Effect of Plant age and environment on foliar epicuticularwax in *Sabal minor* and *Sabal palmetto*

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Abstract:

The foliar epicuticular wax is located on the outside of the leaf cuticle. Epicuticular wax is important because it is an added barrier of protection from the environment. Epicuticular wax protects the plant leaf from salt spray from the ocean, insects, water, and any other foreign objects that may damage or penetrate the leaf. The epicuticular wax may contain different patterns, shapes and structures. The structure and composition depend on both genetic and environmental factors. There should exist a correlation between the age, structure, pattern and abundance of epicuticular wax between Sabal palmetto and Sabal minor leaves.

In order to analyze the structure of the foliar epicuticular waxes, micrographs were taken using a Scanning Electron Microscope (SEM), JEOL JSM-6010LA. This particular SEM used fresh material, whereas most other SEMs required dried specimens. The youngest leaf on each plant and the oldest leaf on each plant were used for analysis. The data show us that not only does the environment of the plant influence the amount of wax a leaf may have, but that the age of the leaf also determines how much wax a leaf will have. As the plant ages, the wax does not leave. If there is less wax in older leaves this indicates that a major environmental event has taken place.

Keywords:
foliar, epicuticular wax, micrographs, cuticle, structure, composition

1. Introduction:

The *Sabal minor* and Sabalplmetto palms both thrive in warm humid climates, but the *Sabal palmetto* is only native to southern east coast, while the *Sabal minor* is native to the gulf coast and the southern east coast. This is especially surprising because both palms are native to similar climates and the *S.palmetto* is able to thrive in a variety of soils as opposed to the *S.minor* that prefers moist soil. While the *S.palmetto* is native to the southern east coast, it can only survive in other regions if it is planted in its native region, then transported to a foreign region. Through the analysis of the foliar epicuticular wax on the adaxial side of the leaves, a set of methods could be established that could genetically modify the *S.palmetto*, so that it could be planted and raised in various regions. Foliar epicuticular waxes were chosen for analysis because the most photosynthetictissues are located there and compared to the rest of the plant they are the most susceptible to environmental damage (Jenks et. al,1995). The adaxial side of the leaf is analyzed because it is the side of the leaf that is naturally exposed to sunlight (Buschhaus,et.al, 2007).

The epicuticular wax is composed of superimposed lipids and cutin and is located on the very surface of most complex plant structures.(Gonzalez andAyerbe, 2009; and Koch, and Ensikat,2007). Epicuticular wax serves as a barrier against any foreign articles that could potentially penetrate the plant's surface. Epicuticular wax is hydrophobic, and therefore increases the hydrophobicity,wettability, and self cleaning behavior of the plant's surface. (Koch, and Ensikat,2007). Environmental changes and conditions influence the epicuticular wax as well. For example, a study revealed that the amount of epicuticular wax produced increases as it is exposed to terminal water stress.(Gonzalez andAyerbe, 2009)

Research has revealed that there are many intricate structures located in the epicuticular wax . This was determined through scanning electron microscopy(Jenks et. al,1995). There are many methods that could be used but the most effective while being the least invasive is the use of hexane for solvent extraction. (Morrison et.al, 2006). The results of both of these methods from previous research revealed results that could be used to aid various plant in their resistance to pathogenic and environmental stress. Though aspects of our research are derived from previous studies, we would like to branch out and determine if there is a consistent correlation between the composition and the structure of epicuticular wax. If certain structures can be matched with results from chemical analysis, and a consistent correlation is found, the structures of the epicuticular wax could be changed with genetic modification.

2. Methodology:

2.1 Chemical extraction of the waxes using hexane:

Samples of both palms from the youngest and oldest leaf were collected and labeled. Test tubes were labeled and filled with 10 mL of hexane. The leaves were folded so that the ends of the leaves were not exposed to the hexane. The samples were capped and sealed with parafilm to prevent the hexane from evaporating. The samples were placed in a chemical fume hood and left to sit for various periods of time. One test tube was filled with solely hexane was the control.

2.2 Wax analysis using the GENESYS 20 visible Spectrophotometer:

A blank hexane solution was placed in a cuvette. The cuvette was placed in the spectrophotometer. The spectrophotometer was set certain wavelengths to analyze the absorbances of the waxes. The previous steps were repeated with each sample.

2.3 Structural analysis using the Scanning Electron Microscope JEOL JSM-6010LA

The leaves were cut to fit around the gold plate. The leaf was tapped with dual sided tape so that the adaxial side of the leaf was visible. The gold plate was placed in the sample holder. The sample holder was placed on the stage of the SEM. The chamber was evacuated to make a vacuum. Images were taken at 100x, 500x, and 1000x.

3. Results and Conclusion:

Age	Absorbance	Wavelength
Youngest leaf	**-0.016 A**	**340 nm**
Oldest Leaf	**0.011 A**	**340 nm**
Youngest Leaf	**0.0 A**	**350 nm**
Oldest Leaf	**0.013 A**	**350 nm**

Figure 1.The spectrophotometer gives a quantitative reading of how much light was absorbed by the epicuticular wax in *S. minor* at certain wavelengths. The more wax present the higher the reading.

Age	Absorbance	Wavelength
Youngest Leaf	**0.026 A**	**340 nm**
Oldest Leaf	**0.087 A**	**340 nm**
Youngest Leaf	**0.030 A**	**350 nm**
Oldest Leaf	**0.066 A**	**350 nm**

Figure 2. The spectrophotometer gives a quantitative reading of how much light was absorbed by the epicuticular wax in *S. palmetto* at certain wavelengths. The more wax present the higher the reading.

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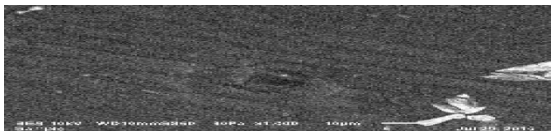
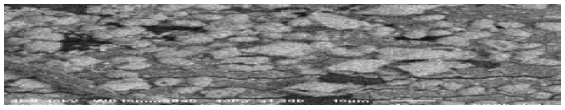
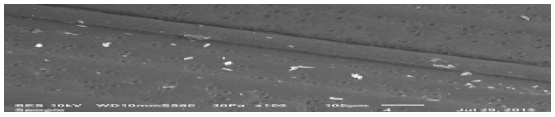
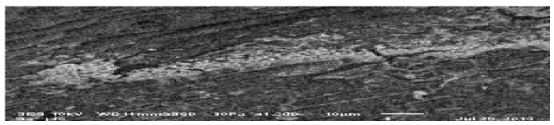
Plant Type	Micrographs	Sheets of waxes	Vertical Plates	Horizontal plates	Globular Units
Sabal palmetto **(Youngest leaf)**		+	-	-	-
Sabal palmetto **(Oldest leaf)**		+	+	+	+
Sabal minor **(Youngest leaf)**		+	-	-	-
Sabal minor (Oldest leaf)		+	+	+	-

Figure 3. The plus sign indicates that a structure is present and the minus sign indicates that a structure is not present

The results display that there is a correlation between the complexity of the wax structures and the amount of wax that is present on the leaf of the palm. There is also a correlation between the absorbance readings and complexity of wax structures between the two types of palms. The *S.minor* has the least complicated wax structures and the lowest absorbance readings and the *S. palmetto* had the most complex wax structures and the highest absorbance readings. This data reveals that the *S.palmetto* has more protection from adverse conditions. This is because the epicuticular wax serves as a barrier and the larger quantity of the wax indicates that there is a stronger barrier.

The evidence shows that there is epicuticular wax present on the young and old leaves. As the leaf gets older more wax is added. It does not lose wax as it gets older it gains wax. Young leaves do not have globular units of wax. This tells us that not only does the environment of the plant influence the amount of wax a leaf may have, but that the age of the leaf also determines how much wax a leaf will have. As the plant ages, the wax does not leave. If there is less wax in older leaves this indicates that a major environmental event has happened to take away the wax. This event could be severe wind damage or an animal etc. Age and environmental cues determine foliar epicuticular wax structure/pattern and abundance.

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