RESEARCH PROJECT

Development of growth rings in roots of dicotyledonous perennial herbs: experimental analysis of ecological factors

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Summary

1 Plant age is an important but less accessible parameter in population ecology and life-history strategy. For most plants, notably perennial herbaceous species, the possibility to determine calendar age has been regarded to be restricted to only exceptional cases, if at all possible. We therefore have only a poor understanding of plant age and life-span for many species, which renders quantitative comparisons of life-history strategies difficult.

2 Our recent studies suggest that annual growth rings in the roots of perennial dicotyledonous herbs are far more widespread than hitherto accepted and could be used for age determination (herb-chronology). Furthermore, as in trees, variations in annual ring width may indicate ontogenetic changes in growth and fluctuations in past growth conditions that could be used in ecological studies.

3 In this project we experimentally test the following hypotheses: (i) growth rings in the stem bases or roots of perennial forbs are truly formed annually, irrespective of growth conditions and (ii) xylem growth responds sensitively to variations in relevant environmental factors so that the patterns of growth ring width can be used to compare growth conditions among sites and to infer changes in past growth conditions.

4 The development of growth rings will be analyzed in a multi-year field experiment at two locations differing in climatic conditions. The experiment will involve competition intensity and simulated herbivory (clipping) as treatments and will include nine species of perennial forbs from different plant families, which differ in the clarity of growth rings and in longevity.

5 We expect our project to yield the first "hard" results on the factors determining the development of growth rings in the roots of perennial herbs. These results are indispensable for a sound evaluation of the potentials and limitations of herb-chronology in current and future studies. Analysis of annual rings can generally contribute to our understanding of processes in plant ecology for a wide variety of forb species and may also be valuable in taxonomic studies.

Keywords: Growth plasticity, growth rings, perennial forbs, plant age, population ecology, reconstructive method

Bulletin of the Geobotanical Institute ETH (2001), **67**, 97–105

Introduction

USES AND PROBLEMS OF PLANT AGE IN ECOLOGY

Plant populations are commonly characterized by a variety of parameters describing population structure and development. While parameters describing the present state of plant populations (e.g. population density and size or stage structure) can be easily measured, parameters reflecting the dimension of time, i.e. rates of birth, growth and death and the resulting age structure or age-dependent size structure are generally harder to obtain (e.g. Harper 1977; Vorontzova & Zaugolnova 1985; Menges 2000). To record parameters of population development, populations are often analysed repeatedly, usually during several consecutive years (e.g. Sarukhán & Harper 1973; During *et al.* 1985; Ehrlén 1995; Fowler 1995; Meyer & Schmid 1999). This method yields accurate data for the explanation of population development or the creation of models of future population growth but it is very time-consuming and therefore poses problems (cf. Menges 2000).

In populations of many woody plants, patterns of annual rings in the secondary xylem can be used to analyse population age structure and past growth rates of individual plants (dendroecology, see e.g. Hett & Loucks 1976; Johnson & Fryer 1989; Veblen *et al.* 1991; Schweingruber 1996). This reconstructive approach allows certain inferences on past population development. In this static analysis not only the number of annual rings can be used (for age determination). There are also fluctuations in the width of annual rings and in the density of vessels, parenchyma or fibres and variations in cell wall thickness which are related to the development of plant size and form or to environmental factors that have influenced plant growth, such as climatic fluctuations, pollution effects and anthropogenic disturbances (Schweingruber 1996).

In contrast to trees, age determination in perennial herbs has been generally regarded as problematic or impossible in most cases (e.g. Harper 1977; Vorontzova & Zaugolnova 1985; Hanzawa & Kalisz 1993). In some cases morphological markers can be used for determination of calendar age, e.g. old leaf or bud scale scars (Callaghan & Collins 1976; Anderson et al. 1993). However, anatomical markers in perennial plant parts, such as main roots, mesocorms or rhizomes, that resemble the annual rings in woody plants have been regarded as exceptional or disregarded (e.g. Harper 1977; Barbour et al. 1980).

GROWTH RINGS IN PERENNIAL FORBS

Our recent research strongly suggests that annual rings are far more widespread in perennial herbs than hitherto accepted and may therefore represent a particularly underutilized resource for research in plant population ecology. In a first systematic survey in central Germany, the permanent main roots from 23 out of 35 dicotyledonous perennial forb species (representing 16 plant families) showed clear or relatively clear growth rings in the secondary xylem (Dietz & Ullmann 1997). The annual nature of the observed growth rings could be verified for ten of these species where individuals with known age were available. Phenological observations provided further evidence for the annual periodicity in the formation of growth rings (Dietz & Ullmann 1997). Growth rings in the roots could also be found in two thirds of 60 species of perennial forbs that were sampled in Michigan (USA) in 1999 and included native as well as non-native species (Dietz & Schweingruber, *unpublished data*). In a further study almost 300 forb species sampled along a climatic gradient from the Canary Islands to sub-alpine areas of the Swiss Alps were analysed. In this sample clearly or relatively clearly demarcated growth rings in the roots or stem bases were present in 50% to 85% of the species, depending on the climatic zone (Schweingruber & Dietz 2001).

We have also observed strong fluctuations in the width of growth rings in perennial forbs. These may be related to differences in climatic conditions (Schweingruber & Dietz 2001). Fluctuations of annual ring widths may also represent sensitive records of past (micro-) site conditions in general. For example, in the invasive forb *Bunias orientalis* (Brassicaceae) within-population changes in the patterns of annual ring width matched a gradient of light supply that developed by growth of Blackthorn scrub (*Prunus spinosa*) gradually overshadowing the population of *B. orientalis* from one side (Dietz & Ullmann 1998).

Other studies that report annual rings in the secondary xylem of dicotyledonous forbs and that make use of them in plant ecology are extremely scarce and are restricted to case studies of single species. Humulum (1981) used annual rings in the secondary xylem of the arctic-alpine forb *Oxyria digyna* to classify populations as increasing, stable or decreasing. Likewise, by analysis of annual rings Boggs & Story (1987) were able to infer that populations of the invasive forb *Centaurea maculata* were stable or still expanding in Montana (USA). They also demonstrated that plant fecundity increased with age and could reject former views that *C. maculosa* is very short-lived.

Research questions and hypotheses

The objective of our research project is to obtain a detailed understanding, in terms of both phenology and ontogeny, of the formation of growth rings in roots of forbs growing under varying conditions. In particular, we will test the assumed correspondence between the annual seasonal climatic periodicity and the number of growth rings. We further want to investigate ecological factors that constrain plant growth and size and their influence on patterns of annual growth increments in the xylem. Our research will focus on two main questions and the related hypotheses.

1. Are the growth rings in dicotyledonous perennial herbs invariably formed on an annual basis?

Our hypothesis is that growth rings in the stem bases or roots of perennial forbs are truly formed annually, irrespective of factors that might influence the root anatomy (other than the seasonal climate). This hypothesis is supported by phenological investigations on growth of the root xylem (Dietz & Ullmann 1997) and by a small set of species where individuals of known age were available for verification of the annual nature of the growth rings (see Humulum 1981; Boggs & Story 1987; Dietz & Ullmann 1997). However, an experimental test of the development of growth rings in the secondary root xylem is still lacking. An experimental approch is also indispensable to evaluate variations in anatomical patterns (Fig. 1, see also Dietz & Ullmann 1997; Schweingruber & Dietz 2001) that may be caused by phylogenetic, ontogenetic and environmental factors.

In this project we will monitor the development of growth rings in a set of 9 forb species from different plant families over several years. The species were chosen such as to represent a wide variety of anatomical patterns and (presumed) longevity. The individuals were grown from seeds and will be cultured for up to 11 years so that we will be able to analyse samples of the plants at all times during the life-span of probably most of the

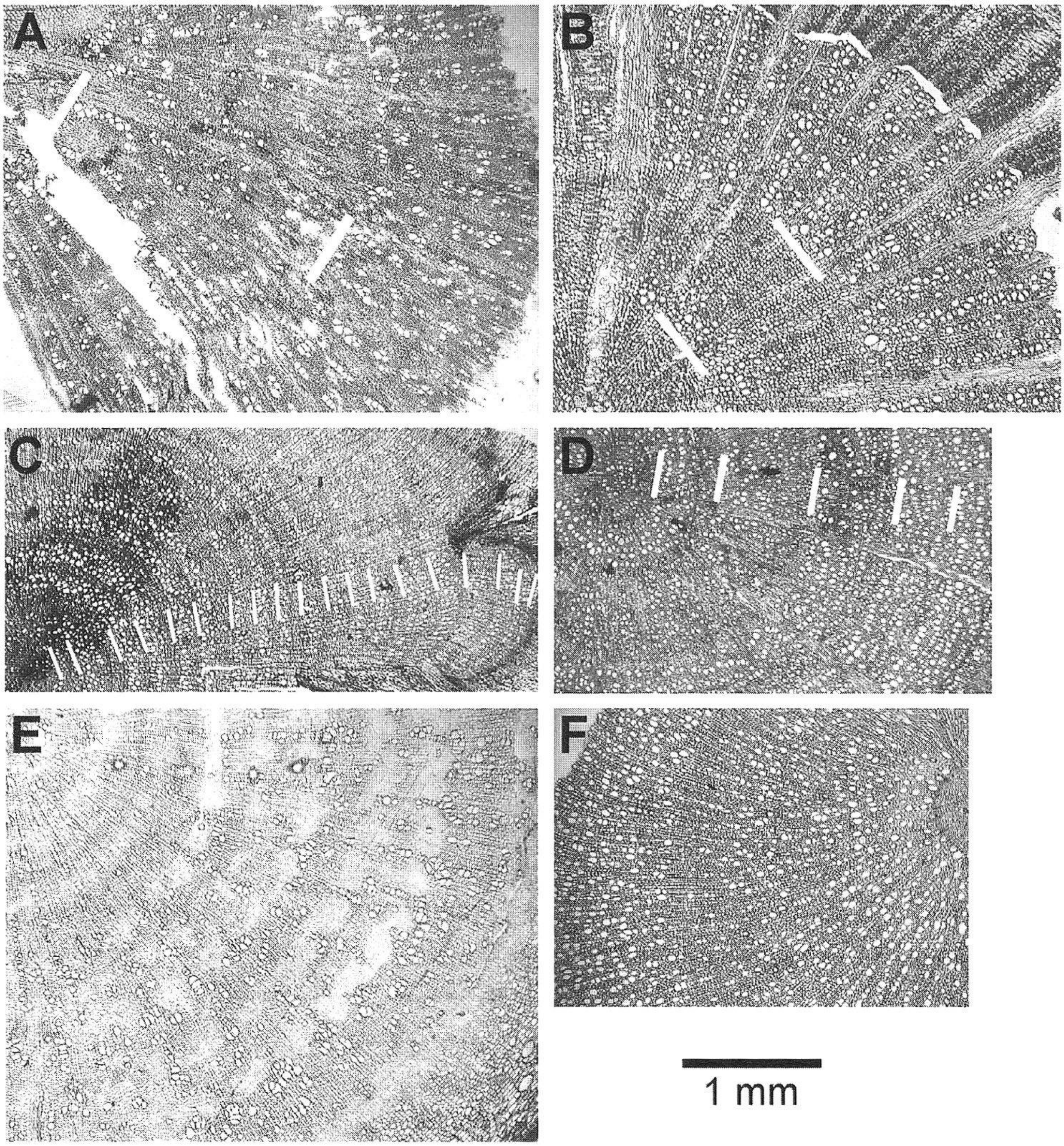

Fig. 1. *Patterns of annual rings in the secondary xylem near the proximate end of the main root in 6 species that are used in this project. The samples shown were collected at typical field sites in 1999 in Michigan, USA and near Würzburg, Germany (*Sanguisorba minor*). White markers indicate presumed transitions from latewood of the previous growing period to earlywood of the following one. Markers are not shown for species with problematic anatomical patterns (E, F). Short-lived species: A,* Cichorium intybus; *B,* Trifolium pratense; *long-lived species: C,* Digitalis grandiflora; *D,* Sanguisorba minor; *species with problematic anatomy: E,* Euphorbia esula*; F,* Hypericum perforatum. Nomenclature follows Tutin *et al.* (1964–1980).

individuals. A sub-year sampling interval in the first years will allow a phenological analysis of xylem growth. Variation in climatic and soil conditions is introduced by replication of a part of the experimental setup at two sites, one of them north and the other south of the Alps.

2. Are growth ring widths (i.e. annual radial increments) sensitively and consistently dependent on microsite conditions?

Our hypothesis is that xylem growth responds sensitively to variations in relevant environmental factors such that the patterns of growth ring width can be used to compare growth conditions among sites and to infer changes in past growth conditions.

As in dendroecology (e.g. Schweingruber 1996) poor growth conditons may be reflected by narrow rings and good growth conditions by wide rings (e.g. Dietz & Ullmann 1998). Growth ring sequences in perennial forbs may show particularly narrow or wide rings interspersed among 'normal' rings. These irregular rings may indicate year-specific events (disturbances in the widest sense) that either curbed (e.g. herbivory) or furthered annual growth (e.g. release from competition). In addition, growth ring widths may gradually increase or decrease due to improving or deteriorating growth conditions, respectively, or to ontogenetic factors. Hence, fluctuations in annual ring width may be used for a posteriori analyses to (i) infer differences in microsite conditions (comparison of plants within a population), (ii) compare growth conditions between sites or (iii) compare plant growth between different species. In particular, analysis of growth ring widths may be used to examine the effects of herbivory or resource competition on plant growth.

In this project we use the experiment outlined above to compare fluctuations of annual ring widths within and between the study species and in response to different treatment factors. We test the effects of strongly increasing intra- and interspecific competition by growing individuals in high density. The high density treatment is expected to result in the development of size hierarchies in the test plant cohorts so that influences of variations in growth on the development of growth rings in the secondary xylem can be analysed independent of plant age. In another treatment plants are relatively widely spaced but they will be clipped to simulate the effects of strong herbivory. One purpose of this treament is to test whether serious impacts like herbivore attacks can produce false rings. In a third treatment the plants are also grown widely spaced but will be left untreated and spontaneous competing neighbours will be removed (control). The analysis of anatomical patterns in relation to plant size and fitness and comparisons between species and treatments will indicate how responsive xylem growth increments are to common factors restricing plant growth, how growth ring patterns change in response to these factors and whether species differ considerably in their responses.

Methods

Material

Nine species of perennial herbs from different families are used for the experiment (Table 1). Most of the species are widespread so that the results of this project can easily be expanded by investigations of natural populations in the field. The species all have permanent main roots to allow age-determination and analysis of annual ring width over a period of several years. Three categories of longevity and patterns of growth rings (Fig. 1), are equally represented by the chosen species (3 species per

Table 1. *Species selected for the project and their attributes. Characterisation of plant size and habitat preference follows Oberdorfer (1990).*

Species	Family	Life-form	Habitat	Category
Anchusa officinale L.	Boraginaceae	Semi-rosette forb, deep-rooting, up to 80 cm tall	Ruderal and other disturbed sites	Short-lived
Cichorium intybus L.	Asteraceae	Semi-rosette forb, deep-rooting, up to 120 cm tall	Ruderal and other disturbed sites	Short-lived
Trifolium pratense L.	Fabaceae	Low-growing forb, up to 40 cm tall	Ruderal sites, meadows, pastures	Short-lived
Digitalis grandiflora Mill	Scrophulariaceae	Tall-growing forb, up to 120 cm tall	Tall herb communities, forest edges	Long-lived
Sanguisorba minor L.	Rosaceae	Semi-rosette forb, up to 100 cm tall	Open vegetation, often on raw, calcareous soils	Long-lived
Silene vulgaris Garcke	Caryophyllaceae	Semi-rosette forb, up to 50 cm tall	Open vegetation, often on raw soils	Long-lived
Euphorbia esula L.	Euphorbiaceae	Clonal forb with lateral roots, deep-rooting, up to 60 cm tall	Ruderal sites with relatively low soil moisture	Problematic pattern
Galium verum L.	Rubiaceae	Clonal forb with lateral roots, deep-rooting, up to 70 cm tall	Meadows and pastures on calcareous soils	Problematic pattern
Hypericum perforatum L.	Hypericaceae	Medium-sized forb, up to 60 cm tall	Meadows, pastures, road verges etc. on nutrient-poor soils	Problematic pattern

category): short-lived species with clearly delineated growth rings, long-lived species with clearly delineated growth rings, and long-lived species with problematic anatomical patterns that resemble growth rings but need further analysis.

EXPERIMENTAL DESIGN

The development of the growth rings in the selected species will be analysed over a period of up to 11 years in controlled field experiments run at two different sites in Switzerland, at the Hönggerberg area of the Swiss Federal Institute of Technology in Zürich (north of the Alps) and near Oggio in the canton Ticino (south of the Alps). The Zürich site has a colder climate and soil moisture is usually higher as compared to the southern site near Oggio, although mean annual precipitation is higher south of the Alps (Table 2). The different treatments are applied to distinct plots within a common experimental area at

Table 2. Characterisation of the two experimental sites in northern (Hönggerberg) and southern (Oggio) Switzerland. At both sites all individuals were planted in September 2000. Climatic data from Imhof (1965-1978).

	Main site (Hönggerberg)	Replicate site (Oggio)
Location	47°26'N, 8°30'E	46°05'N, 8°59'E
Altitude	520 m asl	590 m asl
Site characteristics	Flat, sun-exposed area	Sun-exposed terrace on south-facing slope
	Loamy soil	Rich forest soil with very good drainage
Climatic region	Temperate	Temperate-mediterranean
Mean annual temperature	9 °C	11 °C
Mean annual precipitation	1100 mm	1800 mm

each site. At both sites two treatments (simulated herbivory (clipping) and control) and the six species with clearly delineated growth rings are used while the competition treatment and and the three species with problematic anatomical patterns are only used at the Zürich site.

In the competition treatments each individual is assigned an area of 100 cm^2 (8 individuals per species per plot) intended to result in considerable (intra)specific competition as compared to the other treatments where individuals were assigned an area of more than 600 cm^2 (3 individuals per species per plot). Herbivory will be simulated by clipping the plants once in 2001 and in 2002. In the control plots the plants will be left untreated.

DATA COLLECTION

The plots of a given treatment are replicated so as to provide one plot per harvest date. In the first two years (2002-2003) there will be 3 to 4 harvests per growth period ([April], June, August and October). Afterwards, the plots will be harvested every second year in October.

Every year in June or July and at each harvest date the number of shoots per plant and mean shoot length of all (remaining) individuals in all treatments will be measured (as a surrogate for aboveground biomass, cf. Dietz *et al.* 2001). In addition, the reproductive effort will be determined for each generative individual to examine whether growth increments are reduced due to allocation to sexual reproduction. At harvest time aboveground dry mass will be determined and, as a cumulative size parameter, we will determine the mean root diameter of the main root at 10 cm depth and in the crown region.

The main roots of the sampled individuals will be cut at 10 cm depth and close to the transition to the stem. The cuttings will be dyed with Phloroglucinol-HCl (Trendelenburg & Mayer-Wegelin 1955; Dietz & Ullmann 1997) that cause reddish colouring of lignified cell walls. Upon application of Phloroglucinol-HCl growth rings appear in good contrast in most plant species. The number of growth rings in the secondary xylem will be counted in each cutting. The growth ring increments (widths) will be measured along multiple radii and the total area associated with each growth ring will be determined using specialized image-analysis equipment.

Relevance of the research project for plant ecology

Our research project is indispensable for further development of herb-chronology because it will yield the necessary set of results on the development of growth rings in the roots of perennial herbs under various (controlled) conditions that is still missing. It will provide the first experimental verification of the annuality of growth rings in the secondary root xylem of perennial forbs, and will provide the first results on ontogenetic and environmentally-induced plasticity in the formation of annual growth ring sequences. For that our experiments include a range of test factors, that are generally regarded as crucial determinants for the development of plant populations, i.e. environmental gradients (climatic conditions), competition intensity and herbivory (removal of biomass). These experimental data will complement previous correlative results and, in conjunction with data from anatomical screenings, will form the basic reference for evaluation of growth rings in ongoing and future studies using herb-chronology as a reconstructive method in plant ecology.

Our results obtained so far suggest that there are thousands of species of dicotyledonous perennial herbs worldwide which are candidates for herb-chronology. Thus, herb-chronology may be an efficient reconstructive approach for the analysis of population development of a very wide variety of forb species that, if applicable, can generally contribute to our understanding of processes in plant ecology.

Acknowledgements

We thank M. Kammerlander who helped in obtaining seed material from different central european Botanical Gardens and T. Fotsch for his advice and technical assistance while growing the plants from seed and setting up the field site. S. Güsewell and an anonymous reviewer made valuable suggestions to improve the manuscript.

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Received 15 May 2001
Revised version accepted 2 July 2001