altitude of 150 m. The vegetation is preserved despite the cultivation of sugar cane in the areas around the station (OLIVEIRA et al., 2006).

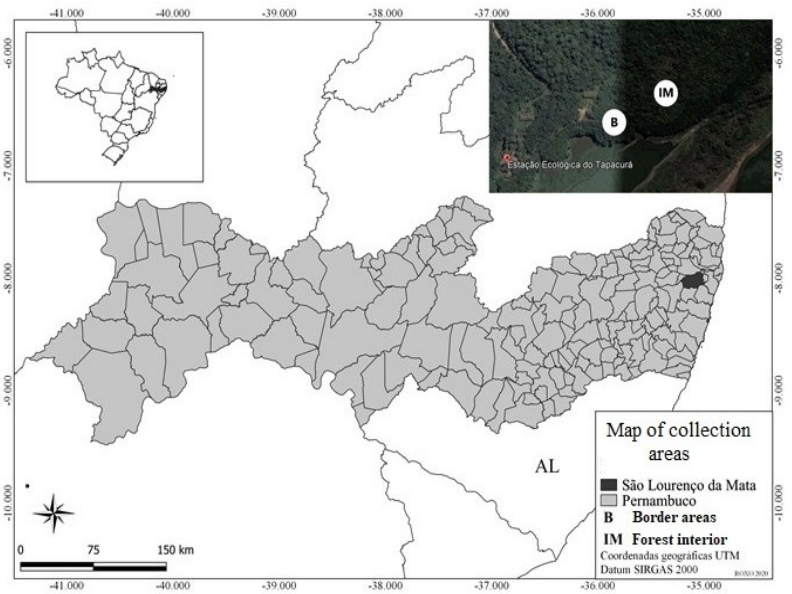


**Figure 1**. Tapacurá Ecological Station, municipality of São Lourenço da Mata - PE, with emphasis on the areas of the edge (B) and interior of the forest (IM) where the leaves were collected for analysis.

The TES is a Semideciduous Lowland Seasonal Forest Area and has an As' type climate with an average annual precipitation of 1300 mm per year and six months with less than 100 mm. It features predominantly arboreal dry forest vegetation, reaching about 30 m in height (MOURA et al., 2012).

Sampling was carried out by removing leaves from adult trees of *V. guianensis* with satisfactory phytosanitary conditions, located in two areas, one in the center and the other on the edge of the fragment. The collection sites were identified and georeferenced using GPS (Garmin-Etrex Legend). Leaves of *V. guianensis* were collected in May 2014. Leaves with pathogens or ectoparasites were eliminated. The material was stored in paper bags and sent to the Laboratory of Natural Products of the Department of Biochemistry of the Federal University of Pernambuco (UFPE), for the extraction of epicuticular wax. Botanical identification was performed following the APG III classification. Its specimen is listed in the Herbarium UFP – Geraldo Mariz, located in the Department of Botany at UFPE with registration number UFP 76600.

Each individual (n = 9) had ten leaves introduced separately per replication into a Petri dish containing 30 mL of chloroform, hexane, and dichloromethane, and gently agitated for 30 seconds. The resulting solutions (wax plus solution) were filtered using filter paper and transferred to a 50 mL volumetric flask, where they were evaporated using a rotary evaporator to obtain the solid residue (wax). To determine the amount of wax per unit of leaf surface, the leaves were scanned and digitized, and the images were used to calculate the leaf area using Image Pro-Plus software version 4.1 for Windows®. The wax quantification was expressed as the amount of wax per unit of leaf area (μg/cm²) (HAMILTON, 1995).

The chromatographic analyses were conducted using a gas chromatography-mass spectrometry (GC-MS) instrument, model PQ 5050ª (Shimadzu). A DB-1 capillary column (30 m length, 0.25 mm internal diameter, and 0.25 μm film thickness) was used, and helium was used as the carrier gas. The injector temperature was set at 290°C, with an initial temperature of 80°C for five minutes, followed by an increase from 80 to 285°C at a rate of 4°C per minute. The final temperature was maintained at 285°C for 40 minutes. The detector temperature was set at 290°C, and the interface temperature of the GC-MS system was also 290°C. The mass detector operated with electron impact ionization (70 eV) and scanned mass range from 30 to 600 Daltons. A 1 μL volume of each sample was injected, and compound identification was performed by analyzing the fragmentation patterns, retention indices, and comparing with the WILEY library of the spectrometer.

The areas were analyzed using a completely randomized design with a 3x9 factorial arrangement (edges x individuals), with 10 replications. The obtained means were subjected to analysis of variance (ANOVA), and the results were further subjected to Tukey's test at a 5% probability level using AgroEstat® software (BARBOSA; MALDONADO JÚNIOR, 2015).

## RESULTS AND DISCUSSION

The content of cuticular wax extracted from *V. guianensis* varied among edges and individuals, ranging from 33.69 to 631.57 μg/cm². The highest values were found in individuals from the interior of the forest, as illustrated in Figure 2. Thicker cuticles can reduce transpiration, providing advantages for individuals in areas subject to higher evapotranspiration (KOTTAPALLI et al., 2009). The influence of the edge effect mitigated the amount of cuticular wax. Individual six (6), located at the forest edge, however, had the highest content of cuticular wax. Edge two was similar to the interior edge (edge 3), while edge one had a lower content of cuticular wax. The interior edge area exhibited the highest content of cuticular wax. The levels of cuticular wax may indicate the influence of the edge effect and contamination from pesticides used in agriculture. Plants

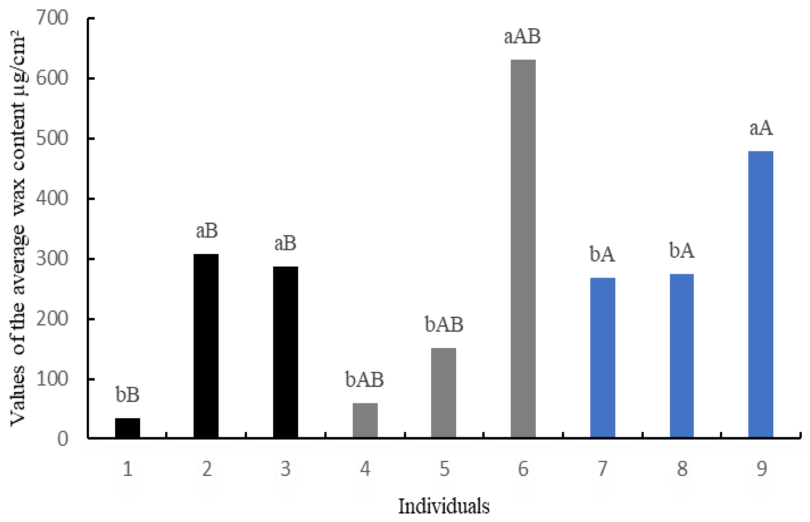


**Figure 2**. Values of the average wax content per unit of leaf surface of *Vismia guianensis* collected at the Tapacurá Ecological Station, in border areas (individual 1 to 3 – Weir border in black; and 4 to 6 – matrix border in light gray) and forest interior (individuals from 7 to 9 – blue). Equal lowercase letters for areas in edges and interior and uppercase letters between clearings do not differ from each other by Tukey's test at 5% probability.

near contaminated areas or subjected to the use of chemical pesticides may undergo phytochemical alterations and serve as environmental bioindicators of pollution (ZHOU et al., 2008).

The edge environment is subject to higher radiation, temperature, anthropogenic effects, and edge effects compared to individuals in the interior of the forest fragment. Environmental factors can affect wax production and content (KAPOS et al., 1997). Solar radiation promotes plant growth and induces and regulates plant metabolism. In response to radiation, plants adapt by releasing or accumulating various secondary metabolites with economic value and antioxidant properties, such as flavonoids and phenolic compounds (YANG et al., 2018). The epicuticular wax serves as protection, mitigating excessive transpiration, which is an important adaptive strategy for species in semi-arid climates (SILVA et al., 2018). The area surrounding the edge may be more exposed to pathogens and contamination, which can influence biodiversity and chemical composition. Clearings, compared to preserved areas, experience higher solar radiation, increased temperatures, water deficits (JARDIM; QUADROS, 2016), and promote the growth of lianas. Clearings are also susceptible to anthropogenic pressure from the surrounding environment.

The edge fragment bordered by sugarcane cultivation may be influenced by the application of chemical compounds, which can affect the chemical composition of the wax. There are studies on wax in agricultural areas, where the application of chemical inputs is likely to occur. Lichston and Godoy (2006) when analyzing coffee leaves, found that the amount of epicuticular wax significantly decreased with fungicide application. Considering the leaf's ability to absorb substances sprayed on them (THOMAZIELLO et al., 2000), agricultural pesticides may be affecting the synthesis of epicuticular leaf wax in *V. guianensis*. However, there is limited research on native species, and little is known about their biodiversity, which may offer compounds for use as phytotherapeutic agents. The Areas of Permanent Preservation (APP) and Legal Reserves (RL) are close to areas used in agriculture, and their management can influence them. Scientific studies evaluating the influence of the edge effect on native species are scarce. Understanding these adaptive mechanisms of species helps develop conservation plans aimed at preserving biodiversity.

In most species, the amounts of epicuticular wax range from 10 to 200 μg/cm², although quantities above 300 μg/cm² have been reported. Therefore, the quantities observed in this study are consistent with previous research on epicuticular wax quantification (Mc WHORTER; OUZTS, 1993). The proportion of waxes may be related to various biotic and abiotic factors. The content of epicuticular wax varies according to environmental conditions, and it may increase in areas with high temperatures, solar radiation, vapor pressure deficit, and water deficit (SAMDUR et al., 2003). Climate change can influence the composition and population distribution, but little is known about the behavior and vulnerability of species native from Brazil. The wax content in conserved areas showed higher levels compared to edge effect areas. The interaction with the environment mitigated wax production, except for matrix number 6, where the epicuticular wax under the edge effect showed a higher number of n-alkanes.

The long-chain fatty acids present in the wax can include alkanes and alcohols with carbon chains ranging from 20 to 34 in length. The wax composition can vary according to species, organ, and development stage (LIU et al., 2014). The epicuticular wax is composed by n-alkanes, esters, alcohols, and fatty acids, but the chemical composition can vary depending on the phylogenetic group (MONQUERO et al., 2004), as well as external influences, pathogens, and environmental factors. Thus, the variation in these chemical compounds contributes to the formation of distinct morphological structures, such as crystalline and amorphous forms, including hydrocarbons and primary alcohols that crystallize in the form of plates, and secondary alcohols, ketones, and betadiketones that form tubules.

Nine long-chain alkanes ($C_{27}$ to $C_{35}$) were identified in the cuticular wax composition of *V. guianensis* (Table 1). The alkane nonacosane ($C_{29}$) was the major compound in the epicuticular wax composition of all *V. guianensis* individuals. Individuals from the interior of the fragment exhibited lower compound diversity compared to individuals from the edge (Table 1). Generally, *n*-alkanes occur in a homologous series ranging from $C_{17}$ to $C_{35}$ in the epicuticular wax of modern plants, with a predominance of $C_{29}$ and/or $C_{31}$ alkanes as major components (BASU; SINHABABU, 2013). The higher diversity of *n*-alkanes in the edge areas may be influenced by edaphoclimatic factors and pathogens. In the interior edge, the compound $C_{29}$ alkane showed greater abundance (Table 1).

**Table 1.** Percentage of n-alkane composition in cuticular wax extracts from *Vismia guianensis* in edge and interior areas of Tapacurá Ecological Station, Pernambuco, Brazil.

| *n*-Alkane (number of carbons) | Weir Edge | | | | Matrix Edge | | Interior | | |
|---|---|---|---|---|---|---|---|---|---|
| | Individuals | | | | | | | | |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| $C_{27}$ | – | – | – | – | – | 1.49 | – | – | – |
| $C_{28}$ | – | – | – | – | – | 13.71 | – | – | – |
| $C_{29}$ | 62.35 | 59.86 | 25.44 | 43.27 | 20.66 | 15.34 | 75.54 | 74.09 | 72.95 |
| $C_{30}$ | 14.78 | 14.06 | 5.99 | 5.39 | 3.70 | 18.45 | 11.48 | 10.53 | 13.74 |
| $C_{31}$ | 22.88 | 26.07 | 22.22 | 36.50 | 17.05 | 17.24 | 12.99 | 15.38 | 13.31 |
| $C_{32}$ | – | – | 19.66 | 9.34 | 19.03 | 12.36 | – | – | – |
| $C_{33}$ | – | – | 15.58 | 5.50 | 20.38 | 8.23 | – | – | – |
| $C_{34}$ | – | – | 11.10 | – | 10.89 | 8.39 | – | – | – |
| $C_{35}$ | – | – | – | – | 8.28 | 4.79 | – | – | – |

The alkanes play an important role in wax composition, contributing to the hydrophobic properties of the cuticle, which serves as a barrier against the external environment and its pathogens (KHELIL et al., 2016), acting as a protective layer. The alkanes present in the wax can help mitigate water loss (WU et al., 2018), and areas affected by edge effects are more open and experience higher solar radiation and temperatures. The presence of long-chain *n*-alkanes in the epicuticular wax may be a strategy to cope with water stress (RIBEIRO et al., 2021), reducing water loss through evapotranspiration. The epicuticular wax covers most above-ground tissues, protecting the plant against environmental factors (LIU et al., 2015). The greater abundance of n-alkane distributions in plants affected by edge effects may indicate that these plants are under adverse conditions, leading to an increase in carbon number and distribution compared to plants in the preserved area (interior). The application of agricultural pesticides for pest management in the neighboring sugarcane plantation may be influencing the content of epicuticular wax in the studied species. Further studies are necessary to confirm the impact of agricultural pesticides.

## CONCLUSION

The production of epicuticular waxes in *Vismia guianensis* in the edge and interior environments of the fragment highlights the edge effect on the species, resulting in changes in the distribution of *n*-alkanes and wax content per unit leaf surface area.

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