

The Study of Annual Rings in *Lespedeza cuneata* in the Tallgrass Prairie of Oklahoma

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Abstract

Herb chronology is the study of the ring like growth in the secondary xylem in the taproots of perennial herbaceous plants. Not much is known about the growth rings of perennial herbaceous species, especially in the tallgrass prairie system of the Great Plains. Herbs were sampled from the Cross Timbers Experimental Range (CTER) in Stillwater, OK. Taproots of five of the most common herbaceous species of CTER were excavated. These taproots were prepared for analysis in the laboratory. The taproots were sliced into thin slices using a mini sledge microtome, and the sections were then dehydrated. Lastly, the sections were stained to differentiate between the lignified and non-lignified cells. The secondary root xylem is then examined under a microscope. Most of the species collected displayed growth rings. If these growth rings are later determined to be annual, the age of the herb can be determined, and the widths of each ring can be analyzed to determine if it correlates with yearly precipitation, average temperature, or land history such as fire.

Key Words: Growth rings, herb-chronology perennial herbs, secondary root xylem.

Introduction

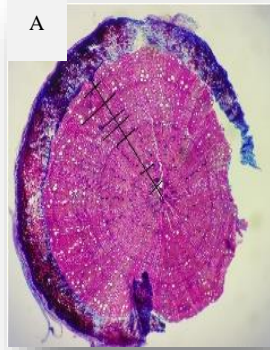
Herb chronology is the study of growth rings in the taproot of perennial forb species. Key goals of this research are to find the extent to which herb species have rings, whether they are annual, and if annual ring width variation varies with certain yearly environmental factors. These annual growth rings are potentially able to tell us a lot about climatic variation, climatic events, growing conditions, and of course age. With annual ring widths we can determine how the herbaceous species compete with other plant species, and how it may respond to climate change within its ecosystem. The annual rings structures are very similarly resemble the patterns of semi-ring porous woods with wider vessels (Dietz and Schweingruber 2002). Dendrochronology, the study of woody species, also uses determined annual growth rings to evaluate climatic factors, growing conditions, and age over a large period of time. Perennial herbs can be used more effectively to evaluate climatic factors, and growing

conditions for shorter periods of time since on average they have much shorter life spans than woody species. Perennial herbs are more sensitive, and more directly responsive to short term climatic events (Dietz and Von Arx 2005). These herbs can be used to monitor climatic events such as droughts, floods, thunderstorms, snow storms, etc..

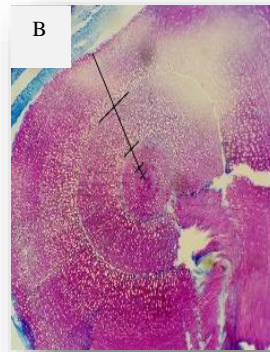
Herb chronology is a new and upcoming field of study within ecology. Little is known about these annual growth rings in the perennial herbs that are measured in herb chronology. The use of annual rings widths can be used to indicate the success of a growing season (Dietz and Ullmann 1997). Analysis of Annual growth rings, which is already common practice among ecological tree researchers (Schweingruber 1996), could maintain the ecological interpretation of stand structure

and its development for perennial herbs (Dietz and Ullmann 1997). Studies suggest that the use of herb chronology may be a valuable asset for many different types of

investigations on plant populations. First, and foremost the age structure of a population can be determined. The age distribution patterns of a population may be



Lespedeza capitata demonstrating 7 discernable growth rings



Hedyotis nigricans, demonstrating 4 discernable growth rings.



Asclepias viridis demonstrating 7 discernible growth rings



Lespedeza cuneata demonstrating 3 discernible growth rings



Psoraleidium tenuiflorum demonstrating no discernible growth rings.

used to determine the development of a population and be able to predict further population growth (Dietz and Ullmann 1997). The phenomenon of growth rings have only been studied in Michigan & Oregon in the United States, and Europe to our knowledge (Dietz and Schweingruber 2002; Perkins 2006). The research data collected provided scientists an opportunity to compare the patterns and the presence of perennial herbs growth rings. Between the two studies, nearly two thirds of species investigated had discernible growth rings within the taproot.

Since herb-chronology has not ever been conducted in the tall grass prairie region, this experiment will establish a basis for herb chronology in this widespread type of grassland. There are hundreds of long-lived perennial herb species that are native to the region. This is a great region to establish research from many perennial species, and see if the growth rings correlate with climate. The tall grass prairie region experiences a fluctuating climate and is thus a fantastic region to determine if the growth rings correlate with the changes in mean annual temperature and rainfall. The objective with this experiment is to demonstrate the presence of growth rings in at least 20 of the most dominant tallgrass prairie perennial herbs, and also determine if growth rings of species analyzed above are annual by utilizing individuals of a known age from a local plant nurseries and counting their rings. We hypothesized that growth rings will be present in most perennial herbaceous species because studies in Michigan, and Europe have found significant presences of growth rings in the amount of herb species investigated.

Methods

Specimens of five species of perennial herbs (*Lespedeza capitata*, *Hedyotis nigricans*, *Asclepias viridis*, *Lespedeza cuneata*, and *Psoraleidum*

tenuiflorum) were excavated from the Cross Timbers Experimental Range. These species were pre-determined to be the most dominant from different burn units. A shovel was used to cut deep below the soil surface. The tap root is then cut close to the proximal end using clippers. Samples are then taken back to a lab on the OSU campus. Using a mini sledge microtome we produced 15-30 µm thick sections of the tap root. The section was then soaked in bleach for 3-5 minutes on a microscope slide. The sections were then rinsed with water, and put into stain for 3-6 minutes. Saffarin and Astra Blue were used to stain lignified cells red and non-lignified cells blue to help aid in detection of growth rings. The section was then once again rinsed off with water. Next the section was dehydrated with 66% alcohol, and then 90% alcohol. Xylene was then used to detect if the sections need further dehydration. Canadian Balsam was lastly used to help affix a cover slip and preserve the section for future observation.

Results

Of the five dominant species analyzed four showed distinct growth rings. Each growth ring is able to be determined because of the ring like structure of the secondary root xylem, and the large amount of vessels that are at the beginning of each ring. Through this process a *Lespedeza capitata* specimen (A) was found to have seven discernible growth rings, a *Hedyotis nigricans* specimen (B), had four discernible growth rings, an *Asclepias viridis* specimen (C) had seven distinguishable growth rings, and a *Lespedeza cuneata* specimen (D), shows three growth rings. *Psoraleidum tenuiflorum* (E) was the only species encountered without discernible growth rings.

Discussion

Throughout sampling these five species we also aim to find the extent to

which the occurrence of growth rings are consistent between different plant lineages. So far, we have found that among legumes species have a tendency to form growth rings. *Lespedeza capitata* and *Lespedeza cuneata* contain obvious growth rings. However, we could not find growth rings in the legume *Psoraleidum tenuiflorum*. . *Asclepias viridis*, commonly known as antelope milkweed, is another abundant herb species that has growth rings.

In order to continue the research at least 60 species will be excavated and analyzed to determine if they also have any distinct growth rings. Also, while collecting more species their secondary root xylem will be observed for the presence of starch. This part of the experiment will be to determine if the amount of starch per ring affects its radial width. This is important because it can create an understanding of how growth rings in the root may be a product of aboveground biomass. Overall, the research will establish a broader basis for the applicability of herb-chronology in the tallgrass prairie region, while also providing answers for determining the utility of this technique within plant ecology.

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Literature Cited

Dee, J. R. and Palmer, M. W. (*Manuscript in preparation*)

Fertilization, along with growing season temperature and precipitation, has differential effects on the growth of annual rings in the roots of perennial forbs: Application of herb-chronology in the tallgrass prairie region of the central U.S.

Dietz, H. & Schweingruber, F.H. 2002. Annual rings in native and introduced forbs of lower Michigan, USA. Canadian Journal of Botany-Revue 80:642-649.

Dietz, H., and Ullmann, I. 1997. Age determination of dicotyledonous herbaceous perennials by means of annual rings: Exception or rule? Ann. Bot. 80: 377-379.

Dietz, H., and Ullmann, I. 1998. Ecological application of 'herb-chronology': comparative stage and structure analyses of invasive plant *Bunias orientalis* L. Ann. Bot. 82: 471-480.

Dietz, H. and von Arx, G. 2005. Climate fluctuation causes large scale synchronous variation in radial root increments of perennial forbs. Ecological Society of America. 86: 2. 327-333

Perkins, D., Parks, C., Dwire, K., Endress, B., and Johnson, K. 2006. Age structure and age related performance of sulfur cinquefoil (*Potentilla recta*). 54: 87-93.