HERB-CHRONOLOGY AS A TOOL FOR DETERMINING THE AGE OF PERENNIAL FORBS IN TROPICAL CLIMATES

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ABSTRACT

Age in wild plant populations is one of the most elusive developmental parameters in plant biology. Several approaches take advantage of plant morphological traits to determine developmental stages or plant age. Annual growth rings forming in woody tissues of perennial plants are one of the traits that have been widely used to determine the age of trees (dendrochronology) and, more recently, herbaceous perennials (herb-chronology). In temperate, alpine, and arctic climates, it has been reported that seasonal climatic variations lead to the formation of annual growth rings in herbaceous perennial forbs; however, to date, no similar studies have been carried out on plants from tropical regions. We have investigated the applicability of herb-chronology on the tropical plant *Pentalinon andrieuxii*, a native vine of the Yucatan peninsula. Our results show that herb-chronology is a potentially useful tool in determining the age of plants growing in tropical climates.

Keywords: Herb-chronology, Tropical climate, Plant age, Apocynaceae, *Pentalinon andrieuxii*.

1 Introduction

2 The importance of determining plant age cannot be overestimated since it allows to draw
3 many conclusions in terms of plant ecology, climate change, ecosystem recovery and
4 plant secondary metabolite accumulation (Dietz and Ullmann 1998; Liu et al. 1998; Dietz
5 and Fattorini 2002; Alexander et al. 2009). Traditionally, the tools most commonly used for
6 classification of wild plant individuals when age is unknown are developmental stages,
7 plant size (Law 1983), and morphological markers (Olesen and Ehlers 2001; Ehrlén and
8 Lehtilä 2002). However, accuracy is still a concern in these cases, since plant
9 developmental stages are usually assigned arbitrarily and plant morphology and plant size
10 (height or girth) might not always be as closely related to plant development as presumed
11 (Dietz and Ullmann 1998; Carlquist 2001).

12 One approach that has gained recent importance in determining the age of herbaceous
13 plants is herb-chronology (Dietz and Ullmann 1997), which focuses on the growth rings
14 observed in the roots and stems of perennial forbs; these rings are recognizable because
15 of differences between wide lumina vessels in earlywood and narrow lumina vessels in
16 latewood (Dietz and von Arx 2005; von Arx and Dietz 2006; Nobis and Schweingruber
17 2013; Dee and Palmer 2016; Shi et al. 2016). Herb-chronology has proved useful in
18 determining the age of plants growing in temperate (von Arx and Dietz 2006; Liu and
19 Zhang 2007; Olano et al. 2013; Eugenio et al. 2014) and alpine regions (Kuen and
20 Erschbamer 2002; Erschbamer and Retter 2004; von Arx et al. 2012), where the periodical
21 repetition of seasonal events such as cold winters and warm springs results in the
22 development of root-growth rings that can be associated to plant age (Carlquist 2001;
23 Schweingruber and Poschlod 2005). However, and although there are reports about
24 dendrochronological studies carried out in tropical forest trees (Worbes 2002; Roig et al.

2005), to date no herb-chronological studies have been reported on plants growing in tropical regions where the differences in temperature between seasons can be relatively small, but the differences in water availability can be significant. It has been reported that water availability influences the size of vessels due to the plasticity of the root tissue, i.e. an excess in water availability induces the appearance of larger vessels and the opposite occurs during dry periods (von Arx et al. 2006; 2012). It remains unclear, however, if the periodicity and the differences in water availability are sufficient to allow the formation of discernable annual growth rings in perennial forbs in these latitudes (Dietz and Schweingruber 2002).

In this study we explore the relevance and potential applicability of herb-chronology as a tool for determining the age of tropical forbs by evaluating the appearance of discernable growth rings, resulting from seasonal differences in water availability in the tropical forb *Pentalinon andrieuxii* (Müll. Arg.) B.F. Hansen & Wunderlin, (Apocynaceae) (Fig. 1), a perennial vine growing from southern Florida to Nicaragua (Morales 2006; 2009).

Materials and Methods

Study site

This study was carried out on a population of *P. andrieuxii* growing wild in the state of Campeche, Mexico (19°46'0.895"N; 90°80'0.156"W). The climate at the study site, like the rest of the Yucatan peninsula, is mostly warm and sub-humid (Orellana et al. 1999; Bautista et al. 2011). Mean values of precipitation and temperature for each month were analyzed taking the Gaussen's xerothermic index (Gaussen and Bagnouls 1952) into account. Two very distinct seasons were observed: a rainy season from June to October,

and a dry season from November to May, with average monthly precipitations of 185.2 mm and 36.6 mm, respectively (mean annual precipitation: 98.5 mm) (Fig. 2) (Márdero et al. 2012).

Plant Material

Plant samples were selected at the site and separated into developmental stages. The developmental stages were defined by taking very distinct morphological characteristics into account. Morphological and size-related parameters are summarized in Table 1.

A total of 36 plant samples were collected just after the end of both the rainy (17 samples; December 2013) and the dry (19 samples; May 2014) seasons. The top 5-10 cm of the upper section of the taproot connected to the stem of each individual were cut and stored in 75% ethanol until processed. A voucher specimen was deposited at the herbarium of “Unidad de Recursos Naturales CICY” (Collection code: Calvo, Dzib, Hiebert 334).

Plant measurements

Three growth indicators were used: categorical developmental stages (Table 1), stem diameter at ground level (SDGL), and plant height. Developmental stages were defined by taking distinct morphological characteristics into account; stem diameter was measured using a Digimatic CD-6 Caliper (Mitutoyo Corp.) and plant height was measured with the aid of a fiberglass telescoping measuring rod, using the tallest visible part of the plant as a reference point. Each measurement was made at the site before plant collection.

Herb-chronological analyses

Herb-chronological analyses were carried out following the methodology described by Dietz and Fattorini (2002). Fresh taproot cross-sections (45-50 μm) from each plant sample were obtained using a Leica RM2125RT microtome. Each cross-section was

1 stained using phloroglucinol/HCl (Wiesner reaction), resulting in lignified structures (walls
2 of xylem vessels, fibers and parenchyma cells) having a reddish color. Stained cross-
3 sections were photographed using a Zeiss Stemi 2000-C Stereo-Microscope and a
4 Moticam 2000 digital camera. Growth ring patterns were then visually analyzed using
5 digital images. Two main criteria were used to identify the distinct concentric earlywood-
6 latewood transitions characteristic for growth rings: 1) Difference in vessel size, where
7 larger vessels presumably correspond to earlywood formed during the rainy season, while
8 smaller vessels are probably related to latewood that appeared during the dry season; 2)
9 spatial distribution of vessels, with larger vessels expected to cluster as concentric rings at
10 the beginning of a growth ring (Fig. 3).

11 *Statistical analysis*

12 Statistical analyses were carried out using the SPSS Statistics software version 17.0.1.
13 Data (categorical developmental stages, SDGL, plant height and number of growth rings)
14 were analyzed in order to determine the significance of differences in the average number
15 of detected growth rings among development stages. Means were compared by a One-
16 Way ANOVA and Tuckey post-hoc tests with a significance threshold of $p \leq 0.05$. A linear
17 correlation analysis, using the Pearson coefficient, was performed in order to establish the
18 relationship between the number of detected growth rings and the size-related
19 developmental stage indicators.

21 **Results**

22 Clarity of ring separation in *P. andrieuxii* was found to be variable, thus the ring structure
23 observed in this investigation corresponds to the semi-ring-porous type usually observed

in perennial forbs (Carlquist 2001). Growth rings were found in the interior of the xylematic tissue in 35 out of the 36 collected samples (Table S1) (Fig. 4). Samples collected at the end of the rainy season presented large vessels in the outmost cell layers of xylem, and samples collected after the dry season presented several rows of relatively narrow vessels between the end of xylem and the last growth ring (Fig. 5A, 5B).

The data showed a statistically significant increase in the average number of growth rings across developmental stages [young = 2.88 (± 0.60), intermediate = 4.40 (± 0.52), adults = 6.38 (± 0.52)], clearly indicating a relationship between the development stages of *P. andrieuxii* and the number of growth rings in the root tissue of the different plants (Fig. 6A) ($F_{(2, 32)} = 107.58$, $p \leq 0.05$). These findings were confirmed by the statistically significant correlation between the number of growth rings and size-related variables such as SDGL and plant height (SDGL: $r_{(34)} = 0.847$, $p \leq 0.01$; height $r_{(34)} = 0.756$, $p \leq 0.01$) (Fig. 6B, 6C).

Discussion

Seasonal events need to repeat periodically for growth rings to be associated to time and plant development (Carlquist 2001; Schweingruber and Poschlod 2005). Since the relationship between climatological conditions and vessel morphology can be observed by analyzing the newest layers of xylem cells (von Arx et al. 2012), the clustering of large vessels at the cambial end of the xylem of the root tissue of *P. andrieuxii* during the rainy season, and their absence during the dry season, confirm the relationship between seasonality and differences in the root hydraulic structures of the plant. This demonstrates that periodic rainy and dry seasons with little differences in temperature, such as those occurring in the Yucatan peninsula, can produce discernable growth rings (Fig. 5). This

1 clear correlation between plant development and the number of growth rings in the roots of
2 *P. andrieuxii* confirms the original assumption that differences in water availability can
3 result in the formation of root-growth rings in herbaceous perennials growing in tropical
4 climates, and that herb-chronology can be a useful tool to study the age of plant
5 populations in tropical regions.

6 Even though our results coincide with those reported from dendrochronological studies of
7 native trees grown in the Yucatán peninsula, which showed that the clear seasonality of
8 the Yucatan peninsula allowed the observation of growth rings (Roig et al. 2005), it is
9 important to keep in mind that atypical seasons of extended rainy or dry periods can be a
10 source of irregularities in the appearance of growth rings and in its relationship to plant
11 development (Schweingruber 1988). This problem has been limited or overcome, in both
12 dendrochronology and herb-chronology studies, by cross-referencing the observed
13 number of growth rings of several species with the historic registry of precipitation (Rigling
14 et al. 2001; von Arx and Dietz 2006).

15 The results of our investigation confirm the importance of herb-chronology as a potentially
16 useful tool to establish the age of tropical forbs, as well as to better understand the
17 intricate dynamics of plant populations in wild forests, its adaptations to climate change,
18 and its capabilities to recover from human driven activities. Still, a controlled study
19 monitoring plants of different species and of known age, in several study sites, and with a
20 thorough registry of water availability, should be performed to deepen the understanding of
21 the dynamics of growth ring formation in herbaceous plants growing in the tropics.

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Table 1. Parameters describing developmental stages established for *P. andrieuxii*.

Developmental stage (No of individuals)	Plant habit	Plant height (m)	SDGL (mm)
Young (16)	Self-supporting	<0.5	<4
Intermediate (11)	Beginning of non-selfsupporting	0.6-1.9	4-10
Adult (9)	Non-selfsupporting, flowering, fruits	>2	>10

Fig. 1. Flower, leaves, and pod of *Pentalinon andrieuxii* (Müll. Arg.) b.f. Hansen & Wunderlin (Apocynaceae) (Magnification 3x).

Fig. 2. Climatogram corresponding to the historical monthly mean values of precipitation and temperature registered at the study site. Graph constructed with historical data (1950 – 2000) using the DIVA-Gis software (Hijmans et al., 2005).

Fig. 3. Growth rings (highlighted with yellow lines) in root cuttings of *P. andrieuxii* (Magnification 10x). Sample stained with fluoroglucinol/HCl (A) and toluidine blue (B) for contrast.

Fig. 4. Growth rings in stained root cuttings corresponding to young (A), intermediate (B) and adult (C) individuals of *P. andrieuxii*. Arrows indicate large vessels appearing during the rainy season and yellow lines emphasize the concentric arrangement of large vessels.

Fig. 5. Root cuttings stained with phloroglucinol/HCl from plants of *P. andrieuxii* collected during the rainy (A) and dry (B) seasons. Black lines highlight the appearance of large vessels forming a new growth ring due to an increase in water availability. Double arrows indicate the distance between the new growth ring and the end of xylem in plants collected during the dry season (not observed in plants collected during the rainy season).

Fig. 6. A) Number of growth rings observed at different developmental stages of *P. andrieuxii*, each letter indicates statistical difference at $p \leq 0.05$. B) and C) Correlation of the number of observed growth rings with the SDGL and plant height, respectively.

Fig. 1. Flower, leaves, and pod of *Pentalinon andrieuxii* (Müll. Arg.) b.f. Hansen & Wunderlin (Apocynaceae) (Magnification 3x).

157x117mm (300 x 300 DPI)

Fig. 2. Climatogram corresponding to the historical monthly mean values of precipitation and temperature registered at the study site. Graph constructed with historical data (1950 – 2000) using the DIVA-Gis software (Hijmans et al., 2005).

89x49mm (300 x 300 DPI)

Fig. 3. Growth rings (highlighted with yellow lines) in root cuttings of *P. andrieuxii* (Magnification 10x). Sample stained with fluoroglucinol/HCl (A) and toluidine blue (B) for contrast.

110x92mm (300 x 300 DPI)

Fig. 4. Growth rings in stained root cuttings corresponding to young (A), intermediate (B) and adult (C) individuals of *P. andrieuxii*. Arrows indicate large vessels appearing during the rainy season and yellow lines emphasize the concentric arrangement of large vessels.

143x94mm (300 x 300 DPI)

Fig. 5. Root cuttings stained with phloroglucinol/HCl from plants of *P. andrieuxii* collected during the rainy (A) and dry (B) seasons. Black lines highlight the appearance of large vessels forming a new growth ring due to an increase in water availability. Double arrows indicate the distance between the new growth ring and the end of xylem in plants collected during the dry season (not observed in plants collected during the rainy season).

168x49mm (300 x 300 DPI)

Draft

Fig. 6. A) Number of growth rings observed at different developmental stages of *P. andrieuxii*, each letter indicates statistical difference at $p \leq 0.05$. B) and C) Correlation of the number of observed growth rings with the SDGL and plant height, respectively.

106x227mm (300 x 300 DPI)